CONTENTS

1. CELL STRUCTURE INTODUCTION

THE CELL AND ITS STRUCTURAL ORGANIZATION

The living world comprises of around four million different species of various living forms. These include bacteria, protozoa, plants and animals that differ in morphology, function as well as behavior. The cells and molecules constitute the building blocks of all the forms of life. Cell is referred to as the basic unit of life which is a microscopic structure. It remains almost same in all except for in few specialized forms and comprises of almost similar functions. It is also confirmed that a single cell can perform all the activities as a multicellular organism in which the cells are grouped and differentiated into tissues and organs. A cell comprises of few basic structures which include- nuclei, cytoplasmic matrix, plastids, mitochondria, endoplasmic reticulum, golgi apparatus, plasma membrane etc, all with regulated functions at molecular level. Biochemists have found that cells comprise of all the elements of inorganic world organized into more complex molecules such as proteins, fats, polysaccharides and nucleic acids.

With the advent of Electron microscope, the sub cellular organization of a cell upto molecular level was made possible. Apart from these methods of cell fractionation has paved the way for separating cell organelleles and these have led to the branches of Biochemistry and Molecular Biology. Various levels of organization of biological structures are mentioned in the following table:

	Dimensions	**Biological field**	**Structure**	**Method of study**
1.	0.1mm or 100μm or larger	Anatomy	Organs	Eyes and simple lenses
2.	100μm to 10μm	Histology	Tissues	Various types of light microscopes, x-ray microscope
3.	10μm to 0.2μm (200nm)	Cell Biology	Cell, Bacteria	
4.	200nm to1nm	Submicroscopic morphology	Cell components, Viruses	Polarization microscopy, Electron microscopy
5.	Smaller than 1nm ($10A^{o}$)	Ultra structure molecular and atomic structure	Arrangements of atoms	x-ray diffraction

History of Cell Biology: It dates back to 384-322 B.C, during which the Greek Philosophers such as Aristotle and Paracelsus have concluded that "all animals and plants, however, complicated, are constituted of a few elements which are repeated in each of them". Many centuries later, magnifying lenses were discovered which allowed viewing microscopic structures of plant parts. Da Vinci (1485) recommended use of lenses for viewing small objects. With the developments in optical lenses and construction of compound microscopes, development of the field of cell Biology took place.

Francis Janssen and Zacharias Janssen invented the first useful microscope in 1590 that had a magnifying power of around 10X to 30X. In 1610 Galilio Galilei (1564-1642) invented simple microscope with a single lense that could be used to view the compound eye of insects. Later, Italian Micro anatomist Marcello Malpighi (1628-1694) was the first to use simple microscope to observe slices of animal tissues. He also studied plant cells and referred to the structural units as 'Utricles'. Robert Hooke (1635 -1703), the English Microscopist has coined the term, "Cell" in 1655, (L., Cella= Hollow space), after this observation of slices of dried cork (Figure1). The cells in the fork are the empty spaces left after disintegration of living matter of the cells. Dutch Microscopist, Anton Van Leeuwen Hoek (1632 – 1723), had succeeded in improving the art of polishing lenses and the magnification could be improved to 300X (figure 2). He was the first to observe free living cells. He collected rain water into tubes that were inserted in soil, and this was used for his microscopic studies. His sketches of microorganisms include many bacteria, protozoan's, rotifers, hydra etc. He also extended his studies and described sperm cells of human, dogs, rabbits, fish etc., and he also observed movement of blood cells of mammals, birds, amphibians and fish. He observed the central body of a cell (nucleus) in various shapes which was revealed in his writings to the royal Society of London during 1675-1683. Nehamiah Grew (1641- 1721) studied sections of plants, flowers, roots, stems etc. in 1831, English Botanist Robert Brown (1771- 1858), discovered nucleus and named it so. 19th century has witnessed various inventions in Cell Biology that led to the formulation of theories such as cell theory and protoplasm theory.

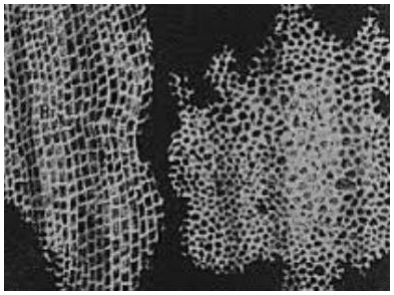

Figure 1. Image of hollow spaces in dried cork

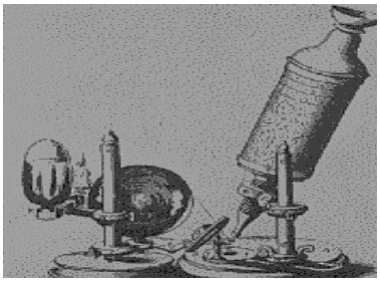

Figure 2. Compound microscope used by Anton Van Leeuwen Hoek

Theories of Cell Formation:

Cell theory: Mathias Jacob Schleiden and Theodor Schwan, 1839 postulated that the cell is the basic unit of structure and function in all forms of life. Schleiden was the first to describe the nucleoli, and stated that each cell leads a double life, one which involved its own development and the other as an integral part of multicellular plant. Schwann studied both plant and animal tissues that led to modification of cell theory to include the idea that living things are composed of both cells and the products or secretions of the cells. He introduced the term metabolism to describe the activities of the cells.

K.Nageli (1817-1891) showed in 1846, that plant cells arise from the division o f pre- existing cells. German Pathologist Rudolf Virchow (1821- 1902) confirmed Nageli's proposal of cell life's continuity. Virchow established the significance of cell division in the reproduction of organisms. He also established that cells were the primary sites of disease and cancer. In 1865, Louis Pasteur (1822- 1895) gave practical evidence in support of Virchow's extension of cell theory.

Modern version of Cell Theory:

1. All living organisms are made up of one or more cells and cell products.
2. All metabolic reactions in unicellular and multi cellular organisms take place in cells.
3. No cell can originate spontaneously de novo, but only from previously existing cells.
4. The smallest defined unit of life is the cell.

With further advancements in techniques, sub-cellular structures such as ribosomes, lysosomes, mitochondria, chloroplasts etc., indicated that cell is not the only basic unit of cell. Exception to cell theory:

All kinds of cells share the three characteristics mentioned below:

1. Cell activities and replication are regulated by a set of genes.
2. Plasma membrane regulates exchange of materials of cells with outside environment.

3. Metabolic machinery is involved in sustaining all activities of life like growth, reproduction and repair of parts.

These do not apply for viruses which reproduce inside the host cells. But they definitely carry all the genetic material required for their organization and reproduction. Even organisms such as the Protozoans Paramecium, the fungus Rhizopus and the algae Vaucheria, do not fit the above hypothesis as they lack cell sort of definite organization.

Protoplasm Theory:

With the increase in knowledge about subcellular components, emphasis was given to contents of protoplasm. Felix Dujardin (1835) coined the term "Sarcode" to refer jelly like substance in Protozoans. Czech Biologist J. E. Purkinje coined the term protoplasm and Von Mohl (1846) used the above term to describe contents of animal embryonic cells. In 1892, O.Hertiwig came up with Protoplasm theory. The theory states that all living matter, out of which animals and plants are formed, is the protoplasm. The cell accumulates it and is limited by an outer membrane and has a nucleus inside. The protoplasm in nucleus is called as the Nucleoplasm while that which exists between nucleus and outer membrane is called the Cyto plasm. With the advent of techniques like Electromicroscopy, X-ray diffraction, Immunochemical and cytochemistry, Ultra centrifuge etc., and even freeze fractionation, micro manipulation, chromatography, electrophoretic and spectrophotometric techniques, the world of cell was explored. This has resulted in another new theory called Organismal theory.

Organismal Theory:

This theory states that multicellular organisms have a continuous mass of protoplasm which remains divided incompletely into cells for performing biological activities. This theory fails to answer the phenomenon of viral replication.

PRACTICE QUESTIONS

1. Cell theory was proposed by _____ and _____.
(A) Mathew Jacob Schwan and Theodar Scheilden
(B) Theodar Schwan and James Scheilden
(C) M.T.Scheilden and T.Schwan
(D) Mathias Jacob Scheilden and Theodar Schwan

2. Which among the following regarding Modern vision of cell theory are false?
1. All living organisms are made up of one or more cells and cell products.
2. All metabolic reactions in unicellular organisms are different from multicellular organisms.
3. No cell can originate spontaneously de novo, but only from previously existing cells.
4. The smallest defined unit of life is the cell.
(A) 1 (B) 2 (C) 3 (D) 4

3. Which among the following statement regarding characteristic of a cell is false:
1. Cell activities and replication are regulated by a set of genes.
2. Plasma membrane regulates exchange of materials of cells with outside environment.
3. Metabolic machinery is involved in sustaining all activities of life like growth, reproduction and repair of parts.
4. Environment primary role and stimulates gene transcription
(A) 1 (B) 2 (C) 3 (D) 4

4. Which among the following organisms have the characteristic of cell theory?
(A) Virus (B) Rhizopus (C) Liches (D) Paramecium

5. The term Sarcode was coined by_____.
(A) Robert Hook (B) Leeuwen Hoek (C) Dujardin (D) Schwan

6. Match the following

a.	Galilio Galilei	**1.**	Cell
b.	Robert Hooke	**2.**	Simple Microscope
c.	Anton Van Leeuwen Hoek	**3.**	Cell

(A) a-1, b-2, c-3 (B) a-2, b-3, c-1 (C) a-3, b-1, c-2 (D)

7. The technique that have contributed to the strengthening of Molecular Biology include-
(A) Light Microscopy and Electron microscopy
(B) Polarization Microscopy and Electron microscopy
(C) X-ray diffraction
(D) Polarization Microscopy, Electron microscopy and X-ray diffraction

8. Which among the following statements is wrong?

(a) Protoplasm comprises of Nucleoplasm and protoplasm

(b) Protoplasm is cytoplasm and nucleoplasm combined

(c) Cell accumulates protoplasm and cytoplasm separately

(d) Cell initially completes formation of cytoplasm from which nucleoplasm is extracted

(A) a, b, d (B) a, c, d (C) a, c (D) b,c,d

9. What does Organismal theory state?

(A) It states that there are no cells, but a multicellular organism is made up of mass of protoplasm.

(B) It states that multicellular organisms are made up of many cells

(C) It describes that multicellular animals are made up of a continuous mass of protoplasm that is divided incompletely

(D) It indicates that cell are unique and distinct with no communication with each other.

10. The following statements are about which theory of cell formation?

1. All living organisms are made up of one or more cells and cell products.

2. All metabolic reactions in unicellular and multi cellular organisms take place in cells.

3. No cell can originate spontaneously de novo, but only from previously existing cells.

4. The smallest defined unit of life is the cell.

(A) Protoplasm theory

(B) Cell theory and protoplasm theory

(C) Protoplasm theory and organismal theory

(D) Cell theory modern version

ANSWER KEYS

1	D	**2**	B	**3**	D	**4**	C	**5**	C	**6**	B	**7**	D
8	B	**9**	C	**10**	D								

EXPLANATIONS

1. Cell theory was proposed by Mathias Jacob Scheilden and Theodar Schwan

2. Modern version of Cell Theory:
- **1.** All living organisms are made up of one or more cells and cell products.
- **2.** All metabolic reactions of unicellular and multicellular organisms take place in cells.
- **3.** No cell can originate spontaneously de novo, but only from previously existing cells.
- **4.** The smallest defined unit of life is the cell.

3. The three characteristics include:
- **1.** Cell activities and replication are regulated by a set of genes.
- **2.** Plasma membrane regulates exchange of materials of cells with outside environment.
- **3.** Metabolic machinery is involved in sustaining all activities of life like growth, reproduction and repair of parts.

4. The characteristic listed for a typical cell do not fit for viruses, Protzoans like Paramecium, fungus Rhizopus and the algae Vaucheria etc.

5. Felix Dujardin coined the term Sarcode to refer to protoplasm the jellylike substance in Protozoans

6. Explanation:

Galilio Galilei	-	Simple Microscope
Robert Hooke	-	Cell
Anton Van Leeuwen Hoek	-	Observed living cells

7. The advent of polarization microscopy, electron microcopy have resulted in the study of cell components. X-ray diffraction has contributed to the study of Nucleic acid and hence the three contribute to strengthening of molecular biology.

8. Protoplasm comprises of both cytoplasm and nucleoplasm

9. Organismal Theory:
This theory states that multicellular organisms have a continuous mass of protoplasm whichremains divided incompletely into cells for performing biological activities. This theory fails toanswer the phenomenon of viral replication.

10. The statements are about Modern version of Cell Theory:

1. All living organisms are made up of one or more cells and cell products.

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2. TECHNIQUES IN CELL BIOLOGY

TECHNIQUES IN CELL BIOLOGY

Advancements in Cell Biology are due to introduction of new tools and techniques in the study of cell. Few are them are discussed below.

1. Microscopy: The diameter of most cells fall in the size range of 0.2 □to 50□m, which is much below the resolving power of human eye. Limit of resolution (l) is defined as the smallest distance that may separate two points on an object and still permit their observation as distinct separate points. Magnification of images/objects increases resolution limits and human eye cannot see objects such low diameters. A light compound microscope having many lenses can magnify objects upto 1500 times. The limit of resolution (l) of an optical instrument is given by Abbe's relationships:

$$\text{Resolution(l)} = \frac{\text{Wavelength}(\lambda)}{\text{Numerical aperature } (n \sin \alpha)}$$

Where, n = refraction index of material

Sin □ = Sine of the angle of aperture of the first lense

Refractive index is increased by using immersion oil which are transparent. A good light microscope, with a numerical aperture of 1.4 and using light of short wave length (0.4□ □m) will resolve two points at ~0.17 □m separation.

Light Microscopy:

Compound Light Microscope uses visible light for illuminating objects. They contain glass lenses that magnify objects and focus light on the retina of eye of observer. The lense closer to eye is called the Eye Piece or Ocular lense and the lense closer to object is called the Objective lense (figure 2.1). A condenser lense between light source and object serves to focus light on the object.

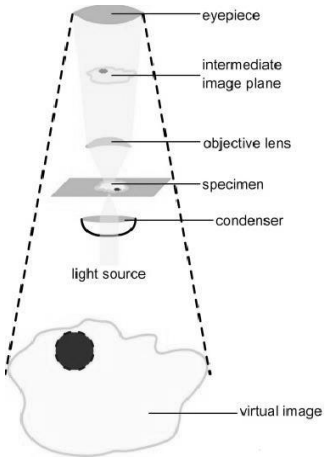


Figure 2.1: Principle of Light Microscope

Improved Types of Light Microscopes:

1. Dark Field Microscope or Ultra microscope: It is useful in viewing suspensions of bacteria. In this Microscopy, the object is viewed using oblique rays and light rays scattered by objects are viewed. Hence the image appears bright in dark background (Figure 2.2).

2. Phase Contrast Microscope: Different parts of the cell have different densities and hence different refractive indices. In the regions where refractive index changes, the light tends to bend. These bent rays are used to from patterns of destructive interference, yielding sharp contrast images. This microscopy is widely used to observe actively dividing cells which are stained.

Interference Microscope is based on the principle of Phase contrast microscopy and it permits detection of small and continuous changes in refractive cells.

3. Polarization Microscope: This is used for viewing objects such as crystals or bundles of parallel filaments etc., using polarized light. If the material is isotropic, the polarized light is propagated through it with the same velocity, independent of the impinging direction. These substances are characterized by having the same index of refraction in all directions. In the case of Anisotropic materials, the velocity of propagation of polarized light varies. These are called Birefringent materials as they present two different indices of refraction corresponding to the respective different velocities of transmission. In this microscopy, the specimen is placed between two closed

polarizers and visible birefringent portions of the sample acct life polarizing films, and hence, these portions of the sample are seen as bright objects on dark back ground.

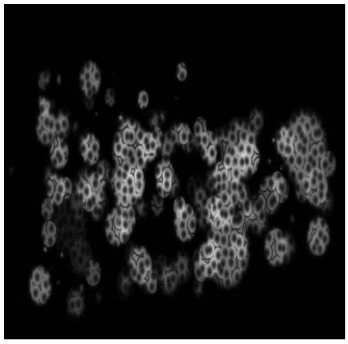


Figure 2.2: Image seen using

Ultramicroscope

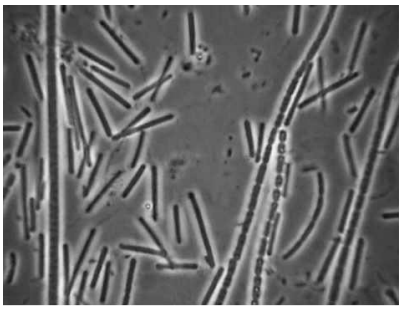


Figure 2.3: Image seen using

Phase Contrast Microscope

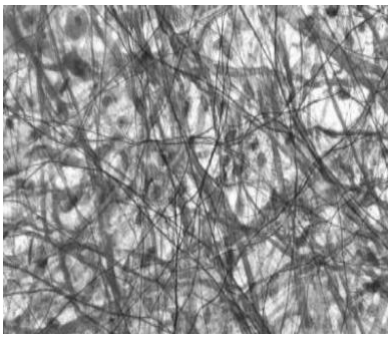


Figure 2.4: Image seen using

Polarization Microscope

Sample preparation for Light microscopy:

The steps involved in sample Preparation for light microscopy includes: Killing cells, Fixation, Dehydration, Embedding, Sectioning, Staining and mounting of slides. Stains used in these studies are of significance as they make the parts stand out among others. These are of two types, acid stains- which include Eosin, Orange G, Aniline Blue and Fast green. Basic stains include- Methylene Blue, Crystal violet, Haematoxylin, Basic Fuchsin etc. In addition, there are certain specific stains called as Cytochemical stains, that selectively bind to specific groups of macromolecules in a cell. These include- Millon reaction, Diazonium reaction and Naphthol Yellow for proteins; Alkaline fast green for Histone proteins, Feulgen reaction for DNA, Methylene Green- pyronine stain to distinguish DNA and RNA (DNA stains green and RNA stains red)(Figure 2.5), Acetocarmine to stain chromosomes in dividing cells (Figure 2.6), Sudan Black and Sudan Blue for lipids etc.

