**M.Sc. Previous Year**

**Zoology, Paper - III**

# ENDOCRINOLOGY AND MOLECULAR CELL BIOLOGY

मध्यप्रदेश भोज (मुक्त) विश्वविद्यालय – भोपाल

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# SYLLABI-BOOK MAPPING TABLE

## Endocrinology and Molecular Cell Biology

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   E. Cellulose fibril synthesis and orientation
2. **Organization of Viral DNA, Bactrial DNA, Eukaryotic DNA, Palindromes, Split Genes, Transposons.**
3. **Gene Concepts and Genetic Code**
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   C. Membrance proteins, Golgi Sorting, Post translational Modifications.
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# INTRODUCTION

NOTES

Endocrinology is the study of the endocrine system in the human body. This is a system of glands that secrete hormones. Hormones are chemicals that affect the actions of different organ systems in the body. The endocrine system involves a number of feedback mechanisms, so that often one hormone (such as thyroid stimulating hormone) will control the action or release of another secondary hormone (such as thyroid hormone).

Hormones are essential for our every-day survival. They control our temperature, sleep, mood, stress, growth and more. Hormones are found in all organisms, i.e. plants and animals. They influence or control a wide range of physiological activities, such as, growth, development, puberty, level of alertness, sugar regulation and appetite, bone growth, etc. The way hormones work contribute to some of the major diseases of mankind like diabetes, thyroid conditions, pituitary conditions, appetite and obesity, bone problems, cancer, etc.

Cell and Molecular Biology studies the structure and function of the cell, which is the basic unit of life. Cell biology is concerned with the physiological properties, metabolic processes, signaling pathways, life cycle, chemical composition and interactions of the cell with their environment. This is done both on a microscopic and molecular level as it encompasses prokaryotic cells and eukaryotic cells. Molecular biology plays a critical role in the understanding of structures, functions, and internal controls within individual cells, all of which can be used to efficiently target new drugs, diagnose disease, and better understand cell physiology. Some clinical research and medical therapies arising from molecular biology are covered under gene therapy whereas the use of molecular biology or molecular cell biology in medicine is now referred to as molecular medicine.

This book is divided into four units that attempt to give the students a in depth knowledge of aims and scope of endocrinology, ontogeny and phylogeny of endocrine glands, neuroendocrine system and neurosecretion, general principles of hormone action, hormone structure and evolution, biosynthesis and secretion of hormones, hormone behaviour, role of hormone in development, and reproduction, bio membrane, cytoskeleton, cell - cell signalling, cell - cell adhesion and communication, cell cycle, cell matrix adhesion, organization of viral DNA, bacterial DNA, eukaryotic DNA, palindromes, split genes, transposons ,gene concepts and genetic code.The book follows the Self-Instructional Mode or SIM format wherein each unit begins with an 'Introduction' to the topic followed by an outline of the 'Objectives'. The detailed content is then presented in a simple and structured manner interspersed with Answers to ‘Check Your Progress’ questions. A list of ‘Key Terms’, a 'Summary' and a set of ‘Self-Assessment Questions and Exercises’ is also provided at the end of each unit for effective recapitulation.

# UNIT 1 ENDOCRINOLOGY - I

**Structure**



NOTES

## 1.0 INTRODUCTION

Endocrinology is a branch of biology and medicine dealing with the endocrine system, its diseases, and its specific secretions known as hormones. It is also concerned with the integration of developmental events proliferation, growth, and differentiation, and the psychological or behavioral activities of metabolism, growth and development, tissue function, sleep, digestion, respiration, excretion, mood, stress, lactation, movement, reproduction, and sensory perception caused by hormones.

The endocrine system consists of several glands, all in different parts of the body, that secrete hormones directly into the blood rather than into a duct system. Therefore, endocrine glands are regarded as ductless glands. Hormones have many different functions and modes of action; one hormone may have several effects on different target organs, and, conversely, one target organ may be affected by more than one hormone.

Hormones are found in all organisms with more than one cell, and so they are found in plants and animals. They influence or control a wide range of physiological activities, such as growth, development, puberty, level of alertness, sugar regulation and appetite, bone growth, etc.

In this unit you will study about aims and scope of endocrinology, phylogeny and ontogeny of endocrine glands, neuroendocrine system and neurosecretion, and general principles of hormone action.

NOTES

## 1.1 OBJECTIVES

After going through this unit, you will be able to:

- Understand the aims and scope of endocrinology.
- Explain phylogeny and ontogeny of endocrine glands.
- Comprehend neuroendocrine system and neurosecretion.
- Elaborate on general principles of hormone action.

## 1.2 ENDOCRINOLOGY: AN INTRODUCTION

Endocrinology (from endocrine + -ology) is a branch of biology and medicine dealing with the endocrine system, its diseases, and its specific secretions known as hormones. It is also concerned with the integration of developmental events proliferation, growth and differentiation, psychological or behavioral activities of metabolism, growth and development, tissue function, sleep, digestion, respiration, excretion, mood, stress, lactation, movement, reproduction, and sensory perception caused by hormones. Specializations include behavioral endocrinology and comparative endocrinology.

The endocrine system consists of several glands, all in different parts of the body, that secrete hormones directly into the blood rather than into a duct system. Therefore, endocrine glands are regarded as ductless glands. Hormones have many different functions and modes of action; one hormone may have several effects on different target organs, and, conversely, one target organ may be affected by more than one hormone.

### The Endocrine System

Endocrinology is the study of the endocrine system in the human body. This is a system of glands which secrete hormones. Hormones are chemicals that affect the actions of different organ systems in the body. Examples include thyroid hormone, growth hormone, and insulin. The endocrine system involves a number of feedback mechanisms, so that often one hormone (such as thyroid stimulating hormone) will control the action or release of another secondary hormone (such as thyroid hormone). If there is too much of the secondary hormone, it may provide negative feedback to the primary hormone, maintaining homeostasis.

In the original 1902 definition by Bayliss and Starling, they specified that, to be classified as a hormone, a chemical must be produced by an organ, be released (in small amounts) into the blood, and be transported by the blood to a distant organ to exert its specific function. This definition holds for most "classical" hormones, but there are also paracrine mechanisms (chemical communication between cells within a tissue or organ), autocrine signals (a chemical that acts on the same cell), and intracrine signals (a chemical that acts within the same cell). A neuroendocrine signal is a "classical" hormone that is released into the blood by a neurosecretory neuron (see article on neuroendocrinology).

## 1.2.1 Aims and Scope of Endocrinology

NOTES

Endocrinology is a branch of medicine that involves the study of hormones and its disorders. The endocrine system consists of several glands located in different parts of the body, that secrete hormones.

The endocrine disorders are a varied group of diseases that usually occur due to hypo-functioning or hyper-functioning of these glands.

Diabetes, the most common endocrine disorder encountered in clinical practice, occurs due to decreased secretion of insulin, a hormonal product from pancreas, an endocrine gland. The common endocrine disorders are given below.

1. **Diabetes**
2. **Thyroid Disorders** – Hypothyroidism, Hyperthyroidism, Thyroid Tumors
3. **Pituitary Disorders**– Pituitary tumors, raised pituitary hormone levels (for example Prolactin, GH, etc.), deficiency of pituitary hormones, diabetes insipidus
4. **Adrenal Disorders**– Adrenal tumors, Cushing's Syndrome, Pheochromocytoma, Adrenal failure
5. Polycystic Ovary Syndrome and other causes of Hirsutism and Virilisation
6. **Male Reproductive Disorders**– Male infertility, erectile dysfunction, Hypogonadism
7. **Disorders of Growth**– Growth hormone deficiency and other causes of short stature, growth hormone excess and other causes of excessive gain in height
8. **Disorders of Puberty**– Delayed puberty, precocious (premature) puberty
9. **Disorders of Sexual Development**– Small penis, hidden testis, genital ambiguity, gynecomastia (development of breast tissue in males), Turner's Syndrome, Klinefelter's Syndrome
10. **Metabolic Bone Disorders**– Osteoporosis, vitamin D deficiency, Rickets, Osteomalacia, Hyperparathyroidism
11. Obesity and overweight
12. **Dyslipidemia**– Disorders related to cholesterol
13. **Other Hormonal Disorders**– Insulinoma, Neuroendocrine Tumors, MEN Syndrome, PGA Syndrome, etc.

### What Does the Endocrine System Do?

Endocrine glands release hormones into the bloodstream. This lets the hormones travel to cells in other parts of the body.

The endocrine hormones help control mood, growth and development, the way our organs work, metabolism, and reproduction.

The endocrine system regulates how much of each hormone is released. This can depend on levels of hormones already in the blood, or on levels of other substances in the blood, like calcium. Many things affect hormone levels, such as, stress, infection, and changes in the balance of fluid and minerals in blood.

Too much or too little of any hormone can harm the body. Medicines can treat many of these problems.

**What Are the Parts of the Endocrine System?**

NOTES

While many parts of the body make hormones, the major glands that make up the endocrine system are given below:

- Hypothalamus
- Pituitary
- Thyroid
- Parathyroids
- Adrenals
- Pineal Body
- Ovaries
- Testes

The pancreas is a part of the endocrine system both and the digestive system both. That's because it secretes hormones into the bloodstream, and makes and secretes enzymes into the digestive tract.

**Hypothalamus:** The hypothalamus is in the lower central part of the brain. It links the endocrine system and nervous system. Nerve cells in the hypothalamus make chemicals that control the release of hormones secreted from the pituitary gland. The hypothalamus gathers information sensed by the brain (such as, the surrounding temperature, light exposure, and feelings) and sends it to the pituitary gland. This information influences the hormones that the pituitary makes and releases.

**Pituitary:** The pituitary gland is at the base of the brain, and is no bigger than a pea. Despite its small size, the pituitary is often called the "master gland." The hormones it makes control many other endocrine glands.

The pituitary gland makes many hormones, such as:

- Growth hormone, which stimulates the growth of bone and other body tissues and plays a role in the body's handling of nutrients and minerals
- Prolactin which activates milk production in women who are breastfeeding
- Thyrotropin which stimulates the thyroid gland to make thyroid hormones
- Corticotropin , which stimulates the adrenal gland to make certain hormones
- Antidiuretic hormone, which helps control body water balance through its effect on the kidneys
- Oxytocin, which triggers the contractions of the uterus that happen during labor pain in women

The pituitary also secretes endorphins, chemicals that act on the nervous system and reduce feelings of pain. The pituitary also secretes hormones that signal the reproductive organs to make sex hormones. The pituitary gland also controls ovulation and the menstrual cycle in women.

**NOTES**

**Thyroid:** The thyroid is situated at the front part of the lower neck. It's shaped like a bow tie or butterfly. It makes the thyroid hormones thyroxine and tri-iodo-thyronine. These hormones control the rate at which cells burn fuels from food to make energy. The more thyroid hormone there is in the bloodstream, the faster chemical reactions happen in the body.

Thyroid hormones are important because they help kids' and teens' bones to grow and develop, and they also play an important role in the development of the brain and nervous system.

**Parathyroids:** Attached to the thyroid are four tiny glands that work together called the parathyroids. They release parathyroid hormone, which controls the level of calcium in the blood with the help of calcitonin, which the thyroid makes.

**Adrenal Glands:** These two triangular adrenal glands sit on top of each kidney. The adrenal glands have two parts, each of which makes a set of hormones and has a different function:

1. The outer part is the **adrenal cortex**. It makes hormones called corticosteroids that help control salt and water balance in the body, the body's response to stress, metabolism, the immune system, and sexual development and function.
2. The inner part is the **adrenal medulla**. It makes catecholamines such as epinephrine. Also called adrenaline, epinephrine increases blood pressure and heart rate when the body is under stress.

**Pineal:** The pineal body, also called the pineal gland, is in the middle of the brain. It secretes melatonin, a hormone that may help to regulate when we sleep at night and wake in the morning.

**Reproductive Glands:** The gonads are the main source of sex hormones. In boys the male gonads, or testes are in the scrotum. They secrete hormones called androgens the most important of which is testosterone. In a male these hormones determine that when it's time to make the changes associated with puberty, like penis and height growth, deepening voice, and growth in facial and pubic hair. Working with hormones from the pituitary gland, testosterone also tells a boy's body when it's time to make sperm in the testes.

A girl's gonads, the ovaries, are in her pelvis. They make eggs and secrete the female hormones estrogen and progesterone. Estrogen is involved when a girl starts puberty. During puberty, a girl will have breast growth, start to accumulate body fat around the hips and thighs, and have a growth spurt. Estrogen and progesterone are also involved in the regulation of a girl's menstrual cycle. These hormones also play a role in pregnancy.

**Pancreas:** The pancreas makes insulin and glucagon, which are hormones that control the level of glucose, or sugar, in the blood. Insulin helps keep the body supplied with stores of energy. The body uses this stored energy for exercise and activity, and it also helps organs work as they should.

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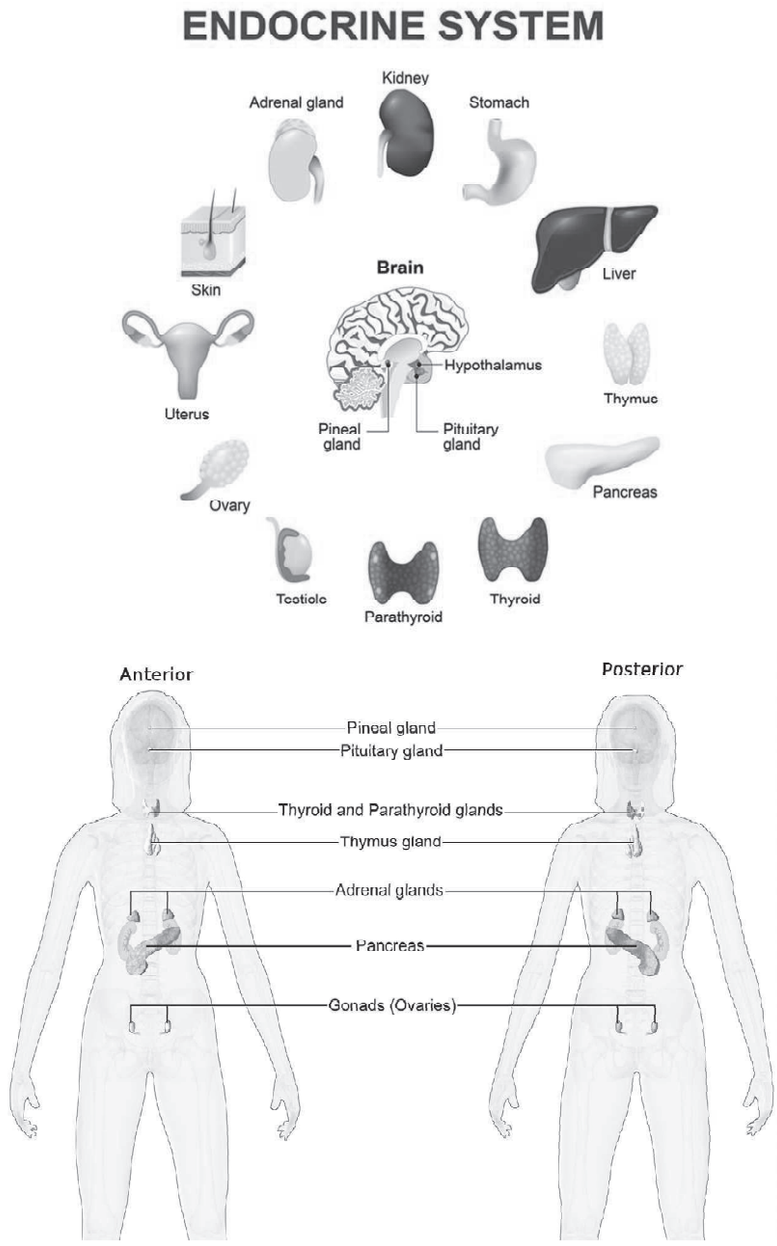
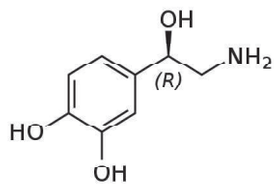



***Fig. 1.1** Endocrine System*

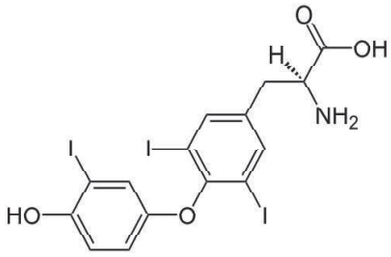
## Hormones

Griffin and Ojeda identify three different classes of hormones based on their chemical composition:

## Amines



**Norepinephrine**




*Triiodothyronine*

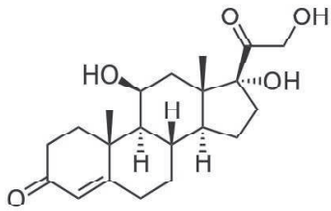
*Examples of Amine Hormones*

Amines, such as norepinephrine, epinephrine, and dopamine (catecholamines), are derived from single amino acids, in this case tyrosine. Thyroid hormones such as 3,5,3'-triiodothyronine (T3) and 3,5,3',5'-tetraiodothyronine (thyroxine, T4) make up a subset of this class because they derive from the combination of two iodinated tyrosine amino acid residues.

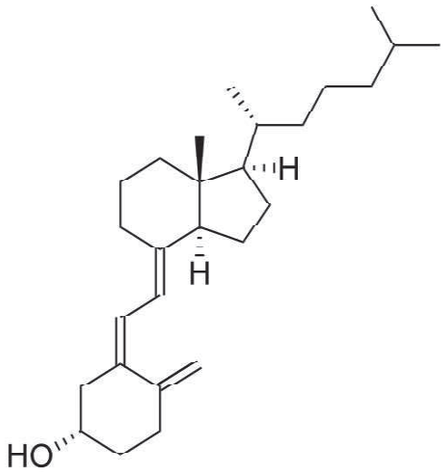
**Peptide and Protein**

Peptide hormones and protein hormones consist of three (in the case of thyrotropin-releasing hormone) to more than 200 (in the case of follicle-stimulating hormone) amino acid residues and can have a molecular mass as large as 31,000 grams per mole. All hormones secreted by the pituitary gland are peptide hormones, as are leptin from adipocytes, ghrelin from the stomach, and insulin from the pancreas.

**Steroid**




**Cortisol**




**Vitamin D3**

*Examples of Steroid Hormones*

**NOTES**

NOTES

Steroid hormones are converted from their parent compound, cholesterol. Mammalian steroid hormones can be grouped into five groups by the receptors to which they bind: glucocorticoids, mineralocorticoids, androgens, estrogens, and progestogens. Some forms of vitamin D, such as, calcitriol, are steroid-like and bind to homologous receptors, but lack the characteristic fused ring structure of true steroids.

### 1.2.2 Hormones as Messengers

Hormones are chemical in nature so they are called chemical messengers. They travel in bloodstream to tissues or organs. They work slowly, over time, and affect many different processes, including endocrine glands, which are special groups of cells which make hormones. The major endocrine glands are the pituitary, pineal, thymus, thyroid, adrenal glands, and pancreas. On the ventral side of diencephalon hypothalamus is present. It consists of scattered masses of grey matter in the white matter. The neurons present in grey matter are secretory in nature and are called hypothalmic nuclei. These nuclei secrete several hormones called neurohormones or Releasing Factors (RF) in the blood. Blood carries these hormones to the anterior pituitary where the neurohormones stimulate pituitary to release its hormone. In this way the neurohormones transmit the information from hypothalamus to the pituitary gland and thus act as messenger. The following neurohormones which are secreted by hypothalamus act as messenger:

1. Thyrotropin Releasing Hormone (TRH OR TRF)
2. Prolactin Releasing Hormone (PRH)
3. Corticotropin Releasing Hormone (CRH)
4. Prolactin Inhibiting Hormone (PIH)(Dopamine)
5. Growth Hormone Releasing Hormone (GHRH)
6. Gonadotropin Releasing Hormone (GnRH)
7. Growth Hormone Inhibiting Hormone (GHIH)(Somatostatin)

The aforesaid hormones after being released from hypothalamus reach their target organ, the adenohypophysis and then the adenohypophysis releases the hormones which in turn reach their target organ and induce it to release their own hormones. For example the TRH after being release from hypothalamus reaches the adenohypophysis of pituitary gland and induces it to secrete Thyroid Stimulating Hormone (TSH). This hormone reaches thyroid gland through blood and induces it to release thyroxin.

**First Messenger:** Protein and peptide hormones catecholamines like epinephrine and eicosanoids such as prostaglandins find their receptors present on plasma membrane of the target cells. Binding the hormone to receptor of plasma membrane initiates a series of events which lead to activate the so called second messenger. So, the hormones which lead to activation of second messenger are called first messenger.

**Hormones as Messengers:** On the ventral side of diencephalon (last part of fore brain) hypothalamus is present. It consists of number of scattered masses of grey matter in the white matter. Masses of grey matter containing neurons form hypothalamic nuclei. The neurons (neuro secretory cells) of hypothalamic nuclei

secrete several hormones called neuro hormones (releasing factors) into the blood. Blood carries these neuro hormones to the anterior pituitary where the neuro hormones stimulate pituitary to release various hormones. Thus, neuro hormones act as messengers.

NOTES

### Hormones as Regulators (Feedback Control)

This mechanism is known as homeostasis. Blood carries hormones from the endocrine gland to the target organ. Decrease or increase in the amount of hormone in the blood has an effect on the concerned gland (secreting gland) to reduce or increase the secretion of the specific hormone. This mechanism is known as feedback control.

It is of two types:

1. Positive Feedback Control
2. Negative Feedback Control

To understand the mechanism involved in feedback control, we can take case of thyroxine secretion.

### 1. Positive Feedback Control

Thyroxine hormone is secreted by thyroid gland. The Thyrotropin Releasing Hormone (TRH) from the hypothalamus stimulates the anterior pituitary to secrete Thyroid Stimulating Hormone (TSH). TSH in turn stimulates the thyroid gland to secrete thyroxine. If the level of thyroxine in blood is less than normal, this low thyroxine level stimulates hypothalamus to secrete more TRH. This results in increased secretion of TSH which in turn stimulates increased secretion of thyroxine. Such regulatory mechanism is called positive feedback control.

### 2. Negative Feedback Control

If the level of thyroxine in blood is more than the normal, this high thyroxine level produces an inhibitory effect on hypothalamus. As a result, less TRH and then less TSH are produced by hypothalamus and anterior pituitary respectively. This results a decrease in thyroxine. This is called negative feedback control.

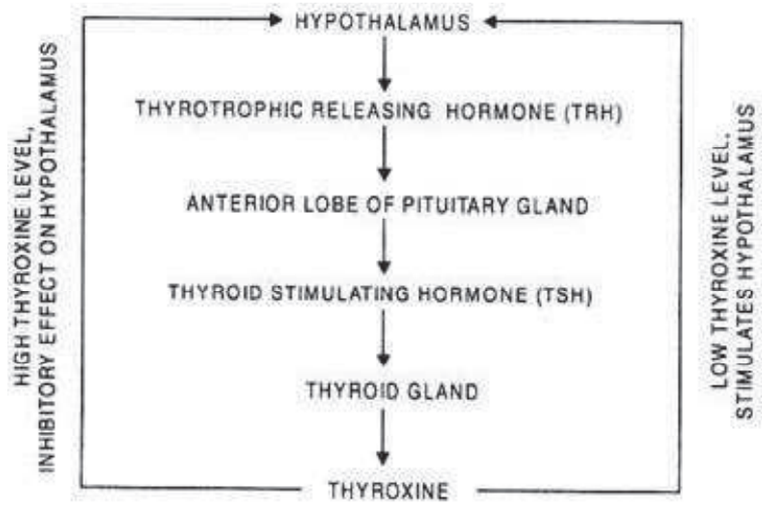



***Fig. 1.2*** *Feedback Control*

NOTES

Hormones regulate internal functions from metabolism and growth to sexual development and the induction of birth. They circulate through the bloodstream, bind to target cells, and adjust the function of whole tissues and organs. It all starts with the hypothalamus and the pituitary gland, the masters of the endocrine system. The hormones they release control the secretions of the other endocrine glands and most endocrine functions. Throughout the body, hormones enable reactions to stress and other outside changes and keep regular processes running smoothly.

### 1.2.3 Hormones and Eukaryotic Metabolic Regulation

Blood glucose levels vary widely over the course of a day as periods of food consumption alternate with periods of fasting. Insulin and glucagon are the two hormones primarily responsible for maintaining homeostasis of blood glucose levels. Additional regulation is mediated by the thyroid hormones.

#### Regulation of Blood Glucose Levels: Insulin and Glucagon

Cells of the body require nutrients in order to function. These nutrients are obtained through feeding. In order to manage nutrient intake, storing excess intake, and utilizing reserves when necessary, the body uses hormones to moderate energy stores. Insulin is produced by the beta cells of the pancreas, which are stimulated to release insulin as blood glucose levels rise (for example, after a meal is consumed). Insulin lowers blood glucose levels by enhancing the rate of glucose uptake and utilization by target cells, which use glucose for ATP production. It also stimulates the liver to convert glucose to glycogen, which is then stored by cells for later use. As insulin binds to its target cell via insulin receptors and signal transduction, it triggers the cell to incorporate glucose transport proteins into its membrane. This allows glucose to enter the cell, where it can be used as an energy source. These actions mediated by insulin cause blood glucose concentrations to fall, called a hypoglycemic, or "low sugar" effect, which inhibits further insulin release from beta cells through a negative feedback loop.

Impaired insulin function can lead to a condition called diabetes mellitus, which has many effects on the body. It can be caused by low levels of insulin production by the beta cells of the pancreas, or by reduced sensitivity of tissue cells to insulin. This prevents glucose from being absorbed by cells, causing high levels of blood glucose, or hyperglycemia (high sugar). High blood glucose levels make it difficult for the kidneys to recover all the glucose from nascent urine, resulting in glucose being lost in urine. High glucose levels also result in less water being reabsorbed by the kidneys, causing high amounts of urine to be produced; this may result in dehydration. Over time, high blood glucose levels can cause nerve damage to the eyes and peripheral body tissues, as well as damage to the kidneys and cardiovascular system. Oversecretion of insulin can cause hypoglycemia, low blood glucose levels. This causes insufficient glucose availability to cells, often leading to muscle weakness. It can sometimes cause unconsciousness or death if left untreated.

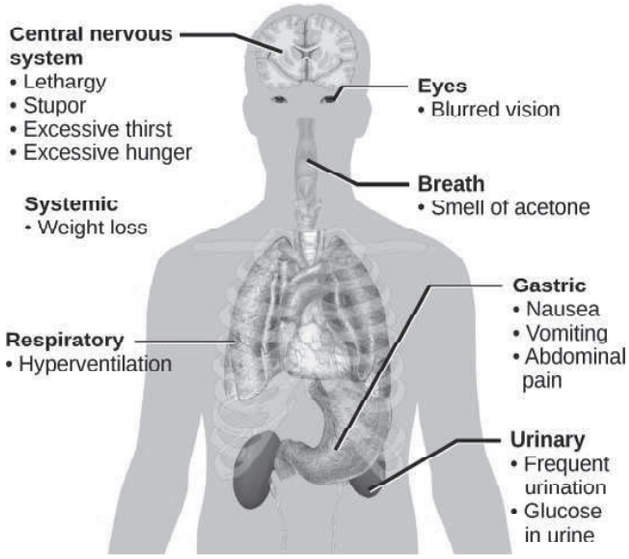



***Fig. 1.3*** *Diabetes Mellitus*

**Diabetes Mellitus:** Diabetes mellitus can cause a wide range of symptoms, including nausea, vomiting, blurred vision, lethargy, a frequenturination, and high levels of glucose in the urine.

When blood glucose levels decline below normal levels, for example between meals or when glucose is utilized during exercise, the hormone glucagon is released from the pancreas. Glucagon raises blood glucose levels, eliciting what is called a hyperglycemic effect, by stimulating the breakdown of glycogen to glucose in skeletal muscle cells and liver cells in a process called glycogenolysis. Glucose can then be utilized as energy by muscle cells and released into circulation by the liver cells. Glucagon also stimulates absorption of amino acids from the blood by the liver, which then converts them to glucose. This process of glucose synthesis is called gluconeogenesis. Rising blood glucose levels inhibit further glucagon release by the pancreas via a negative feedback mechanism. In this way, insulin and glucagon work together to maintain homeostatic glucose levels.

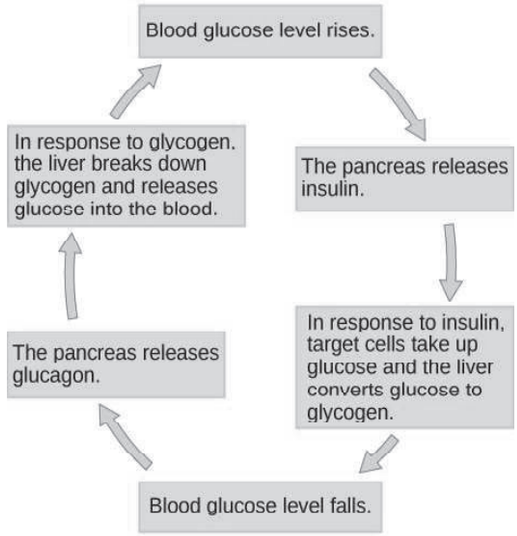



***Fig. 1.4*** *Regulation of Blood Glucose Levels by Insulin and Glucagon*

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**Regulation of Blood Glucose Levels by Insulin and Glucagon**: As the levels of glucose in the blood rise, insulin stimulates the cells to take up more glucose and signals the liver to convert the excess glucose to glycogen, a form in which it can be stored for later use. When the levels of glucose in the blood fall, glucagon responds by stimulating the breakdown of glycogen into glucose and signals the production of additional glucose from amino acids.

**Regulation of Blood Glucose Levels: Thyroid Hormones**

The basal metabolic rate, which is the amount of calories required by the body at rest, is determined by two hormones produced by the thyroid gland: thyroxine, also known as tetraiodothyronine or T4, and tri-iodothyronine, also known as T3. T3 and T4 release from the thyroid gland are stimulated by Thyroid-Stimulating Hormone (TSH), which is produced by the anterior pituitary. These hormones affect nearly every cell in the body except for the adult brain, uterus, testes, blood cells, and spleen. They are transported across the plasma membrane of target cells where they bind to receptors on the mitochondria, resulting in increased ATP production. In the nucleus, T3and T4activate genes involved in energy production and glucose oxidation. This results in increased rates of metabolism and body heat production. This is known as the hormone's calorigenic effect.

Disorders can arise from both the underproduction and overproduction of thyroid hormones. Hypothyroidism, underproduction of the thyroid hormones, can cause a low metabolic rate leading to weight gain, sensitivity to cold, and reduced mental activity, among other symptoms. In children, hypothyroidism can cause cretinism, which can lead to mental retardation and growth defects. Hyperthyroidism, the overproduction of thyroid hormones, can lead to an increased metabolic rate, which may cause weight loss, excess heat production, sweating, and an increased heart rate.

Hormones regulate metabolic activity in various tissues. They are one kind of mechanism for signalling among cells and tissues. Hormones can be defined as signalling molecules that one cell releases into the peripheral fluid or bloodstream, which alter the metabolism of the same or another cell. Hormones are distinguished from communication mechanisms that depend on direct cell cell contact through gap junctions. Hormones are also distinguished from neurotransmitters, although this distinction is somewhat artificial. Neurotransmitters can act as hormones and vice versa.

Hormones act by binding to receptors, which are usually protein molecules. Receptors have two functions: first, they bind the hormone, and secondly, they transduce (change the type of) the signal to affect the metabolism of the recipient cell. The ability of a cell to respond to a hormone depends on two properties of the receptor molecule: how many of them are on a particular cell, and how well they bind the hormone. The first property is called the receptor number, and the second is called the affinity of the receptor for the hormone. The biochemical responsiveness of a cell to a hormone (or a drug, or a neurotransmitter) depends on the number of occupied receptor son the responsive cell.

## 1.2.4 Classification of Hormones

The following three categories of classification of hormones:

1. According to Chemical Nature
2. According to Origin
3. According to Nature of Action

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### 1. According to Chemical Nature

The hormones are classified into following main classes according to the chemical structures:

### A. Steroid Hormones

Steroid hormones are the hormones which are the derivative of cholesterol which includes sex hormones and hormones of the adrenal cortex. They are comprised of three groups which include glucocorticoids, mineralcorticoids and sex hormones (Testosterone, Estrogen, and Progesterone).

Steroids also play roles in inflammatory responses, stress responses, bone metabolism, cardiovascular fitness, behavior, cognition, and mood.

### B. Amine Hormones

Amino acid these are hormones derived from amino acids. Many of the amino acid hormones are neurotransmitters. The hormones derived from amino acids are thyroid hormones (T3, T4) and the hormones of the adrenal medulla (epinephrine, norepinephrine).

### C. Peptide Hormones

Protein Hormones or peptide hormones are prepared from polymers of amino acids. Most of these hormones encourage other glands to create hormones. They are also significant in regulation of metabolism e.g., Oxytocin and vasopressin.

### 2. According to Origin

Mostly reproductive hormones are primarily derived from four major organs or system (Hypothalamus, Anterior and posterior lobe of pituitary gland, Gonads (testis and ovary including their interstitial tissues and corpus luteum), Placenta and Uterus.

### 3. According to Nature of Action

a) **General Hormones:** Growth hormone influence nearly all the body tissues, similar is the case with Thyroid and Insulin hormones, hence they fall in general category.

b) **Specific Hormones:** These hormones affect functions of specific organs, e.g., FSH and androgens.

c) **Local Hormones:** Prostaglandins, Acetyl cholin, Histamine act locally to their site of production.

## Chemical Classification of Hormones

The hormones are classified into following main classes according to the chemical structures:

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### Pitutary Hormones

- **Oxytocin:** The basic functions of oxytocin are as follows:
  - It causes uterine contraction.
  - It causes milk ejection in lactating females.
  - It responds to suckling reflex and estradiol.
  - It lowers steroid synthesis in testes.
- **Vasopressin (Antidiuretic Hormone, ADH):** The major functions of vasopression are as follows:
  - It responds to osmoreceptor, which senses extracellular [$Na^+$].
  - It regulates blood pressure.
  - It increases $H_2O$ re-absorption from distal tubules in kidney.
- **Melanocyte-Stimulating Hormones (MSH):** The major function of melanocyte is of pigmentation.
- **Corticotropin (Adrenocorticotropin, ACTH)L:** The major functions of corticotrophin are as follows:
  - It stimulates cells of adrenal gland.
  - It increases the steroid synthesis and secretion.
- **Lipotropin (LPH):** Lipotropin basically increases the fatty acid release from the adipocytes.
- **Thyrotropin (Thyroid-Stimulating Hormone, TSH):** It acts on thyroid follicle cells to stimulate thyroid hormone synthesis.
- **Growth Hormone (GH):** The major functions of the growth hormone are as follows:
  - It acts as a general anabolic stimulant.
  - It increases the release of insulin-like growth factor-I (IGF-I).
  - It leads to cell growth and bone sulfation.
- **Prolactin (PRL):** The major functions of prolactin are as follows:
  - It stimulates the differentiation of secretory cells of mammary gland.
  - It stimulates the milk synthesis.
- **Luteinizing Hormone (LH):** The major functions of luteinizing hormones are as follows:
  - It increases the ovarian progesterone synthesis.
  - It acts on Leydig cells of testes to increase the testosterone synthesis.
  - It releases and increases the interstitial cell development.
- **Follicle-Stimulating Hormone (FSH):** The major functions of follicle stimulating hormone are as follows:

- It assists in the ovarian follicle development and ovulation.
- It increases the estrogen production.
- It acts on Sertoli cells of semi-ferous tubule to increase spermatogenesis.

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**Hypothalamic Hormones**

- **Somatostatin (SIF):** It inhibits GH and TSH secretion.

**Thyroid hormones**

- **Thyroxine and Triiodothyronine:** It responds to TSH and stimulates oxidations in many cells.
- **Calcitonin:** It is produced in parafollicular C cells of the thyroid and it regulates $Ca^{2+}$ and Pi metabolism.
- **Calcitonin Gene-Related Oeptide (CGRP):** It acts as a vasodilator.

**Parathyroid hormone**

- **Parathyroid Hormone (PTH):** The basic functions of parathyroid hormone are as follows:
  - It regulates $Ca^{2+}$ and Pi metabolism.
  - It stimulates bone resorption.
  - It increasesng serum [$Ca^{2+}$].
  - It stimulates Pi secretion through the kidneys.

**Adipose Tissue Hormones**

- **Leptin:** The major functions of leptin are as follows:
  - It regulates the overall body weight by limiting the food intake.
  - It increases the energy expenditure.
  - It regulates the neuroendocrine axis, inflammatory responses, blood pressure and bone mass.
- **Adiponectin:** This hormone assists in increasing the major biological actions of insulin sensitivity and fatty acid oxidation.
- **Resistin:** It induces the insulin resistance.

**Hormones and peptides of the gut**

- **Glucagon-Like Peptide 1 (GLP-1):** The major functions of Glucagon are as follows:
  - It potentiates the glucose-dependent insulin secretion.
  - It inhibits glucagon secretion.
  - It inhibits gastric emptying.
- **Glucose-Dependent Insulinotropic Polypeptide (GIP):** It inhibits secretion of gastric acid and it enhances the insulin secretion.
- **Ghrelin:** The major functions of ghrelin are as follows:
  - It assists in appetite stimulation.
  - It stimulates the NPY release.

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- It regulates the energy homeostasis, glucose metabolism, gastric secretion and emptying.
- It helps in insulin secretion.

- **Obestatin:** It acts in the opposition to ghrelin action on appetite.
- **Gastrin:** It is produced by stomach antrum and it stimulates acid and pepsin secretion and it also stimulates the pancreatic secretions.
- **Secretin:** It is secreted from duodenum at pH values below 8.5 and it stimulates pancreatic acinar cells to release bicarbonate and $H_2O$.
- **Cholecystokinin (CCK):** It stimulates gallbladder contraction and bile flow and it increases the secretion of digestive enzymes from pancreas.
- **Motilin:** It controls gastrointestinal muscles.
- **Vasoactive Intestinal Peptide (VIP):** It is produced by hypothalamus and GI tract, and it relaxes the GI. It even inhibits the acid and pepsin secretion and acts as a neurotransmitter in peripheral autonomic nervous system. It increases the secretion of $H_2O$ and electrolytes from the pancreas and gut.
- **Somatostatin:** It inhibits the release and action of numerous gut peptides and it also inhibits the insulin and glucagon secretion from the pancreas.
- **Peptide Tyrosine (PYY):** It inhibits gastric motility by inhibiting cholinergic neurotransmission and it inhibits the gastric acid secretion.
- **Neuro-Peptide Tyrosine (NPY):** It effects the hypothalamic function of appetite and it controls the feeding behaviour and energy homeostasis.

**Pancreatic Hormones**

- **Insulin:** It is produced by β-cells of the pancreas and it increases the glucose uptake and utilization and lipogenesis.
- **Glucagon:** It is produced by α-cells of the pancreas and it increases lipid mobilization and glycogenolysis in order to increase the blood glucose levels.
- **Pancreatic polypeptide:** It increases glycogenolysis and it regulates the gastrointestinal activity.
- **Somatostatin:** It assists in the inhibition of glucagon and somatotropin release.

**Placental Hormones**

- **Estrogens:** It helps in the maintenance of pregnancy.
- **Progestins:** It mimics the action of progesterone.
- **Chorionic Gonadotropin:** Its activities are similar to luteinizing hormone.
- **Placental Lactogen:** It acts like prolactin and GH.
- **Relaxin:** It is produced in ovarian corpus luteum and it inhibits myometrial contractions.

**Gonadal Hormones**

- **Androgens (Testicular):** It helps in the maturation and function of male secondary sex organs.
- **Inhibins A and B:** It inhibits FSH secretion.

**Adrenal Cortical Hormones**

- **Glucocorticoids:** It has diverse effects on inflammation and protein synthesis.
- **Mineralocorticoids:** It helps in maintaining salt balance.

**Adrenal Medullary Hormones**

- **Epinephrine (Adrenalin):** It increases glycogenolysis, lipid mobilization, smooth muscle contraction, cardiac function.
- **Norepinephrine (Noradrenalin):** It assists in lipid mobilization, arteriole contraction and it also acts as neurotransmitter in the CNS.

**Liver Hormones**

- **Angiotensin II:** It is responsible for the essential hypertension through stimulated synthesis.

**Kidney Hormones**

- **Calcitriol [1,25-(OH)2-vitamin D3]:** It is responsible for maintenance of calcium and phosphorous homeostasis and it increases the intestinal $Ca^{2+}$ uptake and regulates the bone mineralization.

**Cardiac Hormones**

- **Atrial Natriuretic Peptide (ANP):** It is released from heart atria in response to hypovolemia and it also acts on the outer adrenal cells to decrease the aldosterone production.

**Pineal Hormones**

**Melatonin:** It regulates the circadian rhythms.

**General Considerations of Hormones**

The hormones generally act as a body catalyst like enzymes. However, they differ from the enzymes in the following ways:

- They are not always protein in nature.
- The hormones are secreted in the blood stream prior to use, because, circulating levels can indicate, the activity of endocrine gland and the exposure of target organs.

Some other features of hormones are as follows:

- **Action in Low Concentration:** Hormones act in a very low concentration like vitamins.
- **Storage Destruction and Excretion:** Hormones are not ordinarily stored, except in the gland, of origin. They do not have any cumulative action, because they are destroyed and excreted as soon as their functions are over. Some hormones work quickly and are destroyed quickly like thyroxine.

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## Mode of Action Role of C-AMP and Hormone Action

3'–5' c-AMP plays a unique role in the action of many protein hormones. Its level may be decreased or increased by hormonal action as the effect varies depending on the tissue. The hormones such as glucagon, catecholarnines, PTH, etc. act by influencing a change in the intracellular c-AMP concentration through the adenylate cyclase- c-AMP system. Different types of membrane receptors remain associated with either Gs or Gi type of GTP dependent trimeric nucleotide regulatory complexes of the membrane. Both Gs and Gi are made up of three subunits: Gs contains as 3y while Gi contains a 3y. Formation of the receptor-hormone complex promotes the binding of GTP with the subunit of either Gs or Gi. When a GTP is released, it binds the adenylate cyclase located on the cytoplasmic surface of the membrane and changes its conformation to activate it. However, in some cells calmodulin 4 Ca+" is also required for activation. Adenylate cyclase catalyzes the conversion of ATP to c-AMP thus increasing the intracellular concentration of the latter.

On the other hand, aI-GTP inhibits adenylate cyclase by binding it. This lowers the intracellular concentration of c-AMP.

## Role of Calcium in Hormone Action

The action of most of the protein hormones are inhibited in the absence of calcium even though ability to increase or decrease c-AMP is comparatively unimpaired. Thus, calcium may have more terminal signal for hormone action than c-AMP. It is suggested that the ionized calcium of the cytosol is the important signal. The source of this calcium may be extracellular fluid or it may arise from mobilization of intracellular tissue bound calcium. The hormone receptor binding may directly inhibit the 'Ca++-_ATPase.' It may also directly open up voltage-independent Ca++ channels in the membrane to increase the diffusion of Ca++ in the cell down its inward concentration, which then acts as a second messenger to affect the cellular activities. The receptor-hormone complex may produce ITP, which in turn can increase cytosolic $Ca^{l+}$ concentration by enhancing the mobilization of Ca from mitochondrial and endoplasmic reticular pools. Calcium is involved in the regulation of several enzymes. All these enzymes have special biochemical metabolic roles. Ca++ also changes membrane permeability. Many of its effects are mediated through its binding to -Ca++-dependent regulatory proteins like calmodulin and troponin.

## Regulation of Hormone Secretion

Hormone secretion is strictly under control of several mechanisms.

### Neuroendocrinal Control Mechanism

Nerve impulses control some endocrine secretions. Cholinergic sympathetic fibers stimulate catecholamine secretion from adrenal medulla. Centers in the midbrain, brainstem, hippocampus, etc. can send nerve impulses. At the terminations of these neurons they release acetylcholine and biogenic amines to regulate the

secretions of hypophysiotropic peptide hormones from hypothalamic peptidergic neurons. Some of the endocrine releases are controlled by either stimulatory or inhibitory hormones from a controlling gland, e.g., corticosteroids are controlled by corticotropin and thyroid hormones are controlled by thyrotopin from anterior pituitary. The tropins are further regulated by hypothalamic releasing hormones.

**Feedback Control Mechanism**

It is mainly due to negative feedback that such control is brought about. When there is a high blood level of target gland hormones, it may inhibit the secretion of the tropic hormones stimulating that gland. Adrenal cortex secretes a hormone called cortisol, which brings about the inhibition of secretion of corticotropin from the anterior pituitary and corticotropin releasing hormone from the hypothalamus by a long-loop feedback. This leads to the reduction in cortisol secretion.

## 1.2.5 Discovery of Hormones

A hormone (from the Greek, "Setting in Motion") is any member of a class of signalling molecules in multicellular organisms that are transported to distant organs to regulate physiology and behavior. Hormones are required for the correct development of animals, plants and fungi. The lax definition of a hormone (as a signalling molecule that acts distant from its site of production) means that many different classes of molecule can be defined as hormones. Among the substances that can be considered hormones, are eicosanoids (e.g., prostaglandins and thromboxanes), steroids (e.g., oestrogen and brassinosteroid), amino acid derivatives (e.g., epinephrine and auxin), protein / peptides (e.g. insulin and CLE peptides) and gases (e.g., ethylene and nitrous oxide).

Hormones are used to communicate between organs and tissues. In vertebrates, hormones are responsible for the regulation of many physiological processes and behavioral activities such as digestion, metabolism, respiration, sensory perception, sleep, excretion, lactation, stress induction, growth and development, movement, reproduction, and mood manipulation. In plants, hormones modulate almost all aspects of development, from germination to senescence.

Hormones affect distant cells by binding to specific receptor proteins in the target cell, resulting in a change in cell function. When a hormone binds to the receptor, it results in the activation of a signal transduction pathway that typically activates gene transcription, resulting in increased expression of target proteins. Hormones can also act in rapid, non-genomic pathways that can be synergistic with genomic effects. Water-soluble hormones (such as peptides and amines) generally act on the surface of target cells via second messengers. Lipid soluble hormones, (such as steroids) generally pass through the plasma membranes of target cells (both cytoplasmic and nuclear) to act within their nuclei. A notable exception to this are brassinosteroids in plants, which despite being lipid soluble, still bind to their receptor at the cell surface.

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In vertebrates, endocrine glands are specialized organs that secrete hormones into the endocrine signalling system. Hormone secretion occurs in response to specific biochemical signals and is often subject to negative feedback regulation. For instance, high blood sugar (serum glucose concentration) promotes insulin synthesis. Insulin then acts to reduce glucose levels and maintain homeostasis, leading to reduced insulin levels. Upon secretion water soluble hormones are readily transported through the circulatory system. Lipid-soluble hormones must bond to carrier plasma glycoproteins (e.g., Thyroxine-Binding Globulin (TBG)) to form ligand-protein complexes. Some hormones are completely active when released into the bloodstream (as is the case for insulin and growth hormones), while others are prohormones that must be activated in specific cells through a series of activation steps that are commonly highly regulated. The endocrine system secretes hormones directly into the bloodstream, typically via fenestrated capillaries, whereas the exocrine system secretes its hormones indirectly using ducts. Hormones with paracrine function diffuse through the interstitial spaces to nearby target tissue.

Plants lack specialized organs for the secretion of hormones, although there is special distribution of hormone production. For example, the hormone auxin is produced mainly at the tips of young leaves and in the shoot apical meristem. The lack of specialised glands means that the main site of hormone production can change throughout the life of a plant, and the site of production is dependent on the plant's age and environment.

## Discovery

### Arnold Adolph Berthold (1849)

Arnold Adolph Berthold was a German physiologist and zoologist, who, in 1849, had a question about the function of the testes. He noticed that in castrated roosters that they did not have the same sexual behaviors as roosters with their testes intact. He decided to run an experiment on male roosters to examine this phenomenon. He kept a group of roosters with their testes intact, and saw that they had normal sized wattles and combs (secondary sexual organs), a normal crow, and normal sexual and aggressive behaviors. He also had a group with their testes surgically removed, and noticed that their secondary sexual organs were decreased in size, had a weak crow, did not have sexual attraction towards females, and were not aggressive. He realized that this organ was essential for these behaviors, but he did not know how. To test this further, he removed one testis and placed it in the abdominal cavity. The roosters acted and had normal physical anatomy. He was able to see that location of the testes do not matter. He then wanted to see if it was a genetic factor that was involved in the testes that provided these functions. He transplanted a testis from another rooster to a rooster with one testis removed, and saw that they had normal behavior and physical anatomy as well. Berthold determined that the location or genetic factors of the testes do not matter in relation to sexual organs and behaviors, but that some chemical in the testes being secreted is causing this phenomenon. It was later identified that this factor was the hormone testosterone.

### Charles and Francis Darwin (1880)

Although known primarily for his work on the Theory of Evolution, Charles Darwin was also keenly interested in plants. Through the 1870s, he and his son Francis studied the movement of plants towards light. They were able to show that light is perceived at the tip of a young stem (the coleoptile), whereas the bending occurs lower down the stem. They proposed that a 'transmissible substance' communicated the direction of light from the tip down to the stem. The idea of a 'transmissible substance' was initially dismissed by other plant biologists, but their work later led to the discovery of the first plant hormone. In the 1920s Dutch scientist Frits Warmolt Went and Russian scientist Nikolai Cholodny (working independently of each other) conclusively showed that asymmetric accumulation of a growth hormone was responsible for this bending. In 1933 this hormone was finally isolated by Kögl, Haagen-Smit and Erxleben and christened 'auxin'.

### Bayliss and Starling (1902)

William Bayliss and Ernest Starling, a physiologist and biologist, respectively, wanted to see if the nervous system had an impact on the digestive system. They knew that the pancreas was involved in the secretion of digestive fluids after the passage of food from the stomach to the intestines, which they believed to be due to the nervous system. They cut the nerves to the pancreas in an animal model and discovered that it was not nerve impulses that controlled secretion from the pancreas. It was determined that a factor secreted from the intestines into the bloodstream was stimulating the pancreas to secrete digestive fluids. This factor was named secretin: a hormone, although the term hormone was not coined until 1905 by Starling.

**Check Your Progress**

1. Define endocrinology.
2. What is a hormone?
3. Which is the most common endocrine disorder?
4. What is the work of endocrine glands?

## 1.3 PHYLOGENY OF ENDOCRINE GLANDS

Endocrine glands are ductless glands of the endocrine system that secrete their products, hormones, directly into the blood. The major glands of the endocrine system include the pineal gland, pituitary gland, pancreas, ovaries, testes, thyroid gland, parathyroid gland, hypothalamus and adrenal glands. The hypothalamus and pituitary glands are neuroendocrine organs.

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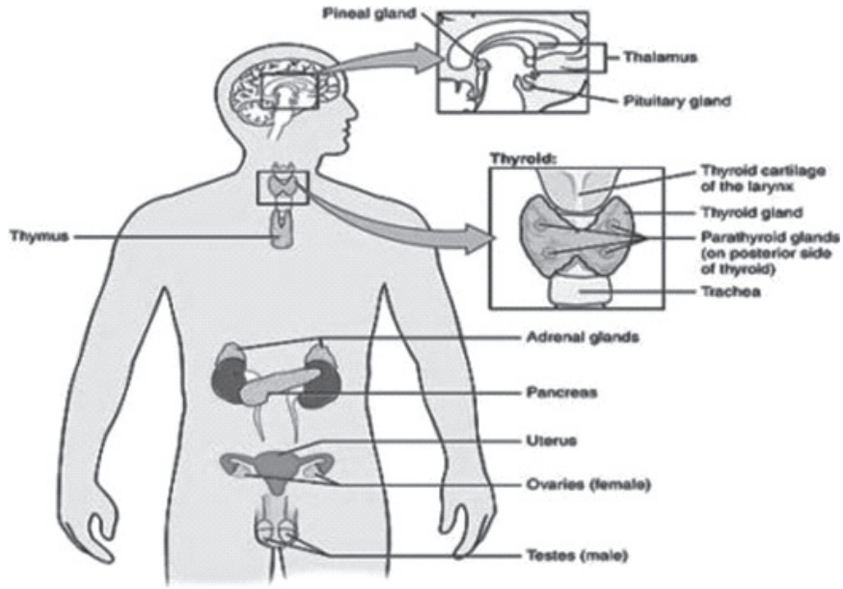


***Fig. 1.5*** *Endocrine Glands*

## Pituitary Gland

The pituitary gland hangs from the base of the brain by the pituitary stalk, and is enclosed by bone. It consists of a hormone-producing glandular portion of the anterior pituitary and a neural portion of the posterior pituitary, which is an extension of the hypothalamus. The hypothalamus regulates the hormonal output of the anterior pituitary and creates two hormones that it exports to the posterior pituitary for storage and later release.

Four of the six anterior pituitary hormones are tropic hormones that regulate the function of other endocrine organs. Most anterior pituitary hormones exhibit a diurnal rhythm of release, which is subject to modification by stimuli influencing the hypothalamus.

Somatotropic Hormone or Growth Hormone (GH) is an anabolic hormone that stimulates the growth of all body tissues especially skeletal muscle and bone. It may act directly, or indirectly via Insulin-like Growth Factors (IGFs). GH mobilizes fats, stimulates protein synthesis, and inhibits glucose uptake and metabolism. Secretion is regulated by Growth Hormone-Releasing Hormone (GHRH) and Growth Hormone-Inhibiting Hormone (GHIH), or somatostatin. Hypersecretion causes gigantism in children and acromegaly in adults; hyposecretion in children causes pituitary dwarfism.

Thyroid-stimulating hormone promotes normal development and activity of the thyroid gland. Thyrotropin-releasing hormone stimulates its release; negative feedback of thyroid hormone inhibits it.

Adrenocorticotropic hormone stimulates the adrenal cortex to release corticosteroids. Adrenocorticotropic hormone release is triggered by corticotropin-releasing hormone and inhibited by rising glucocorticoid levels.

The gonadotropins—follicle-stimulating hormone and luteinizing hormone regulate the functions of the gonads in both the sex. Follicle-stimulating hormone

stimulates sex cell production; luteinizing hormone stimulates gonadal hormone production. Gonadotropin levels rise in response to gonadotropin-releasing hormone. Negative feedback of gonadal hormones inhibits gonadotropin release.

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Prolactin promotes milk production in human females. Its secretion is prompted by prolactin-releasing hormone and inhibited by prolactin-inhibiting hormone.

The intermediate lobe of the pituitary gland secretes only one enzyme that is melanocyte stimulating hormone. It is linked with the formation of the black pigment in our skin called melanin.

The neurohypophysis stores and releases two hypothalamic hormones:

- Oxytocin stimulates powerful uterine contractions, which trigger labour and delivery of an infant, and milk ejection in nursing women. Its release is mediated reflexively by the hypothalamus and represents a positive feedback mechanism.
- Antidiuretic hormone stimulates the kidney tubules to reabsorb and conserve water, resulting in small volumes of highly concentrated urine and decreased plasma osmolality. Antidiuretic hormone is released in response to high solute concentrations in the blood and inhibited by low solute concentrations in the blood. Hyposecretion results in diabetes insipidus.

### Thyroid Gland

The thyroid gland is located in the front of the neck, attached with thyroid cartilage, and is shaped like a butterfly, with two wings connected by a central isthmus. Thyroid tissue consists of follicles with a stored protein called colloid, containing (thyroglobulin), a precursor to other thyroid hormones, which are manufactured within the colloid.

The thyroid hormones increase the rate of cellular metabolism, and include thyroxine (T4) and triiodothyronine (T3). Secretion is stimulated by the thyroid-stimulating hormone, secreted by the anterior pituitary. When thyroid levels are high, there is negative feedback that decreases the amount of Thyroid-stimulating hormone secreted. Most T4 is converted to T3 (a more active form) in the target tissues.

Calcitonin, produced by the parafollicular cells of the thyroid gland in response to rising blood calcium levels, depression blood calcium levels by inhibiting bone matrix resorption and enhancing calcium deposit in bones. Excessive secretion cause hyperthyroidism and deficiency cause hypothyroidism.

### Parathyroid Glands

The parathyroid glands, of which there are 4–6, are found on the back of the thyroid glands, and secrete parathyroid hormone, this causes an increase in blood calcium levels by targeting bone, the intestine, and the kidneys. The parathyroid hormone is the antagonist of calcitonin. Parathyroid hormone release is triggered by falling blood calcium levels and is inhibited by rising blood calcium levels.

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## Adrenal Glands

The adrenal glands are located above the kidneys in humans and in front of the kidneys in other animals. The adrenal glands produce a variety of hormones including adrenaline and the steroids aldosterone cortisol and Dehydroepiandrosterone Sulfate (DHEA). Adrenaline increases blood pressure, heart rate, and metabolism in reaction to stress, the aldosterone controls the body's salt and water balance, the cortisol plays a role in stress response and the dehydroepiandrosterone sulfate (DHEA) produces aids in production of body odor and growth of body hair during puberty.

## Pancreas

The pancreas, located in the abdomen, below and behind the stomach, is both an exocrine and an endocrine gland. The alpha and beta cells are the endocrine cells in the pancreatic islets that release insulin and glucagon and smaller amounts of other hormones into the blood. Insulin and glucagon influence blood sugar levels. Glucagon is released when the blood glucose level is low and stimulates the liver to release glucose into the blood. Insulin increases the rate of glucose uptake and metabolism by most body cells.

Somatostatin is released by delta cells and acts as an inhibitor of GH, insulin, and glucagon.

## Gonads

The ovaries of the female, located in the pelvic cavity, release two main hormones. Secretion of estrogens by the ovarian follicles begins at puberty under the influence of follicle-stimulating hormone. Estrogens stimulate the maturation of the female reproductive system and the development of secondary sexual characteristics. Progesterone is released in response to high blood levels of luteinizing hormone. It works with estrogens in establishing the menstrual cycle.

The testes of the male begin to produce testosterone at puberty in response to luteinizing hormone. Testosterone promotes maturation of the male reproductive organs, development of secondary sex characteristics such as increased muscle and bone mass, and the growth of body hair.

## Pineal Gland

The pineal gland is located in the diencephalon of the brain. It primarily releases melatonin, which influences daily rhythms and may have an antigonadotropic effect in humans. It may also influence the melanotropes and melanocytes located in the skin.

## Other Hormone-Producing Structures

Many body organs not normally considered endocrine organs contain isolated cell clusters that secrete hormones. Examples include the heart (atrial natriuretic peptide); gastrointestinal tract organs (gastrin, secretin, and others); the placenta (hormones of pregnancy—estrogen, progesterone, and others); the kidneys (erythropoietin and renin); the thymus; skin (cholecalciferol); and adipose tissue (leptin and resistin).

**Check Your Progress**

5. Which gland is situated at the front part of the lower neck?
6. Why are the hormones called chemical messengers?
7. What is the effect of diabetes mellitus?
8. How does the parathyroid hormone effect human body?

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## 1.4 ONTOGENY OF ENDOCRINE GLANDS

Ontogeny is development of a single individual, or a system within the individual, from the fertilized egg to death (Smith, 1960), i.e., a total life history including embryonic and postnatal (Gould, 1977). This term should be distinguished from phylogeny, which is a type of development involving modification of a species or a group of species, i.e., 'the family history' or the evolutionary history of a lineage (Gould, 1977).

Endocrine system, any of the systems found in animals for the production of hormones, substances that regulate the functioning of the organism. Such a system may range, at its simplest, from the neurosecretory, involving one or more centres in the nervous system, to the complex array of glands found in the human endocrine system.

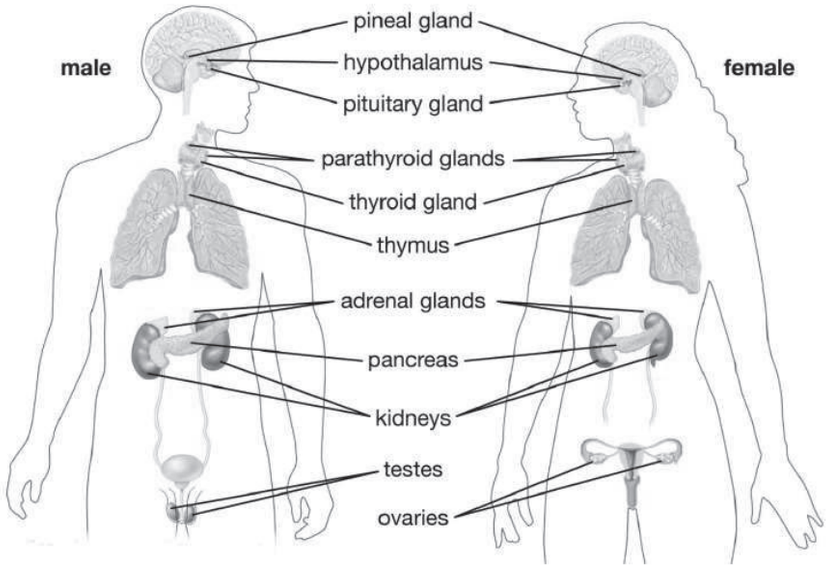


***Fig. 1.6*** *Endocrine Gland in Male and Female*

Comparative endocrinologists investigate the evolution of endocrine systems and the role of these systems in animals' adaptation to their environments and their production of offspring. Studies of nonmammalian animals have provided information that has furthered research in mammalian endocrinology, including that of humans. For example, the actions of a pituitary hormone, prolactin, on the control of body water and salt content were first discovered in fishes and later led to the demonstration of similar mechanisms in mammals. The mediating

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role of local ovarian secretions (paracrine function) in the maturation of oocytes (eggs) was discovered in starfishes and only later extended to vertebrates. The important role of thyroid hormones during embryonic development was first studied thoroughly in tadpoles during the early 1900s. In addition, the isolation and purification of many mammalian hormones was made possible in large part by using other vertebrates as bioassay systems; that is, primitive animals have served as relatively simple, sensitive indicators of the amount of hormone activity in extracts prepared from mammalian endocrine glands. Finally, some vertebrate and invertebrate animals have provided "model systems" for research that have yielded valuable information on the nature of hormone receptors and the mechanisms of hormone action. For example, one of the most intensively studied systems for understanding hormone actions on target tissues has been the receptors for progesterone and estrogens (hormones secreted by the gonads) from the oviducts of chickens.

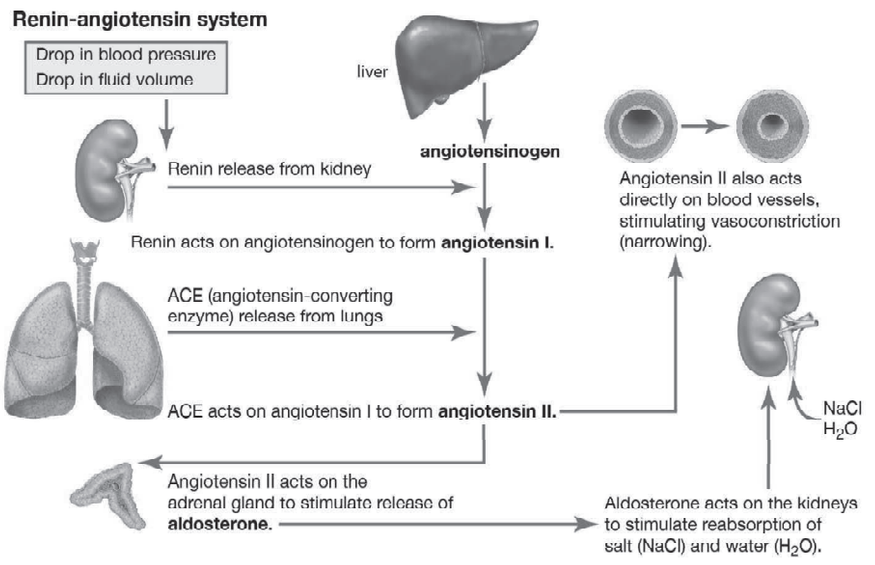


***Fig. 1.7** Renin-angiotensin System*

An understanding of how the endocrine system is regulated in nonmammals also provides essential information for regulating natural populations or captive animals. Artificial control of salmon reproduction has had important implications for the salmon industry as a whole. Some successful attempts at reducing pest insect species have been based on the knowledge of pheromones. Understanding the endocrinology of a rare species may permit it to be bred successfully in captivity and thus prevent it from becoming extinct. Future research may even lead to the reintroduction of some endangered species into natural habitats.

From an evolutionary point of view, the neuroendocrine cells of the gastrointestinal tract are closely related to the peptide—hormone-producing neurons of the central (and peripheral) nervous system and to those of the parenchyma of the classic endocrine glands. Their phylogeny and ontogeny give significant information regarding such concepts as the brain-gut axis, the enteroinsular axis, the pathogenesis of gastrointestinal carcinoids, islet cell tumors, syndromes

of multiple endocrine neoplasia, and neuroendocrine differentiation in traditionally nonendocrine carcinomas.

Transgenic tools help to monitor the ontogeny of endocrine function both under normal conditions, and when an embryo experiences embryonic stressors as can occur in complicated human pregnancies. The effects of altered thyroid hormone levels on thyrotrope development can be examined and also the establishment of negative feedback regulation within the Hypothalamic Pituitary Thyroid (HPT) axis. The Hh signalling may affect pituitary function and due to the consequences of this the endocrine regulation may cause osmotic balance.

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The term Gastro-enteropancreatic (GEP) system is commonly used which emphasize a system of endocrine cells involving the stomach, intestine, and pancreas. The term EnteroPancreatic (EP) system is applied to the endocrine cells of the intestine and pancreas of lampreys and hagfish, for both of these agnathans lack a stomach (Youson, 1999). The EP system is more appropriate for other fishes without a stomach.

**Check Your Progress**

9. Define ontogeny.
10. Define Gastro-Entero Pancreatic (GEP) system

## 1.5 NEUROENDOCRINE SYSTEM AND NEUROSECRETION

Neuroendocrinology is the branch of biology (specifically of physiology) which studies the interaction between the nervous system and the endocrine system, i.e., how the brain regulates the hormonal activity in the body. The nervous and endocrine systems often act together in a process called neuroendocrine integration, to regulate the physiological processes of the human body. Neuroendocrinology arose from the recognition that the brain, especially the hypothalamus, controls secretion of pituitary gland hormones, and has subsequently expanded to investigate numerous interconnections of the endocrine and nervous systems.

The neuroendocrine system is the mechanism by which the hypothalamus maintains homeostasis, regulating reproduction, metabolism, eating and drinking behaviour, energy utilization, osmolarity and blood pressure.

### Neuroendocrine System

Major neuroendocrine systems include the following:

1. Hypothalamic–Pituitary–Adrenal Axis (HPA Axis)
2. Hypothalamic–Pituitary–Thyroid Axis (HPT Axis)
3. Hypothalamic–Pituitary–Gonadal Axis (HPG Axis)
4. Hypothalamic–Neurohypophyseal System

### Hypothalamus

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The endocrine system consists of numerous glands throughout the body that produce and secrete hormones of diverse chemical structure, including peptides, steroids, and neuroamines. Collectively, hormones regulate many physiological processes.

Oxytocin and vasopressin (also called anti-diuretic hormone), the two neurohypophysial hormones of the posterior pituitary gland (the neurohypophysis), are secreted from the nerve endings of magnocellular neurosecretory cells into the systemic circulation. The cell bodies of the oxytocin and vasopressin neurons are in the paraventricular nucleus and supraoptic nucleus, respectively, and the electrical activity of these neurons is regulated by afferent synaptic inputs from other brain regions. By contrast, the hormones of the anterior pituitary gland (the adenohypophysis) are secreted from endocrine cells that, in mammals, are not directly innervated, yet the secretion of these hormones (adrenocorticotrophic hormone, luteinizing hormone, follicle-stimulating hormone, thyroid-stimulating hormone, prolactin, and growth hormone) remains under the control of the hypothalamus. The hypothalamus controls the anterior pituitary gland via releasing factors and release-inhibiting factors; these are blood-borne substances released by hypothalamic neurons into blood vessels at the base of the brain, at the median eminence. These vessels, the hypothalamo-hypophysial portal vessels, carry the hypothalamic factors to the anterior pituitary, where they bind to specific receptors on the surface of the hormone-producing cells.

For example, the secretion of growth hormone is controlled by two neuroendocrine systems: the Growth Hormone-Releasing Hormone (GHRH) neurons and the somatostatin neurons, which stimulate and inhibit GH secretion, respectively. The GHRH neurones are located in the arcuate nucleus of the hypothalamus, whereas the somatostatin cells involved in growth hormone regulation are in the periventricular nucleus. These two neuronal systems project axons to the median eminence, where they release their peptides into portal blood vessels for transport to the anterior pituitary. Growth hormone is secreted in pulses, which arise from alternating episodes of GHRH release and somatostatin release, which may reflect neuronal interactions between the GHRH and somatostatin cells, and negative feedback from growth hormone.

The neuroendocrine systems control reproduction in all its aspects, from bonding to sexual behaviour. They control spermatogenesis and the ovarian cycle, parturition, lactation, and maternal behaviour. They control the body's response to stress and infection. They regulate the body's metabolism, influencing eating and drinking behaviour, and influence how energy intake is utilised, that is, how fat is metabolised. They influence and regulate mood, body fluid and electrolyte homeostasis, and blood pressure.

The neurons of the neuroendocrine system are large; they are mini factories for producing secretory products; their nerve terminals are large and organised in coherent terminal fields; their output can often be measured easily in the blood; and what these neurons do and what stimuli they respond to are readily open to hypothesis and experiment. Hence, neuroendocrine neurons are good "model systems" for studying general questions, like "how does a neuron regulate the synthesis, packaging, and secretion of its product?" and "how is information encoded in electrical activity?"

## Pituitary Gland

The pituitary gland is divided into two sections: the anterior pituitary and the posterior pituitary. The hypothalamus controls the anterior pituitary's hormone secretion by sending trophic hormones down the hypothalamohypophysial portal system. For example, thyrotropin-releasing hormone stimulates the secretion of thyroid-stimulating hormone by the anterior pituitary.

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The posterior pituitary is innervated by the hypothalamus; the hormones oxytocin and vasopressin are synthesized by neuroendocrine cells in the hypothalamus and stored at the nerves' ends in the posterior pituitary. They are secreted directly into systemic circulation by the hypothalamic neurons.

## Neuroendocrine Control Mechanisms

Neuroendocrine control mechanisms are observed in all animals that possess a nervous system. Recent analyses of neuroendocrine functions in invertebrate model systems reveal a great degree of similarity between phyla as far apart as nematodes, arthropods, and chordates. Developmental studies that emphasize the comparison between different animal groups will help to shed light on questions regarding the evolutionary origin and possible homologies between neuroendocrine systems.

## Endocrine and Neuroendocrine Cells

Cells in multicellular animals communicate through signalling mechanisms that take place at direct intercellular contacts, or that involve signals released systemically into the extracellular space where they diffuse over large distances and are able to affect targets far removed from the signalling source. The first mechanism, communication of cells that are in direct contact, is developed to a state of high complexity in the nervous system. Here, a multitude of signals in the form of neurotransmitters chemically couple networks of neurons at specialized cell–cell contacts, the synapses. The second mechanism of cell–cell communication defines the endocrine system. It involves secreted signals, hormones that affect target cells in a less directed way, since all cells expressing receptors for a given hormone will react when that hormone is released. The endocrine system in bilaterian animals consists of multiple specialized cell populations, sometimes compacted into glands that are found in all parts of the body, and are derived from all three germ layers (Tombes 1970, Highnam & Hill 1977, Gorbman et al. 1983, Laufer & Downer 1988). Endocrine glands regulate a large number of homeostatic mechanisms. They include the activity of neurons, muscles, and pigment cells during specific behaviors (food intake, fight and flight, and reproduction), the activity of visceral muscle and exocrine glands (digestion), the control of major metabolic pathways (synthesis, storage, and release of carbohydrates and lipids), the control of the ionic milieu through absorption and excretion, the formation and maturation of gametes, and growth and regeneration of the body. In many instances, endocrine glands form an integrated system in which hormonal production and release is controlled through feedback loops.

Most hormones found throughout the animal kingdom are short polypeptides, produced by proteolytic cleavage from larger precursor proteins, called prohormones. Similar to other secreted proteins, peptide (pro) hormones are

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produced in the rough endoplasmic reticulum, processed through the Golgi apparatus, and stored in membrane-bound vesicles. These vesicles, 100–300 nm in size, give peptide hormone-producing cells their characteristic granular appearance (Golding & Pow 1988, Thorndyke & Georges 1988). Peptide hormone receptors belong to the class of seven pass transmembrane, G-protein-coupled receptors. Beside peptides, lipids and amino acid derivatives act as hormones. The steroid hormones (e.g., cortisone or estrogen in vertebrates and ecdysone in arthropods) are derived from the lipid cholesterol. Juvenile hormone in insects is an ether derivative of a polyunsaturated fatty acid. Like other lipids, these hormones are synthesized in the smooth ER and are not stored in vesicles. Steroid hormone receptors belong to a class of transcription factors, called nuclear receptors that are localized in the cytoplasm in their inactive state; upon ligand binding, they will enter the nucleus and bind to DNA, thereby modulating gene expression (Schulster et al. 1976).

### Neurosecretion

Neurosecretion is the storage, synthesis and release of hormones from neurons. These neurohormones, produced by neurosecretory cells, are normally secreted from nerve cells in the brain that then circulate into the blood. These neurohormones are similar to nonneural endocrine cells and glands in that they also regulate both endocrine and nonendocrine cells. Neurosecretion cells synthesize and package their product in vesicles and exocytose them at axon endings just as normal neurons do, but release their product farther from their target than normal neurons (which release their neurotransmitters short distances at synapses), typically releasing their neurohormones into the circulatory system to reach their distant targets.

### Discovery

In 1928, Ernst Scharrer hypothesized that neurosecretory neurons in the hypothalamus of teleost fish, *Phoxinus laevis*, had secretory activity similar to that of endocrine gland cells. As more became known about neurosecretory cells, the difference between the actions of nerve communication and endocrine hormone release become less clear. Like the average neuron, these cells conduct electrical impulses along the axon but unlike these neurons, neurosecretion produces neurohormones that are released into the body's circulation. Combining the properties of the nervous and endocrine, these cells have the capacity to affect nerves through chemical messengers. Neurosecretion is a broad area of study and must be further observed to be better understood.

### Insects

Insects play a large role in what is known about neurosecretion. In simpler organisms neurosecretion mechanisms regulate the heart, the process of metamorphosis, and directly influences the development of the gonadal function. In more advanced organisms the gonadal function is manipulated by the intermediary endocrine processes. Axons from neurosecretory cells trace to 'Corpora Cardiaca' and 'Corpora Allata' and produce and secrete a brain hormone which insect

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physiologists suspect is bound to a large carrier protein. Although the function is unknown, there are a multitude of these cells found in the ventral ganglia of the nerve cord. Neurosecretory cells, found in clusters in the medial and lateral parts of the brain, control corpora allata activity by producing juvenile hormone during the larval or nymphal instars, the phase between periods of molting in insects. The production of this hormone inhibits the insect during the conversion to maturity and reactivating once the fully-grown adult is prepared for reproduction.

In insect physiology and anatomy, the corpus allatum (plural: corpora allata) is an endocrine gland that generates juvenile hormone; as such, it plays a crucial role in metamorphosis. Surgical removal of the corpora allata (an allatectomy) can cause an immature larva to pupate at its next molt, resulting in a miniature adult. Similarly, transplantation of corpora allata from a young larva to a fully mature larva can greatly extend the larval stage, resulting in an equivalent to gigantism.

In many Diptera species, the corpus allatum is fused with the corpus cardiacum, forming a "ring gland", also known as Weismann's ring.

In Lepidoptera species, the corpus allatum acts as a release site for prothoracicotropic hormone which is generated by the brain.

**Check Your Progress**

11. What is neuroendocrinology?
12. How do the cells in multicellular animals communicate?
13. What is neurosecretion?

## 1.6 GENERAL PRINCIPLES OF HORMONE ACTION

Hormones are chemical messengers that enter the blood directly upon their secretion from endocrine glands. A single gland or cell may secrete multiple hormones and multiple glands may secrete the same single hormone. Hormones, produced by glands or cells, are messengers which act locally or at a distance to coordinate the function of cells and organs. The hormones are basically categorised in the following two broad types:

1. **Peptides (Hypothalamic Releasing Factors) and Proteins (Insulin, Growth Hormone):** These generally interact with membrane receptors located on the cell surface, causing activation of downstream signalling pathways leading to alteration in gene transcription or modulation of biochemical pathways to affect a physiological response.
2. **Steroids (Cortisol, Progesterone, Testosterone, Oestradiol) and Other Lipophilic Substances (Vitamin D, Retinoic Acid, Thyroid Hormone):** These act by crossing the plasma membrane to interact with intracellular receptors, with hormone action via nuclear receptors altering cellular gene expression directly.

The endocrine system is comprised of a system releasing hormones that act as chemical messengers. The major classes of hormones include the following:

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**Amine Hormones** – Amine hormones are derivative of the amino acid tyrosine.

**Thyroid Hormones** – The thyroid gland is located in the lower neck, wrapped around the trachea. Consists of follicles that secrete the two iodine-containing hormones Thyroxine (T4) and Triiodothyronine (T3). These Thyroid Hormones (TH) regulate oxygen consumption, growth and brain development. T4 is secreted in larger amounts but is mostly converted to T3, the more active form.

**Adrenal Medullary Hormones** – There are 2 adrenal glands, one on top of each kidney. Each gland has 2 endocrine parts, an inner adrenal medulla, and a surrounding adrenal cortex. The medulla secretes Epinephrine (E) and NorEpinephrine (NE) which are catecholamines. These hormones exert actions similar to those of sympathetic nerves. More epinephrine than norepinephrine is secreted.

**Peptide Hormones** – The majority of hormones. Initially synthesized as larger preprohormones that are then cleaved to prohormones in the ER. The prohormone is then cleaved to the active hormone in the Golgi. These hormones may also serve as neurotransmitters. Calcitonin, a peptide hormone secreted by parafollicular cells of the thyroid gland, participates in the regulation of blood $Ca^{2+}$ level.

**Steroid Hormones** – Steroid hormones are produced by the adrenal cortex and the gonads.

**Adrenal Cortex Hormones** – Aldosterone participates in mineral balance (mineralocorticoid) by controlling the handling of $Na^+$, $K^+$, and $H^+$ ions by the kidney. Cortisol and corticosterone affect the metabolism of glucose (glucocorticoid) and other organic nutrients. Cortisol also affects stress responses and regulation of the immune system. Adrenal androgens are less potent than the other androgen, testosterone.

**Hormones of the Gonads** – Testosterone is the major androgen secreted by the testes. The major female hormone, 'Estradiol' is secreted by the ovaries and is derived from androgens.

### Hormone Transport in Blood

Water-soluble hormones are transported dissolved in blood plasma while others circulate in blood, bound to plasma proteins. The free hormone diffuses across capillary walls to encounter its target cells.

### Hormone Metabolism and Excretion

The concentration of a hormone in plasma depends upon its rate of secretion and rate of removal. Hormones are either excreted by kidneys or metabolized in blood or the target cells.

### Mechanisms of Hormone Action

A hormone is a secreted chemical messenger that enables communication between cells and tissues throughout the body. Hormones reach all tissues via blood but only cells that have receptors to bind the hormone act as target cells for the hormone. A low concentration of hormones is compensated for by an increase in the number of receptors – up-regulation while a high concentration of hormones leads to a decrease in the number of receptors – down-regulation. A hormone can reduce

the number of receptors available for a second hormone, resulting in decreased effectiveness of the second hormone 'Antagonism'. A hormone can also induce an increase in the number of receptors for a second hormone, increasing the latter's effectiveness (permissiveness).

- Hormones are released into the bloodstream through which they travel to target sites.
- The target cell has receptors specific to a given hormone and will be activated by either a lipid-soluble (permeable to plasma membrane) or water-soluble hormone (binds to a cell-surface receptor).
- Lipid-soluble hormones diffuse through the plasma membrane to enter the target cell and bind to a receptor protein.
- Water-soluble hormones bind to a receptor protein on the plasma membrane of the cell.
- Receptor stimulation results in a change in cell activity, which may send feedback to the original hormone-producing cell.

A hormone is a chemical messenger that enables communication between cells. Hormones are secreted by the glands of the endocrine system and they serve to maintain homeostasis and to regulate numerous other systems and processes, including reproduction and development.

**Hormone Signalling**

The glands of the endocrine system secrete hormones directly into the extracellular environment. The hormones then diffuse to the bloodstream via capillaries and are transported to the target cells through the circulatory system. This allows hormones to affect tissues and organs far from the site of production or to apply systemic effects to the whole body.

Hormone-producing cells are typically specialized and reside within a particular endocrine gland, such as thryocytes in the thyroid gland. Hormones exit their cell of origin through the process of exocytosis or by other means of membrane transport.

Cellular recipients of a particular hormonal signal may be one of several cell types that reside within a number of different tissues. This is so in the case of insulin, which triggers a diverse range of systemic physiological effects. Different tissue types may also respond differently to the same hormonal signal. As a result, hormonal signalling is elaborate and hard to dissect.

Hormones activate target cells by diffusing through the plasma membrane of the target cells (lipid-soluble hormones) to bind a receptor protein within the cytoplasm of the cell, or by binding a specific receptor protein in the cell membrane of the target cell (water-soluble proteins). In both cases, the hormone complex will activate a chain of molecular events within the cell that will result in the activation of gene expression in the nucleus.

The reaction of the target cells may then be recognized by the original hormone-producing cells, leading to a down-regulation in hormone production. This is an example of a homeostatic negative feedback loop.

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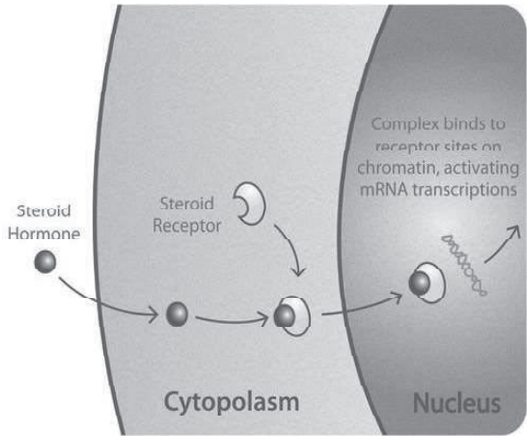


***Fig. 1.8*** *Hormone Signalling*

**Lipid-Soluble Hormone Receptor Activation**: Nuclear hormone receptors are activated by a lipid-soluble hormone, such as estrogen, binding to them inside the cell. Lipid-soluble hormones can cross the plasma membrane.

Steps of Hormonal Signalling are as follows:

- Biosynthesis of a particular hormone in a particular tissue
- Storage and secretion of the hormone
- Transport of the hormone to the target cells, tissues, or organs
- Recognition of the hormone by an associated cell membrane or an intracellular receptor protein
- Relay and amplification of the received hormonal signal via a signal transduction process
- Potential feedback to a hormone-producing cell

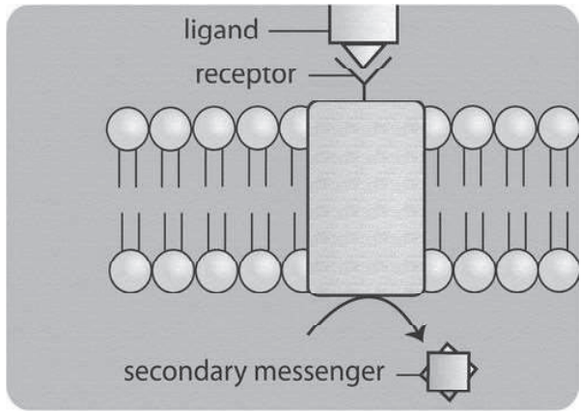


***Fig. 1.9*** *Lipid- Soluble Hormone Receptor Activation*

**Water-Soluble Hormone Receptor Activation**: Water-soluble hormones, such as epinephrine, bind to a cell-surface localized receptor, initiating a signalling cascade using intracellular second messengers.

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## Hormone Classes

Hormones are typically divided into three classes:

1. **Peptide:** Hormones that are modified amino acids or short (peptide) or long (protein) chains of amino acids. Additionally, they can contain carbohydrate moieties.
2. **Lipid:** Steroid hormones that contain lipids synthesized from cholesterol and eicosanoids that contain lipids synthesized from the fatty acid chains of phospholipids found in the plasma membrane.
3. **Monoamine:** Hormones derived from aromatic amino acids such as phenylalanine, tyrosine, and tryptophan.

## Events Elicited by Hormone-Receptor Binding

Receptors for peptide hormones and catecholamines are present on the extracellular surface of the plasma membrane while those for steroid and thyroid hormones are mainly present on the intracellular surface of the membrane. Hormone-receptor binding influences ion channels, enzyme activity (part of the receptor), activity of JAK kinases and G proteins and second messengers. Genes could also be activated or inhibited, causing a change in the synthesis rate of proteins coded for by these genes.

## Control of Hormone Secretion

- **Control by Plasma Concentrations of Specific Substances:** Plasma concentrations of specific ions or nutrients may control the secretion of a hormone and the hormone may, in turn, control the concentration of its regulators in a negative feedback manner.
- **Control by Neurons:** Secretion of some hormones may be under the control of autonomic or central nervous system.
- **Control by Other Hormones:** Hormones called *tropic hormones* may control the secretion of other hormones.
- **Control System Involving the Hypothalamus and Pituitary:** Pituitary gland (hypophysis) lies below the hypothalamus and is connected to it by the infundibulum.

The pituitary gland has the following two lobes:

1. **Posterior Pituitary (Neurohypophysis)** – A neural extension of the hypothalamus. Posterior pituitary hormones are actually produced in the hypothalamus but are stored in the posterior pituitary. The hormones, namely oxytocin stimulates contraction of smooth muscles in breasts and uterus of females and (b) vasopressin (antidiuretic hormone or ADH) participates in the control of water excretion and regulates blood pressure.
2. **Anterior Pituitary (Adenohypophysis)** – Connected to the hypothalamus by blood vessels called hypothalamo-pituitary portal vessels. Hypothalamic hormones called hypophysiotropic hormones control the secretion of anterior pituitary hormones (all peptides), which in turn control the secretion of other hormones from other endocrine glands. The adaptive value of such a chain of control is that it allows more precise feedback control.

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Thyroid-Stimulating Hormone (TSH, Thyrotropin) induces secretion of T4 and T3 from the thyroid. Adreno CorticoTropic Hormone (ACTH) stimulates the secretion of cortisol by the adrenal cortex. The gonadotropins, Follicle-Stimulating Hormone (FSH) and Luteinizing Hormone (LH) stimulate secretion of Estradiol and Progesterone from ovaries and testosterone from testes, as well as regulate the growth and development of ova and sperm. Growth Hormone (GH) stimulates the liver to secrete a growth hormone called Insulin-like Growth Factor I (IGF-I), and exerts direct effects on metabolism. Prolactin does not exert control over the secretion of another hormone but stimulates the development of mammary glands and milk production in females.

### Hypophysiotropic Hormones

These hormones are secreted by neurons in response to action potentials. Each of these hormones is named after the anterior pituitary hormone that it controls. Corticotropin-Releasing Hormone (CRH) stimulates the secretion of ACTH. Growth Hormone-Releasing Hormone (GHRH) stimulates the secretion of GH. Thyrotropin-Releasing Hormone (TRH) stimulates the secretion of TSH or Thyrotropin. Gonadotropin-Releasing Hormone (GnRH) stimulates the secretion of Gonadotropins (FSH and LH). SomatoStatin (SS) inhibits the secretion of GH. Prolactin-Inhibiting Hormone (PIH) inhibits the secretion of Prolactin.

### Feedback Control of the Hypothalamus and Anterior Pituitary

If the last hormone in a chain of control can exert negative feedback on the Hypophysio-Pituitary System, then it is considered as long-loop negative feedback. If an anterior pituitary hormone exerts a negative feedback effect on the hypothalamus, then it is considered as short-loop negative feedback.

### Candidate Hormones

Candidate hormones do not follow the classical description of hormones because:

- Their functions are not conclusively documented, e.g., melatonin produced by the pineal gland probably plays an important part in circadian rhythms and sleep.
- They act as agents, but it is not certain if they reach the target cells through paracrine/autocrine blood, e.g., Growth Factors (GF).

### Endocrine Disorders

- **Hyposecretion** – If a gland is secreting too little hormone because it itself is unable to function normally, the disorder is called primary hyposecretion. Causes could be genetic lack of an enzyme, dietary deficiency of a precursor, infection, etc. If a gland is secreting too little hormone because there is too little tropic hormone to stimulate it, the disorder is called secondary hyposecretion.
- **Hypersecretion** – Primary hypersecretion is a gland itself secreting too much hormone while secondary hypersecretion is the excessive stimulation of a gland by its tropic hormone.

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- **Hyporesponsiveness** – Target cells do not respond to the hormone due to a deficiency of receptors, a defect in the signal transduction mechanism or a deficiency of an enzyme that catalyses the activation of the hormone. In diabetes mellitus, the target cells of the hormone insulin are hyporesponsive.
- **Hyperresponsiveness** – Hypersecretion of thyroid hormones can lead to hyperresponsiveness to epinephrine and a consequent increase in heart rate.

## 1.6.1 Nature of Hormone Action

A hormone mediate its effects by binding to its receptor molecules present on a target cell. Hormone receptors fall into two general classes: transmembrane receptors and intracellular receptors (that belong to the nuclear hormone receptor family). The receptors for lipid-soluble hormones are located inside target cells. However, the receptors for water-soluble hormones are part of the plasma membrane of target cells.

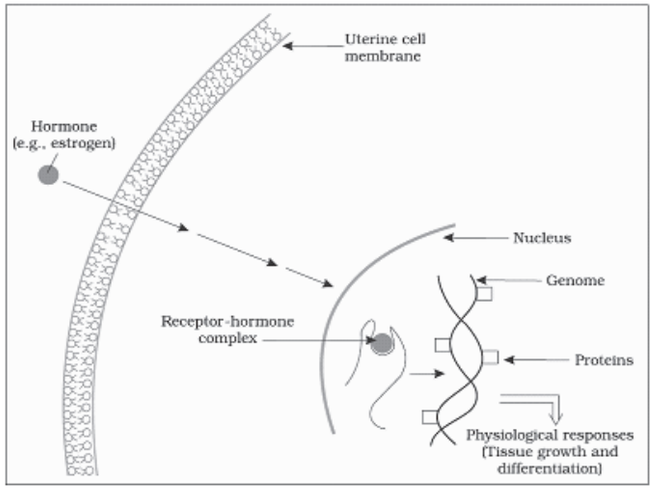


***Fig. 1.10*** *Hormone Action*

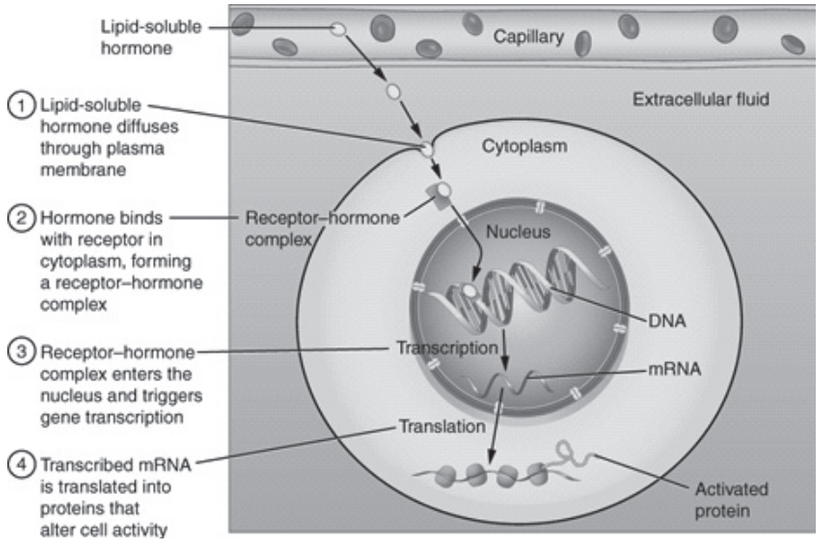


***Fig. 1.11*** *Lipid Soluble Hormone Action*

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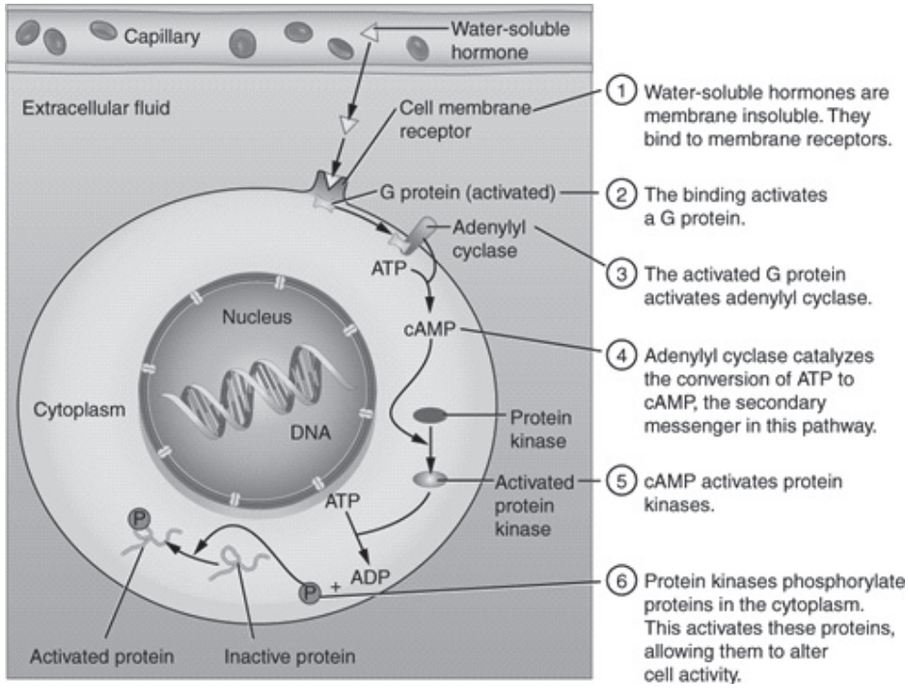


***Fig. 1.12*** *Water Soluble Hormone Action*

### Outline of Lipid-Soluble Hormone Action

Their mechanism of action can be described in a following sequence:

- **Diffusion through Plasma Membrane**: A free lipid-soluble hormone molecule diffuses from the blood, through interstitial fluid, and through the lipid bilayer of the plasma membrane into a cell.
- **Binding with Cytosolic or Nuclear Receptor**: If the cell is a target cell, the hormone binds to and activates receptors located within the cytosol or nucleus.
- **Alteration of Gene Expression**: The activated receptor–hormone complex then alters gene expression: It turns specific genes of the nuclear DNA on or off.
- **Message Processing through Transcription and Translation**: As the DNA is transcribed, new messenger RNA (mRNA) forms, leaves the nucleus, and enters the cytosol. There, it directs synthesis of a new protein, often an enzyme, on the ribosomes.
- **Physiological Response**: The new proteins alter the cell's activity and cause the responses typical of that hormone.

### Outline of Water-Soluble Hormone Action

The water soluble hormones cannot diffuse through the lipid bilayer of the plasma membrane, instead they bind to receptors that are located on the target cell surface. The receptors are integral transmembrane proteins in the plasma membrane. The action of a typical water-soluble hormone can be summed up in following steps:

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**Step 1:** Hormone binding to its receptor located at plasma membrane. A water-soluble hormone diffuses from the blood through interstitial fluid and bind its receptor located on plasma membrane. The hormone itself acts as a first messenger.

**Step 2:** Receptor binding followed by a conformational shift that extends to the cytosolic domain. The conformational shift may result in one or more of the following:

- o Activation of a guanine exchange function of a receptor
- o Homodimerization and/or heterodimerization of receptors to other receptors or co-receptors within the membrane
- o Recruitment and activation of signaling proteins by the cytosolic domain.

**Step 3:** Multiple, hierarchal steps in which downstream effector proteins are dependent on and driven by upstream receptors and signalling molecules and effector proteins. This means that loss or inactivation of one or more components within the pathway leads to hormonal resistance, whereas constitutive activation or overexpression of components can provoke a cellular response in a hormone-independent, unregulated manner.

**Step 4:** Amplification of the initial hormone receptor binding–induced signal. It usually by inclusion of an enzymatic step within a signaling pathway. Amplification can be so great that maximal response to a hormone is achieved upon hormone binding to a fraction of available receptors.

**Step 5:** Activation of multiple divergent or convergent pathways from one hormone receptor–binding event. For example, binding of insulin to its receptor activates three separate signaling pathways.

**Step 6:** Antagonism by constitutive and regulated negative feedback reactions. This means that a signal is dampened or terminated by opposing pathways. Gain of function of opposing pathways can result in hormonal resistance.

### Signalling Pathways in Case of Transmembrane Receptors

It use several common modes of informational transfer, i.e., intracellular messengers and signalling events. These include the following:

- **Conformational Shifts**: Many signalling components are proteins and have the ability to toggle between two (or more) conformational states that alter their activity, stability, or intracellular location. As discussed previously, signaling begins with hormone receptor binding that induces a conformational change in the receptor.
- **Covalent Phosphorylation of Proteins and Lipids**: Enzymes that phosphorylate proteins or lipids are called kinases, whereas those that catalyse dephosphorylation are called phosphatases. Protein kinases and phosphatases can be classified as either tyrosine-specific kinases and phosphatases or serine/threonine-specific kinases and phosphatases. There are also mixed function kinases and phosphatases that recognize all three residues. An important lipid kinase is phosphatidylinositol-3-kinase. The phosphorylated state of a signaling component can alter the following:
  - o **Activity:** Phosphorylation can activate or deactivate a substrate, and proteins often have multiple sites of phosphorylation that induce

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quantitative and/or qualitative changes in the protein's activity.

- **Stability:** For example, phosphorylation of proteins can induce their subsequent ubiquitination and proteasomal degradation.
- **Subcellular Location:** For example, the phosphorylation of some nuclear transcription factors induces their translocation to and retention in the cytoplasm.
- **Recruitment and Clustering of Other Signalling Proteins:** For example, phosphorylation of the cytosolic domain of a transmembrane receptor often induces the recruitment of signaling proteins to the receptor where they are phosphorylated. Recruitment happens because the recruited protein harbours a domain that specifically recognizes and binds to the phosphorylated residue.

- **Non-Covalent GTP Binding to G Proteins:** Non-covalent Guanosine Nucleotide Triphosphate (GTP) binding to GTP-binding proteins (G proteins) G proteins represent a large family of molecular switches, which are latent and inactive when bound to GDP, and active when bound to GTP.
- **Non-Covalent Binding of Cyclic Nucleotide-Monophosphates to their Specific Effector Proteins**: Cyclic Adenosine Monophosphate (cAMP) is generated from Adenosine Triphosphate (ATP) by adenylyl cyclase, which is primarily a membrane protein. Adenylyl cyclase is activated and inhibited by the G proteins, $G_s$-$\alpha$ and $G_i$-$\alpha$, respectively. There are three general intracellular effectors of cyclic AMP (cAMP) 1) Protein kinase A (PKA), Exchange protein activated by cAMP (Epac) and ion channels.
- Generation of lipid informational molecules, which act as intracellular messengers. These include Di Acyl Glycerol (DAG) and Inositol 1, 4, 5-triphosphate (IP3), which are cleaved from Phosphatidylinositol 4,5-bisphosphate (PIP2) by membrane-bound PhosphoLipase C (PLC).
- **Non-Covalent $Ca^{2+}$ Binding**: Cytosolic levels of $Ca^{2+}$ are maintained at very low levels (i.e., $10^{7}$ to $10^{-8}$ M), by either active transport of $Ca^{2+}$ out of the cell, or into intracellular compartments, for example endoplasmic reticulum. The ligand-receptor interaction leads to an increase in $Ca^{2+}$ binding directly to numerous specific effector proteins, which leads to a change in their activities. Additionally, $Ca^{2+}$ regulates several effector proteins indirectly, through binding to the messenger protein, calmodulin. Several of the $Ca^{2+}$/calmodulin targets are enzymes, which amplify the initial signal of increased cytosolic $Ca^{2+}$. The $Ca^{2+}$-dependent message is terminated by the lowering of cytosolic $Ca^{2+}$ by cell membrane and endoplasmic reticular $Ca^{2+}$ ATPases (i.e., $Ca^{2+}$ pumps).

## 1.6.2 Hormone Receptors - Signal Transduction Mechanisms

The hormone binding with its receptor is a type of signal which is transduced into the activation of one or more intracellular signalling molecules. Signalling molecules then act on effector proteins, which in turn, modify specific cellular functions. The combination of hormone receptor binding (signal), activation of signalling molecules (transduction), and the regulation of one or more effector proteins is referred to as

a signal transduction pathway and the final integrated outcome is referred to as the cellular response.

As mentioned above, hormone receptors fall into two general classes: transmembrane receptors and intracellular receptors that belong to the nuclear hormone receptor family.

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### Signal Transduction through Transmembrane Protein Receptors

Transmembrane receptors are proteins that contain three domains (proceeding from outside to inside the cell):

- An extracellular domain that has a high-affinity binding site for a specific hormone.
- One to seven hydrophobic, transmembrane domains that span the cell membrane.
- A cytosolic domain that is linked to signaling proteins.

Hormone binding to a transmembrane receptor induces a conformational shift in all three domains of the receptor protein. Major types of transmembrane hormone-receptors and their signal transduction mechanisms are described below:

### G Protein-Coupled Receptors (GPCRs)

Most peptide hormones, glucagon, hypothalamic and pituitary hormones; epinephrine and non-epinephrine transduce their signals through these receptors. GPCR are so named because their cytoplasmic end interact with G proteins.

As mentioned earlier, G Proteins are proteins which have binding sites for guanine nucleotides, either GDP or GTP. They are heterotrimeric, i.e., consist of three different polypeptide subunits, called $\alpha$, $\beta$ and $\gamma$. They are held at the plasma membrane by lipid chains that are covalently attached to the $\alpha$ and $\gamma$ subunits. The signal transduction mechanism with GPCRs can be summed up in following steps:

**Step 1:** Hormone binding induces a conformation change in the GPCR to activate it. This in turn leads to an increase in the affinity of the receptor for G protein that is present on the cytoplasmic surface of the plasma membrane. As a consequence, the hormone bound receptor forms a receptor–G protein complex.

**Step 2:** The interaction with the receptor induces a conformational change in the a subunit of a G protein, causing the release of GDP, which is followed by binding of GTP. In its GTP-bound conformation, the $G_{bg}$ subunit has a low affinity for $G_a$ leading to its dissociation from the complex.

**Step 3:** Each dissociated $G_a$ subunit (with GTP attached) activate an effector protein, such as adenylyl cyclase, phospholipase C-b or cyclic GMP phosphodiesterase.

**Step 4:** The activation of the effector molecule leads to the production of the second messenger cAMP, phosphatidylinositol derived molecules or cGMP respectively.

**Step 5:** Second messengers, in turn, activate one or more intra-cellular signalling pathways.

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**Termination of the Response:** G-protein signaling is terminated by intrinsic GTPase activity, converting GTP to GDP. This returns the G protein to an inactive state (bound to GDP). Another termination mechanism involves desensitization and endocytosis of the GPCR. The desensitization takes place in two steps. In the first step, the cytoplasmic domain of the activated GPCR is phosphorylated by a specific type of kinase, called G protein-coupled receptor kinase (GRK), a serine-threonine protein kinase. The second step is binding of protein arrestins to GPCR. Arrestin binding prevents the further activation of additional G proteins. This action is termed desensitization because the cell stops responding to the stimulus, while that stimulus is still acting on the outer surface of the cell.

Arrestin molecules may bind to clathrin molecules that are situated in clathrin-coated pits. This binding promotes the uptake of phosphorylated GPCRs into the cell by endocytosis. The endocytosed GPCRs either dephosphorylated to return to the plasma membrane or they are degraded in the lysosomes.

### Downstream Signalling of GPCR Through Second Messengers

A second messenger is a molecule that is capable of diffusing to other sites within the cell. The synthesis of second messenger follows the binding of a first messenger—a hormone or other ligand—to a receptor at the outer surface of the cell. Whereas the first messenger binds exclusively to a single receptor species, the second messenger often stimulates a variety of cellular activities.

As a result, second messengers enable cells to mount a large-scale, coordinated response following stimulation by a single extracellular ligand. There are several second messengers working downstream the GPCRs like cAMP, $Ca^{2+}$, phosphoinositides, inositol trisphosphate, diacylglycerol, cGMP, and nitric oxide.

### Cyclic AMP (cAMP)

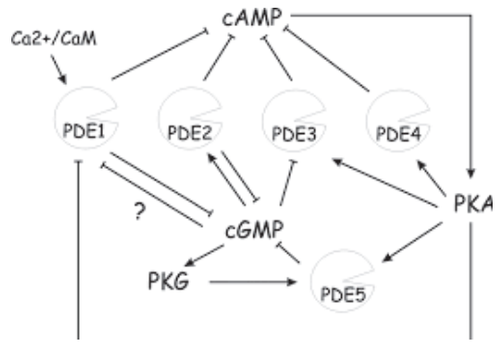


***Fig 1.13*** *Cyclic AMP*

Cyclic AMP is formed from ATP by the action of adenylyl cyclase and degraded to AMP by cAMP phosphodiesterase. Some GPCR are coupled to adenylyl cyclase via a G protein, upon binding a hormone the confirmation change in receptor stimulates enzymatic activity, thereby increasing the intracellular concentration of cAMP. There are a number of ways in which cAMP modify the downstream effector molecules, in different cells and results in a physiological outcome as discussed below:

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**Camp Mediate the Effects upon Binding with an Enzyme Protein Kinase A (PKA)**

- The inactive form of protein kinase A is a tetramer consisting of two catalytic and two regulatory subunits.
- Cyclic AMP binds to the regulatory subunits, leading to their dissociation from the catalytic subunits.
- The free catalytic subunits are then enzymatically active and able to phosphorylate serine residues on their target proteins.
- In many animal cells, the free catalytic unit of PKA enter the nucleus and phosphorylates transcription factor called CREB (for CRE-binding protein), leading to the recruitment of coactivators and transcription of cAMP-inducible genes.

**A Second Effector of cAMP is EPAC (Exchange Protein Activated by cAMP)**

The EPAC has two isoforms. These proteins act as GEFs (Guanine exchange factors) for small G proteins (called Raps). Raps in turn control a wide array of cell functions, including formation of cell-cell junctional complexes and cell-matrix adhesion, $Ca^{2+}$ release from intracellular stores (especially in cardiac muscle) and in the augmentation of glucose-dependent insulin secretion by glucagon-like peptide-1 in pancreatic islet b cells.

**cAMP can Directly Regulate Ion Channels, Independent of Protein Phosphorylation**

Cyclic AMP functions in this way as a second messenger involved in sensing smells. Many of the odorant receptors in sensory neurons in the nose are G protein-coupled receptors that stimulate adenylyl cyclase, leading to an increase in intracellular cAMP. Rather than stimulating protein kinase A, cAMP in this system directly opens $Na^+$ channels in the plasma membrane, leading to membrane depolarization and initiation of a nerve impulse.

Examples of hormones that use cAMP as a second messenger include calcitonin, which is important for bone construction and regulating blood calcium levels; glucagon, which plays a role in blood glucose levels; and thyroid-stimulating hormone, which causes the release of T3 and T4 from the thyroid gland.

**Cyclic GMP (cGMP)**

It is also an important second messenger in animal cells, although its roles are not as clearly understood as those of cAMP. Cyclic GMP is formed from GTP by guanylyl cyclases and degraded to GMP by a phosphodiesterase. Guanylyl cyclases are activated by peptide ligands through GPCR as well as by nitric oxide and carbon monoxide. Stimulation of these guanylyl cyclases leads to elevated levels of cGMP, which then mediate biological responses, such as blood vessel dilation. The action of cGMP is frequently mediated by activation of cGMP-dependent protein kinases, Protein Kinase G (PKG), which phosphorylates and regulates numerous proteins. cGMP also regulates ion channels and phosphodiesterases.

**Phosphatidylinositol-Derived Second Messengers:** The phospholipids of cell membranes are structural molecules that made membranes cohesive and

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impermeable to aqueous solutes. Additionally, these molecules also form the precursors of a number of second messengers. Phospholipids of cell membranes are converted into second messengers by a variety of enzymes that are regulated in response to extracellular signals. These enzymes include:

- Phospholipid kinases (lipid-phosphorylating enzymes)
- Phospholipid phosphatases (lipid-dephosphorylating enzymes)
- Phospholipases (lipid-splitting enzymes)-Phospholipases are enzymes that hydrolyze specific ester bonds that connect the different building blocks that make up a phospholipid molecule.

The most common membrane lipids which are modified by these enzymes are phosphatidylinositol and sphingomyelin.

Guanylate cyclase (GC) catalyzes cGMP synthesis. This enzyme converts GTP to cGMP. Peptide hormones such as the atrial natriuretic factor activate membrane-bound GC, while soluble GC (sGC) is typically activated by nitric oxide to stimulate cGMP synthesis.

Adrenaline Hormone act by cAMP and increase heartbeat while Acetylcholine act by cGMP which decrease heartbeat.

**Phosphatidylinositol Phosphorylation**

- The inositol ring, which resides at the cytoplasmic surface of the bilayer, has six carbon atoms.
- Carbon number 1 is involved in the linkage between inositol and diacylglycerol. The 3, 4, or 5 carbons can be phosphorylated by specific phosphoinositide kinases present in cells and generating PIP2, PIP3.
- The phospholipid species thus generated remain in the cytoplasmic leaflet of the plasma membrane; they are membrane-bound second messengers.
- The phosphorylated inositol rings of phosphoinositides form binding sites for several lipid-binding domains found in proteins. Best known is the PH domain which has been identified in over 150 different proteins.
- Binding of a protein by its PH domain to PIP2 or PIP3 typically recruits the protein to the cytoplasmic face of the plasma membrane where it can interact with other membrane-bound proteins, including activators, inhibitors, or substrates.

**Phosphatidylinositol Phosphatases**

Just as there are lipid kinases to add phosphate groups to phosphoinositides, there are lipid phosphatases to remove them. The activity of these kinases and phosphatases are coordinated so that specific phosphoinositides appear at specific regions of the membrane at specific times after a signal has been received.

**Phospholipase C (PLC)**

- PLC is situated at the inner surface of the membrane, bound there by the interaction between its PH domain and a phosphoinositide embedded in the bilayer.

- PLC catalyzes a reaction that splits PIP2 into two molecules, Inositol 1,4,5-trisphosphate (IP3) and Diacylglycerol (DAG) both of which play important roles as second messengers in cell signalling as described below:

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**Diacylglycerol (DAG)**

Diacylglycerol is a lipid molecule that remains in the plasma membrane following its formation by PLC. There it recruits and activates effector proteins that bear a DAG-binding C1 domain. The best-studied of these proteins is Protein Kinase C (PKC), which phosphorylates serine and threonine residues on a wide variety of target proteins.

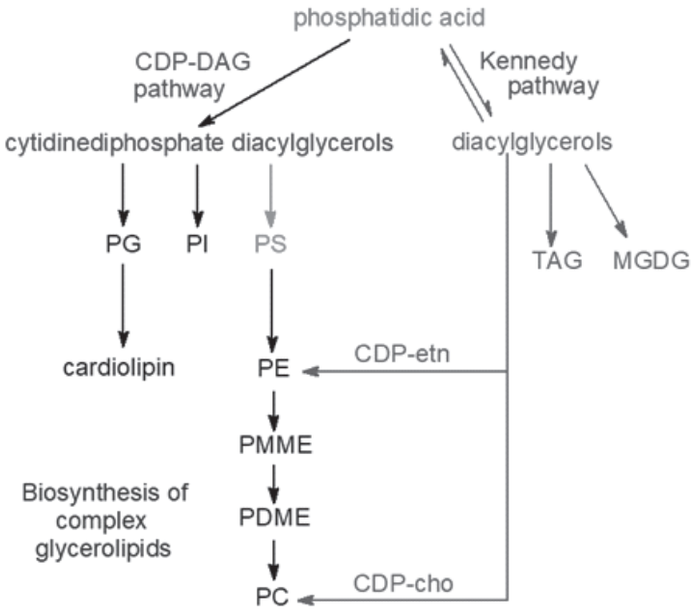


***Fig. 1.14*** *DAG Pathway*

Protein kinase C has a number of important roles in cellular growth and differentiation, cellular metabolism, cell death, and transcriptional activation.

The apparent importance of protein kinase C in growth control is seen in studies with a group of powerful plant compounds, called phorbol esters that resemble DAG. These compounds activate protein kinase C in a variety of cultured cells, causing them to lose growth control and behave temporarily as malignant cells. When the phorbol ester is removed from the medium, the cells recover their normal growth properties. In contrast, cells that have been genetically engineered to constitutively express protein kinase C exhibit a permanent malignant phenotype in cell culture and can cause tumors in susceptible mice. Finally, application of phorbol esters to the skin in combination with certain other chemicals will cause the formation of skin tumors.

**Inositol 1,4,5-Trisphosphate (IP3)**

- Inositol 1,4,5-trisphosphate (IP3) is a sugar phosphate—a small, water-soluble molecule capable of rapid diffusion throughout the interior of the cell.

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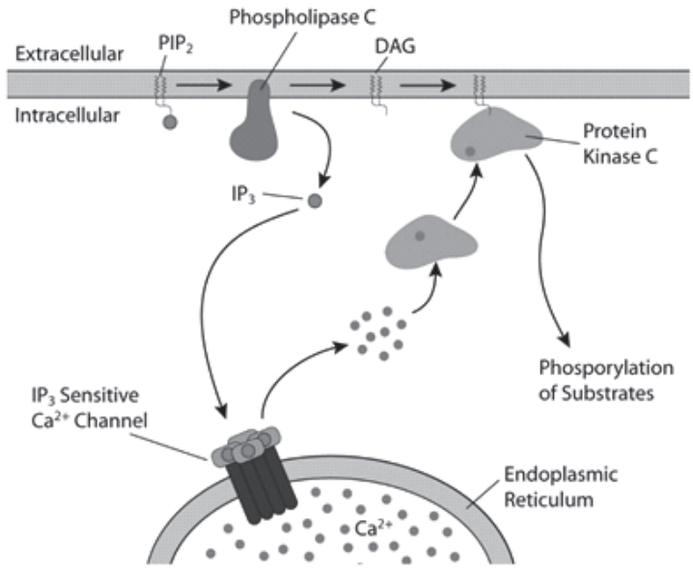


***Fig. 1.15*** *Diagrammatic Representation of Hormone Action by IP3 and $Ca^{++}$*

- IP3 molecules formed at the membrane diffuse into the cytosol and bind to a specific IP3 receptor located at the surface of the smooth endoplasmic reticulum.
- The smooth endoplasmic reticulum is a site of calcium storage in a variety of cells. The IP3 receptor also functions as a tetrameric $Ca^{2+}$ channel.
- Binding of IP3 opens the channel, allowing $Ca^{2+}$ ions to diffuse into the cytoplasm. Calcium ions can also be considered as intracellular or second messengers because they bind to various target molecules, triggering specific responses.

## Calcium Ions ($Ca^{2+}$)

The role of calcium ions as second messenger working downstream the GPCR are discussed below:

- Some members of the protein kinase C family require $Ca^{2+}$ as well as diacylglycerol for their activation.
- In most cells, the transient increase in intracellular $Ca^{2+}$ (by downstream signalling through GPCR via IP3, as mentioned above) triggers a more sustained increase caused by the entry of extracellular $Ca^{2+}$ through channels in the plasma membrane. This entry of $Ca^{2+}$ from outside the cell serves both to prolong the signal initiated by release of $Ca^{2+}$ from the endoplasmic reticulum and to allow the stores of $Ca^{2+}$ within the endoplasmic reticulum to be replenished.
- The entry of extracellular $Ca^{2+}$ is particularly important in the electrically excitable cells of nerve and muscle in which voltage-gated $Ca^{2+}$ channels in the plasma membrane are opened by membrane depolarization.
- The resulting increases in intracellular $Ca^{2+}$ then trigger the further release of $Ca^{2+}$ from intracellular stores by activating distinct $Ca^{2+}$ channels known as ryanodine receptors.

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- One effect of increases in intracellular $Ca^{2+}$ in neurons is to trigger the release of neurotransmitters, so $Ca^{2+}$ plays a critical role in converting electric to chemical signals in the nervous system.
- In muscle cells $Ca^{2+}$ is stored in the sarcoplasmic reticulum from which it is released by the opening of ryanodine receptors in response to changes in membrane potential. This release of stored $Ca^{2+}$ leads to large increases in cytosolic $Ca^{2+}$, which trigger muscle contraction.
- Cells thus utilize a variety of mechanisms to regulate intracellular $Ca^{2+}$ levels, making $Ca^{2+}$ a versatile second messenger that controls a wide range of cellular processes.
- Many of the effects of $Ca^{2+}$ are mediated by the $Ca^{2+}$-binding protein **calmodulin**, which is activated when the concentration of cytosolic $Ca^{2+}$ increases to about 0.5mM. $Ca^{2+}$/calmodulin then binds to a variety of target proteins, including protein kinases.
- One example of such a $Ca^{2+}$/ calmodulin-dependent protein kinase is myosin light-chain kinase, which signals actin-myosin contraction by phosphorylating one of the myosin light chains.
- Other protein kinases that are activated by $Ca^{2+}$/calmodulin include members of the CaM kinase family, which phosphorylate a number of different proteins, including metabolic enzymes, ion channels, and transcription factors.
- Also, the cAMP and $Ca^{2+}$ signalling pathways function co-ordinately to regulate many cellular responses.

### Receptor Protein-Tyrosine Kinases

In contrast to the GPCRs, the cytoplasmic domain of these cell surface receptors possess intrinsic protein tyrosine kinase activity. This family includes the receptors for insulin and growth factors.

Receptor protein-tyrosine kinases in general consist of single polypeptides, although the insulin receptor are dimers consisting of two polypeptide chains.

**Step 1**: The first step in signalling from most receptor protein-tyrosine kinases is hormone-induced receptor dimerization. However, in case of insulin receptor which already exist as a dimer, hormone binding results in a confirmation change of receptor polypeptide chains.

**Step 2**: This leads to activation of cytosolic kinase domains resulting in auto cross-phosphorylation at tyrosine residues of receptors polypeptide chains. The phosphorylated tyrosine residues of the catalytic domain increases protein kinase activity. While the phosphorylation of tyrosine residues outside of the catalytic domain creates specific binding sites for effector molecules that transmit intracellular signals downstream of the activated receptors.

The association of these downstream signaling molecules with receptor protein-tyrosine kinases is mediated by protein domains (like SH2 and PTB) that bind to specific phosphotyrosine-containing peptides.

**Step 3**: The resulting association of SH2- or PTB-containing proteins with activated receptor protein-tyrosine kinases work in four different ways as describe below:

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- **Adaptor Proteins** function as linkers that enable two or more signaling proteins to become joined together as part of a signaling complex. Adaptor proteins contain an SH2 domain and one or more additional protein–protein interaction domains. For instance, the adaptor protein Grb2 contains one SH2 and two SH3 (Src-homology 3) domains.SH3 domains bind to proline-rich sequence motifs. The SH3 domains of Grb2 bind constitutively to other proteins, including Sos and Gab. The SH2 domain binds to phosphorylated tyrosine residues within a Tyr-X-Asn motif. Consequently, tyrosine phosphorylation of the Tyr-X-Asn motif on an RTK results in translocation of Grb2-Sos or Grb2-Gab from the cytosol to a receptor, which is present at the plasma membrane.
- **Docking Proteins**, such as IRS, supply certain receptors with additional tyrosine phosphorylation sites. Docking proteins contain either a PTB domain or an SH2 domain and a number of tyrosine phosphorylation sites. Binding of an extracellular ligand to a receptor leads to autophosphorylation of the receptor, which provides a binding site for the PTB or SH2 domain of the docking protein. Once bound together, the receptor phosphorylates tyrosine residues present on the docking protein. These phosphorylation sites then act as binding sites for additional signaling molecules. Docking proteins provide versatility to the signaling process, because the ability of the receptor to turn on signaling molecules can vary with the docking proteins that are expressed in a particular cell.
- **Transcription Factors,** some SH2 domain proteins work as transcription factors, for instance, that belong to the STAT family play an important role in the function of the immune system. STATs contain an SH2 domain together with a tyrosine phosphorylation site that can act as a binding site for the SH2 domain of another STAT molecule. Tyrosine phosphorylation of STAT SH2 binding sites situated within a dimerized receptor leads to the recruitment of STAT proteins. Upon association with the receptor complex, tyrosine residues in these STAT proteins are phosphorylated. As a result of the interaction between the phosphorylated tyrosine residue on one STAT protein and the SH2 domain on a second STAT protein, and vice versa, these transcription factors form dimers. Dimers, but not monomers, move to the nucleus where they stimulate the transcription of specific genes involved in an immune response.
- **Signaling Enzymes**: Various molecules with SH2/PTB domains on binding with the RTKs get activated, and serve as protein kinases, protein phosphatases, lipid kinases, phospholipases, and GTPase activating proteins. Three general mechanisms have been identified by which these enzymes are activated following their association with a receptor.
    - o Enzymes can be activated simply as a result of translocation to the membrane, which places them in close proximity to their substrates.
    - o Enzymes can also be activated through an allosteric mechanism, in which binding to phosphotyrosine results in a conformational change in the SH2 domain that causes a conformational change in the catalytic domain, resulting in a change in catalytic activity.
    - o Finally, enzymes can be regulated directly by phosphorylation.

**Step 4:** Binding and activation of SH2/PTB containing domains is followed by the downstream intracellular transmission of signals mediated through a number of effector molecules which are discussed below in detail.

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**Signal Termination by RTKs:** The signal is usually terminated by internalization of the receptor. Exactly what causes receptor internalization remains an area of active research. One mechanism involves a receptor-binding protein named Cbl. When RTKs are activated by ligands, they autophosphorylate tyrosine residues, which can act as a binding site for Cbl, which possesses an SH2 domain. Cbl then associates with the receptor and catalyzes the attachment of a ubiquitin molecule to the receptor. Ubiquitin is a small protein that is linked covalently to other proteins, thereby marking those proteins for internalization or degradation. Binding of the Cbl complex to activated receptors is followed by receptor ubiquitination, internalization, and in most cases degradation in a lysosome.

**Downstream Signal transduction by RTKs/ RAS-MAP**

Ras-MAP kinase cascade is one of the common RTKs downstream signal transduction pathway regulating cell proliferation and differentiation. The sequential steps of the pathway are given below:

- This pathway is activated when a growth factor, such as EGF or PDGF, binds to the extracellular domain of its RTK.
- Many activated RTKs possess phosphorylated tyrosine residues that act as docking sites for the adaptor protein Grb2.
- Grb2, in turn, binds to Sos, which is a Guanine Nucleotide Exchange Factor (a GEF) for Ras. Creation of a Grb2-binding site on an activated receptor promotes the translocation of Grb2-Sos from the cytoplasm to the cytoplasmic surface of the plasma membrane, placing Sos in close proximity to Ras. Simply bringing Sos to the plasma membrane is sufficient to cause Ras activation.
- Interaction with Sos opens the Ras nucleotide binding site.
- As a result, GDP is released and is replaced by GTP.
- Exchange of GDP for GTP in the nucleotide-binding site of Ras results in a conformational change and the creation of a binding interface for a number of proteins, including an important signalling protein called Raf.
- Raf is then recruited to the inner surface of the plasma membrane where it is activated by a combination of phosphorylation and dephosphorylation reactions.
- Raf is a serine-threonine protein kinase. One of its substrates is the protein kinase MEK.
- MEK, which is activated as a consequence of phosphorylation by Raf, goes on to phosphorylate and activate two MAP kinases named ERK1 and ERK2.
- Over 160 proteins that can be phosphorylated by these kinases have been identified, including transcription factors, protein kinases, cytoskeletal proteins, apoptotic regulators, receptors, and other signalling proteins.

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- Once activated, the MAP kinase is able to move into the nucleus where it phosphorylates and activates a number of transcription factors and other nuclear proteins.
- Eventually, the pathway leads to the activation of genes involved in cell proliferation, including cyclin D1, which plays a key role in driving a cell from G1 into S phase.

Ras proteins are part of a superfamily of more than 150 small G proteins including the Rabs, Sar1, and Ran. These proteins are involved in the regulation of numerous processes, including cell division, differentiation, gene expression, cytoskeletal organization, vesicle trafficking, and nucleocytoplasmic transport. Some more information about Ras proteins:

- Ras is a small GTPase that is anchored at the inner surface of the plasma membrane by a lipid group that is embedded in the inner leaflet of the bilayer.
- Ras is functionally similar to the heterotrimeric G proteins. However, unlike heterotrimeric G proteins Ras consists of only a single small subunit.
- Ras proteins are present in two different forms: an active GTP-bound form and an inactive GDP-bound form. Ras-GTP binds and activates downstream signaling proteins. Ras is turned off by hydrolysis of its bound GTP to GDP.

  ***Note:*** Mutations in the human RAS gene that lead to tumor formation prevent the protein from hydrolyzing the bound GTP back to the GDP form which keep the cell in the proliferative mode.

- The cycling of monomeric G proteins, such as Ras, between active and inactive states is aided by accessory proteins that bind to the G protein and regulate its activity. These accessory proteins include:
  - **GTPase-Activating Proteins (GAPs):** Most monomeric G proteins possess some capability to hydrolyze a bound GTP, but this capability is greatly accelerated by interaction with specific GAPs. Because they stimulate hydrolysis of the bound GTP, which inactivates the G protein, GAPs dramatically shorten the duration of a G protein mediated response.
  - **Guanine Nucleotide-Exchange Factors (GEFs):** An inactive G protein is converted to the active form when the bound GDP is replaced with a GTP. GEFs are proteins that bind to an inactive monomeric G protein and stimulate dissociation of the bound GDP. Once the GDP is released, the G protein rapidly binds a GTP, which is present at relatively high concentration in the cell, thereby activating the G protein.
  - **Guanine Nucleotide-Dissociation Inhibitors (GDIs):** GDIs are proteins that inhibit the release of a bound GDP from a monomeric G protein, thus maintaining the protein in the inactive, GDP-bound state.
  - The activity and localization of these various accessory proteins are tightly regulated by other proteins, which thus regulates the state of the G protein.
- Ras-GTP can interact directly with several downstream targets.

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### Non-Receptor Tyrosine Kinases

Non-Receptor tyrosine kinases, the cytokine receptor superfamily. Growth hormone, prolactin, erythropoietin and leptin mediate their function through these receptor types. These receptors exist as dimers but do not have intrinsic protein kinase activity. Instead they are non-covalently associated with intracellular protein tyrosine kinases. Their signal transduction mechanism is analogous to that of receptor tyrosine kinase as mentioned below:

**Step 1**: The first step is hormone induced receptor dimerization and cross-phosphorylation of the associated non-receptor intracellular protein-tyrosine kinases.

**Step 2**: These activated kinases then phosphorylate the receptor, providing phosphor-tyrosine-binding sites for the recruitment of downstream signaling molecules that contain SH2 domains.

The kinases associated with cytokine receptors belong to the Janus kinase (or JAK) family and Src family.

### Receptor Serine/Threonine Kinase

This group of transmembrane receptors are bound and activated by members of the transforming growth factors, anti-mullerian hormone and inhibin. Unbound receptors exist as dissociated heterodimers, called RI and RII. Hormone binding to RII induces dimerization of RII with RI, and RII activates RI by phosphorylation. RI then activates latent transcription factors called Smads. Activated Smads heterodimerize with a Co-Smad, enter the nucleus, and regulate specific gene expression.

### Signal Transduction through Intracellular Protein Receptors

Steroid hormones and thyroid hormones bind to intracellular receptors. These receptors are structurally similar and are members of the nuclear hormone receptor superfamily that also includes receptors for lipid-soluble vitamins, Peroxisome Proliferator–Activated Receptors (PPARs), and other metabolic receptors (liver X receptor, farnesyl X receptor).

Nuclear hormone receptors act as transcriptional regulators. This means that the signal of hormone receptor binding is transduced ultimately into a change in the transcriptional rate of a subset of the genes that are expressed within a differentiated cell type. One receptor binds to a specific DNA sequence, called a hormone response element, often close to the promoter of one gene, and influences the rate of transcription of that gene in a hormone-dependent manner.

Nuclear hormone receptors have three major structural domains:

- An Amino Terminus Domain (ABD)-contains a hormone-independent transcriptional activation domain.
- A middle DNA-Binding Domain (DBD) - contains two zinc finger motifs, which represent small loops organized by Zn2þ binding to four cysteine residues at the base of each loop. The two zinc fingers and neighbouring amino acids confer the ability to recognize and bind to specific DNA sequences, which are called Hormone-Response Elements (HREs).

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- A carboxyl terminus Ligand-Binding Domain (LBD) - contains several subdomains:
  - o Site of hormone recognition and binding
  - o Hormone-dependent transcriptional activation domain
  - o Nuclear translocation signal
  - o Binding domain for heat-shock proteins
  - o Dimerization subdomain

There are numerous variations in the details of nuclear receptor mechanisms of action. Two generalized pathways by which nuclear hormone receptors increase gene transcription are the following:

**Pathway 1**: This pathway is common to most of the steroid hormones.

**Step 1**: The non-activated receptor is located in the cytoplasm associated with chaperones (also called as heat shock proteins). Chaperone proteins maintain the stability of the nuclear receptor in an inactive configuration. Hormone binding induces a conformational change in the receptor, causing its dissociation from heat-shock proteins.

**Step 2**: This exposes the nuclear localization signal and dimerization domains, so receptors dimerize and enter the nucleus.

**Step 3**: Once in the nucleus, these receptors bind to their respective Hormone Response Elements (HREs). The HREs for the Progesterone Receptor (PR), Glucocorticoid Receptor (GR), Mineralocorticoid Receptor (MR), and Androgen Receptor (AR) are inverted repeats with the recognition sequence, AGAACANNNTGTTCT. Specificity is conferred by neighbouring base sequences and possibly by receptor interaction with other transcriptional factors in the context of a specific gene promoter. For example, the ER usually binds to an inverted repeat with the recognition sequence, AGGTCANNNTGACCT. The specific HREs are also referred to as an Estrogen-Response Element (ERE), Progesterone-Response Element (PRE), Glucocorticoid-Response Element (GRE), mineralocorticoid- response element (MRE), and androgen-response element (ARE).

**Step 4**: Once bound to their respective HREs, these receptors recruit other proteins, called co-regulatory proteins, which are either co-activators or co-repressors.

- o Co-activators act to recruit other components of the transcriptional machinery and probably activate some of them. Co-activators also possess intrinsic Histone Acetyltransferase *(HAT)* activity, which acetylates histones in the region of the promoter. Histone acetylation relaxes chromatin coiling, making that region more accessible to transcriptional machinery.
- o Co-repressors possess Histone Deacetylase (HDAC*)* activity. In contrast to histone acetylation, histone deacetylation allows tighter coiling of chromatin, which makes promoters in that region less accessible to the transcriptional machinery.

**Pathway 2**: This pathway is used by the thyroid hormone receptors (THRs), vitamin D receptors, PPARs, and retinoic acid receptors. This pathway is different from pathway 1 in following manner.

One, receptor is always located in the nucleus. For example, thyroid hormone receptor (which is present as a heterodimer with retinoic acid receptor) are bound to Thyroid Hormone Response Elements (TREs) in the absence of hormone. It maintains the expression of neighbouring genes at a 'repressed' level.

Two, they do not recruit but exchanges co-repressors with co-activators on hormone binding. For example, thyroid hormone (and other ligands of this class) can readily move into the nucleus and bind to their receptors. Thyroid hormone binding induces dissociation of co-repressor proteins, thereby increasing gene expression to a basal level. The hormone receptor complex subsequently recruits co-activator proteins, which further increase transcriptional activity to the 'stimulated' level.

## 1.6.3 Hormones and Homeostasis

Hormonal effects are dependent on where they are released, as they can be released in different manners. Not all hormones are released from a cell and into the blood until it binds to a receptor on a target. The major types of hormone signalling are:

***Table 1.1*** *Types of Hormone Signalling*

| Types | Description |
|---|---|
| Endocrine | It acts on the target cells after being released into the bloodstream. |
| Paracrine | It acts on the nearby cells and does not have to enter general circulation. |
| Autocrine | It affects the cell types that secreted it and causes a biological effect. |
| Intracrine | It acts intracellularly on the cells that synthesized it. |

### Chemical Classes

As hormones are defined functionally, not structurally, they may have diverse chemical structures. Hormones occur in multicellular organisms (plants, animals, fungi, brown algae, and red algae). These compounds occur also in unicellular organisms and may act as signalling molecules however there is no agreement that these molecules can be called hormones.

### Homeostasis

In biology, homeostasis is the state of steady internal, physical, and chemical conditions maintained by living systems. This is the condition of optimal functioning for the organism and includes many variables, such as, body temperature and fluid balance, being kept within certain pre-set limits (homeostatic range). Other variables include the pH of extracellular fluid, the concentrations of sodium, potassium and calcium ions, as well as that of the blood sugar level, and these need to be regulated despite changes in the environment, diet, or level of activity. Each of these variables is controlled by one or more regulators or homeostatic mechanisms, which together maintain life.

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The word homeostasis uses combining forms of 'homeo-' and '-stasis', New Latin from Greek: 'Similar' and 'Stasis', 'Standing Still', yielding the idea of 'Staying the Same'.

Homeostasis is brought about by a natural resistance to change when already in the optimal conditions, and equilibrium is maintained by many regulatory mechanisms. All homeostatic control mechanisms have at least three interdependent components for the variable being regulated: a receptor, a control centre, and an effector. The receptor is the sensing component that monitors and responds to changes in the environment, either external or internal. Receptors include thermoreceptors, and mechanoreceptors. Control centres include the respiratory centre, and the renin-angiotensin system. An effector is the target acted on, to bring about the change back to the normal state. At the cellular level, receptors include nuclear receptors that bring about changes in gene expression through up-regulation or down-regulation, and act in negative feedback mechanisms. An example of this is in the control of bile acids in the liver.

Some centers, such as the renin-angiotensin system, control more than one variable. When the receptor senses a stimulus, it reacts by sending action potentials to a control center. The control center sets the maintenance range-the acceptable upper and lower limits-for the particular variable, such as temperature. The control center responds to the signal by determining an appropriate response and sending signals to an effector, which can be one or more muscles, an organ, or a gland. When the signal is received and acted on, negative feedback is provided to the receptor that stops the need for further signalling.

The Cannabinoid Receptor Type 1 (CB1), located at the presynaptic neuron, is a receptor that can stop stressful neurotransmitter release to the postsynaptic neuron; it is activated by Endo Cannabinoids (ECs), such as Anandamide (N-Arachidonoyl EthanolAmide; AEA) and 2-ArachidonoylGlycerol (2-AG) via a retrograde signalling process in which these compounds are synthesized by and released from postsynaptic neurons, and travel back to the presynaptic terminal to bind to the CB1 receptor for modulation of neurotransmitter release to obtain homeostasis.

The Polyunsaturated Fatty Acids (PUFAs) are lipid derivatives of Omega-3 (DocosaHexaenoic Acid, DHA, and EicosaPentaenoic Acid, EPA) or of Omega-6 (Arachidonic Acid, ARA) are synthesized from membrane phospholipids and used as a precursor for EndoCannabinoids (ECs) mediate significant effects in the fine-tune adjustment of body homeostasis.

The concept of the regulation of the internal environment was described by French physiologist Claude Bernard in 1849, and the word homeostasis was coined by Walter Bradford Cannon in 1926. In 1932, Joseph Barcroft a British physiologist, was the first to say that higher brain function required the most stable internal environment. Thus, to Barcroft homeostasis was not only organized by the brain-homeostasis served the brain. Homeostasis is an almost exclusively biological term, referring to the concepts described by Bernard and Cannon, concerning the constancy of the internal environment in which the cells of the body live and survive. The term cybernetics is applied to technological control systems such as thermostats, which function as homeostatic mechanisms, but is often defined much more broadly than the biological term of homeostasis.

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The metabolic processes of all organisms can only take place in very specific physical and chemical environments. The conditions vary with each organism, and with whether the chemical processes take place inside the cell or in the interstitial fluid bathing the cells. The best-known homeostatic mechanisms in humans and other mammals are regulators that keep the composition of the extracellular fluid or the 'Internal Environment' constant, especially with regard to the temperature, pH, osmolality, and the concentrations of sodium, potassium, glucose, carbon dioxide, and oxygen. However, a great many other homeostatic mechanisms, encompassing many aspects of human physiology, control other entities in the body. Where the levels of variables are higher or lower than those needed, they are often prefixed with hyper- and hypo-, respectively, such as hyperthermia and hypothermia or hypertension and hypotension.

If an entity is homeostatically controlled it does not imply that its value is necessarily absolutely steady in health. Core body temperature is, for instance, regulated by a homeostatic mechanism with temperature sensors in, amongst others, the hypothalamus of the brain. However, the set point of the regulator is regularly reset. For instance, core body temperature in humans varies during the course of the day, i.e., has a circadian rhythm, with the lowest temperatures occurring at night, and the highest in the afternoons. Other normal temperature variations include those related to the menstrual cycle. The temperature regulator's set point is reset during infections to produce a fever. Organisms are capable of adjusting somewhat to varied conditions such as temperature changes or oxygen levels at altitude, by a process of acclimatisation.

### 1.6.4 Hormonal Regulation of Carbohydrate, Protein and Lipid Metabolism

Variety of hormones and other molecules regulate the carbohydrates metabolism. Some of these have already been cited in previous sections. The appropriate functions of the body are dependent on precise control of the glucose concentration in the blood. 70-90 mg/100 ml is the normal fasting level of glucose in the blood. A condition called hyperglycemia results if the concentration of glucose in blood is too high (above 120 mg/100 ml). Hyperglycemia may temporarily exist as a result of eating a meal rich in carbohydrates. When the concentration of glucose is too low (below 70 mg/100 ml) the condition is called as hypoglycemia. Hypoglycemia is characterized by general weakness, trembling, headache, profuse perspiration, drowsiness, rapid heartbeat, and possible loss of consciousness.

The liver and skeletal muscle self-regulate carbohydrate metabolism in basically the same way as do other cells. Yet, these cell types are also required to respond to external signals by altering their carbohydrate metabolism. These two tissues, along with adipose tissue (which is primarily involved in lipid metabolism), act as the major regulators of nutrient levels in circulation during most metabolic conditions.

The regulation has following two goals:

1. Maintenance of normal circulating glucose levels in the face of changing conditions, and when necessary

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2. Support of physical activity

These tissues are tightly controlled by external signals: the levels of the pancreatic hormones insulin and glucagon, the adrenal hormones epinephrine and cortisol. In the case of skeletal muscle, the neuronal signals govern muscle contraction. In general, glucagon and epinephrine result in phosphorylation of regulatory enzymes, whereas insulin results in removal of the phosphate; calcium usually increases phosphorylation (one major exception is the mitochondrial enzyme pyruvate dehydrogenase, wherein calcium stimulates phosphate removal).

Some of the hormones, especially cortisol and insulin, and to a lesser extent glucagon, alter the amounts of the enzymes present in the cell. The phosphorylation and dephosphorylation events occur quickly, though effects on enzyme concentration are relatively slow processes. The cells of the liver and muscle must also use the same feedback regulatory metabolites as do "normal cells"; these effects interact with the hormonal signals to result in the overall metabolic changes that occur within these cells.

### 1. Carbohydrate Metabolism

Carbohydrate metabolism is the whole of the biochemical processes responsible for the metabolic formation, breakdown, and interconversion of carbohydrates in living organisms.

Carbohydrates are central to many essential metabolic pathways. Plants synthesize carbohydrates from carbon dioxide and water through photosynthesis, allowing them to store energy absorbed from sunlight internally. When animals and fungi consume plants, they use cellular respiration to break down these stored carbohydrates to make energy available to cells. Both animals and plants temporarily store the released energy in the form of high-energy molecules, such as ATP, for use in various cellular processes.

Humans can consume a variety of carbohydrates, digestion breaks down complex carbohydrates into a few simple monomers (monosaccharides) for metabolism: glucose, fructose, mannose and galactose. Glucose is distributed to cells in the tissues, where it is broken down or stored as glycogen. In aerobic respiration, glucose and oxygen are metabolized to release energy, with carbon dioxide and water as end-products. Most of the fructose and galactose travel to the liver, where they can be converted to glucose and fat.

Some simple carbohydrates have their own enzymatic oxidation pathways, as do only a few of the more complex carbohydrates. The disaccharide lactose, for instance, requires the enzyme lactase to be broken into its monosaccharide components, glucose and galactose.

### Metabolic Pathways

### Glycolysis

Glycolysis is the process of breaking down a glucose molecule into two pyruvate molecules, while storing energy released during this process as ATP and NADH. Nearly all organisms that break down glucose utilize glycolysis. Glucose regulation and product use are the primary categories in which these pathways differ between organisms. In some tissues and organisms, glycolysis is the sole method of energy production. This pathway is common to both anaerobic and aerobic respiration.

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Glycolysis consists of ten steps, split into two phases. During the first phase, it requires the breakdown of two ATP molecules. During the second phase, chemical energy from the intermediates is transferred into ATP and NADH. The breakdown of one molecule of glucose results in two molecules of pyruvate, which can be further oxidized to access more energy in later processes.

Glycolysis can be regulated at different steps of the process through feedback regulation. The step that is regulated the most is the third step. This regulation is to ensure that the body is not over-producing pyruvate molecules. The regulation also allows for the storage of glucose molecules into fatty acids. There are various enzymes that are used throughout glycolysis. The enzymes upregulate, downregulate, and feedback regulate the process.

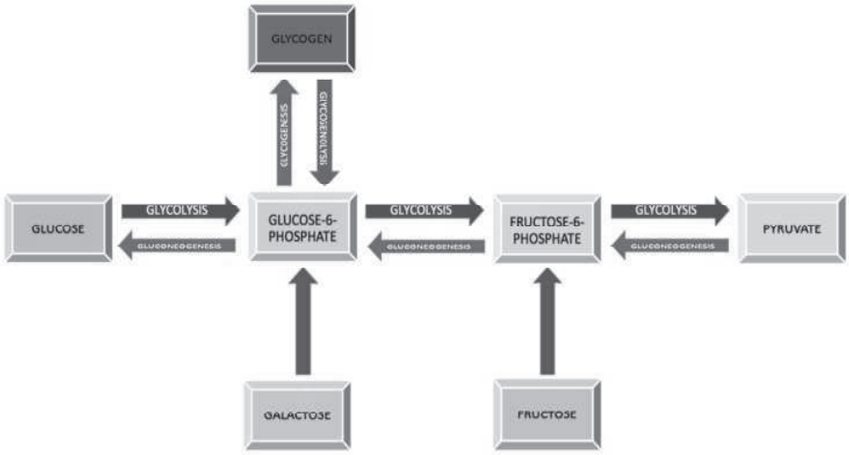


***Fig. 1.16*** *Overview of Connections Between Metabolic Processes*

## Gluconeogenesis

Glucogeogenesis (GNG) is a metabolic pathway that results in the generation of glucose from certain non-carbohydrate carbon substrates. It is a ubiquitous process, present in plants, animals, fungi, bacteria, and other microorganisms. In vertebrates, gluconeogenesis occurs mainly in the liver and, to a lesser extent, in the cortex of the kidneys. It is one of two primary mechanisms – the other being degradation of glycogen (glycogenolysis) - used by humans and many other animals to maintain blood glucose levels, avoiding low levels (hypoglycemia). In ruminants, because dietary carbohydrates tend to be metabolized by rumen organisms, gluconeogenesis occurs regardless of fasting, low-carbohydrate diets, exercise, etc. In many other animals, the process occurs during periods of fasting, starvation, low-carbohydrate diets, or intense exercise.

In humans, substrates for gluconeogenesis may come from any non-carbohydrate sources that can be converted to pyruvate or intermediates of glycolysis (see Figure). For the breakdown of proteins, these substrates include glucogenic amino acids (although not ketogenic amino acids); from breakdown of lipids (such as triglycerides), they include glycerol, odd-chain fatty acids (although not even-chain fatty acids, see below); and from other parts of metabolism they include lactate from the Cori cycle. Under conditions of prolonged fasting, acetone derived from ketone bodies can also serve as a substrate, providing a pathway

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from fatty acids to glucose. Although most gluconeogenesis occurs in the liver, the relative contribution of gluconeogenesis by the kidney is increased in diabetes and prolonged fasting.

The gluconeogenesis pathway is highly endergonic until it is coupled to the hydrolysis of ATP or GTP, effectively making the process exergonic. For example, the pathway leading from pyruvate to glucose-6-phosphate requires 4 molecules of ATP and 2 molecules of GTP to proceed spontaneously. These ATPs are supplied from fatty acid catabolism via beta oxidation.

### Glycogenolysis

Glycogenolysis refers to the breakdown of glycogen. In the liver, muscles, and the kidney, this process occurs to provide glucose when necessary. A single glucose molecule is cleaved from a branch of glycogen, and is transformed into glucose-1-phosphate during this process. This molecule can then be converted to glucose-6-phosphate, an intermediate in the glycolysis pathway.

Glucose-6-phosphate can then progress through glycolysis. Glycolysis only requires the input of one molecule of ATP when the glucose originates in glycogen. Alternatively, glucose-6-phosphate can be converted back into glucose in the liver and the kidneys, allowing it to raise blood glucose levels if necessary.

Glucagon in the liver stimulates glycogenolysis when the blood glucose is lowered, known as hypoglycemia. The glycogen in the liver can function as a backup source of glucose between meals. Liver glycogen mainly serves the central nervous system. Adrenaline stimulates the breakdown of glycogen in the skeletal muscle during exercise. In the muscles, glycogen ensures a rapidly accessible energy source for movement.

### Glycogenesis

Glycogenesis refers to the process of synthesizing glycogen. In humans, glucose can be converted to glycogen via this process. Glycogen is a highly branched structure, consisting of the core protein Glycogenin, surrounded by branches of glucose units, linked together. The branching of glycogen increases its solubility, and allows for a higher number of glucose molecules to be accessible for breakdown at the same time. Glycogenesis occurs primarily in the liver, skeletal muscles, and kidney. The Glycogenesis pathway consumes energy, like most synthetic pathways, because an ATP and a UTP are consumed for each molecule of glucose introduced.

### Pentose Phosphate Pathway

The pentose phosphate pathway is an alternative method of oxidizing glucose. It occurs in the liver, adipose tissue, adrenal cortex, testis, mammary glands, phagocytes, and red blood cells. It produces products that are used in other cell processes, while reducing NADP to NADPH. This pathway is regulated through changes in the activity of glucose-6-phosphate dehydrogenase.

### Fructose Metabolism

Fructose must undergo certain extra steps in order to enter the glycolysis pathway. Enzymes located in certain tissues can add a phosphate group to fructose. This phosphorylation creates fructose-6-phosphate, an intermediate in the glycolysis

pathway that can be broken down directly in those tissues. This pathway occurs in the muscles, adipose tissue, and kidney. In the liver, enzymes produce fructose-1-phosphate, which enters the glycolysis pathway and is later cleaved into glyceraldehyde and dihydroxyacetone phosphate.

### Galactose Metabolism

Lactose, or milk sugar, consists of one molecule of glucose and one molecule of galactose. After separation from glucose, galactose travels to the liver for conversion to glucose. Galactokinase uses one molecule of ATP to phosphorylate galactose. The phosphorylated galactose is then converted to glucose-1-phosphate, and then eventually glucose-6-phosphate, which can be broken down in glycolysis.

### 2. Protein Metabolism

The synthesis of cellular proteins is boosted by growth hormone. Protein synthesis requires the hormone insulin. It is essentially non-existent in the absence of insulin. Insulin may stimulate protein synthesis by speeding up the transport of some amino acids into cells. It also increases glucose availability to cells, reducing the need for amino acids for energy. Most tissue proteins are degraded by glucocorticoids.

The adrenal cortex's glucocorticoids reduce the amount of protein in most tissues while raising the amino acid content in the plasma, as well as increasing both liver and plasma proteins. The glucocorticoids accelerated the breakdown of extra hepatic proteins, resulting in more amino acids being accessible in the bodily fluids. This helps the liver to produce more hepatic cellular proteins and plasma proteins, according to the theory. Testosterone, the male sex hormone, produces an increase in protein deposition in tissues all over the body, particularly in muscle contractile proteins (30 to 50 per cent increase). Growth hormone causes tissues to continue growing almost indefinitely, whereas testosterone causes the muscles and, to a much lesser extent, some other protein tissues to enlarge for only several months. Estrogen, the main female sex hormone, also stimulates protein deposition, but its influence is minor in comparison to that of testosterone.

Thyroxin raises the metabolic rate of all cells, which has an indirect effect on protein metabolism. When there are not enough carbohydrates or lipids to go around, thyroxin triggers rapid protein degradation and consumes it for energy. Thyroxin, on the other hand, can actually increase the rate of protein synthesis if sufficient amounts of carbs and lipids are present, as well as excess amino acids in the extracellular fluid.

### 3. Lipid Metabolism

Lipid metabolism is the synthesis and degradation of lipids in cells, involving the breakdown or storage of fats for energy and the synthesis of structural and functional lipids, such as those involved in the construction of cell membranes. In animals, these fats are obtained from food or are synthesized by the liver. Lipogenesis is the process of synthesizing these fats. The majority of lipids found in the human body from ingesting food are triglycerides and cholesterol. Other types of lipids found in the body are fatty acids and membrane lipids. Lipid metabolism is often considered as the digestion and absorption process of dietary fat; however, there are two sources of fats that organisms can use to obtain energy: from consumed dietary fats and from stored fat. Vertebrates (including humans) use both sources of fat to

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produce energy for organs such as the heart to function. Since lipids are hydrophobic molecules, they need to be solubilized before their metabolism can begin. Lipid metabolism often begins with hydrolysis, which occurs with the help of various enzymes in the digestive system. Lipid metabolism also occurs in plants, though the processes differ in some ways when compared to animals. The second step after the hydrolysis is the absorption of the fatty acids into the epithelial cells of the intestinal wall. In the epithelial cells, fatty acids are packaged and transported to the rest of the body.

### Lipid Digestion

Digestion is the first step to lipid metabolism, and it is the process of breaking the triglycerides down into smaller monoglyceride units with the help of lipase enzymes. Digestion of fats begin in the mouth through chemical digestion by lingual lipase. Ingested cholesterol is not broken down by the lipases and stays intact until it enters the epithelium cells of small intestine. Lipids then continue to the stomach where chemical digestion continues by gastric lipase and mechanical digestion begins (peristalsis). The majority of lipid digestion and absorption, however, occurs once the fats reach the small intestines. Chemicals from the pancreas (pancreatic lipase family and bile salt-dependent lipase) are secreted into the small intestines to help breakdown the triglycerides, along with further mechanical digestion, until they are individual fatty acid units able to be absorbed into the small intestine's epithelial cells. It is the pancreatic lipase that is responsible for signalling for the hydrolysis of the triglycerides into separate free fatty acids and glycerol units.

### Lipid Absorption

The second step in lipid metabolism is absorption of fats. Short chain fatty acids can be absorbed in the stomach, while most absorption of fats occurs only in the small intestines. Once the triglycerides are broken down into individual fatty acids and glycerols, along with cholesterol, they will aggregate into structures called micelles. Fatty acids and monoglycerides leave the micelles and diffuse across the membrane to enter the intestinal epithelial cells. In the cytosol of epithelial cells, fatty acids and monoglycerides are recombined back into triglycerides. In the cytosol of epithelial cells, triglycerides and cholesterol are packaged into bigger particles called chylomicrons which are amphipathic structures that transport digested lipids. Chylomicrons transported via bloodstream and enter adipose and other tissues in the body.

### Lipid Transportation

Due to the hydrophobic nature of membrane lipids, triglycerides and cholesterols, they require special transport proteins known as lipoproteins. The amphipathic structure of lipoproteins allows the triglycerols and cholesterol to be transported through the blood. Chylomicrons are one sub-group of lipoproteins which carry the digested lipids from small intestine to the rest of the body. The varying densities between the types of lipoproteins are characteristic to what type of fats they transport. For example, Very-Low-Density Lipoproteins (VLDL) carry the synthesized triglycerides by our body and Low-Density Lipoproteins (LDL) transport cholesterol to our peripheral tissues. A number of these lipoproteins are

synthesized in the liver, but not all of them originate from this organ.

### Lipid Metabolism Disorders

Lipid metabolism disorders (including inborn errors of lipid metabolism) are illnesses where trouble occurs in breaking down or synthesizing fats (or fat-like substances). Lipid metabolism disorders are associated with an increase in the concentrations of plasma lipids in the blood, such as LDL cholesterol, VLDL, and triglycerides which most commonly lead to cardiovascular diseases. A good deal of the time these disorders are hereditary, meaning it's a condition that is passed along from parent to child through their genes. Gaucher's Disease Types I, II, and III, Niemann–Pick disease, Tay–Sachs disease, and Fabry's disease are all diseases where those afflicted can have a disorder of their body's lipid metabolism. Rarer diseases concerning a disorder of the lipid metabolism are sitosterolemia, Wolman's disease, Refsum's disease, and cerebrotendinous xanthomatosis.

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### 4. Fatty Acid Metabolism

Fatty acid metabolism consists of various metabolic processes involving or closely related to fatty acids, a family of molecules classified within the lipid macronutrient category. These processes can mainly be divided into catabolic processes that generate energy and anabolic processes where they serve as building blocks for other compounds.

In catabolism, fatty acids are metabolized to produce energy, mainly in the form of Adenosine TriPhosphate (ATP). When compared to other macronutrient classes (carbohydrates and protein), fatty acids yield the most ATP on an energy per gram basis, when they are completely oxidized to $CO_2$ and water by beta oxidation and the citric acid cycle. Fatty acids (mainly in the form of triglycerides) are therefore the foremost storage form of fuel in most animals, and to a lesser extent in plants.

In anabolism, intact fatty acids are important precursors to triglycerides, phospholipids, second messengers, hormones and ketone bodies. For example, phospholipids form the phospholipid bilayers out of which all the membranes of the cell are constructed from fatty acids. Phospholipids comprise the plasma membrane and other membranes that enclose all the organelles within the cells, such as the nucleus, the mitochondria, endoplasmic reticulum, and the Golgi apparatus. In another type of anabolism, fatty acids are modified to form myriad other compounds. For example, some second messengers and local hormones. The prostaglandins made from arachidonic acid stored in the cell membrane, are probably the most well-known group of these local hormones.

## 1.6.5 Hormones and Behaviour

Each hormone has a different effect on different cells. Instead, every given hormone can only have a direct effect on cells that have specialised hormone receptors for that hormone. The hormone's target cells are cells that contain these unique receptors.

We shall discuss this topic in the next unit in detail.

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### 1.6.6 Termination of Hormone Action

Termination of hormone action includes the following:

1. Regulation system stops the secretion of hormone.
2. Hormones are isolate from the receptor to stop the signal transducer process.
3. Hormones are degraded by endocytosis at their target cells.
4. Hormones are degraded in liver and blood circulation.
5. Hormones are degraded by oxidation-reduction or deaminization.

**Check Your Progress**

14. Why are hormones called chemical messengers?
15. Define a target cell.
16. How do hormones activate target cells?
17. What is homeostasis?
18. Define glycogenesis.

## 1.7 ANSWERS TO 'CHECK YOUR PROGRESS'

1. Endocrinology is a branch of biology and medicine dealing with the endocrine system, its diseases, and its specific secretions known as hormones.
2. Hormones are chemicals that affect the actions of different organ systems in the body.
3. Diabetes, the most common endocrine disorder encountered in clinical practice, occurs due to decreased secretion of insulin, a hormonal product from pancreas, an endocrine gland.
4. Endocrine glands release hormones into the bloodstream. This lets the hormones travel to cells in other parts of the body.
5. The thyroid gland is situated at the front part of the lower neck.
6. Hormones are chemical in nature so they are called chemical messengers.
7. Diabetes mellitus can cause a wide range of symptoms, including nausea, vomiting, blurred vision, lethargy, a frequent urination, and high levels of glucose in the urine.
8. Parathyroid hormone, causes an increase in blood calcium levels by targeting bone, the intestine, and the kidneys. The parathyroid hormone is the antagonist of calcitonin. Parathyroid hormone release is triggered by falling blood calcium levels and is inhibited by rising blood calcium levels.
9. Ontogeny is development of a single individual, or a system within the individual, from the fertilized egg to death.
10. The term Gastro-enteropancreatic (GEP) system is commonly used which emphasize a system of endocrine cells involving the stomach, intestine, and pancreas.

11. Neuroendocrinology is the branch of biology (specifically of physiology) which studies the interaction between the nervous system and the endocrine system, i.e., how the brain regulates the hormonal activity in the body.
12. Cells in multicellular animals communicate through signalling mechanisms that take place at direct intercellular contacts, or that involve signals released systemically into the extracellular space where they diffuse over large distances and are able to affect targets far removed from the signalling source.
13. Neurosecretion is the storage, synthesis and release of hormones from neurons.
14. A hormone is called a secreted chemical messenger that enables communication between cells and tissues throughout the body. Hormones reach all tissues via blood but only cells that have receptors to bind the hormone act as target cells for the hormone.
15. The target cell is the cell which has receptors specific to a given hormone and will be activated by either a lipid-soluble (permeable to plasma membrane) or water-soluble hormone (binds to a cell-surface receptor).
16. Hormones activate target cells by diffusing through the plasma membrane of the target cells (lipid-soluble hormones) to bind a receptor protein within the cytoplasm of the cell, or by binding a specific receptor protein in the cell membrane of the target cell (water-soluble proteins).
17. Homeostasis is the state of steady internal, physical, and chemical conditions maintained by living systems.
18. Glycogenesis refers to the process of synthesizing glycogen.

## 1.8 SUMMARY

- Endocrinology is a branch of biology and medicine dealing with the endocrine system, its diseases, and its specific secretions known as hormones.
- The endocrine system consists of several glands, all in different parts of the body that secrete hormones directly into the blood.
- Diabetes, the most common endocrine disorder encountered in clinical practice, occurs due to decreased secretion of insulin, a hormonal product from pancreas, an endocrine gland.
- Endocrine glands release hormones into the bloodstream. This lets the hormones travel to cells in other parts of the body.
- Too much or too little of any hormone can harm the body. Medicines can treat many of these problems.
- The pancreas is part of the endocrine system and the digestive system. That's because it secretes hormones into the bloodstream, and makes and secretes enzymes into the digestive tract.
- Hormones are chemical in nature so they are called chemical messengers.

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- The neurons present in grey matter are secretory in nature and are called hypothalmic nuclei.
- Thyroxine hormone is secreted by thyroid gland. The Thyrotropin Releasing Hormone (TRH) from the hypothalamus stimulates the anterior pituitary to secrete Thyroid Stimulating Hormone (TSH).
- If the level of thyroxine in blood is more than the normal, this high thyroxine level produces an inhibitory effect on hypothalamus.
- Blood glucose levels vary widely over the course of a day as periods of food consumption alternate with periods of fasting.
- Cells of the body require nutrients in order to function. These nutrients are obtained through feeding.
- The basal metabolic rate, which is the amount of calories required by the body at rest, is determined by two hormones produced by the thyroid gland: thyroxine, also known as tetraiodothyronine or T4, and triiodothyronine, also known as T3.
- The intermediate lobe of the pituitary gland secretes only one enzyme that is melanocyte stimulating hormone. It is linked with the formation of the black pigment in our skin called melanin.
- The adrenal glands are located above the kidneys in humans and in front of the kidneys in other animals.
- The pancreas, located in the abdomen, below and behind the stomach, is both an exocrine and an endocrine gland.
- The ovaries of the female, located in the pelvic cavity, release two main hormones.
- The pineal gland is located in the diencephalon of the brain.
- Hormones act by binding to receptors, which are usually protein molecules.
- Steroid hormones are the hormones which are the derivative of cholesterol which includes sex hormones and hormones of the adrenal cortex.
- The action of most of the protein hormones are inhibited in the absence of calcium even though ability to increase or decrease c-AMP is comparatively unimpaired.
- A hormone mediate its effects by binding to its receptor molecules present on a target cell.
- The hormone binding with its receptor is a type of signal which is transduced into the activation of one or more intracellular signalling molecules.
- Neuroendocrinology is the branch of biology (specifically of physiology) which studies the interaction between the nervous system and the endocrine system, i.e., how the brain regulates the hormonal activity in the body.
- The endocrine system consists of numerous glands throughout the body that produce and secrete hormones of diverse chemical structure, including peptides, steroids, and neuroamines.
- The neuroendocrine systems control reproduction in all its aspects, from bonding to sexual behaviour.

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- Most hormones found throughout the animal kingdom are short polypeptides, produced by proteolytic cleavage from larger precursor proteins, called prohormones.
- Neurosecretion is the storage, synthesis and release of hormones from neurons.
- Insects play a large role in what is known about neurosecretion. In simpler organisms neurosecretion mechanisms regulate the heart, the process of metamorphosis, and directly influences the development of the gonadal function.
- The glands of the endocrine system secrete hormones directly into the extracellular environment.
- Nuclear hormone receptors are activated by a lipid-soluble hormone, such as estrogen, binding to them inside the cell.
- If a gland is secreting too little hormone because it itself is unable to function normally, the disorder is called primary hyposecretion.
- In biology, homeostasis is the state of steady internal, physical, and chemical conditions maintained by living systems.
- The word homeostasis was coined by Walter Bradford Cannon in 1926.
- The metabolic processes of all organisms can only take place in very specific physical and chemical environments.
- The liver and skeletal muscle self-regulate carbohydrate metabolism in basically the same way as do other cells.
- Carbohydrate metabolism is the whole of the biochemical processes responsible for the metabolic formation, breakdown, and interconversion of carbohydrates in living organisms.
- Glycolysis is the process of breaking down a glucose molecule into two pyruvate molecules, while storing energy released during this process as ATP and NADH.
- Gluconeogenesis (GNG) is a metabolic pathway that results in the generation of glucose from certain non-carbohydrate carbon substrates.
- Glycogenolysis refers to the breakdown of glycogen.
- The pentose phosphate pathway is an alternative method of oxidizing glucose.
- Lipid metabolism often begins with hydrolysis, which occurs with the help of various enzymes in the digestive system.

## 1.9 KEY TERMS

- **Endocrinology:** Endocrinology is a branch of biology and medicine dealing with the endocrine system, its diseases, and its specific secretions known as hormones.
- **Hypothalamus:** The hypothalamus is in the lower central part of the brain.

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- **Thyroid:** The thyroid gland is situated at the front part of the lower neck. It's shaped like a bow tie or butterfly.
- **Parathyroids:** Attached to the thyroid are four tiny glands that work together called the parathyroids.
- **Steroid Hormones:** Steroid hormones are the hormones which are the derivative of cholesterol which includes sex hormones and hormones of the adrenal cortex**.**
- **Protein Hormones**; Protein hormones or peptide hormones are prepared from polymers of amino acids.
- **Pituitary Gland:** The pituitary gland hangs from the base of the brain by the pituitary stalk, and is enclosed by bone.
- **Thyroid Gland**: The thyroid gland is located in the front of the neck, attached with thyroid cartilage, and is shaped like a butterfly, with two wings connected by a central isthmus.
- **Adrenal Glands:** The adrenal glands are located above the kidneys in humans and in front of the kidneys in other animals.
- **Pancreas:** The pancreas, located in the abdomen, below and behind the stomach, is both an exocrine and an endocrine gland.
- **Pineal Gland:** The pineal gland is located in the diencephalon of the brain.
- **Neurosecretion:** Neurosecretion is the storage, synthesis and release of hormones from neurons.
- **Amine Hormones**: Amine hormones are derivative of the amino acid tyrosine.
- **Steroid Hormones**: Steroid hormones are produced by the adrenal cortex and the gonads.
- **Hyposecretion**: If a gland is secreting too little hormone because it itself is unable to function normally, the disorder is called primary hyposecretion.
- **Hypersecretion**: Primary hypersecretion is a gland itself secreting too much hormone while secondary hypersecretion is the excessive stimulation of a gland by its tropic hormone.
- **Hyporesponsiveness**: Target cells do not respond to the hormone due to a deficiency of receptors, a defect in the signal transduction mechanism or a deficiency of an enzyme that catalyses the activation of the hormone.
- **Homeostasis**: In biology, homeostasis is the state of steady internal, physical, and chemical conditions maintained by living systems.
- **Carbohydrate metabolism:** Carbohydrate metabolism is the whole of the biochemical processes responsible for the metabolic formation, breakdown, and interconversion of carbohydrates in living organisms.
- **Glycolysis**: Glycolysis is the process of breaking down a glucose molecule into two pyruvate molecules, while storing energy released during this process as ATP and NADH.

- **Gluconeogenesis**: Gluconeogenesis (GNG) is a metabolic pathway that results in the generation of glucose from certain non-carbohydrate carbon substrates.

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## 1.10 SELF ASSESSMENT QUESTIONS AND EXERCISES

**Short Answer Questions**

1. What is the condition a substance needs to fulfil to be classified as a hormone?
2. How do the hormones control body functions of humans?
3. Name the major glands that make up the endocrine system.
4. Why is the pituitary considered as a master gland?
5. Which hormones control the level of glucose, or sugar, in the blood? Name the gland that secretes these hormones.
6. What is the effect of increased the level of thyroxine in blood?
7. How do hormones regulate the blood glucose levels in humans?
8. How are hormones classified according to their nature of action?
9. Name the major neuroendocrine systems.
10. Write the process of termination of hormone action.

**Long Answer Question**

1. Discuss some common endocrine disorders in detail.
2. What is feedback control? Explain the mechanism of feedback control.
3. Comprehend the classification of hormones according to chemical nature.
4. Explain endocrine disorder in detail with examples.
5. Explain the role of calcium ions as second messenger working.
6. Describe homeostasis in detail.
7. Briefly describe the outline of water-soluble hormone action.

## 1.11 FURTHER READING

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# UNIT 2 ENDOCRINOLOGY - II

NOTES

**Structure**



## 2.0 INTRODUCTION

The endocrine system uses hormones to control and coordinate your body's internal metabolism (or homeostasis) energy level, reproduction, growth and development, and response to injury, stress, and environmental factors.

Hormone are organic substance secreted by endocrine glands that functions in the regulation of physiological activities and in maintaining homeostasis. Hormones carry out their functions by evoking responses from specific organs or tissues that are adapted to react to minute quantities of them. The classical view of hormones is that they are transmitted to their targets in the bloodstream after discharge from the glands that secrete them. This mode of discharge (directly into the bloodstream) is called endocrine secretion.

The earliest study of endocrinology began in China. The Chinese were isolating sex and pituitary hormones from human urine and using them for medicinal purposes by 200 BC. They used many complex methods, such as sublimation of steroid hormones. Hormones are used to communicate between organs and tissues. In vertebrates, hormones are responsible for the regulation of many physiological processes and behavioral activities such as digestion, metabolism, respiration, sensory perception, sleep, excretion, lactation, stress induction, growth and development, movement, reproduction, and mood manipulation. In plants, hormones modulate almost all aspects of development, from germination to senescence. Sometimes, hormone levels can be too high or too low. When this happens, it can have a number of effects on health. The signs and symptoms depend on the hormone that's out of balance.

In this unit you will study about hormone structure and evolution, biosynthesis and secretion of hormones, hormone in circulation and other body fluids, biosynthesis of steroid hormones de-novo, hormones and behaviour, hormones, growth and development, hormones and reproduction

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## 2.1 OBJECTIVES

After going through this unit, you will be able to:

- Understand hormone structure and evolution
- Analyze biosynthesis and secretion of hormones
- Explain hormone glands in circulation and other body fluids
- Describe biosynthesis of steroid hormones de-novo
- Explain hormones and their effect on behaviour, growth and development

## 2.2 HORMONE STRUCTURE AND EVOLUTION

The homeostatic adaptations an organism makes to a constantly changing environment are in large part accomplished through alterations of the activity and amount of proteins. Hormones provide a major means of facilitating these changes. A hormone-receptor interaction results in generation of an intracellular signal that can either regulate the activity of a select set of genes, thereby altering the amount of certain proteins in the target cell, or affect the activity of specific proteins, including enzymes and transporter or channel proteins. The signal can influence the location of proteins in the cell and can affect general processes such as protein synthesis, cell growth and replication, perhaps through effects on gene expression. Other signaling molecules include cytokines, interleukins, growth factors and metabolites. Excessive, deficient or inappropriate production and release of hormones and of other regulatory molecules are major causes of disease.

All the steroid hormones are derived from cholesterol except Vitamin D. They all consist of the same cyclopentanophenanthrene ring and atomic numbering system as cholesterol does. Conversion of C27 cholesterol to the 18, 19 and 21 carbon steroid hormones consists of the rate-limiting, irreversible cleavage of a 6-carbon residue from cholesterol, producing pregnenolone (C21) and isocaproaldehyde. Popular names of steroid hormones are quite popular, but the systematic nomenclature is just now gaining acceptance and familiarity with the nomenclatures. Steroids that have 21 carbon atoms are systematically known as pregnanes, whereas those steroids that contain 19 and 18 carbon atoms are termed as androstanes and estranes, respectively.

**Structure and Function of Hormones**

Integration of body functions in humans and other higher organisms is carried out through the nervous system, the immune system and the endocrine system. The endocrine system consists of a number of tissues that secrete their products in the circulatory system and from there they get disseminated throughout the body, regulating the function of distant tissues and maintaining the process of homeostasis. In a separate but related system, exocrine tissues secrete their products in the ducts and then outside the body or towards the intestinal tract. Basically, the

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endocrine hormones are derived from the amino acids, peptides or sterols and to act at sites distant from their tissue of origin. However, the latter definition has started to blur as it is found that some secreted substances act at a distance (classical endocrines), closer towards the cells, which secrete them (paracrines) or directly on the cell that secreted them (autocrines). Insulin like growth factor -1(IGF-1) that behaves as an endocrine, paracrine and autocrine, acts as a prime example of this difficulty.

Hormones are usually present in the plasma and interstitial tissue at concentrations in the range of 10–7M to 10–10M. Due to these low physiological concentrations, sensitive protein receptors have come up in target tissues for sensing the presence of weak signals. In addition, the systemic feedback mechanisms have also evolved to regulate the endocrine hormone production.

Once a hormone gets secreted by an endocrine tissue, it usually binds with a specific plasma protein carrier, with the complex being disseminated to distant tissues. Plasma carrier proteins exist for all the classes of endocrine hormones. Carrier proteins for peptide hormones prevent hormone destruction by plasma proteases. Carriers for steroid and thyroid hormones permit these very hydrophobic substances to be present in the plasma at concentrations several hundred-fold greater than their solubility in water might permit. Carriers for small, hydrophilic amino acid-derived hormones prevent their filtration through the renal glomerulus, prolonging their circulating half-life greatly.

Those tissues that are capable of responding to endocrines have two properties in common. These properties are as follows:

- They posses a receptor having very high affinity for hormone.
- The receptor is coupled to a process that regulates metabolism of the target cells.

Receptors for most of the amino acid-derived hormones and all peptide hormones are usually located on the plasma membrane. Activation of these receptors by hormones (the first messenger) leads towards the intracellular production of a second messenger, such as cAMP. It initiates the intracellular biological response. Steroid and thyroid hormone are hydrophobic and diffuse from their binding proteins in the plasma, across the plasma membrane to intracellularly localize the receptors. The resultant complex of steroid and receptor bind to response elements of nuclear DNA, regulating the production of mRNA for specific proteins.

**Check Your Progress**

1. What are all steroid hormones derived from?
2. Name the body system that carries out the integration of body function in higher organisms.
3. Where are hormones present in body?
4. How do the hormones change the cell functions?

## 2.3 BIOSYNTHESIS AND SECRETION OF HORMONES

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A hormone is any member of a class of signalling molecules in multicellular organisms that are transported to distant organs to regulate physiology and behaviour. Hormones are required for the correct development of animals, plants and fungi. The lax definition of a hormone, as a signalling molecule that acts distant from its site of production, means that many different classes of molecule can be defined as hormones. Among the substances that can be considered hormones, are eicosanoids (e.g., prostaglandins and thromboxanes), steroids (e.g., oestrogen and brassinosteroid), amino acid derivatives (e.g., epinephrine and auxin), protein / peptides (e.g., insulin and CLE peptides) and gases (e.g., ethylene and nitrous oxide).

Hormones are used to communicate between organs and tissues. In vertebrates, hormones are responsible for the regulation of many physiological processes and behavioural activities, such as digestion, metabolism, respiration, sensory perception, sleep, excretion, lactation, stress induction, growth and development, movement, reproduction, and mood manipulation. In plants, hormones modulate almost all aspects of development, from germination to senescence.

Hormones affect distant cells by binding to specific receptor proteins in the target cell, resulting in a change in cell function. When a hormone binds to the receptor, it results in the activation of a signal transduction pathway that typically activates gene transcription, resulting in increased expression of target proteins. Hormones can also act in rapid, non-genomic pathways that can be synergistic with genomic effects. Water-soluble hormones (such as, peptides and amines) generally act on the surface of target cells via second messengers. Lipid soluble hormones, (such as, steroids) generally pass through the plasma membranes of target cells (both cytoplasmic and nuclear) to act within their nuclei. A notable exception to this is brassinosteroids in plants, which despite being lipid soluble, still bind to their receptor at the cell surface.

In vertebrates, endocrine glands are specialized organs that secrete hormones into the endocrine signalling system. Hormone secretion occurs in response to specific biochemical signals and is often subject to negative feedback regulation. For instance, high blood sugar (serum glucose concentration) promotes insulin synthesis. Insulin then acts to reduce glucose levels and maintain homeostasis, leading to reduced insulin levels. Upon secretion, water soluble hormones are readily transported through the circulatory system. Lipid-soluble hormones must bond to carrier plasma glycoproteins (e.g., Thyroxine-Binding Globulin (TBG)) to form ligand-protein complexes. Completely active hormones can be released into the bloodstream (as seen in insulin and growth hormones), but some travel as prohormones that must be activated in specific cells through a series of activation steps that are commonly highly regulated. The endocrine system secretes hormones directly into the bloodstream, typically via fenestrated capillaries, whereas the exocrine system secretes its hormones indirectly using ducts. Hormones with paracrine function diffuse through the interstitial spaces to nearby target tissue.

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Hormonal signalling involves the following steps:

- Biosynthesis of a particular hormone in a particular tissue
- Storage and secretion of the hormone
- Transport of the hormone to the target cell(s)
- Recognition of the hormone by an associated cell membrane or intracellular receptor protein
- Relay and amplification of the received hormonal signal via a signal transduction process: This then leads to a cellular response. The reaction of the target cells may then be recognized by the original hormone-producing cells, leading to a downregulation in hormone production. This is an example of a homeostatic negative feedback loop.
- Breakdown of the hormone

Hormone producing cells are found in the endocrine glands, such as the thyroid gland, ovaries, and testes. Exocytosis and other methods of membrane transport are used to secrete hormones when the endocrine glands are signalled. The hierarchical model is an oversimplification of the hormonal signalling process. Cellular recipients of a particular hormonal signal may be one of several cell types that reside within a number of different tissues, as is the case for insulin, which triggers a diverse range of systemic physiological effects. Different tissue types may also respond differently to the same hormonal signal.

**Synthesis of Hormones**

Like all other proteins, peptide and protein hormones are synthesised in the rough endoplasmic reticulum. Their amino acid sequences are determined by specific mRNA having definite nucleotide sequences dictated by specific gene. Translation of such mRNA results in the ribosomal synthesis of a protein, usually larger than the mature hormone.

This precursor is referred to as prohormone or preprohormone. The prohormone is extended at their amino termini by a hydrophobic amino acid sequence called leader or signal peptide. Preprohormones in addition to signal peptides, also contain internal cleavage sites that yield different bioactive peptides upon enzymatic action.

With the help of hydrophobic leader pep-tide, the hormone precursors move across the ER membrane to be transported to golgi complex. The leader sequence is removed before the synthesis of polypeptide chain has ended and this permits the protein to gain its secondary structure during its transport to golgi.

Following its arrival at golgi the prohormones may be processed by proteolytic enzyme to generate mature hormones, and /or by other enzymes that add non-protein residues such as carbohydrates in case of glycoprotein hormones, for example Follicle-Stimulating Hormone (FSH) and Luteinizing Hormone (LH).

Whatever the case, the hormone stored in vesicles will fuse the cell membrane dur-ing their release and their contents will be extruded into the extracellular perivascular space. This process is called **exocytosis**, which is the most common process of hormone release.

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The synthesis of amine and steroid hor-mones are different from that of peptide hormones. Amine and steroid hormones originate from the precursor molecule tyrosine and cholesterol, respectively.

Within the cells of synthesis, these precursor molecules are subjected to the sequential action of sev-eral enzymatic catalysis resulting in the formation of various intermediate products that themselves may be hormones. In con-trast to peptide hormones, thyroid hormone and steroids, once produced, can freely cross the cell membrane without having to be packed in granules and are actively exocytosed.

There are ample examples of hormones that are produced at sites other than those in which their precursor is formed. In some cases, a hormone with less activity is converted into an active form by the action of enzymes in the circulation or other tissues.

## Synthesis of Hormones

Hormones are involved in coordination of series of physiological events. The synthesis of all hormones is determined at the genetic level.

**1. Synthesis of Peptide Hormones:** Peptide hormones are synthesized by translational method. Several steps are involved in the transfer of information (gene expression) encoded in the polynucleotide language of DNA to the poly-amino acid language of biologically active proteins (hormones).

The formation of polypeptide hormones involves the initial formation of a parent molecule, called prohormone. As a result of post translational changes, such as cleavage by enzymes, the prohormone is broken up to form hormone itself. It is observed that according to genetic code protein synthesis takes place in ribosomes after transcription. This is known as prohormone which is extended at their amino termini by a hydrophobic amino acid sequence, called signal peptide or leader peptide.

With the help of signal peptide prohormone passes into the Golgi complex via endoplasmic reticulum. The prohormone is then converted into proper hormone by enzymic action and finally secreted by exocytosis. Following schematic representation shows the steps in cellular synthesis of peptide hormone (Figure 2.1).

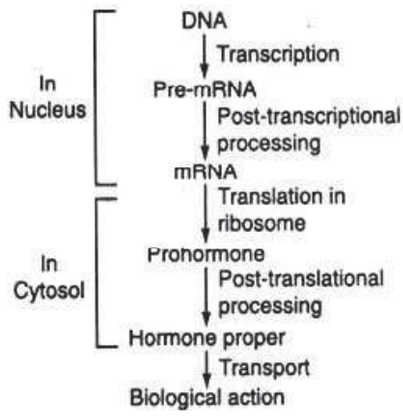


***Fig. 2.1*** *Flowchart Showing Synthesis of Protein Hormone*

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**2. Synthesis of Non-Peptide Hormone:** The synthesis of non-peptide hormones, such as, thyroid hormones, adrenal medullary hormones, steroid hormones involve the action of multiple enzymes. Thyroid and medullary hormones are basically derived from the amino acid tyrosine. All the steroid hormones are synthesized from precursor cholesterol molecule. Synthesis of non-peptide hormones occurs within the cells from their precursor molecules by sequential actions of several enzymes. They may be synthesized within mitochondria or within endoplasmic reticulum. They are then secreted either by active exocytosis or by simple diffusion.

**Transport and Metabolism of Hormones**

Most of hormones after secretion are directly poured into the blood stream and reach the target organ via circulation or it may be bound to a carrier protein. Sometimes hormones are transported through intercellular fluid, especially in paracrine secretion.

Hormones are metabolically degraded after their action. During metabolism of hormones, the molecule may be altered, consumed at the site of action. Usually degraded in the liver and kidney, then excreted through urine. Peptide hormones are digested and converted into individual amino acids in lysosomes.

**Transport of Hormones**

Hormones may be transported from the site of synthesis to their target organ or cell by one of following several ways:

(i) Endocrine, when the hormones are released into circulation directly

(ii) Paracrine, when the released hormones diffuse to its adjacent target cells through immediate extracellular space

(iii) Autocrine, when following release, the hormone is feed backed on the cell of origin

(iv) Neuroendocrine, when the hormone released by a nerve is blood borne

(v) Neurocrine, when a neuron releases its hormone into a synaptic cleft between two adjacent cells

(vi) Luminal, when a hormone is released into the lumen of the gut

**Transport and Metabolism of Hormones**

Following the release to outside from site of synthesis, hormones may circu-late freely into the bloodstream or it may be bound to a carrier protein. In general, amines, peptides and protein hormones circulate in free form, whereas steroids and thyroid hormones are bound to transport proteins (carrier proteins).

An exception to this rule is provided by insulin-like growth factors which despite being polypeptide, circulate tightly attached to specific proteins. Most common carrier proteins include Thyroid Hormone-Binding Globulin (TBG) that carry thyroid hormone, Testosterone-Binding Globulin (TeBG) and Cortisol-Binding Globu-lin (CBG) that carry testosterone and Cortisol, respectively.

Hormones, either initiate immediate target tissue responses or set in motion

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more long-term effects. In either case, hormones must be continuously inactivated or the cellular response would be continuously acti-vated. The Metabolic Clearance Rate (MCR) of a hormone defines quantitatively its removal from plasma.

Under steady state conditions, the MCR represents the volume of plasma cleared of the hormone per unit of time. The plasma half-life of a hormone is inversely related to MCR (Refer Table 2.1). Only a little portion of circulating hormones is removed from the circulation by most target tissues.

***Table 2.1:*** *Half Life of Protein, Amine and Steroid Hormones in Plasma*

| Hormone | Half-life |
|---|---|
| Amines | 2–3 min. |
| Thyroid hormones | |
| $T_4$ | 6–7 days |
| $T_3$ | 0.75 days |
| Polypeptides | 4–40 min. |
| Proteins | 15–170 min. |
| Steroids | 4–120 min. |

The bulk of hormone clearance is done by the liver and the kidneys where a number of enzymatic degradations occur that include hydrolysis, oxidation, hydroxylation, methylation, decarboxylation, sulfation and glucoronidation. In general, only a small fraction (<1%) of any hormone is excreted as such through urine or feces.

### Patterns of Hormone Secretion

The basal secretion of most hormones is not a con-tinuous process but rather has a pulsatile nature. The pulsatile pattern of hormone secretion is characterised by episodes of release that can be as frequent as every 5-10 minutes; each episode is followed by a quies-cent period during which plasma levels of hormone fall toward basal values.

Another discharge then occurs, and the cycle repeats itself, often varying in both amplitude and fre-quency of the pulses. In the case of hormones subjected to negative feedback control, removal of the inhibitory feedback signal results in a marked enhancement of the ampli-tude and frequency of episodes of secretion.

Most prominent episodes of release may occur with a frequency of about an hour, this mode of release is called circhoral; when episodes of release occur at intervals longer than an hour but less than 24 hours, the rhythm is called ultradian; if the periodicity is of about a day, the rhythm is called circadian and if it occurs every day, then it is called quotidian or diurnal (e.g., Adreno CorticoTropic Hormone or ACTH).

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Some hormones may have a much less periodicity. For example, the monthly pre-ovulatory discharge of gonadotropins recurs about every 30 days, a pattern of release known as circatrigintan. Thyroxine exhibits changes in plasma levels that occur over months. If the changes take place on a yearly basis, the rhythm is called circannual or seasonal.

**Control of Hormone Secretion**

Following are the controls of hormone secretion:

**(i) Neural Control**

Nerve impulses control some endocrine secretions. For exam-ple, during stress and emotion, splanchnic nerve stimulates the synthesis and release of catecholamines from adrenal medulla. Nerve impulses from hypothalamic osmoreceptors evoke secretion of vasopressin from neuro--hypophyseal axon-terminals. Impulses from brain, hippocampus, amygdala and other limbic system areas may cause release of acetylcholine and biogenic amines at their axon-terminals that in turn, regulate the release of different hypophysiotropic hor-mones, such as Growth Hormone-Releasing Hormone (GHRH), Corticotropin-Releasing Hormone (CRH), and Thyrotropin-Releasing Hormone (TRH) from hypothalamus.

**(ii) Endocrine Control**

Hormones often regulate hormone secretion from other endocrine glands. For example, secretion of adrenocortical, gonadal and thyroid hor-mones are stimulated respectively by cortico-tropin Adreno CorticoTropic Hormone (ACTH), Gonadotropins (GTH) and Thyrotropin (TSH), which are as such tropic hormones of anterior pituitary that in turn are regulated by hypothalamic hypophysio-tropic hormones, such as Corticotropin-Releasing Hormone (CRH), Gonadotropin-Releasing Hormone (GnRH) and Thyrotropin-Releasing Hormone (TRH), respectively.

**(iii) Feedback Controls**

The secretion of a hormone may be stimulated or inhibited by the feedback effect of some other hormone or metabolites.

**1. Negative Feedback Control:** A high blood level of a hormone may inhibit the secretion of that hormone. It is shown by tar-get gland hormones that inhibit the secretion of their tropic hormones. For example, high levels of Cortisol from adrenal cortex may inhibit the secretion of pituitary-corticotropin release and Corticotropin-Releasing Hormone (CRH) from hypothalamus through long-loop feedback, all these lead to decline in Cortisol secretion itself.

High levels of tro-pic hormones sometimes inhibit the secretion of corresponding releasing factor from hypothalamus through short-loop feedback (Figure 2.2). Sometimes, blood level of some ions or metabolites also shows feedback con-trol over hormone release. For example, a rise in serum $Ca^{2+}$ causes rectilinear fall in para-thyroid hormone; rise in blood glucose cau-ses a decline in glucagon secretion.

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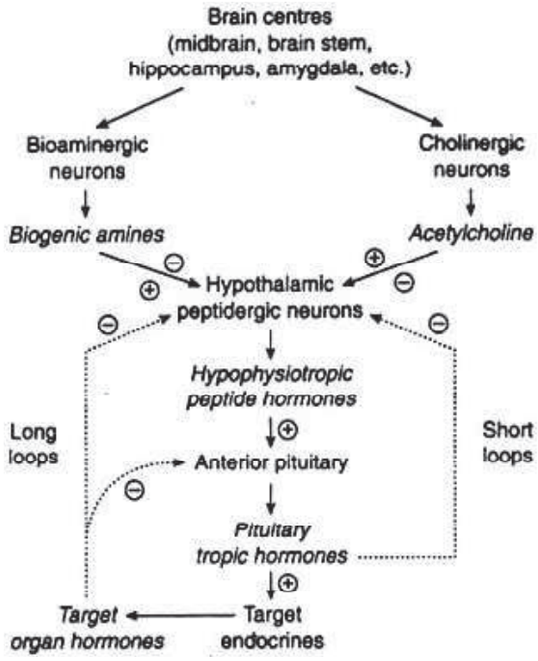


***Fig.2.2*** *Positive and Negative Feedback Control of Hormones*

**2. Positive Feedback Control:** Hormone secretion may be stimulated by a positive feedback effect of another hormone or ion or metabolite. For example, sharp preovulatory rise in LH secretion from anterior pituitary occurs in response to high blood level of estradiol; elevated serum calcium level cau-ses stimulation of calcitonin release from thyroid gland.