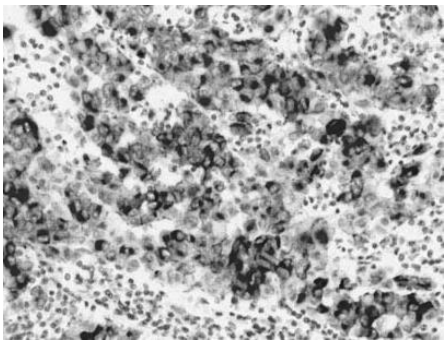


Figure 2.5: Staining of cells by Methylene Green-Pyronine showing DNA in green and RNA in red

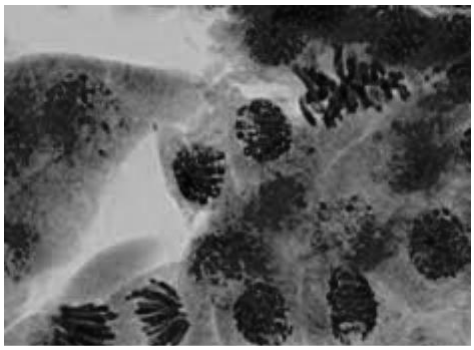


Figure 2.6: Chromosomes stained by acetocarmin

Electron Microscopy:

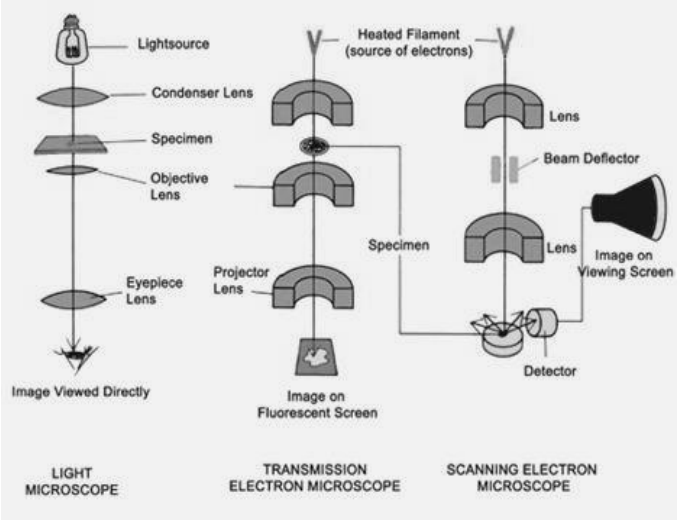
The Electron microscopy uses short wave lengths of electron to view sample and achieve a resolution as low as 3Ao (Figure 2.7). This technique uses Electromagnetic coils that are used to control and focus a beam of electrons accelerated from a heated metal wire by supplying high voltage in the range of 20,000 to 1,00,000 volts. The wave length of electrons depends on the magnitude of voltage applied and the wave length is around 0.01Ao. These electrons are scattered by specimen, which is placed in the path of beam. Various imaging principles are used to generate final image of the specimen the sample scatters the electrons and this scattering depends on density of regions within it. Therefore, regions which are of less density appear as dark regions and those of high density as lighter regions.

Specimen preparation is important in this transmission electron microscopy and they are Ultra thin sections of 10nm to 100nm thick (Figure 2.8). The samples are generally viewed directly, but to increase contrast, electron stains such as Urynyl acetate, Uranyl citrate, Lead citrate, Osmium tetroxide etc are used. To view small particles life viruses, negative staining is done using Phospho tungstic acid that penetrates into all empty spaces of the specimen and increases the contrast.

Scanning Transmission Electron Microscopy (STEM):

It has less resolution power than TEM (~200Ao) and is an effective tool used to study surface topography of specimens. A narrow beam of light is scanned rapidly over specimen, and it results in 3d image from the differential scattering of electron by different part of specimen.

Figure 2.7: Comparison of Light Microscope, Transmission Electron microscope and Scanning Transmission Electron Microscope



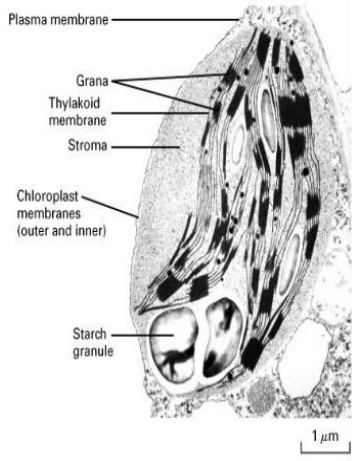


Figure 2.8: Specimen viewed by TEM

X-ray Diffraction Analysis:

This technique is used to analyze 3d structure of DNA molecule and many proteins such as myoglobin, Haemoglobin, Collagen, Myelin sheath of nerve cell etc. This method depends on principle of scattering or diffraction of X-rays by atoms of substance.

If the material has an ordered crystalline atomic structure, the resulting X-ray diffraction pattern is also ordered and reflects the 3d arrangements of atoms in crystals (Figure 2.9).

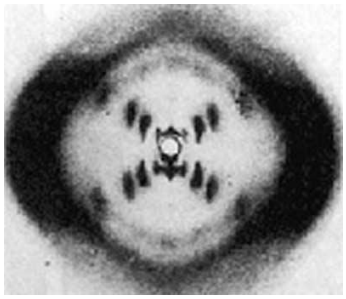


Figure 2.9: X-ray diffraction pattern of DNA

Cell Fractionation:

These techniques are used to study subcellular components, study tissues, cells etc. The procedure involves homogenization, destruction of cell boundaries, followed by sub cellular fractionation based on mass, surface and specific gravity by centrifuges. The centrifuges used in Cell Biology are Analytical Ultracentrifuge that provides information pertaining to mass and shape of molecules(Figure 2.10), while the other is Preparative Ultracentrifuge that can be used for separating molecules.

An ultracentrifuge with rotor of high rpm of around 70,000 revolutions per minute is used. Analytical ultracentrifuges contain optical system, allowing one to observe changes in solute of the solution. As they spin at high speed, the rotors are placed in vacuum to prevent generation of heat.

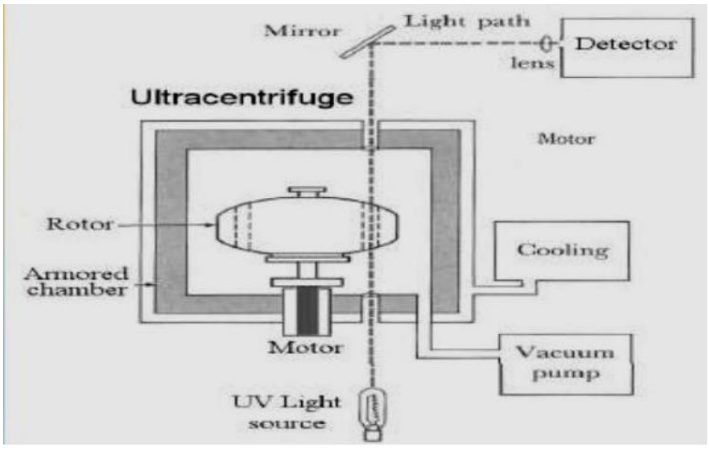


Figure2.10: Schematic representation of Analytical Centrifuge

The Procedure involves cell fractionation, tissue disruption or cell disruption and homogenization. The homogenate is then subjected to differential centrifugation in sucrose solution with increasing velocities (Figure 2.11). The principle in differential centrifugatio n is that at each step, particles settle down based on their rates of sedimentation. After all the organelleles settle down, the residual solution is called Cytosol which contains the soluble macromolecules of cells. These can be isolated using biochemical techniques like chromatography, dialysis and electrophoresis.

Density Gradient Centrifugation is best suitable for all fractionation. In this method, anincreasing gradient of solution is layered in centrifuge tube using Cesium Chloride, Sucrose, Heavy water or albumin. As the homogenate is layered on top and subjected to centrifugation, particles tend to migrate to various distances along the tube based on their buoyant densities (Figure 2.12). This type of separation is called Equilibrium density or Isopycnic centrifugation.

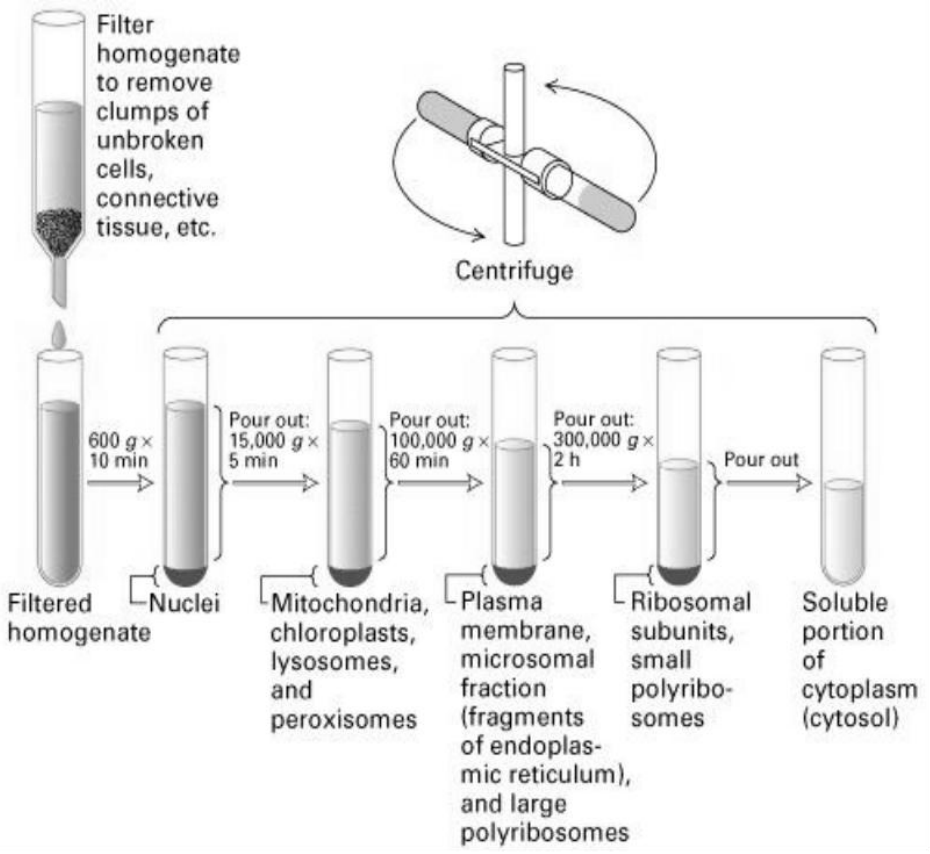


Figure 2.11: Step by step procedure of cell fractionation by

Differential Centrifugation

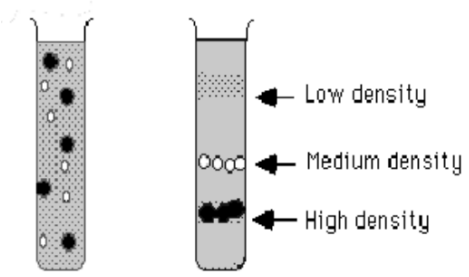


Figure 2.12: Isopycnic centrifugation of protein that get separated based on their buoyant densities.

Cell Culture:

Biological studies required growing cells outside their natural existence, like plants and animal cells and this is called Cell Culturing. It involves providing favorable conditions invitro for proper growth of cells. Cell cultures are of three types: Primary cell cultures, Secondary cell cultures and established cell lines. Primary cell cultures are obtained from plant or animal tissues or organs. A free cell suspension is prepared by using appropriate disaggregation methods, for example in the case of animal cell culture; the tissue is subjected to trypsinization. These are grown in Petri plates and then subjected to another cycle of trypsinization and plating in fresh medium. The resultant culture is called Secondary culture (figure 2.13). Prolonged growth of cells in culture will result in cell lines, examples of which include He La cells (Obtained from Human carcinoma), the L & 3T3 cells (obtained from mouse embryo) and the BHK cells (from Baby Hamster Kidney).

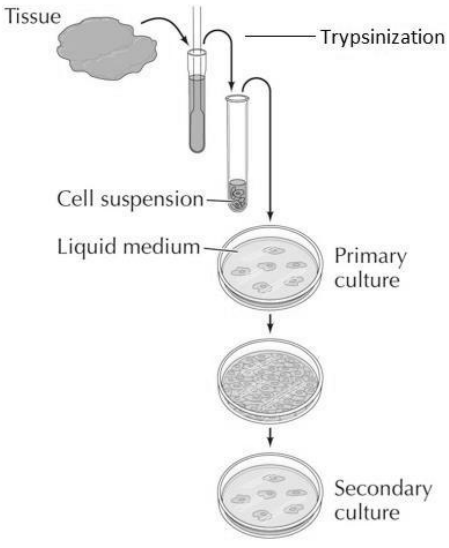


Figure2.13: Formation of Primary and Secondary cell cultures from animal tissue

Chromatography:

These techniques are used to separate mixture of molecules into their component. The following are the commonly used chromatography techniques in Cell Biology.

(A) Paper Chromatography: It is a planar chromatography that uses the principle of partition coefficient. In this technique high quality Whatman filter paper is used to separate samples Figure 2.14). Based on the solubility of sample between stationary phase and mobile phase the same migrates to varied distances on the paper. Water which adheres to the cellulose and act as stationary phase while mobile phase is any solvent that offers solubility to solutes under study. There are several types of paper Chromatography, like the Ascending chromatography, Descending chromatography and radial chromatography. 2-dimensional chromatography enables to separation of complex mixture of samples and is conducted in two dimensions using two different samples. This procedure involves determination of Rf values that are constant for a sample under standard conditions and helps to identify unknown solutes in the test.

Thin layer chromatography is another planar chromatography which used thin layer of Activated alumina or silica gel-G as stationary phase (figure 2.15). These materials are coated on to a plane surface of glass or aluminum sheet and samples are applied. A closed chamber having solvent system is taken and the plates are placed in it. As the mobile phase ascends the samples migrate based on adsorption. Paper chromatography is used for separation of amino acids, Nucleotides, pigments carbohydrates and low molecular weight solutes, while TLC can be used for rapid separation of saturated and unsaturated fatty acids, triglycerides, phospholipids, steroids, peptides, nucleotides etc.

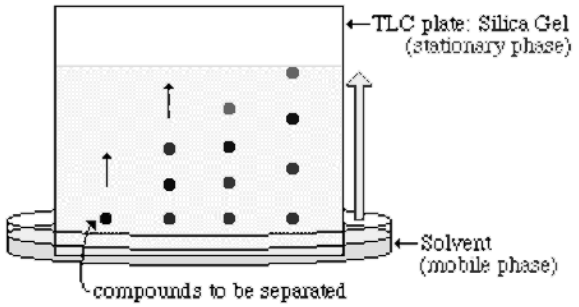


Figure 2.14: 2-D paper chromatography peocedure

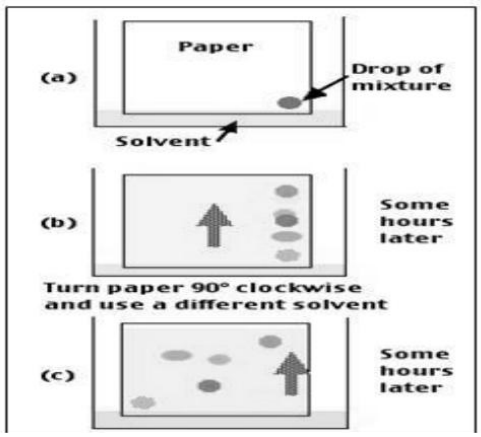


Figure 2.15: Thin layer chromatography

(B) **Column Chromatography:** In these set of chromatographic techniques columns packed with various materials are used for separation of mixtures. The general components of column chromatographies include- column packed with matrix (which varies based on principle), solvent reservoir, suitable solvent, fraction collection system followed by analyzer (Figure 2.16). These enable qualitative analysis of mixtures as well as for preparative purposes. Based on requirement the column dimensions vary. In case of GC they are long and narrow, while in gel permeation and Ion exchange chromatographies it is much larger.

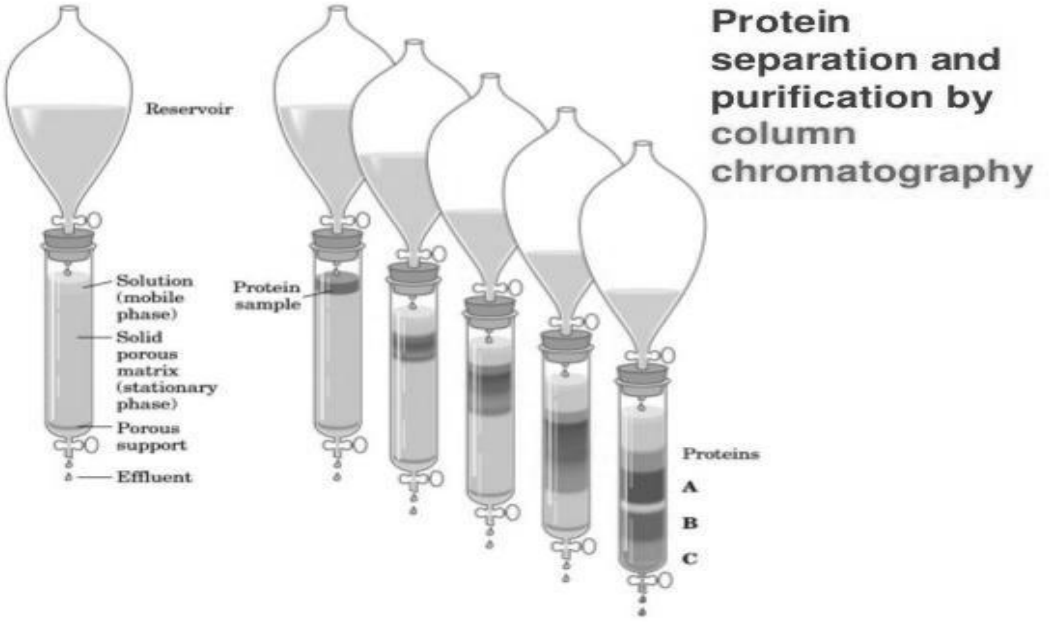


Figure 2.16: Column chromatography illustrating separation of protein mixture

The most used column chromatographies include:

1. Ion exchange chromatography: Used for the separation of charged molecules like Proteins, RNA and DNA.
2. Affinity chromatography: Used to separate molecules having specific binding like,Immunoglobulins, Enzymes, mRNA's etc.
3. Gel permeation chromatography: Separates molecules based on size, and is used to separate proteins, Nucleic acid.
4. Gas chromatography: Uses gas as a mobile phase and is used to separate lipids, oligosaccharides and aminoacids.

Electrophoresis:

It is one of the important tools in cell biology which is rapid, inexpensive that is used to separate charged molecules. This method used support medium which is a porous gel. As molecules like DNA, RNA or proteins move through the gel, based on charge or charge to mass ration mixture gets separated (Figure 2.17). The separated samples are visualized using appropriate methods of visualization. This method has several variations which include;

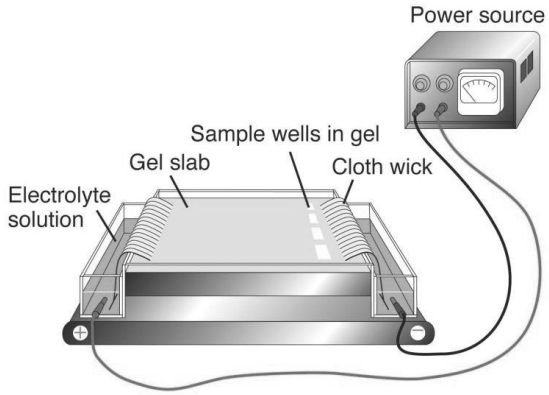


Figure 2.17: Outline view of components of Gel Electrophoresis.

1. Moving Boundary Electrophoresis: Separation of samples takes place in solvent system, and is used for separation of proteins.
2. **Gel or Zone electrophoresis**- used for separation of proteins and nucleic acids
3. Discontinuous Electrophoresis- for isolation of proteins from plasma membrane
4. SDS-PAGE- Separation of proteins based on size, as charge is masked by SDS (sodiumodecyl sulphate- polyacrylamide gel electrophoresis).
5. Maxam-Gilbert technique- used for separation of polynucleotide fragments during their sequence determination.
6. Immuno electrophoresis: Used for separation of antigens and antibodies.

Dialysis:

It is the process of separating molecules in solution based on difference in their rate of diffusion across a semipermeable membrane (Figure 2.18). This laboratory technique employs usage of dialysis bag having a pore size which determines the diffusivity of molecules. The molecules whose diameter is less that the pore size of the membrane will diffuse out of the bag. It is used in protein purification, removal of salts from precipitates, removal of unwanted small ions, salts, sugars, reducing agents or dyes from larger molecules like proteins, polysaccharides or DNA.

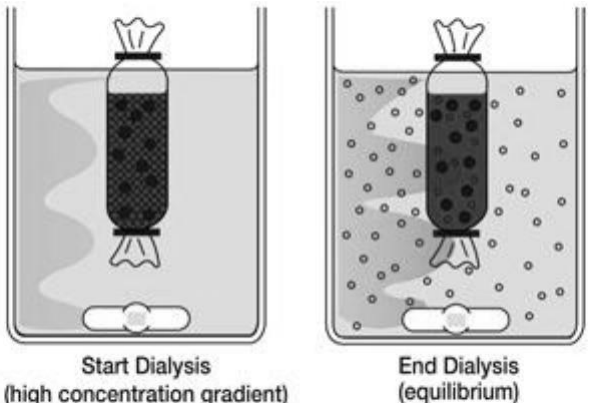


Figure 2.18: Principle of dialysis

PRACTICE QUESTIONS

1. What does the term Limits of resolution mean?
(A) It is used for distinguishing object in space
(B) It is the smallest distance that may separate two points on an object and still permit their observation as distinct separate points.
(C) It is defined as the smallest distance that may separate two objects
(D) It is the maximum distance that enables them to be seen as two separate points.

2. Path of light before image formation by compound light microscope is-
(A) Condenser - Specimen - Objective lens - Ocular lens
(B) Glass plane - Condensor - Collimator - Specimen - objective lens - Ocular lens
(C) Glass plane - Condensor - Collimator - Specimen - occular lens - Objective lens
(D) Glass plane - Collimator - Specimen - Occular lens - Objective lens

3. The property of Birefringence is exhibited by
(A) Isotrophic materials
(B) Anisotrophic materials
(C) Both
(D) None

4. Which among the following is true about scanning electron microscope?
(A) SEM detects secondary electrons emitted by atoms excited by the electron beam
(B) SEM detects primary electrons emitted by atoms excited by the electron beam
(C) SEM detects secondary electrons emitted by atoms excited by radio waves
(D) SEM detects secondary electrons emitted by atoms excited by visible light radiation

5. Which among the following is a cell fractionation method?
(A) TLC
(B) Gel electrophoresis
(C) Mass spectroscopy
(D) Ultracentrifuge

6. Match the following

a.	Lipids	1.	Acetocarmine
b.	Carbohydrates	2.	Naphthol Yellow
c.	Proteins	3.	Sudan black
d.	Dna	4.	Schiff's stain

(A) a-1, b-2, c- 3, d-4
(B) a- 2, b-3, c- 4, d- 1
(C) a - 3, b - 4, c - 2, d – 1
(D) a-4, b - 3, c- 2, d - 1

7. Cell culture used to culture animal cells invitro for conducting biochemical studies involves trypsinization of tissues. This is done for which among the following purposes.
(a) Used for Proteolysis of proteins to isolate cell surface proteins
(b) Used to separate proteins from carbohydrates

(c) Enables cell separation by breaking down bound proteins
(d) Used to separate cells from vessels during their passage
(e) Used to obtain free cells from tissues
(A) All of them (B) a,b,c,d (C) b,c,d,e (D) c, d, e

8. Full form of BHK is
(A) Baby Hamster Kidney cells (B) Baren Hamster Kite cells
(C) Boiled Hen Kidney cells (D) Boston Hamster Kidney cells

9. Match the following techniques with their applications

Column-A		Column-B	
a.	Ion exchange chromatography	**1.**	To separate proteins and nucleic acids based on size
b.	Affinity Chromatography	**2.**	Usa Usage of mobile phase to separatelipids, ligosaccharides and aminoacids
c.	Gel permeation chromatography	**3.**	For separation of charged molecules
d.	Gas chromatography	**4.**	To separate antibodies, enzymes etc

(A) a- 1, b - 2, c - 3, d - 4 (B) a- 4, b - 2, c - 3, d - 1
(C) a- 3, b -2, c - 1, d - 4 (D) a- 3, b - 4, c - 1, d - 2

10. Which among the following statements are true about dialysis?
(a) It separates molecules based on their diffusivity across a membrane
(b) It is used for removing salts from proteins
(c) It is used to remove dyes from large macromolecules
(d) Best separation is seen when desired molecule is large and contaminant is much small
(e) The membranes used have a cut off capacity
(A) All the statements (B) b, c, d, e (C) a, c, d, e (D) a, b, d, e

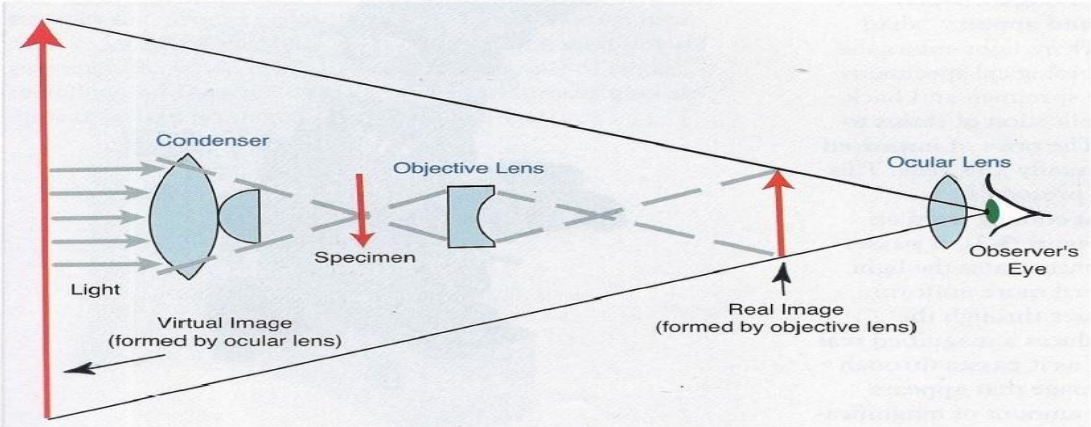
ANSWER KEYS

1	B	**2**	B	**3**	B	**4**	A	**5**	D	**6**	C	**7**	D
8	A	**9**	D	**10**	A								

EXPLANATIONS

1. Limit of resolution (l) is defined as the smallest distance that may separate two points on an object and still permit their observation as distinct separate points.

2. Path of light in compound light microscope



3. Anisotrophic materials exhibit the property of birefringence, which is an optical property.

4. Scanning Electron Microscopy detects secondary electrons emitted by atoms excited by the electron beam.

5. Ultra centrifuge is used for cell fractionation. The other ones include, density gradient centrifugation using zonal rotars for preparative purposes.

6. Stains used for macromolecules.

a.	Lipids	**3.**	Sudan black
b.	Carbohydrates	**4.**	Schiff's stain
c.	Proteins	**2.**	Naphthol Yellow
d.	DNA	**1.**	Acetocarmine

7. Cell culture used to culture animal cells invitro for conducting biochemical studies involves trypsinization of tissues. This is done for which among the following purposes-
– Enables cell separation by breaking down bound proteins.
– Used to separate cells from vessels during their passage.
– Used to obtain free cells from tissues.

8. BHK stands for Baby Hamster Kidney cells that are used in cell culture.

9.

Column-A	Column-B
a. Ion exchange chromatography	**3.** For separation of charged molecules
b. Affinity Chromatography	**4.** To separate antibodies, enzymes etc
c. Gel permeation chromatography	**1.** To separate proteins and nucleic acids based on size
d. Gas chromatography	**2.** Usage of mobile phase to separate lipids, oligosaccharides and aminoacids

10. Some points about dialysis
(**a)** It separates molecules based on their diffusivity across a membrane
(**b)** It is used for removing salts from proteins
(**c)** It is used to remove dyes from large macromolecules
(**d)** Best separation is seen when desired molecule is large and contaminant is much small
(**e)** The membranes used have a cut off capacity

3. CELL STRUCTURE

THE CELL

Living organisms based on the number of cells are of two types- unicellular and multicellular organisms. The cellular organisms are further grouped into Prokaryotes and eukaryotes. The terms were suggested by Hans Ris, in 1960.

PROKARYOTIC CELL:

The Prokaryotic (Gr., pro= primitive; karyon =nucleus) are small, simple and most primitive. They are the probably first ones to come into existence some3.5 billion years ago. Eg: Stomatolites Blue - green algae was found in Australia and it has dated back to 3.5 billion years. Eukaryotes are the evolved cells (Gr., Eu= well; kayon = nucleus). These cells have evolved some 1.4 billion years ago and are most primitive from morphological point of view. These cells have one envelope system, which consists of central nuclear components (i.e., DNA, RNA and nuclear proteins) surrounded by cytoplasmic substances. All the enzymes required for replication and metabolism are present attached to the inner surface of plasma membrane i.e., they do not have separate systems to enclose them as in eukaryotic cells. The cytoplasm of eukaryotes also lacks well defined organelles like Endoplasmic membrane, Golgi apparatus, Mitochondria, centrioles etc. they lack nucleoli, cytoskeleton, centrioles and basal bodies. Examples of prokaryotes include: Mycoplasma, bacteria and blue-green algae.

TYPICAL BACTERIAL CELL STRUCTURE:

Bacteria are the smallest organisms. They are primitive, simple, unicellular prokaryotic microorganisms. Though all bacteria are alike, variations prevail due to biochemical activities, ecological niche for which they get equipped with the metabolic specializations etc.

(1) Occurance : Bacteria occur in air, water, soil and also inside others. They are also found in stagnant water, running streams, rivers, sea waters, food, crude petroleum, municipality wastes, sewage and dead and decaying matter. They can thrive in all sorts of climatic conditions and in all temperatures.

Classification of bacteria based on Nutrition: Bacteria are classified into two types – autotrophs and heterotrophs (Figure 3.1).

1. **Autotrophs :** These synthesize their own food and are again of two types-photosynthetic bacteria that can synthesize their food from sunlight and chemosynthetic organisms that derive energy for synthesizing food by breaking chemical bonds.

2. **Heterotrophs :** These cannot synthesize their food indeed depend on others. These are of two types, saprophytes which depend on dead and decaying organic matter, while parasites which depend on other living organisms and may even damage them.

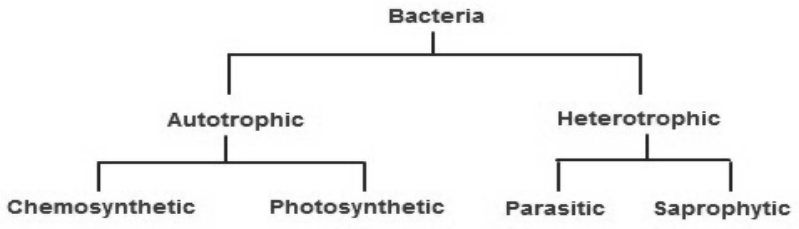


Figure 3.1: Classification of Bacteria based on nutrition

Because of high surface area to volume ratio, absorb nutrients through their cell membrane and exhibit high metabolic activities, hence have a fast rate of replication.

CHARACTERISTICS OF BACTERIA:

1. **Size of Bacteria (Figure 3.2):** It ranges from1µm to 3µm and hence can be seen using light microscope. The smallest bacterium is Dialister pneumosintes (0.15µm) and largest is Spirillum volutans (13 to 15 µm length).

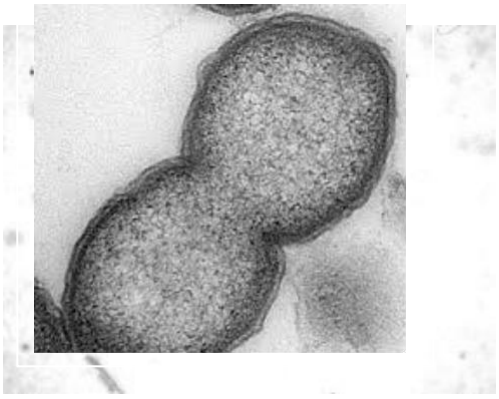


Figure 3.2 Structure of Dialister and Spirillum
the smallest and largest bacteria

2. Forms of Bacteria(Figure 3.3):

(A) Cocci (Singular Coccus): These are spherical or round in shape. These may occur as single bacteria (Mono/micrococcus), in pairs (Diplococcus eg. Diplococcus pneumonia), in groups of four (Tetracocci), in cubic arrangements comprising of eight (Sarcinae), as irregular clumps or bunches of grapes (Staphylococci eg.Staphylococcus aureus) or as beads of chains (Streptococci eg. Streptococci pyrogenes).

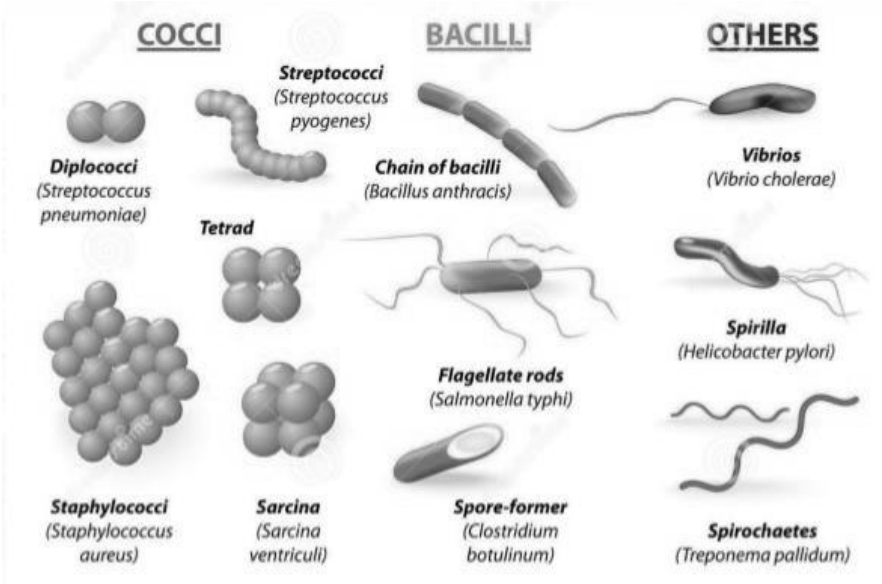


Figure 3.3: Shapes of Bacteria

(B) Bacilli (Singular Bacillus): These are rod shaped bacteria and may occur as pairs (diplococcic) or chains (Streptobacilli). Most disease causing bacilli Eg: Bacillus tuberculosis causes tuberculosis, Clostridium tetani-Tetanus, Solmonella typhosis- Typhoid, Clostridium botulinum-dysentery and food poisoning, Bacillus anthacis- anthrax etc.

(C) Spirilla: Singular spirillum (Spirochetes), these are motile and spiralshaped bacterium.

(D) Vibrios (Single vibrio): They are comma shaped and bent rod shaped bacteria (Vibrio cholera)

3. Gram negative and gram positive bacteria: Based on the stain ability of cell wall by Gram stain bacteria are classified as Gram positive and Gram negative bacteria. This method is called as gram staining method named after Christian Gram of Denmark (1884).

Procedure of staining involves, heat fixing of bacteria and treatment with basic dye, crystal violet which turns bacteria into blue or purple. These are then treated

with iodine solution (KI solution), which fixes stain in cells. Then the smear is treated with alcohol. Those which loose stain after alcohol treatment are called Gram negative bacteria (**Eg:** Escherichia coli, Simonsiella, Cynobacteria) while those which retain stain are called Gram positive bacteria (**Eg:** Bacillus subtilis, Staphylococcus aureus). The cells which have lost stain can be viewed using a counter stain like safranin. The difference in both the cell type is due to the lipid content. High lipid content in Gram negative bacterial cell wall gets dissolved due to alcohol and causes leaching of stain. Gram positive bacteria have a peptidoglycan layer that prevents penetration of solvent inside the cell (Figure 3.4).