Furthermore, some hormones may exert negative feedback on the cells within which they are synthesised by a so-called autoinhibition.

**Regulation**

The rate of hormone biosynthesis and secretion is often regulated by a homeostatic negative feedback control mechanism. Such a mechanism depends on factors that influence the metabolism and excretion of hormones. Thus, higher hormone concentration alone cannot trigger the negative feedback mechanism. Negative feedback must be triggered by overproduction of an 'Effect' of the hormone.

Hormone secretion can be stimulated and inhibited by:

- Other hormones (*stimulating*- or *releasing* -hormones)
- Plasma concentrations of ions or nutrients, as well as binding globulins
- Neurons and mental activity
- Environmental changes, e.g., of light or temperature

One special group of hormones is the tropic hormones that stimulate the hormone production of other endocrine glands. For example, Thyroid-Stimulating Hormone (TSH) causes growth and increased activity of another endocrine gland, the thyroid, which increases output of thyroid hormones.

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To release active hormones quickly into the circulation, hormone biosynthetic cells may produce and store biologically inactive hormones in the form of pre- or prohormones. These can then be quickly converted into their active hormone form in response to a particular stimulus.

Eicosanoids are considered to act as local hormones. They are considered to be 'Local' because they possess specific effects on target cells close to their site of formation. They also have a rapid degradation cycle, making sure they do not reach distant sites within the body.

Hormones are also regulated by receptor agonists. Hormones are ligands, which are any kinds of molecules that produce a signal by binding to a receptor site on a protein. Hormone effects can be inhibited, thus regulated, by competing ligands that bind to the same target receptor as the hormone in question. When a competing ligand is bound to the receptor site, the hormone is unable to bind to that site and is unable to elicit a response from the target cell. These competing ligands are called antagonists of the hormone.

### 2.3.1 Hormone Glands in Circulation and other Body Fluids

Hormones are chemical messengers that are secreted directly into the blood, which carries them to organs and tissues of the body to exert their functions. There are various types of hormones that have different bodily functions and processes.

Hormones circulate throughout the body and stimulate a response in cells that have receptors able to bind with them. In our body, hormones are produced by the specific glands, which are either called hormone producing glands or hormone glands which are scientifically termed as endocrine glands.

The functioning and roles of hormones and the glands is discussed below in detail.

A gland is an organ which produces and releases substances that perform a specific function in the body. There are two types of glands, endocrine glands are ductless glands and release the substances that they make hormones directly into the bloodstream. There are another type of glands called exocrine glands (e.g., sweat glands, lymph nodes). These are not considered as a part of the endocrine system as they do not produce hormones and they release their product through a duct.

Endocrine glands, such as the pancreas and thyroid gland, use the bloodstream to monitor the body's internal environment and to communicate with each other through substances called hormones, which are released into the bloodstream.

The adrenal glands are small structures attached to the top of each kidney. The human body has two adrenal glands that release chemicals called hormones into the bloodstream. These hormones affect many parts of the human body.

The endocrine glands secrete hormones into the surrounding interstitial fluid; those hormones then diffuse into blood and are carried to various organs and

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tissues within the body. The endocrine glands include the pituitary, thyroid, parathyroid, adrenal glands, gonads, pineal, and pancreas.

The pituitary gland, sometimes called the hypophysis, is located at the base of the brain. It is attached to the hypothalamus. The posterior lobe stores and releases oxytocin and antidiuretic hormone produced by the hypothalamus. The anterior lobe responds to hormones produced by the hypothalamus by producing its own hormones, most of which regulate other hormone-producing glands.

The pituitary gland, sits at the base of the brain, just above the brain stem. It is lobe-shaped and hangs down from the hypothalamus, to which it is connected to via a narrow stalk. The anterior part of the pituitary is toward the front, and the posterior end is toward the back. The parathyroid glands, are round structures located on the surface of the right and left lobes of the thyroid gland. In the illustration shown, there are two parathyroid glands on each side, and one is located above the other. The adrenal glands are lumpy, irregular structures located on top of the kidneys. The pancreas is a flattened, elongated lumpy organ, narrower at one end; and is tucked between the stomach and intestine.

The anterior pituitary produces six hormones, namely Growth Hormone, Prolactin, Thyroid-Stimulating Hormone (TSH), Adreno CorticoTropic Hormone (ACTH), Follicle-Stimulating Hormone (FSH), and Luteinizing Hormone (LH). Growth hormone stimulates cellular activities like protein synthesis that promote growth. Prolactin stimulates the production of milk by the mammary glands. The other hormones produced by the anterior pituitary regulate the production of hormones by other endocrine tissues. The posterior pituitary is significantly different in structure from the anterior pituitary. It is a part of the brain, extending down from the hypothalamus, and contains mostly nerve fibers that extend from the hypothalamus to the posterior pituitary.

The endocrine system produces hormones that function to control and regulate many different body processes. The endocrine system coordinates with the nervous system to control the functions of the other organ systems. Cells of the endocrine system produce molecular signals called hormones. These cells may compose endocrine glands, may be tissues or may be located in organs or tissues that have functions in addition to hormone production. Hormones circulate throughout the body and stimulate a response in cells that have receptors able to bind with them. The changes brought about in the receiving cells affect the functioning of the organ system to which they belong. Many of the hormones are secreted in response to signals from the nervous system, thus the two systems act in concert to effect changes in the body.

## Hormones

Maintaining homeostasis within the body requires the coordination of many different systems and organs. One mechanism of communication between neighboring cells, and between cells and tissues in distant parts of the body, occurs through the release of chemicals called hormones. Hormones are released into body fluids,

usually blood, which carries them to their target cells where they elicit a response. The cells that secrete hormones are often located in specific organs, called endocrine glands, and the cells, tissues, and organs that secrete hormones make up the endocrine system. Examples of endocrine organs include the pancreas, which produces the hormones insulin and glucagon to regulate blood-glucose levels, the adrenal glands, which produce hormones such as epinephrine and norepinephrine that regulate responses to stress, and the thyroid gland, which produces thyroid hormones that regulate metabolic rates.

Steroid hormones play an essential role in regulating water and salt balance, metabolism and stress response, and in initiating and maintaining sexual differentiation and reproduction. Researchers investigating steroid-related endocrine conditions have measured alterations in the steroid metabolome for several decades. While clinical laboratories have traditionally measured changes in individual diagnostic marker steroids, the quantification of steroid panels are now gaining widespread traction due to advances in technology, further driven by the emerging diagnostic power of steroid metabolomics, i.e., the combination of mass spectrometry-based steroid profiling with unbiased data analysis by machine learning approaches.

In most cases, alterations in steroid profiles associated with endocrine disorders were identified long before the responsible enzymes were identified or characterized following the advent of modern molecular techniques. While the biochemical pathways for the biosynthesis and metabolism of steroid hormones are now mostly well defined, a gulf still exists with regard to the application of this knowledge to the interpretation of the measured multi-steroid profiles in serum and urine. Researchers and clinicians are increasingly dependent on results obtained by steroid metabolome analysis but are often unfamiliar with the metabolic pathways resulting in the observed steroid profile and the distinct metabolic pathways explaining the differences between serum and urine steroid metabolomes.

### 2.3.2 Biosynthesis of Steroid Hormones De-Novo

Steroid hormones are produced through de novo steroidogenesis in the adrenal cortex, the gonads and the placenta. In addition, a range of neuro steroids are produced in the brain, however these are beyond the scope of this review. Steroidogenic tissues are unique in their ability to utilize cholesterol as starting material for the mitochondrial biosynthesis of pregnenolone, the precursor steroid in the biosynthesis of all steroid hormones. Cholesterol can be obtained from multiple sources including de novo biosynthesis from acetate in the Endoplasmic Reticulum (ER) the hydrolysis of cholesteryl esters stored in lipid droplets by cholesteryl ester hydrolases, exogenous lipoprotein-derived cholesterol esters from LDL receptor-mediated endocytic and/or SR-BI-mediated uptake pathways, and free cholesterol residing in the plasma membrane. All three primary steroidogenic organs, namely the adrenal cortex, gonads and placenta, can biosynthesize cholesterol de novo under the regulation of tropic hormones and plasma lipoproteins are widely accepted as the principal source of cholesterol used for steroid biosynthesis.

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Steroid hormones are bind to ligand-activated proteins, which regulates to the transcription of selected genes. They are found in nucleus and cytosol and they belong to the steroid and thyroid hormone receptor super-family of proteins, which includes not just the receptors for steroid hormones, but also for thyroid hormone (TR), vitamin D, retinoic acid (RAR) and glucocorticoids (GR). This large class of receptors is termed as the nuclear receptor.

When these receptors bind the ligand they undergo a conformational change, which renders them activated to recognize and bind to particular nucleotide sequences. These particular nucleotide sequences in the DNA are termed as Hormone-Response Elements (HREs). When the ligand-receptor complexes interact with the DNA they alter the transcriptional level of the associated gene. Thus, the steroid-thyroid family of receptors all has three distinct domains as follows:

- A ligand-binding domain
- A DNA-binding domain
- A transcriptional regulatory domain

Although there is the commonly observed effect of altered transcriptional activity in response to hormone-receptor interaction, there are family member-specific effects with ligand-receptor interaction. Binding of thyroid hormone to its receptor, results in the release of the receptor from the DNA. Several receptors are induced for interacting with other transcriptional mediators in response to the ligand binding. Binding of glucocorticoid leads to the translocation of the ligand-receptor complex from the cytosol to the nucleus.

The receptors for the retinoids are recognized as RARs and they exist in at least three subtypes, namely, RARα, RARβ and RARγ. In addition, there is another family of nuclear receptors known as the retinoid X receptors (RXRs), which represent a second class of retinoid-responsive transcription factors. The RXRs enhance the DNA-binding activity of RARs and the thyroid hormone receptors (TRs). The RXRs depict a class of receptors, which bind the retinoid 9-cis-retinoic acid. They serve as obligatory heterodimeric partners for numerous members of the nuclear receptor family. RXRα is expressed with highest levels like liver, kidney, spleen, placenta and skin. The critical role for RXRα in development is explained by the fact that null mice are embryonic lethals. RXRβ is crucial for spermatogenesis and RXRγ has a limited expression in both brain and muscle. The major difference between the RARs and RXRs is that the former exhibits highest affinity for all-trans-retinoic acid (all-trans-RA) and the latter for 9-cis-RA.

Additional super-family members are the peroxisome proliferator-activated receptors (PPARs). The PPAR family consists of PPARα, PPARβ/δ and PPARγ. All these receptors form a heterodimer with the RXRs. The first family member identified was PPARα and it was found by the virtue of it binding with the fibrate class of anti-hyperlipidemic drugs or peroxisome proliferators. Consequently, it was shown that PPARα is the endogenous receptor for polyunsaturated fatty acids. Expression of PPARα is also seen in macrophage foam cells and vascular endothelium. Its role in these cells is thought to be the activation of anti-inflammatory and anti-atherogenic effects. PPARγ is a master regulator of adipogenesis and is

most abundantly expressed in the adipose tissue. Low levels of expression are also observed in liver and skeletal muscle. PPARγ was identified as the target of the thiazolidinedione (TZD) class of insulin sensitizing drug. The mechanism of action of the TZDs is a function of the activation of PPARγ activity and the consequent activation of adipocytes leading to increased fat storage and secretion of insulin-sensitizing adipocytokines such asadiponectin. PPARδ is expressed in most tissues and is involved in the promotion of mitochondrial fatty acid oxidation, energy consumption and thermogenesis. PPARδ serves as the receptor for polyunsaturated fatty acids and VLDLs. Current pharmacologic targeting of PPARδ is aimed at increasing HDL levels in humans since experiments in animals have shown that increased PPARδ levels result in increased HDL and reduced levels of serum triglycerides.

Mutations in the gene for PPARγ have been correlated with the insulin resistance. It is unclear as to how the impaired PPARγ signaling can affect the sensitivity of the body to insulin or indeed if the observed mutations are a direct or indirect reasons for the symptoms of insulin resistance.

**Check Your Progress**

5. Where are hormone producing cells present in human body?
6. What is negative feedback control?
7. How is steroid hormones produced in cells?

## 2.4 HORMONES AND BEHAVIOUR

To understand the hormone-behaviour relationship, it is important briefly to describe hormones. Hormones are organic chemical messengers produced and released by specialized glands called endocrine glands. Hormones are released from these glands into the blood, where they may travel to act on target structures at some distance from their origin. Hormones are similar in function to neurotransmitters, the chemicals used by the nervous system in coordinating animals' activities. However, hormones can operate over a greater distance and over a much greater temporal range than neurotransmitters. Examples of hormones that influence behaviour include steroid hormones, such as testosterone (a common type of androgen), estradiol (a common type of estrogen), progesterone (a common type of progestin), and cortisol (a common type of glucocorticoid) (Table 2.2, A-B). Several types of protein or peptide (small protein) hormones also influence behaviour, including oxytocin, vasopressin, prolactin, and leptin.

***Table 2.2 A:*** *Prominent Hormones that Influence Behaviour*

| **Steroid Hormones** | |
|---|---|
| Cortisol | Increases carbohydrate metabolism; mediates stress responses |
| Estradiol | Uterine and other female tissue development; regulates sexual motivation and performance in females and males |
| Testosterone | Promotes sperm production and male secondary sexual characteristics; promotes sexual motivation and behavior, typically by being converted to estradiol |

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***Table 2.2 B:*** *Prominent Hormones that Influence Behaviour*

| Peptides and Protein Hormones | |
|---|---|
| Oxytocin | Stimulates milk letdown and uterine contractions during birth; Promotes social bonding |
| Prolactin | Many actions relating to reproduction, water balance, and behavior associated with parental care |
| Thyroxine | Increases oxidation rates in tissue and affects neural development |
| Vasopressin | Increases water reabsorption in the kidney and affects learning and memory |

Hormones coordinate the physiology and behaviour of individuals by regulating, integrating, and controlling bodily functions. Over evolutionary time, hormones have often been co-opted by the nervous system to influence behaviour to ensure reproductive success. For example, the same hormones, testosterone and estradiol, that cause gamete (egg or sperm) maturation also promote mating behaviour. This dual hormonal function ensures that mating behaviour occurs when animals have mature gametes available for fertilization. Another example of endocrine regulation of physiological and behavioural function is provided by pregnancy. Estrogens and progesterone concentrations are elevated during pregnancy, and these hormones are often involved in mediating maternal behaviour in the mothers.

Not all cells are influenced by each and every hormone. Rather, any given hormone can directly influence only cells that have specific hormone receptors for that particular hormone. Cells that have these specific receptors are called target cells for the hormone. The interaction of a hormone with its receptor begins a series of cellular events that eventually lead to activation of enzymatic pathways or, alternatively, turns on or turns off gene activation that regulates protein synthesis. The newly synthesized proteins may activate or deactivate other genes, causing yet another cascade of cellular events. Importantly, sufficient numbers of appropriate hormone receptors must be available for a specific hormone to produce any effects. For example, testosterone is important for male sexual behaviour. If men have less testosterone, then sexual motivation may be low, and it can be restored by testosterone treatment. However, if men have normal or even elevated levels of testosterone yet display low sexual drive, then it might be possible for a lack of receptors to be the cause and treatment with additional hormones will not be effective.

## 2.5 HORMONES, GROWTH AND DEVELOPMENT

Growth Hormone (GH) or somatotropin, also known as Human Growth Hormone (hGH or HGH) is a peptide hormone that stimulates growth, cell reproduction, and cell regeneration in humans and other animals. It is thus important in human development. GH also stimulates production of IGF-1 and increases the concentration of glucose and free fatty acids. It is a type of mitogen which is

NOTES

specific only to the receptors on certain types of cells. GH is a 191-amino acid, single-chain polypeptide that is synthesized, stored and secreted by somatotropic cells within the lateral wings of the anterior pituitary gland.

A recombinant form of hGH called somatropin (INN) is used as a prescription drug to treat children's growth disorders and adult growth hormone deficiency. The efficacy and safety of this use for HGH has not been tested in a clinical trial. Many of the functions of hGH remain unknown.

The names somatotropin (STH) or somatotropic hormone refer to the growth hormone produced naturally in animals and extracted from carcasses. Hormone extracted from human cadavers is abbreviated hGH. The main growth hormone produced by recombinant DNA technology has the approved generic name (INN) somatropin and the brand name Humatrope and is properly abbreviated rhGH in the scientific literature.

The main hormones concerned with growth are pituitary growth hormone, thyroid hormone, the sex hormones testosterone and estrogen, and the pituitary gonadotropic (sex-gland-stimulating) hormones.

Pituitary growth hormone, a protein with molecular weight of 21,600 and of known amino-acid composition, is secreted by the pituitary gland throughout life. Exactly what its function is in the adult is not clear, but in the child it is necessary for growth; without it dwarfism results. During fetal life it seems not to be necessary, though normally present. It is not secreted at a constant rate all day but in small bursts of activity. Secretion by the pituitary is controlled by a substance sent to it from an adjacent part of the brain. The normal stimulus for secretion is not certain, but a sharp and unnatural lowering of blood sugar will cause growth hormone to be secreted, and this is used as a test. The hormone decreases the amount of fat and causes protein to be laid down in muscles and viscera. Children who lack it are fat as well as small; when given it by injection, they lose fat and grow rapidly.

Human Growth Hormone (GH) is a substance that controls your body's growth. GH is made by the pituitary gland, located at the base of the brain. GH helps children grow taller (also called linear growth), increases muscle mass, and decreases body fat.

In both children and adults, GH also helps control the body's metabolism—the process by which cells change food into energy and make other substances that the body needs.

If children or adults have too much or too little GH, they may have health problems. Growth hormone deficiency (too little GH) and some other health problems can be treated with synthetic (manufactured) GH. Sometimes GH is used illegally for non-medical purposes.

In children, GH is used to treat:

- Growth hormone deficiency.
- Conditions that cause short stature (being shorter than children of the same age), such as chronic kidney disease, Turner syndrome, and Prader-Willi syndrome.

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In adults, GH is used to treat:

- Growth hormone deficiency.
- Muscle wasting (loss of muscle tissue) from HIV.
- Short bowel syndrome.

**Function**

Effects of growth hormone on the tissues of the body can generally be described as anabolic (building up). Like most other protein hormones, GH acts by interacting with a specific receptor on the surface of cells.

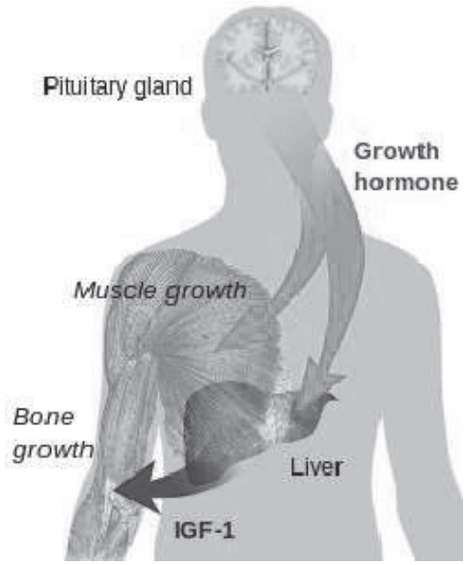


***Fig. 2.3*** *Main Pathways in Endocrine Regulation of Growth*

Increased height during childhood is the most widely known effect of GH. Height appears to be stimulated by at least two mechanisms:

1. Because polypeptide hormones are not fat-soluble, they cannot penetrate cell membranes. Thus, GH exerts some of its effects by binding to receptors on target cells, where it activates the MAPK/ERK pathway. Through this mechanism GH directly stimulates division and multiplication of chondrocytes of cartilage.
2. GH also stimulates, through the JAK-STAT signalling pathway, the production of insulin-like growth factor 1 (IGF-1, formerly known as somatomedin C), a hormone homologous to proinsulin. The liver is a major target organ of GH for this process and is the principal site of IGF-1 production. IGF-1 has growth-stimulating effects on a wide variety of tissues. Additional IGF-1 is generated within target tissues, making it what appears to be both an endocrine and an autocrine/paracrine hormone. IGF-1 also has stimulatory effects on osteoblast and chondrocyte activity to promote bone growth.

In addition to increasing height in children and adolescents, growth hormone has many other effects on the body:

- Increases calcium retention and strengthens and increases the mineralization of bone
- Increases muscle mass through sarcomere hypertrophy
- Promotes lipolysis
- Increases protein synthesis
- Stimulates the growth of all internal organs excluding the brain
- Plays a role in homeostasis
- Reduces liver uptake of glucose
- Promotes gluconeogenesis in the liver
- Contributes to the maintenance and function of pancreatic islets
- Stimulates the immune system
- Increases deiodination of T4 to T3

**Excess**

The most common disease of GH excess is a pituitary tumour composed of somatotroph cells of the anterior pituitary. These somatotroph adenomas are benign and grow slowly, gradually producing more and more GH. For years, the principal clinical problems are those of GH excess. Eventually, the adenoma may become large enough to cause headaches, impair vision by pressure on the optic nerves, or cause deficiency of other pituitary hormones by displacement.

Prolonged GH excess thickens the bones of the jaw, fingers and toes, resulting in heaviness of the jaw and increased size of digits, referred to as acromegaly. Accompanying problems can include sweating, pressure on nerves (e.g., carpal tunnel syndrome), muscle weakness, excess Sex Hormone-Binding Globulin (SHBG), insulin resistance or even a rare form of Type 2 diabetes and reduced sexual function.

GH-secreting tumors are typically recognized in the fifth decade of life. It is extremely rare for such a tumour to occur in childhood, but, when it does, the excessive GH can cause excessive growth, traditionally referred to as pituitary gigantism.

**Deficiency**

The effects of Growth Hormone (GH) deficiency vary depending on the age at which they occur. Alterations in somatomedin can result in growth hormone deficiency with two known mechanisms; failure of tissues to respond to somatomedin, or failure of the liver to produce somatomedin. Major manifestations of GH deficiency in children are growth failure, the development of a short stature, and delayed sexual maturity. In adults, somatomedin alteration contributes to increased osteoclast activity, resulting in weaker bones that are more prone to pathologic fracture and osteoporosis. However, deficiency is rare in adults, with the most common cause being a pituitary adenoma. Other adult causes include a continuation of a childhood problem, other structural lesions or trauma, and very rarely idiopathic GHD.

NOTES

Adults with GHD tend to have a relative increase in fat mass and a relative decrease in muscle mass and, in many instances, decreased energy and quality of life.

Diagnosis of GH deficiency involves a multiple-step diagnostic process, usually culminating in GH stimulation tests to see if the patient's pituitary gland will release a pulse of GH when provoked by various stimuli.

**Check Your Progress**

8. How do the hormones coordinate the physiology and behaviour of individuals?
9. Name the hormones involved in mediating maternal behaviour in mothers.
10. Define target cells.
11. Which hormone is known as growth hormone?

## 2.6 HORMONES AND REPRODUCTION

Just like any other physiological process of our body, reproduction is also controlled by hormones. It is mainly regulated by the interaction of hormones from the hypothalamus and anterior pituitary with hormones from reproductive tissues and organs. The hypothalamus mediates the neuroendocrine control of reproduction in both the sexes through pulsatile secretion of GnRH. In turn, the anterior pituitary gland secrete the gonadotropins LH and FSH in a physiologic fashion.

### Sex Differences in GnRH Secretion Pattern

The main differences between the secretion patterns of GnRH by the female and male animal is that the female secretes high frequency pulses of GnRH in the tonic centre and a large surge of GnRH by the ovulatory centre. Males continuously secrete small amplitude low frequency pulses of GnRH. The ability of the higher centres to release these sex linked GnRH secretion patterns is determined at the time of sexual differentiation and reinforced throughout the adult life. By default, the animal's brain functions as a female's brain. It manifests a cycling pattern with surges, unless it is forced to perform in a male-like fashion that is to maintain continuous secretion.

The transformation from a female to a male pattern of secretion is under testosterone control, at the time of gonad formation during sexual differentiation. At this time, testosterone penetrates the blood-brain barrier; it is aromatized to estrogen and, as such, primes the ovulatory centre to not produce surges of GnRH, in response to high estrogen concentrations. To maintain the male pattern of secretion, the brain has to be reinforced during the early adult life of the animal by exposing the brain to elevated levels of testosterone.

Although estrogen is the hormone that primes the brain to behave like a male, the same phenomenon does not take place in the female. The estrogens circulating in the female organism at the time of sexual differentiation are bound to a much larger carrier, a glycoprotein called alpha fetoprotein ($\alpha$-FP) which cannot cross the blood brain barrier as testosterone does.

## Types of Reproductive Cycles in Females

There are two types of reproductive cycles in females; Mestrual cycle and Estrous cycle. The main differences between two cycles are given below.

NOTES

| Estrous Cycle | Estrous Cycle |
|---|---|
| It occurs in primates (man, apes and monkeys) only. | It occurs in non-primate mammals like cow, dogs, etc. The length of estrous cycle varies widely among species. |
| Shed the endometrium through menstruation if conception does not occur. | Resorption of the endometrium occurs if conception does not occur during that cycle. There is no bleeding phase. |
| Females can be sexually active at any time in their cycle, even when they are not about to ovulate. | Females are generally sexually active during the estrous phase of their cycle. This sexually active period is also known as 'Heat period'. |
| There is no sexually unreceptive phase in animals which undergo menstrual cycle. It is called bleeding phase. | Sexually receptive period is interrupted by periods in which the female is not fertile and sexually unreceptive. During this period estrous cycle is absent and the animal is said to be in 'anestrous' phase. |
| There are four stages of menstrual cycle.<br>(i) Menstrual phase<br>(ii) Proliferative phase<br>(iii) Ovulatory phase<br>(iv) Secretory phase | There are four stages of estrous cycle.<br>(i) Proestrous<br>(ii) Estrous<br>(iii) Metestrous<br>(iv) Diestrous |

## Hypothalamus-Hypophyseal-Testis Axis in Male

The testis is regulated by an endocrine axis involving hypothalamic Gonadotropin-Releasing Hormone (GnRH)-secreting neurons and pituitary gonadotropes that produce both Luteinizing Hormone (LH) and Follicle Stimulating Hormone (FSH). The LH and FSH are pituitary glycoprotein hormones. They are heterodimers, composed of a common α-subunit—the α-Glycoprotein Subunit (αGSU)—and a specific β-subunit (either LH-β or FSH-β).

## Regulation of Leydig Cell Function

The Leydig cell expresses the LH receptor. The LH receptor is coupled to a Gs-cyclic adenosine monophosphate cAMP-PKA signalling pathway. This results in following events:

- Rapid effects include hydrolysis of cholesterol esters and new expression of Steroidogenic activating regulatory protein (StAR).
- Less acute effects include an increase in steroidogenic enzyme gene expression and in the expression of the LDL receptor, SR-BI and testosterone synthesis.
- Over the long-term, LH promotes Leydig cell growth and proliferation.

## Negative Feedback by Testosterone

Testosterone has a negative feedback effect on LH production by the pituitary gonadotrope as testosterone, DHT, and estradiol-17β. All three steroid hormones inhibit the expression of LH-β, the GnRH receptor, and to a lesser extent, FSH-β. Although testosterone, DHT, and estrogen exert negative feedback on both LH and FSH, they selectively inhibit LH more effectively than FSH.

These steroids also inhibit the release of GnRH by the hypothalamic neurons.

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## Regulation of Sertoli Cell Function

The Sertoli cell is stimulated by both testosterone and FSH. The FSH receptor also is coupled primarily to a Gs-cAMP-PKA pathway and involved in following events:

- In stimulating the synthesis of proteins involved in the nurse cell aspect of Sertoli cell function (for example, ABP),
- FSH also stimulates the synthesis of the dimeric protein inhibin. Inhibin has a common $\alpha$-subunit, coupled with either a $\beta$A-subunit, called inhibin A, or a $\beta$B-subunit, called inhibin B. Only inhibin B is expressed in men. Inhibin B expression is stimulated by FSH, and inhibin B exerts a negative feedback on the gonadotrope to selectively inhibit FSH production.

## Hypothalamus-Hypophyseal-Ovarian Axis

The hypothalamic neurons secrete Gonadotropin-Releasing Hormone (GnRH) in a pulsatile manner. GnRH, in turn, stimulates LH and FSH production by pituitary gonadotropes. A high frequency of GnRH pulses selectively promotes LH production, whereas a slow frequency selectively promotes FSH production.

A major difference between the male and the female reproductive axes is the mid-cycle gonadotropin surge in females, which is dependent on a high level of estrogen over a specific duration coming from the mature follicle/s. A highly dynamic conversation occurs among the ovary, pituitary, and hypothalamus, which orchestrates the events of the menstrual cycle in primates and estrous cycle in non-primate females. The main points of events involving the ovary and pituitary gonadotrope that regulate the reproductive cycle, with an overview of hypothalamic involvement are given below.

Beginning with the ovary at the end of the luteal phase of a previous, non-fertile cycle:

**Event 1:** In the ovary, the absence of fertilization and implantation, the corpus luteum regresses and dies (a phenomenon called luteolysis). This leads to a drastic decline in the levels of progesterone, estrogen, and inhibin A.

**Event 2:** The pituitary gonadotrope perceives the end of luteal function as a release from negative feedback. This permits a rise in FSH. The basis for the selective increase in FSH is incompletely understood but may result from the slow frequency of GnRH pulses during the luteal phase, which is due to high progesterone levels.

**Event 3:** The rise in FSH levels recruits a crop of large (2- to 5-mm) antral follicles to begin rapid, highly gonadotropin-dependent growth. These follicles produce low levels of estrogen and inhibin B.

**Event 4:** The gonadotrope responds to the slowly rising levels of estrogen and inhibin B by decreasing FSH secretion. Loss of high levels of progesterone and estrogen causes an increase in the frequency of GnRH pulses, thereby selectively increasing LH synthesis and secretion by the gonadotrope. It results in gradual increase in LH/FSH ratio.

**Event 5:** The ovary's response to declining FSH levels is follicular atresia of all the recruited follicles, except for one or more dominant follicles. Thus, the

process of selection is driven by an extreme dependency of follicles on FSH in the face of declining FSH secretion.

This mature follicle/s produces increasing amounts of estradiol-17β and inhibin B during the second half of the follicular phase. FSH also induces the expression of LH receptors in the mural granulosa cells of the dominant follicle.

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**Event 6:** Once the circulating estrogen levels rise above normal, estrogen exerts a positive feedback on the gonadotrope, producing the LH surge. This is enhanced by the small amount of progesterone starting to be made at just around LH surge. The exact mechanism of the positive feedback is unknown, but it occurs largely at the level of the pituitary.

GnRH receptors and the sensitivity to GnRH signaling increase dramatically in the gonadotropes. The hypothalamus contributes to the gonadotropin surge by increasing the frequency of GnRH pulses. There is some evidence that other neurons in the hypothalamus (for example, kisspeptin neurons; respond to high levels of estrogen by increasing the frequency and amount of GnRH released.

**Event 7:** The LH surge drives three general events in the ovary:

- The primary oocyte completes meiosis I and arrests at metaphase of meiosis II. This is associated with Germinal Vesicle Breakdown (GVBD); the germinal vesicle refers to the nucleus of the oocyte), which is the dissolution of the nuclear membrane and interphase nuclear structure.
- The wall of the follicle and of the ovary at the stigma is broken down, and the free-floating cumulus-oocyte complex is extruded from the ovary, i.e., ovulation.
- The mural granulosa cells and theca cells are restructured to form the corpus luteum. This involves direct vascularization of the granulosa cells and their differentiation into progesterone- and estrogen-producing cells. The granulosa cells also secrete inhibin A. The small amount of progesterone secreted during the periovulatory period contributes to the magnitude of the LH surge.

**Event 8:** Rising levels of progesterone, estrogen, and inhibin A by the mature corpus luteum negatively feedback on the pituitary gonadotrope. Even though estrogen levels in the circulation are still high for positive feedback, the high progesterone levels block any positive feedback. Consequently, both FSH and LH levels decline to basal levels.

**Event 9:** Basal levels of LH (but not FSH) are absolutely required for normal corpus luteum function. The corpus luteum becomes progressively insensitive to LH signalling, however, and dies unless LH-like activity, i.e., hCG from an implanted embryo increases. In a non-fertile cycle, the corpus luteum will regress, and progesterone and estrogen levels will start to decline.

**Event 10:** Removal of negative feedback causes an increase in FSH at the end of the cycle, and the entire process begins again.

From this sequence of events, it is evident that the ovary is the primary clock for the reproductive cycle. The timing of the two main pituitary-based events— the transient rise in FSH that recruits large antral follicles and the LH surge that induces ovulation—is determined by two respective ovarian events:

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Highly regular life span of a corpus luteum, its demise and Growth of the dominant follicle to a point at which it can maintain a sustained high production of estrogen that induces a switch to positive feedback at the pituitary.

The hypothalamic release of GnRH changes over the cycle. The frequency of GnRH pulses increases during the growth of the follicles and decreases during the luteinization.

### Hormonal Regulation During the Menstrual Cycle

In general, estrogen secreted during the follicular phase regulate following:

- Increases endosalpinx epithelial cell size and height
- Increases blood flow to the lamina propria of the oviducts
- Increases the production of oviduct specific glycoproteins (whose functions are poorly understood)
- Increases ciliogenesis throughout the oviduct
- Also, promotes the secretion of a thick mucus in the isthmus and increases tone of the muscularis of the isthmus, thereby keeping the cumulus oocyte complex at the ampullary-isthmus junction for fertilization.

High progesterone, along with estrogen, during the early to mid-luteal phase decreases epithelial cell size and function.

Progesterone promotes deciliation. It also decreases the secretion of thick mucus and relaxes the tone in the isthmus.

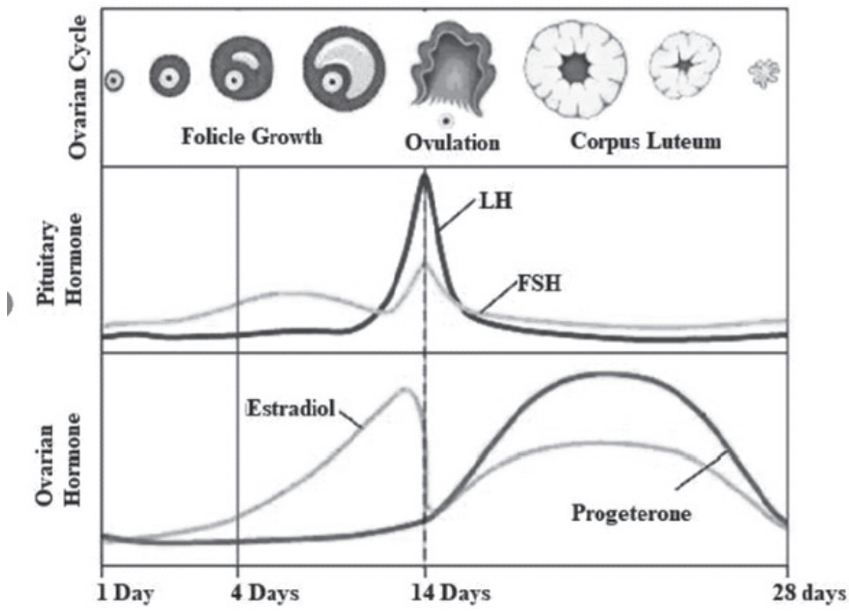


***Fig. 2.4*** *Hormonal Regulation of Menstrual Cycle*

### Types of Breeding Animals

Under natural conditions, most animal species breed during a time which will allow them to give birth when food is more plentiful, in order to improve the chances of survival. Given that different species have different gestation lengths, the breeding

time takes place at different times of the year. This has generated several types of animals as mentioned below and summarized in Table 2.3.

**Continuous Breeders** are animal species in which reproductive cycles occur consecutively throughout the year independent of environmental cues. Cows, sows, rats, and primates (humans, apes and monkeys) belong to this group.

**Seasonal Breeders** are animal species in which reproductive cycle occur during a specific season. They start cycling in response to specific environment cues, such as an increase or decrease of day light. These times of year allow for the optimization of survival of young due to factors, such as ambient temperature, food and water availability, and changes in the predation behaviours of other species. Related sexual interest and behaviours are expressed and accepted only during this period. Female seasonal breeders will have one or more estrus cycles only when she is 'in season' or fertile and receptive to mating. The female seasonal breeders with only one cycle per year are termed **monoestrous**, for example, the wolf and the fox. The female seasonal breeders with more than one reproductive cycles per year are called **polyoestrous**, for example the dog. At other times of the year, they will be anoestrus with no reproductive cycles. Unlike reproductive cyclicity, seasonality is described in both males and females. Male seasonal breeders may exhibit changes in testosterone levels, testes weight, and fertility depending on the time of year. They are further classified as: short and long day breeders.

- **Short Day Breeders** are animal species, such as the ewe, nanny and doe, start cycling as the days get shorter in the fall.
- **Long Day Breeders**, on the other hand, are animal species, such as the mare that start cycling as the days are getting longer in the spring.

Cats also tend to cycle when days are getting longer in the early spring. Cats however, are **induced ovulators**, which means that the release of the LH surge that triggers ovulation only takes place as a result of copulation.

***Table 2.3*** *Types of Breeding Animals*

| Type Of Breeding Animals | | Type Of Reproductive Cycle | Examples |
|---|---|---|---|
| Continuous Breeders | | Menstrual cycle | Primates (humans, apes and monkeys) |
| | | Estrous cycle (Polystrous continuous breeders) | Non-primates like cattle, pigs, mouse and rats. |
| Seasonal Breeders | Long Day Breeders | Polyestrous | Lemurs, horses, hamsters, groundhogs and mink. |
| | | Monoestrous | Bear |
| | Short Day Breeders | Polyestrous | Sheep, goat. deer, elk |
| | | Monoestrous | Dog |

### (a) Reproductive Cycles in Continuous Breeders

Among animals breeding throughout the year, two different types of reproductive cycles could be seen in females: Menstrual and estrous cycle.

**Menstrual Cycle:** It is a regular cyclic hormonal change which coordinate changes in the ovary and internal reproductive tract.

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## (b) Reproductive Physiology of Seasonal Breeders

In seasonal breeders, the nonbreeding (anoestrous) season is characterized by a reduction in the pulsatile secretion of GnRH. The two main reasons for this are: (1) an increased negative feedback of estrogen, involving hypothalamo-hypophyseal-gonadal axis and (2) change in duration of daylight, involving SCN-pineal-hypothalamic axis.

### Role of Estrogen

The primary mechanism in seasonal breeding is the neural control of pattern of GnRH release from hypothalamus. In non-breeding season, a negative feedback effect of estrogen is there on GnRH and gonadotrophin secretion. This results in reduced frequency of GnRH pulses which is insufficient to stimulate the development of follicles in the gonads. While in breeding season, the sustained increase in estrogen activates the surge center of the hypothalamus thereby creating a positive feedback effect on the hypothalamus causing high magnitude pulsatile release of GnRH. This in turn increases the LH and FSH from the pituitary thereby activating the gonads. The seasonal change in sensitivity to oestrogen is the major mechanism for shift from breeding to non-breeding season.

Pre-pubertal period of continuous breeders (primates) is comparable to non-breeding or anestrous period while the puberty is comparable to the onset of breeding season. Similar kind of transition occurs in both the cases. In pre puberty, the small content of estrogen from the developing follicle exerts a negative feedback effect on the hypothalamus, while at puberty the consistently high estrogen exerts a positive feedback effect there by activating the surge center of the hypothalamus. This results in LH surge which causes the maturation of the follicle.

### Duration of Photoperiod

Onset of breeding season depends on duration of photo period. The changing photoperiod acts as a bio-regulator of reproductive activity and fertility through the mediation of central nervous system, hypothalamus, adenohypophysis and the pineal gland. The pineal gland translates the visual signal into hormonal signal thereby showing fluctuation in secretion of melatonin which alters the pulsatile secretion of GnRH/LH, thereby controlling the reproductive function. The orbiting of the tilted earth around the sun causes circadian changes in the length of the daylight or duration of photoperiod at different seasons of the year.

### Role of Pineal Gland-Neural Control

The external light influence the neural control of endocrine glands via the pineal gland. It causes the variability in reproductive cycles of seasonal breeders through the secretion of melatonin. The reproduction of long-day breeders is repressed by melatonin however, the reproduction of short-day breeders is stimulated by melatonin.

The following steps describe the pathway of melatonin secretion and associated endocrine changes in long day breeders:

- Change in photoperiod from longer days to reduced day (increase in darkness) is sensitized by the photo receptors of the eye, which transmits information to the Suprachiasmatic Nuclei (SCN).

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- The suprachiasmatic nucleus have an inhibitory projection to the paraventricular nucleus of the hypothalamus.
- In turn, the sympathetic nerve fibres of paraventricular nucleus releases the neurotransmitter nor-epinephrine.
- The nor-epinephrine binds to the receptors on the cells of pineal gland.
- The pinealocytes through a series of steps causes formation of N-Acetyl Transferase (NAT), a major regulatory enzyme involved in the secretion of melatonin. It converts serotonin to N-acetyl Seratonin.
- Melatonin is then released in to the circulation from pineal gland.
- Melatonin reaching the Suprachiasmatic Nucleus (SCN) of the hypothalamus inhibits the frequency and magnitude of the pulsatile secretion of hypothalamic hormones (GnRH) thereby inhibiting the release of pituitary hormones (FSH/ LH) essential for initiation of reproductive activity in long day breeders.
- Once the season changes with increasing photoperiod into long days, the photo receptors of the eye sensitize the same and the information is passed to the pineal gland. As a result it becomes less active and thereby the secretion of melatonin is also reduced considerably. The inhibitory influence on the hypothalamus is removed. This results in the secretion of hypothalamic releasing factors and pituitary hormones with higher frequency and magnitude to initiate the reproductive process for the onset of breeding. The young ones are produced during spring and summer.

**For Short Day Breeders**

Changing photoperiod from longer day light to shorter days with more periods of darkness initiates the reproductive activity in sheep and goat due to increase in melatonin secretion. The increased melatonin secretion reaching the hypothalamus, then stimulates the pulsatile secretion of GnRH. Subsequently, FSH and LH secretion from the pituitary gland increases, which in turn results in the onset of ovarian activity and the commencement of the breeding season. They experience an annual period of reproductive quiescence in response to increased photoperiod during the late-winter into spring and renaissance during the late summer.

Although photoperiod is considered the primary environmental cue to seasonal infertility, other environmental factors also seem to interact with season to affect the infertility. These include housing (group vs. individual), feeding level, light conditions, boar exposure, group size, ambient temperature and interactions between females. The significance of seasonal infertility has increased in recent years as group housing of dry sows has become more common.

**Check Your Progress**

12. Name the hormone that primes the brain to behave like a male.
13. Which are the two types of reproductive cycles in females?
14. Define continuous breeders.
15. What is the difference between monoestrous and polyestrous?

NOTES

## 2.7 ANSWERS TO 'CHECK YOUR PROGRESS'

1. All the steroid hormones are derived from cholesterol except Vitamin D. They all consist of the same cyclopentanophenanthrene ring and atomic numbering system as cholesterol does.
2. Integration of body functions in humans and other higher organisms is carried out through the nervous system, the immune system and the endocrine system.
3. Hormones are usually present in the plasma and interstitial tissue at concentrations in the range of 10-7M to 10-10M. Due to these low physiological concentrations, sensitive protein receptors have come up in target tissues for sensing the presence of weak signals.
4. Hormones affect distant cells by binding to specific receptor proteins in the target cell, resulting in a change in cell function.
5. Hormone producing cells are found in the endocrine glands, such as, the thyroid gland, ovaries, and testes.
6. A high blood level of a hormone may inhibit the secretion of that hormone. It is shown by target gland hormones that inhibit the secretion of their tropic hormones. This is known as negative feedback control.
7. Steroid hormones are produced through de novo steroidogenesis in the adrenal cortex, the gonads and the placenta.
8. Hormones coordinate the physiology and behaviour of individuals by regulating, integrating, and controlling bodily functions.
9. Estrogens and progesterone concentrations are elevated during pregnancy, and these hormones are often involved in mediating maternal behaviour in the mothers.
10. Not all cells are influenced by each and every hormone. Rather, any given hormone can directly influence only cells that have specific hormone receptors for that particular hormone. Cells that have these specific receptors are called target cells for the hormone.
11. Growth Hormone (GH) or somatotropin, also known as Human Growth Hormone (hGH or HGH) in its human form, is a peptide hormone that stimulates growth, cell reproduction, and cell regeneration in humans and other animals.
12. Estrogen is the hormone that primes the brain to behave like a male, the same phenomenon does not take place in the female.
13. There are two types of reproductive cycles in female's menstrual cycle and estrous cycle.
14. Continuous breeders are animal species in which reproductive cycles occur consecutively throughout the year independent of environmental cues. Cows, sows, rats, and primates (humans, apes and monkeys) belong to this group.
15. The female seasonal breeders with only one cycle per year are termed monoestrous, for example, the wolf and the fox. The female seasonal breeders with more than one reproductive cycles per year are called polyoestrous, for example the dog.

## 2.8 SUMMARY

NOTES

- Integration of body functions in humans and other higher organisms is carried out through the nervous system, the immune system and the endocrine system.
- Hormones are usually present in the plasma and interstitial tissue at concentrations in the range of 10–7M to 10–10M.
- Once a hormone gets secreted by an endocrine tissue, it usually binds with a specific plasma protein carrier, with the complex being disseminated to distant tissues.
- Receptors for most of the amino acid-derived hormones and all peptide hormones are usually located on the plasma membrane.
- A hormone is any member of a class of signaling molecules in multicellular organisms that are transported to distant organs to regulate physiology and behaviour.
- Hormones affect distant cells by binding to specific receptor proteins in the target cell, resulting in a change in cell function.
- In vertebrates, endocrine glands are specialized organs that secrete hormones into the endocrine signaling Hormone producing cells are found in the endocrine glands, such as the thyroid gland, ovaries, and testes.
- The synthesis of amine and steroid hormones are different from that of peptide hormones.
- Hormones are involved in coordination of series of physiological events.
- The synthesis of non-peptide hormones, such as, thyroid hormones, adrenal medullary hormones, steroid hormones involve the action of multiple enzymes.
- Following the release to outside from site of synthesis, hormones may circu-late freely into the bloodstream or it may be bound to a carrier protein.
- The basal secretion of most hormones is not a con-tinuous process but rather has a pulsatile nature.
- Nerve impulses control some endocrine secretions. For exam-ple, during stress and emotion, splanchnic nerve stimulates the synthesis and release of catecholamines from adrenal medulla.
- The secretion of a hormone may be stimulated or inhibited by the feedback effect of some other hormone or metabolites.
- The rate of hormone biosynthesis and secretion is often regulated by a homeostatic negative feedback control mechanism.
- Steroid hormones are produced through de novo steroidogenesis in the adrenal cortex, the gonads and the placenta.
- The receptors for the retinoids are recognized as RARs and they exist in at least three subtypes, namely, RARa, RARb and RARg.

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- Mutations in the gene for PPARg have been correlated with the insulin resistance.
- Hormones coordinate the physiology and behaviour of individuals by regulating, integrating, and controlling bodily functions.
- Growth Hormone (GH) or somatotropin, also known as Human Growth Hormone (hGH or HGH) in its human form, is a peptide hormone that stimulates growth, cell reproduction, and cell regeneration in humans and other animals.
- The main hormones concerned with growth are pituitary growth hormone, thyroid hormone, the sex hormones testosterone and estrogen, and the pituitary gonadotropic (sex-gland-stimulating) hormones.
- Human Growth Hormone (GH) is a substance that controls your body's growth. GH is made by the pituitary gland, located at the base of the brain.
- If children or adults have too much or too little GH, they may have health problems.
- There are two types of reproductive cycles in females; Mestrual cycle and Estrous cycle.
- Testosterone has a negative feedback effect on LH production by the pituitary gonadotrope as testosterone, DHT, and estradiol-17b.
- The hypothalamic neurons secrete Gonadotropin-Releasing Hormone (GnRH) in a pulsatile manner.
- Under natural conditions, most animal species breed during a time which will allow them to give birth when food is more plentiful, in order to improve the chances of survival.
- Continuous breeders are animal species in which reproductive cycles occur consecutively throughout the year independent of environmental cues.
- Seasonal breeders are animal species in which reproductive cycle occur during a specific season.
- Among animals breeding throughout the year, two different types of reproductive cycles could be seen in females: Menstrual and estrous cycle.
- The primary mechanism in seasonal breeding is the neural control of pattern of GnRH release from hypothalamus.
- Onset of breeding season depends on duration of photo period.

## 2.9 KEY TERMS

- **Hormone**: A hormone is any member of a class of signalling molecules in multicellular organisms that are transported to distant organs to regulate physiology and behaviour.
- **Endocrine Glands**: Endocrine glands are specialized organs that secrete hormones into the endocrine signalling system.

NOTES

- **Peptide Hormones:** Peptide hormones are synthesized by translational method.
- **Circatrigintan:** the monthly pre-ovulatory discharge of gonadotropins recurs about every 30 days, this pattern of release known as circatrigintan.
- **Hormone-Response Elements (HREs):** When the receptors bind the ligand they undergo a conformational change, which renders them activated to recognize and bind to particular nucleotide sequences. These particular nucleotide sequences in the DNA are termed as hormone-response elements (HREs).
- **Growth Hormone**: Growth Hormone (GH) or somatotropin, also known as Human Growth Hormone (hGH or HGH) is a peptide hormone that stimulates growth, cell reproduction, and cell regeneration in humans and other animals.
- **Continuous Breeders:** Continuous breeders are animal species in which reproductive cycles occur consecutively throughout the year independent of environmental cues.
- **Seasonal Breeders**: Seasonal breeders are animal species in which reproductive cycle occur during a specific season.
- **Short day Breeders**: Short day breeders are animal species, such as the ewe, nanny and doe, start cycling as the days get shorter in the fall.
- **Long day Breeders**: Long day breeders are animal species, such as the mare that start cycling as the days are getting longer in the spring.
- **Menstrual Cycle**: It is a regular cyclic hormonal change which coordinate changes in the ovary and internal reproductive tract.

## 2.10 SELF ASSESSMENT QUESTIONS AND EXERCISES

### Short-Answer Questions

1. What are the properties of the tissues that are capable of responding to endocrine?
2. In higher vertebrates hormones are responsible for the regulation of which physiological processes and behavioral activities?
3. Write the steps involved in hormonal signaling.
4. Define the process of exocytosis.
5. Define the steps in cellular synthesis of peptide hormone.
6. How are the hormones transported from the site of synthesis to their target organ or cell?
7. What do you understand by antagonists of the hormone?

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8. What are effects of growth hormone on the tissues of the body?
9. Write the difference between menstrual cycle and estrous cycle.
10. Write the steps to describe the pathway of melatonin secretion and associated endocrine changes in long day breeders.

**Long-Answer Questions**

1. Explain the structure of hormones in detail.
2. Describe the secretion of hormone in response to specific biochemical signal by giving suitable examples.
3. Explain the synthesis of hormones.
4. Elaborate the patterns of hormone secretion in the cell.
5. Discuss some prominent hormones that influence the behaviour of organisms.
6. Describe the hormonal regulation during the menstrual cycle in females.
7. Explain the role of estrogen in reproductive physiology of seasonal breeders.

## 2.11 FURTHER READING

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# UNIT 3 MOLECULAR CELL BIOLOGY - I

**Structure**



NOTES

## 3.0 INTRODUCTION

Cell is the basic unit of life that responsible for living and functioning of organisms. Cell biology is the study of structural and functional unit of cell. Cell biology encompasses both prokaryotic and eukaryotic cells and include the study of cell metabolism, cell communication, cell cycle, biochemistry, and cell composition. Cell and molecular biology encompasses study of the structure and function of organisms and biological processes at the level of cells, and the macromolecules that define them (DNA, RNA, proteins, lipids, and carbohydrates). Cell molecular biology includes many disciplines like Cancer Biology, Neurobiology, and Developmental Biology. Cell and Molecular Biology studies the structure and function of the cell, which is the basic unit of life. Cell biology is concerned with the physiological properties, metabolic processes, signaling pathways, life cycle, chemical composition and interactions of the cell with their environment. This is done both on a microscopic and molecular level as it encompasses prokaryotic cells and eukaryotic cells. Knowing the components of cells and how cells work is fundamental to all biological sciences.

In this unit you will study about bio membranes, cytoskeleton, structure and dynamics of microfilaments and microtubules, cell - cell signaling, cell - cell adhesion and communication, and cell cycle.

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## 3.1 OBJECTIVES

After going through this unit, you will be able to:

- Understand the composition of biomembranes
- Analyse cytoskeleton and its dynamics
- Discuss the concept of cell-cell signalling
- Explain cell-cell adhesion and communication development
- Describe the cell cycle

## 3.2 BIOMEMBRANES

A biological membrane or biomembrane is an enclosing or separating membrane that acts as a selectively permeable barrier within living things. Biological membranes, in the form of eukaryotic cell membranes, consist of a phospholipid bilayer with embedded, integral and peripheral proteins used in communication and transportation of chemicals and ions. The bulk of lipid in a cell membrane provides a fluid matrix for proteins to rotate and laterally diffuse for physiological functioning. Proteins are adapted to high membrane fluidity environment of lipid bilayer with the presence of an annular lipid shell, consisting of lipid molecules bound tightly to surface of integral membrane proteins. The cell membranes are different from the isolating tissues formed by layers of cells, such as mucous membranes, basement membranes, and serous membranes.

Biological molecules are amphiphilic or amphipathic, i.e., are simultaneously hydrophobic and hydrophilic. The phospholipid bilayer contains charged hydrophilic head groups, which interact with polar water. The lipids also contain hydrophobic tails, which meet with the hydrophobic tails of the complementary layer. The hydrophobic tails are usually fatty acids that differ in lengths. The interactions of lipids, especially the hydrophobic tails, determine the lipid bilayer physical properties, such as fluidity.

Membranes in cells typically define enclosed spaces or compartments in which cells may maintain a chemical or biochemical environment that differs from the outside. For example, the membrane around peroxisomes shields the rest of the cell from peroxides, chemicals that can be toxic to the cell, and the cell membrane separates a cell from its surrounding medium. Peroxisomes are one form of vacuole found in the cell that contain by-products of chemical reactions within the cell. Most organelles are defined by such membranes, and are called 'membrane-bound, organelles.

### 3.2.1 Molecular Composition and Arrangement Functional Consequences of Biomembrane

Plasma membrane is the outer membrane covering of cell protoplasts discovered by Schwann (1838). It was called cell membrane by Nageli and Cramer (1855). The name plasmalemma of plasma membrane was given to it by Plowe (1931).

Biomembranes are not visible under the light microscope because their thickness is below the resolving power of the microscope. Under electron microscope biomembranes appear to be trilaminar or tripartite. Freeze etching technique has shown that a membrane possesses particles of different sizes. Membrane occur inside the cytoplasm. Internal membranes are rare in prokaryotes. They occur in eukaryotic cells as covering of several cell organelles like nucleus, mitochondria, plastids, lysosomes, Golgi bodies, peroxisomes, etc. Vacuoles are separated from cytoplasm by a membrane called tonoplast. All membranes whether external or internal are called cell membranes or biomembranes. Average thickness is 75 Å. Biomembranes are selectively permeable for solutes but semipermeable for water.

The asymmetric functioning of such system is of little value to cells. It was unclear for many years as to how phospholipid bilayer only 5 nm thick could be strong enough to withstand stress on the plasma membrane of most cells. The size of most animal cells are small enough for adhesive forces between water molecules to maintain spherical cell shape inside a lipid bilayer surface. Plasma membrane is able to lie passively as unreinforced thin lipid barriers at the surface of cell. Monocytes surrounded by thin membranes are deformed as they migrate between vascular endothelial cells in response to injury. Cell membrane face continuous challenge to their integrity from within. An area of membrane about equal to area of entire cell surface turns over every 30 min in many cells due to endocytosis and exocytosis. Endocytosis and exocytosis depend on the cytoskeleton for movement of vesicles to and from plasma membrane and cytoskeletal infrastructure influences the motion of membrane. The first proposal that cellular membranes might contain a lipid bilayer was made in 1925 by two Dutch scientists E. Gorter and F. Grendel. They extracted the lipid from human blood cells and measured the amount of surface area the lipid would cover. Mammalian red blood cell lack both nuclei and cytoplasmic organelles, plasma membrane is the only lipid containing structure and all of the lipids extracted from the cells can be assumed to have resided in the cells plasma membranes. Experiments conducted in 1960 led to the concept of membrane structure known as fluid mosaic model proposed in 1972 by S. Jonathan Singer and Garth Nicolson of University of California, San Diego.

## Composition of Bio Membrane

The lipid bilayer is fundamental structure of most biomembranes. Non lamellar lipid structure also form important components of biomembranes. The phospholipids that are present in biomembranes are highly amphipathic, phosphate containing groups esterified that have long chain fatty acyl groups. The phosphate containing groups are electrically charged and are hydrophilic whereas the hydrocarbon side chain of fatty acyl groups are hydrophobic. In the phospholipid bilayer, these hydrophobic side chains extend within each leaflet toward each other to form hydrophobic interior of biomembranes. In contrast, hydrophilic phosphate containing group seek position in bilayer between hydrophobic interior and either of two external aqueous phases. Animal biomembrane also contain other lipids, such as cholesterol in addition to phospholipids. Cholesterol is less amphipathic than are phospholipids because hydrophilic portion of cholesterol is due to its uncharged and small hydroxyl group rather than to electrically charged and larger phosphate containing group. The protein content of biomembranes varies from

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20% to more than 70% depending on the membrane. The ratio of lipid to protein in membrane varies, depending on type of cell membrane (plasma/ endoplasmic/ Golgi), type of organism (bacteria/plant/animal) and type of cell (cartilage/ muscle /liver). Inner mitochondrial membrane has a very high ration of protein / lipid in comparison to red blood cell plasma membrane, which is high in comparison to membranes of the myelin sheath that form wrapping around nerve cell. Inner mitochondrial membrane contains protein carriers of the electron transport chain and relative to other membranes, lipid is diminished.

In contrast, myelin sheath act as electrical insulation for nerve cell it encloses, a function that is carried by thick lipid layer of high resistance. Biomembranes transport is believed to be catalyzed by integral membrane proteins, though their activities are influenced by peripheral proteins. The tertiary and quaternary structure of integral membrane proteins has been studied. For example, aquaporin-1 (CHIP28) monomer, a member of protein family forms water channel in many epithelial and non-epithelial tissue is observed using cryo electron crystallography to form tetramers of four water channel pathways through the plasma membrane. Biomembranes structure is asymmetric as well as heterogeneous (Refer Figure 3.1).

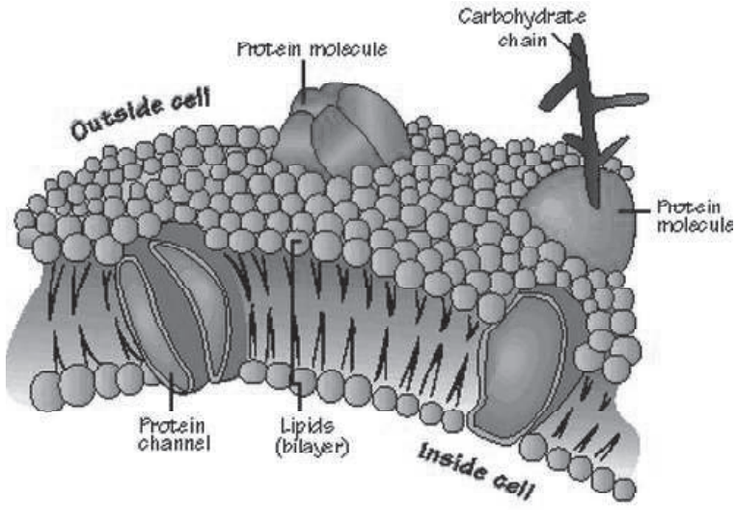


***Fig. 3.1*** *Biomembranes Structure*

**Proteins:** Proteins constitute 20 to 70 % of the membrane by mass. They occur in different ways and forms in different sites of plasma membrane. Two distinct types are common. Extrinsic proteins and intrinsic proteins. Extrinsic proteins are attached to the periphery of the lipid bilayer and can be separated from the membrane. Peripheral proteins are mostly made up of amino acids having hydrophilic side chains which act with the polar heads of lipid molecules or with surrounding water. Intrinsic proteins are partly or wholly embedded in the lipid bilayer. The constituent amino acids of these proteins form hydrophobic bonds with fatty acids of lipid molecules. These proteins are soluble in organic solvents. Due to differential distribution of peripheral proteins, two surfaces of a membrane is always asymmetric. Most integral membrane proteins are inserted into membranes in the endoplasmic reticulum by a process that involves amino acid

residue signaling sequences. Integral proteins are transmembrane proteins that is they pass entirely through lipid bilayer and have domains that protrude from both extracellular and cytoplasmic side of membrane (Refer Figure 3.2).

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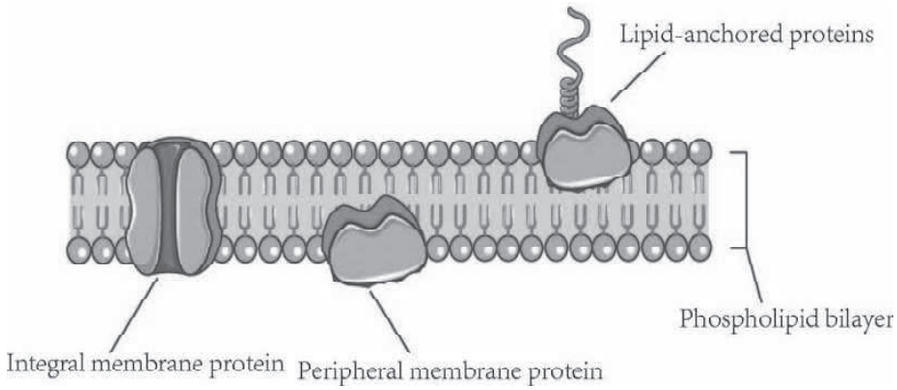


***Fig. 3.2** Protein Membrane*

Some membrane transport proteins undergo large conformational changes during processing and functioning. Peripheral proteins that are located entirely outside the lipid bilayer on either of cytoplasmic or extracellular side, and are associated with surface of membrane by noncovalent bonds. Lipid anchored proteins are located outside the lipid bilayer on either the extracellular surface but are covalently linked to lipid molecule situated within the bilayer. Besides structural proteins, the plasma membrane possess several enzymes which regulate cellular metabolism. More than 30 different types of enzymes are known to occur in association with plasma membrane. The carrier proteins embedded in the membrane help in transport of solutes.

**Lipids:** Lipids have asymmetric concentration across biomembranes, for example, out of four most abundant category of phospholipid in plasma membrane, the anionic one (phosphatidylserine) and a zwitterionic one (phosphotidylethanolamine) are more concentrated in the inner than the outer leaflet of bilayer. Free energy is required to move these phospholipids against their concentration gradients. The asymmetric distribution of lipids across the plasma membrane is scrambled by normal and artificial process. Cellular activation by stimuli is associated with increase in the cytosolic free $Ca^{2+}$ concentration. Membrane lipids contain wide variety of lipids all of which are amphipathic, .i.e., they contain hydrophilic and hydrophobic regions.

There are three types of membrane lipids:

- Phosphoglycerides
- Sphingolipids
- Cholesterol

**Phosphoglycerides:** Phospholipids constitute a major portion of lipids (55-57%) in plasma membranes. They belong to mainly two categories. Neutral phospholipids and acidic phospholipids. Examples of neutral phospholipids are lecithin, cephalin, sphingomyelin, etc. Most membrane lipids contain phosphate group which make them phospholipid, most membrane phospholipids are built on glycerol backbone called phosphoglycerides.

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Unlike triglycerides, which have three fatty acid and are not amphipathic, membrane glycerides are diglycerides-only two of the hydroxyl group of glycerol are esterified to fatty acid, third is esterified to hydrophilic phosphate group. These phospholipid molecules constitute the structural framework of plasma membrane.

**Sphingolipids:** A less abundant class of membrane lipids are sphingolipids, are derivatives of sphingosine, an amino alcohol that contain a long hydrocarbon chain. Sphingolipid consists of sphingosine linked to a fatty acid by its amino group. This molecule is ceramide. The various sphingosine based lipids have additional group esterified to terminal alcohol of sphingosine. If substitution is phosphorylcholine, molecule is sphingomyelin, which is only phospholipid of membrane (Refer Figure 3.3).

**Cholesterol**: It is an amphipathic molecule like phospholipids having both hydrophilic and a hydrophobic portion. Cell membrane would be too fluid and too permeable to some molecules without cholesterol. Cholesterol is part of steroid ring that is closely attracted to part of fatty acid chain on the nearest phospholipid.

Cholesterol is sterol lipid derived from squalene, forming a major component of animal cell membrane. It is absent in higher plant membranes and bacteria. In animal cells, it affect membrane fluidity.

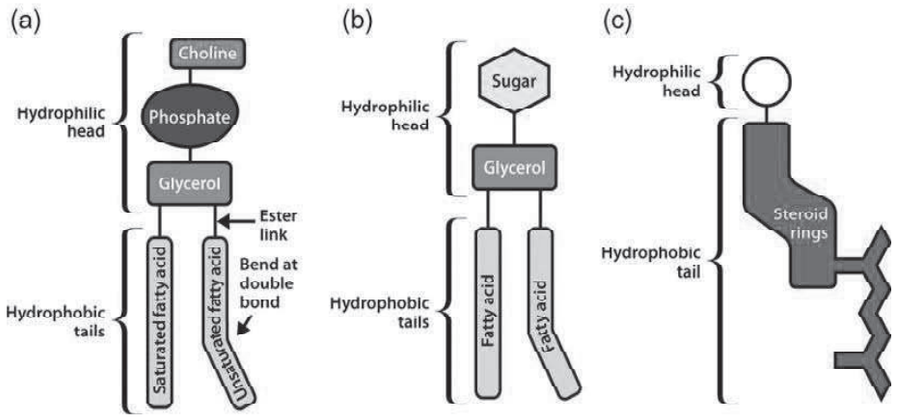


***Fig. 3.3*** *Structure of Membrane Lipid*

## Phospholipid Bilayer

Membrane formation is due to a self-assembly process which a consequence of amphipathic nature of phospholipid molecules is. The polar hydrophilic heads of phospholipids come in contact with water while their hydrophobic tails cannot do so. There are two methods of arrangement pf phospholipids in contact with water-micelle ( globule) and bilayer (bimolecular sheet) in which hydrophobic tails are inside and the hydrophobic heads are towards outside. The micelle or globular form would be mechanically unsound as the bulky double tails would not be able to get adjusted in the center of globule. The driving force for the formation of lipid bilayer is hydrophobic interaction amongst the hydrocarbon tails of lipid molecules. Vander walls forces help in close packing of tails. Polar heads of phospholipids are held to water molecules by electrostatic attractions and hydrogen bonds. Lipid bilayers are also called cooperative structures because their molecules are held

together by many reinforcing non-covalent interactions which are predominantly hydrophobic. Because of these interactions lipid bilayers have a tendency to become extensive, close on themselves and the ability of self-sealing (Refer Figure 3.4).

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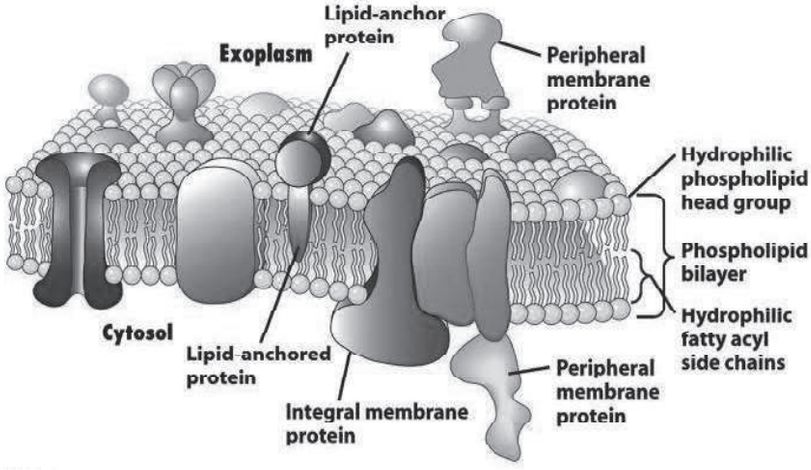


***Fig. 3.4*** *Lipid Bilayer showing Inner and Outer Leaflets*

**Carbohydrates:** More than 90% of the membrane carbohydrate is covalently linked to proteins to form glycoproteins Their peripheral association is highly asymmetric.. The remaining carbohydrates are covalently linked to lipids to form glycolipids. Unlike proteins and lipids, carbohydrates are not integral component of membranes. Their peripheral association is highly asymmetric. Oligosaccharides are covalently bounded to membrane lipids and integral proteins on their non-cytosolic sides. Oligosaccharides are assembled and transferred to membrane proteins and lipids and modified in the lumen of endoplasmic reticulum and Golgi apparatus. No mechanism is known for assembly and attachment of molecules to segments of proteins and lipids at cytosolic surface of membranes. The carbohydrate rich zone on the surface of cell is known as glycocalyx. Specific glycosylation of some integral membrane proteins appear to be essential for their transport activities.

Proteins penetrate through membranes rather than remaining external to lipid bilayer was derived from results of a technique called freeze fracture replication. Here tissue is frozen solid and struck with blade, which fractures block into two pieces. Once the membrane are split, metals are deposited on their exposed surface to form replica which is viewed in the electron microscope (Refer Figure 3.5).

## Functions of Biological Membrane

- Membranes are continuous and enclose compartments. The plasma membrane encloses the contents of cell, whereas the nuclear and cytoplasmic membranes enclose diverse intracellular spaces. The various membrane bound compartments possess different contents. It allows specialized activities to proceed without external interference and enable cell activity to be regulated independently.
- Membrane prevent the unrestricted exchange of molecules from one side to another. Membranes provide the means of communication between compartments.

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- They form outer boundaries around the cells to maintain environment inside the cell different from those of outside environment.
- Membrane prevent the unrestricted exchange of molecules from one side to the other. At the same time, membrane provide the communication between the compartments they separate.
- The plasma membrane contain machinery for physically transporting substance from one side of membrane to another from region where solute is at low concentration to a region where solute is at higher concentration. The plasma membrane is able to transport ions thereby establishing ionic gradients across itself.
- The plasma membrane of organism mediates the interaction between a cell and its neighbours. The plasma membrane allow cells to recognize and signal one another, to adhere when appropriate and to exchange material and information.
- Membranes are involved in the process by which one type of energy is converted to another type (energy transduction). Membranes are involved in transfer of chemical energy from carbohydrates and fats to ATP.
- Plasma membrane plays a role in response of cell to external stimuli, a process known as signal transduction. Membranes possess receptors that combine with specific molecules. The interaction of a plasma membrane receptor with external ligand cause the membrane to generate signal that stimulate or inhibit internal activities.
- Boundaries round distinct sub-cellular compartments (Nucleus, Mitochondria, Lysosomes, Golgi bodies, etc.), compartmentalize and segregate intracellular events, and separate cells from one another.
- Membranes mediate regulation of cellular functions by: acting as selective barriers, allowing inside environment of cells or organelles to differ from outside.
- Plasma membrane is selectively permeable outer boundary of cell.
- Plasma membrane contains: Specific systems; Pumps, Channels, Transporters used for exchange of nutrients and other materials with the environment.
- Normal cellular function starts with normal cell membrane, i.e., damage to membrane can affect water balance and ion influx and therefore grossly alter most processes within the cell.
- In nerve cells, the cell membrane takes part in transmission of impulses.
- They provide sheaths for cilia and flagella.
- Secretory, excretory and waste products are thrown out by plasma membrane through exocytosis.

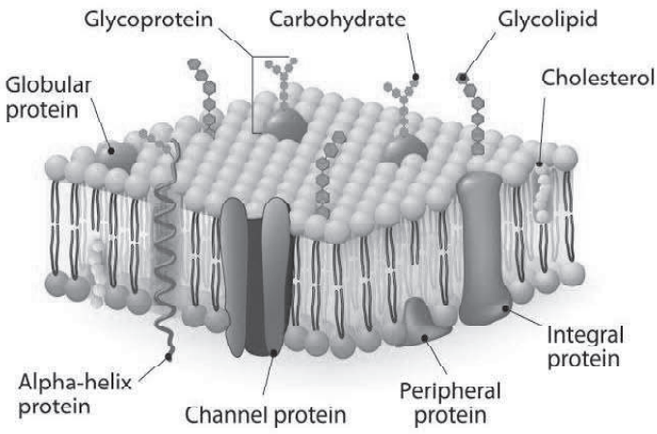


*Fig. 3.5 Biological Membrane with Different Protein Membranes*

## 3.2.2 Transport Across Cell Membrane

In the fluid mosaic plasma membrane, there is not complete and independent freedom of movement for its different component molecules. The mobility of some part of lipid molecules is constrained since that remains tightly bound to some of the integral membrane proteins. For example, the mobility of lipid molecules surrounding **cytochrome oxidase** (an enzyme involved in the synthesis of ATP) are immobilized by the enzyme and makes boundary lipid layer. The immobilized boundary lipid makes 30 per cent of membrane lipid in the mitochondrial membrane.

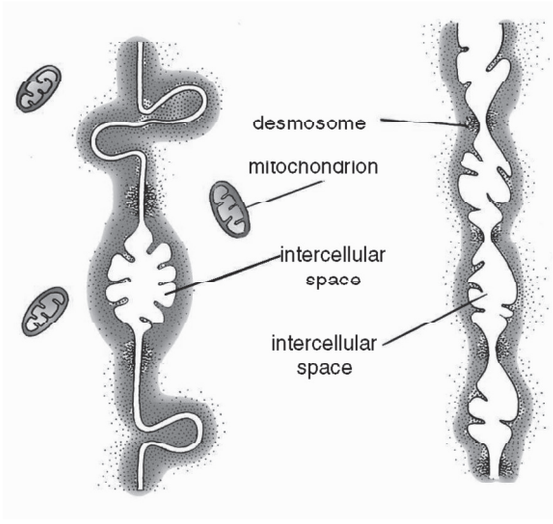


***Fig. 3.6** Inter-cellular Space.*

In contrast to lipids, the mobility and distribution of protein molecules in the membrane is controlled by various ways : (1) Certain proteins of membrane are constrained by protein-protein interactions to form specialized ordered regions, representing 2 to 20 per cent of the membrane of a system, *e.g.*, gap junctions, synapsis of neurons and plaques of halobacteria. (2) Certain peripheral proteins (endoproteins) may form a bridge-like lattice work between integral proteins and restrict their lateral mobility, *e.g.*, spectrin-ankyrin-actin cytoskeletal meshwork

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provides a rigidity to the membrane of human erythrocytes and does not permit the clustering or **capping** of integral proteins when the appropriate antibodies or lectins are added. (3) In nucleated eukaryotic cells, the mobility of the peripheral endoproteins and integral proteins is restrained by their attachment to the ectoplasmic cytoskeleton. The cytoskeleton is extensive, including **myosin** filaments, **actin** filaments and **microtubules** (Fig. 5.10). Rearrangement of cytoskeletal components just below the cell surface manifests in the distribution of integral membrane proteins and also in the cellular motions, endocytosis and exocytosis.

**Inter-Cellular Space:** In the tissues of multicellular animals, the plasma membranes of two adjacent cells usually remain separated by a space of 10 to 150 Aº wide. This inter-cellular space is uniform and contains a material of low electron density which can be considered as a cementing substance. This substance is found to be a mucopolysaccharide (Fig. 3.6).

## Origin of Plasma Membrane

There is hardly any cell structure more important to the immediate health of the cell than the plasma membrane. If it is weakened or injured, the cell loses its ability to maintain gradients, to carry out the selective transport of nutrients, and to contain the pool of enzymes and organelles essential for the homeostasis. In consequence, new membranes may be added to existing membranes without altering the functions as a barrier and selective transporter. Also for maintaining the characteristic membrane asymmetry, the membrane must be assembled with precisely the correct moleular topography.

Thus, all cellular membranes grow from pre-existing membranes which act as **templates** for the addition of new precursors. All cells divide, daughter cells receive a full complement of membrane systems which undergo growth until the next division, to be passed on to subsequent progeny. Meanwhile the molecules within the membrane undergo continuous replacement.

The protein molecules of the plasma membrane are synthesized on both attached and free ribosomes. Proteins synthesized by free ribosomes may be inserted into the plasma membrane following their completion and release from the ribosomes. Proteins of plasma membrane synthesized on attached ribosomes of rough ER are **inserted** first into the membrane of RER and then **transferred** to the Golgi apparatus, **processed** there (*e.g.*, glycosylation) and ultimately are dispatched to the plasma membrane via the secretory vesicles. Likewise, the synthesis of phospholipid molecules of the plasma membrane takes place by the smooth ER (SER). Like the proteins, newly synthesized lipids are inserted into SER membranes, then they are passed to Golgi apparatus for the processing and ultimately are dispatched to the plasma membrane via small secretory vesicles. The cytosol also contains a number of **phospholipid transport proteins** that function to transfer phospholipid molecules from one cellular membrane to another (*e.g.*, from ER membranes to plasma membranes) (see **Sheeler** and **Bianchi**, 1987).

In fact, the process of glycosylation (or glycosidation, *i.e.*, addition of oligosaccharides containing the sugars such as galactose, fucose and/ or sialic

acid, to the molecules of proteins and phospholipids of the plasma membrane) is completed at the level of Golgi apparatus. However, some sugars are added to the proteins in the lumen of RER.



### Functions of Plasma Membrane

The plasma membrane acts as a thin barrier which separates the intra-cellular fluid or the cytoplasm from the extra-cellular fluid in which the cell lives. In case of unicellular organisms (Protophyta and Protozoa) the extra-cellular fluid may be fresh or marine water, while in multicellular organisms the extra-cellular fluid may be blood, lymph or interstitial fluid. Though the plasma membrane is a limiting barrier around the cell but it performs various important physiological functions which are as follows :

**1. Permeability:** The plasma membrane is a thin, elastic membrane around the cell which usually allows the movement of small ions and molecules of various substances through it. This nature of plasma membrane is termed as permeability. According to permeability following types of the plasma membranes have been recognised :

**(i) Impermeable Plasma Membranes:** The plasma membrane of the unfertilized eggs of certain fishes allows nothing to pass through it except the gases. Such plasma membranes can be termed as impermeable plasma membranes.

**(ii) Semi-Permeable Plasma Membranes:** The membranes which allow only water but no solute particle to pass through them are known as semi-permeable membranes. Such membranes have not so far been recognised in animal cells.

**(iii) Selective Permeable Plasma Membranes:** The plasma membrane and other intra-cellular membrane are very selective in nature. Such membranes allow only certain selected ions and small molecules to pass through them.

**(iv) Dialysing Plasma Membranes:** The plasma membranes of certain cells have certain extraneous coats around them. The basement membranes of endothelial cells are the best examples of extraneous coats. This type of plasma membrane having extraneous coats around it, acts as a dialyzer. In these membranes the water molecules and crystalloids are forced through them by the hydrostatic pressure forces.

### Mode of Transport Across Plasma Membrane

The plasma membrane acts as a semipermeable barrier between the cell and the extracellular environment. This permeability must be highly **selective** if it is to ensure that essential molecules such as glucose, amino acids and lipids can readily enter the cell, that these molecules and metabolic intermediates remain in the cell, and that waste compounds leave the cell. In short, the **selective permeability** of the plasma membrane allows the cell to maintain a constant internal environment (**homeostasis**). In consequence, in all types of cells there exists a difference in ionic concentration with the extracellular medium. Similarly, the organelles within the cell often have a different internal environment from that of the surrounding cytosol and organelle membranes maintain this difference. For example, in lysosomes the concentration of protons ($H^+$) is 100 to 1000 times that of the cytosol. This gradient is maintained solely by the lysosomal membrane.

Transport across the membrane may be passive or active. It may occur via the phospholipid bilayer or by the help of specific integral membrane proteins, called **permeases** or **transport proteins.**

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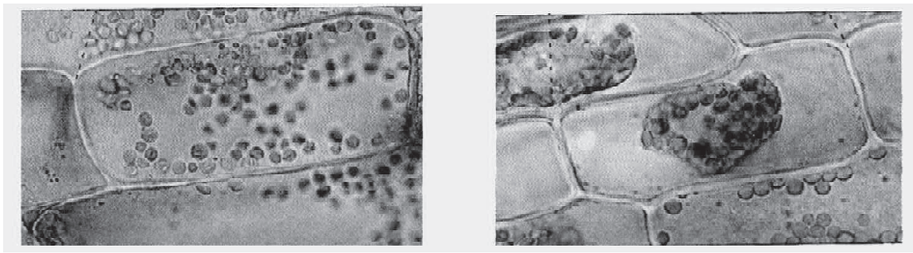
***Table 3.1*** *Comparison of ion concentration inside and outside of a typical mammalian cell (Source : Maclean and Hall, 1987).*

| Component | Intracellular concentration (mM) | Extracellular concentration (mM) |
|---|---|---|
| **Cations :** | | |
| $Na^+$ | 5-15 | 145 |
| $K^+$ | 140 | 5 |
| $Mg^{2+}$ | 30 | 1–2 |
| $Ca^{2+}$ | 1-2 | 2.5–5 |
| $H^+$ | $4\times10^{-5}$ (pH 7.4) | $4\times10^{-5}$ (pH 7.4) |
| **Anions* :** | | |
| $Cl^-$ | 4 | 110 |

**A. Passive transport:** It is a type of **diffusion** in which an ion or molecule crossing a membrane moves down its electrochemical or concentration gradient. *No metabolic energy is consumed in passive transport.* Passive transport is of following three types :



*(a)* *(b)*

***Fig. 3.7*** *The effects of osmosis on a plant cell. (a) hypotonic : normal turgor pressure; (b) hypertonic : no turgor pressure.*

**(a) Osmosis.** The plasma membrane is permeable to water molecules. The to and fro movement of water molecules through the plasma membrane occurs due to the differences in the concentration of the solute on its either sides. The process by which the water molecules pass through a membrane from a region of higher water concentration to the region of lower water concentration is known as **osmosis** (Gr., *osmos*=pushing). The process in which the water molecules enter into the cell is known as **endosmosis,** while the reverse process which involves the exit of the water molecules from the cell is known as **exosmosis**. In plant cells due to excessive exosmosis the cytoplasm along with the plasma membrane shrinks away from the cell wall. This process is known as **plasmolysis** (Gr., *plasma*=molded, *lysis*=loosing).

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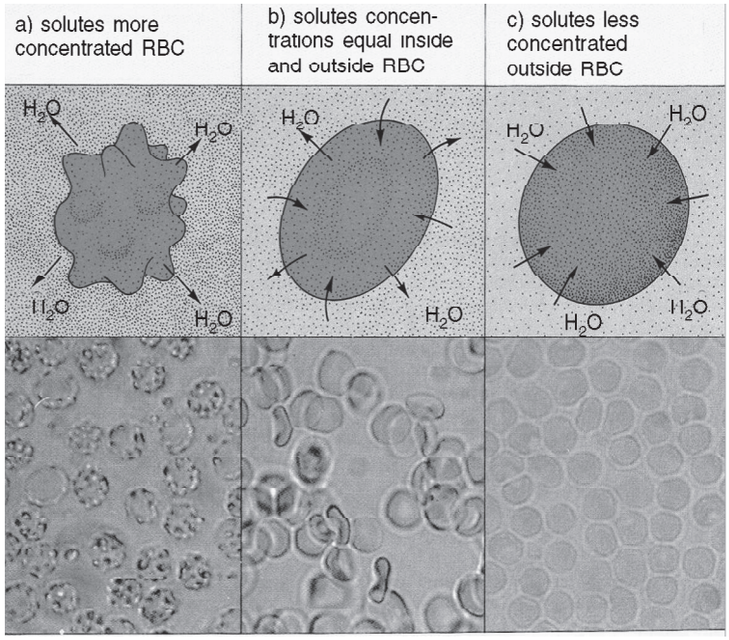


***Fig. 3.8*** *Osmosis and RBC*

A cell contains variety of solutes in it, for instance, the mammalian erythrocytes contain the ions of potassium ($K^+$), calcium ($Ca^+$), phosphate ($PO_4^-$), dissolved haemoglobin and many other substances. If the erythrocyte is placed in a 0.9% solution of sodium chloride (NaCl), then it neither shrinks nor swells.

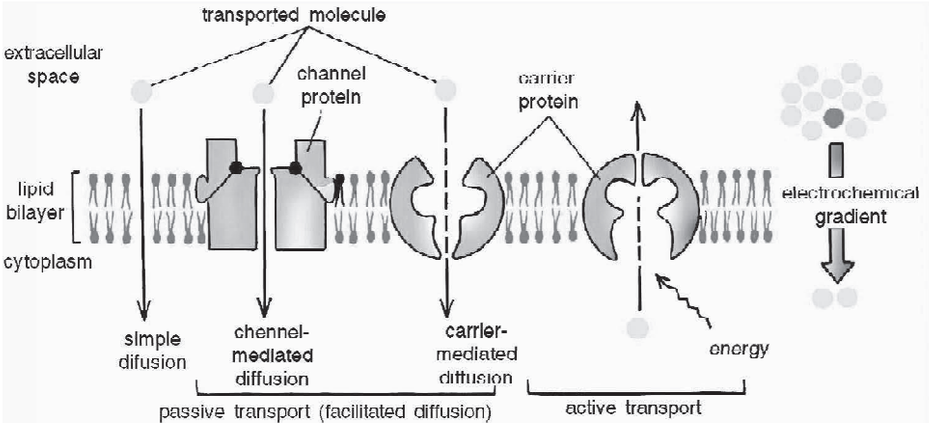


***Fig. 3.9*** *Schematic diagram showing various types of transports across the membrane : simple diffusion, passive transport (down an electrochemical gradient) and active transport (against an electro-chemical gradient) (after Alberts,* et al.*, 1989).*

In such case, because the intra-cellular and extra-celluar fluids contain same concentration and no osmosis takes place. This type of extra-cellular solution or fluid is known as **isotonic solution** or **fluid.** If the concentration of NaCl solution is increased above 0.9% then the erythrocytes are shrinked due to excessive exosmosis. The solutions which have higher concentrations of solutes than the intracellular fluids are known as **hypertonic solutions.** Further, if the concentration of NaCl solution decreases below 0.9% the erythrocytes will swell up due to endosmosis. The extra-cellular solutions having less concentration of the solutes than the cytoplasm are known as **hypotonic solutions.**

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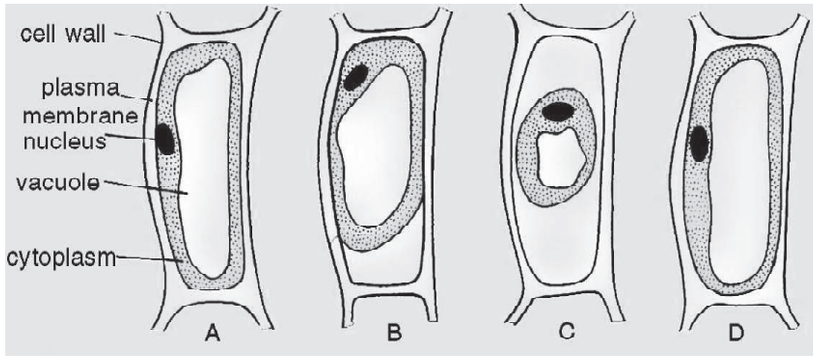


***Fig. 3.10*** *Plasmolysis and deplasmolysis in plant cells. Plasmolysis occurs when a normal plant cell (A) is placed in a hypertonic solution. Water leaves the cell and the plasma membrane shrinks away from the cell wall (B,C). If solutes can penetrate the plasma membrane, the cell will eventually regain water—a process termed* ***deplasmolysis*** *(D) (after De Witt, 1977).*

Due to endosmosis or exosmosis the water molecules come in or go out of the cell. The amount of the water inside the cell causes a pressure known as **hydrostatic pressure.** The hydrostatic pressure which is caused by the osmosis is known as **osmotic pressure.** The plasma membrane maintains a balance between the osmotic pressure of the intra-cellular and inter-cellular fluids.

**(b) Simple Diffusion.** In simple diffusion, transport across the membrane takes place unaided, *i.e*., molecules of gases such as oxygen and carbon dioxide and small molecules (*e.g*., ethanol) enter the cell by crossing the plasma membrane without the help of any permease. During simple diffusion, a small molecule in aqueous solution dissolves into the phospholipid bilayer, crosses it and then dissolves into the aqueous solution on the opposite side. There is little specificity to the process. The relative rate of diffusion of the molecule across the phospholipid bilayer will be proportional to the concentration gradient across the membrane.

**(c) Facilitated Diffusion.** This is a special type of passive transport, in which ions or molecules cross the membrane rapidly because specific permeases in the membrane facilitate their crossing. Like the simple diffusion, facilitated diffusion does not require the metabolic energy and it occurs only in the direction of a concentration gradient. Facilitated diffusion is characterized by the following special features: (1) the rate of transport of the molecule across the membrane is far greater than would be expected from a simple diffusion. (2) This process is specific; each facilitated diffusion protein (called **protein channel**) transports only a single species of ion or molecule. (3) There is a maximum rate of transport, *i.e.*, when the concentration gradient of molecules across the membrane is low, an increase in concentration gradient results in a corresponding increase in the rate of transport. Currently, it is believed that transport proteins form the **channels** through the membrane that permit certain ions or molecules to pass across the latter (see **Darnell** *et al*., 1986).

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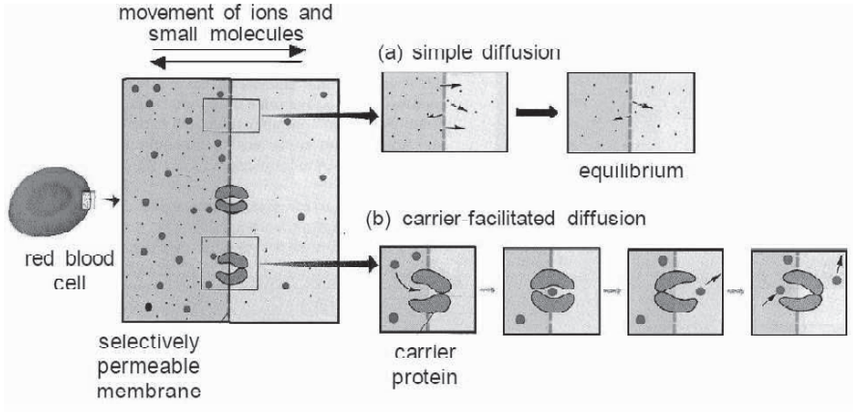


*Diffusion of Cell*

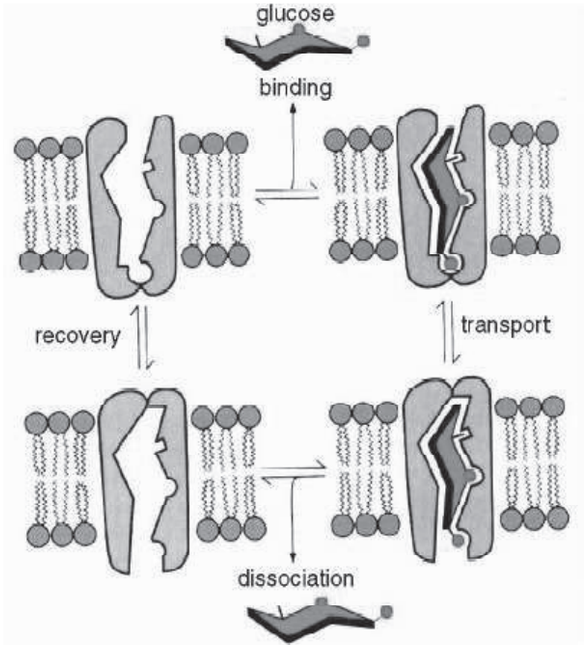


***Fig. 3.11** Facilitated diffusion*

## Examples of Facilitated Diffusion

**Ionic Transport Through Charged Pores:** Nerve conduction is propagated along the axonal membrane by **action potential** which regulates opening and closing of two main types of **ion channels** (*i.e.*, channel proteins with water filled pores) : **$Na^+$ channels** (or **voltage-gated $Na^+$ channels**) and **$K^+$ channels** (or **$k^+$ leak channels**). At the point of stimulation there is a sudden and several hundred fold increase in permeability to Na+, which reaches its peak in 0.1 millisecond (*i.e.*, the membrane potential may depolarise from –90 mV and overshoot to + 50 mV). At the end of the period, the membrane again becomes essentially impermeable to $Na^+$, but the $K^+$ permeability increases and this ion leaks out of the cell, repolarising the nerve fibre. In other words, during the rising phase of the spike, $Na^+$ enter through the $Na^+$ channels, and in descending phase $K^+$ is extruded through the $K^+$ channels.

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Such ion channels also occur in other types of cells such as muscle, sperm and unfertilized ovum. They are not coupled to an energy source (ATP), so the transport they mediate is always passive ("down hill"), allowing specific ions mainly $Na^+$, $K^+$, $Ca^{2+}$ and $Cl^-$ to diffuse down their electrochemical gradient across the lipid bilayer (**Hille**, 1984). Further, an ion channel is made of integral proteins of neural membrane. This protein has two functional elements : (1) a **selective filter** which determines the kind of ion that will be transported ; (2) a **gate** which by opening and closing the channel, regulates the ion flow. In both $Na^+$- and $K^+$-channels, the gating mechanism is electrically driven and is controlled by the membrane potential, without the need of other energy source. In the resting condition (steady state) both $Na^+$ and $K^+$ channels are closed. With depolarisation, the $Na^+$ channel is opened and during repolarisation, it closes again and $K^+$ channel opens.