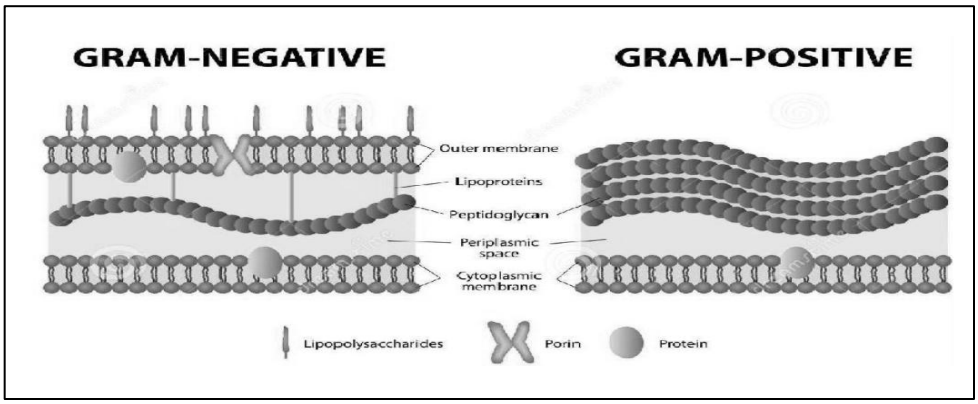


Figure 3.4: Structure of Bacterial Outer membrane

4. Structure of Bacteria: A typical bacterial cell has the following components (Components of Prokaryotic cell-Figure 3.5):

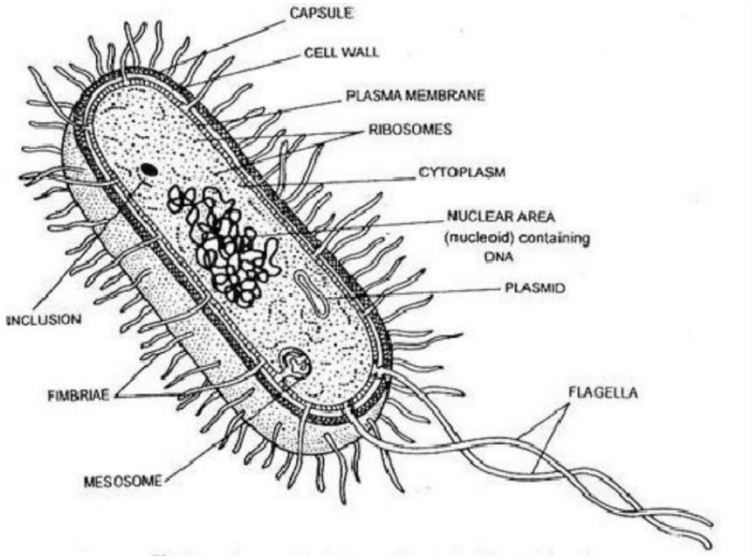


Figure 3.5: Typical Electron Microscopic structure of a bacterial cell

(A) Outer Covering: Bacterial outer covering of cell wall has 3 layers,

1. Plasma Membrane
2. Cell wall
3. Capsule

1. Plasma Membrane: Plasma membrane of bacterial cell wall is ultra thin (6-8nm thick) and is dynamic in nature. It is made of lipids and proteins as per the Fluid Mosaic Model and is a lipid bilayer sheath made up of phospholipids (Figure 3.6).

Some proteins are found embedded within the layer while few are located outside based upon their functionality. Eg: Transmembrane proteins like Carrier Proteins (Permeases), which carry selective molecules and ions into the cell from outside environment and vice versa. Certain proteins act as enzymes and carriers for electron flow in respiration leading to oxidative Phosphorylation (ADP -> ATP). Few of them act as sites for specific binding of bacterial DNA. Plasma membrane intrusions: These are in foldings of plasma membrane in all gram positive bacteria and in few gram negative bacteria.

(a) Mesosomes (Chondroids): These are convoluted extensions of plasma membrane. They are seen in photosynthetic as well as in chemosynthetic bacteria. They are involved in cross wall (Septum) formation during the division of cell.

(b) Chromatophores: These are photosynthetic pigments bearing membranous structures existing in various forms (vesicles, tubes, bundled tubes, stalks, thylakoids etc) in photosynthetic bacteria.

2. Cell Wall: Cell wall covers the plasma membrane. It is strong and rigid offering protection to the cell and also gives the bacterium its shape. Bacterial cell wall is composed of protein, lipid, polysaccharides at times chitin and rarely cellulose.

Gram negative cell wall has two layers:

1. Gel, proteoglycan or peptidoglycan containing periplasmatic space and
2. Outer membrane that consists of lipid bilayer traversed by channels of porin polypeptide, which allow diffusion of solutes. This layer has phospholipids and lipopolysaccharides. While in Gram positive bacteria it is thicker and is made up of many layers of peptidoglycan and proteins, neutral polysaccharides and poly phosphor polymers, such as teichoic acids and teichuronic acid.

3. Capsule: Some bacteria have an additional layer above cell wall which is slimy mucilaginous layer called as the capsule. It is secreted by Plasma membrane.

It offers protection to bacteria from viruses. It also helps in regulating the concentration and uptake of few essential ions and water.

(**B**) **Cytoplasm:** Also called as hyaloplasm in the matrix of substance enclosed by plasma membrane which is he seat for all metabolic activities. The cytosol has seat for all metabolic activities. The cytosol has water, proteins (including enzymes), lipids, carbohydrates different kinds of RNA molecules and other inclusions. The cytosol is differentiated into two distinct zones; one is the less dense nuclear area and a very dense area. The dense areas have thousands of ribosomes which are composed of RNA and proteins. These are the sites for protein synthesis and are of 70S type. Each ribosome has two subunits, the larger 50S subunit and the smaller 30S subunit. During protein synthesis they are found adhered to mRNA reading the codes and forming polyribosomes or polysomes, while the nonfunctional ones remain suspended in cytosol (figure 3.7).

Bacteria also have storage granules called as inclusion bodies. These are of 3 types.

(i) Those which are organic polymer: Poly- beta hydroxyl butyric acid, it is a storage form of carbon or granulose, a storage form of glucose.

(ii) Large reserves of inorganic phosphate, in the form of volutin granules, a meta Phosphate polymer.

(iii) Reserve material for elemental sulphur: special droplets of H_2S in sulphur bacteria as energy reserve.

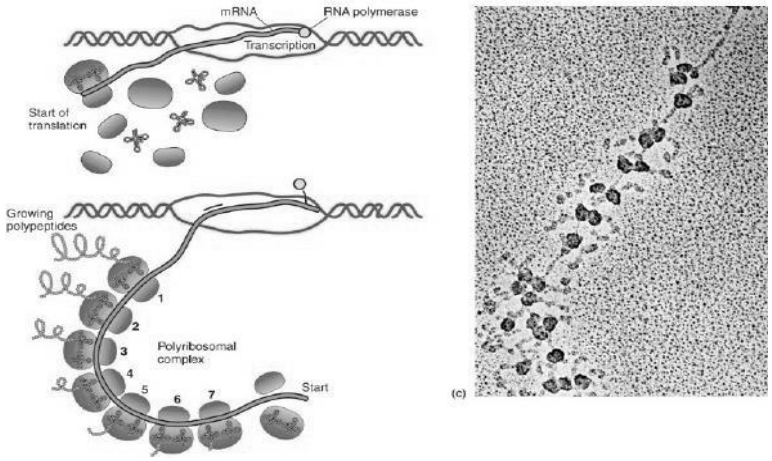


Figure 3.6: Bacterial simultaneous Transcription and translation

(C) Nucleoids: The nuclear material is a single, circular double stranded naked DNA molecule called the Bacterial chromosoe. The clear area of cytosol has the nuclear material and is called Nucleoid. The nuclear material is seen adhered to Plasma membrane along with small heat stable proteins (analogues to histones in eukaryotes).

RNA polymerase present in cytosol forms mRNA, tRNA and rRNA. mRNA formed by transcription from nuclear material is directly ready for translation by ribosomes of cytosol. The proteins which are required for cellular activities are translated by ribosomes of cytosol, while those which are required to be transported are synthesized by ribosomes attached to plasma membrane.

Plasmids: These are extra chromosomal genetic elements present in many bacteria in the form of small, circular closed DNA molecules. Some plasmids have Colcinogenic factors that are involved in antibiotic production. Few have fertility factor (F Factor) which stimulates bacterial conjugation. Few bacterial plasmids have R factor that offers them resistance to drugs such as Chloramphenicol, Neomycin, Penicillin, Streptomycin, Sulphonamides and Tetracycline.

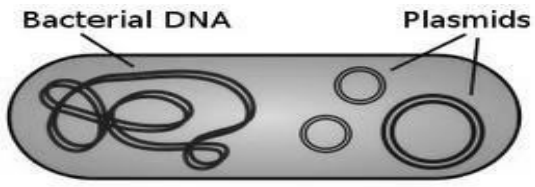


Figure 3.7: Bacterial DNA and plasmid

(D) Flagella and other structures: Flagella enable locomotion of bacteria. Bacterial flagella are smaller than eukaryotic flagella and are much simpler in organization (Figure 3.8). They have helical tube which contains single protein subunit called flagellin. It is attached at its base by a flexible, rotating hook and is propelled by a flagellar rotator motor called Basal body. The flagellar motor comprises of 4 distinct parts, rotor (or ring), Stator, Bearing (S ring) and rod. The rotor is a protein disc integrated into plasma membrane. It is driven by energy stored in the transmembrane proton H^+ gradient and rotates rapidly (~ 100 revolutions/second), in the lipid bilayer against another protein disc called the stator. A rod links the rotor to a hook and flagellum thereby causing them to rotate. The protein bearing serves to seal the outer membrane of the cell wall as the rotating rod passed through hit. The stator and bearing remains stationary.

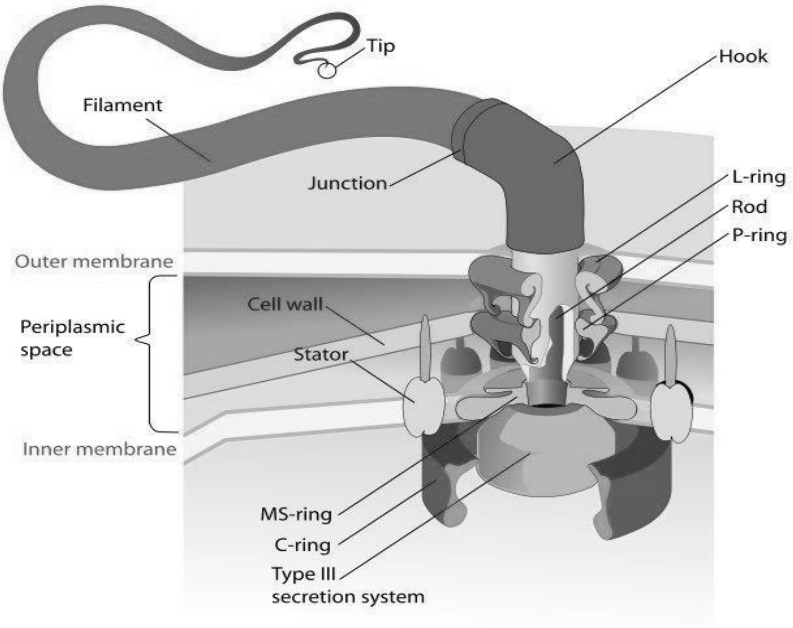


Figure 3.8: Bacterial flagellar organization

Based on number and arrangement of flagella, bacteria are classified as follows (figure 3.9):

(a) Monotrichous – Single flagellum at one pole of the cell
(b) Lophotrichous – Several flagella at one pole
(c) Amphitrichous – The bacterial cell has one flagellum at each pole
(d) Peritrichous – Flagella are present all over the surface of the cell.

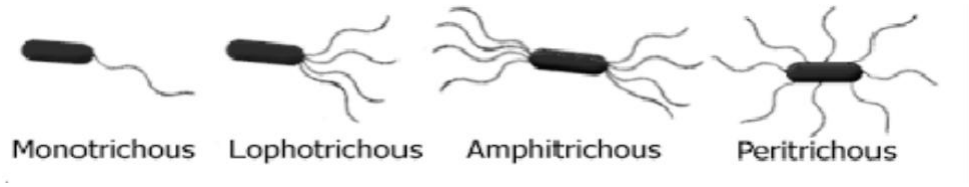


Figure 3.9: Classification of bacteria based on arrangement of flagella

Fimbriae or Pili: Some bacteria (Gram negative) have extremely fine appendages called Fimbriae or Pili. These are non-motile, adhesive structures, which enable bacteria to stick firmly to surfaces or to eukaryotes like to molds, plants and animals (RBC, Epithelial cells, Respiratory tract, Urinary tract etc). F- pili or sex pili present in male bacteria are long and help in bacterial conjugation, for pathogenesis and also act as specific sites for attachment of bacteriophages. Pili are coded by genes present on plasmids.

Spinae: These are present in some gram positive bacteria that help them to tolerate environmental conditions such as salinity, pH, temperature etc. they are tubular, rigid, pericellular appendages made up of a protein called Spinin.

5. **Nutrition in Bacteria:** Bacteria exhibit large variations in their nutritional aspects. They may be autotrophic (Photosynthetic or chemosynthetic) or heterotrophs (Saprophytes/Parasitic).
6. **Respiration:** Bacteria are both aerobic as well as anaerobic. Anaerobic bacteria are being exploited by man for their useful end products that are produced during their course of metabolic activities (acids/ alcohols).

EULARYOTIC CELL

The Eukaryotic cells (Gr., Eu = good, Karyotic = nucleated) are essentially two envelope systems. Apart from plasma membrane, they have secondary membranes that envelope nucleus and other internal organs that are present in cytoplasm like the Endoplasmic reticulum, Golgi complex etc. eukaryotic cells are seen in plants (Algae to Angiosperms) and in animals (Protozoa to Mammals).

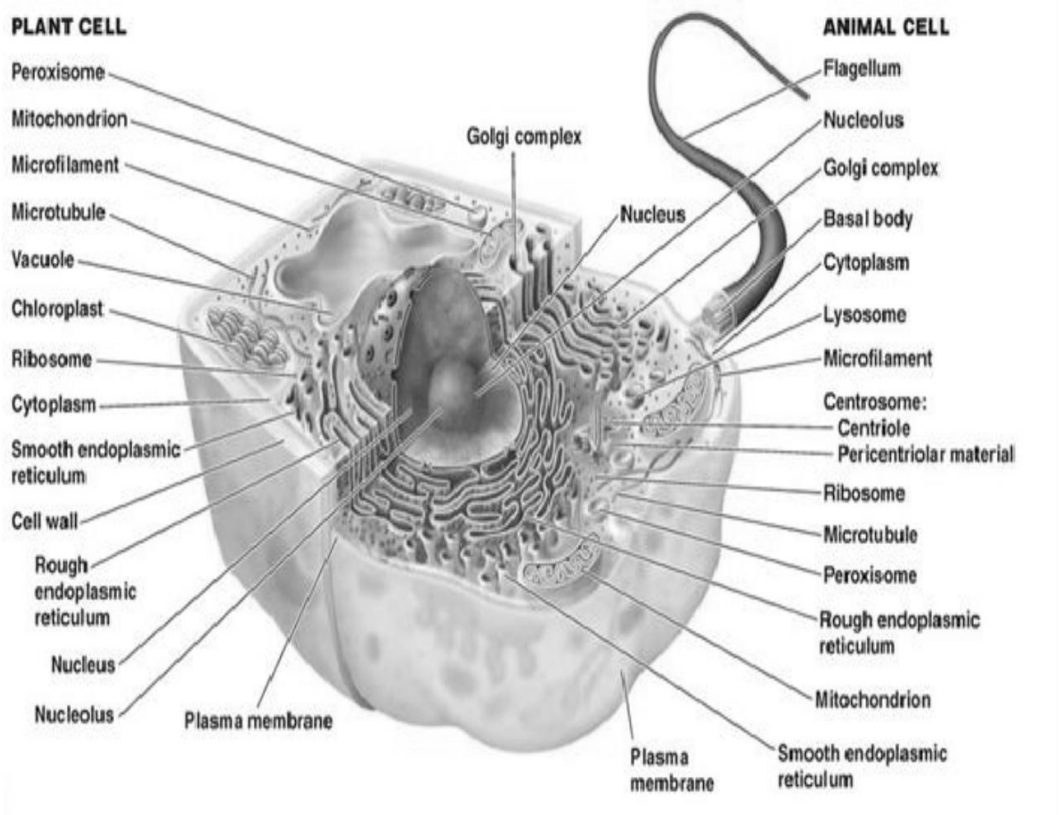


Figure 3.10 Structure of a typical Eukaryotic cell

They belong to the taxa Eukaryota. Eukaryotes appear to be monophyletic (organisms that form a clade) and make up one of the three domains of life. The two other domains, Bacteria and Archaea, are prokaryotes and have none of the above features. Eukaryotes represent a tiny minority of all living things; even in a human body there are 10 times more microbes than human cells. However, due to their much larger size their collective worldwide biomass is estimated at about equal to that of prokaryotes. Cell division involves separating of the genome which is in the form of tightly packed condensed structure known as the chromosomes, through movements directed by the cytoskeleton.

An eukaryotic cell consists of following components:

(A) Cell wall and Plasma membrane

(B) Cytoplasm and

(C) Nucleus

(A) Cell Wall and Plasma Membrane:

1. Cell Wall: The outermost structure ofmost plant cells is a dead and rigid layer called the Cell wall.

The cell wall serves a variety of purposes including:

- Maintaining/determining cell shape (analogous to an external skeleton for every cell). Since protoplasts are invariably round, this is good evidence that the wall ultimately determines the shape of plant cells.
- Support and mechanical strength (allows plants to get tall, hold out thin leaves to obtain light).
- Prevents the cell membrane from bursting in a hypotonic medium (*i.e.*, resists water pressure).
- Controls the rate and direction of cell growth and regulates cell volume.
- Has a metabolic role (*i.e.*, some of the proteins in the wall are enzymes for transport, secretion)
- Physical barrier to: (a) pathogens; and (b) water in suberized cells. However, remember that the wall is very porous (~4nm) and allows the free passage of small molecules, including proteins up to 60,000 MW.
- Carbohydrate storage - the components of the wall can be reused in other metabolic processes (especially in seeds). Thus, in one sense the wall serves as a storage repository for carbohydrates
- Signaling - fragments of wall, called oligosaccharins, act as hormones. Oligosaccharins, which can result from normal development or pathogen attack, serve a variety of functions including:

  (a) stimulate ethylene synthesis.

(b) induce phytoalexin (defense chemicals produced in response to a fungal/bacterial infection) synthesis.

(c) induce chitinase and other enzymes.

(d) increase cytoplasmic calcium levels and

(e) cause an "oxidative burst". This burst produces hydrogen peroxide, superoxide and other active oxygen species that attack the pathogen directly or cause increased cross-links in the wall making the wall harder to penetrate.

Cell wall Components: The main ingredient in cell walls are polysaccharides (or complex Carbohydrates or complex sugars) which are built from monosaccharides (or simple sugars). Eleven different monosaccharides are common in these polysaccharides including glucose and galactose.

(A) Cellulose: It is made up of as many as 25,000 individual glucose molecules. Every other residues is "upside down". *Cellobiose* (glucose-glucose disaccharide) is the basic building block. Cellulose readily forms hydrogen bonds with itself (*intra-molecular H-bonds*) and with other cellulose chains (*inter-molecular H-bonds*). A singlecellulose chain forms hydrogen bonds with about 36 other chains to yield a *microfibril*. Microfibrils are 5-12 nm wide and gives wall strength - they have a tensile strength equivalent to steel. Some regions of the microfibrils are highly crystalline while others are more "amorphous".

(B) Cross-linking Glycans (=Hemicellulose): They are linear (straight), flat, with a β-1,4 backbone, relatively short side chains and are of two types- xyloglucans and glucuronarabinoxylans. Other less common ones include glucomannans, galactoglucomannans, and galactomannans. The main feature of this group is that they don't aggregate to form microfibrils. However, they form hydrogen bonds with cellulose and are called as "*cross-linking glycans*". Each chain may end up with fucose sugar which may help keep the molecules planar by interacting with other regions of the chain.

(C) Pecticpolysaccharides: These are extracted from the wall with hot water or dilute acid or calcium chelators (like EDTA). They form gels (*i.e.*, used in jelly making) and are a diverse group of polysaccharides which are rich in galacturonic acid (galacturonans = pectic acids). They are polymers of primarily β 1,4 galacturonans (=polygalacturonans) are called homogalacturons (HGA). These are helical in shape. Pectic polysaccharides can also be cross- linked by dihydrocinnamic or diferulic acids. The HGA's (galacturonans) are initially secreted from the golgi as methylated polymers; the methyl groups are removed by pectin methylesterase to initiate calcium binding.

Other pectic acids include Rhamnogalacturonan II (RGII) which features rhamnose and galacturonic acid in combination with a large diversity of other sugars in varying linkages. Dimers of RGII can be cross-linked by boron atoms linked to apiose sugars in a side chain. Although most pectic polysaccharides are acidic, others are composed of neutral sugars including arabinans and galactans. The pectic polysaccharides serve a variety of functions including determining wall porosity, providing charge to wall surface for cell-cell adhesion (i.e,. middle lamella), cell-cell recognition, pathogen recognition etc.

(D) **Protein:** Wall proteins are typically glycoproteins which rich in the amino acids hydroxyproline (hydroxyproline-rich glycoprotein, HPRG), proline (proline-rich protein, PRP), and glycine (glycine-rich protein, GRP). These proteins form rods (HRGP, PRP) or beta-pleated sheets (GRP). *Extensin* is a well-studied HRGP which is induced by wounding and pathogen attack. They appears to be cross-linked to pectic substances and may have sites for lignification. The proteins serve as the scaffolding used to construct the other wall components. Another group of wall proteins are heavily glycosylated with arabinose and galactose. These arabinogalactan proteins, or AGP's, are tissue specific and may function in cell signaling. They may be important in embryogenesis, growth and guidance of the pollen tube.

(E) **Lignin :** It is a polymer of phenolics (phenylpropanoids). Lignin is primarily a strengthening agent in the wall which resists fungal/pathogen attack.

(F) **Suberin, Wax, Cutin:** These are the lipids associated with the wall for strength and waterproofing.

(G) **Water:** The wall is highly hydrated and comprises of water between 75-80%. This is responsible for some of the wall properties. For example, hydrated walls have greater flexibility and extensibility than non-hydrated walls.

Morphology of the Cell Wall - There cell wall has three major regions:

Middle lamella - outermost layer, glue that binds adjacent cells, composed primarily of pectic polysaccharides.

Primary wall - wall deposited by cells before and during active growth. It is composed of pectic polysaccharides (ca. 30%), cross-linking glycans (hemicellulose; ca 25%), cellulose (15-30%) and protein (ca. 20%). The actual content of the wall components varies with species and age. All plant cells have a middle lamella and primary wall.

Secondary Wall - Some cells deposit additional layers inside the primary wall. This occurs after growth stops or when the cells begin to differentiate. The secondary

cell wall is mainly for support and is comprised primarily of cellulose and lignin. Often can be distinguished into distinct layers, S1, S2 and S3, which differ in the orientation, or direction, of the cellulose microfibrils.

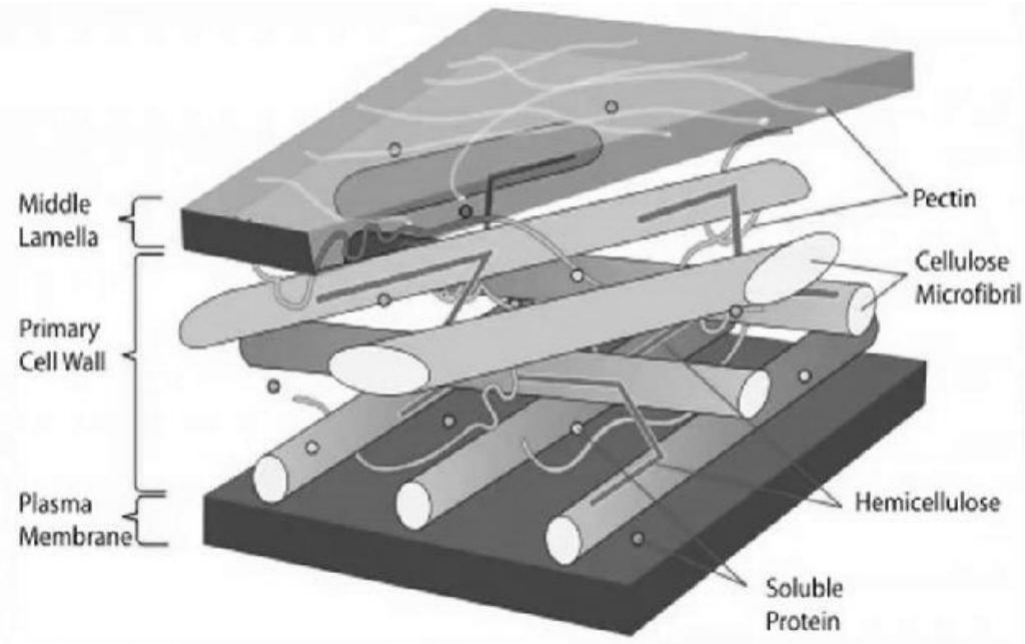


Figure 3.11: Structure of plant cell wall

Plasma membrane: Every kind of animal cell has is bounded by a living, extremely thin and delicate membrane called Plasma lemma, cell membrane or plasma membrane. In plant cells, plasma membrane occurs just next to cell wall, bounding the cytoplasm. It is the most extensively studied membrane which is a Phospholipid bilayer that forms a stable barrier between two aqueous components (cytosol and outside environment).

Function of Plasma Membrane:

- It separates the contents of the cell from its outside environment and it regulates what enters and exits the cell.
- Plasma membrane plays a vital role in protecting the integrity of the interior of the cell by allowing only selected substances into the cell and keeping other substances out.
- It also serves as a base of attachment for the cytoskeleton in some organisms and the cell wall in others. Thus the cell membrane supports the cell and helps in maintaining the shape of the cell.

- The cell membrane is primarily composed of proteins and lipids. While lipids help to give membranes their flexibility and proteins monitor and maintain the cell's chemical climate and assist in the transfer of molecules across the membrane.
- The lipid bilayer is semi-permeable, which allows only selected molecules to diffuse across the membrane.

Composition of Plasma Membrane: Cell membrane is composed of mainly lipids and proteins .

1. **Lipids:** Plasma membrane has three classes of amphipathic lipids which include- phospholipids, glycolipids and sterols. The amount of lipid content varies from cell to cell while the most abundant among the three is the phospholipid. The phospholipids and glycolipids have fatty acids containing even number of carbon atoms (16 to 20) and are saturated or unsaturated fatty acids. The configuration of double bonds is always "cis". The length of the fatty acid and the degree of unsaturation affects the membrane fluidity. The plasma membranes of animal cells contain four major phospholipids (phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, and sphingomyelin), which together account for more than half of the lipid in most membranes. These phospholipids are asymmetrically distributed between the two halves of the membrane bilayer. The outer leaflet of the plasma membrane consists mainly of phosphatidylcholine and sphingomyelin, whereas phosphatidylethanolamine and phosphatidylserine are the predominant phospholipids of the inner leaflet. A fifth phospholipid, phosphatidylinositol, is also localized to the inner half of the plasma membrane. Although phosphatidylinositol is a quantitatively minor membrane component, it plays an important role in cell signaling (Figure 3.13), Cholesterol, another lipid composed of four fused carbon rings, is found alongside phospholipids in the core of the membrane.

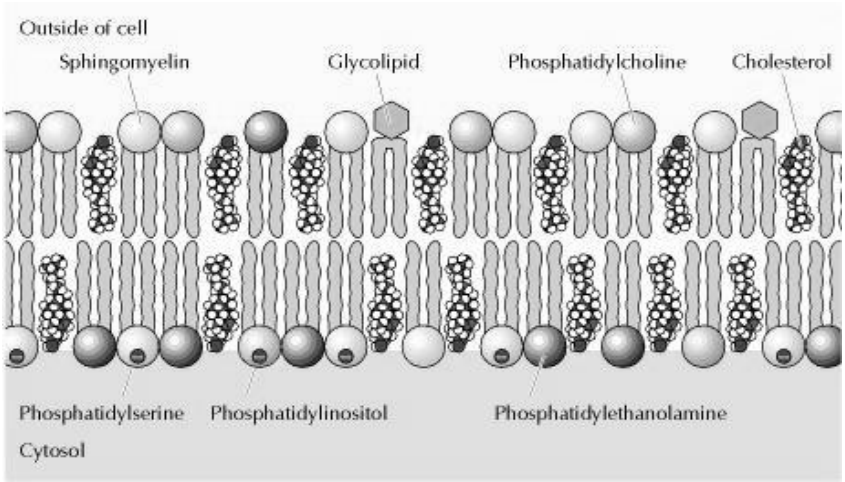


Figure 3.12: Lipid composition of Plasma membrane

Carbohydrates: Plasma membranes also contain carbohydrates, predominantly glycoproteins, but with some glycolipids (cerebrosides and gangliosides). For the most part, no glycosylation occurs on membranes within the cell; rather generally glycosylation occurs on the extracellular surface of the plasma membrane. The glycocalyx is an important feature in all cells, especially epithelia with microvilli. Recent data suggest the glycocalyx participates in cell adhesion, lymphocyte homing, and many others. The penultimate sugar is galactose and the terminal sugar is Sialic acid, as the sugar backbone is modified in the Golgi apparatus. Sialic acid carries a negative charge, providing an external barrier to charged particles.

Proteins: The proteins of the cell membrane account for 50% (w/w) of the membrane and occur in two forms, viz. integral proteins which traverse the thickness of the membrane and peripheral proteins which are adsorbed to the outer or inner surfaces of the membrane (Figure 3.13). While the peripheral proteins are involved in cell recognition and interactions, integral proteins regulate passage of material and active transport of specific molecules across the membrane.

Integral membrane proteins are, as their name suggests, are present integrated into the membrane: they have at least one hydrophobic region that anchors them to the hydrophobic core of the phospholipid bilayer. Some span only part of the membrane, associating with a single layer, while others stretch from one side of the membrane to the other and are exposed on either side (transmembrane proteins). The portions of an integral membrane protein found inside the membrane are hydrophobic, while those that are exposed to the cytoplasm or extracellular fluid tend to be hydrophilic. Transmembrane proteins may cross the membrane just once, or may have as many as twelve different membrane-spanning sections. A typical membrane-spanning segment consists of 20-25 hydrophobic amino acids arranged in an alpha helix, although not all transmembrane proteins fit this mode Integral membrane proteins are diverse and play a number of important roles in the cell. Some act as ion channels or transporters, selectively allowing certain molecules to pass through the plasma membrane. Others act as receptors, detecting a signal on the outside of the cell and undergoing a conformational change (change in shape) that transmits the signal to the inside of the cell, activating pathways that generate a cellular response.

Still others function in cell-cell recognition, help the cell stick to its neighbors or the surrounding matrix, or play structural roles.

Peripheral membrane proteins are found on the outside and inside surfaces of membranes, attached either to integral proteins or to phospholipids.

Unlike integral membrane proteins, peripheral membrane proteins do not extend into the hydrophobic core of the membrane, and they tend to be more loosely attached. Peripheral membrane proteins play a number of important roles, like providing attachment sites for cytoskeletal fibers and relaying signals from receptor proteins.

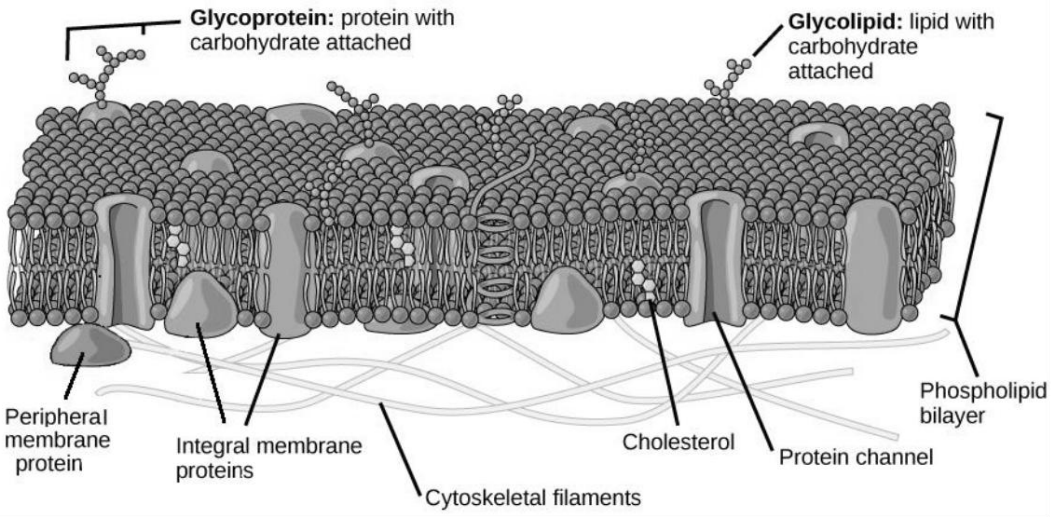


Figure 3.13: Protein components of Plasma membrane

Cytoplasm: The plasma membrane is followed by the cytoplasm, which is distinguished into the following structures:

(A) Cytosol: The plasma membrane is followed by the colloidal organic fluid called matrix or cytosol. It is the aqueous portion of the cytoplasm and the nucleoplasm. It fills all the spaces of the cell. Cytosol is rich in differentiating cells and many fundamental properties of cell are because of this part. It serves to dissolve or suspend the great variety of small molecules concerned with cellular metabolism, eg/. glucose, amino acids, nucleotides, vitamins, minerals, oxygen and ions. Cytosol contains soluble proteins and enzymes form 20 to25% of the total soluble protein content of the cell. In many cells cytosol is differentiated into two parts:

(i) Ectoplasm or cell cortex, which is the peripheral layer and is non-granular, viscous, clear and rigid,

(ii) Endoplasm is the inner portion of cytosol which is granular and less viscous. Cytoskeleton and microtrabecular lattice: Cytosol has fibres that help to maintain cell shape and mobility and that probably provide anchoring point for other cellular structures. These fibres are collectively termed as cytoskeleton.

Three categories of fibres have been identified.

1. Microtubules, which are thick (20nm diameter) and consists of the protein tubulin. Their function is in transportation of water, ions and small molecules, promote cytoplasmic streaming, formation of mitotic and meiotic spindles during cell division. They are also part of structural units like centrioles, basal granules, cilia and flagella.
2. Microfilaments are thinner filaments (7nm diameter) which are solid and are principally formed of actin protein. They maintain the shape of cell and form contractile component of cells, mainly the muscles.
3. Intermediary filaments (IFs) are middle order fibres of around 10nm diameter. They have been classified based on constituent proteins as desmin filaments, keratin filaments, neuro filaments, vimentin and glial filaments.

Cytoplasm is filled with a three-dimensional network of interlinked filaments of cytoskeletal fibres, called microtra-becular lattice that anchors various cell organelles like ribosomes, lysosomes etc. The lattice is flexible and changes its shape during cell movement.

(B) Cytoplasmic Structures: The cytoplasmic matrix has both non-living and living structures. The non-living structures are called paraplasm or inclusions while the living are called organoids or organelles.

(a) Cytoplasmic Inclusions: The include stored food and secretory substances which are suspended in the cytoplasmic matrix. Eg: oil droplets, triacylglycerol (seen in adipose tissues), Yols granules (seen in egg cells), secretory granules, glycogen granules (in liver and muscle cells) and starch granules(in plant cells).

(b) Cytoplasmic Organelles: Cell matric has several membrane bound organelles.

1. Endoplasmic Reticulum (ER): (Figure 3.14)

The endoplasmic reticulum (ER) is a large, continuous membrane-bound organelle comprised of functionally and structurally distinct domains including the nuclear envelope, peripheral tubular ER, peripheral cisternae, and numerous membrane contact sites at the plasma membrane, mitochondria, Golgi, endosomes, and peroxisomes. These domains are required for multiple cellular processes, including synthesis of proteins and lipids, calcium level regulation, and exchange of macromolecules with various organelles at ER-membrane contact sites.

There are two types of endoplasmic reticulum, rough and smooth. The outer (cytosolic) face of the rough endoplasmic reticulum is studded with ribosomes

that are the sites of protein synthesis. The rough endoplasmic reticulum is especially prominent in cells such as hepatocytes. The smooth endoplasmic reticulum lacks ribosomes and functions in lipid manufacture and metabolism, the production of steroid hormones, and detoxification. The smooth ER is especially abundant in mammalian liver and gonad cells. The lacy membranes of the endoplasmic reticulum were first seen in 1945 using electron microscopy.