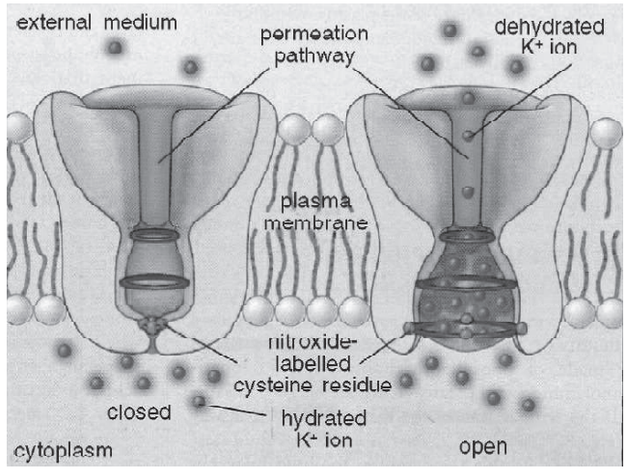


***Fig. 3.12*** *Use of EPR spectroscopy to monitor changes in conformation of a bacterial $K^+$ ion channel as it opens and closes.*

**Calcium Ion Channels (Ca$^{2+}$- Channels)** occur in axonal membranes and other membranes for the entrance of $Ca^{2+}$ ions in the cell. $Ca^{2+}$ ions have a fundamental role in many cellular activities such as exocytosis, endocytosis, secretion, cell motility, cell growth, fertilization and cell division. In the neuronal membrane, there are a number of $Ca^{2+}$ channels that are driven by the membrane potential and are essential in the release of neurotransmitters (acetylcholine).

**2. D-hexose Permease of Erythrocyte:** The plasma membrane of mammalian erythrocytes and other body cells, contain specific channel proteins for the facilitated diffusion of glucose into the cells. They are called **glucose transporter**, **glucose permease** or **D-hexose permease**. After the glucose is transported into the erythrocyte, it is rapidly phosphorylated (by **hexokinase** enzyme and ATP) to form **glucose-6-phosphate**. Once phosphorylated, the glucose no longer leaves the cell; moreover, the concentration of the simple glucose in the cell is lowered. As a result, the concentration gradient of glucose across the membrane is increased, allowing the facilitated diffusion to continue to import glucose. Since no cellular membrane (except the mitochondrial membranes) contains any permease for facilitated diffusion of phosphorylated compounds, so a cell can retain any type of molecule by **phosphorylating** them, *e.g.*, ATP and

phosphorylated nucleosides are never released from the cells containing a normal intact plasma membrane. However, permeases for ATP and ADP do exist in a mitochondrial membrane to allow these molecules to move across it.

The D-hexose permease of the erythrocyte is an integral and transmembrane protein of 45,000 daltons M.W. It accounts for 2 per cent of erythrocyte membrane protein. D-hexose permease, most probably operates in the following way : the binding of glucose to a site on the exterior surface of the permease triggers a conformational change in the polypeptide. This change somehow generates a pore in the protein that allows the bound glucose to pass through the membrane.

**3. Anion Exchange Permease of Erythrocyte:** Band 3 polypeptide of plasma membrane of the erythrocytes and other cells is an **ion exchange permease protein** which catalyzes an one-for-one exchange of anions such as chloride ($Cl^-$) and bicarbonate ($HCO_3^-$) across the membrane (called **chloride shift**; erythrocyte has 100,000 times more permeability of $Cl^-$ than other cells). The rapid flux of anions in the erythrocyte facilitates the transport in the blood of $CO_2$ from the tissues to the lungs. Waste $CO_2$ that is released from cell into the capillary blood, diffuses across the membrane of erythrocyte. In its gaseous from, $CO_2$ dissolves poorly in aqueous solutions such as blood plasma, but inside the erythrocyte the potent enzyme **carbonic anhydrase** converts it into a bicarbonate anion :

$$CO_2 + H_2O \underset{\text{anhydrase}}{\overset{\text{carbonic}}{\rightleftharpoons}} \underset{\text{proton}}{H^+} + \underset{\text{bicarbonate}}{HCO_3^-}$$

This process occurs while the haemoglobin in the erythrocyte is releasing its oxygen into the blood plasma. The removal of oxygen from haemoglobin induces a change in its conformation that enables a globin histidine (amino acid) side chain to bind to the proton produced by carbonic anhydrase enzyme. The bicarbonate anion formed by carbonic anhydrase is transported out of the erythrocyte in exchange for a chloride ($Cl^-$) anions:

| $HCO_3^-$ | + | $Cl^-$ | | $HCO_3^-$ | + | $Cl^-$ |
|---|---|---|---|---|---|---|
| (in) | | (out) | | (out) | | (in) |

As the total volume of the blood plasma is about twice that of the total erythrocyte cytoplasm, this exchange triples the amount of bicarbonate that can be carried by blood as a whole. Without the presence of an anion exchange protein (*i.e.*, band 3 protein), bicarbonate anions generated by carbonic anhydrase would remain within the erythrocyte and blood would be unable to transport all of the $CO_2$ produced by tissue. The entire exchange process is completed within 50 millisecond (ms) during which time $5 \times 10^9$ $HCO_3^-$ ions are exported from the cell. The process is reversed in the lungs : $HCO_3^-$ diffuses into the erythrocyte in exchange for a $Cl^-$. Oxygen binding to haemoglobin causes release of the proton from haemoglobin. The $CO_2$ diffuses out of the erythrocyte and is eventually expelled in breathing. The exact mechanism of anion transport by the Band 3 protein is still unknown.

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**B. Active Transport:** Active transport uses specific transport proteins, called **pumps**, which use metabolic energy (ATP) to move ions or molecules against their concentration gradient. For example, in both vertebrates and invertebrates, the concentration of sodium ion is about 10 to 20 times higher in the blood than within the cell. The concentration of the potassium ion is the reverse, generally 20 to 40 times higher inside the cell. Such a low sodium concentration inside the cell is maintained by the sodium-potassium pump. There are different types of pumps for the different types of ions or molecules such as calcium pump, proton pump, etc.

**Examples of Active Transport**

**(i) $Na^+$- $K^+$- ATPase:** It is an **ion pump** or **cation exchange pump** which is driven by energy of one ATP molecule to export three $Na^+$ ions outside the cell in exchange of the import of two $K^+$ ions inside the cell. Electrical organs of eels are found to be very rich in this enzyme or pump. $N^+$- $K^+$- ATPase is a transmembrane protein which is a dimer having two subunits : one smaller unit which is a glycoprotein of 50,000 daltons M.W., having a unknown function ; and another larger unit having 1,20,000 daltons M. W. The larger subunit of $Na^+$- $K^+$- ATPase performs the actual function of cation transport. It has three sites on its extracytoplasmic surface : two sites for $K^+$ ions and one site for the inhibitor **ouabain.** On its cytosolic side, the larger subunit contains three sites for three $Na^+$ ions and also has one catalytic site for a ATP molecule. It is believed that the hydrolysis of one ATP molecule somehow drives conformational changes in the $Na^+$- $K^+$- ATPase that allows the pump to transport three $Na^+$ ions out and two $K^+$ ions inside the cell (Fig. 3.13).

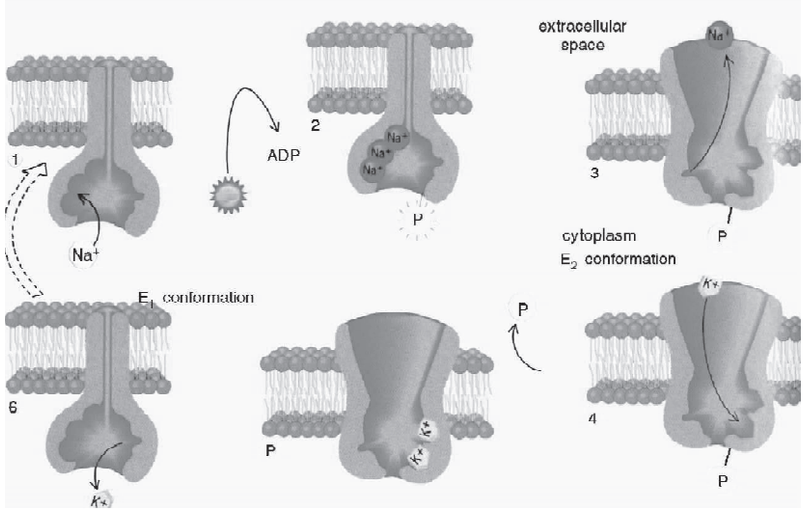


***Fig. 3.13*** *Schematic concept of the $Na^+/K^+$ –ATPase transport cycle*

**(ii) Calcium ATPase:** Calcium pump or $Ca^{2+}$-ATPase pumps $Ca^{2+}$-ions out of the cytosol, maintaining a low concentration of it inside the cytosol. In some types of cells such as erythrocytes, the calcium pumps are located in the plasma membrane and function to transport $Ca^{2+}$ ions out of the cell. In contrast, in muscle cells, $Ca^{2+}$-ion pumps are located in the membrane of ER or **sarcoplasmic**

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**reticulum**. The $Ca^{2+}$-ATPase transports $Ca^{2+}$ from the cytosol to the interior of the sarcoplasmic reticulum for causing the **relaxation** of the muscle cells. Release of $Ca^{2+}$ ions from the sarcoplasmic reticulum into the cytosol of muscle cells causes **contraction** of the muscle cells. Sarcoplasmic reticulum tends to concentrate and store $Ca^{2+}$ ions by the help of following two types of reservoir proteins : (1) **Calsequestrin** (44,000 daltons M.W. ; highly acidic protein) which tends to bind up to 43 $Ca^{2+}$ ions with it. (2) **High affinity $Ca^{2+}$- binding protein** which binds $Ca^{2+}$ ions and also reduces the concentration of free $Ca^{2+}$ ions inside the sarcoplasmic reticulum vesicles and decreases the amount of energy needed to pump $Ca^{2+}$ ions into it from the cytosol.

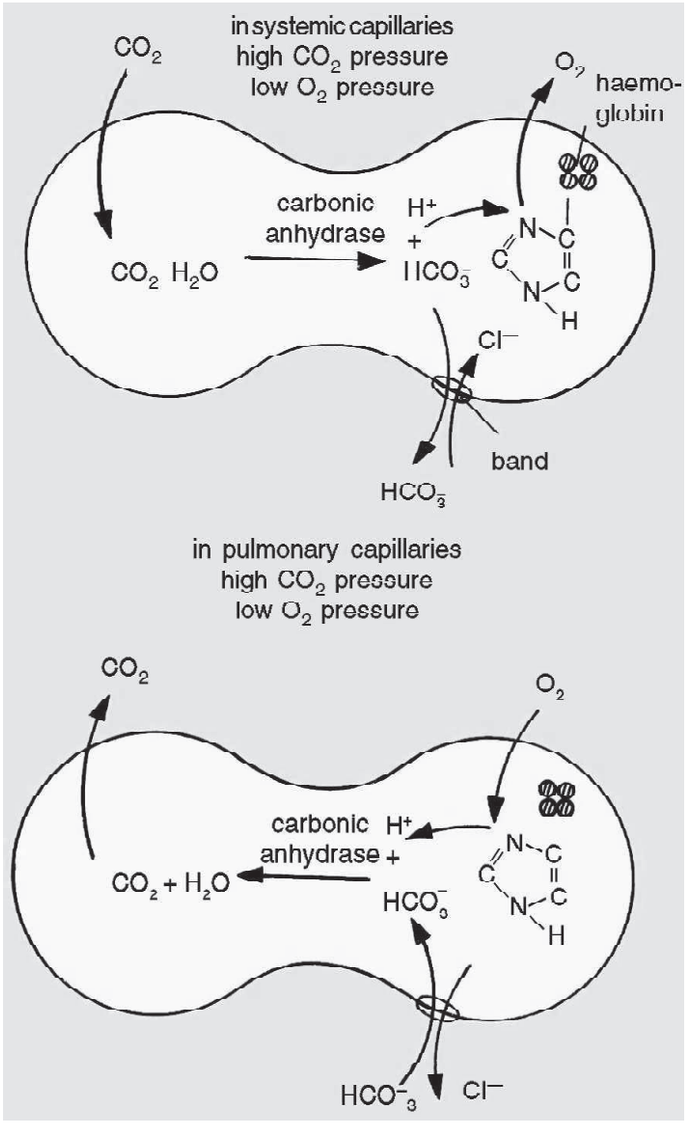


***Fig. 3.14*** *Schematic drawing showing anion transport through the erythrocyte membrane in the capillaries and in the lungs. Band 3 protein (= anion exchange permease) catalyzes the exchange of the anions : $Cl^-$ and $HCO_3^-$ across the erythrocyte membrane (after Darnell* et al.*, 1986).*

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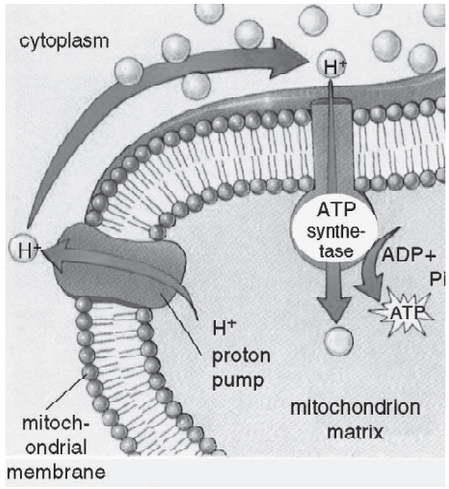


***Fig. 3.15*** *The proton pump*

A calcium pump is a 100,000 M.W., polypeptide, forming 80 per cent of integral membrane protein of sarcoplasmic reticulum. In it, hydrolysis of one ATP molecule transports two $Ca^{2+}$ ions in the counter-transport of one $Mg^{2+}$ ion.

**(iii) Proton Pump or $H^+$- Pump.** The lysosomal membrane contains the ATP-dependent proton pump that transports protons from the cytosol into the lumen of the organelle, keeping the interior of lysosomes very acidic (pH 4.5 to 5.0). The pH of the cytosol is about 7.0.

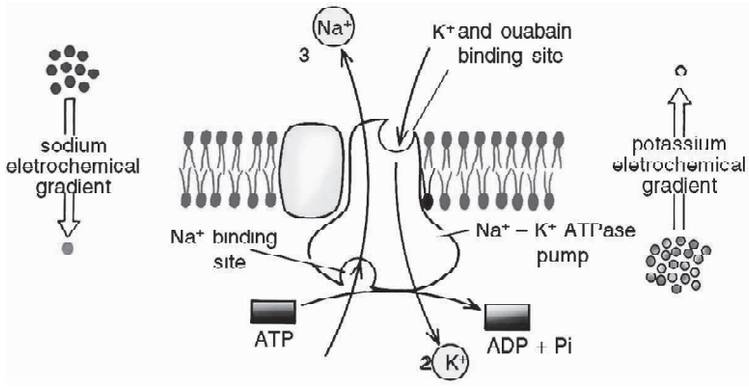


***Fig. 3.16*** *The $Na^+$- $K^+$ ATPase in the plasma membrane actively pumps $Na^+$ out and $K^+$ into a cell against their electrochemical gradients. For every molecule of ATP hydrolyzed inside the cell, 3 $Na^+$ ions are pumped out and 2$K^+$ ions are pumped in (after Alberts* et al.*, 1989).*

Proton pumps also occur in mitochondria and chloroplasts where they participate in the generation of ATP from ADP. They also cause acidification of the mammalian stomach. In the apical membrane of a **parietal cell** or **oxyntic cell** (which sercete HCl or $H^+$ $Cl^-$) are located ATP-dependent proton pumps. Hydrolysis of ATP is coupled to the transport of $H^+$ ions out of the cell (into stomach lumen). HCl production, thus, involves three types of transport proteins : 1. anion-exchange protein; 2. chloride ($Cl^-$) permeases; and 3. ATP- dependant proton ($H^+$) pump.

**Uniport, Symport and Antiport**

Those carrier proteins which simply transport a single solute from one side of the membrane to the other ; are called **uniports.** Others function as **coupled transporters**, in which the transfer of one solute depends on the simultaneous transfer of a second solute, either in the same direction (**symport**) or in the opposite direction (**antiport**). Both symport and antiport collectively form the **cotransport**. Most animal cells, for example, must take up glucose from the extracellular fluid, where the concentration of the sugar is relatively high, by passive transport through the glucose carriers (such as **D-hexose permease**) that operate as the uniports. By contrast, intestinal and kidney cells must take up glucose from the lumen of the intestine and kidney tubules, respectively, where the concentration of the sugar is low. These cells actively transport glucose by symport with $Na^+$ ions whose extracellular concentration is very high. The anion exchange permease of human erythrocytes operates as an antiport to the exchange of $Cl^-$ for $HCO_3^-$ (**Alberts** *et al*., 1989).

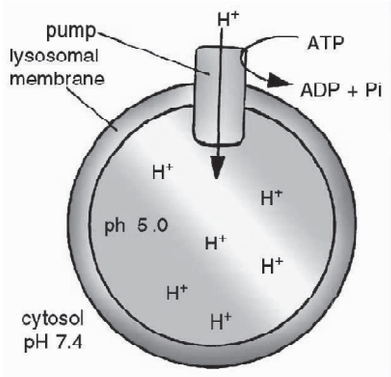


***Fig. 3.17*** *The Proton Pump of the Membrane of the Lysosome (after Darnell* et al.*, 1986)*

**C. Bulk Transport by the Plasma Membrane:** Cells routinely import and export large molecules across the plasma membrane. Macromolecules are secreted out from the cell by **exocytosis** and are ingested into the cell from outside through **phagocytosis** and **endocytosis.**

**1. Exocytosis:** It is also called **emeiocytosis** and **cell vomiting**. In all eukaryotic cells, **secretory vesicles** are continually carrying new plasma membrane and cellular secretions such as proteins, lipids and carbohydrates (*e.g.*, cellulose) from the Golgi apparatus to the plasma membrane or to cell exterior by the process of exocytosis. The proteins to be secreted are synthesized on the rough endoplasmic reticulum (RER). They pass into the lumen of the ER, glycosidated and are transported to the Golgi apparatus by ER-derived **transport vesicles**. In the Golgi apparatus the proteins are modified, concentrated, further glycosidated, sorted and finally packaged into vesicles that pinch off from trans golgi tubules and migrate to plasma membrane to fuse with it and release the secretion to cell's exterior. In contrast, small molecules to be secreted (*e.g.*, histamine by the mast cells) are actively transported from the cytosol (where they are synthesized on the free ribosomes) into preformed vesicles, where they are complexed to specific macromolecules (*e.g.*, a network of proteoglycans, in case of histamine; **Lawson** *et al.*, 1975), so that, they can be stored at high concentration without generating an excessive osmotic gradient.

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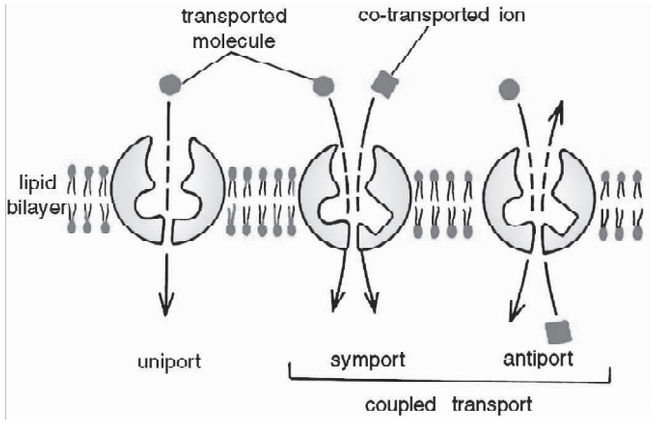



***Fig. 3.18*** *Carrier proteins of membrane functioning as uniports, symports and antiports (After Alberts* et al.*, 1989)*

During exocytosis the vesicle membrane is incorporated into the plasma membrane. The amount of secretory vesicle membrane that is temporarily added to the plasma membrane can be enormous : in a pancreatic acinar cell discharging digestive enzymes, about 900 $\mu m^2$ of vesicle membrane is inserted into the apical plasma membrane (whose area is only 30 $\mu m^3$) when the cell is stimulated to secrete.

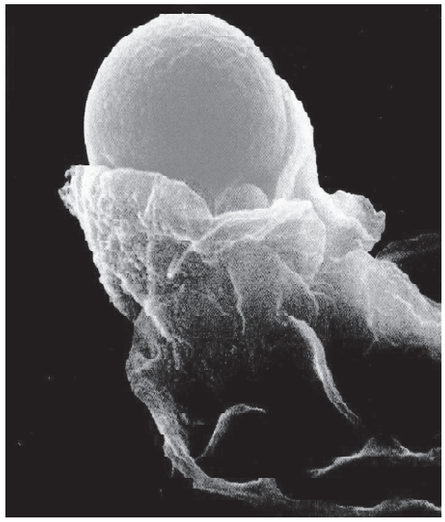


***Fig. 3.19*** *The process of engulfment by a leucocyte ingesting a yeast particle*

**2. Phagocytosis:** Sometimes the large-sized solid food or foreign particles are taken in by the cell through the plasma membrane. The process of ingestion of large-sized solid substances (*e.g.*, bacteria and parts of broken cells) by the cell is known as **phagocytosis** (Gr., *phagein*=to eat, *kytos*=cell or hollow vessel).

**Occurrence of Phagocytosis**

The process of phagocytosis occurs in most protozoans and certain cells of multicellular organisms. In multicellular organisms such as mammals, the phagocytosis occurs very actively in granular leucocytes and in the cells of mesoblastic origin. The cells of the mesoblastic origin are collectively known as the cells of **macrophagic** or **reticuloendothelial system**. The cells of

macrophagic system are histiocytes of the connective tissue, the reticular cells of the hemopoietic organs (bone marrow, lymph nodes and spleen) and the endothelial cells which form the lining of capillary sinusoid of the liver, adrenal gland and hypophysis. The cells of macrophagic system can ingest bacteria, Protozoa, cell debris or even colloidal particles by the process of phagocytosis.

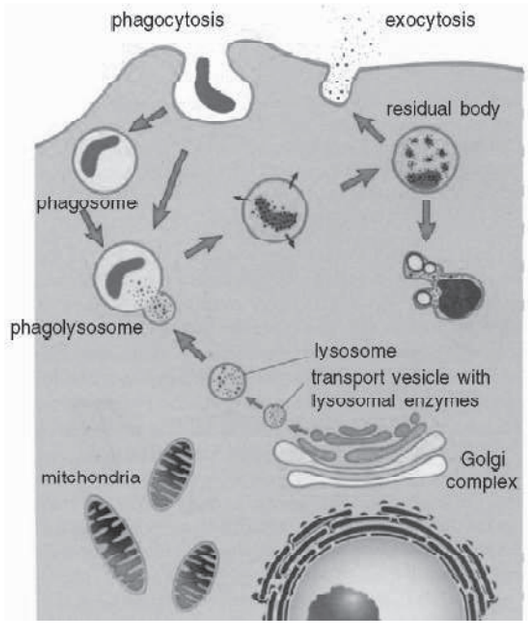
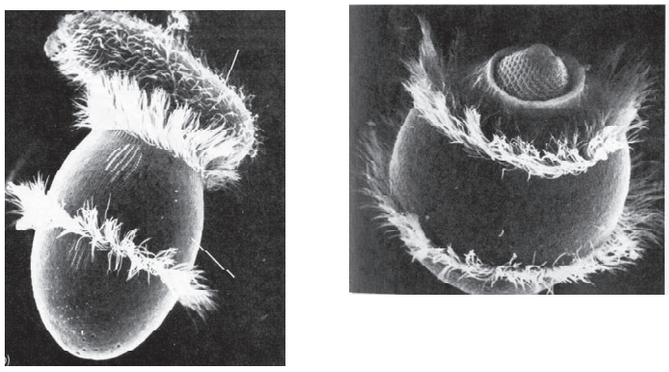



***Fig. 3.20*** *A Summary of the Phagocytic Pathway*



*(a)* *(b)*

*(a) Here an egg-shaped protist* Didinum *illustrates phagocytosis by ingesting the smaller* paramecium.

*(b) The* Didinium's meal *is almost over*

***Fig. 3.21*** *Didinum Illustrating Phagocytosis*

## Process of phagocytosis

In phagocytosis, first the target particle is bound, to the specific receptors on the cell's surface (process is called **adsorption**),then the plasma membrane expands along the surface of the particle and eventually engulfs it. Vesicle formed by phagocytosis is called **phagosome** and it is typically 1 to 2 μm or larger in diameter, much larger than those formed during pinocytosis and receptor-mediated endocytosis. The phagosomes migrate to the interior of the cell and fuse with the pre-existing lysosomes (to form phagolysosome). The food is digested by the

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hydrolytic enzymes (acid hydrolase) of the lysosomes and the digested food is ultimately diffused to the surrounding cytoplasm. In addition to the normal set of lysosomal hydrolases, macrophage's lysosomes contain enzymes that generate hydrogen peroxide ($H_2O_2$) and other toxic chemicals that aid in the killing of the bacteria. The undigested food is expelled from the plasma membrane by the process of **ephagy** or **egestion**. In macrophages, the undigested parts of ingested material such as the cell walls of micro-organisms, accumulate within lysosomes as residual bodies. Accumulation of residual bodies may be one reason why macrophages have a very short life time (*i.e.*, less than a few days).

**Kinds of Phagocytosis:** According to the physical and chemical nature of foreign substance following types of phagocytosis have been recognised :

**(i) Ultraphagocytosis or Colloidopexy:** The process in which plasma membrane ingests smaller colloidal particles is known as **colloidopexy** or **ultraphagocytosis**, *e.g.*, leucocytes and the macrophagic cells of mammals.

**(ii) Chromopexy:** When the cell ingests colloidal chromogen particles phagocytotically the process is known as **chromopexy**, *e.g.*, some mesoblasitc cells.

**3. Endocytosis:** In endocytosis, small regions of the plasma membrane fold inwards or **invaginate**, until it has formed new intracellular membrane limited vesicles. In eukaryotes, the following two types of endocytosis can occur : pinocytosis and receptor-mediated endocytosis.

**(i) Pinocytosis:** Pinocytosis (Gr., *pinein* = to drink; 'cell drinkng') is the non-specific uptake of small droplets of extracellular fluid by **endocytic vesicles** or **pinosomes**, having diameter of about 0.1 μm to 0.2 μm. Any material dissolved in the extracellular fluid is internalized in proportion to its concentration in the fluid. The process of pinocytosis was first of all observed by **Edward** in *Amoeba* and by **Lewis** (1931) in the cultured cells.

The light microscopy has shown that in *Amoeba* tiny **pinocytic channels** are continually being formed at the cell surface by invagination of the plasma membrane. From the inner end of each channel small vacuoles or pinosomes are pinched off, and these move towards the centre of the cell, where they fuse with primary lysosomes, to form **food vacuoles**. Ultimately, ingested contents are digested, small breakdown products such as sugars and amino acids diffuse to cytosol.

**Micropinocytosis:** Electron microscopic observations have been made on the pinocytotic process at sub-cellular or sub-microscopic level in the cells. The pinocytosis which occurs at sub-microscopic level is known as **micropinocytosis**. In the process of micropinocytosis, the plasma membrane invaginates to from small vesicles of 650 Aº diameter. These closed vesicles are not coated by clathrin protein and they move across the cytoplasm of endothelial cells (which line the blood capillaries) to fuse with opposite plasma membrane discharging their contents. This is called **transcytosis** (**Simionescue**, 1980). Such transcellular passage of fluids is also found to occur in other types of cells such as Schawn and satellite cells of nerve ganglion, macrophages, muscle cells and reticular cells, etc., but in them vesicles are coated by clathrin (see **Alberts** *et al*., 1989).

**(ii) Receptor-Mediated Endocytosis.** In this type of endocytosis, a specific receptor on the surface of the plasma membrane "recognizes" an extracellular macromolecule and binds with it. The substance bound with the receptor is called the **ligand**. Examples of ligands may include viruses,

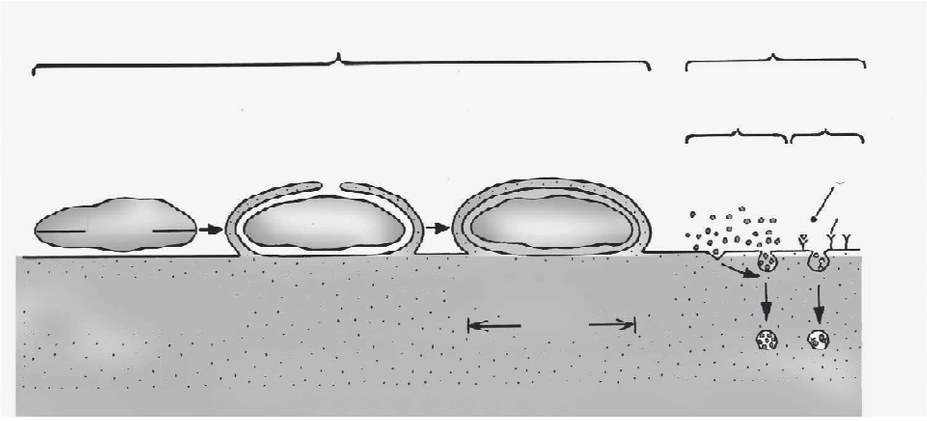

***Fig. 3.22*** *The process of Phagocytosis and Endocytosis (after Darnell* et al.*, 1986)*

small proteins (*e.g.*, insulin, vitellogenin, immunoglobin, transferrin, etc.), vitamin $B_{12}$, cholesterol containing LDL or low density lipoprotein, oligosaccharide, etc. The region of plasma membrane containing the receptor-ligand complex undergoes endocytosis. The whole process of receptor-mediated endocytosis, includes the following events :

**1. Interaction of Ligands and Cell Surface Receptors:** The macromolecules (ligands) bind to complementary cell-surface receptors. There are more than 25 different types of receptors which are involved in receptor-mediated endocytosis of different types of molecules. Such a receptor is a trans-membrane protein which contains two specific binding sites : (1) **ligand-binding site** at the external surface of plasma membrane ; and (2) **coated-pit binding site** at the inner or cytosolic face of the plasma membrane.

**2. Formation of Coated-Pits and Coated-Vesicles:** The endocytic cycle begins at specialized regions of the plasma membrane, called **coated-pits**. Coated-pits are depressions of plasma membrane having a coat of bristle-like structure towards their cytosolic side. The ligand-loaded receptors diffuse into these coated-pits. A coated-pit may accommodate about 1000 receptors of assorted variety. In fact, coated-pits serve as molecular filters and selective concentrating devices, since, they tend to collect certain receptors and leave others. They increase the efficiency of internalization of a particular ligand more than 1000-fold and also carry minor components of extracellular fluid. The life-time of each coated-pit is quite short—within a minute or so of being formed, it invaginates into the cell and pinches off to form the **coated-vesicles**. The coat of coated pits and coated vesicles is made up of protein, called **clathrin** and certain other proteins. A molecule of clathrin is composed of three large polypeptide chains and three smaller polypeptide chains, all of which together form a three-legged structure, called **triskelion**. A number of triskelions assemble into a basket-like network of hexagons and pentagons on the cytoplasmic surface of the membranes (**Pearse** and coworkers, 1981, 1987).

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**3. Fusion of Endocytic Vesicle and Endosome:** Once a coated vesicle is formed, the clathrin and associated proteins dissociate from the vesicle membrane and return to the plasma membrane to form a new coated-pit (**Schmid** and **Rothman**, 1985). The resultant **endocytic vesicle** gets fused with pre-existing endosomes and ultimately its contents are utilized by the cell.

**Check Your Progress**

1. Define biological membrane.
2. Which three types of lipids found in biomembrane?
3. What is inter-cellular space?
4. What do you understand by semi-permeable plasma membranes?
5. Define osmosis.
6. What is phagocytosis?

## 3.3 CYTOSKELETON

### Cytoskeleton

Earlier idea of cell was that it was a collection of some cell organelles suspended in cell sap. But with the advancement of microscopic techniques and the discovery of electron microscopy the idea of cell has been changed radically.

**Definition:** The cytoskeleton is a network of filaments and tubules that extends throughout a cell, through the cytoplasm, which is all of the material within a cell except for the nucleus. It is found in all cells, though the proteins that it is made of vary between organisms. The cytoskeleton supports the cell, gives it shape, organizes and tethers the organelles, and has roles in molecule transport, cell division and cell signalling.

Now it is considered that the cell sap is not a liquid but has network of many interconnected fibres and filaments having similarity with the bony skeleton of the animal body, i.e., an internal scaffolding of the cell.

These thread-like structures can be seen under the electron microscope or under the fluorescence microscope by tagging them with antibodies and fluorescent dyes. This network of fibres found in a cell are known as **cytoskeleton**.

The fibre of the cytoskeleton extends throughout the cell having interconnection with cell membrane and cell organelles. It represents some fibrous proteins of the cytoplasm which help to maintain cell shape and give contractibility to the cell.

It also helps to facilitate communication among intracellular organelles. It also helps in cell locomotion or the movement of protoplasm, i.e., cyclosis. It also helps in the movement of cellular components like chromosomes, membranes and granules, with formation of membrane protrusions (microvilli).

### Components of Cytoskeleton

On the basis of the electron microscopically observations, cytoskeleton components can be divided into following three types:

1. The thickest tubular components are known as microtubules.
2. The thinnest fibres are called microfilaments.
3. The fibres of intermediate thickness are known as Intermediate filaments.

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The network of microtubules becomes denser towards the nucleus, i.e., towards the nuclear envelope and then the fibres radiate towards the surface. Microfilaments, consisting of actin fibres, were found crisscrossing the cell outline.

These microfilaments can be seen by using antibody to actin under immunofluorescence microscopy. Microfilament bundles cross-over each other and also run parallel over long distances. Sometimes these filaments pass over the nucleus.

This type of microfilament organisation is sometimes known as stress fibres. The main function of these microfilaments is to help in the communication between the main cell components.

**1. Microtubules:** Microtubules were first noted in a number of eukaryotic cells by electron microscopic observations. It is a long rod-like structure of 25 nm diameter and up to several millimeters in length.

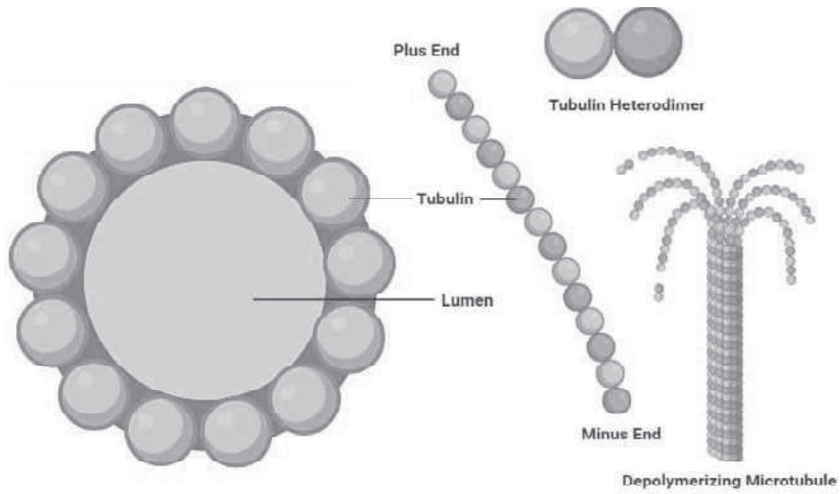


***Fig. 3.23*** *Microtubule*

Microtubules are the largest of the cytoskeleton's fibers at about 23 nm. They are hollow tubes made of alpha and beta tubulin. Microtubules form structures like flagella, which are "tails" that propel a cell forward. They are also found in structures like cilia, which are appendages that increase a cell's surface area and in some cases allow the cell to move. Most of the microtubules in an animal cell come from a cell organelle called the centrosome, which is a MicroTubule Organizing Center (MTOC). The centrosome is found near the middle of the cell, and microtubules radiate outward from it. Microtubules are important in forming the spindle apparatus (or mitotic spindle), which separates sister chromatids so that one copy can go to each daughter cell during cell division. They are also involved in transporting molecules within the cell and in the formation of the cell wall in plant cells.

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## Microtubule Organisation

It has two main characteristics that help to perform diverse type of functions of the cell:

- Long rigid shape facilitates in support-ing and anchoring different cellular constituents.
- Can generate movement in the cellular components as well as in the total cell.

Till the refinement of fixation technique in electron microscopy, microtubules were observed only in some subcellular structures like cilia, flagella, centrioles, mitotic spindle etc. using osmium tetroxide in the fixative. With the use of Glutaraldehyde in the fixative, the network of microtubules in the cytoplasm of the cell, i.e., cytoskeleton, was detected.

Electron microscopy and X-ray diffraction studies show that microtubules contain some longitudinally arranged assemblies of filaments. These filaments are known as protofilaments.

The chemical nature of these protofilaments is of tubulin molecules that are different in their amino acid sequence and are known as $\alpha$ and $\beta$ tubulins of approximately 110,000 molecular weight. These assemblies have an outer diameter of 30 nm and an inner diameter of 14 nm with a wall thickness of 8 nm. This beaded structure of protofilaments can be observed under the electron microscope.

It has also been noted that microtubules remain organised when the tubulin molecules are in equilibrium with non-polymerised tubulin. So the joining and disassembly of microtubule is regulated by changes in this equilibrium.

**2. Microfilaments:** The main component of these filaments is the protein actin, which is usually found in muscle. But these proteins have also been detected in many eukaryotic cells. Some actin like proteins are also found in prokaryotic cells. The non-muscle actins differ from muscle actin by amino acid sequences.

The association of actin into cytoskeleton network has been found to be of four types:

- Association of actin molecules into actin filaments
- Association with non-actin proteins into microfilaments
- Joining of microfilaments with network
- Association of actin fibres with other cell components like membranes.

The role of actin as a supporting aid of various cytoplasmic structures gives an idea that the assembly of actin filaments and their associations with cell components are responsive to cellular controls. Cytochalasins metabolites from fungus Helminthosporium, have a profound effect on the actin filaments. Some proteins inhibit elongation of actin filaments, others promote disassembly and nucleation.

Microfilaments are also called actin filaments because they are mostly composed of the protein actin; their structure is two strands of actin wound in a spiral. They are about 7 nanometers thick, making them the thinnest filaments in the cytoskeleton. Microfilaments have many functions. They aid in cytokinesis, which is the division of a cytoplasm of a cell when it is dividing into two daughter

cells. They aid in cell motility and allow single-celled organisms like amoebas to move. They are also involved in cytoplasmic streaming, which is the flowing of cytosol (the liquid part of the cytoplasm) throughout the cell. Cytoplasmic streaming transports nutrients and cell organelles. Microfilaments are also part of muscle cells and allow these cells to contract, along with myosin. Actin and myosin are the two main components of muscle contractile elements.

**3. Intermediate Filaments:** On the basis of size, another division of cytoskeletal components has been made which has a diameter (10 nm) smaller than that of microtubules. These filaments have a central highly conserved portion of 311-324 amino acids.

Four segments of different size are present in central domain of the microfilaments. These are designated as 1A, IB, 2A and 2B. 1A and 2A are short— 35 residues long. 2A and 2B are large—101 and 121 residues.

Examples of intermediate filaments include Keratin fibres present in epithelial cells, Desmin filaments found in muscle cells, glial filaments and neural filaments in the cells of the nervous system, vimentin filaments present in many types of cells. Intermediate filaments help in the change of cell shape.

Intermediate filaments are about 8-12 nm wide; they are called intermediate because they are in-between the size of microfilaments and microtubules. Intermediate filaments are made of different proteins such as keratin (found in hair and nails, and also in animals with scales, horns, or hooves), vimentin, desmin, and lamin. All intermediate filaments are found in the cytoplasm except for lamins, which are found in the nucleus and help support the nuclear envelope that surrounds the nucleus. The intermediate filaments in the cytoplasm maintain the cell's shape, bear tension, and provide structural support to the cell.

The ability of eukaryotic cells to adopt a variety of shapes and to carry out coordinated and directed movements depends on the **cytoskeleton**. The cytoskeleton extends throughout the cytoplasm and is a complex network of three types of protein filaments : **microtubules**, **microfilaments** (or actinfilaments) and **intermediate filaments** (**IFs**). The cytoskeleton is also can be referred to as **cytomusculature**, because, it is directly involved in movements such as crawling of cells on a substratum, muscle contraction and the many changes in the shape of a developing vertebrate embryo; it also provides the machinery for the cyclosis in cytoplasm. Cytoskeleton is apparently absent from the bacteria; it may have been a significant factor in the evolution of the eukaryotic cells.

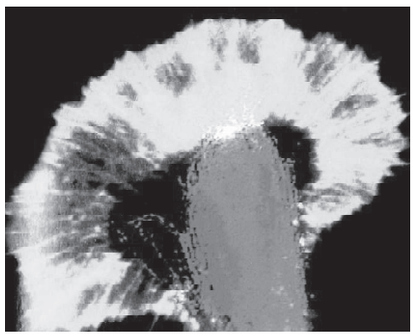

***Fig. 3.24*** *The Terminus of a Growing Axon*

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The existence of an organized fibrous array or cytoskeleton in the structure of the protoplasm was postulated in 1928 by Koltzoff. He conceived of a cytoskeleton that determines both the shape of the cell and the changes in its form.

The main proteins that are present in the cytoskeleton are **tubulin** (in the microtubules),**actin**, **myosin**, **tropomyosin** and other (in the microfilaments) and **keratins**, **vimentin**, **desmin**, **lamin** and others (in intermediate filaments). Tubulin and actin are globular proteins, while subunits of intermediate filaments are fibrous proteins. Great progress has been made in the isolation of these cytoskeletal proteins. In addition, by the production of specific antibodies against these proteins, it has been possible to examine under the light and the electron microscopes the disposition of the microtubules and microfilaments. The use of high-voltage electron microscopy on whole cells has also helped to demonstrate that there is a highly structured, three-dimensional lattice in the ground cytoplasm.

### 3.3.1 Microfilaments and Micratubules: Structure and Dynamics

#### Microtubules

Microtubules were fisrt of all observed in the axoplasm of the myelinated nerve fibres by Robertis and Franchi (1953). They called them neurotubules. The exact nature of microtubules was brought into light when Sabatini, Bensch and Barnett (1963) made use of the glutarldehyde fixative in the electron microscopy. Microtubeles of plant cells were first described in detail by Ledbetter and Porter (1963).

#### Occurrence

With rare exceptions such as the human erythrocytes, microtubules are found in all eukaryotic cells, either free in the cytoplasm or forming part of centrioles, cilia and flagella. The most abundant source of microtubules for the biochemical studies is vertebrate brain— high densities of microtubules exist in axons and dendrites of nerve cells. In the cytoplasm of animal and plant cells, microtubules occur at following seven sites :— 1. cilia and flagella, 2. centrioles and basal bodies, 3. nerve processes, 4. the mitotic apparatus, 5. the cortex of meristematic plant cells, 6. elongating cells such as during the formation of the lens or during spermatogenesis of certain insects. 7. selected structures in Protozoa such as the axostyle of parasitic flagellates, the axoneme of *Echinosphaerium*, the fibre systems of *Stentor*, and the cytopharyngeal basket of *Nassula*.

The stability of different microtubules varies. Cytoplasmic and spindle microtubules are rather labile structures, whereas, those of cilia and flagella are more resistant to various treatments.

#### Structure

Microtubules constitute a class of morphologically and chemically related filamentous rods which are common to both plant and animal cells. A microtubule consists of a long, unbranched, hollow tubules 24–25 nm in diameter, several micrometers long and with 6 nm thick wall having 13 subunits or **protofilaments**. Thus, the wall of the microtubule consists of 13 individual linear or spiralling filamentous structures about 5 nm in diameter, which in turn, are composed of tubulin. These protofilaments have a centre-to-centre spacing of 4.5 nm. Application

of negative staining techniques has shown that microtubules have a lumen 14 nm wide and a protofilament or subunit structure in the wall.

**Chemical Composition**

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Biochemically, a protofilament of microtubule is made of a protein called **tubulin**. Tubulin is an acidic protein with a molecular weight of 55,000 and a sedimentation constant of 6S. It occurs in two different forms, called **α-tubulin** and **β-tubulin**, each containing about 450 amino acids. Both of these proteins have a distinct, though closely related, amino acid sequences and are thought to have evolved from a single ancestral protein. The two proteins show very little divergence from the lowest to the highest eukaryotes ; for example, the β- tubulins of sea urchin flagella and chick brain cells differ only in one amino acid. Similarities such as this suggest that most mutations disrupt the functions of microtubules and are thus lethal and are eliminated by selection (see **King**, 1986).

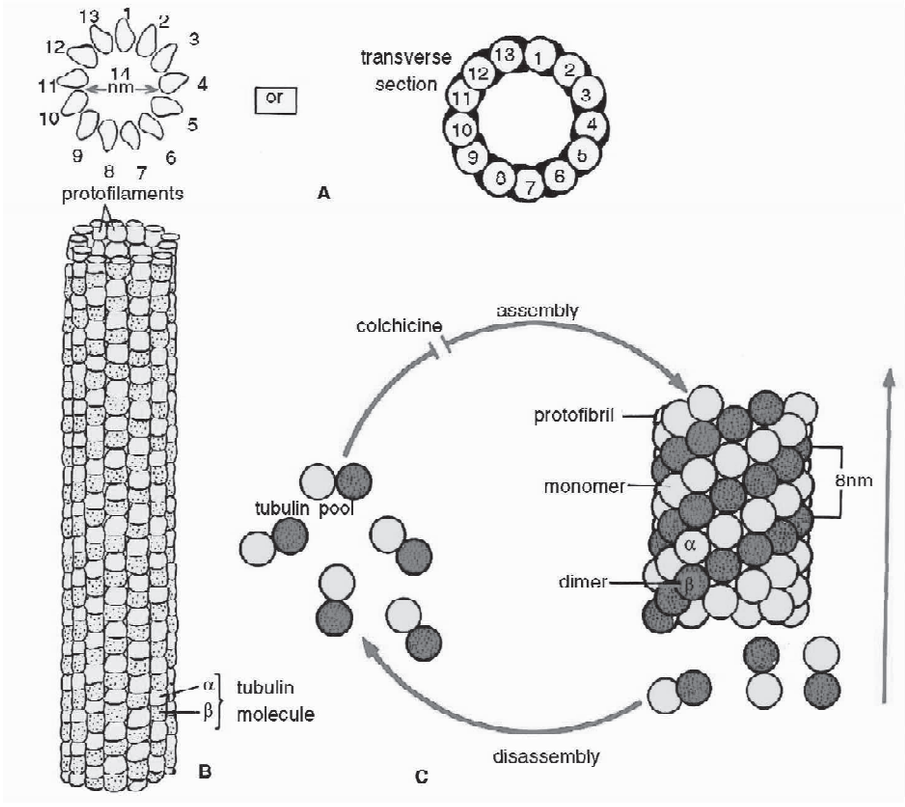


***Fig. 3.25*** *Schematic diagrams of a microtubule, showing how the tubulin molecules pack together to formthe cylindrical wall. A—13 tubulin molecules (subunits of protofilaments) in cross section ; B—Side view of a short section of a microtubule, with the tubulin molecules aligned into rows, or protofilaments. Each of the 13 protofilaments is composed of a series of tubulin molecules, each with an a/b heterodimer ; C—Assembly and disassembly of the microtubule. The microtubule is being disassembled at the bottom while being simultaneoasly assembled at the top. Colchicine, by blocking the assembly process, produces depolymerization of the microtubules (after De Robertis and De Robertis, Jr., 1987 ; Alberts et al., 1989)*

Tubulin in the form of dimers (rather heterodimers of α- and β- tubulins ; each with 115,000 MW, see Berns, 1983) polymerizes into the microtubules. Thus, heterodimers of tubulins assemble to form linear "protofilaments" with the β- tubulin of one dimer in contact with the α- tubulin of the next. Since all the 13 protofilaments are aligned parallely with the same polarity, the microtubules are the polar structurs having a plus or fast growing end and minus or slow-growing

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**end**. The minus ends of cytoplasmic microtubules in cells are bound tightly to **Microtubule Organizing Centres** (**MTOCs**) from which their assembly or polymerization starts. MTOCs also protect the minus ends of the microtubules from the disassembly. Generally, the plus ends of microtubules terminate near cell margins and are protected from disassembly by the **capping proteins** (see **Alberts** *et al.,* 1989).

### Microtubule-Associated Proteins (MAPs)

Recently, a number of proteins have been identified that associate with the surface of microtubules ; these proteins are called **microtubule-associated proteins** or **MAPs**. The following two major classes of MAPs have been isolated from brain in association with microtubules : 1. **HMW proteins** (= high molecular weight proteins) which have molecular weights of 200,000 to 300,000 or more ; 2. **Tau proteins**, with molecular weights of 40,000 to 60,000. Both classes of proteins have two domains, one of which binds to microtubules ; because this domain binds to several unpolymerized tubulin molecules simultaneously, these MAPs tend to speed up the nucleation (= process of grouping around a central mass) step of tubulin polymerization *in vitro*. The other domain is believed to be involved in linking the microtubule to other cell components (Fig. 15.3). Antibodies to HMW and tau proteins show that both proteins bind along the entire length of cytoplasmic microtubules.

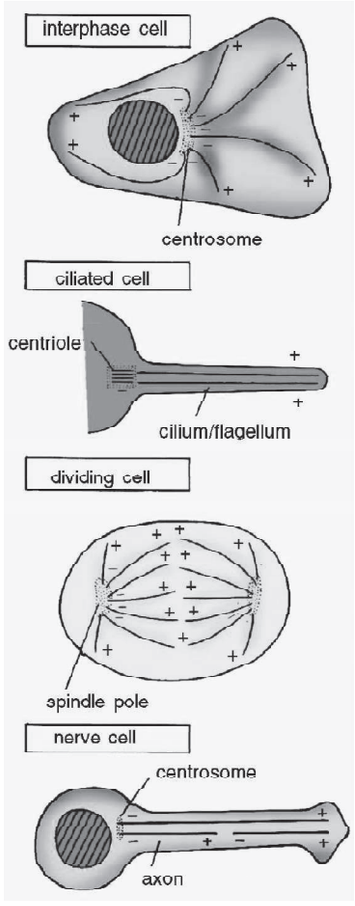


***Fig. 3.26*** *The minus ends of microtubules in cells are generally embedded in a microtubule-organizing centre ; while the plus ends are often near the plasma membrane (after Alberts* et al.*, 1989).*

## Microtubule Organizing Centres (MTOCs)

The microtubules are not found helter-skelter about the cell, but are organized in specific patterns designed to carry out specific function. Spontaneous nucleation, as seen *in vitro*, probably does not occur *in vivo*. Rather, initiation of assembly occurs at **Microtubule Organizing Centres (MTOCs)**. Thus, MTOCs are nucleating centres that serve as templates for the polymerization of tubulin (see **Thorpe**, 1983). MTOCs exist in basal bodies (*e.g., Chlamydomonas*); in centrioles (*e.g.*, most animal cells); at the poles of mitotic spindles in dividing cells that do not have centrioles (*e.g.,* most plant cells); on chromosomes (*i.e.*, **kinetochore**); in membranes and probably many other places as well. Recent studies have revealed that most cytoplasmic microtubules do not arise directly from the centrioles, but from a densely staining **pericentriolar material** that surrounds the centriole (see **King**, 1986).

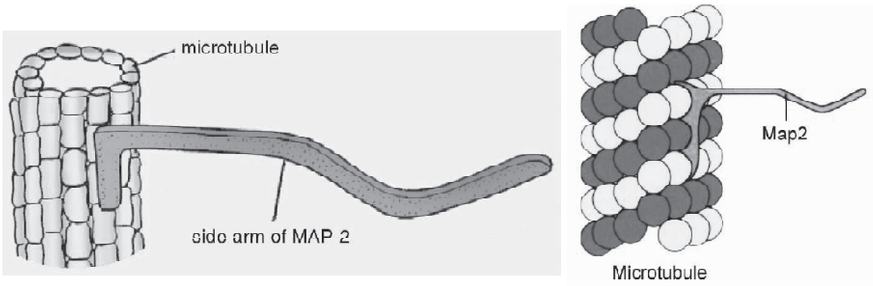


***Fig. 3.27*** *A microtubule associated protein (known as MAP-2) showing its two domains (after Alberts* et al., *1989).*

*MAPs*

Turning on and off of these organizing centres for microtubule assembly at different times in the cell's life are probably regulated by one or all the following factors: changes in nucleation centres, changes in $Ca^{2+}$ concentration and involvement of MAPs.

## Assembly and Disassembly of Microtubules

Cytoplasmic microtubules are highly dynamic structures, constantly forming and disappearing depending on cell activities. They, like the microfilaments, grow by the reversible addition of subunits, accompanied by nucleotide (GTP) hydrolysis and conformational change. The process of polymerization (assembly) and depolymerization (disassembly) of the microtubules appears to be a form of **self-assembly**. The assembly of microtubules from the tubulin dimers is a specifically oriented and programmed process. In the cell, the sites of orientation are MTOCs from which the polymerization is directed. The quantity of polymerized tubulin is high at interphase (cytoplasmic microtubules) and metaphase (spindle microtubules), but low at prophase and anaphase.

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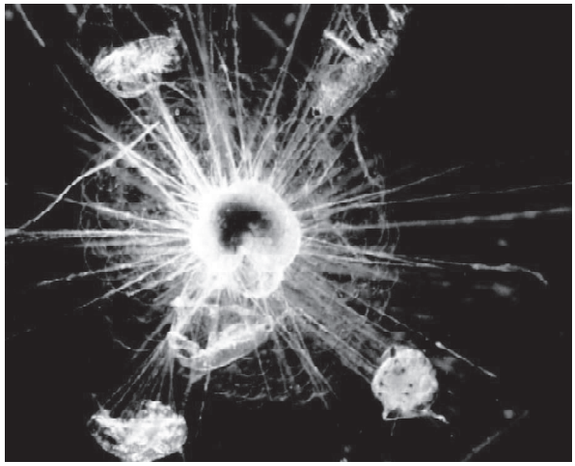

***Fig. 3.28*** *Microtubules as supporting rods.*
*This protist has tentacles projecting from its cell body.*

Within the cell, microtubules are in equilibrium with free tubulin. Phosphorylation of the tubulin monomers by a cyclic AMP-dependent kinase favours the polymerization. A definite relationship has been found between cell shape, the number and direction of microtubules and cAMP. The assembly and disassembly of tubulin constitute a polarized phemomenon. In a microtubule, the assembly of tubulin dimers takes place at one end, while disassembly is common at the other end. If a cell is treated with certain drugs such as **colchicine**, **vincristine** or **vinblastine**, the assembly of the microtubules is inhibited, while the disassembly continues, leading to the disorganization of the microtubule. Further, the assembly is accompanied by the hydrolysis of guanosine triphosphate (GTP) to guanosine diphosphate (GDP) and lack of GTP stops the assembly. *In vivo* control of assembly and disassembly of tubulin involves $Ca^{2+}$ and the calcium-binding protein **calmodulin**. The addition of $Ca^{2+}$ inhibits polymerization of tubulin ; this effect is also enhanced by the addition of calmodulin.

The *in vivo* mechanism involved in self-assembly of the microtubules is still little understood, however, *in vitro* studies have revealed various interesting facts about it. Thus, in a classical study using isolated bovine brain tubulin, **Weingarten** *et al.*, (1975) demonstrated that tubulin alone was not sufficient to bring about *in vitro* assembly into microtubules. Under normal conditions, if brain microtubules are isolated and caused to depolymerize into tubulin subunits, the tubulin molecules will reassemble into microtubules if $Mg^{2+}$ and GTP (an energy source) are added to the mixture. However, according to **King** (1986), *in vitro* assembly of microtubules can occur in the presence of low calcium concentration, MAPs, GTP, and a level of free tubulin monomers above a threshold concentration.

*In vitro* polymerization evidently involves two distinct phases, one of **initiation** and the other of **elongation**. The initiation event seems to involve the formation of some multimeric "**nucleating**" centre, following which the addition of more subunits proceeds rapidly during elongation. Thus, during *in vitro* polymerization of microtubules, $\alpha$- and $\beta$- tubulins combine to form heterodimers. The heterodimers associate to form multimeric **rings**, **spirals** and other intermediate

structures which eventually open up to form strands or protofilaments. Side-by-side assembly of the protofilaments creates sheet-like structures that curl to form a tube. Elongation of this short cylinder occurs by direct addition of new heterodimers at one end of the tubule (*i.e.,* the plus end of tubule). It is believed that during anaphase, addition of dimers to one end of a microtubule is accompanied by the loss of dimers from the other end.

**Functions of Cytoplasmic Microtubules**

Microtubules have several functions in the eukaryotic cells such as follows:

**1. Mechanical Function:** The shape of the cell (*e.g.,* red blood cells of non-mammalian vertebrates) and some cell processes or protuberances such as axons and dendrites of neurons, microvilli, etc., have been correlated to the orientation and distribution of microtubules.

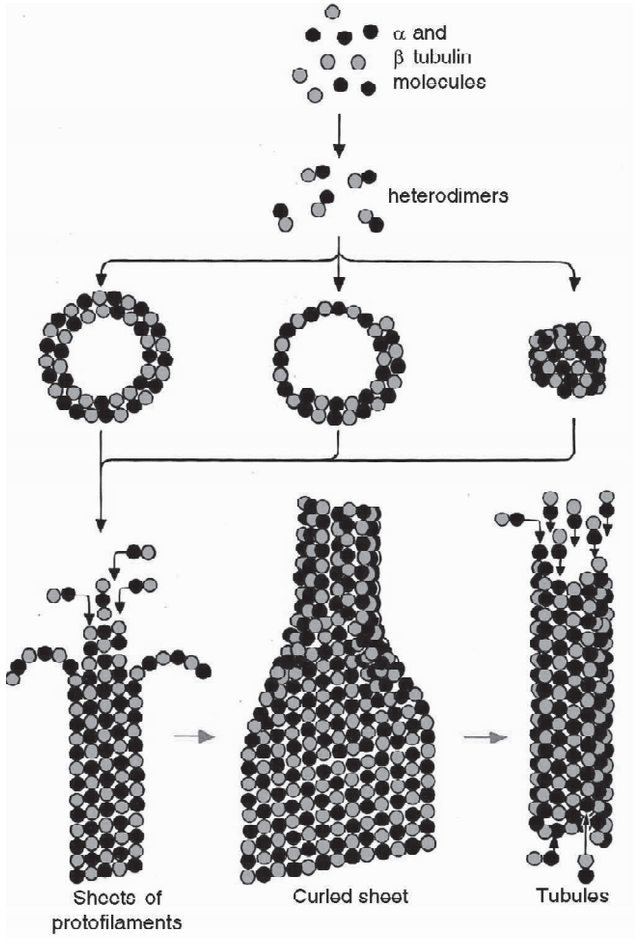


***Fig. 3.29*** *Various steps in* in vitro *polymerization or assembly of the microtubules (After Thorpe, 1984)*

**2. Morphogenesis:** During cell differentiation, the mechanical function of microtubules is used to determine the shape of the developing cells. For example, the enormous elongation in the nucleus of the spermatid during spermiogenesis is accompanied by the production of an orderly array of microtubules that are wrapped around the nucleus in a double helical arrangement. Likewise, the

elongation of the cells during induction of the lens placode in the eye is also accompanied by the appearance of numerous microtubules.

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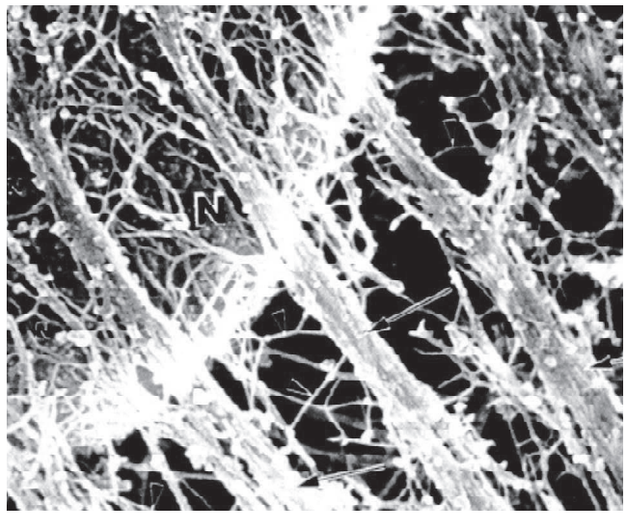


***Fig. 3.30*** *Nucleus (N) of a cell being held in place by a network of cytoskeleton*

**3. Cellular Polarity and Motility**: The determination of the intrinsic polarity of certain cells is also related to the microtubules. Directional gliding of cultured cells is found to depend on the microtubules.

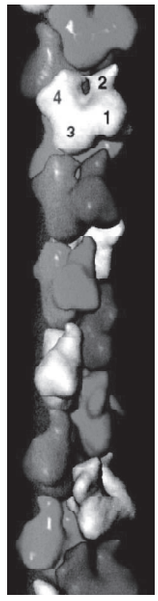


***Fig. 3.31*** *Actin filament structure.*

**4. Contraction:** Microtubules play a role in the contraction of the spindle and movement of chromosomes and centrioles as well as in ciliary and flagellar motion.

**5. Circulation and Transport:** Microtubules are involved in the transport of macromolecules, granules and vesicles within the cell. **Examples**: 1. The

protozoan *Actinosphaerium* (Heliozoa) sends out long, thin pseudopodia within which cytoplasmic particles migrate back and forth. These pseudopodia contain as many as 500 microtubules disposed in a helical configuration. 2. In the protozoan *Nassula*, microtubules drive the food in the gullet. 3. In melanocytes, melanin granules move centrifugally and centripetally with different stimuli. These granules have been observed moving between channels created by the microtubules in the cytoplasmic matrix. 4. In the erythrophores found in fish scales the pigment granules may move at a speed of 25 to 30 μm per second between the microtubules. 5. They have a role in axoplasmic transport of proteins, glycoproteins and enzymes.

### Microfilaments

Thin,solid **microfilaments** of actin protein, ranging between 5 to 7 nm in diameter and indeterminate length, represent the active or motile part of the cytoskeleton. They appear to play major role in cyclosis and amoeboid motion. With high voltage electron microscopy a three-dimensional view of microfilaments has been obtained (*i.e.*, an image of **microtrabecular lattice**). These microfilaments are sensitive to **cytochalasin-B**, an alkaloid that also impairs many cell activities such as beat of heart cell, cell migration, cytokinesis, endocytosis and exocytosis. It is generally assumed that the cytochalasin-B-sensitive microfilaments are the contractile machinery of non-muscle cells.

### Distribution

Microfilaments are generally distributed in the cortical regions of the cell just beneath the plasma membrane. In contrast, intermediate filaments and microtubules are found in subcortical and deeper regions of the cell. Microfilaments also extend into cell processes, especially where there is movement. Thus, they are found in the microvilli of the brush border of intestinal epitheliun and in cell types where amoeboid movement and cytoplasmic streaming are prominent.

### Chemical Composition

**Actin** is the main structural protein of microfilaments. The concentration of actin in non-muscle cells is surprisingly high ; it may account up to 10 per cent of total cell protein. It can be extracted and *in vitro* settings will undergo polymerization reactions from G-actin monomer state to F-actin. In fact, the globular (=G actin) – fibrillar (=F-actin) transition is the basis of the classical sol-gel transition in the cytoplasm of moving cells. Further, there are present three types of actins— α , β and γ. The α- form of actin is found in fully mature muscle tissue. The other two forms are more characteristic of non-muscle cells.

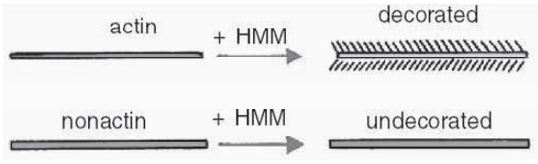


***Fig. 3.32*** *Mode of "Decoration" of Actin Filaments by HMM (after Thorpe, 1984)*

NOTES

In non-muscle cells, microfilaments, being of actin composition, can bind myosin (a contractile protein). *In vitro* and *in situ* mircrofilaments can be coated or "decorated" with heavy myosin (HMM) or $S_1$ heads. This binding results in an arrow-head pattern to the microfilaments in which the arrowheads all point in the same direction. This pattern indicates that microfilaments possess a polarity, a property that is probably crucial to their role in mediating cell movements.

The HMM binding method has become a very useful method for identifying and localizing microfilaments in any type of cell. Intermediate filaments are not decorated by HMM.

### Function

Microfilaments are found to be involved in movement associated with furrow formation in cell division, cytoplasmic streaming in plant cells (*e.g.*, *Nitella* and *Chara*) and cell migration during embryonic development.

### Intermediate Filaments

Intermediate filaments (IFs) are tough and durable protein fibres in the cytoplasm of most higher eukaryotic cells. Constructed like woven ropes, they are typically between 8 nm to 10 nm in diameter, which is "intermediate" between the thin and thick filaments in muscle cells, where they were first described ; their diameter is also between microfilaments (actin filaments) and microtubules. IFs are found resistant to colchicine and cytochalasin B and are sensitive to proteolysis.

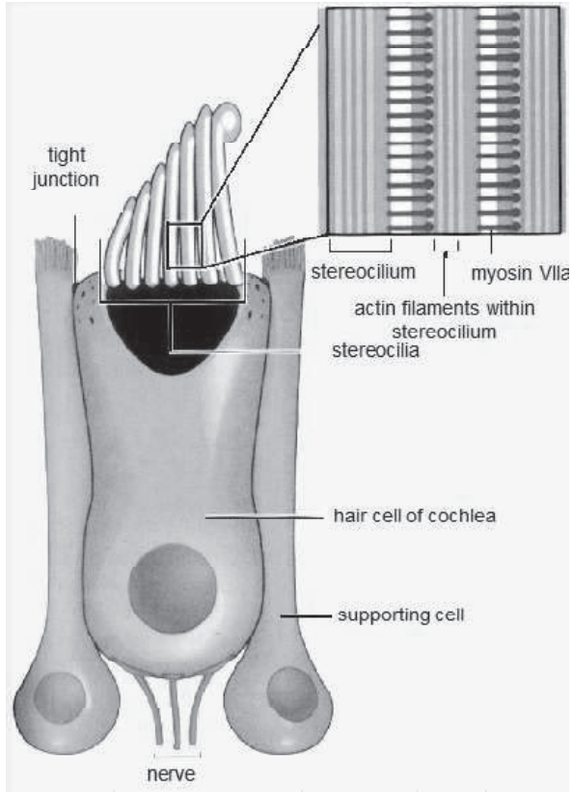


***Fig. 3.33*** *Hair cell of the cochlea.*
*The inset shows a portion of sterocilia, which is composed of actin filaments.*

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In most animal cells IFs form a "basket" around the nucleus and extend out in gentle curving arrays to the cell periphery. IFs are particularly prominent where cells are subjected to mechanical stress, such as in epithelia, where they are linked from cell to cell at desmosomal junctions, along the length of axons, and throughout the cytoplasm of smooth muscle cells. Various names have been attached to the intermediate filaments that have a basis in the cell type in which they are observed. Thus, IFs in epidermal cells are called **tonofilaments**, in nerve cells they are referred to as **neurofilaments** and in neuroglial cells they are designated as **glial filaments**.

In cross-section, intermediate filaments have a tubular appearance. Each tubule appears to be made up of 4 or 5 protofilaments arranged in parallel fashion (**Thorpe**, 1984). IFs are composed of polypeptides of a surprisingly wide range of sizes (from about 40,000 to 130,000 daltons).

**Types of Intermediate Filaments:** The intermediate filaments are very heterogeneous from the point of view of their biochemical properties, but by their morphology and localization can be grouped into following four main types (Table 3.2) :

**1. Type I IF proteins:** They are found primarily in epithelial cells and include two subfamilies of **keratin** (also celled **tono**, **perakeratin** or **cytokeratin**) : acidic keratin and neutral or basic keratin. Keratin filaments are always heteropolymers formed from an equal number of subunits from each of these two keratin subfamilies. The keratins are most complex class of IF proteins, with at least 19 distinct forms in human epithelia and 8 more in the keratins of hair and nails. Mammalian cytokeratin are $\alpha$-fibrous proteins that are synthesized in cells of living layers of the epidermis and form the bulk of the dead layers of **stratum corneum**.

**2. Type II IF proteins:** They include the following four types of polypeptides: vimentin, desmin, synemin and glial fibrillary acidic protein (or glial filaments). **Vimentin** is widely distributed in cells of mesenchymal origin, including fibroblasts, blood vessel endothelial cells and white blood cells, **Desmin** is found in both striated (skeletal and cardiac) and smooth muscle cells. **Glial filaments** occur in some type of glial cells such as astrocytes and some Schwann cells, in the nervous system. **Synemin** is a protein of 230,000 daltons, which is also present in the intermediate filaments of muscle, together with desmin and vimentin. Vimentin and synemin containing IFs can be observed in the chicken erythrocytes.

Each of thse IF proteins tends to assemble spontaneously *in vitro* to form homopolymers and will also co-assemble with the other Types II IF proteins to form **co-polymers** and **heteropolymers**. In fact, co-polymers of vimentin and desmin, or of vimentin and glial fibrillary acidic protein, are found in some type of cells. For example, desmin remains concentrated in the Z-lines and T-tubule system of striated or skeletal system, together with vimentin, synemin and $\alpha$- actinin. Since desmin links actin to plasma memberane, from this fact the name of desmin has been derived by **Lazarides** and coworkers in 1976 (in Greek desmin means link or bond).

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***Table 3.2*** *Characteristics of Four Types of Intermediate Filament Proteins (Source : Alberts et al., 1989).*

| Types of intermediate filaments | Component polypeptide (mass in daltons) | Cellular location |
|---|---|---|
| 1. Type I | Acidic keratins (40,000—70,000) Neutral or basic keratins (40,000—70,000) | Epithelial cells and epidermal derivatives such as hair and nail |
| 2. Type II | Vimentin (53,000) Desmin (52,000) Glial fibrillar acidic protein (glial filaments; 45,000) Synemin (230,000) | Many cells of mesenchymal origin Muscle cells Glial cells (astrocytes and some Schwann cells) Muscle cells |
| 3. Type III | Neurofilament proteins (about 130,000, 100,000 and 60,000) | Neurons |
| 4. Type IV | Nuclear lamins A, B and C (65,000—75,000) | Nuclear lamina of all cells |

**3. Type III IF Proteins:** These IF proteins assemble into **neurofilaments**, a major cytoskeletal element in nerve axons and dendrites, and consequently are called **neurofilament** proteins. In vertebrates, Type III IFs consist of three distinct polypeptides, the so-called neurofilament triplet.

**4. Type IVIF Proteins:** They are the **nuclear lamins** which form highly organized two dimensional sheets of filaments. These filaments rapidly disassemble and reassemble at specific stage of mitosis.

## General Structure of IFs

Despite the large differences in their size, all cytoplasmic IF proteins are encoded by members of the same multigene family. Their amino acid sequences indicate that each IF polypeptide chain contains a homologous central region of about 310 amino acid residues that forms an extended α -helix with three- short— α- helical interruptions (Fig. 3.34).

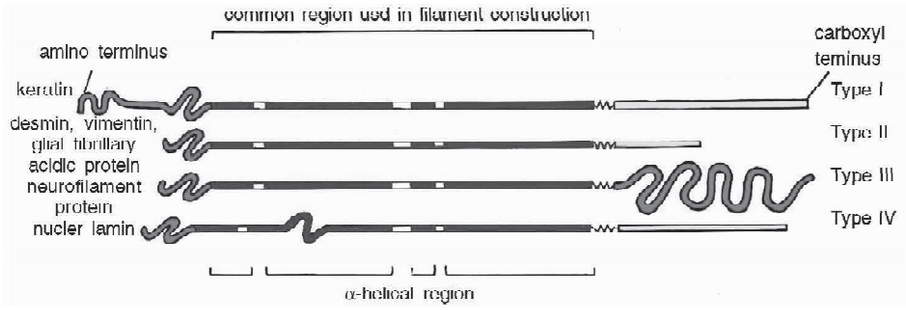


***Fig. 3.34*** *All IF proteins share a similar central region (about 310 amino acid residues) that forms an extended helix with three short interruptions.*

### Assembly of IFs

A current model of assembly of a intermediate filament includes the following steps : 1. Two identical **monomers** pair to form a **dimer** in which the conserved helical central regions are aligned in parallel and are wound together into a coiled coil. 2. Two dimers then line up side-by-side to form a 48 nm by 3 nm **protofilament** containing four polypeptide chains. 3.These protofilaments then associate in a staggered manner to form successively larger structures. 4. The final 10 nm diameter of the intermediate filament is thought to be composed of 8-protofilaments (*i.e.*, 32 polypeptide chains) joined end on end to neighbours by staggered overlap to form the long rope-like filaments. It is still not known whether IFs are polar structures (like actin and tubulin) or non-polar (like the DNA double helix).

## 3.3.2 Microtubules and Mitosis

Mitosis of cultured epithelial cells shows striking changes in intermediate filaments of cytokeratin and vimentin. During prophase the 10 nm filaments unwind into threads of 2 to 4 nm and into spheroidal aggregates containing both types of proteins. At metaphase and anaphase most vimentin and cytokeratin appear as spheroid bodies, while at telophase the filamentous cytoskeleton become gradually reestablished. From these experimental studies, **Franke** (1982) has concluded that the living cells contain factors that promote the reversible disintegration and restoration of intermediate filaments during mitosis.

### Functions of IFs

The main function of most intermediate filaments is to provide mechanical support to the cell and its nucleus. IFs in epithelia form a transcellular network that seems designed to resist external forces. The neurofilaments in the nerve cell axons probably resist stresses caused by the motion of the animal, which would otherwise break these long, thin cylinders of cytoplasm. Desmin filaments provide mechanical support for the sarcomeres in muscle cells, and vimentin filaments surround and probably support the large fat droplets in the fat cells.

### Comparison of Microtubules, Intermediate Filaments and Microfilaments

The three components of the cytoskeleton, namely microtubules, intermediate filaments and microfilaments have been compared in Table 3.3.

***Table 3.3*** *Comparison of Some Properties of Microtubules, Intermediate Filaments and Microfilaments (Source : Thorpe, 1984).*

| Property | Microtubules | Intermediate filaments | Microfilaments |
|---|---|---|---|
| 1. Structure | Hollow with walls made up of 13 protofilaments | Hollow with walls made up of 4 to 5 protofilaments | Solid made up of polymerized actin (F-actin) |
| 2. Diameter (nm) | 24 — 25 | 10 | 7 — 9 |
| 3. Monomer units | α- and β- tubulin | Five types of protein defining five major classes | G-actin |
| 4. ATPase activity | Present in dynein arms | None | None |
| 5. Functions | 1. Motility of eukaryotes | 1. Integrate contractile units in muscle | 1. Muscle contraction |
| | 2. Chromosome movement | 2. Cytoskeletal structural function in cytoplasm | 2. Cell shape changes |

| | | |
|---|---|---|
| | 3. Movements of intracellular materials | 3. Protoplasmic streaming |
| | 4. Contribute toward maintaining cell shape | 4. Cytokinesis |

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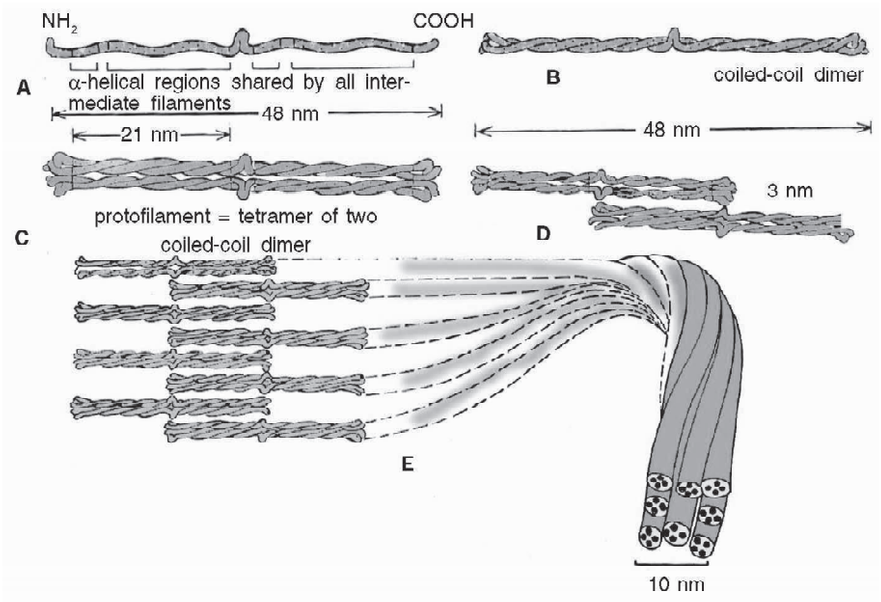


***Fig. 3.35*** *A current model to explain the mode of assembly of a 10-nm thick intermediate filament (after Alberts* et al.*, 1989)*

## 3.3.3 Cell Movements - Intercellular Transport, Role of Kinesin and Dynein

Cells need to be able to transport a variety of molecules, and even entire organelles, around a cell. Because cells are so tiny, many cellular processes use simple random diffusion to get materials from one place to another. For small molecules and proteins, random diffusion is fast enough to get the job done, but for some larger molecules, cells have to take a more active approach. This is where molecular motors come in. Cells drag large cellular objects to their proper destinations by using molecular motors, which produce the force required for transport through the hydrolysis of ATP. Motor proteins play an important role in muscle contraction, cell migration, chromosome segregation, beating of sperms and cilia, transport of intracellular cargoes, etc.

Common properties of motor proteins are:

- They move along the filaments.
- They can bind to specific filament types.
- They hydrolyze ATP.

### Types of Motor Proteins

There are three major types of molecular motor proteins falling under two categories:

- **Actin Based Motor Protein:** Myosin is an actin-based motor protein. It uses the energy of ATP to move along actin filaments.
- **Tubulin Based Motor Protein:** Tubulin is the building block of microtubules. **Kinesin** and **dynein** are tubulin-based motor proteins. They move along the microtubule. Microtubules are polarised (anchored at the minus end, polymerised at the plus end) and microtubular motors are classified as plus-end-directed or minus-end-directed according to the direction of their movement along the microtubule. Kinesin motor moves towards plus (+) end of microtubule, i.e., away from centrosome whereas dynein moves towards minus (–) end of the microtubules, i.e., towards centrosome (Refer Figure 3.36).

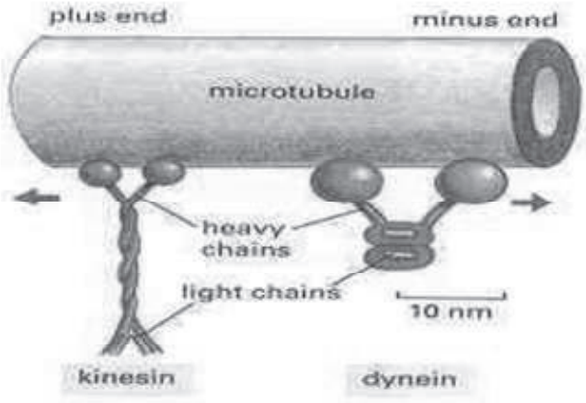


***Fig. 3.36*** *Orientation of Motor Proteins*

In this section, we will mainly take a look at the role of tubulin-based motor proteins, i.e., Kinesins and Dyneins in intra-cellular transport. Transport can be thought of in two forms: Anterograde Transport and Retrograde Transport. Anterograde transport, also known as plus-ended, refers to the transport of cargo from the centre of the cell to the periphery. Meanwhile, retrograde transport, also known as minus-ended, refers to the transport of cargo from the periphery to the centre of the cell. Kinesin motor proteins are mostly associated with anterograde transport. Meanwhile, dynein is more likely to perform retrograde transport, although dynein motor proteins are capable of bidirectional stepping.

### Kinesin

Kinesin is a dimer of two heavy chains, each complexed to a light chain, with a total molecular weight of 380,000. The molecule is organized into three domains, a pair of large globular head domains connected by a long central stalk to a pair of small globular tail domains, which contain the light chains (Refer Figure 3.37). Each domain carries out a particular function: the head domain, which binds microtubules and ATP, is responsible for the motor activity of kinesin, and the tail domain is responsible for binding to membrane vesicles. In light of the transport function of kinesin, a bound membrane vesicle is often referred to as Kinesin's 'Cargo'.

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Kinesins belong to a family of related motor proteins, with more than 12 different family members identified till date. All of them contain the kinesin motor domain, but they differ in their tail domains and several other properties.

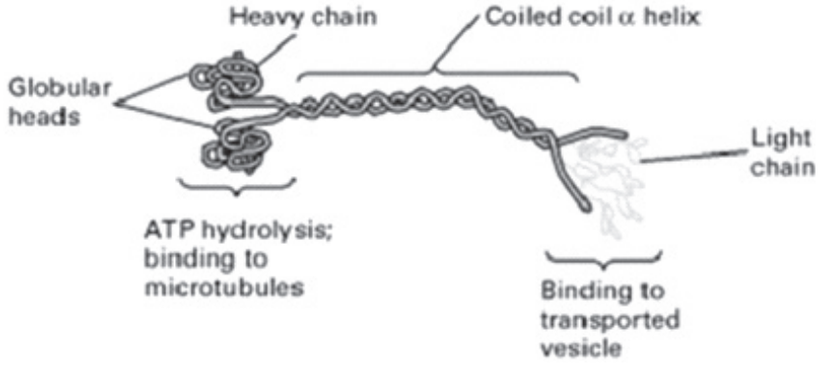


***Fig. 3.37*** *Structure of Kinesin*

In most kinesins, the motor domain is at the N-terminus (N-type), but in some, the motor domain is central (M-type) or at the C-terminus (C-type). Both N and M-type kinesins are (+) end directed motors, whereas C-type kinesins are (–) end directed motors. In addition, some kinesins are monomeric (i.e., have a single heavy chain); however, most are dimeric.

Kinesins can be divided into two wide functional groups based on the nature of the cargo they transport.

**Cytosolic Kinesins**: The functional differences between kinesins may be related to their unique tail domains. Cytosolic kinesins are involved in vesicle and organelle transport. They include the classic axonal kinesin, involved in the transport of lysosomes and other membranous organelles.

**Spindle Kinesins**: They play a role in spindle assembly and chromosome segregation during cell division. These are also known as Kinesin Related Proteins, or KRP motors. This group comprises numerous proteins, including the kinetochore-associated protein CENP-E; the spindle pole protein BimC; and a (–) end directed motor protein.

**Dynein**

Dyneins are multimeric proteins, with molecular weights exceeding 1,000,000. They are composed of two or three heavy chains (MW 470,000 – 540,000) complexed with a poorly determined number of intermediate and light chains. Dyneins are divided into two functional classes:

- **Cytosolic Dynein:** They are found in all eukaryotic cells. Their functional purpose is to traffic cargo along vesicles and to localize the Golgi apparatus to the centre of the cell. Cytosolic dynein is a two-headed molecule, with two identical or nearly identical heavy chains forming the head domains.
- **Axonemal Dynein:** They are specialized to create the sliding movements of microtubules that power the beating of cilia and flagella. Therefore, axonemal dyneins are critical to the motility of single celled organisms.

Unlike kinesin, dynein cannot mediate transport by itself. Rather, dynein-related motility requires a large complex of microtubule-binding proteins that link vesicles and chromosomes to microtubules but by themselves do not exert force to cause movement.

**Functions of Kinesin and Dynein**

Some of the major functions of Kinesin and Nyncin are as follows:

**Organelle Transport:** One of the major roles of microtubules is to transport membrane vesicles and organelles through the cytoplasm of eukaryotic cells. Kinesins and dyneins are usually oriented with their minus end anchored in the centrosome and their plus end extending toward the cell periphery, therefore they transport vesicles and organelles in opposite directions through the cytoplasm. So, conventionally the kinesin family carries out the transport of organelles toward the cell periphery, whereas cytoplasmic dyneins and minus end directed members of the kinesin family transport materials toward the center of the cell. In addition to transporting membrane vesicles in the endocytic and secretory pathways, these proteins are involved in positioning membrane-enclosed organelles (such as the endoplasmic reticulum, golgi apparatus, lysosomes, and mitochondria) within the cell. For example, the Endoplasmic Reticulum extends to the periphery of the cell in association with microtubules. This positioning of the Endoplasmic Reticulum appears to involve the action of kinesin, which pulls the ER along microtubules in the plus end direction, toward the cell periphery. Similarly, kinesin also appears to play a key role in the positioning of lysosomes away from the center of the cell, and three different members of the kinesin family have been implicated in the movements of mitochondria.

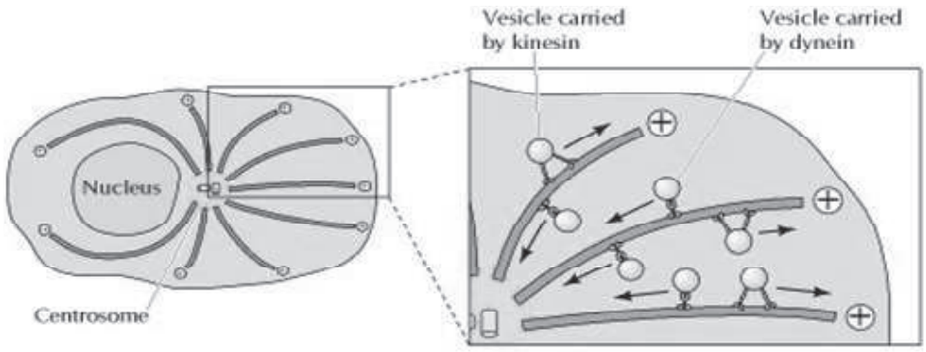


***Fig. 3.38*** *Transport of Vesicles by Kinesin and Dyenin*

On the other hand, cytoplasmic dynein plays a role in positioning the Golgi apparatus. The Golgi apparatus is located in the center of the cell, near the centrosome. It transports Golgi vesicles to the center of the cell, i.e., towards the minus end of microtubules. Movement along microtubules is thus responsible not only for vesicle transport, but also for establishing the positions of membrane-enclosed organelles within the cytoplasm of eukaryotic cells.

**Separation of Mitotic Chromosomes:** Microtubules reorganize at the beginning of mitosis to form the Mitotic Spindle, which plays a central role in cell division by distributing the duplicated chromosomes to daughter nuclei. This critical

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distribution of the genetic material takes place during anaphase of mitosis, when sister chromatids separate and move to opposite poles of the spindle. Chromosome movement proceeds by two distinct mechanisms, referred to as anaphase A and anaphase B, which involve different types of spindle microtubules and associated motor proteins. Anaphase A consists of the movement of chromosomes toward the spindle poles along the kinetochore microtubules, which shorten as chromosome movement proceeds. Cytoplasmic dynein and minus end directed members of the kinesin family play a role in poleward chromosome movement. Anaphase B refers to the separation of the spindle poles and elongation of the polar microtubules. During anaphase B the overlapping polar microtubules slide against one another, pushing the spindle poles apart. This type of movement has been found to result from the action of several plus end directed members of the kinesin family. In addition, the spindle poles are pulled apart by the probable action of cytoplasmic dynein anchored to the cell cortex or another structure in the cytoplasm.

**Difference between the Roles of Kinesin and Dynein**: They have many attributes in common, both being motor proteins dependent on microtubules and ATP to catalyse a cyclic stepping motion to transport cargoes. However, there are unique qualities of each family of motor protein. Dynein has a larger step size than that of kinesin, making dynein a faster motor than kinesin. Although dynein is larger and faster, kinesin is capable of transporting molecules in bulk. Also, kinesin comprises of a more extensive and diverse group of motor proteins, while dynein is relatively limited with respect to number of species in the family and the number of tasks the type of motor protein completes.

### 3.3.4 Signal Transduction Mechanisms

Signalling information has to be transmitted from the receptor in the plasma membrane across the cytoplasm to the nucleus (if gene transcription is the response), the cytoskeleton (if cell movement, or another change to cell morphology, is the response), or various other subcellular compartments. The transmission of a signal must occur in a time-frame appropriate for the cellular response. So, signal transduction needs to take place over both space and time. We have already described a simple signalling model, where a chain of intracellular mediators successively activates the next in the chain until the target is reached. In reality, of course, it is rarely a simple chain, but a branching network, allowing for integration, diversification and modulation of responses. The branched molecular network of activation (and deactivation) of signalling molecules linking receptor activation to the intracellular targets is referred to as a **signal transduction pathway** (or cascade).

Intracellular signalling molecules have particular properties that allow control of the speed, duration and target of the signal, and may be categorized according to these properties. Broadly speaking, intracellular signalling molecules can be divided into two groups on the basis of molecular characteristics, *second messengers* and *signalling proteins*.

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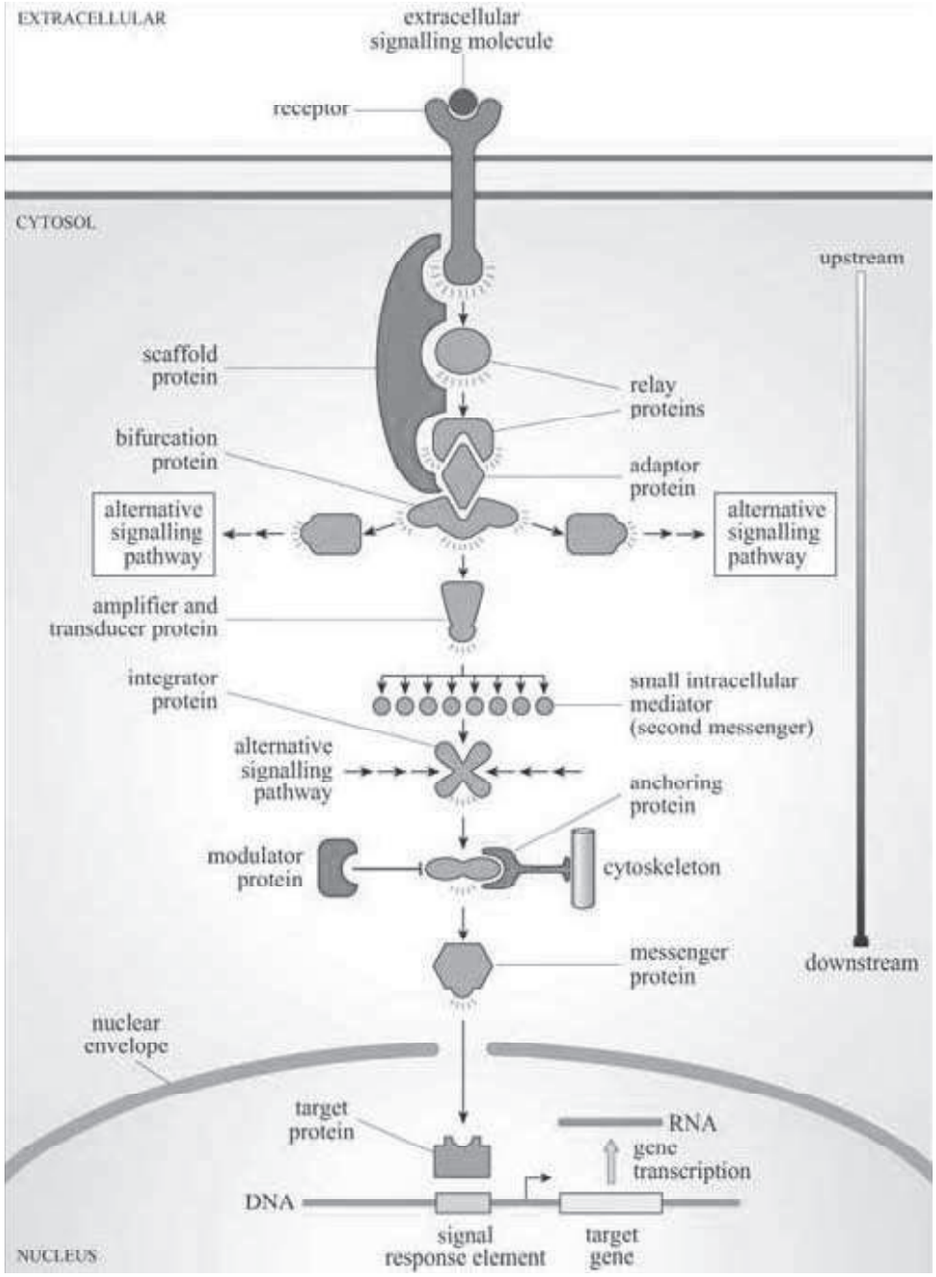


***Fig. 3.39*** *Signal Transduction Pathways*

Figure 3.39 Signal transduction pathways are not simple chains, but highly complex, branching pathways, involving many different types of signalling proteins (including scaffold proteins, relay proteins, bifurcation proteins, adaptor proteins, amplifier and transducer proteins, integrator proteins, modulator proteins, messenger proteins and target proteins) and small intracellular mediators known as second messengers. Interaction involves signalling proteins and second messengers, leading to cellular responses, in this case expression of a target gene and/or changes in the cytoskeleton (via the anchoring protein). A typical signalling pathway will involve many of these components.

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Second messengers are small readily diffusible intracellular mediators, whose concentration inside the cell changes rapidly on receptor activation; in this manner, they regulate the activity of other target signalling molecules. The calcium ion, $Ca^{2+}$, is a classic example of a second messenger, being released in large quantities in response to a signal (so amplifying the signal) and diffusing rapidly through the cytosol. $Ca^{2+}$ ions can therefore broadcast the signal quickly to several distant parts of the cell. For example, $Ca^{2+}$ ions mediate and coordinate contraction of skeletal muscle cells. In general, if a rapid, generalized response is necessary, a second messenger is likely be prominent in the signalling pathway.

Other water-soluble second messengers such as cAMP and cGMP act similarly to $Ca^{2+}$, by diffusing through the cytosol, whereas second messengers such as diacylglycerol (DAG) are lipid-soluble, and diffuse along the inside of the plasma membrane, in which are anchored various other key signalling proteins.

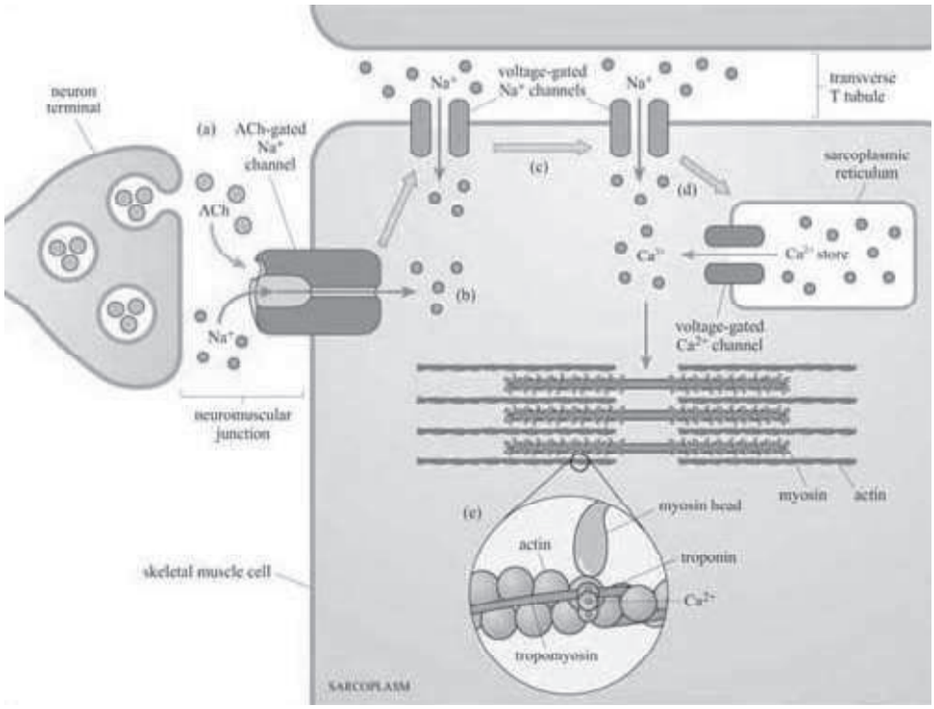


***Fig. 3.40*** *Calcium Ions help to Synchronize the Rapid Contraction of Skeletal Muscle Cells*

Calcium ions help to synchronize the rapid contraction of skeletal muscle cells. (a) Acetylcholine (ACh, shown in pink) is released from the neuron terminal, and binds to ACh-gated $Na^+$ channels on the surface of the muscle cell. (b) These receptors are ion channels, and so promote local depolarization (an increase in membrane potential caused by the entry of sodium ions). (c) Depolarization is propagated in the muscle cell (yellow arrows) by voltage-gated $Na^+$ channels, which allows further $Na^+$ ion entry. (d) This more general depolarization triggers the very rapid release of $Ca^{2+}$ ions into the sarcoplasm (muscle cytoplasm) through voltage-gated $Ca^{2+}$ channels from stores in the sarcoplasmic reticulum; the $Ca^{2+}$ ions

spread through the muscle cell. (e) The increase of $Ca^{2+}$ concentration throughout the sarcoplasm enables the rapid and synchronous contraction of the muscle filaments. $Ca^{2+}$ achieves this by binding to an inhibitory protein complex of tropomyosin and troponin, which under resting conditions prevents actin and myosin filaments from interacting.

Second messengers were the first intracellular signalling molecules to be identified; they were so named because hormones or other extracellular signalling molecules were considered the 'first messengers'. However, the term 'second messenger' seems somewhat outdated, since a signalling pathway can easily involve a sequence of eight or more different messengers, and the 'second messenger' in question could well actually be acting as, say, the fifth messenger.

**Signalling proteins** are the large intracellular signalling molecules that generally, but not exclusively, function by activating the next signalling protein in the signal transduction cascade, or by modifying the concentration of second messengers.

Proteins are much larger and generally less mobile than small water-soluble second messengers, so they are not so useful for the rapid dissemination and amplification of a signal. However, proteins are capable of interacting in a highly specific manner with other proteins, they exhibit binding specificity for ligands and for recognition motifs on other molecules, and their activity can be regulated, for example by allosteric regulation and by phosphorylation. They are therefore able to perform rather more sophisticated signalling roles than water-soluble second messengers.

Attempts have been made to group intracellular signalling proteins according to their function, but you will soon see that there are plenty that have more than one function, making classification into functional groupings difficult. Nevertheless, these descriptions give a flavour of the variety of possible signalling functions. Later in this chapter we shall discuss many examples from these groups.

- **Relay proteins** simply pass the signal on to the next member of the chain.
- **Messenger proteins** carry the signal from one part of the cell to another. For example, activation may cause translocation of the protein from the cytosol to the nucleus.
- **Amplifier proteins** are capable of either activating many downstream signalling proteins or generating large numbers of second messenger molecules; they tend to be enzymes such as adenylyl cyclase, which synthesizes cAMP, or ion channels such as $Ca^{2+}$ channels, which open to release $Ca^{2+}$ ions from intracellular stores.
- **Transducer proteins** change the signal into a different form. Voltage-gated $Ca^{2+}$ channels are examples of signalling proteins, which fall into two of these functional categories, since in addition to their role as an amplifier protein, they detect a change in membrane potential, and transduce it into an increase in the concentration of a second messenger.

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- **Bifurcation proteins** branch the signal to different signalling pathways.
- **Integrator proteins** receive two or more signals from different pathways, and integrate their input into a common signalling pathway.
- **Modulator proteins** regulate the activity of a signalling protein.

Other proteins are involved purely in the correct placement of some signalling molecules:

- **Anchoring proteins** tether members of the signalling pathway in particular subcellular locations, such as the plasma membrane or the cytoskeleton, thereby ensuring that the signal is being relayed to the right place.
- **Adaptor proteins** link one signalling protein with the next at the correct time, without signalling themselves.
- **Scaffold proteins** are proteins that bind several signalling proteins, and may also tether them, forming a much more efficient functional complex. Scaffold proteins may therefore share attributes of both anchoring and adaptor proteins.

**Check Your Progress**

7. Define cytoskeleton.
8. What are the common properties of motor proteins?
9. What is signal transduction pathway?
10. Define second messengers.
11. What is a signaling protein?

## 3.4 CELL - CELL SIGNALLING

Cell signalling can result either from the direct interaction of a cell with its neighbour or from the action of secreted signalling molecules. Signalling by direct cell-cell (or cell-matrix) interactions plays a critical role in regulating the behaviour of cells in animal tissues. Cells express a variety of cell surface receptors that interact with signalling molecules on the surface of neighbouring cells. Signalling via such direct cell-cell interactions plays a critical role in regulating the many interactions between different types of cells that take place during embryonic development, as well as in the maintenance of adult tissues.

Cell signalling is part of a complex system of communication that governs basic cellular activities and coordinates cell actions. The ability of cells to perceive and correctly respond to their microenvironment is the basis of development, tissue repair, and immunity as well as normal tissue homeostasis. Errors in cellular information processing are responsible for diseases, such as cancer, autoimmunity, and diabetes. By understanding cell signalling, diseases may be treated effectively and, theoretically, artificial tissues may be yielded.

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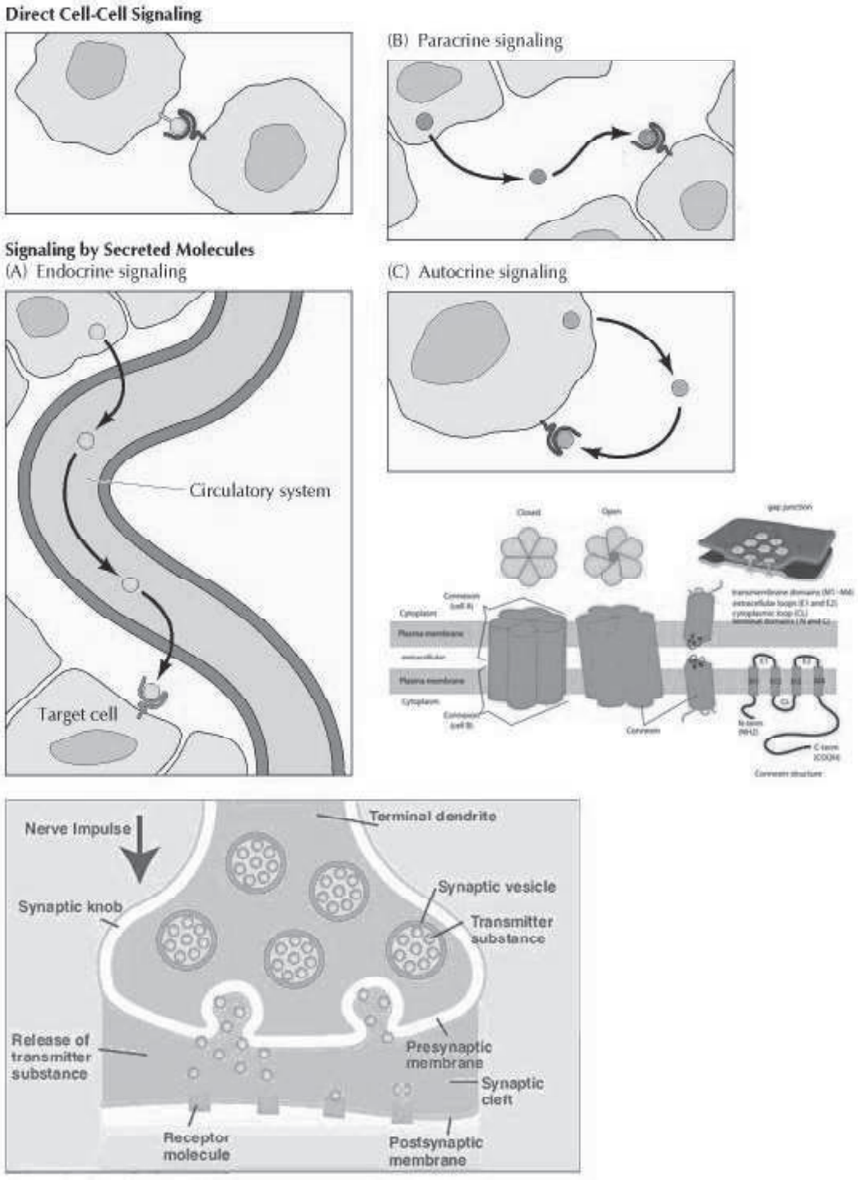


***Fig. 3.41*** *Cell – Cell Signalling*

## Different Types of Signalling

A cell can communicate signals to other cells in the following various ways.

Direct signalling is a transfer of ions or small molecules from one cell to its neighbour through pores in the membrane. Those pores are built out of membrane proteins and are called gap junctions. This is the fastest mode of cell-cell communication and is found in places where extremely fast and well-coordinated activity of cells in needed. An example of this process can be found in the heart. The muscle cells in the heart communicate with each other via gap junctions which allow all heart cells to contract almost simultaneously.

Endocrine signalling utilizes hormones. A cell secretes chemicals into the bloodstream. Those chemicals affect the behaviour of distant target cells.

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Paracrine signalling is a way for a cell to affect the behaviour of neighbouring cells by secreting chemicals into the common intercellular space. This is an important process during embryonic development.

Autocrine signalling is a way for a cell to alter its own extracellular environment, which in turn affects the way the cell functions. The cell secretes chemicals outside of its membrane and the presence of those chemicals on the outside modifies the behaviour of that same cell. This process is important for growth.

The juxtacrine signalling also known as contact dependent signalling in which two adjacent cells must make physical contact in order to communicate. This requirement for direct contact allows for very precise control of cell differentiation during embryonic development. Notch signalling is an example for juxtacrine signalling.

Synaptic signalling is found in the nervous system. It is a highly specific and localized type of paracrine signalling between two nerve cells or between a nerve cell and a muscle cell. We will go into details of synaptic signalling when we cover the human nervous system.

Except autocrine signalling molecules others actively participate in the intercellular signalling process. They are also otherwise called as extracellular signalling because signalling molecule originated from extracellular region.

### 3.4.1 Cell Surface Receptors

Cell surface receptors (membrane receptors, transmembrane receptors) are receptors that are embedded in the plasma membrane of cells. They act in cell signalling by receiving (binding to) extracellular molecules. They are specialized integral membrane proteins that allow communication between the cell and the extracellular space. The extracellular molecules may be hormones, neurotransmitters, cytokines, growth factors, cell adhesion molecules, or nutrients; they react with the receptor to induce changes in the metabolism and activity of a cell. In the process of signal transduction, ligand binding affects a cascading chemical change through the cell membrane.

#### Structure and Mechanism

Many membrane receptors are transmembrane proteins. There are various kinds, including glycoproteins and lipoproteins. Hundreds of different receptors are known and many more have yet to be studied. Transmembrane receptors are typically classified based on their tertiary (three-dimensional) structure. If the three-dimensional structure is unknown, they can be classified based on membrane topology. In the simplest receptors, polypeptide chains cross the lipid bilayer once, while others, such as the G-protein coupled receptors, cross as many as seven times. Each cell membrane can have several kinds of membrane receptors, with varying surface distributions. A single receptor may also be differently distributed at different membrane positions, depending on the sort of membrane and cellular function. Receptors are often clustered on the membrane surface, rather than evenly distributed.

#### Mechanism

Two models have been proposed to explain transmembrane receptors' mechanism of action.

**Dimerization:** The dimerization model suggests that prior to ligand binding, receptors exist in a monomeric form. When agonist binding occurs, the monomers combine to form an active dimer.

**Rotation:** Ligand binding to the extracellular part of the receptor induces a rotation (conformational change) of part of the receptor's transmembrane helices. The rotation alters which parts of the receptor are exposed on the intracellular side of the membrane, altering how the receptor can interact with other proteins within the cell.

Receptors for cell signalling mainly are of two types namely cell surface receptors and intracellular or internal receptors. Those signalling molecules which are capable of diffusing into cytosol of the cell can interact with internal receptors and execute signalling process. Steroid molecules and nitric oxide are examples of signalling molecules which can bind to internal receptors. They participate in intracellular signalling process.

Signalling molecules like proteins which are unable to enter into cells can interact with the cell surface receptors and execute its signalling process. Cell surface receptors are transmembrane proteins whose extracellular portion has the binding site for the signalling molecule and intracellular portion activates proteins in the cytosol that in different ways eventually regulate gene transcription in the nucleus.

### 3.4.2 Second Messenger System

Second messengers are intracellular signalling molecules released by the cell in response to exposure to extracellular signalling molecules—the first messengers. Intracellular signals, a non-local form or cell signalling, encompassing both first messengers and second messengers, are classified as juxtacrine, paracrine, and endocrine depending on the range of the signal. Second messengers trigger physiological changes at cellular level, such as proliferation, differentiation, migration, survival, apoptosis and depolarization. They are one of the triggers of intracellular signal transduction cascades.

Examples of second messenger molecules include cyclic AMP, cyclic GMP, inositol triphosphate, diacylglycerol, and calcium. First messengers are extracellular factors, often hormones or neurotransmitters, such as epinephrine, growth hormone, and serotonin. Because peptide hormones and neurotransmitters typically are biochemically hydrophilic molecules, these first messengers may not physically cross the phospholipid bilayer to initiate changes within the cell directly—unlike steroid hormones. This functional limitation requires the cell to have signal transduction mechanisms to transduce first messenger into second messengers, so that the extracellular signal may be propagated intracellularly. An important feature of the second messenger signalling system is that second messengers may be coupled downstream to multi-cyclic kinase cascades to greatly amplify the strength of the original first messenger signal. For example, RasGTP signals link with the Mitogen Activated Protein Kinase (MAPK) cascade to amplify the allosteric activation of proliferative transcription factors, such as Myc and CREB.

Earl Wilbur Sutherland Jr. discovered second messengers, for which he won the 1971 Nobel Prize in Physiology or Medicine. Sutherland saw that epinephrine would stimulate the liver to convert glycogen to glucose (sugar) in

liver cells, but epinephrine alone would not convert glycogen to glucose. He found that epinephrine had to trigger a second messenger, cyclic AMP, for the liver to convert glycogen to glucose.

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Secondary messenger systems can be synthesized and activated by enzymes, for example, the cyclases that synthesize cyclic nucleotides, or by opening of ion channels to allow influx of metal ions, for example $Ca^{2+}$ signalling. These small molecules bind and activate protein kinases, ion channels, and other proteins, thus continuing the signalling cascade.

### Types of Second Messenger Molecules

There are following three basic types of secondary messenger molecules:

- **Hydrophobic Molecules:** Water-insoluble molecules, such as diacylglycerol, and phosphatidylinositols, which are membrane-associated and diffuse from the plasma membrane into the intermembrane space where they can reach and regulate membrane-associated **effector proteins.**
- **Hydrophilic Molecules:** Water-soluble molecules, such as cAMP, cGMP, $IP_3$, and $Ca^{2+}$, that are located within the cytosol.
- **Gases**: Nitric Oxide (NO), Carbon Monoxide (CO) and Hydrogen Sulfide ($H_2S$) which can diffuse both through cytosol and across cellular membranes.

These intracellular messengers have some properties in common:

- They can be synthesized/released and broken down again in specific reactions by enzymes or ion channels.
- Some (such as, $Ca^{2+}$) can be stored in special organelles and quickly released when needed.
- Their production/release and destruction can be **localized**, enabling the cell to limit space and time of signal activity.

### Common Mechanisms of Second Messenger Systems

There are several different secondary messenger systems (cAMP system, phosphoinositol system, and arachidonic acid system), but they all are quite similar in overall mechanism, although the substances involved and overall effects can vary.

| | cAMP System | Phosphoinositol system | Arachidonic acid system | cGMP System | - |
|---|---|---|---|---|---|
| *Neurotransmitters (Receptor)* | Norepinephrine (α2, β1, β2) Acetylcholine (M2) | Norepinephrine (α1) Acetylcholine (M1, M3) | Histamine (Histamine receptor) | - | - |
| *Hormones* | ACTH, ANP, CRH, CT, FSH, Glucagon, hCG, LH, MSH, PTH, TSH | AGT, GnRH, GHRH, Oxytocin, TRH | - | ANP | INS, IGF |
| *Transducer* | $G_s$ (β1, β2), $G_i$ (α2, M2) | $G_q$ | Unknown G-protein | - | - |
| *Primary effector* | Adenylyl cyclase | Phospholipase C | Phospholipase A | guanylate cyclase | receptor tyrosine kinase |
| *Secondary messenger* | cAMP (cyclic adenosine monophosphate) | $IP_3$ (inositol 1,4,5 triphosphate) and DAG (Diacylglycerol), both from PIP2 | Arachidonic acid | cGMP | - |
| *Secondary effector* | protein kinase A | Ca++ release (see calcium-binding protein) and PKC (protein kinase C) | 5-Lipoxygenase, 12-Lipoxygenase, cycloxygenase | protein kinase G | - |

***Fig. 3.42*** *General Schematic of Second Messenger Mechanism*

In most cases, a ligand binds to a membrane-spanning receptor protein molecule. The binding of a ligand to the receptor causes a conformation change in the receptor. This conformation change can affect the activity of the receptor and result in the production of active second messengers.

In the case of G protein-coupled receptors, the conformation change exposes a binding site for a **G-protein**. The G-protein (named for the GDP and GTP molecules that bind to it) is bound to the inner membrane of the cell and consists of three subunits: alpha, beta and gamma. The G-protein is known as the 'Transducer'.

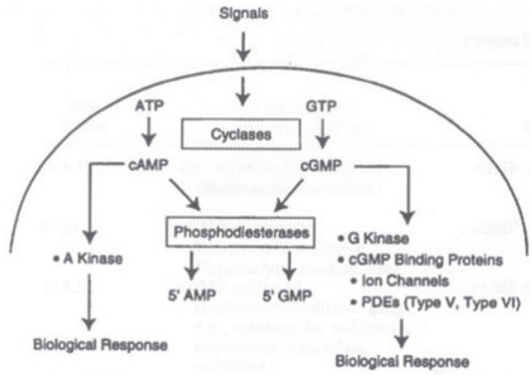


*Fig. 3.43*

When the G-protein binds with the receptor, it becomes able to exchange a GDP (guanosine diphosphate) molecule on its alpha subunit for a GTP (guanosine triphosphate) molecule. Once this exchange takes place, the alpha subunit of the G-protein transducer breaks free from the beta and gamma subunits, all parts remaining membrane-bound. The alpha subunit, now free to move along the inner membrane, eventually contacts another membrane-bound protein - the 'Primary Effector'.

The primary effector then has an action, which creates a signal that can diffuse within the cell. This signal is called the **second (or secondary) messenger**. The secondary messenger may then activate a **secondary effector** whose effects depend on the particular secondary messenger system.

Calcium ions are one type of second messengers and are responsible for many important physiological functions including muscle contraction, fertilization, and neurotransmitter release. The ions are normally bound or stored in intracellular components (such as, the Endoplasmic Reticulum (ER)) and can be released during signal transduction. The enzyme phospholipase C produces diacylglycerol and inositol trisphosphate, which increases calcium ion permeability into the membrane. Active G-protein open up calcium channels to let calcium ions enter the plasma membrane. The other product of phospholipase C, diacylglycerol, activates protein kinase C, which assists in the activation of cAMP (another second messenger).

### 3.4.3 MAP Kinase Pathways

Mitogen-Activated Protein (MAPK or MAP kinase) is a type of protein kinase that is specific to the amino acids serine and threonine (i.e., a serine/threonine-specific protein kinase). MAPKs are involved in directing cellular responses to a diverse array of stimuli, such as mitogens, osmotic stress, heat shock and

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proinflammatory cytokines. They regulate cell functions including proliferation, gene expression, differentiation, mitosis, cell survival, and apoptosis.

MAP kinases are found in eukaryotes only, but they are fairly diverse and encountered in all animals, fungi and plants, and even in an array of unicellular eukaryotes. MAPKs belong to the CMGC (CDK/MAPK/GSK3/CLK) kinase group. The closest relatives of MAPKs are the cyclin-dependent kinases (CDKs). Most MAPKs have a number of shared characteristics, such as the activation dependent on two phosphorylation events, a three-tiered pathway architecture and similar substrate recognition sites. These are the classical MAP kinases. But there are also some ancient outliers from the group as sketched above, that do not have dual phosphorylation sites, only form two-tiered pathways, and lack the features required by other MAPKs for substrate binding. These are usually referred to as 'Atypical' MAPKs. It is yet unclear if the atypical MAPKs form a single group as opposed to the classical ones.

The mammalian MAPK family of kinases includes three subfamilies:

(i) Extracellular Signal-Regulated Kinases (ERKs)

(ii) c-Jun N-terminal Kinases (JNKs)

(iii) p38 Mitogen-Activated Protein Kinases

Generally, ERKs are activated by growth factors and mitogens, whereas cellular stresses and inflammatory cytokines activate JNKs and p38s.

**Activation**

Mitogen-activated protein kinases are catalytically inactive in their base form. In order to become active, they require (potentially multiple) phosphorylation events in their activation loops. This is conducted by specialized enzymes of the STE protein kinase group. In this way protein dynamics can induce a conformational change in the structure of the protein via long-range allostery.

In the case of classical MAP kinases, the activation loop contains a characteristic TxY (threonine-x-tyrosine) motif (TEY in mammalian ERK1 and ERK2, TDY in ERK5, TPY in JNKs, TGY in p38 kinases) that needs to be phosphorylated on both the threonine and the tyrosine residues in order to lock the kinase domain in a catalytically competent conformation. In vivo and in vitro, phosphorylation of tyrosine oftentimes precedes phosphorylation of threonine, although phosphorylation of either residue can occur in the absence of the other.

Members of the MAPK family can be found in every eukaryotic organism examined so far. In particular, both classical and atypical MAP kinases can be traced back to the root of the radiation of major eukaryotic groups. Terrestrial plants contain four groups of classical MAPKs (MAPK-A, MAPK-B, MAPK-C and MAPK-D) that are involved in response to myriads of abiotic stresses. However, none of these groups can be directly equated to the clusters of classical MAPKs found in opisthokonts (fungi and animals). In the latter, the major subgroups of classical MAPKs form the ERK/Fus3-like branch (that is further sub-divided in metazoans into ERK1/2 and ERK5 subgroups), and the p38/Hog1-like kinases (that has also split into the p38 and the JNK subgroups in multicellular animals). In addition, there are several MAPKs in both fungi and animals, whose origins are

less clear, either due to high divergence (e.g., NLK), or due to possibly being an early offshoot to the entire MAPK family (ERK3, ERK4, ERK7). In vertebrates, due to the twin whole genome duplications after the cephalochordate/vertebrate split, there are several paralogs in every group.

### 3.4.4 Signalling from Plasma Membrane to Nucleus

The cell membrane (also known as the Plasma Membrane (PM) or cytoplasmic membrane, and historically referred to as the plasmalemma) is a biological membrane that separates the interior of all cells from the outside environment (the extracellular space) which protects the cell from its environment. The cell membrane consists of a lipid bilayer, including cholesterols (a lipid component) that sit between phospholipids to maintain their fluidity at various temperatures. The membrane also contains membrane proteins, including integral proteins that go across the membrane serving as membrane transporters, and peripheral proteins that loosely attach to the outer (peripheral) side of the cell membrane, acting as enzymes shaping the cell. The cell membrane controls the movement of substances in and out of cells and organelles. In this way, it is selectively permeable to ions and organic molecules. In addition, cell membranes are involved in a variety of cellular processes, such as cell adhesion, ion conductivity and cell signalling and serve as the attachment surface for several extracellular structures, including the cell wall, the carbohydrate layer called the glycocalyx, and the intracellular network of protein fibers called the cytoskeleton.

In order to respond to changes in their immediate environment, cells must be able to receive and process signals that originate outside their borders. Individual cells often receive many signals simultaneously, and they then integrate the information they receive into a unified action plan. But cells are not just targets. They also send out messages to other cells both near and far.

Most cell signals are chemical in nature. For example, prokaryotic organisms have sensors that detect nutrients and help them navigate toward food sources. In multicellular organisms, growth factors, hormones, neurotransmitters, and extracellular matrix components are some of the many types of chemical signals cells use. These substances can exert their effects locally, or they might travel over long distances. For instance, neurotransmitters are a class of short-range signalling molecules that travel across the tiny spaces between adjacent neurons or between neurons and muscle cells. Other signalling molecules must move much farther to reach their targets. One example is follicle-stimulating hormone, which travels from the mammalian brain to the ovary, where it triggers egg release.

**Check Your Progress**

12. What is direct signaling?
13. Define paracrine signalling.
14. What are cell surface receptors?
15. Write some examples of second messenger molecules.
16. Who discovered second messengers?
17. What is Mitogen-Activated Protein Kinase (MAPK or MAP kinase)?

# 3.5 CELL - CELL ADHESION AND COMMUNICATION

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Cell adhesion is the process by which cells interact and attach to neighbouring cells through specialised molecules of the cell surface. This process can occur either through direct contact between cell surfaces such as cell junctions or indirect interaction, where cells attach to surrounding extracellular matrix, a gel-like structure containing molecules released by cells into spaces between them. Cells adhesion occurs from the interactions between Cell-Adhesion Molecules (CAMs), transmembrane proteins located on the cell surface. Cell adhesion links cells in different ways and can be involved in signal transduction for cells to detect and respond to changes in the surroundings. Other cellular processes regulated by cell adhesion include cell migration and tissue development in multicellular organisms. Alterations in cell adhesion can disrupt important cellular processes and lead to a variety of diseases, including cancer and arthritis. Cell adhesion is also essential for infectious organisms, such as bacteria or viruses, to cause diseases.

### General Mechanism

Cell-Adhesion Molecules (CAMs) are classified into four major families: integrins, immunoglobulin (Ig) superfamily, cadherins, and selectins. **Cadherins** and **IgSF** are homophilic CAMs, as they directly bind to the same type of CAMs on another cell, while **integrins** and **selectins** are heterophilic CAMs that bind to different types of CAMs. Each of these adhesion molecules has a different function and recognizes different ligands. Defects in cell adhesion are usually attributable to defects in expression of CAMs.

In multicellular organisms, bindings between CAMs allow cells to adhere to one another and creates structures called cell junctions. According to their functions, the cell junctions can be classified as:

- Anchoring junctions (adherens junctions, desmosomes and hemidesmosomes), which maintain cells together and strengthens contact between cells
- Occluding junctions (tight junctions), which seal gaps between cells through cell–cell contact, making an impermeable barrier for diffusion
- Channel-forming junctions (gap junctions), which links cytoplasm of adjacent cells allowing transport of molecules to occur between cells
- Signal-relaying junctions, which can be synapses in the nervous system

Alternatively, cell junctions can be categorised into two main types according to what interacts with the cell: cell–cell junctions, mainly mediated by cadherins, and cell–matrix junctions, mainly mediated by integrins.

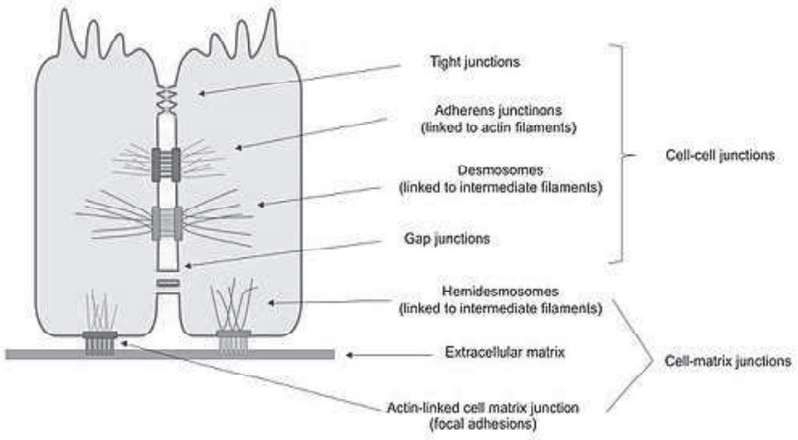


***Fig.3.44*** *Overview Diagram of Different Types of Cell Junctions Present in Epithelial Cells, including Cell–Cell Junctions and Cell–Matrix Junctions*

## Cell–Cell Junctions

Cell–cell junctions can occur in different forms. In anchoring junctions between cells, such as adherens junctions and desmosomes, the main CAMs present are the cadherins. This family of CAMs are membrane proteins that mediate cell–cell adhesion through its extracellular domains and require extracellular $Ca^{2+}$ ions to function correctly. Cadherins forms homophilic attachment between themselves, which results in cells of a similar type sticking together and can lead to selective cell adhesion, allowing vertebrate cells to assemble into organised tissues. Cadherins are essential for cell–cell adhesion and cell signalling in multicellular animals and can be separated into two types: classical cadherins and non-classical cadherins.

## Adherens Junctions

Adherens junctions mainly function to maintain the shape of tissues and to hold cells together. In adherens junctions, cadherins between neighbouring cells interact through their extracellular domains, which share a conserved calcium-sensitive region in their extracellular domains. When this region comes into contact with $Ca^{2+}$ ions, extracellular domains of cadherins undergo a conformational change from the inactive flexible conformation to a more rigid conformation in order to undergo homophilic binding. Intracellular domains of cadherins are also highly conserved, as they bind to proteins called catenins, forming catenin-cadherin complexes. These protein complexes link cadherins to actin filaments. This association with actin filaments is essential for adherens junctions to stabilise cell–cell adhesion. Interactions with actin filaments can also promote clustering of cadherins, which are involved in the assembly of adherens junctions. This is since cadherin clusters promote actin filament polymerisation, which in turn promotes the assembly of adherens junctions by binding to the cadherin–catenin complexes that then form at the junction.

## Desmosomes

Desmosomes are structurally similar to adherens junctions but composed of different components. Instead of classical cadherins, non-classical cadherins, such as, desmogleins and desmocollins act as adhesion molecules and they are linked to intermediate filaments instead of actin filaments. No catenin is present in

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desmosomes as intracellular domains of desmosomal cadherins interact with desmosomal plaque proteins, which form the thick cytoplasmic plaques in desmosomes and link cadherins to intermediate filaments. Desmosomes provides strength and resistance to mechanical stress by unloading forces onto the flexible but resilient intermediate filaments, something that cannot occur with the rigid actin filaments. This makes desmosomes important in tissues that encounter high levels of mechanical stress, such as heart muscle and epithelia, and explains why it appears frequently in these types of tissues.

### 3.5.1 Families of CAMs: $Ca^{++}$ Dependent and $Ca^{++}$ Independent

There are four major superfamilies or groups of CAMs: the immunoglobulin super family of cell adhesion molecules (IgCAMs), Cadherins, Integrins, and the superfamily of C-type of Lectin-Like Domains Proteins (CTLDs). Proteoglycans are also considered to be a class of CAMs.

One classification system involves the distinction between **Calcium-Independent** CAMs and **Calcium-Dependent** CAMs. Integrins and the Ig-superfamily CAMs do not depend on $Ca^{2+}$ while cadherins and selectins depend on $Ca^{2+}$. In addition, integrins participate in cell–matrix interactions, while other CAM families participate in cell–cell interactions.

**1. Calcium Independent - IgSF CAMs**

Immunoglobulin superfamily CAMs (IgSF CAMs) is regarded as the most diverse superfamily of CAMs. This family is characterized by their extracellular domains containing Ig-like domains. The Ig domains are then followed by Fibronectin Type III domain repeats and IgSFs are anchored to the membrane by a GPI moiety. This family is involved in both homophilic or heterophilic binding and has the ability to bind integrins or different IgSF CAMs.

**2. Calcium Dependent - Integrins**

Integrins, one of the major classes of receptors within the ECM, mediates cell–ECM interactions with collagen, fibrinogen, fibronectin, and vitronectin. Integrins provide essential links between the extracellular environment and the intracellular signalling pathways, which can play roles in cell behaviours, such as apoptosis, differentiation, survival, and transcription.

Integrins are heterodimeric, as they consist of an alpha and beta subunit. There are currently 18 alpha subunits and 8 beta subunits, which combine to make up 24 different integrin combinations. Within each of the alpha and beta subunits there is a large extracellular domain, a transmembrane domain and a short cytoplasmic domain. The extracellular domain is where the ligand binds through the use of divalent cations. The integrins contain multiple divalent cation binding sites in the extracellular domain. The integrin cation binding sites can be occupied by $Ca^{2+}$ or by $Mn^{2+}$ ions. Cations are necessary but not sufficient for integrins to convert from the inactive bent conformation into the active extended conformation. Both the presence of cations bound to the multiple cation binding sites is required, along with the direct physical association with ECM ligands for integrins to attain the extended structure and concomitant activation. Thus, rise in extracellular $Ca^{2+}$ ions may serve to prime the integrin heterodimer. The release of intracellular $Ca^{2+}$ have been shown to be important for integrin inside-out activation. However,

extracellular $Ca^{2+}$ binding may exert different effects depending on the type of integrin and the cation concentration. Integrins regulate their activity within the body by changing conformation. Most exist at rest in a low affinity state, which can be altered to high affinity through an external agonist which causes a conformational change within the integrin, increasing their affinity.

## 3.5.2 Gap Junctions and Connexins

Gap junctions are composed of channels called connexons, which consist of transmembrane proteins called connexins clustered in groups of six. Connexons from adjacent cells form continuous channels when they come into contact and align with each other. These channels allow transport of ions and small molecules between cytoplasm of two adjacent cells, apart from holding cells together and provide structural stability like anchoring junctions or tight junctions. Gap junction channels are selectively permeable to specific ions depending on which connexins form the connexons, which allows gap junctions to be involved in cell signalling by regulating the transfer of molecules involved in signalling cascades. Channels can respond to many different stimuli and are regulated dynamically either by rapid mechanisms, such as voltage gating, or by slow mechanism, such as altering numbers of channels present in gap junctions.

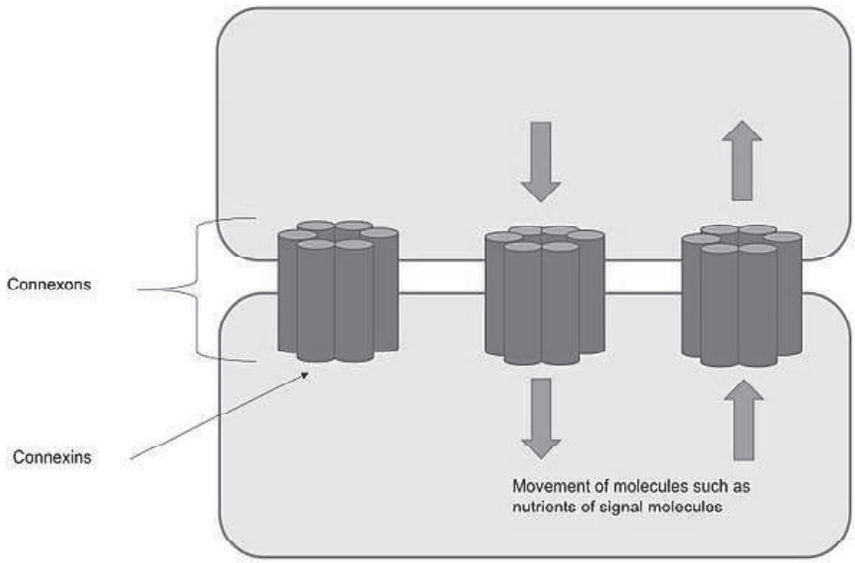


***Fig.3.45*** *Gap Junctions Showing Connexons and Connexins*

**Tight Junctions:** Tight junctions are normally present in epithelial and endothelial tissues, where they seal gaps and regulate paracellular transport of solutes and extracellular fluids in these tissues that function as barriers. Tight junction is formed by transmembrane proteins, including claudins, occludins and tricellulins, that bind closely to each other on adjacent membranes in a homophilic manner. Similar to anchoring junctions, intracellular domains of these tight junction proteins are bound with scaffold proteins that keep these proteins in clusters and link them to actin filaments in order to maintain structure of the tight junction. Claudins, essential for formation of tight junctions, form paracellular pores which allow selective passage of specific ions across tight junctions making the barrier selectively permeable.

NOTES

**Check Your Progress**

18. What is cell adhesion?
19. What are the four major families of Cell-Adhesion Molecules (CAM)?
20. What is the function of adherens junctions ?
21. Define connexins.

## 3.6 CELL CYCLE

A cell cycle is a series of events that takes place in a cell as it grows and divides. A cell spends most of its time in what is called interphase, and during this time it grows, replicates its chromosomes, and prepares for cell division. The cell then leaves interphase, undergoes mitosis, and completes its division. The resulting cells, known as daughter cells, each enter their own interphase and begin a new round of the cell cycle.

The cell cycle, or cell-division cycle, is the series of events that take place in a cell that cause it to divide into two daughter cells. These events include the duplication of its DNA (DNA replication) and some of its organelles, and subsequently the partitioning of its cytoplasm and other components into two daughter cells in a process called cell division.

In cells with nuclei (eukaryotes), (i.e., animal, plant, fungal, and protist cells), the cell cycle is divided into two main stages: interphase and the mitotic (M) phase (including mitosis and cytokinesis). During interphase, the cell grows, accumulating nutrients needed for mitosis, and replicates its DNA and some of its organelles. During the mitotic phase, the replicated chromosomes, organelles, and cytoplasm separate into two new daughter cells. To ensure the proper replication of cellular components and division, there are control mechanisms known as cell cycle checkpoints after each of the key steps of the cycle that determine if the cell can progress to the next phase.

The cell division cycle is a vital process by which a single celled fertilized egg develops into a mature organism, as well as the process by which hair, skin, blood cells, and some internal organs are renewed. After cell division, each of the daughter cells begin the interphase of a new cycle. Although the various stages of interphase are not usually morphologically distinguishable, each phase of the cell cycle has a distinct set of specialized biochemical processes that prepare the cell for initiation of the cell division.

### 3.6.1 Cyclins and Cyclin Dependent Kinases (CDKs)

Cyclins are among the most important core cell cycle regulators. Cyclins are a group of related proteins, and there are four basic types found in humans and most other eukaryotes.

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Cyclin Dependent Kinases (CDKs) are the families of protein kinases first discovered for their role in regulating the cell cycle. They are also involved in regulating transcription, mRNA processing, and the differentiation of nerve cells. They are present in all known eukaryotes, and their regulatory function in the cell cycle has been evolutionarily conserved. In fact, yeast cells can proliferate normally when their CDK gene has been replaced with the homologous human gene. CDKs are relatively small proteins, with molecular weights ranging from 34 to 40 kDa, and contain little more than the kinase domain. By definition, a CDK binds a regulatory protein called a cyclin. Without cyclin, CDK has little kinase activity; only the cyclin-CDK complex is an active kinase but its activity can be typically further modulated by phosphorylation and other binding proteins, like p27. CDKs phosphorylate their substrates on serines and threonines, so they are serine-threonine kinases.

Cyclins and Cyclin-Dependent Kinases (CDKs) are important master regulators of the cell cycle. Their role is to phosphorylate proteins on either S or T amino acids and thereby regulate the activity of those proteins. Yeast have just one CDK (Cdk1), while 'Metazoans' (animals) like humans have nine, of which four are really critical to the cell cycle. CDKs need to hydrolize ATP for energy in order to perform phosphorylation. They have an ATP binding cleft whose ability to bind ATP is regulated by two mechanisms. First, CDKs have a 'flexible T loop' which contains a Threonine (T) residue which normally blocks the ATP binding cleft, but not when the T is phosphorylated. Second, cyclins bind CDKs and induce a conformational change that also helps to expose the ATP binding cleft. Therefore, a fully active CDK is one which is both phosphorylated at the T on the T loop and is bound to a cyclin.

The various activities of the cell cycle, then, are determined by the combination of cyclins and CDKs that are active at each stage, as shown in the Table 3.4.

***Table 3.4*** *Cyclins and CDKs*

| Cell Cycle Stage | Cyclins | CDKs | Comments |
|---|---|---|---|
| G1 | Cyclin D | CDK4&6 | Can react to outside signals such as growth factors or mitogens |
| G1/S | Cyclins E & A | CDK2 | Regulate centrosome duplication; important for reaching START |
| S | Cyclins E & A | CDK2 | Targets are helicases and polymerases |
| M | Cyclins A & B | CDK1 | Regulate G2/M checkpoint. The cyclins are synthesized during S but not active until synthesis is complete. Phosphorylate lots of downstream targets |

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All cyclins contain a conserved 100 amino acid 'Cyclin Box'. Cyclin/CDK complexes regulate the cell cycle both by promoting activities for their respective stages, and by inhibiting activities for future cell cycle stages that must not yet be reached. Therefore, cyclins must be able to be both generated and degraded in order for the cell cycle to proceed.

### 3.6.2 Regulation of CDK-Cycline Activity.

Cyclins drive the events of the cell cycle by partnering with a family of enzymes called the cyclin dependent kinases (CDKs). A lone CDK is inactive, but the binding of a cyclin activates it, making it a functional enzyme and allowing it to modify target proteins.

CDK levels remain relatively constant throughout the cell cycle and most regulation is post-translational. Most knowledge of CDK structure and function is based on CDKs of *S. pombe* (Cdc2), *S. cerevisiae* (CDC28), and vertebrates (CDC2 and CDK2). The four major mechanisms of CDK regulation are cyclin binding, CAK phosphorylation, regulatory inhibitory phosphorylation, and binding of CDK inhibitory subunits (CKIs).

**Cyclin Binding**

The active site, or ATP-binding site, of all kinases is a cleft between a small amino-terminal lobe and a larger carboxy-terminal lobe. The structure of human CDK2 revealed that CDKs have a modified ATP-binding site that can be regulated by cyclin binding. Phosphorylation by CDK-Activating Kinase (CAK) at Thr 161 on the T-loop increases the complex activity. Without cyclin, a flexible loop called the activation loop or T-loop blocks the cleft, and the position of several key amino acid residues is not optimal for ATP-binding. With cyclin, two alpha helices change position to permit ATP binding. One of them, the L12 helix that comes just before the T-loop in the primary sequence, becomes a beta strand and helps rearrange the T-loop, so it no longer blocks the active site. The other alpha helix called the PSTAIRE helix rearranges and helps change the position of the key amino acid residues in the active site.

There is considerable specificity in which cyclin binds with CDK. Furthermore, cyclin binding determines the specificity of the cyclin-CDK complex for particular substrates. Cyclins can directly bind the substrate or localize the CDK to a subcellular area where the substrate is found.

**Phosphorylation**

Full kinase activity requires an activating phosphorylation on a threonine adjacent to the CDK's active site. The identity of the CDK-Activating Kinase (CAK) that performs this phosphorylation varies across the model organisms. The timing of this phosphorylation varies as well. In mammalian cells, the activating phosphorylation occurs after cyclin binding. In yeast cells, it occurs before cyclin binding. CAK activity is not regulated by known cell-cycle pathways and cyclin binding is the limiting step for CDK activation.

Unlike activating phosphorylation, CDK inhibitory phosphorylation is vital for regulation of the cell cycle. Various kinases and phosphatases regulate their phosphorylation state.

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**CDK Inhibitors**

A Cyclin-dependent Kinase Inhibitor (CKI) is a protein that interacts with a cyclin-CDK complex to block kinase activity, usually during G1 or in response to signals from the environment or from damaged DNA. In animal cells, there are two major CKI families: the INK4 family and the CIP/KIP family. The INK4 family proteins are strictly inhibitory and bind CDK monomers. Crystal structures of CDK6-INK4 complexes show that INK4 binding twists the CDK to distort cyclin binding and kinase activity. The CIP/KIP family proteins bind both the cyclin and the CDK of a complex and can be inhibitory or activating. CIP/KIP family proteins activate cyclin D and CDK4 or CDK6 complexes by enhancing complex formation.

Progression through the eukaryotic cell cycle is regulated by cyclin-dependent kinases (cdks) which bind to cyclins to form Cdk-cyclin complexes. At the restriction point (in yeast this point is called start), a **G1-Cdk-cyclin** catalyzes the phosphorylation of the *Rb protein* to trigger passage into S phase (Fig. 3.44). At the $G_2$–M boundary, a mitotic CDK-cyclin triggers entry into mitosis by catalyzing the phosphorylation of various proteins, thereby promoting nuclear envelope breakdown, chromosome condensation and spindle formation and at metaphase-anaphase boundary, mitotic-CDK-cyclin contribute to activation of anaphase-promoting complex, which triggers a protein degradation pathway that initiates chromatid separation. This protein degradation pathway also targets mitotic cyclin for breakdown, leading to an inactivation of mitotic CDK activity that in turn triggers events associated with the exit from mitosis, including chromatin decondensation and assembly of nuclear envelope.

Mitosis depends upon the activation of a pre-existing protein, the M-phase kinase. This protein has two subunits: 1. Cyclin dependent kinase (CDK) which is activated by modification at the start of M-phase and 2. Cyclin which is accumulated during interphase but is destroyed during mitosis.

CDK (kinase subunit) bring about phosphorylation. It associates with cyclin (regulatory subunit) to form a CDK-cyclin complex. This complex is also known as cell-cycle's engine. M-phase kinase acts as Maturation Promoting Factor (MPF) to cause somatic cells to enter M-phase. M-phase protein (dimer) can phosphorylate a variety of protein substrates.

Rb protein is a protein whose phosphorylation controls passage through the restriction point in the cell cycle.

Lastly, cell utilises a series of **checkpoint mechanism** to monitor conditions within the cell and transiently halt the cell cycle if conditions are not suitable for continuing. The DNA replication checkpoint monitors the state of DNA replication to ensure that DNA synthesis is completed prior to permitting the cell to exit from $G_2$ and begin to mitosis. The **p53 protein** plays a central role in a series of DNA

damage check-points that halt the cell cycle at various points of DNA damage. Finally, the *anaphase promoting complex* is involved in the spindle checkpoint, which prevents anaphase chromosome movements from the beginning before the chromosomes are attached to the spindle.

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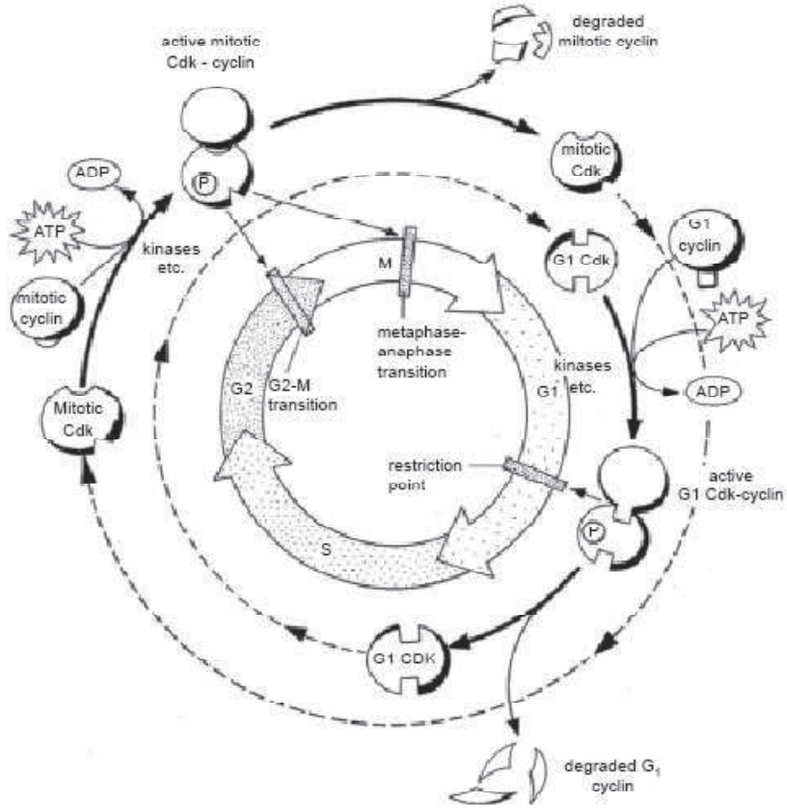


***Fig. 3.46*** *A general model for cell cycle regulation. According to this model, passage through the three main transition points in the cell cycle is triggered by protein complexes made of cyclin and Cdk, whose phosphorylation of other proteins induces progression through the cell cycle (after Becker et. al., 2006).*

**Check Your Progress**

22. What is a cell cycle?
23. What do you understand by Cyclin-dependent Kinase Inhibitor (CKI)?
24. Which are the two subunits of M-phase kinase?

## 3.7 ANSWERS TO 'CHECK YOUR PROGRESS'

1. A biological membrane or bio-membrane is an enclosing or separating membrane that acts as a selectively permeable barrier within living things.
2. There are three types of membrane lipids:
   - Phosphoglycerides
   - Sphingolipids
   - Cholesterol

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3. In the tissues of multicellular animals, the plasma membranes of two adjacent cells usually remain separated by a space of 10 to 150 Aº wide. This intercellular space is uniform and contains a material of low electron density which can be considered as a cementing substance.
4. The membranes which allow only water but no solute particle to pass through them are known as semi-permeable membranes. Such membranes have not so far been recognised in animal cells.
5. The process by which the water molecules pass through a membrane from a region of higher water concentration to the region of lower water concentration is known as osmosis.
6. Sometimes the large-sized solid food or foreign particles are taken in by the cell through the plasma membrane. The process of ingestion of large-sized solid substances (*e.g.*, bacteria and parts of broken cells) by the cell is known as phagocytosis.
7. The cytoskeleton is a network of filaments and tubules that extends throughout a cell, through the cytoplasm, which is all of the material within a cell except for the nucleus.
8. Common properties of motor proteins are:
   - They move along the filaments.
   - They can bind to specific filament types.
   - They hydrolyze ATP.
9. The branched molecular network of activation (and deactivation) of signalling molecules linking receptor activation to the intracellular targets is referred to as a signal transduction pathway (or cascade).
10. Second messengers are small readily diffusible intracellular mediators, whose concentration inside the cell changes rapidly on receptor activation.
11. Signalling proteins are the large intracellular signalling molecules that generally, but not exclusively, function by activating the next signalling protein in the signal transduction cascade, or by modifying the concentration of second messengers.
12. Direct signalling is a transfer of ions or small molecules from one cell to its neighbour through pores in the membrane.
13. Paracrine signalling is a way for a cell to affect the behaviour of neighbouring cells by secreting chemicals into the common intercellular space. This is an important process during embryonic development.
14. Cell surface receptors (membrane receptors, transmembrane receptors) are receptors that are embedded in the plasma membrane of cells.
15. Examples of second messenger molecules include cyclic AMP, cyclic GMP, inositol triphosphate, diacylglycerol, and calcium.
16. Earl Wilbur Sutherland Jr. discovered second messengers, for which he won the 1971 Nobel Prize in Physiology or Medicine.

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17. Mitogen-Activated Protein (MAPK or MAP kinase) is a type of protein kinase that is specific to the amino acids serine and threonine (i.e., a serine/threonine-specific protein kinase).
18. Cell adhesion is the process by which cells interact and attach to neighbouring cells through specialised molecules of the cell surface.
19. Cell-Adhesion Molecules (CAMs) are classified into four major families: integrins, immunoglobulin (Ig) superfamily, cadherins, and selectins.
20. Adherens junctions mainly function to maintain the shape of tissues and to hold cells together.
21. Gap junctions are composed of channels called connexons, which consist of transmembrane proteins called connexins clustered in groups of six.
22. The cell cycle, or cell-division cycle, is the series of events that take place in a cell that cause it to divide into two daughter cells.
23. A Cyclin-dependent Kinase Inhibitor (CKI) is a protein that interacts with a cyclin-CDK complex to block kinase activity, usually during G1 or in response to signals from the environment or from damaged DNA.
24. M-phase kinase protein has two subunits: 1. Cyclin dependent kinase (Cdk) which is activated by modification at the start of M-phase and 2. Cyclin which is accumulated during interphase but is destroyed during mitosis.

## 3.8 SUMMARY

- A biological membrane or bio-membrane is an enclosing or separating membrane that acts as a selectively permeable barrier within living things.
- Plasma membrane is the outer membrane covering of cell protoplasts discovered by Schwann (1838).
- The lipid bilayer is fundamental structure of most biomembranes.
- Phospholipids constitute a major portion of lipids (55-57%) in plasma membranes.
- The driving force for the formation of lipid bilayer is hydrophobic interaction amongst the hydrocarbon tails of lipid molecules
- In the fluid mosaic plasma membrane, there is not complete and independent freedom of movement for its different component molecules.
- The plasma membrane acts as a thin barrier which separates the intra-cellular fluid or the cytoplasm from the extra-cellular fluid in which the cell lives.
- The plasma membrane acts as a semipermeable barrier between the cell and the extracellular environment.
- Calcium ion channels ($Ca^{2}$+- channels) occur in axonal membranes and other membranes for the entrance of $Ca^{2}$+ ions in the cell.
- In all eukaryotic cells, secretory vesicles are continually carrying new plasma membrane and cellular secretions such as proteins, lipids and carbohydrates

(*e.g.*, cellulose) from the Golgi apparatus to the plasma membrane or to cell exterior by the process of exocytosis.

- The ability of eukaryotic cells to adopt a variety of shapes and to carry out coordinated and directed movements depends on the cytoskeleton.
- The cytoskeleton is a network of filaments and tubules that extends throughout a cell, through the cytoplasm, which is all of the material within a cell except for the nucleus.
- Electron microscopy and X-ray diffraction studies show that microtubules contain some longitudinally arranged assemblies of filaments.
- Microtubules were fisrt of all observed in the axoplasm of the myelinated nerve fibres by Robertis and Franchi (1953).
- Cell signalling can result either from the direct interaction of a cell with its neighbour or from the action of secreted signalling molecules.
- Cell surface receptors (membrane receptors, transmembrane receptors) are receptors that are embedded in the plasma membrane of cells.
- The dimerization model suggests that prior to ligand binding, receptors exist in a monomeric form.
- Calcium ions are one type of second messengers and are responsible for many important physiological functions including muscle contraction, fertilization, and neurotransmitter release.
- Protein (MAPK or MAP kinase) is a type of protein kinase that is specific to the amino acids serine and threonine (i.e., a serine/threonine-specific protein kinase).
- Adherens junctions mainly function to maintain the shape of tissues and to hold cells together.
- Microtubules constitute a class of morphologically and chemically related filamentous rods which are common to both plant and animal cells.
- Gap junctions are composed of channels called connexons, which consist of transmembrane proteins called connexins clustered in groups of six.
- A cell cycle is a series of events that takes place in a cell as it grows and divides.
- Progression through the eukaryotic cell cycle is regulated by cyclin-dependent kinases (cdks) which bind to cyclins to form Cdk-cyclin complexes.
- The cell cycle, or cell-division cycle, is the series of events that take place in a cell that cause it to divide into two daughter cells.

## 3.9 KEY TERMS

- **Bio Membranes**: A biological membrane or bio-membrane is an enclosing or separating membrane that acts as a selectively permeable barrier within living things.

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- **Passive Transport.** It is a type of diffusion in which an ion or molecule crossing a membrane moves down its electrochemical or concentration gradient.
- **Osmosis:** The process by which the water molecules pass through a membrane from a region of higher water concentration to the region of lower water concentration is known as osmosis.
- **Facilitated Diffusion:** This is a special type of passive transport, in which ions or molecules cross the membrane rapidly because specific permeases in the membrane facilitate their crossing.
- **Ultraphagocytosis or Colloidopexy:** The process in which plasma membrane ingests smaller colloidal particles is known as colloidopexy or ultraphagocytosis.
- **Microtubules:** Microtubules were first noted in a number of eukaryotic cells by electron microscopic observations. It is a long rod-like structure of 25 nm diameter and up to several millimeters in length.
- **Micropinocytosis.** Electron microscopic observations have been made on the pinocytotic process at sub-cellular or sub-microscopic level in the cells. The pinocytosis which occurs at sub-microscopic level is known as micropinocytosis.
- **Triskelion** : A molecule of clathrin is composed of three large polypeptide chains and three smaller polypeptide chains, all of which together form a three-legged structure, called triskelion.
- **Tight Junctions:** Tight junctions are normally present in epithelial and endothelial tissues, where they seal gaps and regulate paracellular transport of solutes and extracellular fluids in these tissues that function as barriers.

## 3.10 SELF ASSESSMENT QUESTIONS AND EXERCISES

### Short-Answer Questions

1. Define the molecular arrangement of biomembrane.
2. How is the mobility and distribution of protein molecules in the membrane controlled?
3. What is permeability? According to permeability, how many types of the plasma membranes have been recognized?
4. Differentiate between endosmosis, exosmosis and plasmolysis.
5. What are the components of cytoskeleton?
6. What are the functions of microtubules?
7. State the functions of kinesin and dynein.
8. What are the three basic types of secondary messenger molecules?
9. What are cell junctions? Write their classification.

10. State the basis of the classifications of the families of CAMs.
11. Define cyclin binding.
12. Write the process of regulation of CDK-Cycline activity.

**Long-Answer Questions**

1. Explain the composition of biomembrane.
2. Describe the functions of biological membrane.
3. Analyze the mode of transport across plasma membrane.
4. Discuss the process of active transport.
5. Explain the chemical composition of microtubule.
6. Analyze the types of motor proteins.
7. Explain transmembrane receptors' mechanism of action.
8. Elaborate on Mitogen-Activated Protein (MAPK or MAP kinase).
9. Describe the various activities of the cell cycle, determined by the combination of cyclins and CDKs.

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## 3.11 FURTHER READING

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# UNIT 4 MOLECULAR CELL BIOLOGY - II

NOTES

**Structure**



## 4.0 INTRODUCTION

Cell biology is the study of cell structure and function, and it revolves around the concept that the cell is the fundamental unit of life. Focusing on the cell permits a detailed understanding of the tissues and organisms that cells compose. On the whole, cell biology focuses on the structure and function of a cell, from the most general properties shared by all cells, to the

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unique, highly intricate functions particular to specialized cells. Knowing the components of cells and how cells work is fundamental to all biological sciences while also being essential for research in biomedical fields such as cancer, and other diseases. Research in cell biology is interconnected to other fields such as genetics, molecular genetics, biochemistry, molecular biology, medical microbiology, immunology, and cyto-chemistry.

Molecular biology is the study of the molecular underpinnings of the biological phenomena, focusing on molecular synthesis, modification, mechanisms and interactions. Although there are many kinds of molecules in every living thing, most molecular biologists focus on genes and proteins. Proteins perform a huge diversity of functions within living cells and genes contain the information required to make more proteins. Molecular biology is not simply the study of biological molecules and their interactions; rather, it is also collection of techniques developed since the field's genesis which have enabled scientists to learn about molecular processes. Molecular biology also plays a critical role in the understanding of structures, functions, and internal controls within individual cells, all of which can be used to efficiently target new drugs, diagnose disease, and better understand cell physiology.

In this units you will study about cell matrix adhesion, organization of viral DNA, bacterial DNA, eukaryotic DNA, palindromes, split genes, transposons, gene concepts and genetic code, intracellular protein traffic, biology of cancer and biology of aging.

## 4.1 OBJECTIVES

After going through this unit, you will be able to:

- Understand cell matrix adhesion
- Analyze the organization of viral DNA, bacterial DNA and eukaryotic DNA
- Explain palindromes
- Comprehend split genes and transposons
- Discuss gene concepts and genetic code
- Analyze intracellular protein traffic
- Explain biology of cancer
- Understand biology of aging

## 4.2 CELL MATRIX ADHESION

Cell matrix adhesion is the interaction of a cell with the extracellular matrix, mediated by multi-protein adhesion structures, such as focal adhesions, fibrillar adhesions and podosomes. The ECM (ExtraCellular Molecules) is a network of extracellular molecules which are secreted locally to ensure cell and tissue cohesion.

The ECM also serves as a reservoir for extracellular signalling molecules that control cell growth, migration, and differentiation. The major classes of ECM molecules are proteoglycans, **collagens** and multi-adhesive matrix proteins (e.g., laminin, fibronectin). In mammals, the ECM is

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commonly known as connective tissue. ECM components are linked to each other through diverse protein and carbohydrate-binding domains. For stability in tissues, cells are linked to the ECM through cell adhesion receptors (e.g., **integrins**). A specialized form of extracellular matrix that underlies the basal side of polarized epithelial cell sheets to separate them from the underlying connective tissue is the basal lamina.

Basal laminae (plural) also surround individual muscle cells, fat cells, and cells lining peripheral nerve cell axons (i.e., Schwann cells). The basal lamina is thin and flexible, and is composed of closely packed matrix molecules that lack significant volume. The basal lamina components are synthesized and deposited by the cells on either side: the epithelial cells and the cells within the underlying bed of connective tissue (i.e., fibroblasts). The basal laminae forms a cohesive network and mechanical connection between cells and their external environment. Force-driven signals originating between the basal lamina components (i.e., fibronectin) and linked cell adhesion receptors (i.e., integrins) is communicated to the interior of cells through a mechanotransduction system to influence cell polarity, metabolism, fate, and migration.

The key constituents found in the basal lamina are glycoproteins (i.e., laminin, collagen) and proteoglycans (i.e., perlecan), however, the precise composition varies from tissue to tissue and various other molecules (e.g., fibronectin) can also be found.

Cell matrix interactions are mediated by adhesion receptors and lead to the formation of multi-protein adhesion structures that interact with the actin cytoskeleton at the cell interior; collectively, they are called Cell-Matrix Adhesion Complexes (CMACs). The cytoskeletal component composed of actin and associated proteins aids in polymerization-driven cell motility as well as in myosin-mediated cell contractility.

These adhesions act as vital information processing centers that enable cells to sense numerous extracellular signals that convey information about the chemistry, geometry, and physical properties of the ECM.

The association of cells with the ExtraCellular Matrix (ECM) initiates the assembly of specific cell matrix adhesion sites. These sites are involved in physical attachment of cells to external surfaces, which is essential for cell migration and tissue formation as well as for activation of adhesion-mediated signalling events. Key mediators of both matrix attachment and signalling responses are the integrins, which are heterodimeric transmembrane receptors for ECM components (Hynes, 1992; Clark and Brugge, 1995). Following association with their ligands, integrins induce reorganization of the actin cytoskeleton and associated proteins, resulting in the formation of cell-matrix adhesion sites.

The best-known class of matrix adhesions in cultured cells are the Focal Contacts (FCs), which can be visualized by electron microscopy or interference reflection microscopy.

The specific type of integrin present in matrix adhesions can vary, depending on the nature of the underlying ExtraCellular Matrix (ECM).

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In biology, the Extra Cellular Matrix (ECM) is a three-dimensional network consisting of extracellular macromolecules and minerals, such as collagen, enzymes, glycoproteins and hydroxyapatite that provide structural and biochemical support to surrounding cells. Because multicellularity evolved independently in different multicellular lineages, the composition of ECM varies between multicellular structures; however, cell adhesion, cell-to-cell communication and differentiation are common functions of the ECM.

The animal extracellular matrix includes the interstitial matrix and the basement membrane. Interstitial matrix is present between various animal cells (i.e., in the intercellular spaces). Gels of polysaccharides and fibrous proteins fill the interstitial space and act as a compression buffer against the stress placed on the ECM. Basement membranes are sheet-like depositions of ECM on which various epithelial cells rest. Each type of connective tissue in animals has a type of ECM: **collagen fibers** and bone mineral comprise the ECM of bone tissue; reticular fibers and ground substance comprise the ECM of loose connective tissue; and blood plasma is the ECM of blood.

Cells create extracellular matrix by releasing molecules into its surrounding extracellular space. Cells have specific CAMs that will bind to molecules in the extracellular matrix and link the matrix to the intracellular cytoskeleton. Extracellular matrix can act as a support when organising cells into tissues and can also be involved in cell signalling by activating intracellular pathways when bound to the CAMs. Cell–matrix junctions are mainly mediated by integrins, which also clusters like cadherins to form firm adhesions.

### 4.2.1 Integrins

Integrins are transmembrane heterodimers formed by different α and β subunits, both subunits with different domain structures. Integrins can signal in both directions: inside-out signalling, intracellular signals modifying the intracellular domains, can regulate affinity of integrins for their ligands, while outside-in signalling, extracellular ligands binding to extracellular domains, can induce conformational changes in integrins and initiate signalling cascades. Extracellular domains of integrins can bind to different ligands through heterophilic binding while intracellular domains can either be linked to intermediate filaments, forming hemidesmosomes, or to actin filaments, forming focal adhesions.

**Hemidesmosomes:** In hemidesmosomes, integrins attach to extracellular matrix proteins called laminins in the basal lamina, which is the extracellular matrix secreted by epithelial cells. Integrins link extracellular matrix to keratin intermediate filaments, which interacts with intracellular domain of integrins via adapter proteins, such as plectins and BP230. Hemidesmosomes are important in maintaining structural stability of epithelial cells by anchoring them together indirectly through the extracellular matrix.

**Focal Adhesions:** In focal adhesions, integrins attach fibronectins, a component in the extracellular matrix, to actin filaments inside cells. Adapter proteins, such as talins, vinculins, α-actinins and filamins, form a complex at the intracellular domain of integrins and bind to actin filaments. This multi-

protein complex linking integrins to actin filaments is important for assembly of signalling complexes that act as signals for cell growth and cell motility.

## 4.2.2 Collagen

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Collagens are the most abundant protein in the ECM. In fact, collagen is the most abundant protein in the human body and accounts for 90% of bone matrix protein content. Collagens are present in the ECM as fibrillar proteins and give structural support to resident cells. Collagen is exocytosed in precursor form (procollagen), which is then cleaved by procollagen proteases to allow extracellular assembly. Disorders, such as, Ehlers Danlos Syndrome, osteogenesis imperfecta, and epidermolysis bullosa are linked with genetic defects in collagen-encoding genes. The collagen can be divided into several families according to the types of structure they form:

- Fibrillar (Type I, II, III, V, XI)
- Facit (Type IX, XII, XIV)
- Short Chain (Type VIII, X)
- Basement Membrane (Type IV)
- Other (Type VI, VII, XIII)

### Elastin

Elastins, in contrast to collagens, give elasticity to tissues, allowing them to stretch when needed and then return to their original state. This is useful in blood vessels, the lungs, in skin, and the ligamentum nuchae, and these tissues contain high amounts of elastins. Elastins are synthesized by fibroblasts and smooth muscle cells. Elastins are highly insoluble, and tropoelastins are secreted inside a chaperone molecule, which releases the precursor molecule upon contact with a fiber of mature elastin. Tropoelastins are then deaminated to become incorporated into the elastin strand. Disorders, such as cutis laxa and Williams syndrome are associated with deficient or absent elastin fibers in the ECM.

### Extracellular Vesicles

In 2016, Huleihel et al., reported the presence of DNA, RNA, and Matrix-Bound Nanovesicles (MBVs) within ECM bioscaffolds. MBVs shape and size were found to be consistent with previously described exosomes. MBVs cargo includes different protein molecules, lipids, DNA, fragments, and miRNAs. Similar to ECM bioscaffolds, MBVs can modify the activation state of macrophages and alter different cellular properties, such as proliferation, migration and cell cycle. MBVs are now believed to be an integral and functional key component of ECM bioscaffolds.

## 4.2.3 Non-Collagen Components

Non-collagenous components include proteoglycans (such as, decorin, biglycan, fibromodulin, lumican, osteoadherin, and versican) and several glycoproteins, such as osteocalcin, osteonectin, and the SIBLING proteins (i.e., osteopontin, bone sialoprotein, dentin matrix protein-1, dentin sialophosphoprotein, and matrix extracellular phosphoglycoprotein).

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### 4.2.4 Auxin - Cell Expansion

The plant hormone **auxin** is well known to stimulate cell elongation via increasing wall extensibility. Auxin participates in the regulation of cell wall properties by inducing wall loosening.

Plant cells are surrounded by cell walls, which are dynamic structures displaying a strictly regulated balance between rigidity and flexibility. Walls are fairly rigid to provide support and protection, but also extensible, to allow cell growth, which is triggered by a high intracellular turgor pressure. Wall properties regulate the differential growth of the cell, resulting in a diversity of cell sizes and shapes. The plant **hormone auxin** is well known to stimulate cell elongation via increasing wall extensibility. Auxin participates in the regulation of cell wall properties by inducing wall loosening.

Auxin has two phase of response, namely early and late response. Immediately after the application of auxin, it induces cell elongation by loosening the cell wall based on the "Acid Growth Hypothesis". This early response occurs within 10-15 minutes before the starting of traditional signalling events. The early response is mediated through the modification of cell wall. The late auxin response is the TIR1/AFB-mediated degradation of AUX/IAA repressor. Through this pathway, auxin controls the expression of cell wall related genes such as cellulose, expansion, XTH, pectin and peroxidase genes.

### 4.2.5 Cellulose Fibril Synthesis and Orientation

Cellulose is the most abundant biopolymer on earth. It consists of bundles of β-1,4-glucan chains typically organized as microfibrillar structures. It is mostly found in plants, bacteria, oomycetes, and green algae.

Cellulose microfibrils, composed of hydrogen-bonded β-1,4 glucans, are key components for anisotropic growth in plants. Cellulose is synthesized by plasma membrane-localized cellulose synthase complexes. In higher plants, these complexes are assembled into hexagonal rosettes in intracellular compartments and secreted to the plasma membrane. Here, the complexes typically track along cortical microtubules, which may guide cellulose synthesis, until the complexes are inactivated or internalized. Determining the regulatory aspects that control the behaviour of cellulose synthase complexes is vital to understanding directed cell and plant growth and to tailoring cell wall content for industrial products, including paper, textiles, and fuel.

A microfibril is a very fine fibril, or fiber-like strand, consisting of glycoproteins and cellulose. It is usually, but not always, used as a general term in describing the structure of protein fiber, for example hair and sperm tail. Its most frequently observed structural pattern is the 9+2 pattern in which two central protofibrils are surrounded by nine other pairs. Cellulose inside plants is one of the examples of non-protein compounds that are using this term with the same purpose. Cellulose microfibrils are laid down in the inner surface of the primary cell wall. As the cell absorbs water, its volume

increases and the existing microfibrils separate and new ones are formed to help increase cell strength.

Cellulose is synthesized by cellulose synthase or Rosette terminal complexes which reside on a cell's membrane. As cellulose fibrils are synthesized and grow extracellularly they push up against neighbouring cells. Since the neighbouring cell cannot move easily the Rosette complex is instead pushed around the cell through the fluid phospholipid membrane. Eventually this results in the cell becoming wrapped in a microfibril layer. This layer becomes the cell wall. The organization of microfibrils forming the primary cell wall is rather disorganized. However, another mechanism is used in secondary cell walls leading to its organization. Essentially, lanes on the secondary cell wall are built with microtubules. These lanes force microfibrils to remain in a certain area while they wrap. During this process microtubules can spontaneously depolymerize and repolymerize in a different orientation. This leads to a different direction in which the cell continues getting wrapped.

Fibrillin microfibrils are found in connective tissues, which mainly makes up fibrillin-1 and provides elasticity. During the assembly, microfibrils exhibit a repeating stringed-beads arrangement produced by the cross-linking of molecules forming a striated pattern with a given periodicity when viewed stained under an electron microscope. In the formation of elastic fiber, fibrillin microfibrils guides the deposit of tropoelastin and remains in the outer layer of mature elastin fibers. The microfibril is also associated in cell communication. Formation of fibrillin microfibrils in the pericellular region affects the activity of a growth factor called TGFβ.

**Check Your Progress**

1. What is cell matrix adhesion?
2. Which is the best-known class of matrix adhesions in cultured cells?
3. How does cell create extracellular matrix?
4. Which hormone stimulates cell elongation?

## 4.3 ORGANIZATION OF DNA

In this section we will study the organization of DNA in various organisms in detail.

### 4.3.1 Viral DNA

The viral DNA is composed of a genome made of deoxyribonucleic acid (DNA) that is replicated by a DNA polymerase. They can be divided between those that have two strands of DNA in their genome, called double-stranded DNA (dsDNA) viruses, and those that have one strand of DNA in their genome, called single-stranded DNA (ssDNA) viruses. dsDNA viruses primarily belong to two realms: Duplodnaviria and Varidnaviria, and ssDNA viruses are almost exclusively assigned to the realm Monodnaviria, which

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also includes dsDNA viruses. Additionally, many DNA viruses are unassigned to higher taxa. Viruses that have a DNA genome that is replicated through an RNA intermediate by a reverse transcriptase are separately considered reverse transcribing viruses and are assigned to the kingdom Pararnavirae in the realm Riboviria.

DNA viruses are ubiquitous worldwide, especially in marine environments where they form an important part of marine ecosystems, and infect both prokaryotes and eukaryotes. They appear to have multiple origins, as viruses in Monodnaviria appear to have emerged from archaeal and bacterial plasmids on multiple occasions, though the origins of Duplodnaviria and Varidnaviria are less clear. Prominent disease-causing DNA viruses include herpesviruses, papillomaviruses, and poxviruses.

### 4.3.2 Bacterial DNA

Bacteria are very small unicellular organisms which do not contain nuclear envelope, mitochondria, endoplasmic reticulum, mitotic apparatus and nucleolus, etc. The bacteria typically divide by fission. Bacteria have a rigid cell wall which surrounds their cytoplasmic membrane. Their cytoplasm contains ribosomes. mesosomes and several granular inclusions. About 1/5 of the cell volume is occupied by DNA, the genetic material.

According to their external shape, bacteria are grouped into the following two main classes:

(1) Cocci

(2) Bacilli

1. **Cocci:** The 'Cocci' are spherical in shape and they show different patterns when their cells are incompletely separated, for example (i) Diplococcus: Cells in pairs, (ii) Streptococci: Cells chains, (iii) Staphylococci: Cells in clusters, and (iv) Sarcinae: Cells forming tertrads (square) or cubic packets.
2. **Bacilli:** These bacteria are rod-shaped or cylindrical, and are of different types, such as, (i) Coccobacilli: Short elongated cells, (ii) Fusiform Bacilli: Cells tapered at both ends, (iii) Filamentous Bacilli: Long threads, (v) Vibrios: Curved small bacilli, and (v) Spirilla: Long threads curved bacilli.

**Bacterial Nuclear Body**

Bacterial cells do not contain a typical nucleus but as per the 'Feulgen' reaction it shows one, two or more discrete nuclear bodies per cell which are termed as 'Nucleoids'. The bacterial genome is confined to this nucleoid, which is more or less compact structure without any membrane.

When bacterial cells are lysed in the presence of high salt concentration, nucleoids can be recovered intact. The isolated nucleoid may be membrane free or it may be associated with membrane (mesosome). The constituents of the membrane-free nucleoid are DNA (~ 60%) RNA (-30%) and Protein (- 10%) DNA forms 2-3% of the dry weight of a bacterial cell).

## Bacterial Chromosomes

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Bacterial chromosome is a double-stranded circular DNA. In general, bacterial DNA ranges from 1100 pm to 1400 µm in length. An Escherichia coli cell contains 4. 2 x 106 kbp DNA which is about 1.3 mm (1300 µm) in length.

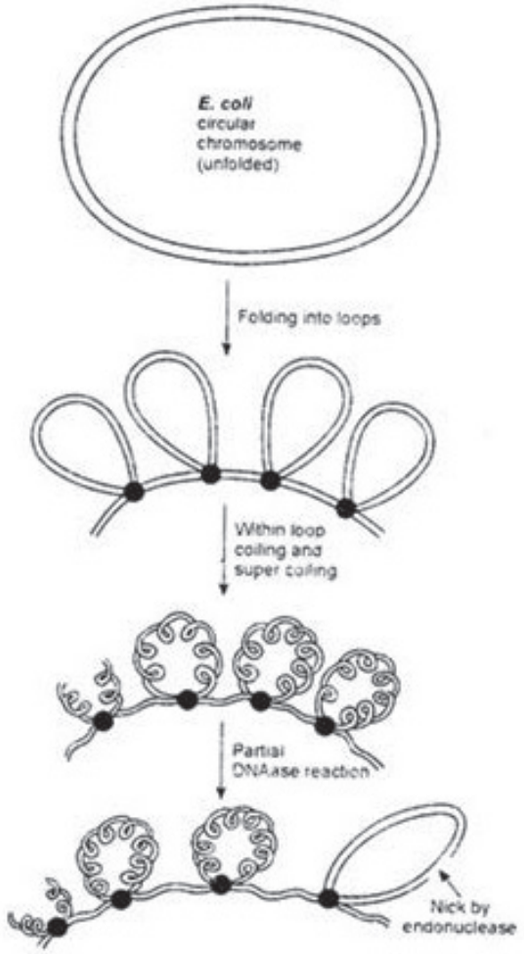


***Fig. 4.1*** *Model of the Genome of Escherichia Coli*

Such a long DNA molecule must be greatly folded to be packaged in a small space of 1.7 x 0.65 µm. The bacterial chromosome is folded into loops or domains which are about 100 in number. A chromosomal domain may be defined as a discrete structural entity within which supercoiling is independent of the other domains.

Thus different domains can maintain different degrees of supercoiling. The DNA chain is coiled on itself to produce supercoiling (Figure Above). The ends of the loops or domains are bound in some way which does not allow rotational events to propagate from one domain to another.

If an endonuclease puts a nick in DNA strand of one domain, this loop becomes larger due to the uncoiling, but the other domains are not affected. Each domain contains about 40 kbp (13 µm) of DNA. The loops are bound by some mechanism that may involve proteins and/or RNA but the mechanism is not clearly understood.

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In *Escherichia coli*, a number of proteins have been isolated which have some similarities with the eukaryotic chromosomal proteins. These proteins are HU, IHF (Integration Host Factor). HI (H-NS) and R It is suspected that HU is involved in the nucleoid condensation.

The protein HI probably has effects on gene expression. The amino acid sequence of P has some similarity with the protamine's (DNA of certain sperms is bound with protamine's).

## Replication of Bacterial Chromosome

Bacterial chromosome is a single replicon. Auto-radiographic studies have shown that it replicates bi-directionally in a semiconservative manner. A theta (0) shaped intermediate is formed during its replication. The rate of replication is 50,000 base pairs per minute which is 25 times faster than that of eukaryotic DNA (2000 bp/minute).

The DNA of bacteria, for example *Escherichia coli*, is a covalently closed circular molecule. It forms the bacterial chromosome, though this chromosome is much simpler in structure and in level of organization than the eukaryotic chromosomes of plants and animals. Also, each bacterial cell normally has a single chromosome containing a single circular DNA molecule.

In *Escherichia coli*, the DNA molecule is 1,300 μm long when fully stretched containing some 4,700 x 103 base-pairs which encode about 4,000 genes. In order to pack this long DNA molecule into a cell measuring only about 1 μm x 3 μm, the molecule has to be highly folded and supercoiled.

### 4.3.3 Eukaryotic DNA

Eukaryotic DNA is bound to proteins known as histones to form structures called nucleosomes. During initiation, the DNA is made accessible to the proteins and enzymes involved in the replication process. There are specific chromosomal locations called origins of replication where replication begins. In some eukaryotes, like yeast, these locations are defined by having a specific sequence of base pairs to which the replication initiation proteins bind. In other eukaryotes, like humans, there does not appear to be a consensus sequence for their origins of replication. Instead, the replication initiation proteins might identify and bind to specific modifications to the nucleosomes in the origin region.

Certain proteins recognize and bind to the origin of replication and then allow the other proteins necessary for DNA replication to bind the same region. The first proteins to bind the DNA are said to recruit the other proteins. Two copies of an enzyme called helicase are among the proteins recruited to the origin. Each helicase unwinds and separates the DNA helix into single-stranded DNA. As the DNA opens up, Y-shaped structures called replication forks are formed. Because two helicases bind, two replication forks are formed at the origin of replication; these are extended in both directions as replication proceeds creating a replication bubble. There are multiple origins of replication on the eukaryotic chromosome which allow

replication to occur simultaneously in hundreds to thousands of locations along each chromosome.

During elongation, an enzyme called DNA polymerase adds DNA nucleotides to the 3′ end of the newly synthesized polynucleotide strand. The template strand specifies which of the four DNA nucleotides (A, T, C, or G) is added at each position along the new chain. Only the nucleotide complementary to the template nucleotide at that position is added to the new strand.

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DNA polymerase contains a groove that allows it to bind to a single-stranded template DNA and travel one nucleotide at time. For example, when DNA polymerase meets an adenosine nucleotide on the template strand, it adds a thymidine to the 3′ end of the newly synthesized strand, and then moves to the next nucleotide on the template strand. This process will continue until the DNA polymerase reaches the end of the template strand.

DNA polymerase cannot initiate new strand synthesis; it only adds new nucleotides at the 3′ end of an existing strand. All newly synthesized polynucleotide strands must be initiated by a specialized RNA polymerase called primase. Primase initiates polynucleotide synthesis and by creating a short RNA polynucleotide strand complementary to template DNA strand. This short stretch of RNA nucleotides is called the primer. Once RNA primer has been synthesized at the template DNA, primase exits, and DNA polymerase extends the new strand with nucleotides complementary to the template DNA.

Eventually, the RNA nucleotides in the primer are removed and replaced with DNA nucleotides. Once DNA replication is finished, the daughter molecules are made entirely of continuous DNA nucleotides, with no RNA portions

Eukaryotic chromosomes have multiple origins of replication, which initiate replication almost simultaneously. Each origin of replication forms a bubble of duplicated DNA on either side of the origin of replication. Eventually, the leading strand of one replication bubble reaches the lagging strand of another bubble, and the lagging strand will reach the 5′ end of the previous Okazaki fragment in the same bubble.

DNA polymerase halts when it reaches a section of DNA template that has already been replicated. However, DNA polymerase cannot catalyse the formation of a phosphodiester bond between the two segments of the new DNA strand, and it drops off. These unattached sections of the sugar-phosphate backbone in an otherwise full-replicated DNA strand are called nicks.

Once all the template nucleotides have been replicated, the replication process is not yet over. RNA primers need to be replaced with DNA, and nicks in the sugar-phosphate backbone need to be connected.

The group of cellular enzymes that remove RNA primers include the proteins FEN1 (Flap Endonulcease 1) and RNase H. The enzymes FEN1 and RNase H remove RNA primers at the start of each leading strand and at the

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start of each Okazaki fragment, leaving gaps of unreplicated template DNA. Once the primers are removed, a free-floating DNA polymerase lands at the 3′ end of the preceding DNA fragment and extends the DNA over the gap. However, this creates new nicks (unconnected sugar-phosphate backbone).

In the final stage of DNA replication, the enyzme ligase joins the sugar-phosphate backbones at each nick site. After ligase has connected all nicks, the new strand is one long continuous DNA strand, and the daughter DNA molecule is complete.

### 4.3.4 Palindromes

A palindromic sequence is a nucleic acid sequence in a double-stranded DNA or RNA molecule whereby reading in a certain direction (for example, 5' to 3') on one strand is identical to the sequence in the same direction (for example, 5' to 3') on the complementary strand. This definition of palindrome thus depends on complementary strands being palindromic of each other.

The meaning of palindrome in the context of genetics is slightly different from the definition used for words and sentences. Since a double helix is formed by two paired antiparallel strands of nucleotides that run in opposite directions, and the nucleotides always pair in the same way (Adenine (A) with Thymine (T) in DNA or Uracil (U) in RNA; Cytosine (C) with Guanine (G)), a (single-stranded) nucleotide sequence is said to be a palindrome if it is equal to its reverse complement. For example, the DNA sequence ACCTAGGT is palindromic because its nucleotide-by-nucleotide complement is TGGATCCA and reversing the order of the nucleotides in the complement gives the original sequence.

A palindromic nucleotide sequence is capable of forming a hairpin. The stem portion of the hairpin is a pseudo-double stranded portion since the entire hairpin is a part of same (single) strand of nucleic acid. Palindromic motifs are found in most genomes or sets of genetic instructions. They have been specially researched in bacterial chromosomes and in the so-called Bacterial Interspersed Mosaic Elements (BIMEs) scattered over them. In 2008, a genome sequencing project discovered that large portions of the human X and Y chromosomes are arranged as palindromes. A palindromic structure allows the Y chromosome to repair itself by bending over at the middle if one side is damaged.

Palindromes also appear to be found frequently in the peptide sequences that make up proteins, but their role in protein function is not clearly known. It has been suggested that the existence of palindromes in peptides might be related to the prevalence of low-complexity regions in proteins, as palindromes are frequently associated with low-complexity sequences. Their prevalence may also be related to the propensity of such sequences to form alpha helices or protein/protein complexes.

**Palindromic Nucleotides in T Cell Receptors**

Diversity of T Cell Receptor (TCR) genes is generated by nucleotide insertions upon V(D)J recombination from their germline-encoded V, D and

J segments. Nucleotide insertions at V-D and D-J junctions are random, but some small subsets of these insertions are exceptional, in that one to three base pairs inversely repeat the sequence of the germline DNA. These short complementary palindromic sequences are called P nucleotides.

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In the DNA molecule a variety of base sequences have been observed. Most of them do not have special features. The repeated sequences are of particular interest because they are the site of enzymatic activity and, sometimes give special features to the nucleic acid. For the first time Wilson and Thomas (1974) used the term palindromic DNA.


***Fig. 4.2*** *Palindromic Sequence*

The DNA molecules containing palindromes and inverted repeats may exist in alternative forms. After separation of complementary strands, the intra-molecular base pairing may result in a double stranded stretch formed between adjacent complementary sequences. This is known as cruciform structure.

Consequently, a palindrome produces an intrastrand double stranded structure which is known as hairpin (Figure Below). The interrupted inverted repeats result in a structure consisting of a double stranded segment with a terminal single-stranded loop. This structure is called stem and loop.

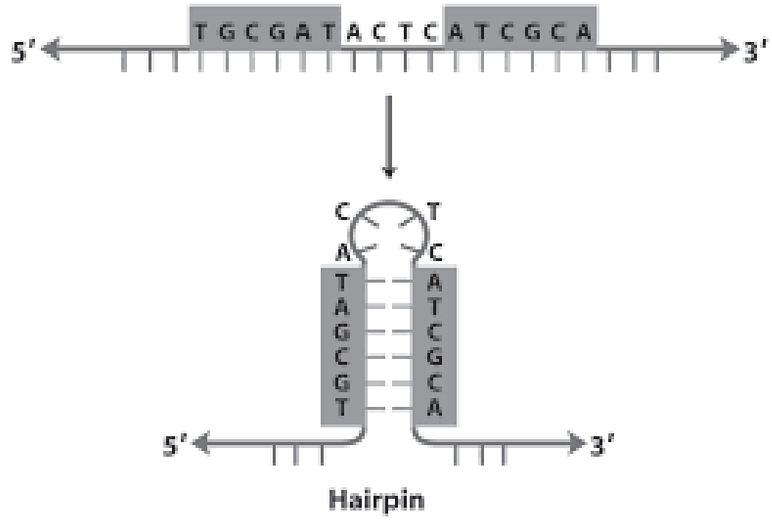


***Fig.4.3*** *Palindrome Double Stranded Structure as Hairpin*

The palindromic DNA or palindromes are the inverted repeats and region of dyad symmetry. The length of palindromes may be short by about 3-10 bases or long by about 50-100 base pairs. As compared to prokaryotic DNA, the eukaryotic DNA contains a large palindrome of about several thousand base pairs. Sometimes a spacer separates the two inverted repeats. The following shows the palindromic DNA sequence.

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Palindromic sequences play an important role in molecular biology. Because a DNA sequence is double stranded, the base pairs are read, (not just the bases on one strand), to determine a palindrome. Many restriction endonucleases (restriction enzymes) recognize specific palindromic sequences and cut them. The restriction enzyme EcoR1 recognizes the following palindromic sequence:

5'- G A A T T C -3'

3'- C T T A A G -5'

The top strand reads 5'-GAATTC-3', while the bottom strand reads 3'-CTTAAG-5'. If the DNA strand is flipped over, the sequences are exactly the same (5'GAATTC-3' and 3'-CTTAAG-5').

### 4.3.5 Split Genes

**Split genes** means that the (DNA) sequences containing actual information of the gene (called **exons**) are interrupted by other sequences (called **introns**) which are spliced out after transcription.

#### A. Discovery of Split Genes

With the discovery of DNA as the genetic material, a gene was regarded as a continuous segment of DNA. In 1964, it was also proved that there was co-linearity between sequence of nucleotides on DNA and sequence of amino acids in corresponding protein. Therefore, there was absolutely no doubt about the continuity of nucleotides in a gene represented by a DNA segment. This was proved to be true both for prokaryotes and eukaryotes. However, a big surprise came in 1977, when geneticists came to know that in some mammals, birds and amphibians, a gene may not be represented by a continuous sequence of nucleotides but may be interrupted by some intervening sequences which are not represented in mRNA transcribed from the gene and utilized for the synthesis of proteins. Such genes with intervening sequences were called **split genes** or **interrupted genes** (Fig. 4.4). Split genes are also called **discontinuous** or **mosaic genes** (see **Brown**, 1998).

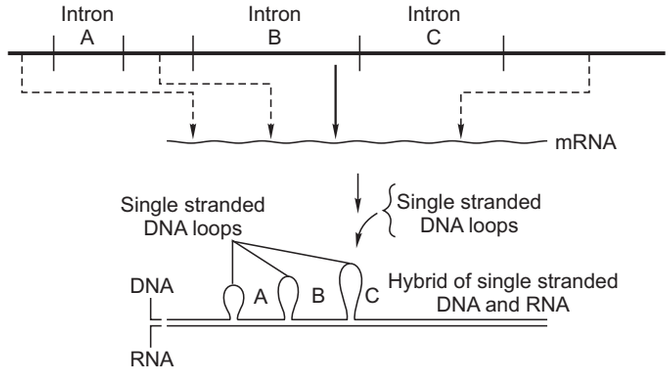


***Fig. 4.4*** *The upper figure shows a DNA sequence representing an split or interrupted gene with their introns (A, B, C) and the synthesis of its mRNA; the bottom figure shows the result of hybridization of mRNA with single stranded DNA obtained after denaturation of native DNA (note the loops formed by three intron genes).*

The discovery of split genes was made in 1977 by various groups of biologists in a variety of materials: (*i*) Two research groups separately headed

by **Philip A. Sharp** and **Richard J. Roberts** studied genes of adenovirus 2. (*ii*) Research groups of **P. Chambon**, **P. Leader** and **R.A. Flavell** studied β globin genes, ovalbummin genes and tRNA genes. In all these cases the genes were found to be interrupted by intervening sequences. The credit for discovery of split genes, however, goes to **Philip Sharp** and **Richard Roberts**, who won in 1993 Nobel Prize for Medicine or Physiology for their independent work on split genes. They analyzed the hybrids of *late mRNA* of adenovirus 2 with the adenovirus genomic DNA. When these mRNA-DNA hybrids were examined under electron microscope, the adjoining sequences of mRNA were found to be hybridized with discontinuous stretches of genomic DNA of adenovirus. The intervening DNA sequences were observed as loops and the phenomenon was later described as **R-looping**.