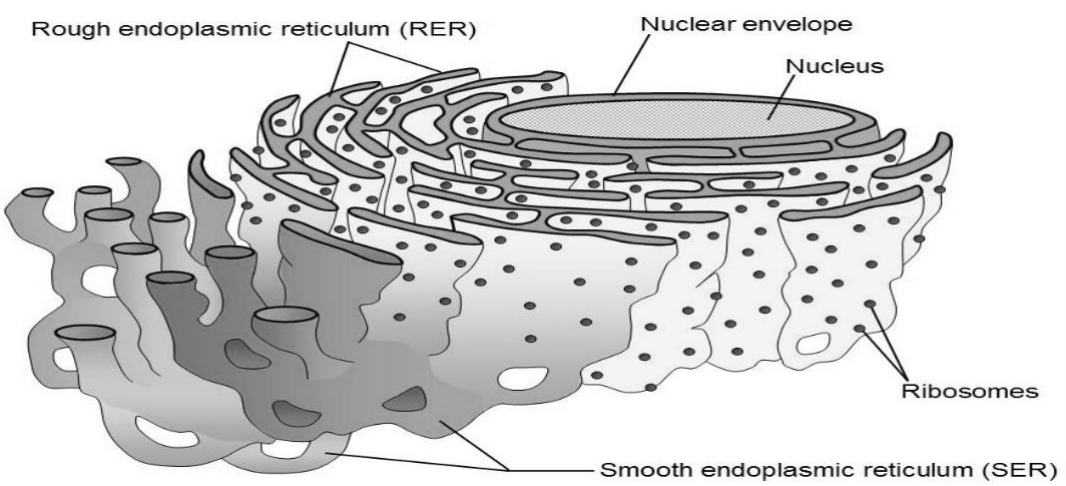


Figure 3.14: Representation of Rough and Smooth endoplasmic reticulum

Rough endoplasmic reticulum:

The surface of the rough endoplasmic reticulum (RER or Rough ER) (also called *ergastoplasm*) is studded with protein-manufacturing ribosomes giving it a "rough" appearance (hence its name). The binding site of the ribosome on the rough endoplasmic reticulum is the translocon. However, the ribosomes bound to it at any one time are not a stable part of this organelle's structure as they are constantly being bound and released from the membrane. A ribosome only binds to the RER once a specific protein-nucleic acid complex forms in the cytosol. This special complex forms when a free ribosome begins translating the mRNA of a protein destined for the secretory pathway. The first 5-30 amino acids polymerized encode a signal peptide, a molecular message that is recognized and bound by a signal recognition particle (SRP). Translation pauses and the ribosome complex binds to the RER translocon where translation continues with the nascent protein forming into the RER lumen and/or membrane. The protein is processed in the ER lumen by an enzyme (a signal peptidase), which removes the signal peptide. Ribosomes at this point may be released back into the cytosol;

however, non-translating ribosomes are also known to stay associated with translocons (Figure:3.15).

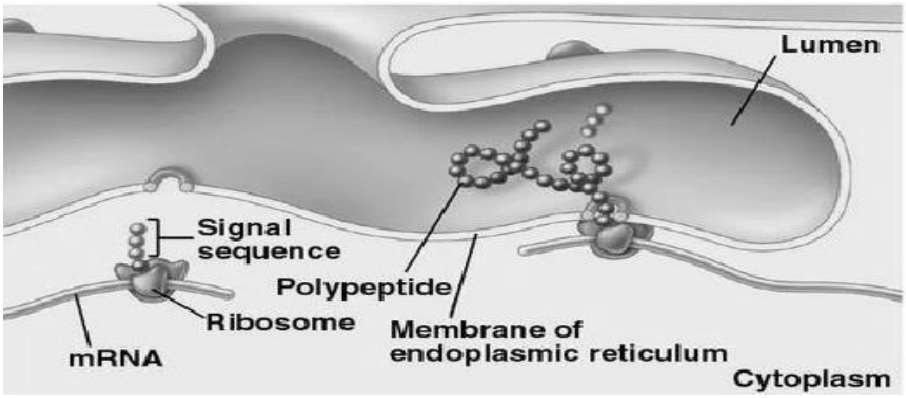


Figure 3.15: Role of Rough endoplasmic reticulum in protein synthesis

The membrane of the rough endoplasmic reticulum forms large double membrane sheets that are located near, and continuous with, the outer layer of the nuclear envelope. Although there is no continuous membrane between the endoplasmic reticulum and the Golgi apparatus, membrane- bound vesicles shuttle proteins between these two compartments. Vesicles are surrounded by coating proteins called COPI and COPII. COPII targets vesicles to the Golgi apparatus and COPI marks them to be brought back to the rough endoplasmic reticulum. The rough endoplasmic reticulum works in concert with the Golgi complex to target new proteins to their proper destinations. A second method of transport out of the endoplasmic reticulum involves areas called membrane contact sites, where the membranes of the endoplasmic reticulum and other organelles are held closely together, allowing the transfer of lipids and other small molecules.

The rough endoplasmic reticulum functions include:

- Manufacture of lysosomal enzymes with a mannose-6-phosphate marker added in the *cis*- Golgi network.
- Manufacture of secreted proteins, either secreted constitutively with no tag or secreted in a regulatory manner involving clathrin and paired basic amino acids in the signal peptide.
- Integral membrane proteins that stay embedded in the membrane as vesicles exit and bind to new membranes. Rab proteins are key in targeting the membrane SNAP and SNARE proteins are key in the fusion event.

- Initial glycosylation as assembly continues. This is N-linked (O-linking occurs in the Golgi).
- N-linked glycosylation: If the protein is properly folded, Oligosaccharyl transferase recognizes the AA sequence NXS or NXT (with the S/T residue phosphorylated) and adds a 14-sugar backbone (2-*N*-acetylglucosamine, 9-branching mannose, and 3-glucose at the end) to the side-chain nitrogen of Asn.

Smooth endoplasmic reticulum:

The smooth endoplasmic reticulum (abbreviated SER) has functions in several metabolic processes. It synthesizes lipids, phospholipids, and steroids. Cells which secrete these products, such as those in the testes, ovaries, and sebaceous glands have an abundance of smooth endoplasmic reticulum. It also carries out the metabolism of carbohydrates, detoxification of natural metabolism products and of alcohol and drugs, attachment of receptors on cell membrane proteins, and steroid metabolism. In muscle cells, it regulates calcium ion concentration. Smooth endoplasmic reticulum is found in a variety of cell types (both animal and plant), and it serves different functions in each. The smooth endoplasmic reticulum also contains the enzyme glucose-6-phosphatase, which converts glucose-6-phosphate to glucose, a step in gluconeogenesis. It is connected to the nuclear envelope and consists of tubules that are located near the cell periphery. These tubes sometimes branch forming a network that is reticular in appearance. In some cells, there are dilated areas like the sacs of rough endoplasmic reticulum.

The network of smooth endoplasmic reticulum allows for an increased surface area to be devoted to the action or storage of key enzymes and the products of these enzymes.

The sarcoplasmic reticulum (SR), is smooth ER found in myocytes (figure 3.16). The only structural difference between this organelle and the smooth endoplasmic reticulum is the medley of proteins they have, both bound to their membranes and drifting within the confines of their lumens. This fundamental difference is indicative of their functions: The endoplasmic reticulum synthesizes molecules, while the sarcoplasmic reticulum stores calcium ions and pumps them out into the sarcoplasm when the muscle fiber is stimulated. After their release from the sarcoplasmic reticulum, calcium ions interact with contractile proteins that utilize ATP to shorten the muscle fiber. The sarcoplasmic reticulum plays a major role in excitation-contraction coupling.

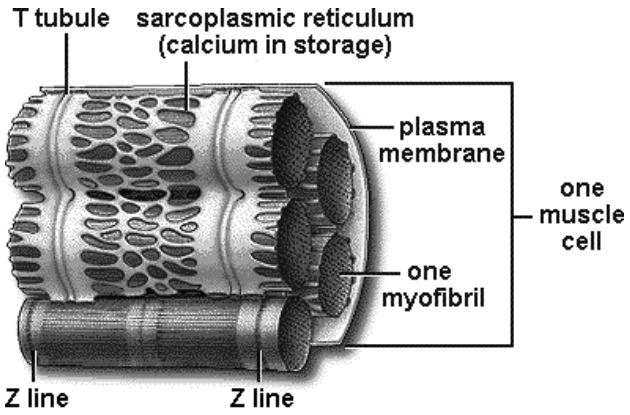


Figure 3.16: Skeletal muscle showing sarcoplasmic reticulum

Plant Cell Endoplasmic Reticulum:

In plant cell, the endoplasmic reticulum acts as a port for the entry of proteins into the membrane. It also plays a vital role in the biosynthesis and storage of lipids. There are number of soluble membrane, which are associated with the enzymes and the molecular chaperones. The general functions of the endoplasmic reticulum in plant cell are protein synthesis and maturation. Endoplasmic reticulum of plant cell possesses some additional functions, which is not found in animal cells. The additional function involves cell to cell communication between specialized cells and also it serves as a storage site for proteins. Endoplasmic reticulum of plant cell contains enzymes and structural proteins, which are involved in the process of oil body biogenesis and lipid storage. In plants, the endoplasmic reticulum is connected between the cells via the plasmodesmata.

Animal Cell Endoplasmic Reticulum

In animal cells, the endoplasmic reticulum is a network of sacs, which play a vital role in manufacturing, processing and transporting different types of chemical compounds for use of both inside and outside of the cell. It is connected to the double-layered nuclear envelope, which provides the pipeline between the nucleus and the cytoplasm of a cell. In animal cells, the endoplasmic reticulum is a multifunctional organelle, which synthesis the membrane lipids, proteins and also regulates the intracellular calcium.

2. **Golgi Apparatus:** These are cup-shaped organelles which are located near the nucleus in many eukaryotic cells. The Golgi complex is referred to as the manufacturing and the shipping center of the eukaryoric cell. The Golgi apparatus or the Golgi body or Golgi complex or Golgi is a cellular organelle present in most of the cells of the eukaryotic organisms. The Golgi

bodies were identified by an Italian biologist Camillo Golgi, in the year 1897 and was named after him in the year 1898. The Golgi complex is responsible inside the cell for packaging of the protein molecules before they are sent to their destination. This organelle helps in processing and packaging the macromolecules like proteins and lipids that are synthesized by the cell, It is known as the 'post office' of the cell. The major function of the Golgi body is to modify, sort and package the macromolecules. It also helps in transportation of lipids around the cell and the creation of lysosomes.

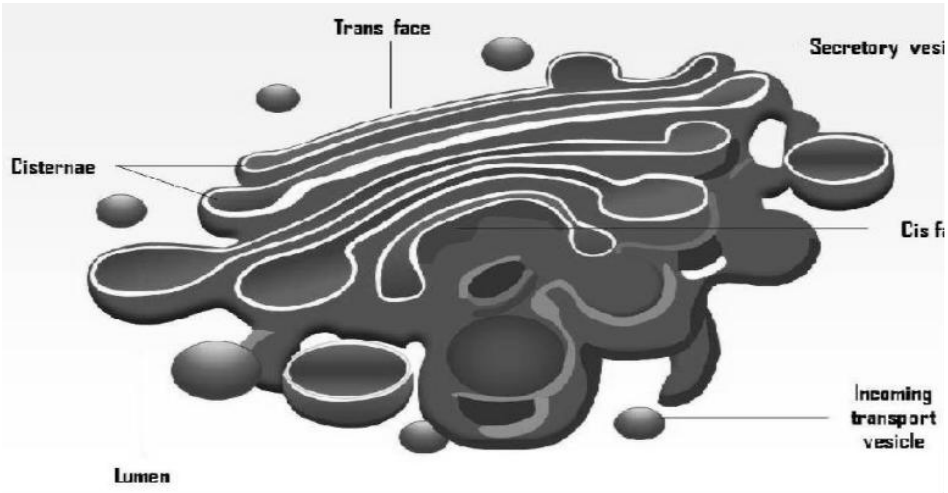


Figure 3.17: Structure of Golgi Apparatus

Golgi apparatus consists of set of smooth cisternae (Closed fluid filled flattened membranous sacs or vesicles) which often are stacked together in parallel rows. They are surrounded by membrane bound vesicles which appear to transport proteins to and from it (Figure: 3.17).Golgi has three distinct classes of Cisternae: Cis Golgi, Median Golgi and Trans Golgi. Each of these are distinct in their activities. Proteins synthesized by Rough Endoplasmic reticulum follow a direction as mentioned below (Figure 3.18) :

RER → cis Golgi → median Golgi → trans Golgi → secretory → vesicles/ corticalgranules/ lysosomes/ peroxisomes

The size of golgi apparatus also indicates the metabolic activity in a cell, mainly active synthesis. In plant cells, freely distributed Golgi are seen which are called as the dictyosomes, which are actively involved in secreting cellulose and pectin for cell wall formation during cell division.

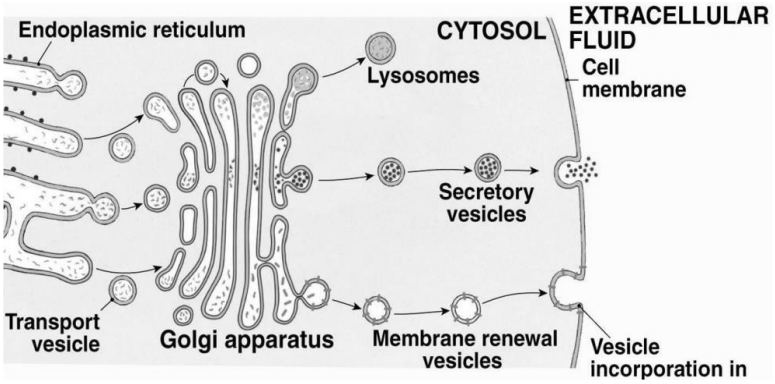


Figure 3.18: Protein transport from ER to golgi apparatus and their secretion

Trafficking between the Endoplasmic Reticulum and the Golgi Complex involves the vesicular coat complexes COPII (coatamer protein II), which mediates the concentration of secretory cargoes into anterograde transport carriers, and COPI (coatamer protein I), which recycles material from post-Endoplasmic Reticulum membranes back to the Endoplasmic Reticulum.
Function of Golgi Apparatus:

1. The cell synthesize a huge variety of macromolecules and the main function of the Golgi apparatus is to modify, sort and package the macromolecules that are synthesized by the cells for secretion purposes or for use within the cell hence it is referred to as the Major Sorting Centre of the cell.

2. It mainly modifies the proteins that are prepared by the rough endoplasmic reticulum.

3. They are also involved in the transport of lipid molecules around the cell.

4. They also producee lysosomes, hence referred as post office where the molecules are packaged, labelled and sent to different parts of the cell.

5. The enzymes in the cisternae have the ability to modify proteins by the addition of carbohydrates and phosphate by the process of glycosylation and phoshphorylation respectively.

6. In order to modify the proteins the golgi complex imports substances like nucleotides from the cytosol of the cell. The modifications brought about by the golgi body might form a signal sequence. This determines the final destination of the protein.

7. The Golgi complex also plays an important role in the production of proteoglycans. The proteoglycans are molecules that are present in the extracellular matrix of the animal cells.
8. It is also a major site of synthesis of carbohydrates. These carbohydratres includes the synthesis of glycoasaminoglycans, Golgi attaches to these polysaccharides which then attaches to a protein produced in the endeoplasmic reticulum to form proteoglycans.
9. The Golgi is involved in the sulfation process of certain molecules.
10. The process of phosphorylation of molecules by the Golgi requires the import of ATP into the lumen of the Golgi.
11. Forms Acrosome in sperm during sperm maturation.
12. In plants they synthesize all polysaccharides such as pectin, hemicelluloses and microfibrills of cellulose.
13. It forms cell plate during cell division at the centre of the spindle.

3. **Lysosomes:** The cytoplasm of animal cells contains many tiny, spheroidal to irregular-shaped, membrane bounded vesicles known as Lysosomes (Figure 3.19). Christian De duve first observed these organelles in animal cells in the year 1949. These originate from Golgi Apparatus and contain many hydrolytic enzymes (more than 50types of enzymes) that are required for intra cellular and extracellular digestion. The enzymes contained within the lysosomes are collectively called as acid hydrolases and they work best in acid environment. The pH within lysosomes is about 4.5 to 5 while that of cytosol is 7.2.

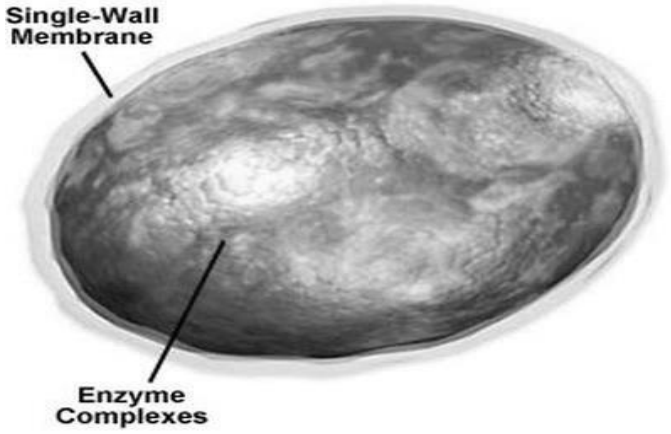


Figure 3.19: Structure of Lysosomes

Functions of Lysosomes:

1. Lysosomal enzymes are involved in degradation of proteins into dipeptides and also carbohydrates into monosaccharides. They cannot digest sucrose and polysaccharides.
2. Autophagy: By this process lysosomes constantly remove cellular components like mitochondria. The cytoplasmic organelles become sourounded by Smooth ER and lysosomes attach to it. They discharge their contents into autophagic vacuole and the organelle is digested.
3. Development process involves shedding and remodeling of tissues. The removed tissues/ cells are removed by lysosomes.
4. Exocytosis: Primary lysosomes at times are released to outside the cells by way of exocytosis. Removal of dead cells and also replacements of tissues involves exocytosis of lysosomes on the tissues.
5. Lysososmes fuse with vesicles and vacuoles: Vacuoles having material to be digested fuses with lysosomes to form an endosome where digestion occurs. **Eg:** in Amoeba, the engulfed food in food vacuole is digested by lysosomes, while in phagocytosis, bacteria engulfed by them are also digested in phagosomes formed by fusion of lysosomes (Figure 3.20).
6. Role in germ cells: Acrosome in sperm cells contains protease, acid phosphatase and

Hyalurodinase. This helps in penetration of egg cells.