**P. Chambon's** group (France) and **B.W. O'Malley's** group (USA) showed that the **ovalbumin gene** of chickens is not contiguous but is made up of pieces scattered in the chromosome. These genes are the *split genes*. Within a gene there may be silent regions which are not represented in the polypeptide chain. The natural ovalbumin gene transcribes precursor RNA which contains intervening sequences not found in mRNA. The precursor RNA loops out these intervening sequences, which are then excised, to form mRNA.

**Chambon's** group compiled sequences of the boundaries of introns from a large number of protein coding eukaryotic genes (not ribosomal RNA or tRNA genes), which revealed the presence of *consensus sequences* at the intron-exon junctions. Of these GT (guanine-thymine) was always found at the 5′ side of the intron (**left splice junction**) and AG (adenine-guanine) at the 3′ side (**right splice junction**). This became popularly known as **GT-AG rule** or **Chambon's rule** (*Note:* Rarely, AT-AC occur at intron-exon junctions). Some of the diseases (*e.g.*, thalessemia) are caused by mutations, which created or abolished these splice junctions.

### Methods of Investigations of Split Genes

A detailed study was conducted on **ovalbumin gene** found in chicken. This ovalbumin gene is responsible for synthesis of a protein, called **ovalbumin** consisting of 386 amino acids and synthesized only by highly specialized tubular gland cells of the oviduct at the time when the hen is laying eggs. The expression of this ovalbumin gene is controlled by some female sex hormones. Chambon and his collegues synthesized artificial ovalbumin gene in order to study its regulation. Such an artificial gene of ovalbumin could be synthesized by using ovalbumin mRNA which could give rise to cDNA (complementary DNA) with the help of enzyme **reverse transcriptase**. This cDNA was inserted into a plasmid and cloned in *E.coli* for its multiplication. When this cDNA was compared with corresponding genomic DNA, it was discovered (through DNA hybridization) that the genomic DNA had additional intervening sequences.

Another ingenious technique used for the study of split gene was the use of **restriction enzymes** which have the property of cleaving DNA at unique sites. More than 100 such restriction endonuclease enzymes are

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now available. In view of this, restriction endonucleases could be used to find out the presence or absence of a certain sequence in a particular gene. For example, when *EcoR1* and *Hind III* enzymes were used with cDNA for ovalbumin, it was found that no cleavage occurred suggesting that the sequences of six base pairs each recognized by these two restriction enzymes were absent. On the basis of this it was expected that if the DNA extracted from oviduct was cleaved by utilizing these two enzymes, the ovalbumin gene will not be broken and the cleavage will occur at other places thus making it possible to isolate ovalbumin gene from the living cells. This DNA segment representing ovalbumin gene was expected to be separated with the help of hybridization with cDNA artificially synthesized. When such hybridization was done with cDNA hybridized with diffferent fragments of DNA rather than with a single fragment. Hybridization between the single stranded DNA having the gene for ovalbumin and its mRNA also showed the formation of distinct loops at specific sites as observed in electron microscope. Such DNA which exists in the loops is obviously missing in mRNA. This kind of conclusion eventually led to the discovery of split genes in 1977.

In subsequent years, it could be proved that split genes are present at least in two more cases, *i.e.*, gene for β–**globin** (a component of haemoglobin molecule) in rabbit and mouse and **immunoglobin gene** (antibody gene). Later on, split genes were reported to occur in higher organisms as a common phenomenon. For understanding the structure of a split gene, we can consider an example of a hypothetical split gene which has a DNA sequence including three pieces, called **exon 1**, **exon 2** and **exon 3**. These three exons are separated by two long intervening sequences, called **intron A** and **intron B**. The terms exon and intron were used by **Walter Gilbert** (1985) for the first time and are being followed ever since.

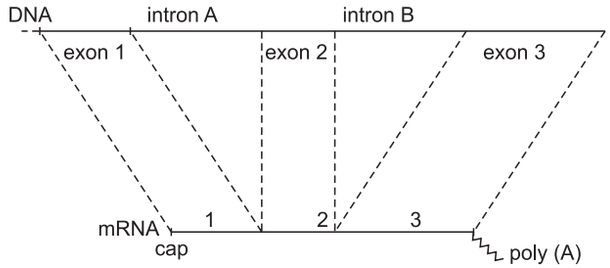


***Fig. 4.5*** *Diagrammatic representation of a hypothetical split gene having three exons (exon 1, exon 2 and exon 3) and two introns (intron A and intron B) and its relationship with mRNA. There are no sequences in mRNA corresponding to those in two introns.*

Figure 4.5 shows that in the transcription of DNA, the ultimate product, *i.e.*, mRNA had only those sequences which correspond to exons, the sequences representing introns being entirely absent. In subsequent years, it is discovered that both exons and introns are first transcribed and this primary transcript in then modified. The sequences corresponding to introns are removed from the transcript and the sequences corresponding to exons are joined together, in correct order to give rise to mRNA. A generalization

has been made that the order of exons on DNA is the same as the order in which they are found in the processed mRNA.

Examples of split genes or interrupted genes occur in a variety of organisms: (*i*) nuclear genes for proteins; (*ii*) nuclear genes for rRNAs; (*iii*) nuclear genes for tRNAs; (*iv*) mitochondrial genes in yeast; (*v*) chloroplast genes in a wide variety of plants; (*vi*) genes in archaebaceria and (*vii*) genes in bacteriophages of *E.coli*. They were initially believed to be entirely absent in eubacterial genomes but in 1990s introns were discovered even in eubacteria.

### B. Examples of Split Genes

**1. Structure of Ovalbumin Split Gene of Hen (Chicken):** In the split gene of ovalbumin there are eight exons and seven intron (Fig. 36.3 and Fig. 36.4). The size of ovalbumin gene is 7,700 base pairs long although the mature mRNA for ovalbumin is only 1,872 nucleotides. The entire ovalbumin gene with its 7700 base pairs is first transcribed to a precursor RNA to which a cap consisting of 7 mG is added at the 5′ end and poly A tail is added at the 3′ end. After the addition of cap and tail, five introns are excised in the first step and the remaining two in second step. The exons produced due to splicing are then joined with the help of enzyme ligase to produce the mature mRNA of ovalbumin gene.

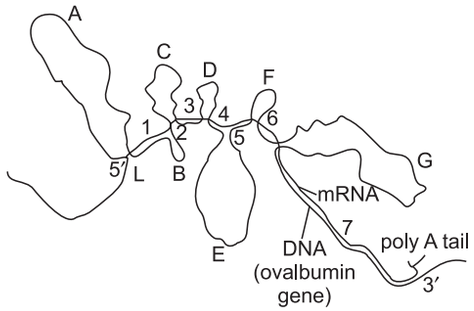


***Fig. 4.6*** *The map of electron micrograph prepared after hybridizing mRNA (thin line) of the ovalbumin gene with its single stranded DNA (thick line). The loops A-G represent 7 different introns and segments L and 1-7 represent 8 exons.*

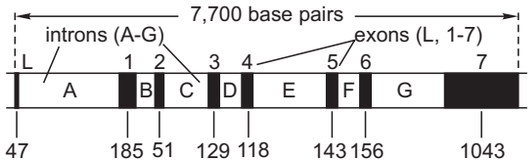


**Fig. 4.7** *Schematic representation of ovalbumin gene showing 7 introns (A-G) and 8 exons (I, 1-7); number of base pairs in each exon are also given.*

**2. Split genes in humans:** In the human gene for the **cystic fibrosis** transmembrane regulator, the introns are much longer than the exons. This gene is 250 kb long and is split into 24 exons and 23 introns. The average length of the exons is 227 bp, so all the exons added together make up only 2.4% of the gene. These exons are scattered throughout the entrie length of gene, separated by introns that range in size from 2 to 35 kb (see **Brown**, 1998).

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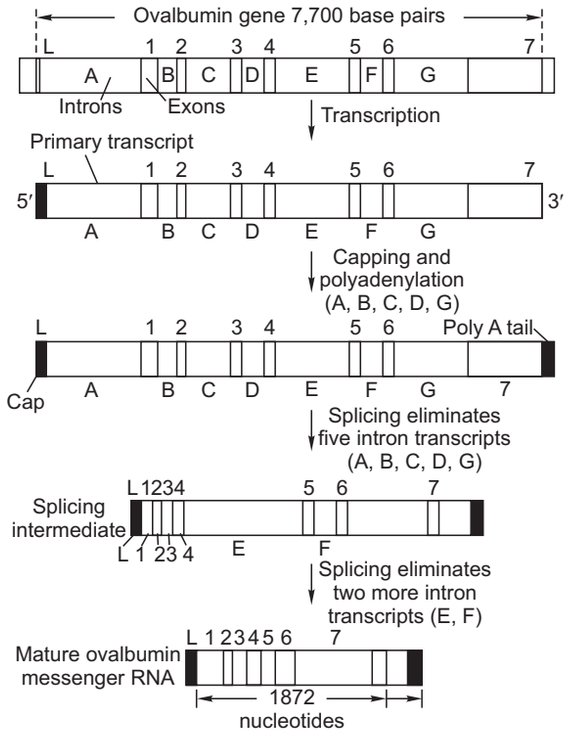


***Fig. 4.8*** *Steps involved in the production of ovalbumin mRNA (1872 nucleotides long) from ovalbumin gene (7700 base pairs long); introns (A-G) and exons (L and 1-7)*

**3. Split Genes in Fungal Mitochondria:** Split genes are also found in the mitochondria. Introns of these split genes in fungal mitochondria are of two types: (*i*) **Group I introns** which are found in majority of the fungal mitochondria split genes, do not carry any conserved sequence, called **internal guide sequence**. By internal pairing, this guide sequence brings the two intron-exon junctions together and help in splicing out the introns. These group I introns are also found in the nuclear gene coding for rRNA in *Tetrahymena* (a ciliate) and *Physarum* (a slime mold). Features similar to those of group I introns are also reported from introns of phage T4 genes. (*ii*) **Group II introns** resemble nuclear genes and have consensus sequences (GT and APy) and a branch sequence that resembles the TACTAAC box. These introns are excised as lariats.

**4. Split Genes in Chloroplasts:** Split genes for ribosomal RNA (rRNA), transfer RNAs (tRNA) and some proteins have also been reported in the chloroplast genomes of several plants including *Chlamydomonas* and *Nicotiana*. Introns found in chloroplast genes can be classified into three groups on the basis of intron boundary sequences. (*i*) *Group I introns* (*e.g.*, in *trnL*) can be folded in a secondary structure similar to self-splicing rRNA precursor of *Tetrahymena*. These can be removed either by self-splicing or by a "**maturase**" enzyme (as in cytochrome b and cytochrome oxidase mRNA precursors). (*ii*) **Group II introns** (*e.g.*, majority of genes including *trnA* and *trnI*) can be folded into a complex secondary structure (as in introns of mitochondrial genes for cytochrome oxidase in maize and yeast). (*iii*) **Group III introns** (*e.g.*, *trnG*, *trnK*, *trnV*, *rpl2*, *rps12*, *rps16*, etc.) have conserved sequences at their borders (GTGCGNY at 5′ end, and ATCNRYY(N)YYAY

at 3′ end), similar to those in the eukaryotic nuclear genes (R = purine; Y = pyrimidine; N = any nucleotide).

Although introns are generally absent in protein-coding genes of bacteria, archaebacteria and some lower eukaryotes, class II type of self-splicing introns have recently been reported even in bacteria. Such a distribution of introns has been used for a study of the role of introns in the evolution of genes or alternatively for a study of the origin of genes or alternatively for a study of the origin of introns themselves.

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### 4.3.6 Transposons

Transposable elements were discovered by B. McClintock through an analysis of genetic instability in maize. The instability involved chromosome breakage and was found to occur at sites where transposable elements were located. Breakage events were detected by following the loss of certain genetic markers. McClintock used a marker that controlled the deposition of pigmentation in the aleurone, the outermost layer of the endosperm of maize kernels. The endosperm triploid being produced by the union of two maternal nuclei and one paternal nucleus. McClintock marker was an allele of the C locus on the short arm of chromosome 9.

**Transposable Elements in Bacteria**

Genetic instabilities have been found in bacteria and in many cases have led to the identification of transposable elements. These transposons were the first to be studied at the molecular level and provided important clues about organization and behavior of eukaryotic transposons. The simplest bacterial transposons are the insertion sequences or IS elements. These are less than 1500 nucleotide pairs long and contain only genes involved in promoting and regulating transposition. Two homologous IS elements combine with other genes to form a composite transposon denoted by Tn. The integrating bacteriophage is also considered to be a transposable element because it can insert itself into bacterial chromosome. This phage represents the upper limit of transposon size and contains many genes that are not necessary for the insertion behavior.

**IS elements**

They are compactly organized. There is a single coding sequence with short, identical or nearly identical sequences at both ends. These terminal sequences are always in inverted orientation with respect to each other, so they are called inverted terminal repeats. Their lengths range from 9 to 40 nucleotide pairs.

When IS elements insert into chromosomes or plasmids, they create a duplication of the DNA sequence at the site of insertion. One copy of the duplication is located on each side of the element. These short (3-12 nucleotide pairs) directly repeated sequences are called target site duplication and thought to arise from staggered breaks in double stranded DNA. IS elements mediate the integration of episomes into bacterial chromosomes. This process involves homologous recombination between IS elements located in the episome and in the chromosome. Composite transposons are created when two IS elements insert near each other. The sequence between them can be transposed by the joint action of the flanking elements.

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## Medical Significance of Bacterial Transposons

Bacterial transposons are responsible for the transposition of genes controlling resistance to antibiotics from one molecule to another. They are believed to play a role in the rapid evolution of R plasmid. All conjugative R plasmid have at least two components, one segment carrying a set of genes involved in conjugative DNA transfer and a second segment carrying antibiotic and drug resistance gene. The segment carrying the transfer genes is called resistance transfer factor component, the segment carrying the resistance gene or genes is called R determinant. The transmissibility of R plasmids, the transposability of the R determinants and the rapid evolution of compound R plasmids which carry genes for resistance to a whole battery of our most effective antibiotics and drugs are of great concern to medical practitioners. Not only are these plasmids rapidly dispersed within a bacterial species but they are also transmitted across species. For example, E. coli R plasmids are known to be transferred to several genera including Proteus, Salmonella, Haemophilus, Pasteurella and Shigella all of which include pathogenic species.

## Transposable Elements in Eukaryotes

**Yeast TY Elements:** The yeast carries Saccharomyces cerevisiae carries about 35 copies of a transposable element called Ty in its haploid genome. These transposons are about 5900 nucleotide pairs long and are bounded at each end by a DNA segment, which is 340 base pairs long.

The genetic organization of the Ty elements resembles that of eukaryotic retroviruses. These single stranded RNA virus synthesizes DNA from their RNA after entering a cell. The DNA then inserts itself into a site in the genome, creating a target site duplication. The inserted material has the same over all structure as a yeast Ty element and is called provirus.

**Maize Transposons:** Transposable elements have been found in several plants, maize and snapdragon. The most extensive investigation involves maize, in which several transposons families have been identified.

**Ac and Ds Elements:** The Ac/ Ds family of maize discovered by McClintock comprises numerous elements scattered throughout the genome. Molecular studies have shown that the functionally autonomous element Ac consists of 4563 nucleotide pairs bounded by an 8 nucleotide pair direct repeat.

**Drosophila Transposons:** Transposable elements have been discovered in many animals but best information comes from the studies with Drosophila in which as much as 15 % DNA is mobile. Several classes of Drosophila transposons have been identified.

**Retrotransposons:** The largest group of Drosophila transposons comprises the retrovirus like elements or retrotransposons. These elements are 5000 to 15000 nucleotides pairs long and resemble the integrated forms of retrovirus much like TY elements of yeast. When retrotransposons inserts into a chromosome, it creates a target site duplication with one copy on each side of the transposon. The size of this duplication is characteristic of each retrotransposon family.

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Transposable elements also produce chromosomal breakage. This is demonstrated by the behavior of the double Ds elements in maize and by the P elements in Drosophila. Breaks can lead to the loss or rearrangement of chromosomal material. Sometimes transposable elements mediate recombination events between DNA molecules. For example, IS mediated insertion of F plasmids into E. coli chromosome. Another is the structural rearrangement of X chromosomes in Drosophila following recombination between homologous transposons that are located in different positions.

**Use in Genetic Analysis**

The natural ability of transposable element to cause mutations has been harnessed in the laboratory. In several organisms, it is feasible to stimulate the transposition of a particular family of elements, thereby increasing mutation rate. This procedure has an advantage over traditional methods of inducing mutations because a transposable element that has caused mutation by inserting into a gene can serve as a landmark. This feature is seen in Drosophila, in which the technique of in-situ hybridization can be used to locate the site of a transposon insertion. In this technique, radioactively labeled transposon sequences are made single stranded and then hybridized to single stranded DNA in the giant chromosomes of the salivary glands. The hybridization reaction takes place on the surface of a microscopic slide, where the chromosomes have been spread by squashing dissected glands. When the hybridization reaction is completed, the location of the radioactive sequences can be determined by autoradiography.

The four transposable genetic elements in prokaryotes are:

- Bacterial Insertion Sequences
- Prokaryotic Transposons
- Insertion-Sequence Elements and Transposons in Plasmids
- Phage mu

**Bacterial Insertion Sequences**

Let us study the bacterial insertion sequences in detail.

**Insertion Sequences or Insertion-Sequence (IS) Elements:** Insertion sequences, or Insertion-Sequence (IS) elements, are now known to be segments of bacterial DNA that can move from one position on a chromosome to a different position on the same chromosome or on a different chromosome. An IS element contains only genes required for mobilizing the element and inserting the element into a chromosome at a new location. Is elements are normal constituents of bacterial chromosome and plasmids.

When IS elements appear in the middle of genes, they interrupt the coding sequence and inactivate the expression of that gene. Owing to their size and in some cases the presence of transcription and translation termination signals, IS elements can also block the expression of other genes in the same operon if those genes are downstream from the promoter of the operon.

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IS elements were first found in E. coli as a result of their affects on the expression of a set of three genes whose products are needed to metabolize the sugar galactose as a carbon source. Careful investigations showed that the mutant phenotypes resulted from the insertion of an approximately 800 base pairs (bp) DNA segment into a gene. This particular DNA segment is now called Insertion Sequence l (IS 1).

**Properties of IS Elements**

Is1 is the genetic element capable of moving around the genome. It integrates into the chromosome at locations with which it has no homology, thereby distinguishing it from recombination. This event is an example of transposition event. There are number of IS elements that have been identified in E. coli, including IS1, IS2, and IS 10, each present in 0 to 30 copies per genome, and each with a characteristic length and unique nucleotide sequence.

IS 1 is 768 bp long, and is present in 4 to 19 copies on the E. coli chromosomes. IS2 is present in 0 to 12 copies on the E. coli chromosome and in one copy on the F plasmid, and IS 10 is found in a class of plasmids called R plasmid that can replicate in E. coli.

Among prokaryotes, the IS elements are normal cell constituents, that is, they are found in most cells. Altogether, IS elements constitute approx. 0.3% of the cell's genome. All IS elements that have been sequenced, end with perfect or nearly perfect Inverted Terminal repeats (IRs) of between 9 and 41 bp. This means that essentially the same sequence is found at each end of an IS but in opposite orientations.

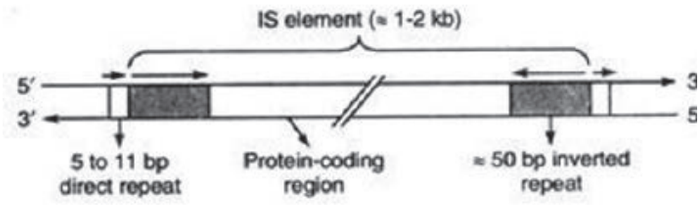


***Fig. 4.9*** *Structure of Bacterial IS Elements*

**IS Transposition**

When transposition of an IS element takes place, a copy of the IS element inserts into a new chromosome location while the original IS elements remains in place. That is, transposition requires the precise replication of the original IS element, using the replication enzymes of the host cell. The actual transposition also requires an enzyme encoded by the IS element called transposase.

The IR sequences are essential for the transposition process, that is, those sequences are recognized by transposase to initiate transposition. Is elements insert into the chromosomes at sites with which they have no sequence homology?

Genetic recombination between non-homologous sequences is called illegitimate recombination. The sites into which IS elements insert are called

target sites. The process of IS insertion into a chromosome. Firstly, a taggered cut is made in the target site and the IS element is then inserted, becoming joined to the jingle-stranded ends.

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The gaps are filled in by DNA polymerase and DNA ligase, producing an integrated IS element with two direct repeats of the target site sequence flanking the IS element. 'Direct' in this case means that the two sequences are repeated in the same orientation. The direct repeats are called target site duplications. The sizes of target site duplication vary with the IS elements, but tend to be small. Integration of some IS elements show preference for certain regions, while others integrate only at particular sequences.

All copies of a given IS element have the same sequence, including that of the inverted terminal repeats. Mutations that affect the inverted terminal repeat sequence of IS elements affect transposition, indicating that the inverted terminal repeat sequences are the key sequences recognized by transposase during a transposition event (Refer Figure 4.10).

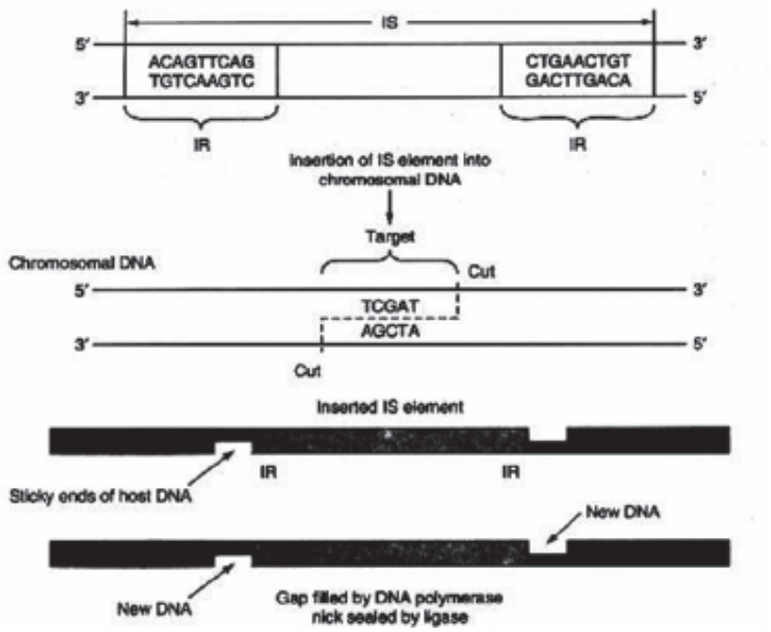


***Fig. 4.10*** *Schematic Representation of the Integration of an IS Elements into Chromosomal DNA*

## Prokaryotic Transposons

A transposon (Tn) is more complex than an IS elements. A transposon is a mobile DNA segment that, like an IS element, contains genes for the insertion of the DNA segment into the chromosome and for the mobilization of the element to other locations on the chromosome. There are two types of prokaryotic transposons: composite transposons and non-composite transposons.

**Composite Transposons:** They are complex transposons with a central region containing genes, for example drug resistance genes, flanked on both sides by IS elements (also called IS modules). Composite transposons may be thousands of base pairs long. The IS elements are both of the same types

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and are called IS-L (for 'left') and IS-R (for 'right'). Depending upon the transposon, IS-L and IS-R may be in the same or inverted orientation relative to each other. Because the ISs themselves have terminal inverted repeats, the composite transposons also have terminal inverted repeats.

The Tn 10 transposon is 9,300 bp long and consists of 6,500 bp of central, nonrepeating DNA containing the tetracycline resistance gene flanked at each end with a 1,400-bp IS element. These IS elements are designated IS10L and IS10R and are arranged in an inverted orientation. Cells containing Tn 10 are resistant to tetracycline resistance gene contained within the central DNA sequence.

Transposition of composite transposon occurs because of the function of the IS elements they contain. One or both IS element supplies the transposase. The inverted repeats of the IS elements at the two ends of the transposon are recognized by transposase to initiate transposition (as with transposition of IS elements).

Transposition of Tn 10 is rare, occurring once in 10 cell generations. This is the case because less than one transposase molecule per cell generation is made by Tn 10 (Refer Figure 4.11). Like IS elements, composite transposons produce target site duplications after transposition.

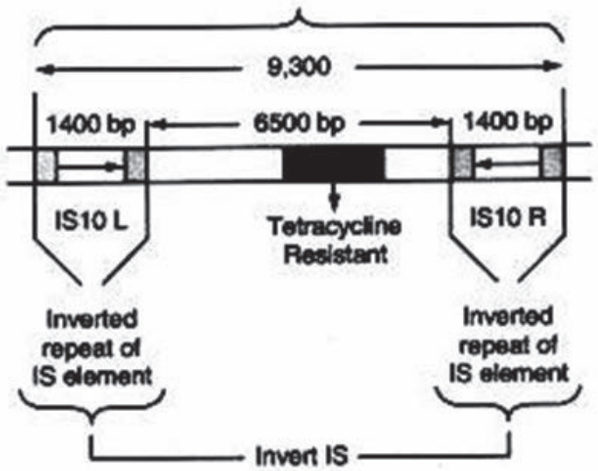


***Fig. 4.11*** *Detailed Structure of Tn10 Transposon*

**Non-Composite Transposons:** They like composite transposons, contain genes, such as those for drug resistance. Unlike composite transposons, they do not terminate with IS elements. However, they do have the repeated sequences at their ends that are required for transposition. Tn3 is a non-composite transposon.

Tn3 has 38 bp inverted terminal repeats and contains three genes in its central region. One of those genes, bla, i.e., b-lactam-resistant, encodes b-lactamase which breaks down ampicillin and therefore makes cells containing Tn3 resistant to ampicillin. The other two genes, transposase A (tnpA) and Transposase B (tnpB), encode the enzymes transposase and resolvase that are needed for transposition of Tn3. Transposase catalyzes insertion of the Tn into new sites, and resolvase is an enzyme involved in the particular re-combinational events associated with transposition.

Resolvase is not found in all transposons. The genes for transposition are in the central region for non-composite transposons, while they are in the terminal IS elements for composite transposons. Non-composite transposons also cause target site duplications when they move.

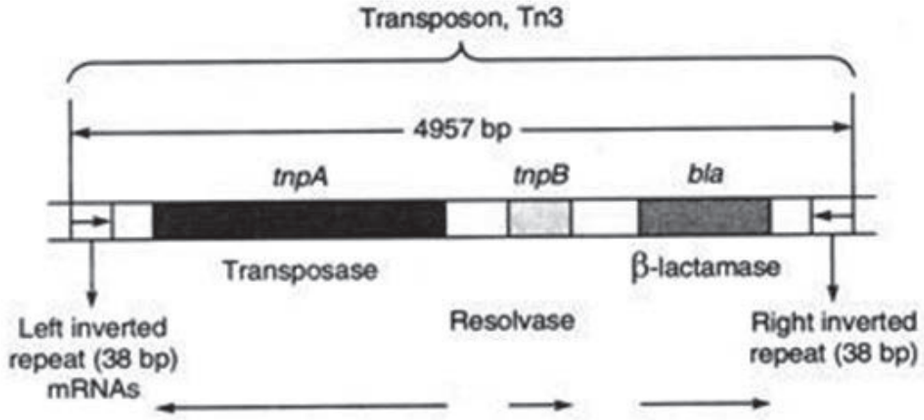


***Fig. 4.12*** *Detailed Structure of Tn3 Transposon*

**Mechanism of Transposition in Prokaryotes**

Several different mechanisms of transposition are employed by prokaryotic transposable elements. And, as we shall see later, eukaryotic elements exhibit still additional mechanisms of transposition. In E. coli, we can identify replicative and conservative (non-replicative) modes of transposition. In the replicative pathway, a new copy of the transposable element is generated in the transposition event. The results of the transposition are that one copy appears at the new site and one copy remains at the old site. In the conservative pathway, there is no replication. Instead, the element is excised from the chromosome or plasmid and is integrated into the new site

**Replicative Transposition:** The transposition of Tn3 occurs in two stages. Firstly, the transposase mediates the fusion of two molecules, forming a structure called cointegrate. During this process, the transposon is replicated, and one copy is inserted at each junction in cointegrate. The two Tn3 are oriented in the same direction. In the second stage of transposition, the tnpR-encoded resolvase mediates a site-specific recombination event between the two Tn3 elements. This event occurs at a sequence in Tn3 called res, the resolution site, and generates two molecules, each with a copy of the transposon.

The tnpR gene-product also has another function, namely, to repress the synthesis of both the transposase and resolvase proteins. This repression occurs because the res site is located in between the tnpA and tnpR genes. By binding to this site, the tnpR protein interferes with the synthesis of both gene-products, leaving them in chronic short supply. Consequently, the Tn3 element tends to remain immobile.

**Conservative Transposition:** Some transposons, such as Tn10, excise from the chromosome and integrate into the target DNA. In these cases, DNA replication of the element does not occur, and the element is lost from the site of the original chromosome. This mechanism is called conservative (non-

replicative) transposition or simple insertion. Tn 10, for example transposes by conservative transposition.

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Insertion of a transposon into the reading frame of a gene will disrupt it, causing a loss of function of that gene. Insertion into gene's controlling region can cause changes in the level of expression of the gene. Deletion and insertion events also occur as a result of activities of the transposons, and from crossing-over between duplicated transposons in the genome.

**IS Elements and Transposons in Plasmids**

The transfer of genetic material between conjugating E. coli is the result of the function of the fertility factor F. The F factor, a circular double stranded DNA molecule, is one of the example of bacterial plasmid. Plasmids, such as F that are also capable of integrating into the bacterial chromosomes are called episomes. F factor consists of 94,500 bp of DNA that code for a variety of proteins.

The important elements are:

- Transfer gene (tra) required for the conjugation transfer of the DNA.
- Genes that encode proteins required for the plasmid's replication,
- Four IS elements, two copies of IS3, one of IS2, and one of an insertion sequence element called gamma- delta.

It is because the E. coli chromosome has copies of these four insertion sequence at various positions that the F factor can integrate into the E. coli chromosome at different sites and in different orientations with homologous sequence of the insertion elements.

Another class of plasmids that has medical significance is the R plasmid group, which was discovered in Japan in the 1950s, during the cure for dysentery. The disease is the result of infection by the pathogenic bacterium Shigella. Shigella was found to be resistant to most of the commonly used antibiotics.

Subsequently, they found that the genes responsible for the drug resistances were carried on R plasmids, which can promote the transfer of genes between bacteria by conjugation, just as the F factor. One segment of an R plasmid that is homologous to a segment in the F factor is the part needed for the conjugal transfer of genes.

That segment and the plasmid-specific genes for DNA replication constitute what is called the RTF (Resistance Transfer Factor) region. The rest of the R plasmid differs from type to type and includes the antibiotic-resistance genes or other types of genes of medical significance, such as resistance to heavy metal ions. The resistance genes in R plasmid are, in fact, transposons, that is each resistance gene is located between flanking, directly repeated segments, such as one of the IS modules. Thus, each transposon with its resistance gene in the R plasmid can be inserted into new location on other plasmids or on the bacterial chromosome, while at the same time leaving behind a copy of itself in the original position.

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**Phage mu:** Phage mu is a normal-appearing phage. We consider it here because, although it is a true virus, it has many features in common with IS elements. The DNA double helix of this phage is 36,000 nucleotides long-much larger than an IS element. However, it does appear to be able to insert itself anywhere in a bacterial or plasmid genome in either orientation. Once inserted, it causes mutation at the locus of insertion-again like an IS element. (The phage was named for this ability: mu stands for 'mutator'.)

Normally, these mutations cannot be reverted, but reversion can be produced by certain kinds of genetic manipulation. When this reversion is produced, the phages that can be recovered showing no deletion, proving that excision is exact and that the insertion of the phage therefore does not involve any loss of phage material either. Each mature phage particle has on each end a piece of flanking DNA from its previous host. However, this DNA is not inserted anew into the next host. Its function is unclear. Phage mu also has an IR sequence, but neither of the repeated elements is at a terminus.

Mu can also act like a genetic snap fastener, mobilizing any kind of DNA and transposing it anywhere in a genome. For example, it can mobilize another phage, such as l or the F factor. In such situations, the inserted DNA is flanked by two mu genomes.

**Check Your Progress**

5. According to their external shape, which are the two classes of bacteria?
6. What are nucleosomes?
7. What is a palindromic sequence?
8. Define split genes.
9. How are the bacterial transposons responsible for the transposition of genes?

## 4.4 GENE CONCEPTS

In biology, a gene is a basic unit of heredity and a sequence of nucleotides in DNA that encodes the synthesis of a gene product, either RNA or protein. Gene, is a unit of hereditary information that occupies a fixed position (locus) on a chromosome. Genes achieve their effects by directing the synthesis of proteins. **Seymour Benzer** in 1950's coined the term *cistron* for the smallest genetic unit (length of genetic material) that exhibits a *cis-trans* position effect. We thus have a new word for the gene, one in which function is more explicit. We have, in principle, refined **Beadle** and **Tatum's** one-gene-one-enzyme hypothesis to a more accurate one-cistron-one-polypeptide concept. The cistron is the smallest unit that codes for a messenger RNA that is then translated into a single polypeptide or expressed directly (transfer RNA or ribosomal RNA).

**Definition:** The functional gene has been called cistron by Benzer because it is a chromosomal segment within which the cis-trans effect operates. The cistron represents a segment of DNA molecule, and consists of a linear sequence of nucleotides which control some cellular functions.

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The cistron starts with an initiation codon and ends with a terminating codon.

**Fine structure mapping:** After Beadle and Tatum established in 1914 that a gene controls the production of an enzyme that then controls a step in biochemical pathway, **Benzer** used analytical techniques to dissect the fine structure of the gene. Fine structure mapping means examining the size and number of sites within a gene that are capable of mutation and recombination. In the late 1950s (*i.e.*, 1955, 1956), when biochemical techniques were not available for DNA sequencing, Benzer used classical recombinational and mutational techniques with bacterial viruses (*i.e.*, rII locus of $T_4$ bacteriophage) to provide reasonable estimates on the details of fine structure and to give insight into the nature of the gene. He coined the terms **muton** for the smallest mutable site and **recon** for the smallest unit of recombination. It is now known that both muton and recon are a single base pair (**Tamarin**, 2002).

**Loci and Alleles.** A gene occupies a definite position within the chromosome. This position is called the **locus** (plural **loci**) (**Demerec** 1955). Chromosomes exist in homologous pairs, each cell contains two kinds of genes which are found in pairs. The two members of a pair of a gene are called **alleles**.

**Gene Regulation. Jacob** and **Monod** (1961) proposed the **operon model** to explain the control of gene activity. It was assumed that there are three kinds of genes: *structural genes*, *operator genes* and *regulator genes*. **Structural gens** are those genes that produce mRNA. **Operator genes** control structural genes by acting as switches in controlling mRNA synthesis by the latter. **Regulator genes** produce substances, called **repressors** which block the operator genes, so that mRNA synthesis by structural genes is prevented. The structural and operator genes, both combine to form a **operon**.

**Overlapping Genes (Genes within Genes)**

According to "one gene, one protein hypothesis" of **Beadle** and **Tatum** (1937–40) each gene is responsible for the coding of one enzyme (protein)/ polypeptide. **Barrell** and coworkers (1976) first gave evidence that suggested the possibility of overlapping of genes in the bacterial virus (= bacteriophage) ϕ×174. The bacteriophage consists of an icosahedral protein capsid containing single-stranded circular DNA. Genetic mapping techniques have shown that the bacteriophage has 9 genes (arranged in the order A–B–C–D–E–J–F–G–H) which codes for 9 proteins. The functions of these genes are as follows:

Gene A = DNA replication
Gene B and Gene D = Assembly of phase particle
Gene C = Function not known
Genes F, G and H = structural proteins of capsid

**Barrell** *et al.*, presented evidence that the proteins coded by genes D and E are specified by the same segment of DNA.

**Fredrick Sanger** and his colleagues (1976, 1977) have mapped the entire nucleotide sequence of ϕ × 174 and phage $G_4$ DNA. The ϕ × 174 DNA contains 5,386 nucleotides. On the basis of triplet code this number should

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code for a maximum of about 1,800 amino acids having a total weight of about 200,000 dalton. Actually, however, the total proteins coded by these nucleotides have a molecular weight of 250,000 daltons. The phage DNA which should have a coding capacity of 5 to 6 average sized protein molecules, in fact code for 9 proteins. The phage DNA is thus apparently 10 to 15% too short for the total protein coded.

A comparison of the DNA base sequence with the amino acid sequences of the protein coded revealed that in two cases the same gene coded for two different proteins. Thus two genes which should normally have coded two proteins actually coded four. Sanger and his coworkers found that gene B (136 nucleotides) was completely contained within gene A (1536 nucleotides). Simiarly, gene E (273 nucleotides) was contained in gene D (1456 nucleotides).

The protein synthesized by gene B is not simply a shorter version of that synthesized by gene A. The two proteins have completely different amino sequnces. Similar is the case with the proteins synthsised by genes D and E. It has been explained that the coding of two proteins by one gene is achieved by a **shift in frame reading**. For example, a reading frame of ...G, AAG, TTA, ACA ... nucleotides codes for the amino acids *lysine*, *leucine* and *threonine*. If the frame is read from one point earlier it becomes ... GAA, GTT, AAC, A ... which codes for *glutamine*, *valine* and *asparagine*. Thus, the same gene can code for two different sequences by a frame shift (overlapping code) and produce two totally different proteins. Since one gene codes for more than one protein is an amendment to the one-gene, one-protein dogma becomes necessary, at least in few cases such as $\phi \times 174$ and G4 phages.

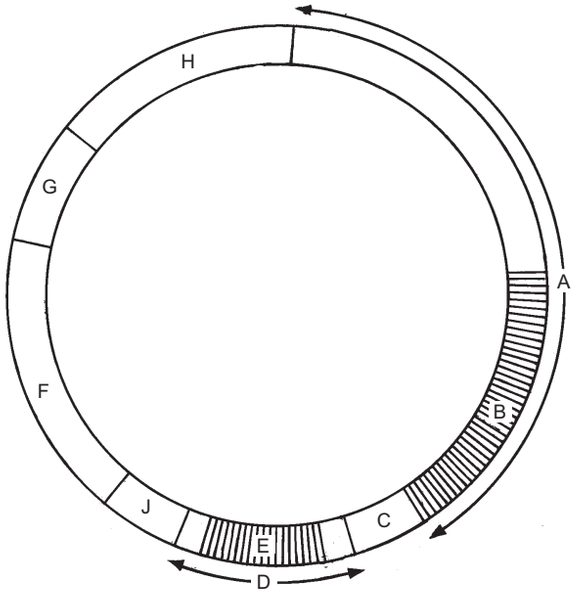


***Fig. 4.13*** *Genetic map of the bacteriophage $\phi \times 174$. Note the overlapping of gene E over D and of gene B over A.*

Another interesting fact that the **termination codon** of gene D and the **initiation codon** of gene J overlap by one nucleotide:

---T—A—A—T—G---

Termination codon of gene D — Initiation codon of gene J

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The bacteriophage G4 which infects *E.coli* also has overlapping genes. Gene B is completely contained within gene A and gene E within gene D. Gene K is made up of the last 86 nucleotides of gene A and the first 89 nucleotides of gene C.

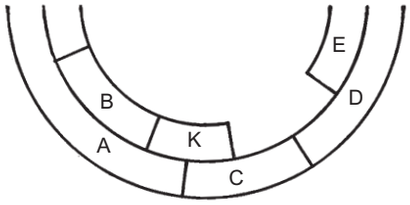


***Fig. 4.14*** *A part of the genetic map of the virus G4 showing overlapping genes.*

The condition of overlapping genes is not of common occurrence. It may have arisen in the smaller viruses because of the limitations of the quantity of DNA that can be enclosed in the small viral capsids. It seems to be an economic device to make better use of genetic information in less DNA.

### 4.4.1 Genetic Code

As DNA is a genetic material, it carries genetic information from cell to cell and from generation to generation. At this stage, an attempt will be made to determine that in what manner the genetic informations are existed in DNA molecule? Are they written in articulated or coded language on DNA molecule? If in the language of codes what is the nature of genetic code?

A DNA molecule is composed of three kinds of moieties: (*i*) phosphoric acid, (*ii*) deoxyribose sugar, and (*iii*) nitrogen bases. The genetic informations may be written in any one of the three moieties of DNA. But the poly-sugar-phosphate backbone is always the same, and it is, therefore, unlikely that these moiteies of DNA molecule carry the genetic informations. The nitrogen bases, however, vary from one segment of DNA to another, so the informations might well depend on their sequences. The sequence of nitrogen bases of a given segment of DNA molecule, actually, has been found to be identical to linear sequence of amino acids in a protein molecule. The proof of such a **colinearity** between DNA nitrogen base sequence and amino acid sequence in protein molecules has first obtained from an analysis of mutants of head protein of bacteriophage $T_4$ (**Sarabhai** *et al.*, 1964) and the A protein of tryptophan synthetase of *Escherichia coli* (**Yanofski** *et al.*, 1964). The colinearity of protein molecules and DNA polynucleotides has given the clue that the specific arrangement of four nitrogen bases (*e.g.*, A, T, C and G) in DNA polynucleotides chains, somehow, determines the sequence of amino acids in protein molecules. Therefore, these four DNA bases can be considered as four alphabets of DNA molecule. All the genetic informations, therefore, should be written by these four alphabets of DNA. Now the question arises that whether the genetic informations are written in articulated language or coded language? If genetic informations might have occurred in an articulated language, the DNA molecule might require various alphabets, a complex system of grammar and ample amount of space on it. All of which might be

practically impossible and troublesome too for the DNA. Therefore, it was safe to conclude for molecular biologists that genetic informations were existed in DNA molecule in the form of certain special language of code words which might utilize the four nitrogen bases of DNA for its symbols. Any coded message is commonly called **cryptogram**.

**Basis of Cryptoanalysis**

The basic problem of such a genetic code is to indicate how information written in a four letter language (four nucleotides or nitrogen bases of DNA) can be translated into a twenty letter language (twenty amino acids of proteins). The group of nucleotides that specifies one amino acid is a **code word** or **codon**. By the **genetic code** one means the collection of base sequences (codons) that correspond to each amino acid and to translation signals. We can consider here the classical but logical reasoning done by **George Gamov** (1954) about the possible size of a codon. The simplest possible code is a **singlet code** (a code of single letter) in which one nucleotide that codes for one amino acid could be specified. A **doublet code** (a code of two letters) is also inadequate, because it could specify only sixteen (4 × 4) amino acids, whereas a **triplet code** (a code of three letters) could specify sixty four (4 × 4 × 4) amino acids. Therefore, it is likely that there may be 64 triplet codes for 20 amino acids. The possible singlet, doublet and triplet codes, which are customarily represented in terms of "**mRNA language**" [mRNA is a complementary molecule which copies the genetic informations (cryptogram of DNA) during its transcription], have been illustrated in Table 4.1.

***Table 4.1*** *Possible Singlet, Doublet and Triplet Codes of mRNA*

| Singlet code (4 words) | Doublet code (16 words) | Triplet code (64 words) |
|---|---|---|
| A<br>G<br>C<br>U | AA AG AC AU<br>GA GG GC GU<br>CA CG CC CU<br>UA UG UC UU | AAA AAG AAC AAU<br>AGA AGG AGC AGU<br>ACA ACG ACC ACU<br>AUA AUG AUC AUU<br>GAA GAG GAC GAU<br>GGA GGG GGC GGU<br>GCA GCG GCC GCU<br>GUA GUG GUC GUU<br>CAA CAG CAC CAU<br>CGA CGG CGC CGU<br>CCA CCG CCC CCU<br>CUA CUG CUC CUU<br>UAA UAG UAC UAU<br>UGA UGG UGC UGU<br>UCA UCG UCC UCU<br>UUA UUG UUC UUU |

The first experimental evidence in support to the concept of triplet code is provided by **Crick** and coworkers in 1961. During their experiments, when they added or deleted single or double base pairs in a particular region of DNA of $T_4$ bacteriophages of *E.coli*, they found that such bacteriophages

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ceased to perform their normal functions. However, bacteriophages with addition or deletion of three base pairs in DNA molecule, had performed normal functions. From this experiment, they concluded that a genetic code is in **triplet** form, because the addition of one or two nucleotides has put the reading of the code out of order, while the addition of third nucleotide resulted in a return to the proper reading of the message.

**Codon Assignment (Cracking the Code or Deciphering the Code)**

The genetic code has been cracked or deciphered by the following kinds of approaches:

**A. Theoretical Approach**

The physicist **George Gamow** proposed the **diamond code** (1954) and the **triangle code** (1955) and suggested an exhaustive theoretical framework to the different aspect of the genetic code. **Gamow** suggested the following properties of the genetic code:

(i) **A triplet codon** corresponding to one amino acid of the polypeptide chain.

(ii) **Direct template translation** by codon-amino acid pairing.

(iii) Translation of the code in an **overlapping** manner.

(iv) **Degeneracy** of the code, *i.e.*, an amino acid being coded by more than one codon.

(v) **Colinearity** of nucleic acid and the primary protein synthesized.

(vi) **Universality** of the code, *i.e.*, the code being essentially the same for different organisms.

Some of these Gamow's proposals have been contradicted by the molecular biologists. For example, **Brenner** (1957) showed that the overlapping triplet code is an impossibility, and subsequent work has shown that the code is a **non-overlapping** one. Similarly, Gamow's idea of direct template relationship between nucleic acid and polypeptide chain was challenged when **Crick** proposed his **adaptor hypothesis**. According to this hypothesis, **adaptor molecules** intervene between nucleic acid and amino acids during translation. In fact, it is now known that tRNA molecules act as adaptors between codons of mRNA and amino acids of the resulting polypeptide chain.

**B. The *in vitro* Codon Assignment**

**1. Discovery and Use of Polynucleotide Phosphorylase Enzyme:** **Marianne Grunberg-Manago** and **Severo Ochoa** isolated an enzyme from the bacteria (*e.g.*, *Azobacter vinelandii* or *Micrococcus lysodeikticus*) that catalyzes the breakdown of RNA in bacterial cells. This enzyme is called **polynucleotide phosphorylase**. **Manago** and **Ochoa** found that outside of the cell (*in vitro*), with high concentrations of ribonucleotides, the reaction could be driven in reverse and an **RNA** molecule could be made (see **Burns** and **Bottino**, 1989). Incorporation of bases into the molecule is random and does not require a DNA template. Thus, in 1955 **Manago** and **Ochoa** made

possible the artificial synthesis of polynucleotides = (mRNA) containing only a single type of nucleotides (U, A, C, or G respectively) repeated many times.

***Table 4.2*** *Configuration of Polynucleotide*

| | Polynucleotide | Configuration |
|---|---|---|
| 1. | Polyuridylic acid or poly (U) | UUUUUU |
| 2. | Polyadenylic acid or poly (A) | AAAAAA |
| 3. | Polycytidylic acid or poly (C) | CCCCCC |
| 4. | Polyguanidylic acid or poly (G) | GGGGGG |

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Thus, the action of polynucleotide phosphorylase can be represented in the following way:

$$(RNA)_n + \text{Ribonucleoside diphosphate} \underset{\text{polynucleotide phosphorylase}}{\rightleftharpoons} (RNA)_{n+1} + Pi$$

The polynucleotide phosphorylase enzyme differs from RNA polymerase used to transcribe mRNA from DNA in that: (*i*) it does not require a template or primer; (*ii*) the activated substrates are ribonucleoside diphosphates (*e.g.*, UDP, ADP, CDP and GDP) and not triphosphates; and (*iii*) orthophosphate (*Pi*) is produced instead of pyrophosphates (*PPi*).

The deciphering of the genetic code was made possible by the use of synthetic (or artificial) polynucleotides and trinucleotides. The different types of techniques used include the use of polymers containing a single type of nucleotide (called **homopolymers**), the use of mixed polymers (**copolymers**) containing more than one type of nucleotides (**heteropolymers**) in random or defined sequences and the use of trinucleotides (or "**minimessengers**") in ribosome-binding or filter-binding.

**2. Codon Assignment with Unknown Sequence: (*i*) Codon Assignment by Homopolymer:** The first clue to codon assignment was provided by **Marshall Nirenberg** and **Heinrich Matthaei** (1961) when they used *in vitro* system for the synthesis of a polypeptide using an artificially synthesized mRNA molecule containing only one type of nucleotide (*i.e.*, homopolymer). Prior to performing the actual experiments, they tested the ability of a cell-free protein synthesizing system to incorporate radioactive amino acids into newly synthesized proteins. Their cell-free extracts of *E.coli* contained ribosomes, tRNAs, aminoacyl-tRNA synthetase enzymes, DNA and mRNA.The DNA of this extract was eradicated by the help of **deoxyribonuclease** enzyme, thus, the template which might synthesize new mRNA was destroyed. When twenty amino acids were added to this mixture along with ATP, GTP, $K^+$ and $MG^{2+}$, they were incorporated into proteins. This incorporation continued so long as mRNA was present in such a cell-free suspension. It also continued in the presence of synthetic polynucleotides (mRNAs) which could be made with the help of polynucleotide phosphorylase enzyme.

The first successful use of this technique was made by **Nirenberg** and **Matthaei** who synthesized a chain of uracil molecules (poly U) as their synthetic mRNA (homopolymer). Poly (U) seemed a good choice; because there could be no ambiguity in a message consisting of only one base. Poly

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(U) was a good choice for other reasons: it binds well to ribosomes and, as it turned out, the product protein was insoluble and easy to isolate. When poly (U) was presented as the message to the cell free system containing all the amino acids, one amino acid was exclusively selected from the mixture for incorporation into the polypeptide, called **polyphenylalanine**. This amino acid was phenylalanine and it could be concluded that some sequence of UUU coded for phenylalanine. Other homogeneous chains of nucleotides (Poly A, Poly C and Poly G) were inactive for phenylalanine incorporation. The mRNA code word for phenylalanine was, therefore, shown to be UUU. Thus, the first code word to be deciphered was UUU.

This discovery was extended in the laboratories of **Nirenberg** and **Ochoa**. The experiment was repeated using synthetic **poly (A)** and **poly (C)** chains, which gave **polylysine** and **polyproline** respectively. Thus, AAA was identified as the code for lysine and CCC as the code for proline. A poly (G) message was found non-functional *in vitro*, since it attains secondary structure and, thus, could not attach the ribosomes. In this way three of 64 codons were easily accounted for.

**(*ii*) Khorana's Copolymers: Dr. H.G. Khorana** devised an ingenious technique for codon assignment by using synthetic DNA. **Har Gobind Khorana** (b. 1922) is a India born US biochemist who shared with Holley and Nirenberg the 1968 Nobel Prize for Physiology or Medicine toward deciphering the Genetic code.

Khorana and his coworkers could prepare polyribonucleotides (RNA) with **known repeating sequences**. A repeating sequence means that, if CU are two bases, these will be repeatedly present throughout the length as follows:

CUCUCUCUCUCUCU

In a similar manner, if ACU are three bases, they will be present repeatedly as follows:

ACUACUACUACUACU

Such copolymers will direct the incorporation of amino acids in a manner which can be theoretically predicted. For instance, in (CU) coplymer: CUC/UCU/CUC/UCU only two codons are possible and these are CUC and UCU. These codon are present in alternating sequence. The result would be the polypeptide formed would have only two amino acids in alternating sequences, *i.e.*, leucine, serine (Table 4.3).

***Table 4.3*** *Assignment of codons, having known sequences, with the help of copolymers having repetitive sequences of two bases.*

| | Copolymers used | Codons in copolymers | Amino acids incorporated | Codon assigned |
|---|---|---|---|---|
| 1. | (CU)n | CUC/UCU/CUC | leucine/serine | CUC/UCU |
| 2. | (UG)n | UGU/GUG/UGU | cysteine/valine | UGU/GUG |
| 3. | (AC)n | ACA/CAC/ACA | threonine/histidine | ACA/CAC |

We may similarly consider a repeating sequence of three bases, *e.g.*, (ACG)n. Depending upon where the reading is started, three kinds of homopolypeptides are expected (Table 4.4).

***Table 4.4*** *Assignment of codons, having known sequences, with the help of copolymers having repetitive sequnces of three bases = (ACG)n.*

| | Codons | Homopolypeptide | Codon assignment |
|---|---|---|---|
| 1. | ACG/ACG/ACG/ACG/ACG = Poly (ACG) | (Threonine)n | ACG = threonine |
| 2. | A/CGA/CGA/CGA/CGA = Poly (CGA) | (Arginine)n | CGA = arginine |
| 3. | AC/GAC/GAC/GAC/GAC = Poly (GAC) | (Aspartic acid)n | GAC = aspartic acid |

**NOTES**

**(*iii*) Codon Assignment by Heteropolymers (Copolymers with Random Sequences):** Further exposition of the genetic code took place by using synthetic messenger RNAs containing two kinds of bases. This technique was used in the laboratories of **Ochoa** and **Nirenberg** and led the deduction of the composition of codons for the 20 amino acids. The synthetic messengers contained the bases at random (called **random copolymers**). For example, in a random copolymer using U and A nucleotides eight triplets are possible, such as UUU, UUA, UAA, UAU, AAA, AAU, AUU and AUA. Theoretically, eight amino acids could be coded by these eight codons. Actual experiments, however, yielded only six, *i.e.*, phenylalanine, leucine, tyrosine, lysine, asparagine and isoleucine. By varying the relative compositions of U and A in the random copolymer and determining the percentage of the different amino acids in the proteins formed, it was possible to deduce the composition of the code for different amino acids.

**3. Assignment of Codons with Known Sequences: (*i*) Use of Trinucleotides or Minimessengers in Filter Binding (Ribosome-Binding Technique):** Ribosome technique of **Nirenberg** and **Leder** (1964) made use of the finding that aminoacyl-tRNA molecules specificially bind to ribosome-mRNA complex. The binding does not require the presence of a long mRNA molecule; in fact, the association of a **trinucleotide** or **minimessenger** with the ribosome is sufficient to cause aminoacyl-tRNA binding. When a mixture of such small mRNA molecules-ribosomes and amino acid-tRNA complexes are incubated for a short time and then filtered through a **nitrocellulose membrane**, then the mRNA-ribosome-tRNA-amino acid complex is retained back and rest of the mixture passes through the filter. By using a series of 20 different amino acid mixtures, each containing one radioactive amino acid at a time, it is possible to find out the amino acid corresponding to each triplet by analysing the radioactivity absorbed by the membrane, *e.g.*, the triplet GCC and GUU retain only alanyl-tRNA and valyl-tRNA respectively. All 64 possible triplets have been synthesized and tested in this way. Forty five of them have given clear-cut results. Later on, with the help of longer synthetic messages it has been possible to decipher 61 out of the possible 64 codons.

### C. The *in vivo* Codon Assignment

The cell free protein synthetic systems, though have proved of great significance in deciphering of the genetic code, but they could not tell us whether the genetic code so deciphered is used in the living systems of all organisms also. Three kinds of techniques are used by different molecular biologists to determine whether the same code is also used *in vivo* (*a*) amino acid replacement studies (*e.g.*, tryptophan synthetase synthesis in *E.coli* (**Yanofsky** *et al.*, 1963) and haemoglobin synthesis in humans), (*b*) frameshift

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mutations (*e.g.*, investigations of **Terzaghi** *et al.*, 1966, on lysozyme enzyme of $T_4$ bacteriophages, and (*c*) comparison of a DNA or mRNA polynucleotide cryptogram with its corresponding polypeptide clear text (*e.g.*, comparison of amino acid sequence of the $R_{17}$ bacteriophage coat protein with the nucleotide sequence of the $R_{17}$ mRNA in the region of the molecule that dictates coat-protein synthesis by **S. Cory** *et al.*, 1970).

Thus, *in vitro* and *in vivo* studies, so far described, gave the way to formulate a code table for twenty amino acids.

***Table 4.5*** *The genetic dictionary. The trinucleotide codons are written in the 5′ → 3′ direction.*

| First base | Second base | | | | Third base |
|---|---|---|---|---|---|
| | U | C | A | G | |
| U | UUU, UUC: Phe<br>UUA, UUG: Leu | UCU, UCC, UCA, UCG: Ser | UAU, UAC: Tyr<br>UAA, UAG: Non-sense† codon | UGU, UGC: Cys<br>UGA: Non-sense† codon<br>UGG: Trp | U<br>C<br>A<br>G |
| C | CUU, CUC, CUA, CUG: Leu | CCU, CCC, CCA, CCG: Pro | CAU, CAC: His<br>CAA, CAG: Gln | CGU, CGC, CGA, CGG: Arg | U<br>C<br>A<br>G |
| A | AUU, AUC, AUA: Ileu<br>AUG*: Met | ACU, ACC, ACA, ACG: Thr | AAU, AAC: Asn<br>AAA, AAG: Lys | AGU, AGC: Ser<br>AGA, AGG: Arg | U<br>C<br>A<br>G |
| G | GUU, GUC, GUA, GUG: Val | GCU, GCC, GCA, GCG: Ala | GAU, GAC: Asp<br>GAA, GAG: Glu | GGU, GGC, GGA, GGG: Gly | U<br>C<br>A<br>G |

*AUG—Met or chain initiation codon; †UAA, UAG, UGA—Stop codon.

***Table 4.6*** *Amino acids and their messenger RNA Codons*

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Alanine (Ala) | – GCA | Glycine (Gly) | – GGA | Leucine (Leu) | – CUA | Threonine (Thr) | – ACA |
| | GCG | | GGG | | CUG | | ACG |
| | GCC | | GGC | | CUC | | ACC |
| | GCU | | GGU | | CUU | | ACU |
| Argine (Arg) | – AGA | Glutamine | – CAA | | UUA | | |
| | AGG | (Gln; Glun) | CAG | | UUG | Tryptophan (Trp) | – UGG |
| | CGA | Glutamic acid | | Methionine (Met) | – AUG | | |
| | CGG | (Glu) | – GAA | (Starting codon) | | Tyrosine (Tyr) | – UAC |
| | CGC | | GAG | | | | UAU |
| | CGU | | | | | | |
| Aspartic acid | – GAC | Histidine (His) | – CAC | Phenylalanine | – UUC | Valine (Val) | – GUA |
| (Asp) | GAU | | CAU | (Phe) | UUU | | GUG* |
| Asparagine | – AAC | Isoleucine (Ile) | – AUC | Proline (Pro) | – CCA | | GUC |
| (Asn; Aspn) | AAU | | AUU | | CCG | | GUU |
| Cysteine | – UGC | | AUA | | CCC | Terminator | – UAA |
| (Cys) | UGU | | | | CCU | (Nonsense | (ocher) |
| | | Lysine (Lys) | – AAA | Serine (Ser) | – AGC | codons) | UAG |
| | | | AAG | | AGU | | (amber) |
| | | | | | UCA | | UGA |
| | | | | | UCG | | (opal) |
| | | | | | UCC | | |
| | | | | | UCU | | |

*GUG is also used as a start codon for some proteins.

## Characteristics of Genetic Code

NOTES

The genetic code has the following general properties:

**1. The Code is a Triplet Codon:** The nucleotides of mRNA are arranged as a linear sequence of codons, each codon consisting of three successive nitrogenous bases, *i.e.*, the code is a triplet codon. The concept of triplet codon has been supported by two types of point mutations: frameshift mutations and base substitution.

**(*i*) Frameshift Mutations:** Evidently, the genetic message once initiated at a fixed point is read in a definite frame in a series of three letter words. The framework would be disturbed as soon as there is a deletion or addition of one or more bases. When such frameshift mutations were intercrossed, then in certain combinations they produce wild type normal gene. It was concluded that one of them was deletion and the other an addition, so that the disturbed order of the frame due to mutation will be restored by the other (Fig. 4.13).

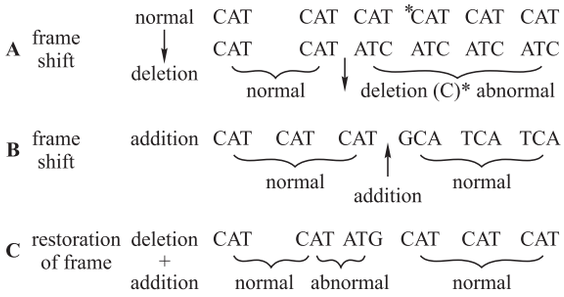


***Fig. 4.15*** *Frame-shift mutations: A—Deletion; B—Addition; C—Restoration of frame.*

**(*ii*) Base Substitution.** If in a mRNA molecule at a particular point, one base pair is replaced by another without any deletion or addition, the meaning of one codon containing such an altered base, will be changed. In consequence, in place of a particular amino acid at a particular position in a polypeptide, another amino acid will be incorporated. For example, due to substitution mutation, in the gene for tryptophan synthetase enzyme in *E.coli*, the GGA codon for glycine becomes a missence codon AGA which codes for arginine. **Missence codon** is a codon which undergoes an alteration to specify another amino acid.

A more direct evidence for a triplet code came from the finding that a piece of mRNA containing 90 nucleotides, corresponded to a polypeptide chain of 30 amino acids of a growing haemoglobin molecule. Similarly, 1200 nucleotides of "satellite" tobacco necrosis virus direct the synthesis of coat protein molecules which have 372 amino acids.

**2. The Code is Non-Overlapping:** In translating mRNA molecules the codons do not overlap but are "read" sequentially (Fig. 4.16). Thus, **a non-overlapping code** means that a base in a mRNA is not used for different codons. In Figure, it has been shown that an


***Fig. 4.16*** *Bases in mRNA are read sequentially in the 5′ to 3′ direction, in group of three bases.*

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overlapping code can mean coding for four amino acids from six bases. However, in actual practice six bases code for not more than two amino acids. For example, in case of an overlapping code, a single change (of substitution type) in the base sequence will be reflected in substitutions of more than one amino acid in corresponding protein. Many examples have accumulated since 1956 in which a single base substitution results into a single amino acid change in insulin, tryptophan synthetase, TMV coat protein, alkaline phosphatase, haemoglobin, etc.

Recently, however, it has been shown that in the bacteriophage $\phi \times 174$ there is a possibility of overlapping of genes and codons (**Barrel** and coworkers, 1976; **Sanger**, *et al.*, 1977).

**3. The Code is Commaless:** The genetic code is commaless, which means that no codon is reserved for punctuations. It means that after one amino acid is coded, the second amino acid will be automatically coded by the next three letters and that no letters are wasted as the punctuation marks.

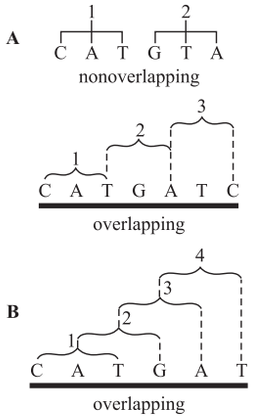


*Fig. 4.17* *A–Non-overlapping codons; B–Overlapping of codon to one base; C–Overlapping of codon due to two bases*

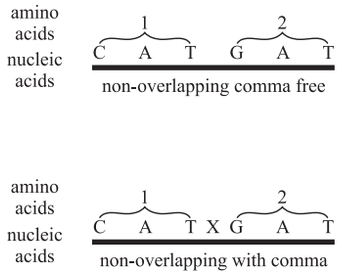


*Fig. 4.18* *Fig. 38.4 Two forms of genetic code. A–Genetic code without comma; B–Genetic code with comma*

**4. The Code is Non-Ambiguous:** Non-ambiguous code means that a particular codon will always code for the same amino acid. In case of ambiguous code, the same codon could have different meanings or in other words, the same codon could code two or more than two amino acids. Generally, as a rule, the same codon shall never code for two different amino acids. However, there are some reported exceptions to this rule: the codons AUG and GUG both may code for *methionine* and as initiating or starting codon, although GUG is meant for *valine*. Likewise, GGA codon codes for two amino acids *glycine* and *glutamic acid*.

### Genetic Code is Degenerate Not Ambiguous

After the start and stop codons, the remaining 60 codons are far more than enough to code for the other 19 amino acids and indeed there are repeats. Thus we say that the genetic code is **degenerate**, that is, an amino acid may be represented by more than one codon. The degeneracy is not evenly divided among the amino acids. For example, methionine and tryptophan

are represented by only one codon each, whereas leucine is represented by six different codons.

The term "degeneracy" should not be confused with "ambiguity". To say that the code was ambiguous would mean that a *single codon could specify either of two* (*or more*) *different amino acids*; there would be doubt whether to put in, say leucine or something else. The genetic code is not ambiguous. Degeneracy in the code means that *there is more than one clear way to say* "*put leucine here*". In other words, a given amino acid may be encoded by more than one codon, but a codon can code for only one amino acid. But just as people in different places prefer different ways of saying the same thing—"Good-bye"! "See you"| "Ciao"! and "So long" ! have the same meaning different organisms prefer one or others of the degenerate codons (Ciao means informal hello or good bye). These preferences are important in genetic engineering.

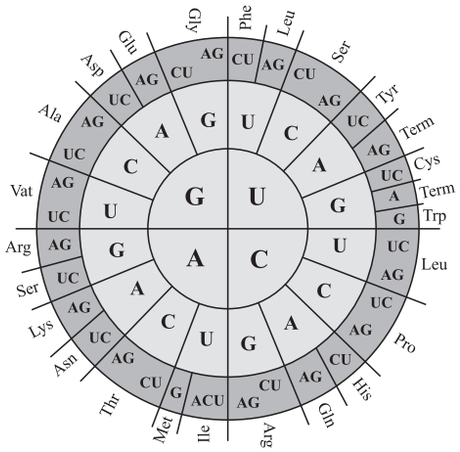


***Fig. 4.19*** *Genetic code degenerate.*

**5. The Code has Polarity:** The code is always read in a fIxed direction, *i.e.*, in the 5′ → 3′ direction. In other words, the codon has a **polarity**. It is apparent that if the code is read in opposite directions, it would specify two different proteins, since the codon would have reversed base sequence:

| **Codon:** | UUG | AUC | GUC | UCG | CCA | ACA | AGG |
|---|---|---|---|---|---|---|---|
| **Polypeptide:** → | Leu | Ile | Val | Ser | Pro | Thr | Arg |
| | Val | Leu | Leu | Ala | Thr | Thr | Gly ← |

**6. The Code is Degenerate:** More than one codon may specify the same amino acid; this is called **degeneracy** of the code. For example, except for *tryptophan* and *methionine*, which have a single codon each, all other 18 amino acids have more than one codon. Thus, nine amino acids, namely *phenylalanine*, *tyrosine*, *histidine*, *glutamine*, *asparagine*, *lysine*, *aspartic acid*, *glutamic acid* and *cysteine*, have two codons each. Isoleucine has three codons. Five amino acids, namely *valine*, *proline*, *threonine*, *alanine* and *glycine*, have four codons each. Three amino acids, namely *leucine*, *arginine* and *serine*, have six codons each.

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The code degeneracy is basically of two types: partial and complete. **Partial degeneracy** occurs when first two nucleotides are identical but the third (*i.e.*, 3′ base) nucleotide of the degenerate codons differs, *e.g.*, CUU and CUC code for leucine. **Complete degeneracy** occurs when any of the four bases can take third position and still code for the same amino acid (*e.g.*, UCU, UCC, UCA and UCG code for serine).

Degeneracy of genetic code has certain biological advantages. For example, it permits essentially the same complement of enzymes and other proteins to be specified by microorganisms varying widely in their DNA base composition. Degeneracy also provides a mechanism of minimizing mutational lethality.

**7. Some Codes Act As Start Codons:** In most organisms, AUG codon is the **start** or **initiation** codon, *i.e.*, the polypeptide chain starts either with **methionine** (eukaryotes) or **N-formylmethionine** (prokaryotes). Methionyl or N-formylmethionyl-tRNA specifically binds to the **initiation site** of mRNA containing the AUG initiation codon. In rare cases, GUG also serves as the initiation codon, *e.g.*, bacterial protein synthesis. Normally GUG codes for valine, but when normal AUG codon is lost by deletion, only then GUG is used as initiation codon.

**8. Some Codes Act As Stop Codons:** Three codons UAG, UAA, UGA are the chain **stop** or **termination** codons. They do not code for any amino acids. These codons are not read by any tRNA molecules (*via* their anticodons), but are read by specific proteins, called **release factors** (*e.g.*, RF-1, RF-2, RF-3 in prokaryotes and RF in eukaryotes). These codons are also called as **nonsense codons**, since they do not specify any amino acid.

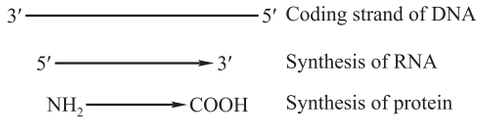
The UAG was the first termination codon to be discovered by **Sidney Brenner** (1965). It was named **amber** after a graduate student named **Bernstein** (= the German word for 'amber' and amber means brownish yellow) who helped in the discovery of a class of mutations. Apparently, to give uniformity the other two termination codons were also named after colours such as **ochre** for UAA and **opal** or **umber** for UGA. (*Ochre* means yellow red or pale yellow; *opal* means milky white and *umber* means brown). The existence of more than one stop codon might be a safety measure, in case the first codon fails to function.

**9. The Code is Universal:** Same genetic code is found valid for all organisms ranging from bacteria to human beings. Such universality of the code was demonstrated by **Marshall**, **Caskey** and **Nirenberg** (1967) who found that *E.coli* (bacterium), *Xenopus laevis* (amphibian) and guinea pig (mammal) amino acyl-tRNA use almost the same code. **Nirenberg** has also stated that the genetic code may have developed 3 billion years ago with the first bacteria, and it has changed very little throughout the evolution of living organisms.

### Codon and Anticodon

The codon words of DNA would be complementary to the mRNA code words (*i.e.*, DNA codes run in 3′ → 5′ direction and mRNA code words run in 5′ →

3′ direction) and so thereby the three bases forming the **anticodon** of tRNA (*i.e.*, bases of anticodons run in 3′ → 5′ direction). Three bases of anticodon




*Directions of synthesis of RNA and protein with respect to the coding strand of DNA (after Freifelder, 1985)*

pair with the mRNA on the ribosomes at time of aligning the amino acids during protein synthesis (translation of mRNA into proteins which proceeds in $NH_2$—COOH direction). For example, one of two mRNA and DNA code words for the amino acid phenylalanine is UUC and AAG respectively, and the corresponding anticodon of tRNA is CAA. This indicates that codon and anticodon pairing is antiparallel. In this case, C pairs with G and U pairs with A.

**Wobble Hypothesis**

The genetic code is a degenerate code, meaning that a given amino acid may have more than one codon. Eight of the sixteen boxes contain just one amino acid per box (*e.g.*, serine, leucine, proline, arginine, threonine, valine, alanine and glycine). (A **box** is determined by the first and second positions, *e.g.*, the UUX box in which X is any of the four bases). Therefore, for these eight amino acids, the codon need only be read in the first two positions because the same amino acid will be represented regardless of the third base of the codon. These eight groups of codons are termed unmixed families of codons. An unmixed family is the four codons beginning with the same two bases that specify a single amino acid. For example the codon family GUX codes for valine.

Mixed families of codons code for two amino acids or for stop signals and one or two amino acids. Six of the mixed-family boxes are split in half so that the codons are differentiated by the presence of a purine or a pyrimidine in the third base. For example, CAU and CAC both code for histidine: in both, the third base, U (uracil) or C (cytosine) is a pyrimidine. Only two of the families of codons are split differently.

The lesser importance of the third position in the genetic code is linked with two facts about transfer RNAs. First, although there would seem to be a need for sixty-two transfer RNAs—since there are sixty-one (*i.e.*, one codons specifying amino acids and an additional codon for initiation) there are actually only about fifty different transfer RNAs in an *E.coli* cell. Second, a rare base such as inosine (I) can appear in the anticodon, usually in the position that is complementary to the third position of the codon. Visualising these two facts researchers believe that some kind of conservation of transfer RNAs is occurring and that rare base may be involved.

At this stage, one point should be very clear that both messenger RNA and transfer RNA bases are usually numbered from the 5′ side. Thus, the number one base of the codon is complementary to the number-three base

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of the anticodon. Thus, the codon base of lesser importance is the number-three base, whereas its complement in the anticodon is the number-one base.

Since the first position of the anticodon (5′) is not as constrained as the other two positions, a given base at that position may be able to pair with any of several bases in the third position of the codon. **Crick** (1966) characterized this ability as **Wobble** (wobble means to sway or move unsteadily). Table 4.7 shows the possible pairings that would produce a transfer RNA system compatible with the known code. For example, if an isoleucine transfer RNA has the anticodons 3′-UAI-5′, it is compatible with the three codons for that amino acid: 5′-AUU-3′, 5′-AUC-3′ and 5′-AUA-3′. That is inosine is the first (5′) position of the anticodon can recognise U, C, or A in the third (3′) position of the codon, and thus one transfer RNA complements with all three codon for isoleucine.

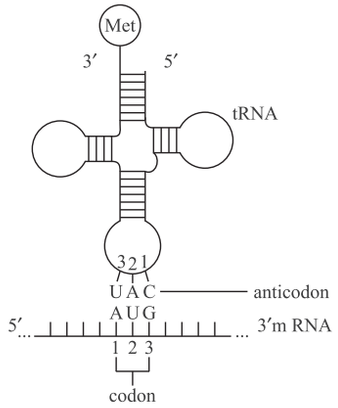


*Fig. 4.20* Codon and anticodon base positions are numbered from the 5′ end. The 3′ position in the codon (5′ in the anticodon) is the wobble base (after Tamarin, 2002).

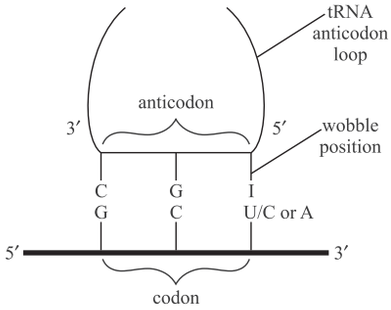


*Fig. 4.21* Wobble hypothesis. In the third site (5′ end) of the anticodon I can take either of three wobble positions, thus being able to pair with U, C or A. This means that a single tRNA species carrying an amino acid can recognize three codons in mRNA: GCU, GCC and GCA (After Suzuki et al.,1986).

***Table 4.7*** *Pairing combinations at the third codon position (Source: Tamarin, 2002)*

| Number-one base in tRNA (5′ end) | Number-three base in mRNA (3′ end) |
|---|---|
| G | U or C |
| C | G |
| A | U |
| U | A or G |
| I | A, U or C |

## Deviations From Universality of Genetic Code

Molecular biologists concluded by 1979 that the genetic code was universal. That is the code dictionary was the same for *E.coli*, human beings and oak trees, as well as other species investigated up to that time. The universality of the genetic code was demonstrated, for example, by taking the ribosomes and messenger RNA from rabbit reticulocytes and mixing them with the aminoacyl-tRNA and other translational components of *E.coli*. Rabbit haemoglobin was synthesized.

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Around 1980, however researchers noted divergence when sequencing mitochondrial genes for structural proteins. It was discovered that there were *two* kinds of deviations from universality in the way mitochondrial transfer RNAs read the code. First, fewer transfer RNAs were needed to read the genetic code, second, there were several cases in which the mitochondrial and cellular systems interpreted a codon differently.

According to Crick's wobble rules, thirty-two transfer RNAs (including one for initiation) can complement all sixty-one non-terminating codons. For example, unmixed families of codons require two transfer RNAs, and mixed families of codons require one, two or three transfer RNAs, depending on the family. The yeast mitochondrial coding system apparently needs only one twenty-four transfer RNAs. The reduction in numbers is attained primarily by having only one transfer RNA recognize each unmixed family. Because mitochondrial transfer RNAs for unmixed families of codons have a U in the first (*i.e.*, wobble) position of the anticodon, apparently, given the structure of the mitochondrial transfer RNAs, the U can pair with U, C, A or G Most probably, evolutionary pressure has minimized the number of transfer RNA genes in the DNA of the mitochondrion, in keeping with its small size. Reduction from thirty-two to twenty-four is a 25% savings. Recent evidence suggests that mammalian mitochondria may need only twenty-two transfer RNAs.

***Table 4.8*** *The genetic code dictionary of yeast mitochondria; anticodons (3′ → 5′) are given within boxes (The ACU Trp anticodon is predicted). (Source: Tamarin, 2002)*

| First Position (5′ end) | Second Position U | Second Position C | Second Position A | Second Position G | Third Position (3′ end) |
|---|---|---|---|---|---|
| U | Phe AAG | Ser AGU | Try AUG | Cys ACG | U C A G |
| | Leu AAU | | *stop* | Trp ACU | |
| C | Thr GAU | Pro GGU | His GUG | Arg GCA | U C A G |
| | | | Glon GUU | | |
| A | IIe UAG | Thr UGU | Asn UUG | Ser UCG | U C A G |
| | Met UAC | | Lys UUU | Arg UCU | |
| G | Val CAU | Ala CGU | Asp CUG | Gly CCU | U C A G |
| | | | Glu CUU | | |

It has also been found that yeast mitochondria read the CUX family (*i.e.*, CUU, CUC, CUA, CUG) as threonine rather than as leucine and the terminator UCA (opal) as tryptophan rather than as termination. However, there have been found differences among mitochondria of different groups of organisms reading the CUX family. Human and *Neurospora* mitochondria appear to read the CUX codons as leucine just as cellular systems do. Of the groups so far analyzed, only yeast reads the CUX family as threonine. Similarly, human and *Drosophila* mitochondria read AGA and AGG as stop signals rather than as arginine.

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***Table 4.9*** *Common and alternative meanings of codons (Source: Tamarin, 2002)*

| Codon | General meaning | Alternative meaning |
|---|---|---|
| 1. CUX | Leu | Thr in yeast mitochondria |
| 2. AUA | Ile | Met in mitochondria of yeast, *Drosophila* and Vertebrates |
| 3. UGA | Stop | Trp in mycoplasmas and mitochondria other than higher plants |
| 4. AGA/ AGG | Arg | *Stop* in mitochondria of yeast and vertebrates; Ser in mitochondria of *Drosophila* |
| 5. CGG | Arg | Trp in mitochondria of higher plants |
| 6. UAA/ UAG | *Stop* | Gln in ciliated protozoa |
| 7. UAG | *Stop* | Ala or Leu in mitochondria of some higher plants |

The ciliated protozoan *Paramecium* species read the UAA and UAG stop codons as glutamine within the cell. In addition, a prokaryote (*Mycoplasma capricolum*) reads UGA codon as tryptophan. We do not yet know how general this finding is : scientists have analysed the genetic code of very few species. We can thus conclude that *the genetic code seems to have universal tendencies among prokaryotes*, *eukaryotes* and *viruses*. Mitochondria however, read the code slightly differently : different wobble rules apply, and mitochondria and cells read at least one terminator and one unmixed family of codons differently.

**Site Specific Variation:** In this type of variation in codon reading, the interpretation of a codon depends on its specific location. For example, codons GUG and, rarely, UUG can serve as prokaryotic initiation codons. This means that they are recognized by $tRNA_f^{Met}$. However, they are not recognised by $tRNA_m^{Met}$. (*i.e.*, GUG and UUG are not misread internally in messenger RNAs). In some cases, two of the termination codons (UGA and UAG, but not UAA) are misinterpreted as codons for amino acids. That is, termination will not occur at the normal place, resulting in a longer-than usual protein. In some cases, these "read-through" proteins are vital—the organism depends on their existence. For example, in the phage θβ, the coat-protein gene is read through about 2% of the time. Without this small number of read through proteins, the phage coat (capsid) cannot be constructed properly.

**Selenocysteine Codon:** Site-specific variation also involves the amino acid selenocysteine (*i.e.*, cysteine with a selenium atom replacing the sulphur). Although many proteins have unusual amino acids, almost all are due to post-translational modifications of normal amino acids. However, the amino acid selenocysteine is inserted directly into some proteins, such as formate dehydrogenase in *E.coli*, which has selenium in its active site. Selenocysteine is inserted into the protein by a new transfer RNA that recognises the termination codon, UGA, if that codon involved in a particular stem-loop secondary structure in the messenger RNA. The selenocysteine transfer RNA is originally charged with a serine that is then modified to a selenocysteine. In addition to the stem-loop structure 3′ (downstream) from

the amber codon (UAG), a selenocysteine elongation factor (SELB) is also needed at the ribosome. This same mechanism may occur in eukaryotes, but not all of the components have yet been recognized.

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### Evolution of Genetic Code

Certain modern theories have suggested that the genetic code has the wobble in it because it originally arose from a code in which only the first two bases were needed for the small number of amino acids in use several billion years ago. As new amino acids with useful properties became available, they were incorporated into proteins by a code modified by the third base, albeit with less specificity. This view has been supported by the fact that codons starting with the same nucleotide come from the same biosynthetic pathway. This indicates that in early evolution, as biosynthetic pathways were extended to new amino acids, the newcomers were incorporated by use of the second and the third bases of the code.

Let us see whether there is a relationship between the codons and the amino acids they code for, or is the code just one of many random/chance possibilities. That is, whether the genetic code is highly evolved or just a "frozen accident". Recent computer simulations of random codes indicate that the current genetic code is far outside the range of random in its ability to protect the organism from mutation. This suggests that the genetic code is not a frozen accident, but rather is highly evolved. Numerous examples in the current code support this view.

For example, in the unmixed family 5′-CUX-3′, any mutation in the third position produces another codon for the same amino acid. Wobble in the third position and codon arrangement ensures that less than half of the mutations in the third codon position result in the specification of a different amino acid.

Some patterns also have been observed in the genetic code. For example, the mutation of one codon to another results in an amino acid of similar properties. A high probability exists that such a mutation will produce a functional protein. All the codons with U as the middle base, for example, are for amino acids that are hydrophobic (*e.g.*, phenylalanine, leucine, isoleucine, methionine and valine). Mutation in the first or third positions for any of these codons still codes a hydrophobic amino acids. Both of the two negatively charged amino acids, aspartic acid and glutamic acid, have codons that start with GA. All of the aromatic amino acids—phenylalanine, tyrosine and tryptophan—have codons that begin with uracil. Such patterns minimize the negative effects of mutation (see **Tamarin**, 2002).

**Check Your Progress**

10. Define gene.
11. What are loci and alleles?
12. What is a missence codon?

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## 4.5 INTRACELLULAR PROTEIN TRAFFIC

Intracellular transport is the movement of vesicles and substances within a cell. Intracellular transport is required for maintaining homeostasis within the cell by responding to physiological signals. Proteins synthesized in the cytosol are distributed to their respective organelles, according to their specific amino acid's sorting sequence. Eukaryotic cells transport packets of components to particular intracellular locations by attaching them to molecular motors that haul them along microtubules and actin filaments. Since intracellular transport greatly relies on microtubules for movement, the components of the cytoskeleton play a vital role in trafficking vesicles between organelles and the plasma membrane by providing mechanical support. Through this pathway, it is possible to facilitate the movement of essential molecules, such as membrane-bounded vesicles and organelles, mRNA, and chromosomes.

The membrane-trafficking system is a characteristic feature of eukaryotic cells. The best-known organelles and major protein families of this system are largely conserved across the vast diversity of eukaryotes. However, the variation exists that specify the evolutionary forces which specifically shaped the endomembrane system in eukaryotes and highlights methods in which membrane trafficking in the protists differs from the standard models of mammalian and yeast cells. Both parasites and free-living protists possess specialized trafficking organelles, some are lineage specific while others are more widely distributed. Novel members of protein families are present across eukaryotes but have been lost in humans.

The organelles of the membrane-trafficking system include the Endoplasmic Reticulum (ER), Golgi Apparatus, early and recycling Endosomes, MultiVesicular Body (MVB or Late Endosome), Lysosome/ Vacuole, and Plasma Membrane. These organelles represent static fixtures or transitional stages in maturing compartments, and the transport of materials between them is essentially mediated by vesicle formation from a donor organelle and vesicle fusion at a subsequent acceptor organelle.

### Protein Targeting

A typical mammalian cell may contain numerous kinds of proteins and numerous individual protein molecules. The eukaryotic cell is a multi-compartmental structure. Considering the various organelles, in which each requires different proteins, except a few of the proteins which are typically synthesized in mitochondria and chloroplasts, all other proteins essential for the cell and the ones to be secreted by the cell are synthesized in the cytosol on free ribosomes and on ribosomes bound to the Endoplasmic Reticulum (ER).

Most proteins are coded by the nuclear genome and synthesized in the cytoplasm. The proteins are present in the ER, mitochondria, chloroplasts,

Golgi, peroxisomes, nucleus, in the cytosol and in the membranes of all these organelles. They are selectively transported into their appropriate organelles inside the cell and across the plasma membrane to be secreted outside the cell.

Some of them are carried into membrane bound vesicles which bud off from one organelle and transported in definite pathways. Various distinct destinations of different proteins require sophisticated system for labelling and sorting newly synthesized proteins and ensuring that they reach their proper places. This transportation of proteins to their final destinations is termed as **protein targeting**.

Proteins destined for cytoplasm and those to be incorporated into mitochondria, chloroplasts and nuclei are synthesized on free ribosomes in the cytoplasm. Proteins destined for cellular membranes, lysosomes and extracellular transport, use a special distribution system. The main structures in this system are the Rough Endoplasmic Reticulum (RER) and Golgi complex.

The RER is a network of interconnected membrane enclosed vesicles or vacuoles. The endoplasmic reticulum is coated with polyribosomes to give it a rough appearance. The Golgi complex is also a stack of membrane bound sacs but they are not interconnected. The Golgi complex acts as a switching center for proteins to various destinations.

Proteins to be directed to their destinations via Golgi complex are synthesized by ribosomes associated with endoplasmic reticulum.

**Protein Traffic**

Protein sorting requires proper address labels which are in the form of peptide signal sequences. A signal sequence that directs the protein to its target is present in the form of 13-35 amino acids in the newly synthesized protein itself. It is the first to be synthesized and is mostly present at the amino N-terminal, sometimes at the carboxyl C- terminal.

It is also sometimes termed as signal sequence or leader sequence. Some proteins are further sorted to a sub-compartment within the target organelle. For this purpose, a second signal sequence is present behind the first signal sequence which is cleaved.

Proteins carried inside the membrane bound vesicles are called cargo proteins. An embedded or integrated protein is carried in the membrane of the vesicle, while secretory protein is carried within the lumen of the vesicle. The vesicle buds off from the donor surface and fuses with the target surface releasing its contents into the target organelle and the membrane protein is incorporated into the membrane of the target organelle. The process is repeated during the passage of protein from ER to Golgi to lysosomes and from Golgi to plasma membrane. Following figure illustrates the nascent proteins targeting to different organelles of the cell and cell secretion.

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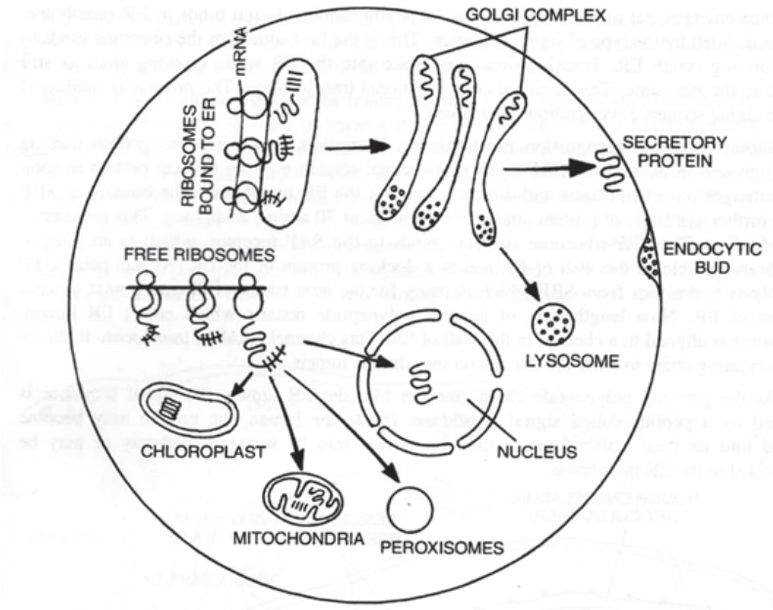


***Fig. 4.22*** *Nascent Proteins Targeting to Different Organelles of the Cell and Cell Secretion*

## Transport of Proteins into ER

A short N-terminus signal sequence at the beginning of the growing nascent protein chain' determines whether a ribosome synthesizing the proteins binds to ER or not. The protein synthesis always begins on free ribosomes. As the signal sequence emerges out of the ribosome, the large ribosomal sub-unit binds to ER membrane. This transportation is characteristically settled by the type of signal sequence. This is the first sorting as the ribosome binds to ER, forming rough ER. Translocation takes place into the ER while growing chain is still bound to the ribosome. This is called co-translational translocation. The process is simplified by the signal sequence recognition mechanism.

## Signal Sequence Recognition Mechanism

It consists of a Signal Recognition Particle (SRP) present in the cytosol. SRP binds to the signal sequence of the nascent protein as soon as it emerges out of ribosome and directs it towards the ER membrane. The binding of SRP stops further synthesis of protein chain when it is about 70 amino acids long.

This prevents it from folding. The SRP-ribosome complex binds to the SAP receptor, which is an integral membrane protein in the wall of ER and is a docking protein of the ER. At this point GTP hydrolysis hydrolyses frees SRP which is ready for the next round of directing next nascent protein of ER.

Then the lengthening of nascent polypeptide restarts which enters ER lumen. Ribosome is aligned to a channel in the wall of ER. This channel is called translocon. It allows the elongating chain to enter the translocon into the ER lumen.

In ER lumen, after glycosylation, many proteins are folded and stabilized by disulphide proteins bonds (-S-S-). This reaction is catalyzed

by an enzyme, Protein Disulphide Isomerase (PDI). Most of human proteins are stabilized by disulphide bonds.

Once inside the lumen of ER, the protein undergoes folding and several modifications for which the ER lumen contains a number of enzymes and chaprone proteins. The most common processing is glycosylation which involves addition of carbohydrates to the protein chain. Glycosylation generally occurs in the ER lumen but sometimes in Golgi also.

**Golgi Complex and Protein Transportation**

The role of Golgi complex is to act as a switching center for proteins to various destinations. Both ER and Golgi apparatus are flattened cisternae. Transport of proteins from one compartment (donor) to the next one (target) is carried out in transport vesicles. The vesicles contain cargo proteins in their lumen and integral membrane proteins in their membranes.

The vesicles bud off from ER and fuse with the cis-compartment or receiving compartment of Golgi. In this process cargo proteins are delivered into the lumen of Golgi and membrane proteins become part of the membrane of the target vesicles. The proteins are glycosylated, folded, modified and sorted in ER. This process of glycosylation, modification and sorting of proteins continues in successive Golgi cisternae.

**Protein Targeting to Chloroplasts**

The newly synthesized proteins by free ribosomes are imported into chloroplasts as in mitochondria. Calvin cycle enzymes fix atmospheric $CO_2$ into carbohydrates during photosynthesis.

**Protein Targeting into Nucleus**

The nuclear envelope consists of outer and inner membranes and has inter membranous space between them. The outer membrane is continuous with ER and has ribosomes on it. Proteins for the nucleus are synthesized on free ribosomes in the cytosol and imported into nucleus through 3000-4000 nuclear pores known as nuclear pore complexes which are special gates.

**Membrane Proteins**

The proteins embedded in different membranes may have single trans-membrane domain which is a segment of 20-25 amino acids. Other proteins may have many trans-membrane domains connected by loops on both sides of the membrane. These proteins are called multi-pass orientation proteins. In photosynthetic bacteria a protein called bacterio-rodospin spans 12-14 times across the lipid bilayer membrane of bacteria. It traps energy from sunlight and uses it to pump protons across the bacterial membrane.

## 4.5.1 Protein Synthesis on Free and Bound Polysomes

Before Studying protein synthesis on free and bound polysomes let us study transcription and translation in brief.

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## Transcription

The process by which the genetic information contained within DNA is re-written into messenger RNA (mRNA) by RNA polymerase is called DNA transcription. The mRNA exits the nucleus, and acts as the basis for the translation of DNA. By controlling the production of mRNA within the nucleus, the cell regulates the rate of gene expression.

Transcription is the process of copying a segment of DNA into RNA. The segments of DNA transcribed into RNA molecules that can encode proteins are said to produce messenger RNA (mRNA). Other segments of DNA are copied into RNA molecules called non-coding RNAs (ncRNAs). The general preponderance of mRNA in cells is valid even though less than 2% of the human genome can be transcribed into mRNA, while at least 80% of mammalian genomic DNA can be actively transcribed (in one or more types of cells.

Both DNA and RNA are nucleic acids, which use base pairs of nucleotides as a complementary language. During transcription, a DNA sequence is read by an RNA polymerase, which produces a complementary, antiparallel RNA strand called a primary transcript.

General Steps of the Process of Transcription

- RNA polymerase, together binds to promoter DNA.
- Then it generates a transcription bubble, which separates the two strands of the DNA helix. This is accomplished by breaking the hydrogen bonds between complementary DNA nucleotides.
- RNA polymerase adds RNA nucleotides (which are complementary to the nucleotides of one DNA strand).
- RNA sugar-phosphate backbone forms with assistance from RNA polymerase to form an RNA strand.
- Hydrogen bonds of the RNA–DNA helix break, freeing the newly synthesized RNA strand.
- If the cell has a nucleus, the RNA may be further processed.
- The RNA may remain in the nucleus or exit to the cytoplasm through the nuclear pore complex.

## Translation in Protein Synthesis

Translation is the process in which ribosomes in the cytoplasm or endoplasmic reticulum synthesize proteins after the process of transcription of DNA to RNA in the cell's nucleus. This process is called gene expression in cell biology.

Translation is the synthesis of protein from RNA. Genetic information is contained in the nucleotide sequence of DNA in a code. The coded information from DNA is copied during transcription into a form of RNA known as messenger RNA (mRNA), which is then translated into chains of amino acids. Amino acid chains are folded into helices, zigzags, and other shapes to form proteins and are sometimes associated with other amino acid chains.

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During this process, messenger RNA (mRNA) is decoded in a ribosome, outside the nucleus, to produce a specific amino acid chain, or polypeptide. The polypeptide then folds into an active protein and performs its functions in the cell. The ribosome helps in decoding by inciting the binding of complementary tRNA anticodon sequences to mRNA codons. The tRNAs bear specific amino acids that are chained together into a polypeptide as the mRNA passes through and is "read" by the ribosome.

**Translation proceeds in three phases:**

Initiation: The ribosome congregates around the target mRNA. The first tRNA is attached at the start codon.

Elongation: The last tRNA substantiate, by the small ribosomal subunit (accommodation) transfers the amino acid carried by it, to the large ribosomal subunit which binds it to the one of the tRNA (transpeptidation). The ribosome then moves to the next mRNA codon to continue the process (translocation), creating an amino acid chain.

Termination: When a stop codon is reached, the ribosome releases the polypeptide. The ribosomal complex remains intact and moves on to the next mRNA to be translated.

The specific amounts and sequence of amino acids in a protein determine unique properties of that specific protein; for example, muscle protein and hair protein contain the same 20 amino acids, but the sequences of these amino acids in the two proteins are quite different. If the nucleotide sequence of mRNA is thought of as a written message, it can be said that this message is read by the translation apparatus in "words" of three nucleotides, starting at one end of the mRNA and proceeding along the length of the molecule. These three-letter words are called codons. Each codon stands for a specific amino acid.

**Polysomes**

A polyribosome or polysome is a group of ribosomes bound to an mRNA molecule like beads on a thread. It consists of a complex of an mRNA molecule and two or more ribosomes that act to translate mRNA instructions into polypeptides. Polysomes are formed during the elongation phase when ribosomes and elongation factors synthesize the encoded polypeptide. Multiple ribosomes move along the coding region of mRNA, creating a polysome.

Ribosomes associate with mRNA to form polysomes, the size of the polysome varies according to the length of the mRNA and the number of attached ribosomes. Polysomes are found either free in the cytoplasm or attached to the surface of membranes of the endoplasmic reticulum (ER) and the nucleus. In animal embryonic cells, most of the polysomes are free and engaged in protein synthesis for internal use, whereas in those differentiated animal cells from which large amounts of protein are exported, most of the polysomes are found attached to the ER. Thus, the generalization arose that membrane-bound ribosomes synthesize protein for export and free ribosomes for intracellular use.

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The way in which ribosomes attach to membranes is not clear; the ribosomes themselves, the membranes and the protein being synthesized, have all been suggested as being involved in the binding. There is evidence from animals that the large subunit is in close contact with the membranes, and Baglioni et al. (1971) have postulated that the large subunit binds directly to the membrane, probably at a specific binding site (Sunshine et al., 1971), and that a protein on Electron micrograph of a thin section of parts of two adjacent cells in a shoot apex of pea showing ribosomes and polyribosomes. The membranes is responsible for the attachment (James et al., 1969). Alternatively, it has been suggested that one of the proteins of the large subunit is responsible, whereas other observations suggest that the binding may be dependent on the nascent polypeptide chain. It now seems likelly that, in vivo, membrane-bound ribosomes synthesize a different class of protein to free ribosomes, whereas in vitro, proteins of both classes are synthesized by both types of ribosomes, suggesting the control is not a function of the ribosome itself.

## 4.5.2 Uptake into ER

### Import of Protein into ER

There are two major categories of hydrophobic signals used in insertion of membrane Proteins. All of these are membrane crossing domains:

### Start Transfer Sequences

These are of following two types:

- N-Terminal Signal Peptide Sequence
- Internal Start Transfer Sequence
- **N-Terminal Signal Peptide Sequence**: A cluster of about 8 hydrophobic Amino Acids at the N-terminal end of a Protein. This sequence remains in the membrane and is cleaved off of the Protein after transfer through the membrane.
- **Internal Start Transfer Sequence**: Similar to a signal sequence, but located internally (not at the N-terminal end of the Protein). It also binds to the SRP and initiates transfer. Unlike the N-terminal signal sequence, it is not cleaved after transfer of the protein.

### Stop Transfer Signal

Stop transfer signal is also a sequence of about 8 hydrophobic Amino Acid residues. It follows either N-terminal signal sequence or a start transfer sequence. The stop transfer signal is a membrane crossing domain. It remains in the membrane. The peptide is not cleaved.

This process of membrane insertion has a very important result: It establishes orientation of membrane Proteins. Recall the earlier discussion of 'Sidedness of Membranes'. This is one of the chief ways that 'Sidedness' happens.

NOTES

### Protein Targeting

Protein targeting or Protein sorting is the biological mechanism by which Proteins are transported to their appropriate destinations in the cell or outside it. Proteins can be targeted to the inner space of an organelle, different intracellular membranes, plasma membrane, or to exterior of the cell via secretion.

Most Mitochondrial Proteins are synthesized as cytosolic precursors containing uptake peptide signals. Cytosolic chaperones deliver preproteins to channel linked receptors in the mitochondrial membrane. The preprotein with presequence targeted for the mitochondria is bound by receptors and the General Import Pore (GIP) (Receptors and GIP are collectively known as Translocase of Outer Membrane or TOM) at the outer membrane. The preprotein is translocated through TOM as hairpin loops.

### Proteins Entering the ER

Synthesis of all Proteins begins in the cytosol compartment. For proteins entering the secretory or lysosomal pathways, the first step is targeting to the endoplasmic reticulum. This targeting relies on a targeting signal encoded in the N-terminal portion of the protein. The targeting signal is recognized by a specific receptor that results in the protein entering the endoplasmic reticulum.

### Targeting of Proteins to the Endoplasmic Reticulum

Synthesis of proteins entering the endoplasmic reticulum is initiated on free ribosomes. A targeting sequence of hydrophobic amino acids near the amino terminal end of the growing polypeptide results in the binding of the ribosome to ER membrane and in insertion of the polypeptide into the Endoplasmic reticulum. Proteins secretory or lysosomal pathways enter the ER and do not come out again. The proteins entering either of these pathways may be of either of two types:

- Proteins that are completely translocated into the Endoplasmic Reticulum. These Proteins are soluble (not membrane Proteins) and are destined for secretion, or for transfer to lysosomes. In all of these cases the Proteins are never part of membranes.
- Proteins that are inserted into membranes, and hence are only partially translocated into the Endoplasmic Reticulum. These Proteins may be destined for ER, membranes of another organelle (Golgi, Lysosomes or Endosomes), or the plasma membrane. In all of these cases the Proteins stay within the membrane once they are inserted into the ER membrane (for example, cellulose synthase).

## Translation of all Proteins begins on Free Ribosomes

Those ribosomes that produce proteins for export through the endoplasmic reticulum become attached to the endoplasmic reticulum as ribosomes of the rough ER. The signal for ER entry is 8 or more hydrophobic Amino Acid residues which rivets the polypeptide to the ER membrane and is also involved in translocation.

NOTES

Whether or not a ribosome becomes attached to the endoplasmic reticulum depends on the nature of the message being translated, the protein being made, and is not an intrinsic property of the ribosome itself. The ribosome and its attached nascent peptide become targeted to the endoplasmic reticulum (Refer Figure 4.23).

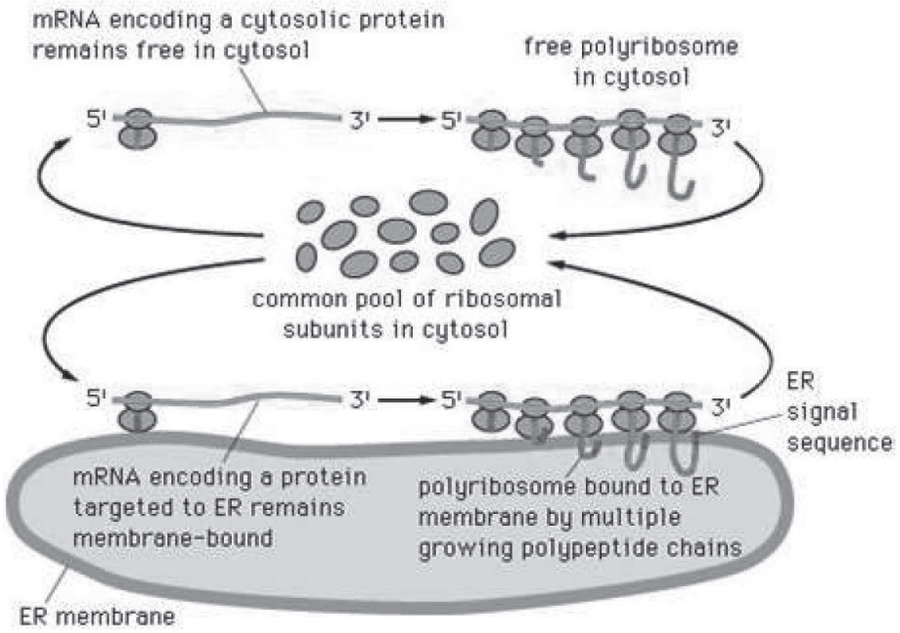


***Fig.4.23*** *Protein Travel through Endomembrane System*

Targeting to the endoplasmic reticulum takes place through the interaction of the **signal peptide sequence** (a sequence of at least eight hydrophobic Amino Acids at the Amino terminal end of the polypeptide. The emerging signal sequence combines with a **'Signal Recognition Particle'** (SRP). This greatly reduces the rate of translocation and allows the ribosome to attach to the Endoplasm Reticulum by means of a special **SRP receptor** in the ER membrane.

The ribosome becomes attached to a **ribosome receptor** that also functions as the **translocation channel** for the newly synthesized polypeptide. As the ribosome becomes attached, the SRP is removed and translation resumes (Refer Figure 4.24).

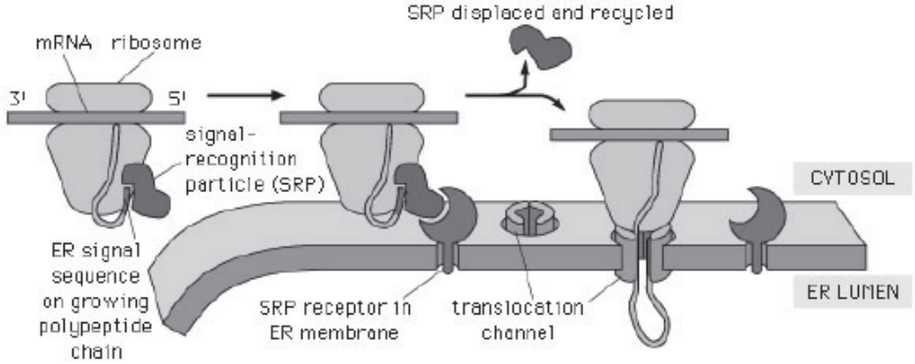


***Fig 4.24*** *ER Signal Sequence*

- There is a Signal Recognition Particle (SRP) in the cytosol. This binds to the ER Signal sequence when it is exposed on the ribosome and slows Protein synthesis long enough to allow the SRP to find the second part, the SRP Receptor.
- The Signal Recognition Particle Receptor (SRPR) which is embedded in the ER membrane. We now have the new polypeptide synthesizing system in place and Protein synthesis speeds up. It seems that the Signal Sequence opens the translocation channel.

Experimental tests show that ER targeting signal is both necessary and sufficient to bring about targeting (Refer Figure 4.25).

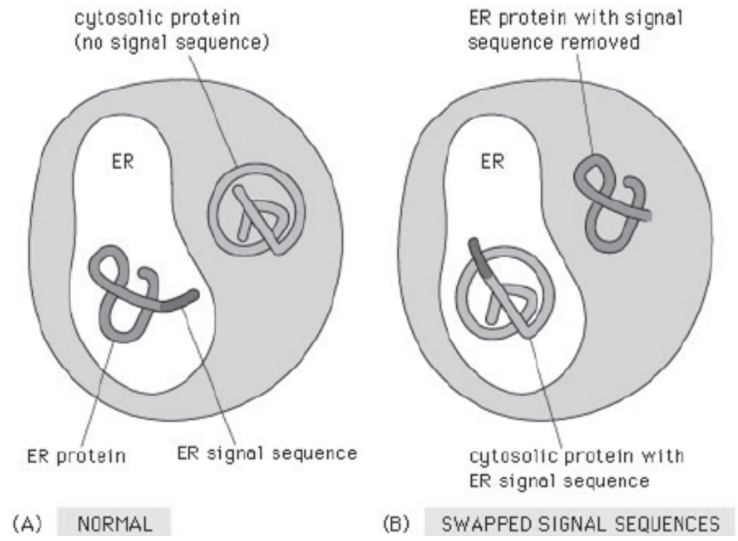


***Fig. 4.25*** *Experimental Test of the Role of Signal Sequences*

## Protein in ER

**Soluble Proteins:** The peptide moves through the translocation channel into the lumen of the ER. The signal peptide sequence remains attached to the membrane. It is later cleaved off by a **signal peptidase**. Leaving the Protein free in the lumen of the ER (Refer Figure 4.26).

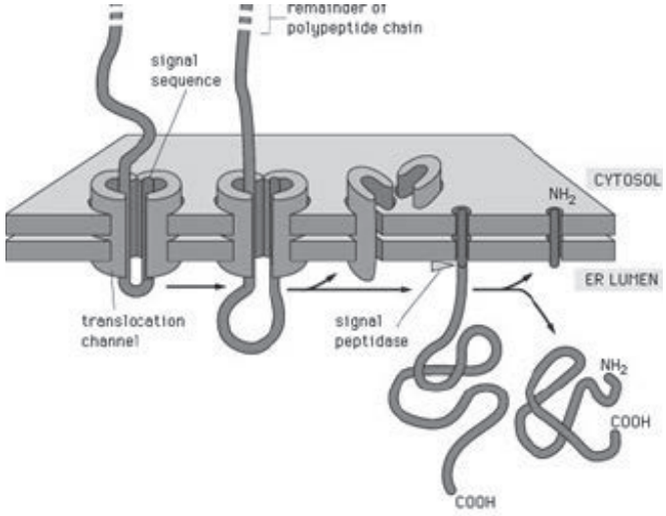


***Fig.4.26*** *Protein Translocation into ER*

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**Membrane Protein:** Orientation of a Protein in the membrane is established when it is first inserted into the membrane. This orientation of the Protein persists all of the way to its final destination. That is, the cytosolic side of membrane remains on the cytosolic side throughout all processes. As membrane Proteins are being translated, they are translocated or transferred into the ER until a hydrophobic membrane crossing domain is encountered. This serves as a 'Stop Transfer' signal and leaves the Protein inserted in the ER membrane.

The hydrophobic trans-membrane domain holds the Protein in the membrane because of the very strong hydrophobic interaction between this part of the Protein and the hydrophobic membrane core.

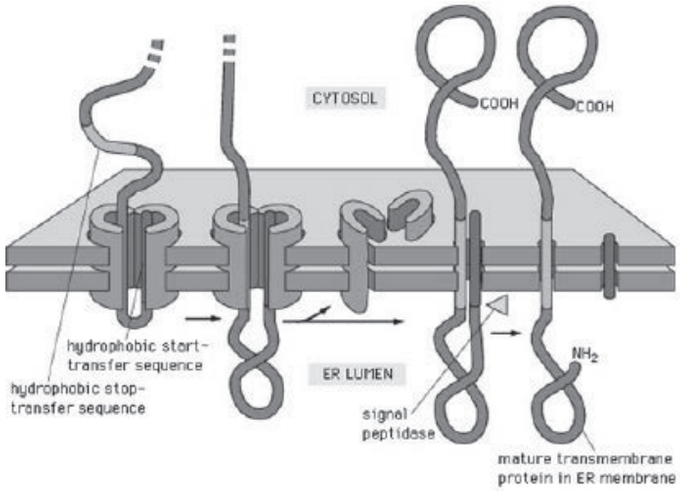


***Fig 4.27** Import of a Membrane Protein*

Import of a membrane Protein. The above Figure 4.27 illustrates the case of a Protein being incorporated in the membrane of the Endoplasmic Reticulum, but import into organellar membranes works much the same way. The blue sheath-like component shown in the figure is the transport complex that moves the Protein through the membrane. This example is a single pass membrane Protein that contains a single membrane crossing domain.

## 4.5.3 Membrane Proteins, Golgi Sorting, Post Translational Modifications

Let us study membrane proteins, golgi sorting and post translational modifications in detail.

### (a) Membrane Proteins

Every cell in our body contains organelles, each of which contributes in its own way to functions well as a whole. To perform various cellular jobs, including the task of producing, packaging, and exporting certain cellular products, a set of three major organelles together form a system within the cell called the endomembrane system. The organelles of the endomembrane system include the Endoplasmic Reticulum, Golgi Apparatus, and Vesicles.

In addition to the role performed by the endomembrane system, the cell has many other important functions. Just as you must consume nutrients to

provide yourself with energy, so must each of your cells take in nutrients, some of which convert to chemical energy that can be used to power biochemical reactions? Just like a factory cannot run without electricity, a cell cannot run without energy. This energy, in the form of ATP, is produced within the mitochondria, which is the second organelle that we will go through in the current unit.

Much like the bony skeleton structurally supports the human body, the cytoskeleton helps the cells to maintain their structural integrity. The cytoskeleton is a group of fibrous proteins that provide structural support for cells in addition to their role in cell motility, cell reproduction, and transportation of substances within the cell.

**(b) Golgi Sorting**

Let us study the Golgi Complex in detail

**Golgi Complex**

The golgi apparatus, or golgi complex, is a membrane-bound, multi-compartment organelle located in the cytoplasm of most Eukaryotic cells, including plants, animals and fungi. golgi apparatus serves as a factory in which proteins received from the Endoplasmic Reticulum (ER) are further processed and sorted for transport to their eventual destinations, i.e., lysosomes, the plasma membrane, or for secretion outside the cell. Also, glycolipids and sphingomyelin are synthesized and packaged in the golgi apparatus, for transport. In plant cells, it is the site at which the complex polysaccharides of the cell wall are synthesized. While many types of cells contain only one or several golgi bodies, plant cells can contain hundred.

As golgi apparatus is involved in processing the broad range of cellular constituents that travel along the secretory pathway, it is more prominent in the cells which are involved in secretion of substances. This unit provides a brief historical account of the discovery of the golgi apparatus, a description of its unique structure and chemical composition, and an overview of its functions in the cell.

**History**

Golgi apparatus was first described by an Italian cytologist, Camillo Golgi, in 1898 as an 'Intracellular Reticular Apparatus' stained by 'Black Reaction' in neuronal cells. Black reaction is a procedure, based on the fixation of nervous tissue blocks in potassium pichromate and impregnation in a solution of silver nitrate that results in black deposits that fill up the neurons completely. The stain used, is now known as the 'Golgi Stain'. In the early 1900s, Golgi, Negri, Cajal and others used black reaction to visualize a similar reticular apparatus in different cells, leading to the view that golgi apparatus is found in nearly all Eukaryotic cells. In 1913, Nusbaum coined the term 'Golgi Apparatus'.

Nassanov in 1924 and Bowen in 1929 described the role of golgi apparatus in secretion and also introduced the term 'Golgi Complex' to emphasize its multi-component nature. However, the reality of the organelle was questioned for decades until 1954, when Felix and Dalton visualized and confirmed the very

existence of Golgi apparatus by viewing it under the newly developed electron microscope. In 1951, Baker coined the term lipochondria for golgi complex. In 1958, Perner coined the term dictyosomes for the golgi bodies in plants.

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## Structure

The basic components of golgi apparatus are described below.

**Cisternae:** A golgi apparatus is composed of flattened, plate or saucer-like, closed, fluid filled sacs known as cisternae (Singular: cisterna). The sacs are stacked in an orderly fashion, typically comprising of fewer than eight cisternae. Each stacked grouping has a membrane that separates its insides from the cell's cytoplasm. Depending on the cell type, an individual cell may contain from a few to several thousand stacks per cell (Refer Figure 4.28).

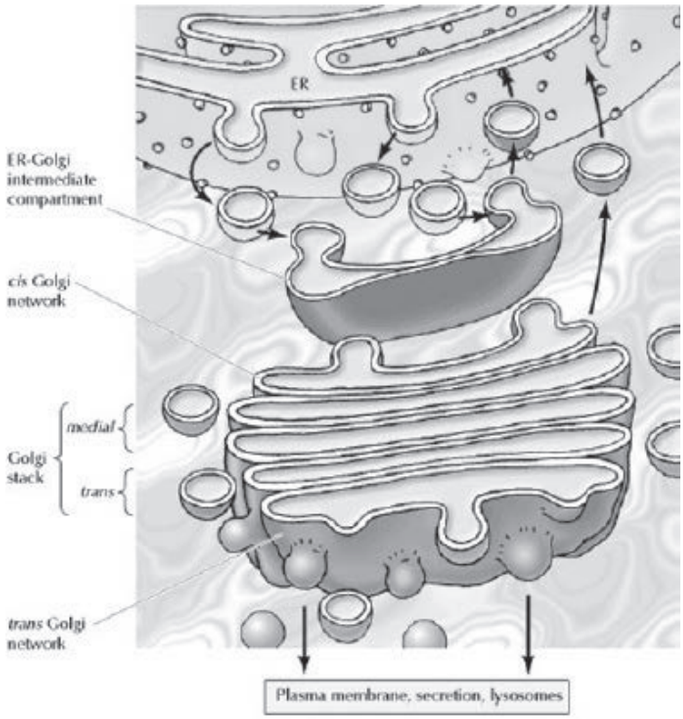


***Fig 4.28*** *Regions of Golgi Apparatus*

Each cisterna is bounded by a double membrane structure enclosing a thin space of about 20Å. In each stack, cisternae are separated by a space of 200 to 300 Å, which may contain rod-like elements or fibres. The cisternae are relatively compressed at their centres and dilated peripherally. The sacs tend to be bowed and have the shape of a saucer.

**Polarity:** A striking feature of the golgi apparatus is its distinct polarity in both structure and function. The cisterna closest to the Endoplasmic Reticulum (ER) is usually convex and is said to be at the cis face or proximal face or forming face (facing towards the nucleus), while the cisterna at the opposite end of the stack is of concave shape and said to be at the trans or distal or maturing face (facing away from the nucleus and towards the plasma membrane). This polarization is called cis-trans axis of the golgi apparatus. proteins and other products of the ER enter the golgi apparatus at the cis face and leave the golgi at the trans face. The products are then exported from the cell via exocytosis or transported to other parts of the cell (Refer Figure 4.29).

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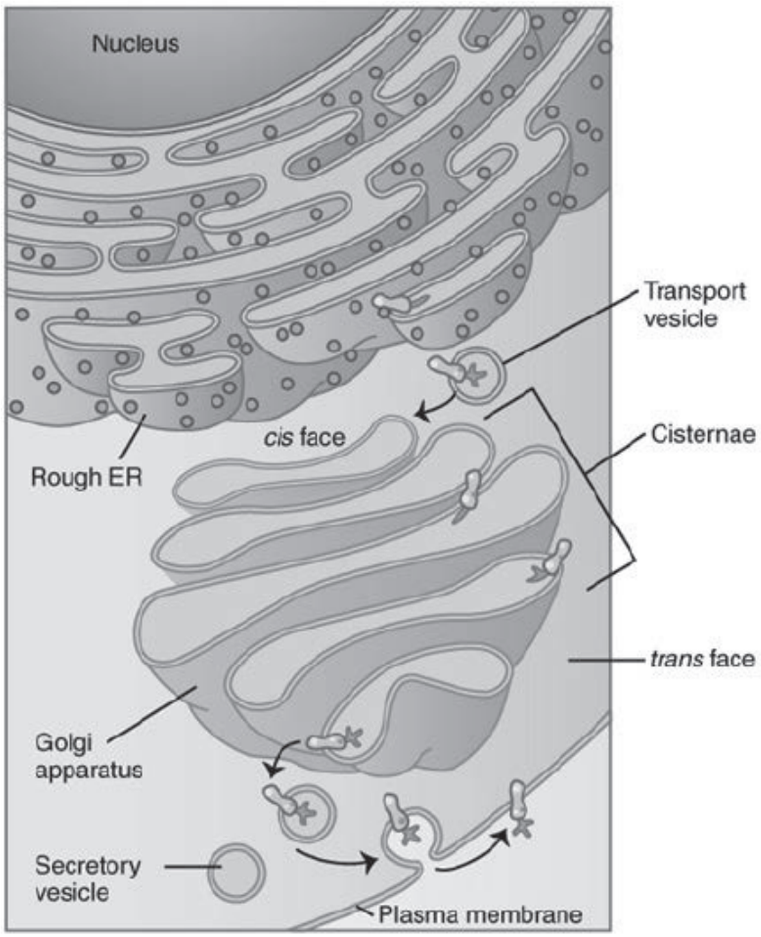


***Fig 4.29*** *Golgi Apparatus*

### Functional Compartments

Distinct processing and sporting events appear to take place in an ordered sequence within different regions of the Golgi complex, so functionally the Golgi complex is divided into following three distinct compartments:

- Cis Golgi Network
- Golgi Stack (which is further divided into the medial and trans sub-compartments)
- Trans Golgi Network

Newly synthesized membrane, secretory and lysosomal proteins from the ER are transported to the ER Golgi intermediate compartment and then enter the golgi apparatus at the **cis golgi network**. They then pass through the compartments of the golgi stack, within which most of the processing events take place. The modified molecules then move to the **trans golgi network**, which acts as a sorting and distribution centre, directing molecular traffic to lysosomes, the plasma membrane, or the cell exterior. The trans Golgi network is also referred to as **GERL region** (**Golgi + Smooth ER + Lysosome**). It is found to be involved in the origin of primary lysosome and melanin granules. It is rich in acid phosphates.

NOTES

**Tubules:** The cis or forming face is characterized by the presence of small transition vesicles or tubules that converge upon the Golgi cisternae, forming a kind of fenestrated plate.

**Vesicles:** They are small structures that bud from the ER or from the cis and medial cisternae of the golgi complex. They vary in size from 40-100 Å. When observed under electron microscope they are seen to be covered by an indistinct fuzzy coat that contains a GTP- binding protein, called ARF (Adenylation Ribose Factor).

In addition to ARF, the vesicles contain a complex of seven distinct, but related COat Proteins (COPs). Vesicles coated with COPs are referred to as non-clathrincoated vesicles. These vesicles, containing the secretory products of Golgi are present towards the concave face and finally get converted into Zymogen Granules or Lysosomes.

Another type of vesicles are clathrincoated vesicles which contain an outer honeycomb like lattice composed of the protein clathrin and an inner shell, composed of protein complexes called adaptors. These vesicles are present at the periphery of the organelle.

### Zones of Exclusion

The golgi complex is surrounded by a differentiated region of cytoplasm where Ribosomes, glycogen and organelles, such as mitochondria are either scarce or absent. This is called zone of exclusion. Coated vesicles are restricted to this region.

### Chemical Composition

The chemical composition of golgi complex varies in different plant and animal cells. The three primary functions of the golgi apparatus are the transport, sorting and modification of both protein and lipid, and the protein composition of the organelle reflects these functions. It is estimated that up to 1000 proteins make up the mammalian golgi apparatus. These include a variety of enzymes, such as Glycosyltransferases (for example, Sialyltransferases), Oxi-reductases (for example, NADH-cytochrome C-reductase), Phosphatases (G-6-Phosphatase), Phospholipase (Phospholipase A), Kinases (casein Phosphokinase), etc. In animal cells, golgi complex contains phospholipids in the form of Phosphatidylcholine, whereas, that of plant cells contains Phosphatidic Acid and Phosphatidylglycerol. Both animal and plant cells have some carbohydrate components in common, such as galactose, glucosamine, mannose, glucose, and fucose, but plants also have some other special sugars.

### Golgi Sorting

Proteins, lipids and polysaccharides, produced within the cell are transported from the golgi apparatus to their final destinations through the secretory pathway. This involves the sorting of proteins into different kinds of transport vesicles, which bud from the trans golgi network and deliver their contents to the appropriate locations. Transport vesicles carrying the proteins and the lipids destined for the plasma membrane or for continuous secretion from the

cell, normally leave the trans golgi network in a steady stream and follow the constitutive secretory pathway where the secretion of components is largely unregulated (Refer Figure 4.30). Specialized secretory cells, however, have a second secretory pathway in which soluble proteins are stored in secretory vesicles for later release. This type of pathway is mainly found in cells specialized for secreting products rapidly in response to environmental signals — release of hormones from endocrine cells, the release of neurotransmitters from neurons, and the release of digestive enzymes from the pancreatic acinar cells.

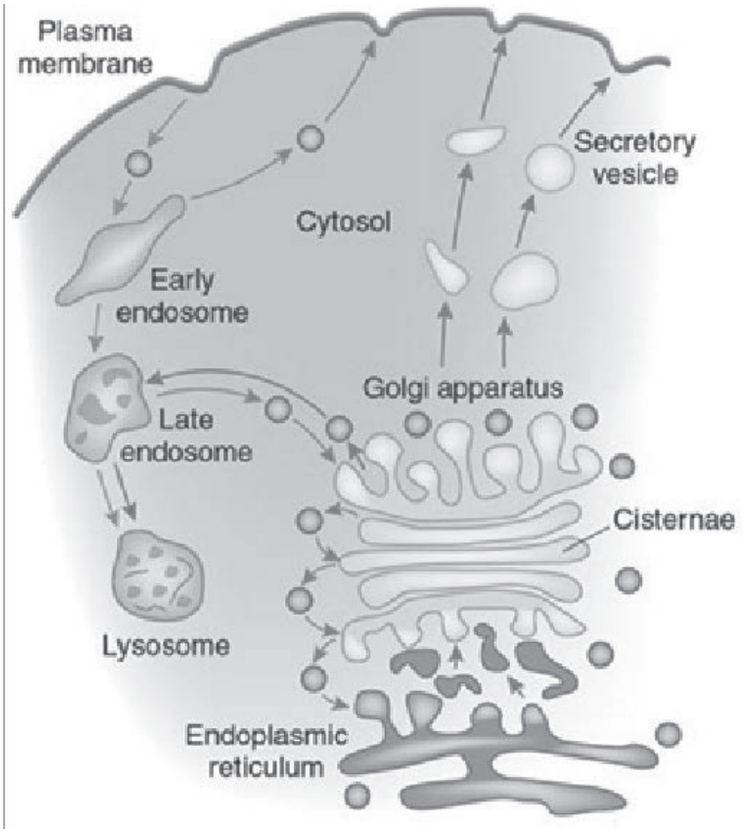


***Fig. 4.30*** *Golgi Apparatus: Modifies and Sorts Proteins for Transport throughout the Cell*

**Membrane Flow:** Membranes do not arise de novo. Actually, membrane components flow by means of vesicles from ER through trans face of Golgi to the plasma membrane. The fusion of the vesicles with the plasma membrane is called **exocytosis**. Likewise, removal of the excess membranes from the apical region of the cell takes place by the invagination of a small portion of the membrane from the surface thereby forming small vesicles that move back into the Golgi via cis face, to be reutilized in the packing of more secretion.

**Acrosome Formation:** The acrosome is a unique membranous organelle located over the anterior part of the sperm nucleus. This part of the sperm contains a number of hydrolytic enzymes, of which hyaluronidase is the most abundant that, when secreted, help the sperm penetrate the egg's coat during fertilization. Active trafficking from the Golgi apparatus is involved in acrosome formation.

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In mammals, acrosome biogenesis begins with the fusion of proacrosomal granules synthesized by the Golgi apparatus in early stages of spermiogenesis. The acrosomal vesicle attaches to the nuclear envelope and grows as a result of the constant arrival and fusion of Golgi-derived vesicles. In the later stages, the acrosomal vesicle flattens and spreads over the nucleus, covering up to two-thirds of its surface. During this process, acrosomal proteins condense and are packed in a paracrystalline structure called the **acrosomal granule**, which eventually forms the acrosomal matrix in mature sperm.

**Functions**

Cells synthesize a large number of different macromolecules required for life. The golgi complex represents a special membranous compartment, interposed between the endoplasmic reticulum and the extracellular space that plays an integral role in modifying, sorting, and packaging the substances, produced by the cell, for cell secretion (exocytosis) or for use within the cell.

**Other Golgi Functions:** Secretion of tropocollagen and collagen; formation of melanin granules and other pigments; formation of yolk and vitelline membrane of growing primary oocytes; formation of cortical granules of a variety of oocytes; secretion of materials of primary and secondary cell walls; formation of cell plate during cell division.

**(c) Post Translational Modifications**

Let us study these modifications in detail.

**Synthesis of Glycoconjugates:** The Golgi apparatus plays a major role in the production of glycoconjugates through the sequential action of glycosyltransferases that add a diverse set of carbohydrates to both proteins and lipids. They could be glycoproteins (mostly protein), proteoglycans (mostly carbohydrate), or glycolipids. One of the major aspects of processing events carried out in the Golgi apparatus for the production of glycoconjugates is the modification of the N-linked oligosaccharides that were added to proteins in the ER. Following synthesis on the membrane-bound ribosomes of the RER, the polypeptides reach the golgi complex, where the terminal side chains of galactose, fucose, and sialic acid are added by the corresponding transferases present in the golgi apparatus. These glycoconjugates are important for many biological and disease processes of an organism for example, proteoglycans, which are the molecules present in the extracellular matrix of animals.

A single species produces a large number of different glycoconjugates, from glycoproteins (mostly protein) to proteoglycans (mostly carbohydrate) to glycolipids, and requires specific glycosyltransferases for this task. Each glycosyltransferase is specific for the sugar it transfers (for example, galactose vs. sialic acid), the linkage used to attach the sugar to a growing polymer, and the substrate receiving the sugar (for example, N- vs O-linked oligosaccharides). Different species express different sets of golgi glycosyltransferases and thus produce different glycoconjugates.

**Proteolytic Processing:** A large number of secreted proteins, such as, insulin, serum albumin, glucagon and several other peptide hormones, are primarily synthesized as high molecular weight precursors called proproteins. These proproteins then undergo a series of proteolytic processing events initiated by cleavage at dibasic sites (Arg-Arg, Arg-Lys or Lys-Lys) within the trans golgi network or in secretory granules formed from the trans golgi network. These endoproteases work along with carboxypeptidases and/or aminopeptidases to process proproteins to their biologically active mature form.

## 4.5.4 Biogenesis of Mitochondria

Regarding the origin of the mitochondria, several hypotheses have been postulated which are as follows:

**1. "De novo" Origin:** According to this hypothesis, the mitochondria are originated "de novo" (L. anew) from the simple building blocks such as amino acids and lipids. But, there is no direct evidence in suppport of "de novo" hypothesis for the origin of the mitochondria therefore, it is discarded now.

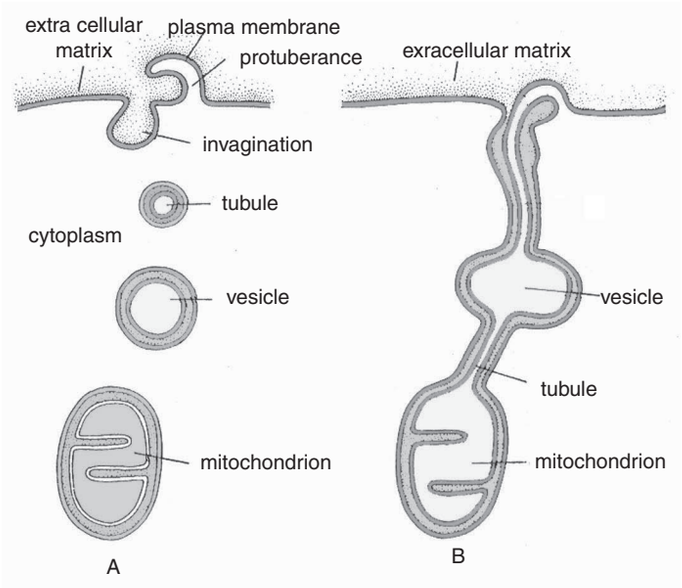


***Fig. 4.31*** *Hypothetical diagrams showing the Origin of Mitochondria from Plasma Membrane.*

**2. Origin from the Endoplasmic Reticulum or Plasma Membrane:** According to **Morrison** (1966) the new mitochondria might have been originated from the endoplasmic reticulum or plasma membrane. This hypothesis also could not provide direct evidences, therefore, it is not well accepted at present time.

**3. Origin by Division of Pre-Existing Mitochondria:** The electron microscopic and radio-autographic observations of the culture cells have shown clearly that the new mitochondria are originated by the growth and division of pre-existing mitochondria. On average, each mitochondrion must double in mass and then divide in half once in each cell generation. Mitochondria are distributed between the daughter cells during mitosis and

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their number increase during interphase. Electron microscopic studies of *Neurospora crassa* (**Luck**, 1963) and HeLa cells (**Attardi** *et al*., 1975) have suggested that organelle division begins by an inward furrowing of the inner membrane, as occurs in cell division in many bacteria. After elongating, one or more centrally located cristae form a partition by growing across the matrix and fusing with the opposite inner membrane. This separates the matrix into two compartments. The outer membrane then invaginates at the partition plane, constricting until there is membrane fusion between the two inner membrane walls. Thus, two separable daughter mitochondria are formed.

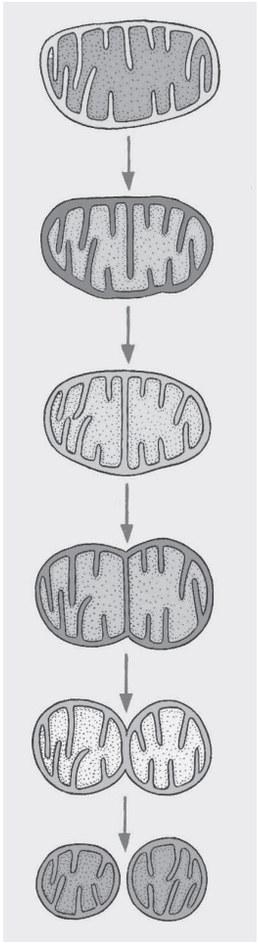

***Fig. 4.32*** *Fission of a mitochondrion by partition formation (after Thorpe, 1984).*

**Mitochondria as Semiautonomous Organelles:** Recently the study of mitochondrial and chloroplast biogenesis became of great interest because it was demonstrated that these organelles contain DNA as well as ribosomes and are able to synthesize proteins. The term **semiautonomous organelles** was applied to the two structures in the recognition of these findings. This term also indicated that the biogenesis was highly dependent on the nuclear genome and the biosynthetic activity of the ground cytoplasm. It is well established now that the mitochondrial mass grows by the integrated activity of both genetic systems, which cooperate in time and space to synthesize the main components. The mitochondrial DNA codes for the mitochondrial, ribosomal and transfer RNA and for a few proteins of the inner membrane. Most of the proteins of the mitochondrion, however, result from the activity of the nuclear genes and are synthesized on ribosomes of the cytosol (cytoplasmic matrix). The cooperation of two genomes has been greatly clarified by studies on the molecular assembly of cytochrome oxidase (**Saltzgaber** *et al*., 1977). This cytochrome, as studied in *Saccharomyces cerevisiae* is made up of seven polypeptide subunits for a combined molecular weight of 139,000 daltons. Three of the polypeptides are coded by mt DNA and assembled on mitochondrial ribosomes. They are very hydrophobic and high in molecular weight (23,000 – 40,000 daltons). The remaining four subunits are coded by nuclear DNA and made on cytoplasmic ribosomes. These are hydrophilic polypeptides of lower molecular weight (4500–14,000 daltons).

**Mitochondrial DNA:** Mitochondrial DNA (mt DNA) molecule is relatively small, simple, double-stranded and except for the DNA of some algae and protozoans, it is circular. The size of mitochondrial genome is very much large in plants than in animals. Thus, mt DNA varies in length from about 5 μm in most animal species to 30 μm or so in higher plants. The mt DNA is

localized in the matrix and is probably attached to the inner membrane at the point where DNA duplication starts. This duplication is under nuclear control and the enzymes used (*i.e*., polymerases) are imported from the cytosol.

**Mitochondrial Ribosomes:** Mitochondria contain ribosomes (called **mitoribosomes**) and polyribosomes. In yeast and *Neurospora*, ribosomes have been ascribed to a 70S class similar to that of bacteria ; in mammalian cells, however, mitoribosomes are smaller and have a total sedimentation coefficient of 55S, with subunits of 35S and 25S (**Attardi** *et al*., 1971). In mitochondria, ribosomes appear to be tightly associated with the inner membrane.

**Mitochondrial Protein Synthesis**

As already described, mitochondira can synthesize about 12 different proteins, which are incorporated into the inner mitochondrial membrane. These proteins are very hydrophobic (*i.e*., they are proteolipids). Thus, on the mitoribosomes are made the following proteins : three largest subunits of cytochrome oxidase (Fig. 10.6), one protein subunit of the cytochrome b-$c_1$ complex, four subunits of ATPase and a few hydrophobic proteins. One of the best known differences between the two mechanisms of protein synthesis (*i.e.,* in the cytosol and in the mitochondrial matrix) is in the effect of some inhibitors. The mitochondrial protein synthesis is inhibited by **chloramphenicol**, while synthesis in the cytosol (cytoplasmic matrix) is not affected by this drug. In contrast, **cycloheximide** has the reverse effect.

**Import Mechanism of Mitochondrial Proteins**

Most mitochondrial proteins are coded by nuclear genes and are synthesized on free ribosomes in the cytosol (cytoplasmic matrix). The import of these polypeptides involves similar mechanism both in mitochondria, and chloroplasts. The transport processes involved have been most extensively studied in mitochondria, especially in yeasts (**Attardi** and **Schatz**, 1988). A protein is translocated into the mitochondrial matrix space by passing through sites of adhesion between the outer and inner membrane, called **contact sites**. Translocation is driven by both ATP hydrolysis and the electrochemical gradient across the inner membrane, and the transported protein is unfolded as it crosses the mitochondrial membranes. Only proteins that contain a specific **signal peptide** are translocated into mitochondria and chloroplasts. The signal peptide is usually located at the amino terminus and is cleaved off after import. Transport to the inner mitochondrial membrane can occur as a second step if a **hydrophobic signal peptide** is also present in the imported protein; this second signal peptide is unmarked when the first signal peptide is cleared. In the case of chloroplasts, import from the stroma into the thylakoid likewise requires a second signal peptide.

**Mitochondrial Lipid Biosynthesis.** The biogenesis of new mitochondria and chloroplasts requires lipids in addition to nucleic acids and proteins. Chloroplasts tend to make the lipids they require. For example, in spinach leaves, all cellular fatty acid synthesis takes place in the chloroplast. The major glycolipids of the chloroplast are also synthesized locally.

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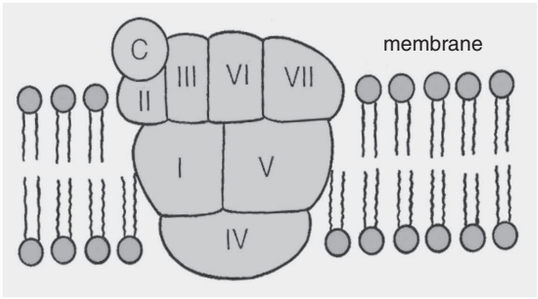


***Fig. 4.33*** *Model of cytochrome oxidase of yeast showing topographical relationship of its seven subunits and its relation with cytochrome c (after Thorpe, 1984).*

Mitochondria, on the other hand, import most of their lipids. In animal cells the phospholipids — **phosphatidyl-choline** and **phosphatidyl-serine**— are synthesized in the ER and then transferred to the outer membrane of mitochondria. The transfer reactions are believed to be mediated by **phospholipid exchange proteins**; the imported lipids then move into the inner membrane, presumably at contact sites. Inside mitochondria, some of the imported phospholipids are decarboxylated and converted into **cardiolipin** (diphosphatidyl glycerol). Cardiolipin is a " double" phospholipid that contains four fatty - acid tails; it is found mainly in the inner mitochondrial membrane, where it constitutes about 20 per cent of the total lipids.

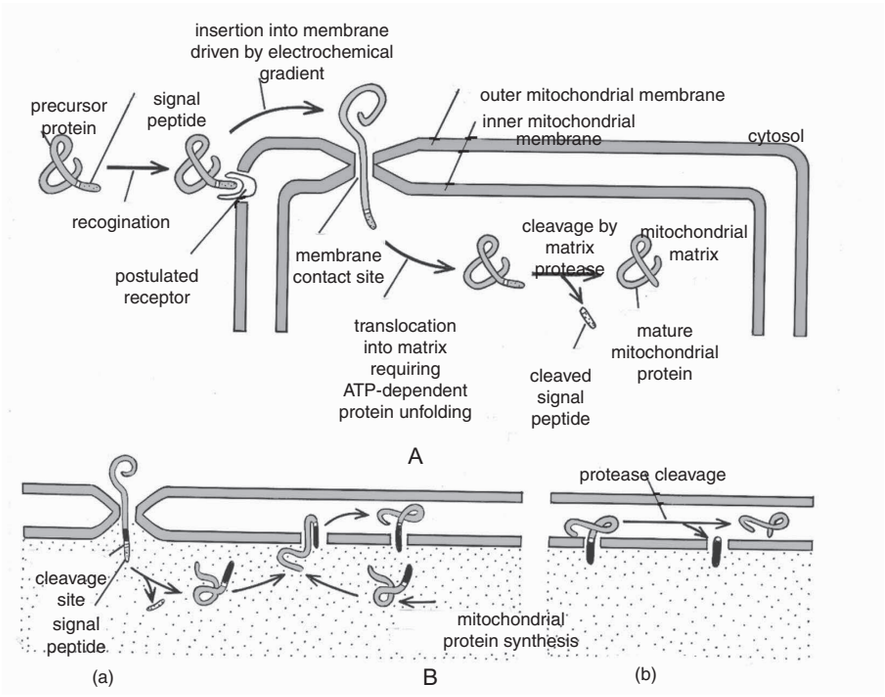


***Fig. 4.34*** *A—Protein import by mitochondrial matrix through single signal; B— Import of proteins from the cytosol (cytoplasmic matrix) to the mitochondrial intermembrane space or inner membrane through two (or multiple) signals (after Albert et al; 1989).*

## Prokryotic Origin or Symbiont Hypothesis

Early cytologists such as **Altmann** and **Schimber** (1890) have suggested the possibility of origin of the mitochondria from the prokaryotic cells. According to their hypothesis, the mitochondria and chloroplasts may be considered

as intra-cellular parasites of the cells which have entered in the cytoplasm of eukaryotic cells in early evolutionary days, and have maintained the symbiotic relations with the eukaryotic cells. The mitochondira are supposed to be derived from the bacterial cells (purple bacteria) while chloroplasts are supposed to be originated from the blue green algae (see **Margulis**, 1981). Due to these reasons **Altmann** suggested the name "**bioblasts**" to the mitochondria and he also hinted about their self-duplicating nature.

Recent cytological findings have also suggested many homologies between the mitochondria and the bacterial cells. The similarities between the two can be summarised as follows :

1. **Similarity in Inner Mitochondrial Membrane and Bacterial Plasma Membrane:** (i) In the mitochondria the enzymes of the respiratory chain are localized on the inner mitochondrial membrane like the bacteria in which they remain localized in the plasma membrane. The bacterial plasma membrane resembles with the inner mitochondrial membrane in certain respects.

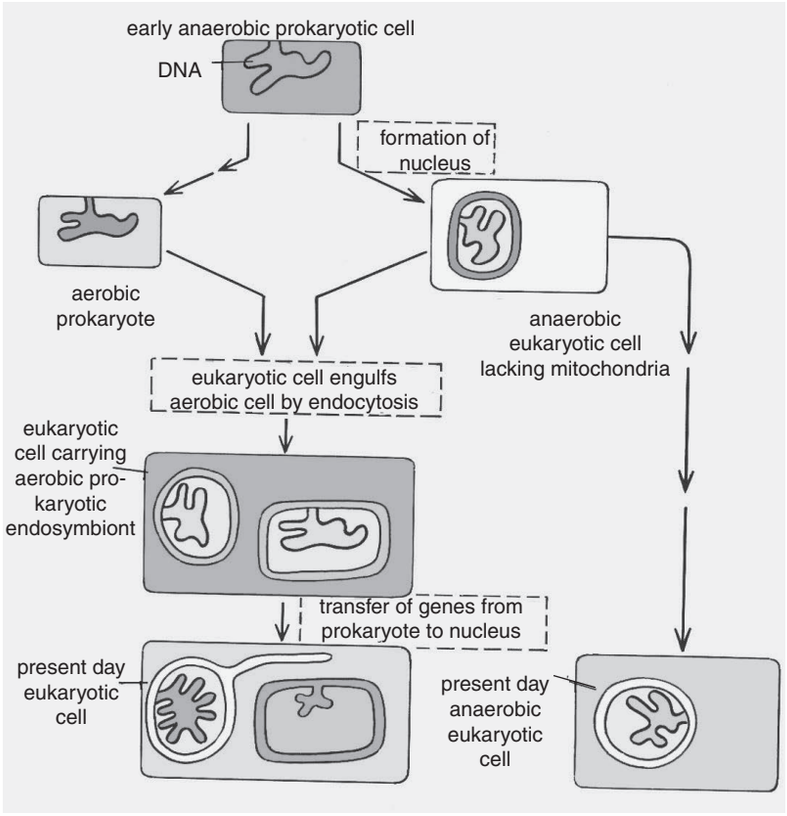


***Fig. 4.35*** *Symbiotic origin of mitochondria and chloroplast (after Alberts et al., 1989).*

(ii) The plasma membrane of certain bacterial cells gives out finger-like projections in the cytoplasm known as mesosomes. The mesosomes can be compared with mitochondrial crests. **Salton** (1962) has reported respiratory chain enzymes in the mesosomes. (iii) Because the outer mitochondrial membrane resembles with the plasma membrane, therefore, it may be assumed that the mitochondrial matrix and the inner mitochondrial membrane represent the

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symbiont which might be enclosed by the membrane of the cellular origin (outer mitochondrial membrane).

2. **Similarity in DNA Molecule:** The DNA molecule of the mitochondria is circular like the DNA molecule of the bacterial cells. Further the replication process of the mitochondrial DNA is also similar to bacterial DNA.
3. **Similarity in Ribosomes:** The mitochondrial ribosomes are small in size and resemble the ribosomes of the bacteria.
4. **Similarity in the Process of Protein Synthesis:** The process of protein synthesis of both mitochondria and bacteria is fundamentally same because in both, the process of protein synthesis can be inhibited by same inhibitor known as chloramphenicol.

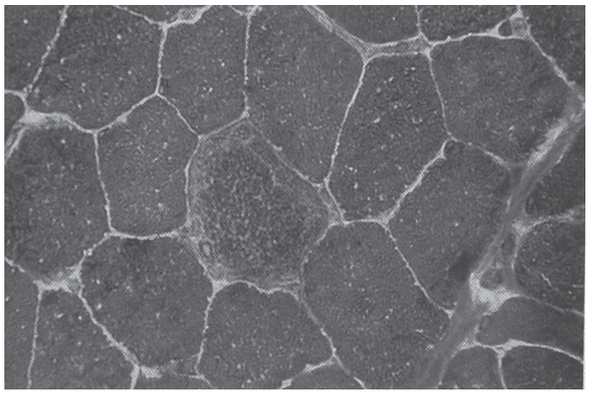

***Fig. 4.36*** *These degenerating muscle fibers are from a biospy of a patient and show accumulations of red staining "blotches" just beneath the cell's plasma membrane, which are due to abnormal proliferation of mitochondria.*

Further, the mitochondria for the process of protein synthesis depend partially on the mitochondrial matrix and DNA and partially on the nucleus and cytoplasm of the eukaryotic cells. This shows the symbiotic nature of the mitochondria.

Due to the above-mentioned similarities between the bacteria and mitochondria, the symbiont hypothesis postulated that the host cell (eukaryotic cell) represented an anaerobic organism which derives the required energy from the oxidations of food by the process of glycolysis. While the mitochondria represent the symbionts which respire **aerobically** and contain the enzymes of **Krebs cycle** and **respiratory chain**. The symbionts seem to be capable to get the energy by **oxidative phosphorylation** from the partially oxidised food (pyruvic acid) of the host cell.

## 4.5.5 Biogenesis of Nuclei

The separation of a cell's genetic material from the surrounding cytoplasm may be the single most important feature that distinguishes eukaryotes from prokaryotes, which makes the appearance of a nuclear envelope a landmark in biological evolution (**Karp** 2010). The **nuclear envelope** consists of two cellular membranes arranged parallel to one another and separated by a 10 to 50 nm distance. The membranes of nuclear envelope serve as a barrier that

keeps ions, solutes and macro-molecules from passing freely between the nucleus and cytoplasm. The two nuclear membranes of the envelope are fused at sites forming circular pores that contain complex assemblies of proteins. The average mammalian cells contain several thousand nuclear pores.

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The outer nuclear membrane is generally studded with ribosomes and is continuous with the membrane of rough endoplasmic reticulum. The space between the nuclear membranes is called **perinuclear space** and it is continuous with the RER lumen.

The inner surface of the nuclear envelope of animal cells is bound by integral membrane proteins to a thin filamentous meshwork, called the **nuclear lamina**. The nuclear lamina provides mechanical support to the nuclear envelope, serves as a site of attachment for chromatin fibers at the nuclear periphery. Nuclear lamina also has a poorly understood role in DNA replication and transcription. The filaments of nuclear lamina are approximately 10 nm in diameter and composed of polypeptides, called **lamins**.

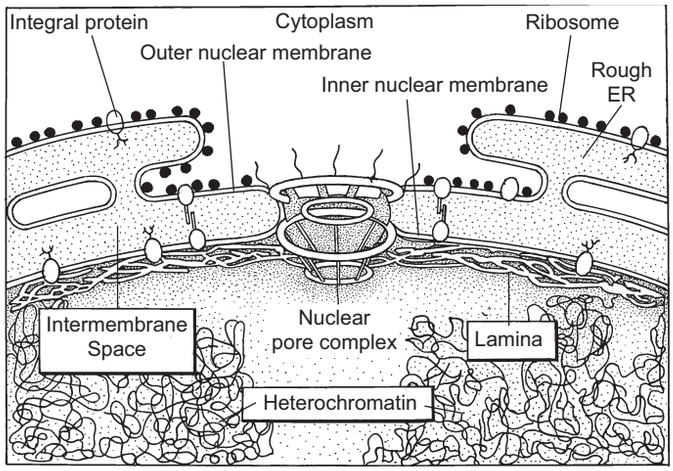


***Fig. 4.37*** *The nuclear envelope. Schematic drawing showing the double membrane, nuclear pore complex, nuclear lamina and the continuity of outer membrane of the nuclear envelope with rough endoplasmic reticulum (RER). Both membranes of nuclear envelope contain their own distinct complement of proteins.*

Lamins are members of the same superfamily of polypeptides that assemble into the 10 nm intermediate filaments of the cytoplasm. As in the cytoplasm, the integrity of intermediate filaments that make up the nuclear lamina is regulated by phosphorylation and dephosphorylation. The disassembly of the nuclear lamina prior to mitosis is induced by phosphorylation of the lamins by a specific protein kinase (enzyme). Nuclear lamina is also called **fibrous lamina**, **zonula nucleum limitans**, **internal dense lamella**, **nuclear cortex** and **lamina densa**.

The nuclear lamina is a protein meshwork which is 10 nm thick. It lines the inside surface of the inner nuclear membrane, except the areas of nucleopores, and consists of a square lattice of intermediate filaments. In mammals, these intermediate filaments are of three types: **lamins A**, **B** and **C** having M.W. 74,000, 72,000 and 62,000 daltons, respectively. The lamins form dimers that have a rod-like domain and two globular heads at

one end. Under appropriate conditions of pH and ionic strength, the dimers spontaneously associate into filaments that have a diameter and repeating structure similar to those of cytoplasmic filaments.

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The nuclear lamina is a very dynamic structure. In mammalian cells undergoing mitosis, the transient phosphorylation of several serine residues on the lamins causes the lamina to reversibly disassemble into tetramers of hypo-phosphorylated lamin A and lamin C and membrane associated lamin B. As a result, lamin A and C become entirely soluble during mitosis, and at telophase they become dephosphorylated again and polymerize around chromatin. Lamin B seems to remain associated with membrane vesicles during mitosis, and these vesicles in turn remain as a distinct subset of membrane components from which nuclear envelope is reassembled at telophase. Inside an interphase nucleus, chromatin binds strongly to the inner part of the nuclear lamina which is believed to interfere with chromosome condensation. In fact, during meiotic chromosome condensation, the nuclear lamina completely disappears by the pachytene stage of prophase and reappears later during diplotene in oocytes, but does not reappear at all in spermatocytes.

The lamins may play a crucial role in the assembly of interphase nuclei. For example, when cells are left for a long time in colchicine (drug which arrests cells in metaphase), the lamins assemble around individual chromosomes, which then surrounded by nuclear envelopes give rise to micronuclei containing only one chromosome. A similar phenomenon occurs during normal amphibian development. In the first few cleavages of amphibian development, the nuclear envelope initially forms around individual chromosomes, forming several vesicles that then fuse together to form a single nucleus. This suggests that chromatin is the nucleating centre for the deposition of a nuclear lamina and envelope.

The nuclear envelope is the barrier between the nucleus and the cytoplasm, and nuclear pores are the gateways across that barrier. Unlike the plasma membrane, which prevents passage of macro-molecules between the cytoplasm and extracellular space, the nuclear envelope is a hub of activity for the movement of RNAs and proteins in both directions between the nucleus and cytoplas. The replication and transcription of genetic material within the nucleus requires the participation of large number of proteins that are synthesized in the cytoplasm and transported across the nuclear envelope. Conversely, the mRNAs, tRNAs and ribosomal subunits that are manufactured in the nucleus must be transported through the nuclear envelope in the opposite direction. Some components, such as snRNAs of the spliceosome move in both directions; they are synthesized in the nucleus, assembled into RNP particles in the cytoplasm and then shipped back to the nucleus where they function in mRNA processing. For appreciating the magnitude of traffic between the two major cellular compartments, **Karp** (2010) has cited the following example: a HeLa cell, which is estimated to contain about 10 million (one crore) ribosomes. To support its growth, a single HeLa cell nucleus must import approximately 560,000 ribosomal proteins and export approximately 14,000 subunits every minute.

The nuclear envelope of a typical mammalian cell contains 3000 to 4000 pores (about 11 pores/$\mu m^2$ of membrane area). If the cell is synthesizing DNA, it needs to import about $10^6$ histone molecules from the cytoplasm every 3 minutes in order to package newly made DNA into chromatin, which means that on an average each pore needs to transport about 100 histone molecules per minute. Further, if the cell is growing rapidly, each nuclear pore needs to export about three newly assembled ribosomes per minute to the cytoplasm, since ribosomes are produced in nucleus but function in the cytoplasm. The export of new ribosomal subunits is particularly problematic since these particles are about 15 nm in diameter and are much too large to pass through the 9 nm channels of nuclear pores, it is believed that they are specifically exported through the nuclear pores by an active transport system. Similarly, mRNA molecules complexed with special proteins to form ribonucleoprotein particles, are thought to be actively exported from the nucleus.

**Number of Nuclear Pores (Pore Density)**

In nuclei of mammals it has been calculated that nuclear pores account for 5 to 15 per cent of the surface area of the nuclear membrane. In amphibian oocytes, certain plant cells and protozoa, the surface occupied by the nuclear pores may be as high as 20 to 36 per cent. The number of pores in the nuclear envelope or **pore density** seems to correlate with the transcriptional activity of the cell. Thus, pore densities as low as ~3 pores/$\mu m^2$ are seen in nucleated red blood cells and lymphocytes (which are inactive in transcription). These cells are highly differentiated but metabolically inactive and they are non-proliferating cells. The majority of proliferating cells have pore densities between 7 and 12 pores/$\mu m^2$. Among cells of a third type, differentiated but highly active, pore densities are often 15 to 20 pores/$\mu m^2$. Liver, kidney and brain cells fall into this category. Still higher pore densities are found in specialized cells, such as salivary gland cells (~40 pores/$\mu m^2$) and the oocytes from *Xenopus laevis* (~50 pores/$\mu m^2$), both of which are very active in transcription.

**Arrangement of Nuclear Pores on Nuclear Envelope**

In somatic cells, the nuclear pores are evenly or randomly distributed over the surface of nuclear envelope. However, pore arrangement in other cell types is not random but rather range from **rows** (*e.g.*, spores of *Eqisetum*) to **clusters** (*e.g.*, oocytes of *Xenopus laevis*) to **hexagonal** (*e.g.*, Malpighian tubules of leaf hoppers) packing order.

**Nuclear Pore Complex (NPC):** Nuclear pores contain an intricate structure, called **Nuclear Pore Complex (NPC)** that appears to fill the pore like a stopper, projecting into both the cytoplasm (Fig. 4.38). The NPC is a huge, supermolecular complex –15 to 30 times the mass of a ribosome that exhibit octagonal symmetry due to the eightfold repitition of a number of structures. Despite their considerable size and complexity, NPCs contain only 30 different proteins, called **nucleoporins**, which are largely conserved between yeast and vertebrates. Each nucleoporin is present in at least eight copies, in keeping with octagonal symmetry of the structure. The NPC is not a static structure, as evidenced by the finding that many of its component

proteins are replaced with new copies over a time period of seconds to minutes.

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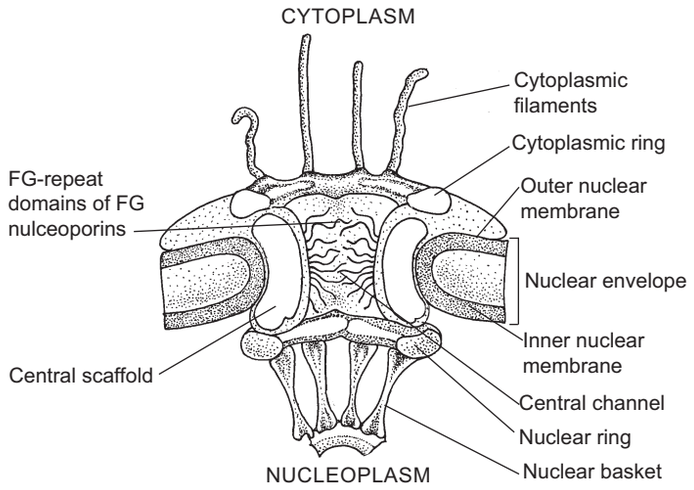


***Fig. 4.38*** *Three dimensional representation of a vertebrate NPC as it is situated within the nuclear envelope. This elaborate structure consists of several parts, including a scaffold that anchors the complex to the nuclear envelope, a cytoplasmic and nuclear ring, a nuclear basket, and eight cytoplasmic filaments. The FG (phenylalanine-glycine)-containing nucleoporins line the channel with this disordered FG-containing domains extending into the opening and forming a hydrophobic meshwork (after Karp 2010).*

The amino acid sequences of some nucleoporin proteins include a large number of phenylalanine-glycine repeat (*i.e.*, FG, by their single letter names). The FG repeats are clustered in a particular region of each molecule called the FG domain. Because of their unusual amino acid composition, the FG domains possess a disordered structure that gives them an extended and flexible organization. The FG repeat containing nucleoporins are thought to line the central channel of the NPC with their filamentous FG domains extending into the heart (center) of the 20 to 30 nm wide channel. The FG domains form a hydrophobic meshwork or sieve that blocks the diffusion of larger macromolecules (greater than about 40,000 Daltons) between the nucleus and cytoplasm.

### Functions of Nuclear Pore: Nuclear Transport

**Laskey** *et al.*, (1982) has found that **nucleoplasmin**, one of the more abundant nuclear protein of amphibian oocytes, contains a stretch of amino acids near its C-terminus that functions as a **nuclear localization signal** (**NLS**). This sequence enables a protein to pass through the nuclear pores and enter the nucleus. The best studied or "classical" NLSs, consist of one or two short stretches of positively charged amino acids. The T antigen encoded by the virus SV40, for example, contains an NLS identified as –Pro–Lys–Lys–Lys–Arg–Lys–Val. If one of the basic amino acids in this sequence is replaced by a nonpolar amino acid the protein fails to become localized in the nucleus. Contrary to this, if this NLS is fused to a nonnuclear protein, such as serum albumin and injected into the cytoplasm, the modified protein becomes concentrated in the nucleus. Thus, targeting of proteins to the nucleus is similar in principle to trafficking of other proteins that are destined for segregation within a particular organelle, such as a mitochondrion, a

chloroplast or a peroxisome. In all of these cases, the proteins possess a specific "address" that is recognized by a specific receptor that mediates its transport into the organelle.

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### Importins and Exportins

Recently, researchers have identified a family of proteins that function as mobile transport receptors, ferrying macromolecules across the nuclear envelope. Within this family, **importins** move macromolecules from the cytoplasm into the nucleus and **exportins** move macromolecules in the opposite direction.

**Nuclear Imports:** During nuclear import of a protein, such as nucleoplasmin, that contains a classical NLS, following steps are involved.

**Step 1.** Import of a protein begins as the NLS-containing cargo protein binds to a heterodimeric, soluble NLS receptor, called **importin** α/β, that resides in the cytoplasm.

**Step 2.** The transport receptor is thought to escort the protein cargo to the outer surface of the nucleus where it likely docks with the cytoplasmic filaments that extend from the outer ring of the NPC.

**Step 3.** The receptor-cargo complex then moves through the nuclear pore by engaging in a series of successive interactions with the FG domains of the FG-containing nucleopores. These interactions are thought to "dissolve" portions of FG-rich meshwork that fills the interior of the channel, allowing passage of the receptor-cargo complex through the NPC.

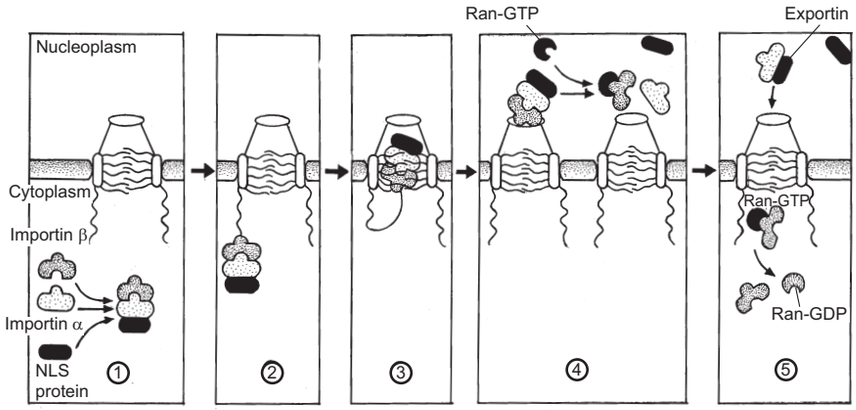


***Fig. 4.39*** *Mechanism of import from the cytoplasm into the nucleus. The protein bearing a NLS (= nuclear localization signal) binds to the heterodimeric receptor (importin α/β) (step 1) forming a complex that associates with a cytoplasmic filament (step 2). The receptor-cargo complex moves through the nuclear pore (step 3) and into nucleoplasm where it interacts with Ran-GTP and dissociates (step 4). The importin β subunit, in association with Ran-GTP, is transported back to the cytoplasm where the Ran-GTP is hydrolyzed (step 5). Ran-GDP is subsequently transported back to the nucleus, where it is converted to Ran-GTP. Conversely, importin α is transported back to cytoplasm (after Karp 2010).*

### Role of Ran Protein in Import

In nucleus, there is a GTP-binding protein called **Ran** which has a role in next two remaining steps of import of proteins into nuclear compartment. Ran can exist in an *active* GTP-bound form or in *inactive* GDP-bound form. Ran's

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role in regulating nucleocytoplasmic transport is based on a mechanism in which the cell maintains a high concentration of Ran-GTP in the nucleus and a very low concentration of Ran-GDP in the cytoplasm. The steep gradient of Ran-GTP across the nuclear envelope depends on the compartmentalization of certain accessory proteins such as RCC1 and RanGAP1. **RCC1** is sequestered in the nucleus where it promotes the conversion of Ran-GDP to Ran-GTP, thus maintaning the high level of Ran-GTP inside nucleus. **RanGAP1** resides in the cytoplasm where it promotes hydrolysis of Ran-GTP to Ran-GDP, thus, maintaining the low cytoplasmic level of Ran-GTP. In this way, the energy released by GTP hydrolysis is used to maintain the Ran-GTP gradient across the nuclear envelope. The Ran-GTP gradient drives nuclear transport by a process that depends only on receptor-mediated diffusion; *no motor proteins or ATPases have been implicated.*

So, when the importin-cargo complex arrives in the nucleus during Step 3, it is by a molecule of Ran-GTP, which binds to the complex and promotes disassembly of complex (Step 4). The imported cargo is released into the nucleoplasm, and one portion of NLS receptor (the importin β subunit) is shuttled back to the cytoplasm together with the bound Ran-GTP (Step 5). Once in the cytoplasm, the GTP molecule bound to Ran is hydrolyzed, releasing Ran-GDP from the importin β subunit. Ran-GDP is returned to the nucleus where it is converted back to the GTP-bound state for additional rounds of activity. Importin α is transported back to the cytoplasm by one of the exportins.

**Nuclear Export.** Ran-GTP plays a key role in the escort of macromolecules from the nucleus, just as it does in their import from the cytoplasm. As it has been mentioned that Ran-GTP is essentially confined to the nucleus. Whereas the Ran-GTP induces the disassembly of imported complexes, it also promotes the assembly of exported complexes. Proteins exported from the nucleus contain amino acid sequences, called **Nuclear Export Signals** or **NES**, that are recognized by the transport receptors that carry them through the nuclear envelope to the cytoplasm. Most of the traffic moving in this direction consists of various types of RNA molecules—especially mRNA, rRNAs and tRNAs—that are synthesized in the nucleus and function in cytoplasm. In most cases, these RNAs move through the NPC as **RiboNucleoProteins** (**RNPs**).

### Export of mRNA

Transport of an mRNP (messenger RNA protein) from the nucleus to cytoplasm is associated with extensive remodeling; certain proteins are removed from the mRNP, while others are added to the complex. Transport of mRNPs does not appear to require Ran protein but does require the activity of an **RNA helicase** located on the cytoplasmic filaments of the NPC. It is speculated that the helicase provides the motive force to move the mRNA into the cytoplasm. Various studies have shown a functional link between pre-mRNA splicing and mRNA export; only mature (*i.e.*, fully processed) mRNAs are capable of nuclear export. If an mRNA still contains an unspliced intron, that RNA is retained in the nucleus.

Lastly, nuclear pores are not the only avenues for nucleocytoplasmic exchanges. For example, small molecules and ions readily permeate both nuclear membranes. Larger molecules and particles may pass through the membrane by formation of small pockets and vesicles that traverse the envelope and empty on the other side.

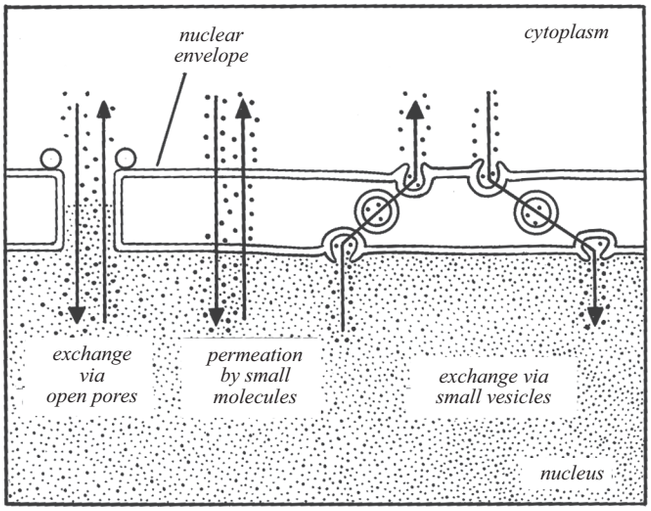


***Fig. 4.40*** *Various avenues of transport of material from the nucleus to the cytosol (cytoplasmic matrix).*

## 4.5.6 Trafficking Mechanisms

The propeptide sequences have significant functions in protein maturation. The key class of cleaved peptide sequences is signal peptides. Signal peptides direct the protein from the cytoplasm into a particular cellular compartment. In the case of prokaryotes, this essentially means the cell membrane, but for eukaryotes, there are specific signal peptides that can direct the protein to the nucleus, to the mitochondria, to the Endoplasmic Reticulum (ER), and other intracellular organelles. The peptides are specifically recognized by receptors on the membranes of particular compartments, which then help to guide the insertion of the protein into or through the membrane. Almost all protein synthesis in eukaryotes is carried out in the cytoplasm, with the exception of a few proteins in the chloroplasts and mitochondria, therefore proteins found in any other compartment or embedded in any membrane must have been targeted and transported into that compartment by its signal sequence.

Even though this is principally considered a eukaryotic process given that there are so many potential targets, prokaryotes do have membrane proteins. In fact, some 800 different ones in *Escherichia coli* comprising ~20% of total protein. The membrane proteins are positioned with the support of insertase enzymes, such as YidC and complexes, such as Sec Translocase. The Sec translocase uses a Signal Recognition Particle (SRP) similar to that in eukaryotes. YidC, which has eukaryotic homologues, for example 'Oxa1 in mitochondria', is a 61 kDa transmembrane protein that is placed in the membrane through an SRP-Sec translocase mechanism. Once transported, YidC interacts with nascent polypeptides (once they reach ~70 amino acids

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long) that have begun to interact with the lipids of the cell membrane and pushes the protein into/through the membrane.

The nucleus is one such compartment, and examples of the proteins found within include DNA and RNA polymerases, transcription factors, and histones. These and other nuclear proteins have an N-terminal signal sequence known as the NLS, or nuclear localization signal. This is a well-studied pathway that involves a set of importin adapter proteins and the nuclear pore complex. Transport into the nucleus is particularly challenging because it has a double membrane, it is contiguous with the endoplasmic reticulum membrane.

Once the nucleoprotein-importin aggregate is moved into the nucleus, Ran-GTP, a small GTPase, causes the aggregate to dissociate. The imported protein is released in the nucleus. The importins are also released in the nucleus, but they are exported back out again to be reused with another protein targeted for the nucleus.

Export from the nucleus to the cytoplasm also occurs through the nuclear pore. The Ran-GTP is also a part of the export complex and in conjunction with an exportin protein and whatever is to be exported, is moved out of the nucleus via the nuclear pore. Once in the cytoplasm, the hydrolysis of GTP to GDP by Ran (activated by Ran-GAP, a cytoplasmic protein) provides the energy to dissociate the cargo (for example, mRNA) from the exporting transport molecules. The Ran-GDP then binds to importins, re-enters the nucleus, and the GDP is exchanged for GTP.

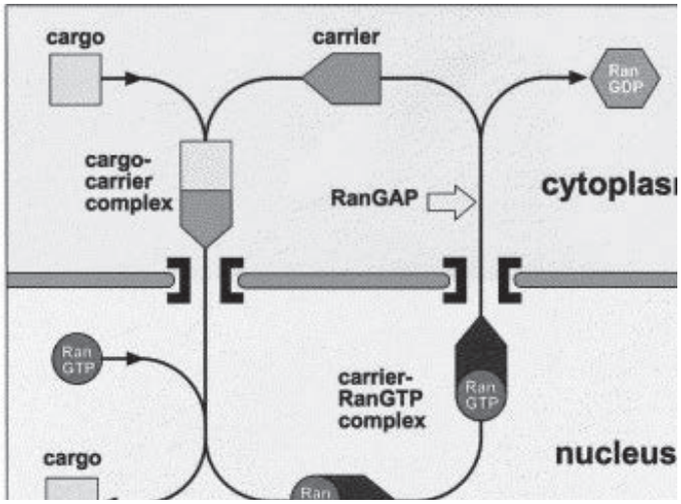


***Fig 4.41*** *Export from the Nucleus to the Cytoplasm*

**Check Your Progress**

13. What is intracellular transport?
14. What are cargo protein?
15. Define a polysome.
16. What is the golgi apparatus?

## 4.6 BIOLOGY OF CANCER

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The proliferation, differentiation and survival of individual cells in multicellular organisms are carefully regulated to meet the needs of the organism as a whole. This regulation is lost in cancer cells, which grow and divide in an uncontrolled manner, ultimately spreading throughout the body and interfering with the function of normal tissues and organs (**Cooper** and **Hausman**, 2007).

Cells which undergo rapid, abnormal and uncontrolled growth at the cost of remaining cells are called **neoplastic cells**. The growths resulting from the division of such cells are called **neoplastic growths** or **tumours**. Thus, any abnormal proliferation of cells is the tumour. Almost all type of differentiated cells of animals can become neoplastic or cancerous. The process of cell change in which a cell loses its ability to control its rate of division, and thus becomes a tumour cell, is called **cell transformation**.

Cancer is a genetic disease because it can be traced to alterations within specific genes, but in most cases, it is not an inherited disease. In an inherited disease, the genetic defect is present in the chromosomes of a parent and is transmitted to the zygote. In contrast, the genetic alterations that lead to most cancers arise in the DNA of a somatic cell during the lifetime of the affected individual. Because of these genetic changes, cancer cells proliferate uncontrollably, producing malignant tumours that invade surrounding healthy tissue (see **Karp**, 2002).

### 4.6.1 Types of Cancer

Cancer can result from abnormal proliferation of any of the different kinds of cells in the body, so there are more than a hundred distinct types of cancers. These cancers can vary substantially in their behaviour and response to treatment. Based on differences in their growth patterns, tumours are classified as either benign or malignant. A **benign tumour** or **primary tumour**, such as a common skin wart, remains confined to its original location. A **malignant tumour** or **secondary tumour**, however, is capable of both invading surrounding normal tissue and spreading throughout the body via the circulatory or lymphatic systems (**metastasis**). Only malignant tumours are properly referred to as cancers and it is their ability to invade and metastasize that makes cancer so dangerous. The term **cancer** refers to any malignant tumour that is capable of spreading from its original location to other sites. Whereas benign tumours can usually be removed surgically, the spread of malignant tumours to distant body sites frequently makes them resistant to such localized treatment.

Both benign and malignant tumours are classified according to the type of cell from which they arise. About two hundred distinct types of cancer have been recognized. These can be grouped into four main types: carcinomas, sarcomas, lymphomas and leukemias.

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The earliest recorded description of cancer is found in an Egyptian papyrus dating back to around 3000 B.C. (Papurus refers to a manuscript written on paper prepared from papyrus plant *Cyperus papyrus*). The term cancer, which means "crab" in Latin, was coined by Hippocrates in the fifth century B.C., to describe diseases in which tissues grow and spread unrestrained throughout the body, eventually choking off life (**Becker** *et al.*, 2006). Hippocrates used the words *carcinus, carcinoma* and *cancer*, which refer to crabs in Latin, to describe the tumours (**Cooper** and **Hausman**, 2007).

**1. Carcinomas:** These cancers account for 90 per cent of all human cancers. Carcinomas arise from the epithelial cells that cover external and internal body surfaces. These epithelial cells may be of ectodermal or endodermal origin. Carcinomas include cervical, breast, skin, brain, lung, colon prostate, kidney, bladder cancers. Carcinomas affect cells of ectodermal or endodermal origin.

**2. Sarcomas:** These cancers account for 4 per cent of all human cancers. Sarcomas are solid tumours of connective tissues such as cancers of muscle, bone, cartilage, fat cells and fibrous tissue. Sarcomas affect the cells of mesodermal origin.

**3. Lymphomas:** These cancers constitute about 4 per cent of human cancers. In lymphomas, there is an excessive production of lymphocytes by the lymph nodes and spleen. Hodgkin's disease is an example of lymphoma (Hodgkin's disease is a neoplastic disease that is characterized by progressive enlargement of lymph nodes, spleen, and liver and by progressive anaemia).

**4. Leukemias:** These cancers form about 4 per cent of human cancers. Leukemias are neoplastic growth of leucocytes (W.B.C.) and are characterized by excessive production of white blood cells. Leukemias affect cells of mesodermal origin and are a class of sarcomas (**Lodish** *et al.* 2004). In addition to the four types of cancer mentioned above there may be **mixed malignant** tumours arising from ectodermal and mesodermal tissues, *e.g.*, tumours arising from ectodermal and mesodermal tissues.

The leukemias grow as individual cells in the blood. The name **leukemia** is derived from the Latin for "white blood": the massive proliferation of leukemic cells can cause a patients blood to appear milky.

Prior to 1900, most human deaths were due to infectious diseases such as pneumonia and tuberculosis, and life expectancy was less than 50 years. Cancer was rare disease that accounted for only a small percentage of deaths.

According to a recent survey conducted by American cancer society in 2005, more than 5 lac Americans die of cancer each year. The four most common cancers accounting for more than half of all cancer cases are those of the prostate, breast, lung, and colon/rectum. Lung cancer, by far the most lethal, is responsible for nearly 30% of all cancer death.

## 4.6.2 Growth of Normal and Cancerous Cells

**1. Growth of Cancer Cells Produce Multilayered Clumps.**

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Information on the behaviour of human cancer cells has come largely from studies on cells grown *in vitro*. At the cellular level, the most important characteristic of a cancer cell—whether residing in the body or on a culture dish—is its loss of growth control. The **capacity** for growth and division is not drastically different between a cancer cell and most normal cells. When normal cells are grown in tissue culture, under conditions that promote cell proliferation, they grow and divide at a rate similar to that of their malignant counterparts. However, when the normal cells proliferate to the point where they cover the bottom of culture dish, their growth rate decreases markedly, and they tend to remain as a single layer (monolayer) of cells. Growth rates drop as normal cells respond to inhibitory influences from their environment. Growth inhibiting influences may arise as the result of depletion of growth factors in the culture medium or from contact with surrounding cells on the dish (called **contact inhibition**). In contrast, when malignant cells are cultured under the same conditions, they continue to grow, piling on top of one another to form clumps or foci (Fig.). It is evident that malignant cells are not responsive to the types of regulatory signals that causes their normal counterparts to cease growth and division.

**Stages of Tumour Development.**

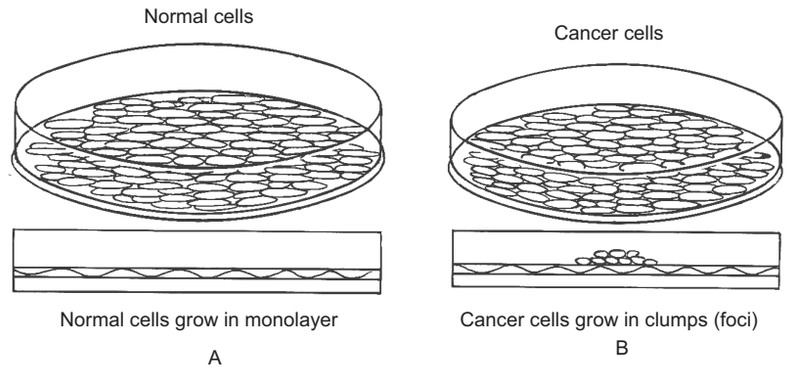


***Fig. 4.42*** *Growth properties of normal and can-cerous cells. A—Normal cells typically grow in a culture dish until they cover the surface as a monolayer. B—In contrast, cells that have been transformed by viruses or carcinogenic chemicals (or malignant cells that have been cultured from tumours) typically grow in multilayered clumps or foci (after Karp, 2002).*

At the cellular level, the development of cancer is viewed as multistep process involving **mutation** and **selection** for cells with progressively increasing capacity for proliferation, survival, invasion, and metastasis .

**1. Initiation:** The step of tumour initiation is thought to be the result of a genetic alteration due to mutation leading to abnormal proliferation of a single cell. Cell proliferation then leads to the outgrowth of population of clonally derived tumour cells.

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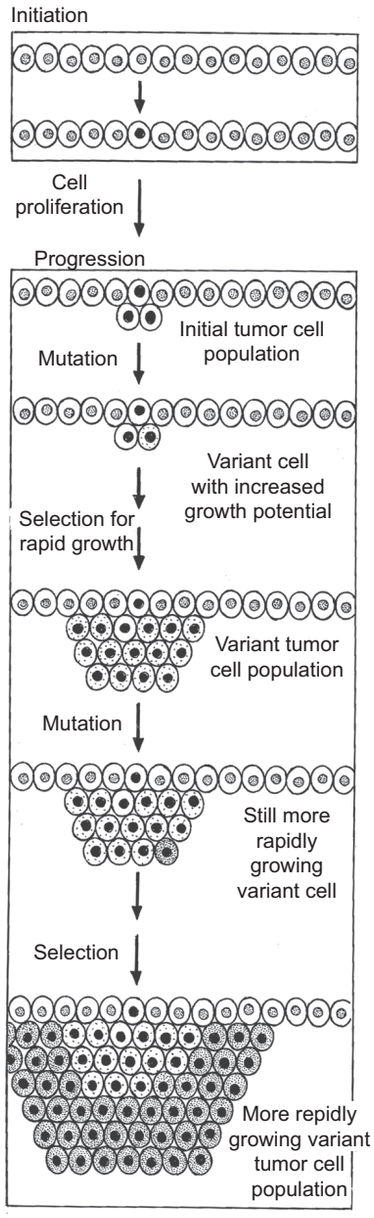


***Fig. 4.43*** *Stages of tumour development. The development of cancer initiates when a single mutated cell begins to proliferate abnormally. Additional mutations followed by the selection for more rapid growing cells within the population then result in progressioin of the tumour to increasingly rapid growth and malignancy (after Cooper and Hausman, 2007).*

**2. Tumour Progression:** This process continues as additional mutations occur within cells of the tumour population. Some of these mutations confer a selective advantage to the cell, such as more rapid growth, and the descendants of a cell bearing such mutations will consequently become dominant within the tumour population. The process is called **clonal selection**, since a new clone of tumour cells has evolved on the basis of its increased growth rate or other properties (such as survival, invasion or metastasis) that confer a selective advantage. Clonal selection continues throughout tumour development, so tumours continuously become more rapid-growing and increasingly malignant.

**Angiogenesis**

Both primary and secondary tumours require *angiogenesis*, the formation of new blood vessels, in order to grow to a large mass. Most tumours induce the formation of new blood vessels that invade the tumour and nourish it, a process called **angiogenesis**. This complex process requires several distinct steps:

1. Degradation of the basal lamina that surrounds a nearby capillary,
2. Migration of endothelial cells lining the capillary into the tumour
3. Division of these endothelial cells
4. Formation of a new basement membrane around the newly elongated capillary

Many tumours produce growth factors that stimulate angiogenesis; other tumours somehow induce surround-ing normal cells to synthesize and secrete such factors. Basic fibroblast growth factors (bFGF), transforming growth factor α (TGFα) and vascular endothelial growth factor (VEGF), which are secreted by many tumours, all have angiogenic properties. New blood vessels nourish the growing tumour, allowing it to increase in size and thus increase the probability that additional harmful mutations will occur (see **Lodish** *et al.*, 2004).

**Example of Development of a Human Cancer: Colon Carcinomas**

Studies of colon carcinoma have provided a clear example of tumour progression during the development of a common human malignancy. The earliest stage in tumour development is increased proliferation of colon epithelial cells. One of the cells within this proliferative cell population is then thought to give rise to a small benign neoplasm (an **adenoma** or **polyp**). Further rounds of clonal selection lead to the growth of adenomas of increasing size and proliferative potential.

## 4.6.3 Characteristics of Cancer Cells

The cancerous cell generally retains the structural and functional characteristics of the normal cell type from which it is derived. For example, the cancerous cells of thyroid gland continue to secrete thyroxin. Neoplastic cells, however, differ from their normal counterparts in several ways.

**1. Immortalization:** Normal cell cultures do not survive indefinitely. For example, human cell cultures die after about 50 generations, and chicken cell cultures have a much shorter life expectancy. In contrast, transformed cell cultures are immortal and can grow indefinitely. A striking example is provided by HeLA cells, which were obtained in 1953 from a uterine cancer diagnosed in a women named **Henreitta Lacks** (hence the term "HeLa" cells). After the tumour was removed by surgeons, some of its cells were placed in culture. The cultured cells quickly began to proliferate and have continued to do so for more than 50 years, dividing more than 18,000 times with no sign of stopping (see **Becker** *et al.*, 2006). Similarly, cell cultures infected with mouse sarcoma virus can be maintained as long as nutrition is provided and overcrowding avoided.

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Cancer cells, like germ cells and stem cells but unlike most differentiated cells, produce **telomerase** enzyme, which prevent shortening of chromosomes during DNA replication and may contribute to their **immortalization**. The absence of telomerase is associated with resistance to generation of certain types of human tumours (*e.g.*, papillomas and colon tumours of mice) (see **Lodish** *et al.*, 2004).

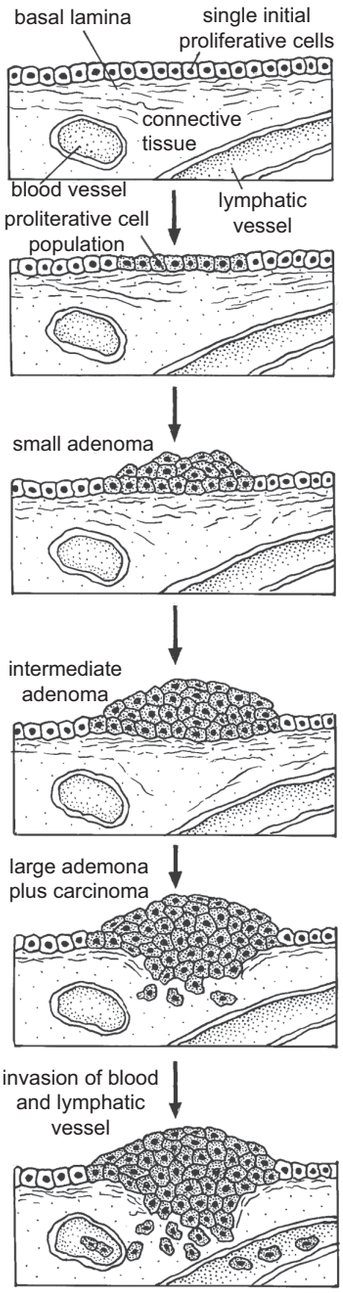


***Fig. 4.44*** *Development of colon carcinomas. A single initially altered cell gives rise to a proliferative cell population, which progresses first to benign adenomas of increasing size and then to malignant carcinoma. The cancer cells invade the underlying connective tissue and penetrate blood and lymphatic vessels, thereby spreading throughout the body (after Cooper and Hausman, 2007).*

**2. Loss of Contact Inhibition:** Normal cells in a culture stop growing when their plasma membranes come into contact with one another. This

inhibition of growth after contact is called **contact inhibition**. Transformed cells are unable to go into a quiescent stage after division. They will grow (*i.e.,* devide) continuously until they kill themselves. Transformed cells apparently undergo a change in the property of their plasma membranes, which become less adhesive. This change enables the cells to dissociate from neighbouring cells and to infiltrate other organs, where they form metastatic tumours. Cancer cells thus, lack proper recognition and communication.

**3. Reduced Cellular Adhesion:** When normal cells become cancerous there is change in the **stickiness** or **adhesiveness** of their plasma membranes. If grown in a nutrient medium kept in a glass vessel, the normal cells stick to the glass rather than float in the medium. Transformed cells show a **decreased adhesiveness** and if grown in solid media, they stick to each other less than do normal cells.

Adhesiveness shows considerable specificity. For example, liver cells tend to stick to other liver cells and not to other cell types, *e.g.*, kidney cells. If the cells of the liver and the kidney are separated by the enzyme trypsin and incubated together, they aggregate to form small pieces of liver tissue and kidney tissue. Thus, kidney cells stick to kidney cells and liver cells to liver cells. Cancerous cells do show this property (*i.e.*, specificity of adhesiveness). Thus, if malignant skin cancer cells are mixed with normal kidney cells then aggregates formed contain both kidney and skin cells mixed together. This probably explains why malignant cell can invade several normal organs.

**4. Invasiveness:** One of the most significant characteristics of transformed cells is their invasiveness, *i.e.*, the ability of cancer cells, to invade other tissues. In contrast to normal cells, transformed cells can penetrate the chorioallantoic membrane of the hen's egg. This invasiveness could be due to changes in the plasma membrane and/or proteases released by the cancerous cells.

**5. Loss of Anchorage Dependence:** Most normal cells must be attached to a rigid substratum (*i.e.*, they must be anchored) in order to grow in a culture medium. Transformed cells can grow even when they are not anchored to the substratum. For example, when transformed cells are suspended in a semisolid medium containing agar or methyl cellulose. This loss of anchorage is most striking characteristic of transformed cells which form malignant tumours. This property is used to select transformed cells from a normal cell population.

**6. Lower Serum Requirements:** Growth of normal cells in tissue culture medium requires a high concentration of serum. Some serum growth factors (*e.g.*, somatomedins) resemble insulin hormone in interacting with external receptors on the plasma membrane to regulate biochemical activities within the cell. Transformed cells can grow in a culture medium containing much less serum than required by normal cells. For example, normal 3T3 cells (established fibroblasts of the mouse line commonly used is tissue culture) grow optimally in 10 per cent foetal calf-serum, while cells transformed by SV40 (simian virus number 40) can grow equally well in 1 per cent of 10 per cent serum. It has been suggested that the lower serum requirement of transformed cells is because of their lesser requirement out side substances

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to lower their intracellular cAMP (cyclic AMP) level to trigger mitosis.