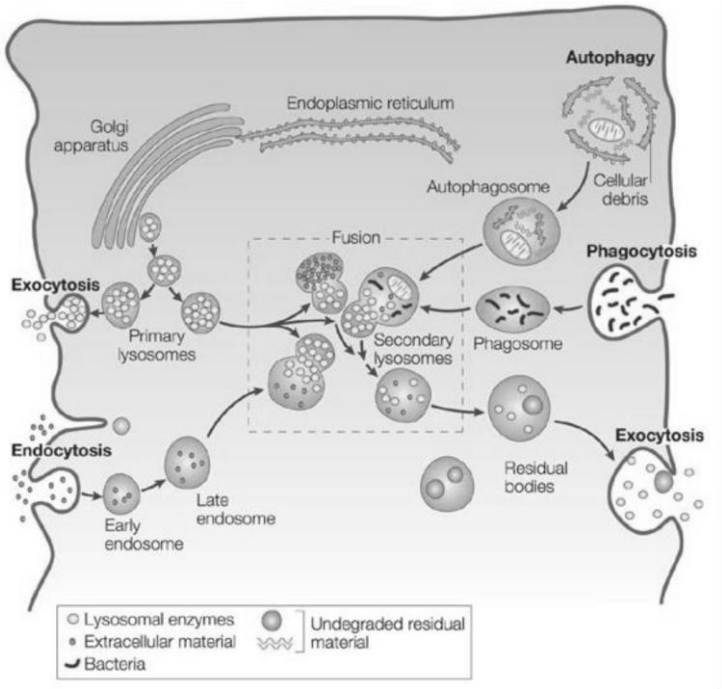


Figure 3.20: Functions of Lysosomes

7. The lysosomes of plant cells are membrane bound and are involved in hydrolysis in large vacuoles of parenchyma cells of corn seedlings, proteins or aleurone bodies and starch granules in cereals and other seeds.
8. Role of lysosomes in diseases: Some of the diseases are caused due to damaging effects of lysosomes as in, Rheumatoid arthritis, silicosis, inflammatory responses, anorexia, myocardial infraction etc.

Significance of Lysosome:

- In WBC or leucocytes: Cells of leucocytes digest foreign protens, bacteria and virus
- In autophagy: During starvation, the lysosomes digest stored food contents such as proteins, fats and glycogen of the cytoplasm and supply the necessary amount of energy to the cell.
- In fertilization: The lysosomal enzymes present in the acrosome of the sperm cells digest the limiting membrane of the ovum. Thus, the sperm is able to enter the ovum and start fertilization.

4. **Cytoplasmic vacuoles:** These are seen in plants and in very few animal cells (lower forms like ciliated protozoans). They are small or larger in size, hollow, liquid filled structures. Vacuoles are supposed to be expanded Endoplasmic reticulum or Golgi apparatus. Vacuoles in animal cells are bounded by a lipoproteinaceous membrane and their function is storage and transmission of the materials and the maintenance of internal pressure. In plants vacuoles are large, bounded by a single semipermeable membrane known as tonoplast (Figure3.22). Proteins found in the tonoplast (aquaporins) control the flow of water into and out of the vacuole through active transport, pumping potassium (K^+) ions into and out of the vacuolar interior. Due to osmosis, water will diffuse into the vacuole, placing pressure on the cell wall. If water loss leads to a significant decline in turgor pressure, the cell will plasmolyze. Turgor pressure exerted by vacuoles is also required for cellular elongation: as the cell wall is partially degraded by the action of expansins, the less rigid wall is expanded by the pressure coming from within the vacuole. Turgor pressure exerted by the vacuole is also essential in supporting plants in an upright position. Another function of a central vacuole is that it pushes all contents of the cell's cytoplasm against the cellular membrane, and thus keeps the chloroplasts closer to light. In plants vacuoles contain water, phenol, flavonols, anthocyanins, alkaloids, storage products like sugars, proteins etc.

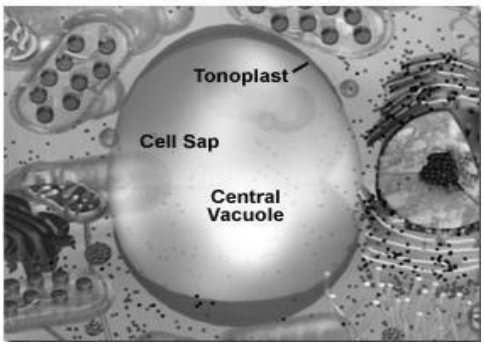


Figure 3.21 Vacuole in plant cell

5. **Peroxisomes:** Peroxisomes are small, membrane-enclosed organelles (Figure 3.22) that contain enzymes involved in a variety of metabolic reactions, including several aspects of energy metabolism. Although peroxisomes are morphologically similar to lysosomes, they are assembled, like mitochondria and chloroplasts, from proteins that are synthesized on free ribosomes and then imported into peroxisomes as completed polypeptide chains. Although peroxisomes do not contain their own genomes, they are similar to mitochondria and chloroplasts in that they replicate by division.

   They are involved in the catabolism of very long chain fatty acids, branched chain fatty acids, D- amino acids, and polyamines,reduction of reactive oxygen species - specifically hydrogen peroxide - and biosynthesis of plasmalogens, i.e. ether phospholipids critical for the normal function of mammalian brains and lungs. They also contain approximately 10% of the total activity of two enzymes in the pentose phosphate pathway, which is important for energy metabolism. It is vigorously debated if peroxisomes are involved in isoprenoid and cholesterol synthesis in animals. Other known peroxisomal functions include the glyoxylate cycle in germinating seeds ("glyoxysomes"), photorespiration in leaves, glycolysis in trypanosomes("glycosomes"), and methanol and/or amine oxidation and assimilation in some yeasts. Peroxisomes were identified as organelles by the Belgian cytologist Christian de Duve in 1967 after they had been first described by a Swedish doctoral student, J. Rhodin in 1954.

   Function of Peroxisomes:

   The main function of peroxisomes is to break down long fatty acid chains through beta-oxidation and synthesize necessary phospholipids (such as plasmologen) that are critical for proper brain and lung function. Furthermore, they aid certain enzymes with energy metabolism in many eukaryotic cells

as well with cholesterol synthesis in animals. Peroxisomes are also involved in germinating seeds in the glyoxylate cycle, photosynt hesis in leaves, and oxidation of amines in various yeasts.

During catabolism of fatty acid chains in animal cells, peroxisomes break down long fatty acids into medium fatty acids which are then transported to mitochondria where the majority of catabolism happens. However, in yeast and plant cells, catabolism of fatty acid chains happens only in the peroxisome and the mitochondria is not involved. The catabolism of fats and fat - soluble vitamins, such as vitamin A and vitamin K, as well as the production of bile acids also takes place in peroxisomes. If this catabolism is not done properly, genetic disorders or skin disorders often result. The synthesis of plasmalogens in animal cells also takes place in peroxisomes. These organelles are very important to the cell because production of plasmalogens is critical to proper functioning of the nervous system since a lack of plasmalogens causes abnormalities in the myelination of nerve cells.

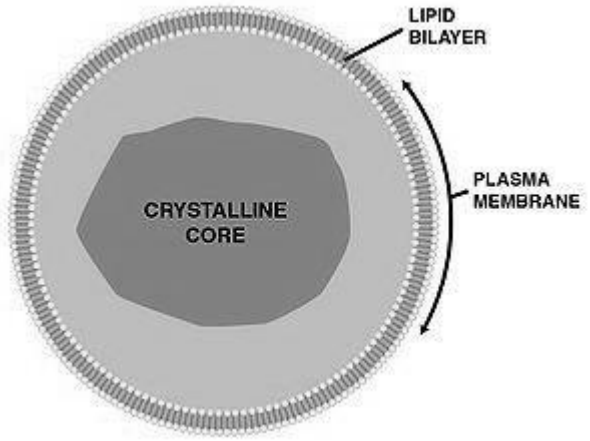


Figure 3.22 Structure of Peroxisome

6. **Glyoxisomes:** In 1967, R. W. Breidenbach and H. Beevers discovered that microbodies in the fat-storing cells of germinating fatty seeds contain enzymes of the glyoxylate cycle in addition to peroxisomal enzymes. They coined the specific term glyoxysomes for these particles, although it is to be understood that glyoxysomes are a form of peroxisome. Glyoxysomes contain not only enzymes specific to the glyoxylate cycle (i.e., isocitrate lyase and malate synthetase) but they also contain several of the essential enzymes of the Krebs cycle, which therefore function simultaneously in both mitochondria and

glyoxysomes. The name "glyoxysome" takes precedence over "peroxisome" whenever glyoxylate cycle enzymes are identified in the microbody. The relationship between the Krebs cycle and the glyoxylate cycle is shown in Figure 3.24. Both cycles employ the same reactions to produce isocitrate from acetyl-CoA and oxaloacetate, but beyond this point the pathways differ. In the Krebs cycle, isocitrate is successively decarboxylated, producing two molecules of carbon dioxide and succinate. In the glyoxylate cy- cle, isocitrate is converted to succinate and glyoxylate. Instead of being lost as two molecules of CO_2, the two-carbon glyoxylate condenses with another acetyl- CoA to form the four-carbon dicarboxylic acid malate.

The four carbon atoms of the two acetyl-CoA molecules are thus conserved as one four-carbon compound that, after conversion to succinate and migration to the mitochondrion, may be converted to oxaloacetate. The oxaloacetate may then be utilized in gluconeogenesis. Oxaloacetate formed in mitochondria from glyoxysomal succinate is presumed to serve as a direct precursor of phosphoenol pyruvate (PEP). The conversion of PEP to glucose and other carbohydrates occurs essentially by the reversal of the steps of glycolysis. Glyoxysome- containing tissues are thus able to convert simple two-carbon sources such as acetate into carbohydrate. In some tissues, such as fat-storing cells in seeds, the acetate is obtained through the degradation of fatty acids; therefore glyoxysomes participate in the conversion of fat to carbohydrate.

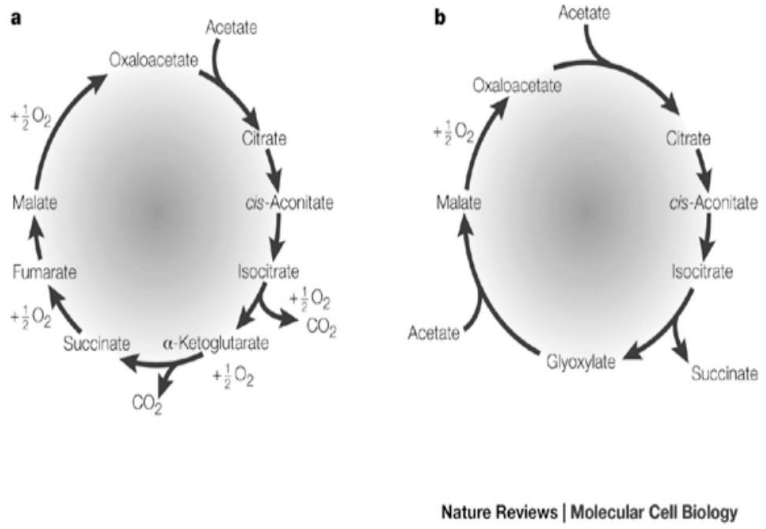


Figure 3.23: Comparision of Krebs cycle (a) and Glyoxylate cycle (b)

Distribution and Origin of Glyoxysomes:

The glyoxylate cycle is especially significant for cells growing exclusively on acetate or fatty acids (e.g., a number of microorganisms), where the cycle acts as a source of four-carbon dicarboxylic acids. Certain microorganisms including Euglena, Chlorella, Neurospora, and Polytomella contain "glyoxysome like" particles, but the term glyoxysome is normally reserved for the organelles of fat-storing endosperm or cotyledons of germinating fatty seeds.

DNA is present in glyoxysomes, indicates the possibility that these organelles possess some degree of autonomy. Glyoxysomes appear to be formed by a process similar to that which gives rise to peroxisomes i.e., by the fission or budding of preexisting glyoxysomes. Most glyoxysomal enzymes are synthesized by free (i.e., unattached) ribosomes in the cytosol and are then translocated into or through the membranes of glyoxysomes already present in the cell.

7. Mitochondria:

The cytoplasm of nearly all eukaryotic cells contains mitochondria, although there is at least one exception, the protist *Chaos (Pelomyxa) carolinensis*. They are especially abundant in cells and parts of cells that are associated with active processes. For example, in flagellated protozoa or in mammalian sperm, mitochondria are concentrated around the base of the flagellum or flagella. In cardiac muscle, mitochondria surround the contractile elements. Hummingbird flight muscle is one of the richest sources of mitochondria known. They are involved in energy production. Multicellular organisms probably could not exist without mitochondria.

Mitochondria are well-defined cytoplasmic organelles of the cell which take part in a variety of cellular metabolic functions. The necessary biological energy of the cell, is obtained by oxidizing the substrates of the Krebs cycle in the mitochondria. Hence, the mitochondria re referred to as the 'power houses' of the cell. These organelles generate most of the energy of the cell in the form of adenosine triphosphate (ATP) and it is used a source of chemical energy. The y are also involved in other cellular activities like signaling, cellular differentiation, cell senescence and also control of cell cycle and cell growth. Mitochondria also affect human health, like mitochondrial disorder and cardiac dysfunction and they also play important role in the aging process. The term 'mitochondrion' is derived from a Greek word 'mitos' which means 'thread' and 'chondrion' which means 'granule'.

In 1890, mitochondria was first described by Richard Altmann and he called them as bioblasts. Benda in the year 1897 coined the term mitochondrion. In

the 1920s, a biochemist Warburg found that oxidative reactions takes place in most tissues in small parts of the cell

Structure of Mitochondria

Mitochondria are rod shaped structure found in both animal and plant cells. It is a double membrane bound organelle. It has the outer membrane and the inner membrane. The membranes are made up of phospholipids and proteins (figure 3.24).

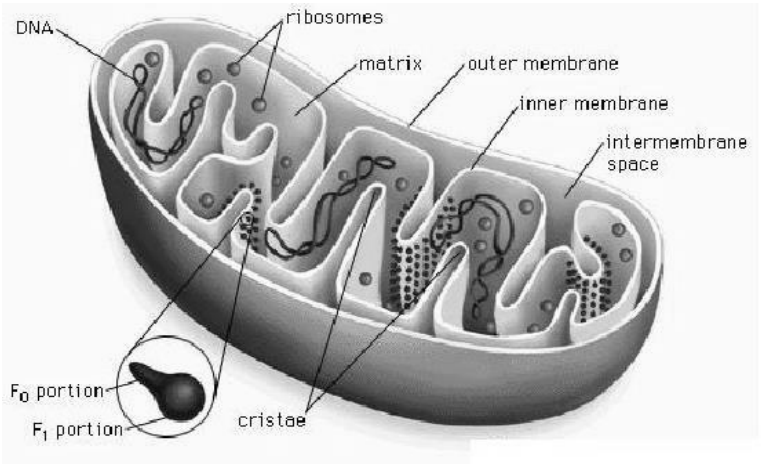


Figure 3.24: Internal structure of Mitochondria

Outer Membrane:

The outer mitochondrial membrane, which encloses the entire organelle, is 60 to 75angstroms (Å) thick. It has a protein-to-phospholipid ratio similar to that of the eukaryotic plasma membrane (about 1:1 by weight). It contains large numbers of integral membrane proteins called porins. These porins form channels that allow molecules of 5000 daltons or less in molecular weight to freely diffuse from one side of the membrane to the other.[9] Larger proteins can enter the mitochondrion if a signaling sequence at their N-terminus binds to a large multisubunit protein called translocase of the outer membrane, which then actively moves them across the membrane. The outer membrane also contains enzymes involved in such diverse activities as the elongation of fatty acids, oxidation of epinephrine, and the degradation of tryptophan. These enzymes include monoamine oxidase, rotenone-insensitive NADH-cytochrome c-reductase, kynurenine hydroxylase and fatty acid Co-A ligase. Disruption of the outer membrane permits proteins in the intermembrane space to leak into the cytosol,

leading to certain cell death. The mitochondrial outer membrane can associate with the endoplasmic reticulum (ER) membrane, in a structure called MAM (mitochondria-associated ER-membrane). This is important in the ER-mitochondria calcium signaling and is involved in the transfer of lipids between the ER and mitochondria. It is freely permeable to nutrient molecules, ions, energy molecules like the ATP and ADP molecules.

Inner membrane : The inner mitochondrial membrane has proteins having five different functions.

1. Those which perform redox reactions of oxidative phosphorylation
2. ATP synthases which generate ATP in the matrix
3. Specific transport proteins that regulate metabolite passage into and out of the matrix
4. Protein import machinery
5. Mitochondrial fusion and fission proteins

It contains more than 151 different polypeptides, and has a very high protein-to-phospholipid ratio. The inner membrane is rich in an unusual phospholipid, cardiolipin. It contains four fatty acids rather than two, and may help to make the inner membrane impermeable. Unlike the outer membrane, the inner membrane doesn't contain porins, and is highly impermeable to all molecules. Almost all ions and molecules require special membrane transporters to enter or exit the matrix. Proteins are transported into the matrix via the translocase of the inner membrane (TIM) complex or via Oxa. In addition, there is a membrane potential across the inner membrane, formed by the action of the enzymes of the electron transport chain.

The inner mitochondrial membrane is compartmentalized into numerous cristae, which expand the surface area of the inner mitochondrial membrane, enhancing its ability to produce ATP. These folds are studded with small round bodies known as F_1 particles or oxysomes. These invaginations of the inner membrane, can affect overall chemiosmotic function.

Intermembrane space:

It is the space between inner and outer membranes of Mitochondria and is also called as perichondrial space. It has same composition as cytosol. Outer membrane is permeable to smaller ions and molecules while large molecule entry requires specific signaling sequences. Oneprotein which is localized to this space is the Cytochorme C.

Matrix:

The matrix is the space enclosed by the inner membrane. It contains about 2/3 of the total protein in a mitochondrion and is important in the production of ATP

with the aid of the ATP synthase contained in the inner membrane. The matrix contains a highly concentrated mixture of hundreds of enzymes, special mitochondrial ribosomes, tRNA, and several copies of the mitochondrial DNA genome. Of the enzymes, the major functions include oxidation of pyruvate and fatty acids, and the citric acid cycle.

Function of Mitochondria:

Functions of mitochondria depends on the cell type in which they are present.

- The most important function of the mitochondria is to produce energy. The simpler molecules of nutrition are sent to the mitochondria to be processed and to produce charged molecules. These charged molecules combine with oxygen and produce ATP molecules. This process is known as oxidative phosphorylation.
- Mitochondria help the cells to maintain proper concentration of calcium ions within the compartments of the cell.
- The mitochondria also help in building certain parts of blood and hormones like testosterone and estrogen.
- The liver cells mitochondria have enzymes that detoxify ammonia.
- The mitochondria also play important role in the process of apoptosis or programmed cell death. Abnormal death of cells due to the dysfunction of mitochondria can affect the function of organ.