**7. Selective Agglutination by Lectins:** Lectins are proteins widely distributed in plants (*e.g.*, legumes) and some animals They have the ability to bind to receptors, which are branched chain sugar molecules (oligosaccharides), on the surface of the plasma membrane. As a result of such binding lectin cause **agglutination** or **clumping** of cells. Due to this property, lectins work as agglutinins.

In normal cells the receptors or agglutin binding sites for lectins lie in a diffuse manner on the cell surface and are immobile. In such cases, lectins make few intercellular bridges, and therefore agglutination is not possible. In transformed cells, the receptors are more mobile within the plasma membrane. Local regions of high binding site concentration are formed. Lectins are thus able to form enough intercellular bridges to result in agglutination in the transformed cells.

**8. Molecular Changes in Components of the Plasma Membrane:** There are several differences between the surface of plasma membranes of normal and transformed cells. The plasma membrane consists of four main types of **phospholipids**, which form the lipid bilayer, with **glycolipids** and **glycoproteins** inserted into this bilayer. Cancerous cells apparently do not differ from normal cells in their relative amounts of phospholipids. However, **gangliosides** (glycolipids which contain sialic acids) become reduced in certain mouse cancer cells. Enzymes involved in biosynthesis of these gangliosides are also reduced. Normal cells possess four types of gangliosides, GM1a, GM1, GM2 and GM3. Tumour cells predominantly contain the simplest type, GM3.

Certain changes have been reported in the **glycoproteins** of plasma membrane of cancerous cells. The surface glycoprotein of MW 46,000 disappears early in transformation to the cancerous condition. There is also a slow disappearance of a major protein called **LETS** (large, external, transforming sensitive) protein (MW 240,000). Probably the most important protein to disappear after transformation is the one having MW 200,000.

**9. Disorganisation of Cytoskeleton:** Normal cells have a well organised cytoskeleton which consists of **microtubules** (of tubulins), **microfilaments** (of actins) and **intermediate filaments** (for example of collagen). The fibre-like proteins have a regular arrangement and bring about coordinated cell movement. In transformed or cancer cells, the fibres are much fewer in number and usually much thinner. In transformed cells the cytoskeleton is found to undergo **depolymerisation**. The microtubules disaggregate. The microfilaments undergo depolymerisation and disappear, but diffuse actin persists. The myosin-like filaments also disappear. Thus, in transformed cells the cytoskeleton proteins become less organised than in normal cells. It has been suggested that due to this disorganisation of the cytoskeleton, there is an increased mobility of plasma membrane proteins.

The disorganisation of the cytoskeleton also affects the cell surface in another way. When cancer cells are touched, there is a constant and uncoordinated throwing out and retraction of **blebs**, **microvilli** and **ruffles**

from the cell surface. Tumour cells have a more ruffled surface than normal cells, with many more surface processes.

**10. Increase in Negative Surface Charge:** Comparisons of surface membrane charge by microelectrophoresis have been made between normal and malignant cells. In malignant cells anodic mobility is usually higher, indicating increase in negative surface charge.

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**11. Defective Electrical Communication:** Electrical connections normally occur between individual normal cells. In some cancer cells, however, it has been reported that such electrical connections are defective.

**12. Increased Sugar Transport:** Tumour cells consume much more glucose than normal cells because they have to grow and multiply. There is a great increase in the rate of sugar transport across the surface membrane (plasma membrane) after transformation of the cell. This tends to increase in sugar intake by malignant cells.

**13. Increased Rate of Glycolysis:** In the 1920's **Warburg** pointed out that oxidative (aerobic) respiration is depressed in tumour cells and that glycolysis (anaerobic respiration) increases. This has been demonstrated by an increase in lactic acid production in cells of solid tumour. There is a corresponding increase in the uptake of glucose.

**14. Appearance of Virus-Specific Transplantation Rejection Antigens:** Plasma membranes of most transformed cells contain **antigens** which are not present in normal cells. Thus in cells transformed by adenoviruses and papovaviruses the **T-antigen** is always present. Similarly all cells transformed by the Epstein-Barr virus (EB virus) contain an antigen called the **EB nuclear antigen** (**EBNA**). Tumour antigens can bring about an **immunity response** against themselves in genetically similar hosts. This is in contrast to normal histocompatibility antigens. The immunity response brought about by the antigens results in recognition and destruction of newly formed cancer cells and their descendants. Such a defence mechanism is called **immunological surveillance** and can lead to the elimination of transformed or cancer cells under favourable conditions. It has been suggested that only in the rare cases when this defence mechanism fails that tumour is formed.

**15. Increased Secretion of Proteolytic Enzymes:** Large amount of proteolytic enzymes are secreted by all types of cancer cells, except those of blood forming tissues. The cancer cell secretes a protease called the **cell factor** (MW ~40,000). The cell factor acts on an inert serum protein **plasminogen** (MW 85,000) to form a **plasmin**, a **proteolytic enzyme** (MW 76,000). It has been suggested that plasmin removes many proteins projecting from the cell surface by enzymatic digestion and signals the cell into division.

If normal cells are treated with proteases they show many of the characteristics of transformed cells. It has been speculated that viral proteins cause the release of extracellular protease. However, there is no direct evidence for this.

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**16. Aldolases:** In most mammalian tissues the enzyme **aldolase** exists in the form of three isozymes A, B and C. Isozymes A and C predominate in embryonic tissues while in adult differentiated tissues the B isozyme is predominant. In some tumours, especially in poorly differentiated and rapidly growing cancers such as **hepatomas**, isozyme B is replaced by isozyme A, the embryonic form.

**17. Most Cancers Develop Later in Life:** Because the multiple mutations that lead to formation of a tumour may require many years to accumulate, most cancers develop later in life. The occurrence of cancer after the age of reproduction may be one reason that evolutionary restraints have not done more to supress cancer. The requirement for multiple mutations also lowers the frequency of cancer compared with what it would be if tumerogenesis were triggered by a single mutation. However, huge numbers of cells are, in essence, mutagenized and tested for altered growth during our lifetime, a sort of evolutionary selection for cells that proliferate. *Fortunately the tumour itself is not inherited* (see **Lodish** *et al.*, 2004).

### 4.6.4 Causes of Cancer

Cancers are caused mainly by environmental agents and lifestyle factors, most of which act by triggering DNA mutations.

**1. Epidemiological Data:** The first indication that a particular agent may cause cancer is usually provided by an approach called **epidemiology,** the branch of medical science that investigates the frequency and distribution of diseases in human populations. Epidemiological studies have revealed that cancers arise with differing frequencies in different parts of the world. For example, stomach cancer is frequent in Japan, breast cancer is prominent in the United States and liver cancer is common in Africa and Southeast Asia. To determine whether differences in heredity or environment are responsible for such differences, scientists have examined cancer rates in people who move from one country to another. For example, in Japan the incidence of stomach cancer is greater and the incidence of colon cancer is lower than in United States. When Japanese families move to the United States, their cancer rates come to resemble the rates in the United States, indicating that cancer rates are determined more by environment and lifestyle factors than by heredity.

Epidemiological data have played an important role in identifying environmental factors that can cause cancer. The most drastic facts involve lung cancer, a disease that has increased over tenfold in frequency in the United states since 1900. When the possible causes for this epidemic of lung cancer were investigated, it was discovered that virtually all lung cancer patients share one trait: a history of smoking cigarettes. As might be expected if cigarettes were responsible, heavy smokers develop lung cancer more frequently than light smokers, and long-term smokers develop lung cancer more frequently than do short-term smokers.

While the association between cigarette smoking and cancer was first detected through epidemiological studies, definitive proof of a cause-and-effect relationship requires direct experimental evidence. In the case of cigarettes

and lung cancer, such evidence has come from studies showing that cigarette smoke contains several dozen chemicals that cause cancer when administered to animals. Such substances that cause cancer are called **carcinogens**.

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**2. Carcinogens:** The idea that certain chemicals, such as those found in tobacco smoke, can cause cancer was first proposed more than 200 year's ago. In 1761 a London doctor, **John Hill**, reported that people who routinely use snuff (a powdered form of tobacco that is inhaled) experience an abnormally high incidence of nasal cancer, suggesting the presence of carcinogen (cancer-causing) chemicals in tobacco. A few years later another British physician, **Percival Pott**, observed an elevated incidence of scrotum cancer among men who had served as chimney sweepers in their youth. It was common practice at the time to employ young boys to clean chimney flues because they fit into narrow spaces more readily than adults. Pott speculated that the chimney soot became dissolved in the natural oils of the scrotum, irritated the skin, and eventually triggered the development of cancer. This theory led to the discovery that scrotum cancer could be prevented among chimney sweepers through the use of protective clothing and regular bathing practices.

In forthcoming years, the list of known and suspected carcinogens has grown to include hundreds of different chemicals. Chemicals are usually labelled as carcinogens because humans or animals develop cancer when exposed to them. This does not mean, however, that each of these substances causes cancer through its own direct action. For example, consider the behaviour of **2-naphthylamine**, a potent carcinogen that causes bladder cancer in industrial workers and is present in tobacco smoke. As might be expected, feeding 2-naphthylamine to laboratory animals induces a high incidence of **bladder cancer**. But if 2-naphthylamine is implanted directly into an animal's bladder, cancer rarely develops. The explanation for the apparent difference is that 2-naphthylamine is ingested (by animals) or inhaled (by humans), it passes through the liver and is metabolically converted into chemical compounds that are actual causes of cancer. Placing 2-naphthylamine directly in an animal's bladder bypass this metabolic activation and consequently cancer does not arise.

**Precarcinogens:** Many carcinogens share the above need for metabolic activation before they can cause cancer. Substances exhibiting such behaviour are more accurately referred to as **precarcinogens**, a term applied to any chemical that is capable of causing cancer only after it has been **metabolically activated**. The activation of most precarcinogens is carried out by liver proteins that are members of cytchrome P450 enzyme family. Members of this enzyme family catalyze the oxidation of ingested foreign chemicals, such as drugs and pollutants, to make the molecules less toxic and easier to excrete from the body. However, in some cases these oxidation reactions accidentally convert foreign chemicals into carcinogens—a phenomenon called **carcinogen activation**.

**Mode of Action of Carcinogens:** Once it has been determined that chemicals can cause cancer, the question arose as to how they work. The

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idea that carcinogenic chemicals act by triggering **DNA mutations** was first proposed around 1950, but there was little supporting evidence at that time because nobody had systemically compared the mutagenic potency of different chemicals with their ability to cause cancer.

**Ames Test:** The need for identifying potential carcinogens, inspired **Bruce Ames** (1973) to develop a simple rapid laboratory test for measuring a chemical's mutagenic activity. The procedure he developed, called the **Ames test**, utilise bacteria as a test organisms because they can be quickly grown in enormous numbers in culture. The bacteria used for the Ames test are a special strain that lack the ability to synthesize the amino acid **histidine**. As shown in Fig. 4.42, the bacteria are placed in a culture dish containing a growth medium without histidine, along with the chemicals being tested for mutagenic activity. Normally, the bacteria would not grow in the absence of histidine. However, it will trigger random mutations, some of which might restore the ability to synthesize histidine. Each bacterium acquiring such a mutation will grow into a visible colony, so that the total number of colonies is a measure of the **mutagenic potency** of the substance being investigated.

Since many chemicals that cause cancer only become carcinogenic after they have been modified by liver enzymes, the Ames test includes a step in which the chemical being tested is first incubated with an extract of liver cells to mimic the reactions that normally occur in the liver. The resulting chemcial mixture is then tested for its ability to cause bacterial mutations. When the Ames test is performed in this way, a strong correlation is observed between a chemical's ability to cause mutations and its ability to cause cancer.

**Cancer Arise Through a Multistep Process:** Chemicals induce the development of cancer through a multistep process involving **initiation, promotion**, and **tumour progression**. **Initiation** is based on DNA mutation, whereas **promotion** involves proliferation of the initiated cells for a prolonged period of time and accompanied by a gradual **selection** of cells exhibiting enhanced growth properties. During **tumour progression**, cells acquire additional mutations and undergo changes in gene expression that produce cells with increasingly observant traits.

**3. Role of Ionizing and Ultraviolet Radiations in Carcinogenesis:** Shortly after the discovery of **X-rays** by **Wilhelm Roentgen** in 1895, it was noticed that people working with this type of radiation developed cancer at abnormally high rates. Animal studies subsequently confirmed that X-rays create DNA mutations and cause cancer in direct proportion to the dose administered.

X-rays type of radiations is emitted by many **radioactive elements.** An early example of the carcinogenic hazards posed by radioactivity occurred in 1920's in a New Jersey factory that produced glow-in-the-dark watch dials. A luminescent paint containing the radioactive element **radium** was used for painting the dials, and this paint was applied with a fine tipped brush that the workers frequently wetted with their tongues. As a result, tiny quantities

of radium were carelessly ingested and became concentrated in their bones, leading to the development of **bone cancer**.

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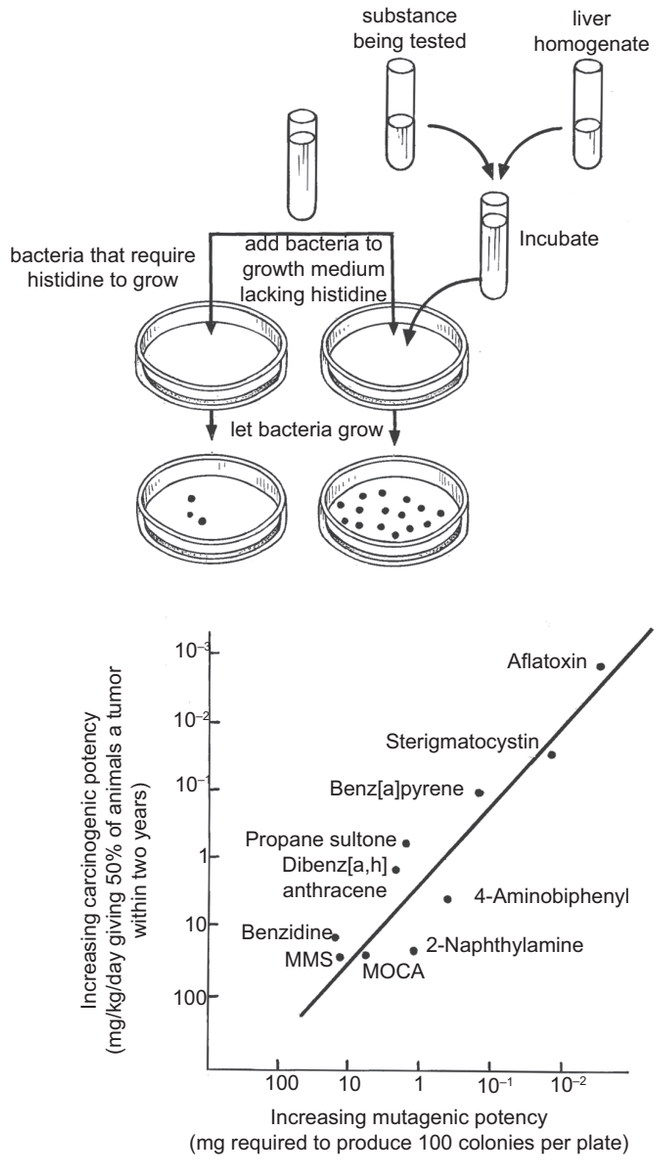


***Fig. 4.45*** *Procedure of Ames test. Ames test is based on the grounds that most carcinogens are mutagens.*
*A—The ability of chemicals to induce mutations is measured in bacteria that lacks the ability to synthesize the amino acid histidine. When placed in a growth medium that lacks histidine, the only bacteria that can grow are those that have acquired a mutation that allows them to make histidine. The number of bacterial colonies that grow is therefore related to the mutagenic potency of the substance being tested. Chemicals being investigated with the Ames test are first incubated with a liver homogenate because many of the chemicals to which humans are exposed only become carcinogenic after they have undergone biochemical modification in the liver. B—The data in the graph reveal that substances that exhibit strong mutagenic activity in the Ames test also tend to be strong carcinogens. Here, aflatoxin is the most potent mutagen and the most potent carcinogen. (Abbreviations: MOCA = 4–4' methylene-bis2-chloroanaline,*
*MMS = methyl methanesulphonate; after Becker et. al., 2006).*

High rates of cancer caused by exposure to radioactivity have also been observed in people exposed to radioactive fallout from nuclear explosions.

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The most dramatic incidents occurred in the Japanese cities of Hiroshima and Nagasaki after atomic bombs were dropped there in 1945. Another incident occurred in the area surrounding the Chernobyl nuclear power plant in the former Soviet Union (now Ukraine), which exploded in 1986.

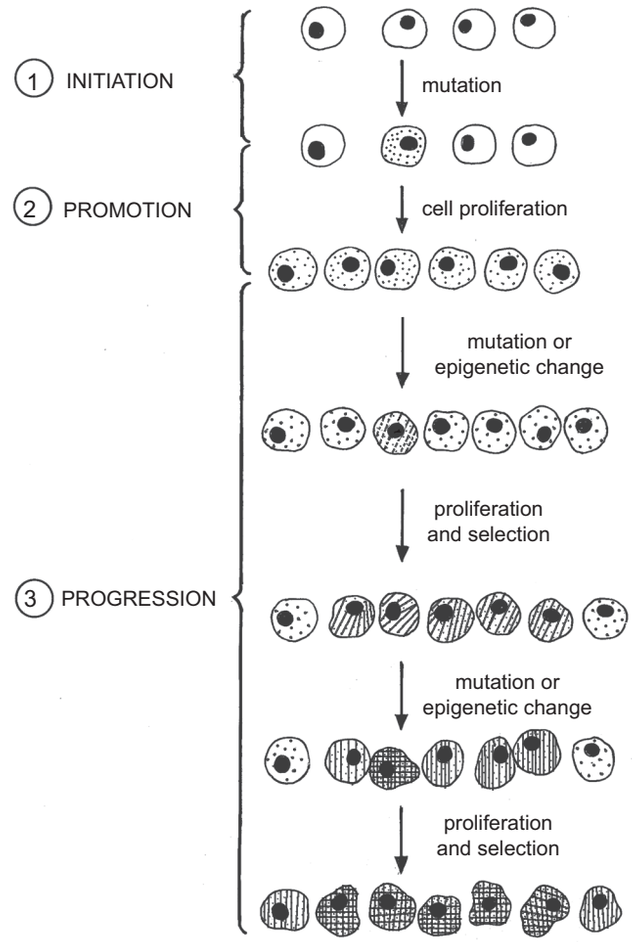


***Fig. 4.46*** *Main stages in the development of cancer. Cancer arises by a complex process involving three main stages. 1. Initiation. It is based on DNA mutation. 2. Promotion. During this stage the initiated cell is stimulated to proliferate. 3. Progression. During tumour progression, further mutations and changes in gene expression create variant cells exhibiting enhanced growth rates or other aggressive properties that give certain cells a selective advantage. Such cells tend to outgrow their companions and become the predominant cell population in the tumour. Repeated cycles of this selection process create a population of cells whose properties gradually change over time (after Becker et al., 2006).*

X-rays and related forms of radiation emitted by radioactive elements, are called **ionizing radiation** because they remove electrons from molecules, thereby generating highly reactive ions that create DNA damage. **Ultraviolet radiation (UV)** is another type of radiation that causes cancer by damaging DNA. The ability of the UV radiation in sunlight to cause cancer was first deduced from the observation that skin cancer is most prevalent in people who spend long hours in the sun, especially in tropical regions where the sunlight is very intense. UV radiation is absorbed mainly by the skin, where it imparts enough energy to trigger **pyrimidine dimer** formation—that is, the

formation of covalent bonds between adjacent pyrimidine bases in DNA. If the damage is not repaired, distortion of the double helix causes improper base-pairing during DNA replication that leads to distinctive mutation patterns. For example, a CC → TT mutation (conversion of two adjacent cytosines to thymines) is a unique product of UV exposure and can therefore be used as a distinctive "signature" to identify mutations caused by sunlight.

The existence of such **signature mutations** provided a way to prove that UV-induced mutations cause skin cancer. One of the first genes studied was the *p53* gene, which is known to be mutated in many human cancers. When the *p53* gene of skin cancer cells, is examined using DNA sequencing techniques, mutations exhibited the distinctive UV signature (such as CC → TT) are frequently observed. In contrast, when *p53* mutations are detected in other types of cancer, they do not exhibit the UV signature Fig.

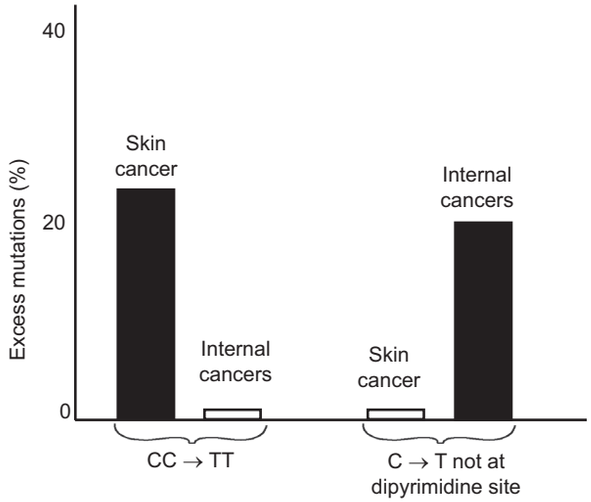


***Fig. 4.47*** *Incidence of two types of p53 mutations in skin cancer and internal cancers. The two bars on the left represent the frequency of CC → TT mutations, which are triggered by UV radiation. The two bars on the right represent the frequency of C → T mutations not located at dipyrimidine sites, which are not caused by UV radiation. Note that the UV-triggered type of mutation is found in the p53 gene of skin cancers (squamous cell carcinomas), but not in cancers of internal organs. Mutations frequencies are plotted relative to what would be expected to occur randomly (after Becker et al., 2006).*

**4. Virus, Bacteria and Other Infectious Agents Cause Some Cancers:** Peyton Rous, in 1911, performed experiments on sick chickens brought to him by local farmers that showed for the first time that cancer can be caused by a virus. These chickens had cancers of connective tissue origin, or **sarcomas**. To investigate the origin of the tumours, Rous ground up the tumour tissue and passed it through a filter, whose pores were so small that not even bacterial cells could pass through them. When he injected the clear cell-free extract into healthy chickens, Rous concluded that sarcomas can be transmitted by an infectious agent that is smaller than a bacterial cell. This was the first time that anyone had demonstrated the existence of an **oncogenic virus—**that is, a virus that can cause cancer.

Rous conclusions were initially greeted with skepticism. It was not until 1966 that an 87-year old Rous finally received the Nobel Prize,

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more than 50 years after his discovery of the first cancer virus. It is now well established that dozens of viruses cause cancer in animals and that a smaller number cause cancer in humans. The first human example was discovered by **Danis Burkitt**, a British surgeon working in Africa in the late 1950's. At certain times of the year, Burkitt noted that a large number of his young patients developed massive lymphocytic cancers of the neck and jaw. Because this kind of cancer, now known as **Burkitt's lymphoma** occurred in periodic epidemics localized to specific geographical regions, Burkitt proposed that it was transmitted by an infections agent.

Burkitt's ideas soon attracted the attention of two virologists, named **Epstein** and **Barr**, whose electron microscopic studies revealed virus particles in Burkitt's lymphoma cells, the virus is now called the **Epstein-Barr virus** or **EBV** in recognition of these virologists.

**Note:** Unlike viruses that cause cancer in animals, it is difficult to prove that a virus such as EBV causes cancers in humans because ethical considerations prevent directly testing the hypothesis by injecting the virus into healthy individuals. Nonetheless, some indirect evidence supports the conclusion that EBV causes Burkitt's lymphomas: 1. DNA sequences and proteins encoded by EBV are detected in tumour cells obtained from patients with Burkitt's lymphoma, but not in normal cells from the same individuals; 2. Adding purified EBV to normal human lymphocyte cultures cause the cells to acquire the properties of cancer cells; and 3. Injecting EBV into monkeys induces the formation of lymphomas.

Following the discovery of EBV, several additional viruses have been identified as causes of human cancer. For example, liver cancer is caused by **Hepatitis B** and **Hepatitis C** viruses and cervical cancer is caused by **Human Papilloma Virus (HPV)**. In addition to viruses, the bacterium *Helicobacter pylori* (*H. pylori*) causes stomach cancer, and parasitic flatworm infections have been linked to a small number of bladder and bile duct cancers.

Infectious agents trigger the development of cancer in two fundamentally different ways. One involves those agents such as the hepatitis B and C viruses, *H. pylori*, and parasitic flatworms that cause tissue destruction and chronic inflammation. Such destruction creates chronic inflammatory conditions in which cells of the immune system infiltrate the tissue and attempt to kill the infectious agent and repair the tissue damage. Unfortunately, the mechanisms used by immune cells to fight infections often produce mutagenic chemicals, such as **oxygen free radicles** (highly reactive forms of oxygen containing an unpaired electrons). This means that proliferation of replacement cells for the injured tissue takes place under conditions in which DNA damage is likely, thereby increasing the likelihood that cancer-causing mutations will arise.

The other way in which infectious agents cause cancer is related to the ability of certain viruses to directly stimulate the proliferation of infected cells. The mechanism by which this is accomplished varies among viruses. In some cases, it involves cancer-causing viral genes, whereas in other cases a virus alters the behaviour of host cell genes. The types of genes involved in such events play a role not just in viral cancers, but in cancers caused by chemicals and radiation as well.

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## 4.6.5 Genes Involved in Cancer

**Oncogenes** are genes whose presence can cause cancer. While they are sometimes introduced into cells by viruses, more often they arise from normal cellular genes called **proto-oncogenes** by *point mutation, gene amplification, chromosomal translocation, local DNA rearrangements* or *insertional mutagenesis.* Most proteins produced by oncogenes are signalling pathway components, such as growth factors, receptors, plasma membrane GTP-binding proteins, non-receptor protein kinases, transcription factors, and cell-cycle or cell-death regulators. Oncogenes code for abnormal forms or excessive quantities of such proteins, thereby leading to excessive stimulation of cell proliferation.

Tumour suppressor genes are genes whose loss or inactivation can lead to cancer. Susceptibility to develop cancer is increased in people who inherit defective tumour suppressor genes. Three important tumour suppressor genes are:

1. The *RB* gene, which produces a protein that restrains passage from $G_1$ into S phase.
2. The *p53* gene, which produces a protein that prevents cells with damaged DNA from proliferating.
3. The *APC* gene, which produces a protein that inhibits the Wnt pathway. The Wnt pathway plays a prominent role in controlling cell proliferation and differentiation during embryonic development. The central component of this wnt pathway is a protein called β**-catenin**.

## 4.6.6 Diagnosis, Screening and Treatment of Cancer

Much progress has been made in recent years in explaining the genetic and biochemical abnormalities that underlie cancer development. One of the hopes for such research is that our growing understanding of the molecular alterations exhibited by cancer cells will eventually lead to improved strategies for cancer diagnosis and treatment.

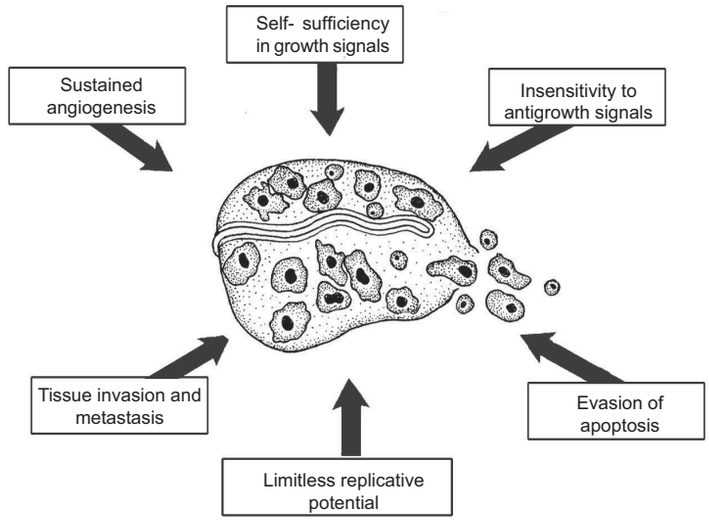


***Fig. 4.48*** *Overview of changes in cells that cause cancer. During carcinogenesis, six fundamental cellular properties are altered, as shown here, to give rise to the complete, most destructive cancer phenotype. Less dangerous tumours arise when only some of these changes occur (after Lodish et al., 2004).*

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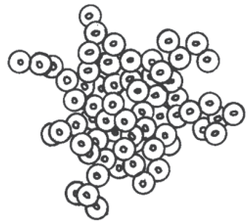
## 1. Microscopic Analysis of Cancer.

Because cancer can arise in almost any tissue, few regular generalizations are possible regarding disease symptoms. A definitive diagnosis typically requires a **biopsy,** which involves surgical removal of a tiny tissue sample for microscopical examination. Under the microscope, cancer cells show a number of features that together indicate the presence of cancer. For example, cancer cells often have large, irregularly shaped nuclei, prominent nucleoli, and a high ratio of nuclear-to-cytoplasmic volume. Cancers also tend to exhibit significant variability in cell size and shape, as well as a loss of normal tissue organisation. To varying extents, cancer cells lose the specialized structural and biochemical properties of the cells normally residing in the tissue of origin. Cancers usually have more dividing cells than normal, which means that the mitotic index will be elevated.

***Table 4.10*** *Some differences in the microscopic appearance of benign and malignant tumours (source: Becker et al., 2006)*

| | Trait | Benign | Malignant |
|---|---|---|---|
| 1. | Nuclear size | Small | Large |
| 2. | N/C ratio (Ratio of nuclear to cytoplasmic volume) | Low | High |
| 3. | Nuclear shape | Regular | Pleomorphic (irregular shape) |
| 4. | Mitotic index | Low | High |
| 5. | Tissue organisation | Normal | Disorganised |
| 6. | Differentiation | Well-differentiated | Poorly differentiated (anaplastic) |
| 7. | Tumour boundry | Well-defined | Poorly-defined |
| | |  |  |

**Note:** Percentage of cells in a population that are in any stage of mitosis at a certain point in time; used to estimate the relative length of the M phase of cell cycle. For example, the mitotic index for cultured mammalian cells is often about 3–5 per cent, which means that M phase lasts less than an hour (usually 30–45 minutes).

### Tumour Grading

If a sufficient number of these seven traits are observed upon microscopic examination of sample, it can be concluded that cancer is present. In other words, the presence of these traits indicates a tumour that, if left untreated will eventually spread by invasion and metastasis. The severity of these observed microscopic, abnormalities varies significantly among cancers, even when they arise from the same cell type and in the same organ. These variability form the basis for **tumour grading**, which is the assignment of numerical grades to tumours based on differences in their microscopic appearance.

Lower numerical grades (*e.g.*, grade 1) are assigned to tumours whose cells exhibit normal differentiated features, divide slowly, and display only modest abnormalities in the traits. Higher numbers (*e.g.,* grade 4) are assigned

to tumours containing rapidly dividing, poorly differentiated cells bear less resemblance to normal cells and exhibit severe abnormalities in the trait. The highest grade cancers contain cells that are **anaplastic**, which means that they are so poorly differentiated and abnormal in appearance and organization that they bear no resemblance to the cells of the tissue in which the tumour arose. Such anaplastic, high-grade cancers tend to grow and spread more aggressively and be less responsive to therapy than lower grade cancers (see **Becker** *et al.,* 2006).

**2. Screening Techniques of Cancer**

When cancer is detected before it has spread, cure rates tend to be very high. Therefore, a great need exists for screening techniques that can routinely detect cancers at an early stage. Some of them are following:

**(*i*) Pap Smear:** It is one of the most successful screening procedures that was developed in the early 1930s by **George Papanicolaou** (for whom it is named). The logic underlying this procedure is that the microscopic appearance of cancer cells is so distinctive that it is possible to detect the likely presence of cancer by simply examining a few isolated cells. A Pap smear is performed by taking a tiny sample of a woman's vaginal secretions and examining it with a microscope. If the cells in the fluid exhibit unusual features, such as large irregular nuclei or prominent variations in cell size and shape, it is a sign that cancer may be present and further tests need to be done. Because a Pap smear allows **cervical cancer** to be detected in its early stages before metastasis has occurred, this procedure has prevented hundreds of thousands of cancer deaths.

**(*ii*) Mammography and Other Techniques:** The success of the Pap smear has led to development of screening techniques for other cancers. For example, **mammography** utilizes a special X-ray technique to look for early signs of **breast cancer** and **colonoscopy** uses a slender fiber-optic instrument to examine the colon (*i.e.*, the part of the large intestine that extends from the cecum to the rectum) for early signs of **colon cancer**. The ideal screening test would allow doctors to detect cancers anywhere in the body with one simple procedure, such as a blood test. **Prostate cancer** is an example of cancer that can sometimes be detected this way. Men over the age of 50 are often advised to get a **PSA test,** which measures how much **prostate-specific antigen (PSA)** is present in the bloodstream. PSA, which is a protein produced by cells of the prostate gland, normally appears in only tiny concentrations in the blood. If a PSA test reveals a high concentration of PSA, it indicates the existence of a prostate problem and further tests are performed to determine whether or not cancer is actually present.

**(*iii*) Proteomic Analysis:** Other cancers also release small amounts of specific proteins into the bloodstream, where their presence might be used to signal the existence of early disease. To investigate such tiny changes in blood proteins, scientists are experimenting with a general approach called **proteomic analysis** to analyse proteins present in the blood (the term proteomic refers to the complete set of proteins produced by an organism's genome). The key to most proteomic techniques is **mass spectrometry**, a

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high speed, extremely sensitive method for identifying proteins based on differences in mass and electrical charge. Because a blood sample contains thousands of different proteins, the data generated by proteomic analysis can be extremely complex. To deal with this problem, artificial intelligence software programs are used to compare the complex protein patterns seen in blood samples from individuals with or without cancer.

One of the first cancer to be investigated by proteomic analysis was **ovarian cancer**. When ovarian cancer is detected before it spreads, the five year survival rate exceeds 95 per cent. Using proteomic analysis, scientists have recently identified a pattern of five proteins in the blood of women with ovarian cancer that is not seen in the blood of other women.

**3. Treatments of Cancer**

People diagnosed with cancer have various treatment options that depend both on the type of cancer involved and how far it has spread.

**(*i*) Surgery:** The most common treatment of cancer involves surgery to remove the primary tumour followed (if necessary) by radiation therapy and/or chemotherapy to destroy any remaining cancer cells.

**(*ii*) Radiation Therapy:** This approach employ's high energy X-rays or other forms of ionizing radiation to kill cancer cells. We already know that DNA damage created by ionizing radiation can cause cancer, but paradoxically, the same type of radiation is also used in higher doses to destroy cancer cells in people who already have the disease. Ionizing radiation kills cells in two different ways. First, DNA damage caused by radiation activates the p53 signaling pathway, which then triggers cell death. However, many cancers have mutations that disable the p53 pathway, so p53-induced apoptosis plays only a modest role in the response of most cancers to radiation treatment. In the second mechanism, radiation kills cells by causing chromosomal damage that is so severe that it prevents cells from progressing through mitosis, and the cells therefore die while trying to divide.

**(*iii*) Chemotherapy:** Most forms of chemotherapy use drugs that, like radiation, are intended to kill dividing cells. Such drugs can be subdividing into four major categories:

**(*a*) Antimetabolites:** They inhibit metabolic pathways required for DNA synthesis by acting as competitive inhibitors that bind to enzyme active sites in place of normal substrate molecules. Examples of such antimetabolites include methotrexate, fluorouracil and mercap-topurine.

**(*b*) Alkylating Agents:** They inhibit DNA function by chemically cross-linking the DNA double helix. Examples of such drugs include cyclophosphamide, chlorambucil and cisplatin.

**(*c*) Antibiotics.** These are substances made by microorganisms that inhibit DNA function by either binding to DNA or inhibiting topoisomerases required for DNA replication. Examples of such antibiotics include bleomycin and doxorubicin.

**(*d*) Plant-Derived Drugs:** These drugs either inhibit topoisomerases or disrupt the microtubules of the mitotic spindle. Examples of such drugs include the topoisomerase-inhibitor **etoposide** and the microtubule disrupting drug, **taxol**.

One problem with such drugs (and radiation therapy) is that they are toxic to normal dividing cells as well as to cancer cells. When cancer arises in a tissue whose growth requires a specific hormone, it may be treated in a less toxic manner using drugs that block the action of that particular hormone. For example, many breast cancers require estrogen for their growth. Estrogens tend to exert their effects by binding to nuclear receptor proteins that activate the expression of specific genes. The drug **tamoxifen**, a common treatment for breast cancer, binds to estrogen receptors in place of estrogen and prevents the receptors from being activated.

Newer treatment approaches include **immunotherapies** that exploit the ability of the immune system to attack cancer cells, **molecular** targeting drugs aimed at proteins that are critical to the cancer cells, and **antiangiogenic agents** that attacks a blood supply of the tumour.

**Check Your Progress**

17. What do you understand by neoplastic cells?
18. Define cell transformation.
19. What is carcinogen?
20. What do you understand by epidemiology?

## 4.7 BIOLOGY OF AGING

Development does not cease once birth has occurred but continues throughout the stages of life: infancy, childhood, adolescence and adulthood. **Aging** or **senescence** encompasses these progressive changes that contribute to an increased risk of infirmity, disease and death. The study of biology of senescence or aging is called **gerontology** (**Mader** 1998).

When entropy wins, aging or senescence sets in (**Gilbert** 2010). Entropy is a measure of the unavailability of a system's thermal energy for conversion into mechanical work. In some contexts, entropy is interpreted as a measure of the degree of disorder or randomness in the system.

Aging is a slow process during which the body undergoes changes that eventually bring about death, even if no marked disease or disorder is present. Medical science is trying to extend the human life span and the health span, the length of time the body functions normally.

A multicellular organism is able to develop and maintain its identity for only so long before deterioration prevails over synthesis, and the organism ages. Aging can be defined as *the time-related deterioration of the physiological functions necessary for survival and fertility* (**Gilbert** 2010). The characteristics of aging—as distinguished from diseases of aging, such as cancer and heart disease—affect all individuals of a species. The aging

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process has two major facets. The first is simply how long an organism lives (maximum life span); the second concerns the physiological deterioration, or senescence, that characterizes old age. These topics are often viewed as being interrelated.

### 4.7.1 Maximum Life Span (Genes and Aging)

Genetic factors play roles both between species and within species. The **maximum life span**, which is the maximum number of years any member of a given species has been known to survive, is characteristic of a species. The maximum human life is estimated to be 121 years (**Arking** 1998). The life spans of some tortoises and lake trout are both unknown but are estimated to be more than 150 years. The maximum life span of a domestic dog is about 20 years and that of a laboratory mouse is 4.5 years. If a *Drosophila* fruit fly survives to eclose (in the wild, more than 90% die as larva), it has a maximum life span of 3 months. (**Note.** *Eclosion* means emergence of a fruit fly or any other holometabolous insect from its puparium; see **Agarwal** 2009).

The species-specific life span appears to be determined by genes that affect a compromise between early growth and reproduction and somatic maintenance (**Kirkwood** 1977). In other words, aging results from natural selection operating more on early survival and reproduction than on having a vigorous post-reproductive life. If longevity is a selectable trait, one should expect to find heritable variation within populations. Recently, long-term studies of wild populations of animals have provided convincing data that there is hereditable variation within a species for aging (**Wilson** *et al*., 2007).

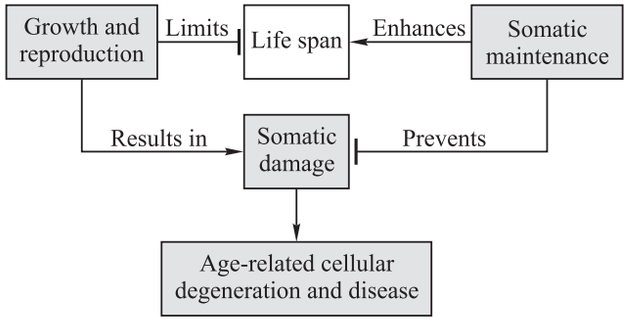


***Fig. 4.49*** *Kirkwood's proposal that organisms have to affect a compromise between the energy allocated to reproduction and growth and the energy allocated to the maintenance and repair of bodily tissues.*

Molecular evidence indicates that certain genetic components of longevity are conserved between species: flies, worms, mammals, and even yeast cells all appear to use the same set of genes to promote survival and longevity (**Kenyon** 2001; **Vijg** and **Campisi** 2008). There are two sets of genes that are well known to be involved in aging and its prevention, and both sets appear to be conserved between phyla and even kingdoms of organisms. These are genes encoding DNA repair enzymes (proteins) and the genes encoding proteins involved in the insulin signalling pathway.

**I. Genes Encoding DNA Repair Proteins**

1. **Efficient DNA Repair Enzymes:** DNA repair and synthesis may be

important in preventing senescence. Individuals of species whose cells have more efficient DNA repair enzymes live longer (**Heart** and **Setlow** 1974; Fig. 4.50).

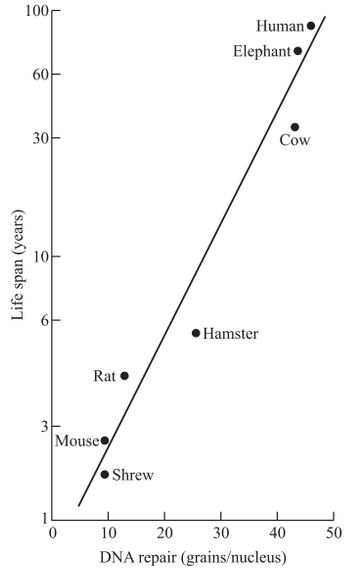


***Fig. 4.50*** *Life span and the aging phenotype. Correlation between life span and the ability of fibroblasts to repair DNA in various mammalian species. Repair capacity is represented in auto-radiography by the number of grains from radioactive thymidine per cell nucleus. Note that y-axis (life span) is logarithmic scale (after Gilbert 2010)*

2. **Premature Aging Syndromes:** Certain premature aging syndromes, called **progerias,** in humans appear to be caused by mutations in certain DNA repair enzymes (**Sun** *et al*., 1998; **Shen** and **Loeb** 2001). In humans, **Hutchinson-Gilford progeria** is a rapid-aging syndrome; children born with this condition age rapidly, dying (usually of heart failure) as early as 12 years of age. Symptoms of this type of progeria include thin skin with age spots, resorbed bone mass, hair loss, and arteriosclerosis—all characteristics of human senescent phenotype. Hutchinson-Gilford progeria is the result of a dominant mutation in the gene that encodes **lamin A**, a nuclear membrane protein, and these same mutations can be seen in age-related senescence (**Scaffidi** and **Misteli** 2006).
3. **Klotho Gene:** Another type of progeria is reported in mice. It is caused by loss-of-function mutations of the *Klotho* gene (**Kuro** *et al*., 1997). Conversely, the same gene's gain-of-function phenotype (causing its overexpression) has been known to prolong a mouse's life by 30% (**Kurosu** *et al*., 2005). Klotho gene appears to encode a hormone that down regulates insulin signaling. The suppression of signaling by insulin and insulin-like growth factor 1 (IGF-1) is one of the ways life span can be extended in many species.
4. **Protein p53:** Protein p53 is a transcription factor and is regarded as one of the most important regulators of cell division. Protein p53 is often called **"guardian of the genome"** (see **Gilbert** 2010) because of its ability to block cancer in several ways. It can stop cell cycle, cause cellular senescence in rapidly dividing cells, instruct the *Bax* genes to initiate cellular apoptosis, and activate DNA repair enzymes. In most

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cells, p53 is bound to another protein that keeps p53 inactive. However, ultraviolet radiation, oxidative stress and other factors that cause DNA damage will also separate and activate p53 protein. The induction of apoptosis by p53 protein can be beneficial (when destroying cancer cells) or deleterious (when destroying neurons or other vital cells). It is possible that animals with high levels of p53 have increased protection against cancer, but they may also age more rapidly (**Tyner** *et al*., 2002). Indeed, p53 can be activated by the absence of lamin A (**Varela** *et al*., 2005), thereby suggesting a mechanism for Hutchinson-Gifford progeria.

5. **Sirtuin Genes:** Sirtuin genes encode histone deacetylation (chromatin-silencing) enzymes. They guard the genome, preventing genes from being expressed at the wrong times and places, and blocking chromosomal rearrangements. Sirtuin proteins are usually found in regions of chromatin (especially repetitive DNA sequences) where such mistaken chromosomal rearrangements can occur. However, when DNA strands break (as inevitably happens as the body ages), sirtuin proteins are called on to fix them. Thus, genes that are usually silenced become active as the cells age. Sirtuin proteins have been found to prevent aging throughout the eukaryotic kingdoms, including in yeasts and mammals (**Howitz** *et al*., 2003; **Oberdoerffer** *et al*., 2008).

## II. Aging and Insulin Signaling Cascade

Certain recent investigations in mice, nematode *Caenorhabditis elegans*, and *Drosophila* suggested that there is a conserved genetic pathway that regulates aging. This genetic pathway was found to be selected for during evolution. This genetic pathway involves the response to insulin hormone or insulin like growth factor. In *C. elegans*, a larva proceeds through four larval stages, after which it becomes an adult. If the nematodes are overcrowded or if there is insufficient food, however, the larva can enter a metabolically dormant **dauer larval stage,** a non-feeding state of **diapause** during which development and aging are suspended. The nematode can remain in the dauer larval stage for upto 6 months, rather than becoming an adult that lives only a few weeks. In this diapausal state, the nematode has increased resistance to oxygen radicals that can cross-link proteins and destroy DNA. The pathway that regulates both dauer larva formation and longevity has been identified as the **insulin signaling pathway**. Favourable environments signal the activation of the insulin receptor homologue DAF-2, and this receptor stimulates the onset of adulthood . Poor environments fail to activate the DAF-2 receptor, and dauer formation happens. While severe loss-of-function alleles in this pathway cause the formation of dauer larvae in any environment, weak mutations in the insulin signaling pathway enable the animals to reach adulthood and live four times longer than wild-type animals.

There are many other functions of **down regulation** of insulin signaling pathway. First, it appears to influence metabolism, decreasing mitochondrial electron transport. When the DAF-2 receptor is not active, organisms have decreased sensitivity to **Reactive Oxygen Species (ROS),** metabolic by-products that can damage cell membranes and proteins and even destroy

DNA. Second, downregulating the insulin pathway increases the production of enzymes that prevent oxidative damage, as well as DNA repair enzymes. Third, this lack of insulin signaling decreases fertility. This increase in DNA synthetic enzymes and in enzymes that protect against ROS is due to the DAF-16 transcription factor. This forkhead-type transcription factor is inhibited by the insulin receptor (DAF-2) signal. When that signal is absent, DAF-16 can function, and this factor appears to activate the genes encoding several enzymes (such as catalase and superoxide dismutase) that are involved in reducing ROS, several enzymes that increase protein and lipid turnover, and several stress proteins.

### Affect of Insulin-Signaling Pathway in Mammal's Longevity

In mammals, insulin and insulin-like growth factor pathways are so deeply integrated with embryonic development and adult metabolism that mutations often have numerous and deleterious effects (such as diabetes or Donahue syndrome). However, there is some evidence that the insulin signaling pathway does affect life-span in mammals. Dog breeds with low levels of insulin-like growth factor 1 (IGF-1) live longer than breeds with higher levels of this factor. Mice with loss-of-function mutations of the insulin signaling pathway live longer than their wild-type litermates. Mice heterozygous for the insulin-like growth factor 1 receptor (IGF-1R) not only lived about 30% longer than their wild-type littermates, they also had greater resistance to oxidative stress. In addition, mice lacking one copy of their IGF-IR gene lived about 25% longer than wild-type mice (and had higher ROS resistance, but otherwise normal physiology and fertility).

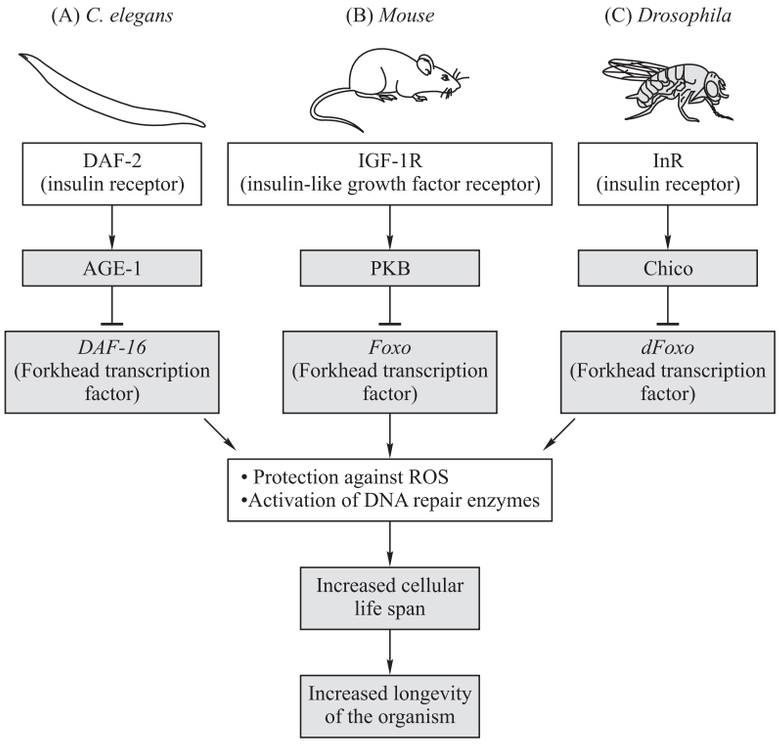


***Fig. 4.51*** *A pathway for regulating longevity. In each case, the insulin signaling pathway inhibits the synthesis of proteins that would otherwise protect cells against oxidative damage caused by reactive oxygen species (ROS) that crosslink proteins and can*

*damage DNA. These protective proteins may be particularly important in mitochondria. When insulin signaling is downregulated, Forkhead transcription factors may activate DNA repair enzymes that may protect against mutations caused randomly by ROS or other agents. Such protection against ROS and mutation may increase the functional life span of the cells and the longevity of the organism (after Gilbert 2010).*

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### Affect of Insulin-Signaling Pathway on *Drosophila's* Longevity

The insulin signaling pathway also regulates life span in *Drosophila*. In 2001, research teams of **Clancy** and of **Tatar** have reported that flies with loss-of-function mutations of the insulin receptor gene or genes in the insulin pathway (such as *Chico*) live nearly 85% longer than wild-type flies. These long-lived mutants are sterile, and their metabolism resembles that of flies that are in diapause. In *Drosophila*, the insulin receptor is thought to regulate a Forkhead transcription factor (dFoxo), similar to the DAF-16 protein of *Caenorhabditis elegans*. When the *dFoxo* gene of *Drosophila* is activated in the fat body; it lengthen the fly's life span. Other studies have shown that when these enzymes (such as superoxide dismutase) are downregulated by mutation or by RNA interference, the resulting flies die early, have increased oxidative stress, and display higher levels of DNA damage. Conversely, over expression of superoxide dismutase genes can lengthen the *Drosophila* life span. While some evidence points to a correlation between longer life span, lower insulin signaling and elevated ROS protection in *Drosophila* (**Broughton** *et al*., 2005), other studies suggest that some flies and other insects can obtain longer life spans without increasing the enzymes known to protect against oxidative stress (**Le Bourg** and **Fournier** 2004; **Parker** *et al*., 2004).

From an evolutionary point of view, the insulin pathway may mediate a compromise between reproduction and survival/maintenance. Many of the long-lived mutants have reduced fertility. Thus, it is interesting that another longevity signal originates in the gonad. When the germline cells are removed from *C. elegans*, the animals live longer. It is thought that the germline stem cells produce a substance that blocks the effects of a longevity-inducing steroid hormone. Conversely, ROS appears to promote germline development at the expense of somatic development in *C. elegans.* The oxidation of certain lipids accelerates germ cell development, while same lipids, in their unoxidized form, prevent germ cell proliferation (**Shibata** *et al*., 2003).

### III. Integrating the Conserved Aging Pathways

An interaction occurs between the proteins involved in the insulin signaling pathway and the DNA repairs pathway (Fig. 52.4). The p53 protein that induces cell cycle arrest also blocks the activity of the receptor for insulin-like growth factor 1. Likewise, sirtuin proteins, in addition to activating Foxo proteins, can also block p53. In some cases, the same protein is involved in both the DNA repair and insulin signaling pathways (**Niedernhofer** *et al*., 2006). This is the case in the protein encoded by the *XPF-ERCC*1 gene in humans. Most people with mutations of this gene have xeroderma pigmentosum, a defect in DNA repair that makes them susceptible to cancers, especially melanomas. However, if mutation occurs in a different part of the same gene, the affected individuals have a premature aging syndrome in which the genes involved in the insulin

signaling pathway are downregulated. It is possible that the enzyme encoded by this gene has two functions. Initially it may be used for DNA repair, but later it might act to prolong life by downregulating the insulin pathway.

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## 4.7.2 Life Expectancy

According to **Gilbert** (2010), most people cannot expect to live 121 years, and most mice in the wild do not live to celebrate even their first birthday. **Life expectancy** means the length of time an average individual of a given species can expect to live. It is not a characteristic of species, but of populations. Life expectancy is usually defined as *the age at which half the population still survives*. A baby born in England during the 1780s could expect to live to be 35 years old. In Massachusetts during that same time, life expectancy was 28 years. These ages represent normal range of human life expectancy for most of the human race throughout recorded history (**Arking** 1998). Even today, in some areas of the world (such as Cambodia, Togo, Afghanistan, and several other countries) life expectancy is less than 40 years. In the United States, a male born in 1986 can expect to live 74 years, while females have a life expectancy of around 80 years.

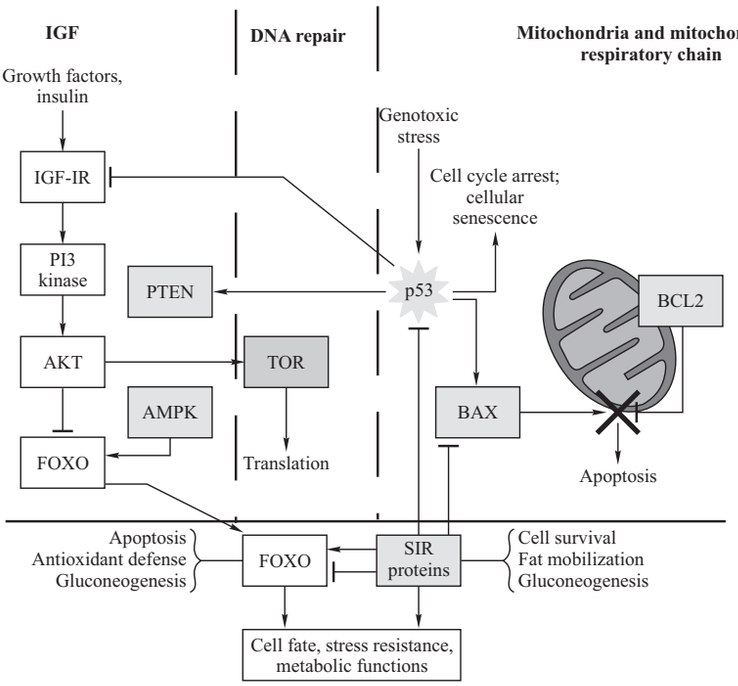


***Fig. 4.52*** *Interactions of different evolutionarily conserved aging pathways. The insulin signaling pathway and mitochondrial pathway for apoptosis (cell death) are indicated. The pro-aging activities of these pathways are conserved across phyla, and energy sensors, such as AMPK, are potentially important integrators of these pathways. Many longevity signals converge on members of the FOXO and sirtuin protein families, which can interact. Note that sirtuin proteins can both activate and repress, FOXO, depending on the context. TOR is a positive regulator of general translational activity. Here, insulin-like factors and DNA-disrupting ("genotoxic") chemicals initiate these pathways (after Gilbert 2010).*

Thus, in 1900, 50% of Americans were dead before the age of 60; in 1950, the comparable age was 72; by 2000, this "median survival" age had climbed to 80 years. A 70 year old person was exceptional in 1900 but is

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commonplace today. People in 1900 did not have the "luxury" of dying from heart attacks or cancers, because these conditions are most likely to affect people over 50. Rather people died (as they are still dying in many parts of the world) from microbial and viral infections. In fact, before the discovery of antibiotics, the death rate of young women due to infections associated with childbirth was high throughout the world. Thus, the phenomena of senescence and diseases of aging are much more common today that they were a century ago. Until recently, relatively few people exhibited the general human senescent phenotype: gray hair, sagging and wrinkling skin, stiff joints, osteoporosis (loss of bone calcium), loss of muscle fibers and muscular strength, memory loss, eyesight deterioration and slowed sexual responsiveness.

The general senescent phenotype is characteristic of each species. But what causes it? This question can be asked at many levels. Here we will look primarily at the cellular level of organization.

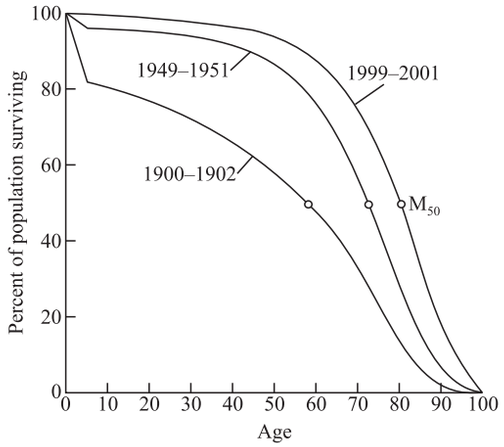


***Fig. 4.53*** *Survival curves for the United States population for the periods 1900–1902, 1949–1951, and 1999–2001. The circles represent M50, the age at which 50% of individuals of that age survived (after Gilbert 2010).*

## 4.7.3 Subcellular Changes due to Aging

Due to the process of aging following changes occur in the different cellular organelles:

**1. Mitochondria: Reiner** (1947) has reported that due to aging the rate of carbohydrate metabolism (Krebs cycle) is decreased. The aging also affects the glycolysis. Such results have also been reported by **Rafsky** (1952) and **Barrows** (1958). **Dampsey** (1956) has shown that the mitochondria are related with the aging process of the animals and in old tissues the mitochondria become degenerated. **Zeuthen** (1947, 1953) has reported that the rate of respiration in multicellular organisms becomes slow and they become somewhat dehydrated.

**2. Endoplasmic Reticulum:** Due to aging the amount of granular endoplasmic reticulum decreases in the cytoplasm of old cells. In the nerves of older animals and human the decrease of Nissl substance (rRNA) have been reported by various workers.

**3. Pigment Accumulation and Lysosomes:** Due to aging various pigmented inclusions such as lipofuscin, yellow pigment, brown degenerations, etc., become accumulated in the cells. The lipofuscin probably is the result of accumulation and autooxidation of lipid components of lysosomes. **Shock** (1962) and **Strehler** (1962, 1963) have reported the accumulation of calcium, various pigments and other inert matter in the aged cell. The inert matter are the accumulated metabolic excretory wastes which might have failed to exit across the plasma membrane.

**4. Nucleus: Minot** (1970) has suggested that the natural death of the cell is a consequence of cellular differentiation of which a change in nucleocytoplasmic ratio is an important index. **Smallwood** and **Philips** (1916) have shown that fatigue and age do not produce significant changes in the nuclear size. **Falzone** (1959) has studied the effect of the age on the ploidy or DNA contents of the nuclei of rat liver cells and reported that no significant effect of aging on the nuclei. However, various modern cytologists have shown that the age affects the nucleus variously.

**5. Nuclear and Plasma Membrane: Lansing** (1942, 1952) has reported that due to aging process the calcium becomes accumulated in the cellular membranes and causes various physiological changes in them.

### 4.7.4 How does Aging Affect Body Systems of Humans?

Some of the effects of aging are as follows:

**1. Effect of Aging on Skin:** As aging occurs, skin becomes thinner and less elastic because the number of elastic fibers decreases and collagen fibers undergo cross-linking. Also, there is less adipose tissue in the subcutaneous layer; therefore, older people are more likely to feel cold. The loss of thickness of skin partially accounts for sagging and wrinkling of the skin.

Homeostatic adjustment to heat is also limited because there are fewer sweat glands for sweating to occur. There are fewer hair follicles, so the hair on the scalp and the extremities thin out. The number of oil (sebaceous) glands is reduced, and the skin tends to crack. Older people also experience a decrease in the number of melanocytes, making hair gray and skin pale. In contrast, some of the remaining pigment cells are larger, and pigmented blotches appear in skin.

**2. Processing and Transporting: Cardiovascular Disorders:** are the leading cause of death among the elderly. The heart shrinks because there is a reduction in cardiac muscle size. This leads to loss of cardiac muscle strength and reduced cardiac output.

Because the middle coat of arteries contains elastic fibers, which most likely are subject to cross-linking, the arteries become more rigid with time, and their size is further reduced by plaque, a buildup of fatty material. Therefore, blood pressure reading gradually rise. There is reduced blood flow to the liver, and this organ does not metabolize drugs as efficiently as before.

Circulatory problems often are accompanied by respiratory disorders, and vice-versa. Growing inelasticity of lung tissues means that ventilation is reduced. Because we rarely use the entire vital capacity, these effects are not noticed unless there is increased demand for oxygen.

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There is also reduced blood supply to the kidneys. The kidneys become smaller and less efficient at filtering wastes. Salt and water balance are difficult to maintain, and the elderly dehydrate faster than young people. Difficulties involving urination include incontinence (lack of bladder control) and the inability to urinate. In men, the prostate gland may, enlarge and reduce the diameter of the urethera, making urination so difficult that surgery is often needed.

The loss of teeth, which is frequently seen in elderly people, is more apt to be the result of long-term neglect than aging. The digestive tract loses tone, and secretion of saliva and gastric juice is reduced, but there is no indication of reduced absorption. Therefore, an adequate diet, rather than vitamin and mineral supplements, is recommended (see **Mader** 1998). There are common complaints of constipation, increased amount of gas, and heartburn, but gastritis, ulcers and cancer can also occur.

**3. Effect of Aging on Integration and Coordination:** It is often mentioned that while most tissues of the body regularly replace their cells, some at faster rate than others, the brain and muscles ordinarily do not. However, contrary to previous opinion, recent studies show that few neural cells of the cerebral cortex are lost during the normal aging process. This means that cognitive skills remain unchanged even though there is characteristically a loss in short-term memory. Although the elderly persons learn more slowly than the young, they can acquire and remember new material. It is noted that when more time is given for the subject to respond, age differences in learning decrease.

Neurons are extremely sensitive to oxygen deficiency, and if neuron death does occur, it may not be due to aging itself but to reduced blood flow in narrowed blood vessels. Specific disorders, such as **depression, Parkinson disease,** and **Alzheimer disease**, are sometimes seen, but they are not common. Reaction time, however, does slow, and more stimulation is needed for hearing, taste, and smell receptors to function as before. After age 50, there is a gradual reduction in the ability to hear tones at higher frequencies and this can make it difficult to identify individual voices and to understand conversation in a group. The lens of the eye does not accommodate as well and may also develop a **cataract**. **Glaucoma**, the buildup of pressure due to increased fluid, is more likely to develop because of a reduction in the size of the anterior cavity of the eye.

Loss of skeletal muscle mass also occurs. There is a reduced capacity to do heavy labour, but routine physical work should be no problem. A decrease in the strength of the respiratory muscles and inflexibility of the rib cage contribute to the inability of the lungs to expand as before, and reduced muscularity of the urinary bladder contributes to difficulties with urination.

Aging is also accompanied by a decline in bone density. Osteoporosis, characterized by a loss of calcium and mineral from bone, is not uncommon, but there is evidence that proper health habits can prevent its occurrence. **Arthritis** which causes pain upon movement of the joint, is also seen.

Weight gain occurs because the basal metabolism decreases and inactivity increases. Muscle mass is replaced by stored fat and retained water.

**4. Effects of Aging on the Reproductive System:** Females undergo menopause, and thereafter the level of female sex hormones in blood falls markedly. The uterus and the cervix are reduced in size, and there is a thinning of the walls of the oviducts and the vagina. The external genitals become less pronounced. In males, the level of androgens fall gradually over the age span of 50–90, but sperm production continues until death.

It is of interest that as a group, females live longer than males. Although their health may be better, it is also possible that the female sex hormone estrogen offers women some protection against circulatory disorders when they are younger. Males suffer a marked increase in heart diseases in their forties, but an increase is not noted in females until after menopause. Then women lead men in the incidence of stroke. Men are still more likely than women to have a heart attack, however (see **Mader** 1998).

## 4.7.5 Theories of Aging

No single theory of aging has gained general acceptance, but the following ones are being intensively investigated (see **Raven** *et al*., 2005).

**1. Accumulated Mutation Theory:** The oldest general theory of aging is that cells accumulate mutations as they age, leading eventually to lethal damage. Careful studies have shown that somatic mutations do indeed accumulate during aging. As cells age, for example, they tend to accumulate the modified base 8-hydroxyguanine in which an OH group is added to the base guanine. There is little direct evidence, however, that these mutations cause aging. No acceleration in aging occurred among survivors of Hiroshima and Nagasaki despite their enormous added mutation load, arguing any general relationship between mutation and aging (see **Raven** *et al*., 2005).

**2. Telomere Depletion Theory**

In an important experiment carried out in 1961, **Leonard Hayflick** demonstrated that fibroblast cells growing in tissue culture will divide only a certain number of time. After about 50 population doublings, cell division stops—the cell cycle is blocked just before DNA replication. If a cell sample is taken after 20 doublings and frozen, when thawed it resumes growth for 30 more doublings, and then stops.

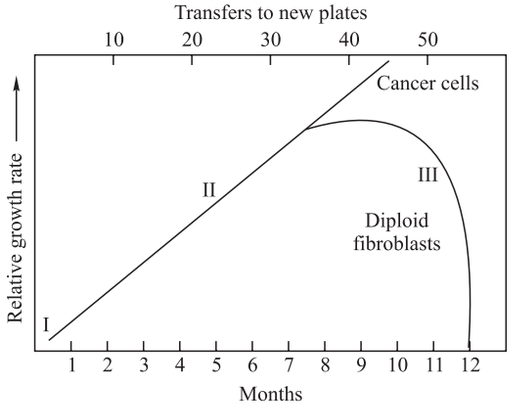


***Fig. 4.54*** *Hayflick's experiment. Fibroblast cells stop growing after about 50 doublings. Growth is rapid in phase I and II but slows in phase III, as the cultures become senescent, until the final doubling. Cancer cells, by contrast, do not age (after Raven el al., 2005).*

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An explanation of the "Hayflick limit" was suggested in 1986 when **Howard Cooke** first glimpsed an extra length of DNA at the ends of chromosomes. These **telomeres**, repeats of the sequence TTAGGG, were found to be substantially shorter in older somatic tissue, and Cooke speculated that a 100-base-pair portion of the telomere cap was lost by a chromosome during each cycle of DNA replication. Eventually, after about 50 replication cycles, the protective telomeric cap would be used up, and the cell line would then enter senescence, no longer able to proliferate. Cancer cells appear to avoid telomere shortening.

Research reported in 1998 has confirmed Cook's hypothesis, providing direct evidence for a causal relationship between telomeric shortening and cell senescence. Using genetic engineering researches transferred into human primary cell cultures a gene that leads to expression of **telomerase**, an enzyme that builds TTAGGG telomeric caps.

The result was clear-cut. New telomeric caps were added to the chromosomes of the cells, and the cells with the artificially elongated telomeres did not age at the Hayflick limit, continue to divide in a healthy and vigorous manner for more than 20 additional generations.

**Evidence Against Telomere Depletion Theory**

In some cases, no correlation between telomere length and the life span of an animal (humans have much shorter telomeres than mice) has been found, nor is there a correlation between human telomere length and a person's age (**Cristofalo** *et al*., 1998, **Rudalph** *et al*., 1999; **Karlseder** *et al*., 2002). Nematodes can have mutations that extend or shorten longevity, and the length to the telomere does not correlate with the age in the roundworms (**Raices** *et al*., 2005). Telomeres appear to be critical in stem cell maintenance, and the telomere-dependent inhibition of cell division might serve primarily as a defense against cancer (**Blasco** 2005; **Flores** *et al.,* 2005).

**3. Wear-and-Tear Theories:** Numerous theories of aging focus in one way or another on the general idea that cells wear out over time, accumulating damage until they are no longer able to function. Loosely dubbed the "wear-and-tear" hypothesis, this idea implies that there is no inherent designed-in limit to aging, just a statistical one—that is, disruption, wear, and damage over time erode a cell's ability to function properly.

Considerable evidence indicates that aging cells do accumulate damage. Some of the most interesting evidence concerns free radicals, fragments of molecules or atoms that contain an unpaired electron. Free radicals are very reactive chemically and can be quite destructive in a cell. Free radicals are produced as natural by-product of oxidative metabolism, but most are mopped up by special enzymes that function to sweep the cell interior free of their destructive effects.

One of the most damaging free radical reactions (oxidative damage) that occurs in cells causes glucose to become linked to proteins, a non-enzymatic process called **glycation**. Two of the most commonly glycated proteins are **collagen** and **elastin,** key components of the connective tissues in our joints.

Glycated proteins can cross-link to one-another, reducing the flexibility of connective tissues in the joints and producing many of the other characteristic symptoms of aging.

**ROS and Aging:** One major theory views metabolism as the cause of aging. According to this theory, aging is a result of metabolism and its by-products, **Reactive Oxygen Species (ROS).** The ROS produced by normal metabolism can oxidize and damage cell membranes, proteins and nucleic acids. Some 2–3% of the oxygen atoms taken up by our mitochondria are reduced insufficiently and form ROS: superoxide ions, hydroxyl ("free") radicals, and hydrogen peroxide. Evidence that ROS molecules are critical in the aging process includes the observation that fruit flies over expressing the enzymes that destroys ROS (Catalase and superoxide dismutase) live 30–40% longer than do control flies. Moreover, flies with mutations in the *methuselah* gene (named after the biblical fellow said to have lived 969 years) live 35% longer than wild-type flies. These mutants have enhanced resistance to ROS (**Lin** *et al*., 1998). In nematode *C. elegans*, too, individuals with mutations that result in either the degradation of ROS or the prevention of ROS formation live much longer than wild-type nematodes (**Feng** *et al.*, 2001). These findings not only suggests that aging is under genetic control, but also provide evidence for the role of ROS in the aging process.

**4. Random Genetic Drift:** A new variant of "wear-and-tear" theories is the hypothesis of **random genetic drift**. Given that appropriate **methylation** is essential for normal development, one can immediately see that diseases would result as a consequence of inappropriate epigenetic methylation. Recent evidences have confirmed that inappropriate methylation can be the critical factor in aging and cancers. Some of the evidences for this hypothesis

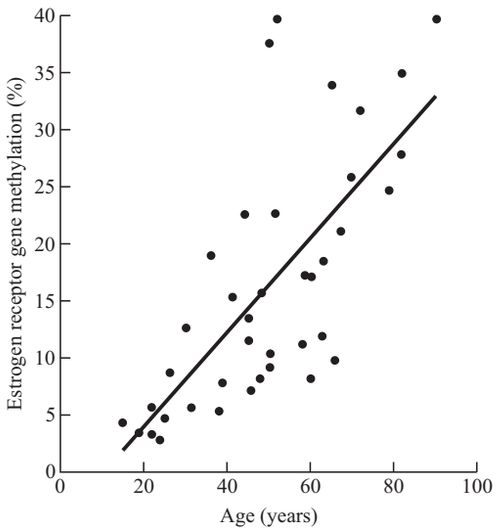


***Fig. 4.55*** *Methylation of the estrogen receptor gene occurs as a function of a normal aging (after Gilbert 2010).*

comes from identical twins. Most "identical" twins start life with very few differences in appearance with age. Experiences counts, and both random events and life styles may be reflected in phenotypes. **Fraga** and colleagues (2005) found that twin pairs were nearly indistinguishable in methylation

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patterns when young but older monozygotic twins exhibited very different patterns of methylation. This affected their gene expression patterns, such that older twin pairs had different patterns of DNA expression, while younger twin pairs had very similar expression patterns. **Fraga** *et al*., (2005) have shown that monozygotic twin pairs start off with identical amounts of methylated DNA and acetylation of histones H3 and H4 (these are three **epigenetic markers**). As twins age, however, both methylation and acetylation increase, but to different extents and at different chromosomal locations in each twin.

The idea that random genetic drift inactivates important genes without any particular environmental cue gives rise to an entirely new hypothesis of aging. Instead of randomly accumulated mutations—which might be due to specific mutagens—we are at the mercy of *chance accumulations of errors* made by the DNA methylating and demethylating enzymes. Indeed, our DNA methylating enzymes, unlike the DNA polymerases, are prone to errors. The enzymes DNA methyltransferases are not the most fastidious of enzymes. At each round of DNA replication, they must methylate the appropriate cytosine residues and leave the others unmethylated. This is not always done properly, and such errors accumulate as we age.

For instance, the methylation of the promoter region of estrogen receptor is known to increase with age (**Issa** *et al*., 1994). There is a linear relationship between the methylation of a promoter region in an estrogen receptor gene and increased age. The methylation of the promoters of the genes for the alpha and beta estrogen receptors increases with age, resulting in the inactivation of this gene in the smooth muscles of the circulatory system. Moreover, methylation of the estrogen receptor genes is even more prominent in the atherosclerotic plaques (thickened artery walls) that occlude the blood vessels. The atherosclerotic plaques show more methylation of estrogen receptor genes than does the tissue around it (**Post** *et al*., 1999; **Kim** *et al*., 2007). Thus, DNA methylation-associated inactivation of the estrogen receptor genes in vascular tissue may play a role in athrogenesis and aging of the vascular system. This potentially reversible defect may provide a new target for intervention in heart disease (**Gilbert** 2010).

**5. Gene Clock Theory:** There is little doubt that atleast some aspects of aging are under the direct control of genes. Just as genes regulate its rate of aging, some genes appear to promote longevity. For example, people over 100 years old are five times as likely to carry a mutation in mitochondrial DNA called **C150T**.

Other genes produce premature aging. For example, in 1996, a gene was identified that is responsible for **Werner's syndrome**, which produces premature aging and affects some 10 people per million worldwide. The syndrome is named after **Otto Werner,** who in 1904 reported in a German family affected by premature aging and believed a genetic component was at work. Werner's syndrome appears in adolescence, usually producing death before age 50 of heart attack or one of a variety of rare connective tissue cancers. Located on the short arm of chromosome 8, the gene seem to affect a helicase enzyme involved in repair of DNA. The genes which

codes for 1432-amino acid protein, has been fully sequenced, and four mutant alleles identified. Helicase enzymes are needed to unwind the DNA double helix whenever DNA has to be replicated, repaired or transcribed. The high incidence of certain cancers among Werner's syndrome patients leads investigators to speculate that the mutant helicase may fail to activate critical tumour suppressor genes. The potential role of helicases in aging is the subject of heated research.

Research on aging in other animals strongly supports the hypothesis that genes regulate the rate of aging. A *Drosophila melanogaster* gene mutation called *Indy* ('I am not dead yet') doubles the fruit fly life span from the usual 37 days to an average of 70 days. When researchers isolated the DNA of the *Indy* gene and compared its DNA sequence with the Human Genome Project sequences, they found that the *Indy* gene is 50% similar to a human gene called *dicarboxylic cotransporter.* In humans, dicarboxylic cotransporter proteins move preliminary products of food metabolism (dicarbxylic acids of the Krebs cycle) across membranes to where the food processing takes place. In mutant *Indy* flies, poor dicarboxylic acid pumping means that less metabolic energy can be gleaned from the fly's food. In essence, the *Indy* mutation is the genetic equivalent of caloric restriction (*i.e*., starving). Starving is known to prolong life in the nematode *Caenorhabditis elegans*, but *Indy's* caloric restriction does not involve the unpleasantness of starving. The *Indy* mutation in effect puts flies on a severe diet, while flies eat as much as normal and lead a normal vigorous life for far longer.

Several interacting agents may promote longevity. These include calorie restriction, protection against oxidative stress, and the factors activated by a suppressed insulin pathway. It is not yet known how these factors interact—whether they are part of a single "longevity pathway", or if they act separately. Moreover, genetics and diet do not appear to be the full answer to aging. Chance it seems, still play a role. When clonally indentical C. elegans (nematodes) are fed an identical diet, some organisms still live longer than others, and different organs deteriorate more rapidly in different individuals (Herndon et al., 2002). Mutations are randomly occurring events, and they may play a role in the aging process.

**6. Diet Caloric Restriction Theory:** It is one of the few known ways of extending mammalian longevity (in fact at the expense of fertility) and it may do so through several routes. First, restricting caloric intake may reduce levels of IGF-1 and of circulating insulin (**Kenyon** 2001; **Roth** *et al*., 2002; **Holzenberger** *et al*., 2003). This association of increased longevity with the down regulation of the insulin pathway through diet is seen in yeast, flies, nematodes, and mice. Dietary restrictions may also work through the sirtuin proteins (**Lamming** *et al*., 2005), thereby uniting the insulin metabolic pathway with the genomic protection hypothesis. The insulin pathway in mammals also negatively regulates *Foxo*, the gene for a transcription factor that activates ROS-protective enzymes (**Essers** *et al*., 2004). Calorie restriction also represses a ribosomal activator whose absence is associated with increased longevity (**Selman** *et al*., 2009).

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**7. Parabiosis Theory (Young Blood: Serum Factors and Progenitor Cells):** One of the distinctive features of aging is the declining ability of stem cells and progenitor cells to restore damaged or non-functioning tissues. A decline in muscle progenitor (satellite) cell activity when Notch signaling is lost results in a significant decrease in the ability to maintain muscle function. Similarly, an age-dependent decline in liver progenitor cell division impairs liver regeneration due to a decline in transcription factor CEBP-α.

The problem seems to exist in the environment of the stem cells. If an aged and young mouse are parabiosed (*i.e*., their circulatory systems are surgically joined so that the two mice share one blood supply), the stem cells of the old mouse are exposed to factors in young blood serum (and vice-versa). This **heteronic parabiosis** has been seen to restore the activity of old stem cells. Notch signalling of the muscle stem cells regained its youthful levels, and muscle cell regeneration was restored. Similarly, liver progenitor cells regained "young" levels of CEBP-α and their ability to regenerate (**Conboy** *et al*., 2005). When young haematopoietic stem cells were placed into "old" stem cell niches that had been exposed to old blood, they did not make many new cells when injected into lethally irradiated mice. However, when these young stem cells were placed into "old" niches that had been exposed by parabiosis to "young" blood cells as the young stem cells that had seen young niches exposed only to young blood.

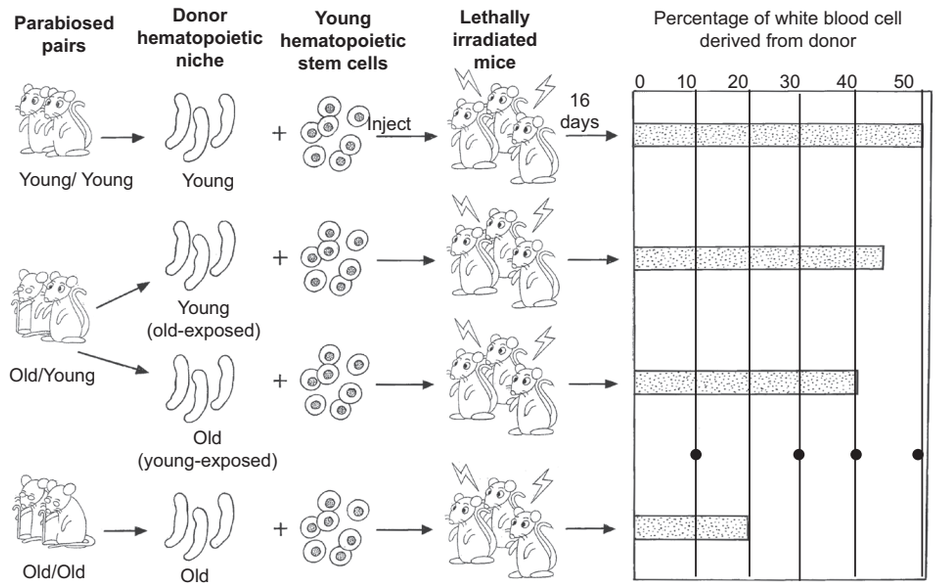


***Fig. 4.56*** *Circulating factors "rejuvenate" haematopoietic niche cells. Haematopoietic niche cells were isolated from parabiosed (surgically conjoined) mouse pairs. The pairs were of either same age/young (2 months); same age/old (21 months); or one young and one old mouse. Niche cells were cultured with young haematopoietic stem cells and injected into lethally irradiated mice (i.e., mice whose own stem cells had been destroyed by radiation). When the white blood cells produced by the injected stem cells were analyzed 16 weeks later, stem cells residing in young niche with young blood produced the most white blood cells; those residing in old niches bathed in old blood had the worst reconstitution. However, old-niche cells that developed in contact with young blood (i.e., those from the "mixed" parabiosed pair) reconstituted the host while cell population almost as well as the "ever-young" cells (after Gilbert 2010).*

According to **Mayack** and coworkers (2010), the factor involved in aging of the niche may be IGF-1 which is produced locally in the niche and is regulated by factors in the blood.

**8. Pace Maker Theories of Aging:**

Evidence is growing that aging is caused, in part, by progressive breakdown in the immunological system. **Burnet** has suggested that the **thymus gland** is the **biological clock** that determines how fast we age. It is the pacemaker for the whole body and its atrophy would be programmed event that leads to aging of the animal. It is well known that the thymus begins to atrophy shortly after puberty in humans.

## 4.7.6 Exceptions to the Aging Rule

There are a few species such as turtles, monarch butterflies and a hydrozoan in which aging seems to be optional, and these may hold some important clues to how animals can live longer and retain their health. Many turtle species not only live a long time, but they do not undergo the typical aging syndrome. In these species, for example, older females lay as many eggs as their younger counterparts. **Miller** (2001) showed that a 60-year old female three toed box turtle (*Terrapene carolina triunguis*) lays as many eggs annually as she ever did. Interestingly, turtles have special adaptations against oxygen deprivation, and these enzymes also protect against ROS (**Congdon** *et al*., 2003; **Lutz** *et al*., 2003).

In monarch butterflies (*Danaus plexippus*), adults that migrate to wintering grounds in the mountains of central Mexico live several months (August–March), whereas their summer counterparts live only about 2 months (May–July). The regulation of this difference appears to be juvenile hormone (**Herman** and **Tatar** 2001). The migrating butterflies are sterile because of suppressed synthesis of juvenile hormone (JH). If migrants are give JH in the laboratory, they regain fertility but lose their longevity. Conversely, when summer monarchs have their corpora allata removed (so they no longer make JH), their longevity increases 100%. Mutations in the insulin signaling pathway of *Drosophila* likewise decrease JH synthesis (**Tu** *et al*., 2005). This decrease in JH makes the flies small, sterile and long-lived, adding to whatever longevity-producing effect protection against ROS might have.

Finally, there may be organisms that have actually cheated death. The hydrozoan cnidarian *Turritopsis nutricula* may be such an immortal animal. Most hydrozoans have a complex life cycle in which a colonial (polyp) stage asexually buds off the sexually mature, solitary, adult medusa (usually called a **jelly fish)**. Eggs and sperm from the medusa develop into an embryo and then a planula larva. Planula larva then forms colonial polyp stage. Medusae, like the polyps have a limited life span, and in most hydrozoans they die shortly after releasing their gametes (**Martin** 1997). *Turritopsis*, however, has evolved a remarkable variation on this theme. The solitary medusa of this species can revert to its polyp stage after becoming sexually mature (**Bavestrello** *et al*., 1992; **Piraino** *et al*., 1996). In the laboratory, 100% of *Turritopsis* medusae undergo this change (see **Gilbert** 2010).

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How does the jellyfish accomplish this feat? Apparently, it can after the differentiated state of a cell, transforming it into another cell type. Such a phenomenon is called **transdifferentiation** and is usually seen only when parts of an organ regenerate. However, it appears to occur normally in the *Turritopsis* life cycle. In the transdifferentiation process, the medusa is transformed into the stolons and polyps of a hydroid colony. These polyps feed on zooplankton and soon are budding off new medusae. Thus, it is possible that a organismic death does not occur in this species.

**Check Your Progress**

21. Define aging.
22. What do you understand by the term maximum life span?
23. What is the effect of aging on skin?
24. What are neoplastic cells?
25. Define invasiveness.

## 4.8 ANSWERS TO 'CHECK YOUR PROGRESS'

1. Cell matrix adhesion is the interaction of a cell with the extracellular matrix, mediated by multi-protein adhesion structures, such as focal adhesions, fibrillar adhesions and podosomes.
2. The best-known class of matrix adhesions in cultured cells are the Focal Contacts (FCs), which can be visualized by electron microscopy or interference reflection microscopy.
3. Cells create extracellular matrix by releasing molecules into its surrounding extracellular space.
4. The plant hormone auxin is well known to stimulate cell elongation via increasing wall extensibility. Auxin participates in the regulation of cell wall properties by inducing wall loosening.
5. According to their external shape, bacteria are grouped into the following two main classes:
   - Cocci
   - Bacilli
6. Eukaryotic DNA is bound to proteins known as histones to form structures called nucleosomes.
7. A palindromic sequence is a nucleic acid sequence in a double-stranded DNA or RNA molecule whereby reading in a certain direction (for example, 5' to 3') on one strand is identical to the sequence in the same direction (for example, 5' to 3') on the complementary strand.
8. Split genes means that the (DNA) sequences containing actual information of the gene (called exons) are interrupted by other sequences (called introns) which are spliced out after transcription.

9. Bacterial transposons are responsible for the transposition of genes by controlling resistance to antibiotics from one molecule to another.
10. A gene is a basic unit of heredity and a sequence of nucleotides in DNA that encodes the synthesis of a gene product, either RNA or protein.
11. A gene occupies a definite position within the chromosome. This position is called the locus (plural loci) (Demerec 1955). Chromosomes exist in homologous pairs, each cell contains two kinds of genes which are found in pairs. The two members of a pair of a gene are called alleles.
12. Missence codon is a codon which undergoes an alteration to specify another amino acid.
13. Intracellular transport is the movement of vesicles and substances within a cell. Intracellular transport is required for maintaining homeostasis within the cell by responding to physiological signals.
14. Proteins carried inside the membrane bound vesicles are called cargo proteins.
15. A polyribosome or polysome is a group of ribosomes bound to an mRNA molecule like beads on a thread.
16. The Golgi Apparatus, or Golgi Complex, is a membrane-bound, multi-compartment organelle located in the Cytoplasm of most Eukaryotic cells, including plants, animals and fungi.
17. Cells which undergo rapid, abnormal and uncontrolled growth at the cost of remaining cells are called neoplastic cells.
18. The process of cell change in which a cell loses its ability to control its rate of division, and thus becomes a tumour cell, is called cell transformation.
19. Substances that cause cancer are called carcinogens.
20. The branch of medical science that investigates the frequency and distribution of diseases in human populations is called epidemiology.
21. Aging is a slow process during which the body undergoes changes that eventually bring about death, even if no marked disease or disorder is present. Medical science is trying to extend the human life span and the health span, the length of time the body functions normally.
22. The maximum life span, which is the maximum number of years any member of a given species has been known to survive.
23. As aging occurs, skin becomes thinner and less elastic because the number of elastic fibers decreases and collagen fibers undergo cross-linking. Also, there is less adipose tissue in the subcutaneous layer; therefore, older people are more likely to feel cold. The loss of thickness of skin partially accounts for sagging and wrinkling of the skin.
24. Cells which undergo rapid, abnormal and uncontrolled growth at the cost of remaining cells are called neoplastic cells.
25. Invasiveness is the ability of cancer cells, to invade other tissues.

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## 4.9 SUMMARY

- Cell matrix adhesion is the interaction of a cell with the extracellular matrix, mediated by multi-protein adhesion structures, such as focal adhesions, fibrillar adhesions and podosomes.
- The specific type of integrin present in matrix adhesions can vary, depending on the nature of the underlying Extra Cellular Matrix (ECM).
- Integrins are transmembrane heterodimers formed by different $\alpha$ and $\beta$ subunits, both subunits with different domain structures.
- Plant cells are surrounded by cell walls, which are dynamic structures displaying a strictly regulated balance between rigidity and flexibility.
- A microfibril is a very fine fibril, or fiber-like strand, consisting of glycoproteins and cellulose.
- A DNA virus is a virus that has a genome made of deoxyribonucleic acid (DNA) that is replicated by a DNA polymerase.
- Bacterial cells do not contain a typical nucleus but as per the 'Feulgen' reaction it shows one, two or more discrete nuclear bodies per cell which are termed as 'Nucleoids'.
- Bacterial chromosome is a single replicon. Auto-radiographic studies have shown that it replicates bi-directionally in a semiconservative manner.
- Eukaryotic DNA is bound to proteins known as histones to form structures called nucleosomes.
- A palindromic nucleotide sequence is capable of forming a hairpin. The stem portion of the hairpin is a pseudo-double stranded portion since the entire hairpin is a part of same (single) strand of nucleic acid.
- In the DNA molecule a variety of base sequences have been observed. Most of them do not have special features.
- Split genes means that the (DNA) sequences containing actual information of the gene (called exons) are interrupted by other sequences (called introns) which are spliced out after transcription.
- Transposable elements were discovered by B. McClintock through an analysis of genetic instability in maize.
- As DNA is a genetic material, it carries genetic information from cell to cell and from generation to generation.
- The genetic code is commaless, which means that no codon is reserved for punctuations.
- The genetic code is a degenerate code, meaning that a given amino acid may have more than one codon.
- Intracellular transport is the movement of vesicles and substances within a cell. Intracellular transport is required for maintaining homeostasis within the cell by responding to physiological signals.

- A typical mammalian cell may contain numerous kinds of proteins and numerous individual protein molecules.
- Transportation of proteins to their final destinations is termed as protein targeting.
- Protein sorting requires proper address labels which are in the form of peptide signal sequences.
- A short N-terminus signal sequence at the beginning of the growing nascent protein chain' determines whether a ribosome synthesizing the proteins binds to ER or not.
- The role of Golgi complex is to act as a switching center for proteins to various destinations.
- The proteins embedded in different membranes may have single trans-membrane domain which is a segment of 20-25 amino acids.
- Aging or senescence encompasses these progressive changes that contribute to an increased risk of infirmity, disease and death.
- The species-specific life span appears to be determined by genes that affect a compromise between early growth and reproduction and somatic maintenance.
- As aging occurs, skin becomes thinner and less elastic because the number of elastic fibers decreases and collagen fibers undergo cross-linking.
- Cancer can result from abnormal proliferation of any of the different kinds of cells in the body, so there are more than a hundred distinct types of cancers.
- The cancerous cell generally retains the structural and functional characteristics of the normal cell type from which it is derived.
- Cancers are caused mainly by environmental agents and lifestyle factors, most of which act by triggering DNA mutations.

## 4.10 KEY TERMS

- **Integrins:** Integrins are transmembrane heterodimers formed by different $\alpha$ and $\beta$ subunits, both subunits with different domain structures.
- **Split Gene:** Split genes means that the (DNA) sequences containing actual information of the gene (called exons) are interrupted by other sequences (called introns) which are spliced out after transcription.
- **Loci:** A gene occupies a definite position within the chromosome. This position is called the locus (plural loci).
- **Alleles:** Chromosomes exist in homologous pairs, each cell contains two kinds of genes which are found in pairs. The two members of a pair of a gene are called alleles.
- **Protein Targeting:** Protein targeting or Protein sorting is the biological

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mechanism by which Proteins are transported to their appropriate destinations in the cell or outside it.

- **Cisternae:** A Golgi apparatus is composed of flattened, plate or saucer-like, closed, fluid filled sacs known as cisternae.
- **Maximum Life Span:** The maximum life span is the maximum number of years any member of a given species has been known.
- **Cell Transformation:** The process of cell change in which a cell loses its ability to control its rate of division, and thus becomes a tumour cell, is called cell transformation.
- **Life Expectancy:** Life expectancy means the length of time an average individual of a given species can expect to live.
- **Cancer:** The term cancer refers to any malignant tumour—that is, any tumour capable of spreading from its original location to other sites.
- **Oncogenes:** Oncogenes are genes whose presence can cause cancer.
- **Tumour Suppressor Genes:** Tumour suppressor genes are genes whose loss or inactivation can lead to cancer.

## 4.11 SELF ASSESSMENT QUESTIONS AND EXERCISES

### Short-Answer Questions

1. What are the non-collagenous components of the cell?
2. Define palindroms.
3. What are transposons? What is the significance of transposons?
4. Write the general properties genetic code.
5. What do you understand by the term ‘protein targeting’?
6. Which are the protein in ER?
7. What is the function of the golgi apparatus?
8. Define life expectancy.
9. Why are the cardiovascular disorders the leading cause of death among the elderly?
10. State the wear and tear theory of aging.
11. Why is cancer considered as a genetic disease?
12. How many groups of cancer are recognized?
13. Write the characteristics of cancer cells.
14. Which genes are involved in cancer?

### Long-Answer Questions

1. Which are the major classes of ECM molecules. Explain in class in detail.
2. Explain the process of replication of bacterial chromosome.

3. Write a short note on discovery of split genes.
4. Elaborate on gene concept.
5. Explain Wobble hypothesis of genetic code.
6. Describe the post translational modifications.
7. Describe the biogenesis of mitochondria.
8. Analyze the subcellular changes due to the process of aging.
9. Explain the various theories of aging.
10. Write a short note on cancer.
11. Explain the process of angiogenesis.

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## 4.12 FURTHER READING

Rastogi, S. C. 2012. *Cell Biology*, 3rd Edition. New Delhi: New Age International (P) Ltd.

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