Mitochondrial DNA:

Mitochondrial DNA or mtDNA or mDNA is the DNA in the mitochondria, Human mitochondrial DNA spans about 16,500 DNA base pairs, it represents a small fraction of the total DNA in cells. The mtDNA contains 37 genes. All these genes are essential for normal function of the mitochondria. These DNA help the mitochondria to divide independently from the cell. mtDNA is maternally inherited. The fact that mt DNA is maternally inherited enables to trace the maternal lineage far back in time. The mt DNA in most multicellualr organisms is circular, covalently closed, double-stranded DNA. mt.DNA is susceptible to free oxygen radicals. Mutations in the mitochondrial DNA leads to a number of illness like exercise intolerance. Human mitochondrial DNA sequence revealed 16,569 base pairs encoding 37 genes:

22 tRNA, 2 rRNA, and 13 peptide genes.[37] The 13 mitochondrial peptides in humans are integrated into the inner mitochondrial membrane, along with proteins encoded by genes that reside in the host cell's nucleus.

8. Plastids: Plastids are large cytoplasmic organelles. Plastids are major organelles found in the cells of plants and algae. Plastids are the site of manufacture and storage of important chemical compounds used by the cell. Plastids often contain pigments used in photosynthesis, and the types of pigments present can change or determine the cell's colour. The term plastid was derived from the Greek word plastikas meaning formed or moulded. This term was coined by Schimper in 1885.

Classification:

On the basis of presence or absence of pigments, and the stage of development, plastids have been classified into proplastids, leucoplasts and chromoplasts.

Proplastids:

Small vesicular structures present in meristematic cells are called proplastids. They are colorless and undeveloped. As cells mature into different cell types, depending upon the organs and presence or absence of light, proplastids undergo transformation and develop into either colorless leucoplasts or colored chromoplasts including green chloroplasts. Proplastids continuously divide and redivide and provide them for cells undergoing differentiation into various types.

Leucoplasts:

Colorless plastids that are found in storage parenchyma and other colorless tissues are refereed to as leucoplasts. Most of them act as storage organelles. Based on the kind of substance they store they are further classified into amyloplasts. If such leucoplasts are exposed to sunlight they will be transformed into colored plastids, which suggest that these plastids have retained all the genetic potentiality to develop and perform photosynthesis.

Chromoplasts:

All plastids containing different colored pigments are grouped under chromoplasts, of which green colored ones are called chloroplasts. Depending upon the dominant pigments present in plastids, they are further classified into Rhodoplasts rich in red pigment i.e. phycoerythrin. Phaeoplasts and Xanthoplasts contain yellow pigments i.e. xanthophylls, carotinoids. Along with the above pigments phycocyanin and other pigments are also present in other colored plastids.

Other plastids:

Such colored plastids, other than chloroplasts are predominantly found in certain class of plants and plant organs including floral parts. Though floral parts are derived from the same set of proplastids, produce different pigments in

petals. The exact process differentiation is not known for different plants do produce different colored petals and it is genetically programmed.

Interconversion: (Figure 3.25)

Proplastids divide and redivide in meristematic cells, and then they are distributed to cell derivatives on exposure to light, depending upon the structures in which they found and also depending upon the intra cellular factors they develop into colorless plastids or colored plastids. Leucoplasts on exposure to light develop into green plastids. Similarly chloroplasts may become leucoplasts; but colored plastids as in petals are mostly terminally differentiated.

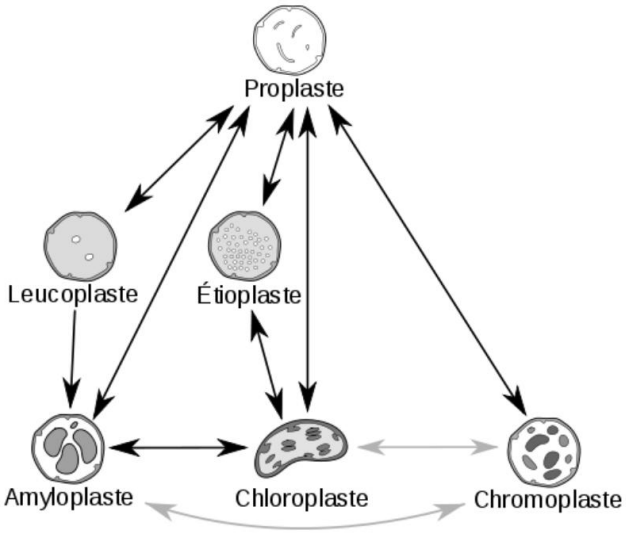


Figure 3.25: Interconversion of Plastids

Chloroplasts:

Chloroplasts are green colored plastids for they contain greater amounts of chlorophyll pigments. They are ubiquitously present in green plants and they are mainly responsible for providing food for themselves and to other animals in the world. These structures are mostly restricted to photosynthetic parts of the plants.

Number and Shapes:

In lower plants, such as Chlamydomonas, spirogyra, diatoms, Hdrodictyon, etc, the number of plastids present in each cell is always constant and characteristic; so also their shapes. Chlorella cells contain a single plate shaped chloroplast; whereas Chlamydomonas possesses only one cup shaped chloroplast. One the other hand chloroplasts in spirogyra are ribbon shaped spirally coiled. But Zygnema cells contain two star shapes chloroplasts. In Hydrodictyon and

cladophora chloroplasts are highly diffused and this is in the form of a network. In all the above said plants, plastids contain pyrenoids. In higher land plants, the number of chloroplasts varies from cell to cell and from organ to organ, i.e. 30-200 per cell and most of them are nearly spherical or ovoidal in shape.

Distribution:

In certain algae like Caulerpa and Vaucheria, chloroplasts are generally clustered in the region of the cell where it is exposed to sunlight. Even in higher plants such as tropical grasses etc, chloroplasts found in bundle sheath cells and those found in the surrounding mesophyll cells are crowded towards each other. Generally the position of chloroplasts in photosynthetic cells is not fixed, because they are constantly swept along with the protoplasmic movement. Size of eukaryotic chloroplasts is 4-5 nm in size but size may vary from plant to plant. Plants growing in shade contain large sized chloroplasts in their cells than that of growing in intense light.

Multiplication of plastids:

Proplastids are constantly dividing in meristematic cells and keep pace with cell division. Once proplastids develop into fully developed chloroplasts, in higher plants, they rarely divide. But in spirogyra and many algae fully developed plastids, divide at the time of cell division and they are equally distributed among the daughter cells. In Chlamydomonas, chloroplasts divide into two four or more at the time of reproduction. The mode of division is typical of bacterial cleavage. The time taken for such division is should take 10-18 hours. In all developing organs or plant structures plastids keep on multiplying.

Detailed structure of Chloroplasts: (figure 3.26)

The chloroplast are double membrane bound organelles and are the site of photosynthesis The chloroplasts have a system of three membranes: the outer membrane, the inner membrane and the thylakoid system. The outer and the inner membrane of the chloroplast enclose a semi-gel- like fluid known as the stroma. This stroma makes up much of the volume of the chloroplast, the thylakoids system floats in the stroma.

Outer membrane : It is a semi-porous membrane and is permeable to small molecules and ions, which diffuses easily. The outer membrane is not permeable to larger proteins.

Intermembrane Space: It is usually a thin intermembrane space about 10-20 nanometers and it is present between the outer and the inner membrane of the chloroplast.

Inner membrane: The inner membrane of the chloroplast forms a border to the stroma. It regulates passage of materials in and out of the chloroplast. In addition of regulation activity, the fatty acids, lipids and carotenoids are synthesized in the inner chloroplast membrane.

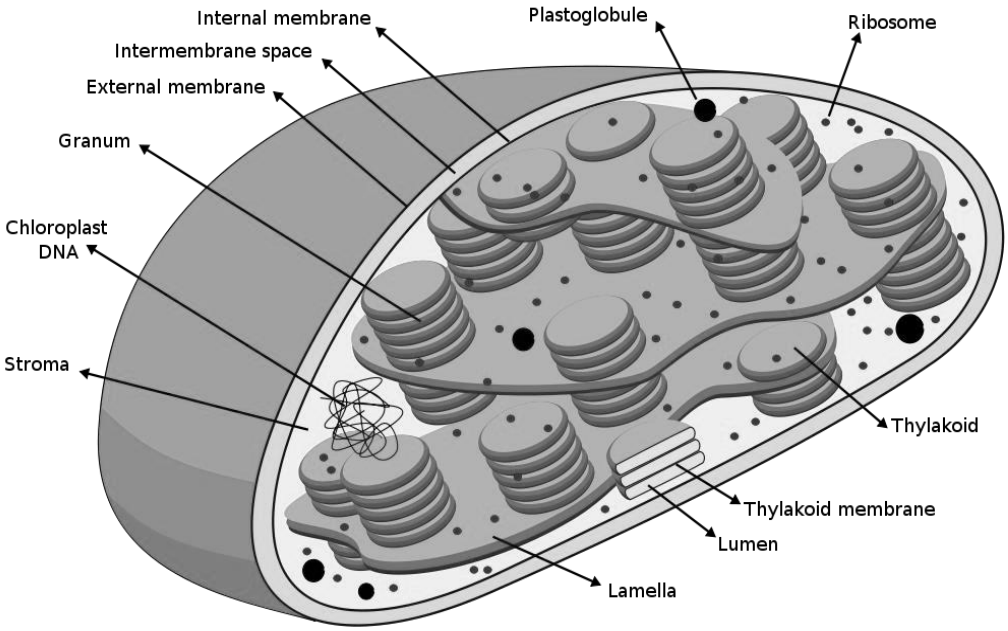


Figure 3.26: Detailed structure of Chloroplasts

Stroma:

Stroma is a alkaline, aqueous fluid which is protein rich and is present within the inner membrane of the chloroplast. The space outside the thylakoid space is called the stroma. The chloroplast DNA chlroplast ribosomes and the thylakoid sytem, starch granules and many proteins are found floating around the stroma.

Thylakoid System:

The thylakoid system is suspended in the stroma. The thylakoid system is a collection of membranous sacks called thylakoids. The chlorophyll is found in the thylakoids and is the sight for the process of light reactions of photosynthesis to happen. The thylakoids are arranged in stacks known as grana. Each granum contains around 10-20 thylakoids. Thylakoids are interconnected small sacks, the membranes of these thylakoids is the site for the light reactions of the photosynthesis to take place. The word 'thylakoid' is derived from the Greek word "thylakos" which means 'sack'.

Important protein complexes which carry out light reaction of photosynthesis are embedded in the membranes of the thylakoids. The Photosystem I and the Photosystem II are complexes that harvest light with chlorophyll and carotenoids, they absorb the light energy and use it to energize the electrons.

The molecules present in the thylakoid membrane use the electrons that are energized to pump hydrogen ions into the thylakoid space, this decrease the pH and become acidic in nature. A large protein complex known as the ATP synthase controls the concentrat ion gradient of the hydrogen ions in the thylakoid space to generate ATP energy and the hydrogen ions flow back into the stroma.

Thylakoids are of two types - granal thylakoids and stromal thylakoids. Granal thylakoids are arranged in the grana are pancake shaped circular discs, which are about 300-600 nanometers in diameter. The stromal thylakoids are in contact with the stroma and are in the form of helicoid sheets. The granal thylakoids contain only photosystem II protein complex, this allows them to stack tightly and form many granal layers wiht granal membrane. This structure increases stability and surface area for the capture of light.

The photosystem I and ATP synthase protein complexes are present in the stroma. These protein complexes acts as spacers between the sheets of stromal thylakoids.

Functions of Chloroplast:

- In plants all the cells participate in plant immune response as they lack specialized immune cells.

  The chloroplasts with the nucleus and cell membrane and ER are the key organelles of pathogen defense.

- The most important function of chloroplast is to make food by the process of photosynthesis.

  Food is prepared in the form of sugars. During the process of photosynthesis sugar and oxygen are made using light energy, water, and carbon dioxide.

- Light reactions takes place on the membranes of the thylakoids.
- Chloroplasts, like the mitochondria use the potential energy of the H+ ions or the hydrogen ion gradient to generate energy in the form of ATP.
- The dark reactions also known as the Calvin cycle takes place in the stroma of chloroplast.

- Production of $NADPH_2$ molecules and oxygen as a result of photolysis of water.
- By the utilization of assimilatory powers the 6-carbon atom is broken into two molecules of phosphoglyceric acid.

9. Ribosomes: Ribosomes are small particles, present in large numbers in all the living cells. They are sites of protein synthesis. The ribosome word is derived - '*ribo*' from ribonucleic acid and '*somes*' from the Greek word '*soma*' which means '*body*'. The ribosomes link amino acids together in the order that is specified by the messenger RNA molecules. The ribosomes are made up of two subunits - a small and a large subunit. The small subunit reads the mRNA while the large subunit joins the amino acids to form a chain of polypeptides. Ribosmal subunits are made of one or more rRNA (ribosomal RNA) molecules and various proteins.

Structure of Ribosome : (Figure 3.28)

- Ribosomes in a cell are located in two regions of the cytoplasm.
- They are found scattered in the cytoplasm and some are attached to the endoplasmic reticulum.
- When the ribosomes are bound to the ER there are known as the rough endoplasmic reticulum.
- The bound and the free ribosomes are similar in structure and are involved in protein synthesis.
- Ribosomes are tiny particles about 200 Ã and composed of both RNA and proteins. About 37 -62% of RNA are made up of RNA and the rest is proteins.
- Ribosome is made up of two subunits. The subunits of ribosomes are named according to their ability of sedimentation on a special gel which the Sevdberg Unit.
- Prokarytotes have 70S ribosomes each subunit consisting of small subunit is of 30S and the large subunit is of 50S. Eukarytotes have 80S ribosomes each consisting of small (40S) and large (60S) subunit.
- The ribosomes found in the chloroplasts of mitochondria of eukaryotes consists of large and small subunits bound together with proteins into one 70S particle.
- The RNA is organized in various tertiary structures. The RNA in the larger ribosomes are into several continuous insertion as they form loops out of the core structure without disrupting or changing it.

- The catalytic activity of the ribosome is carried out by the RNA, the proteins reside on the surface and stabilize the structure.
- The differences between the ribosomes of bacterial and eukaryotic are used to create antibiotics that can destroy bacterial infection without harming human cells.

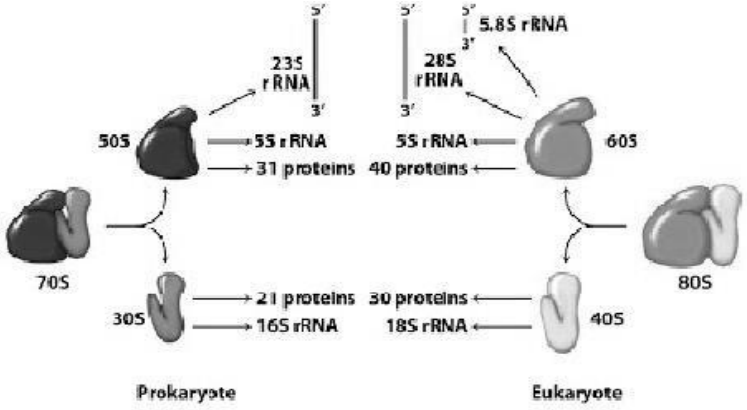


Figure 3.27: Structure and composition of ribosomes

Ribosome Function:

The main functions of ribosmes are :

- They assemble amino acids to form specific proteins, proteins are essential to carry out cellular activities.

The process of production of proteins, the deoxyribonucleic acid produces mRNA by the process of DNA transcription.

The genetic message from the mRNA is translated into proteins during DNA translation. The sequences of protein assembly during protein synthesis are specified in the mRNA.

In the cytoplasm, the two subunits of ribosomes are bound around the polymers of mRNA; proteins are then synthesized with the help of transfer RNA. Ribosomes have A site and P site, aminoacyl t-RNAs are placed initially at A site while the amino acid on the specific-RNA is transferred to another t-RNA having peptide to form peptide bond. This leads to chain elongation of peptide and protein synthesis occurs. The tRNA placed at A site has anticodon sequence complementary to code on mRNA (Figure 3.27)

The proteins that are synthesized by the ribosomes present in the cytoplasm are used in the cytoplasm itself. The proteins produced by the bound ribosomes are transported outside the cell.

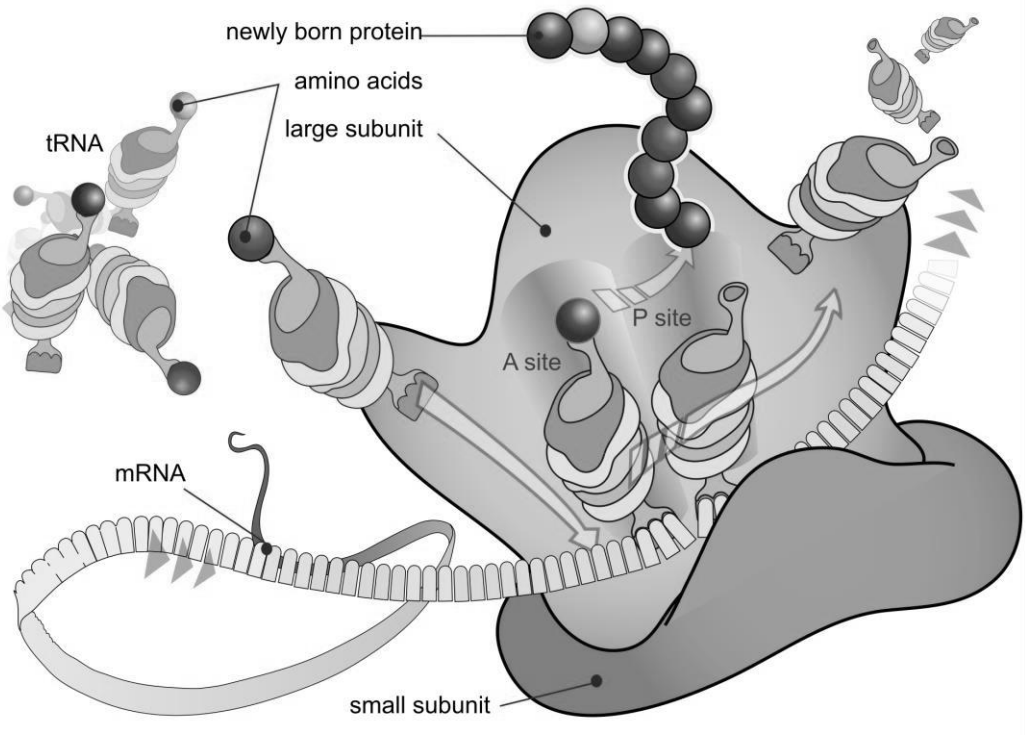


Figure 3.28: Assembly of mRNA, tRNA on ribosomes for protein synthesis

Plant Cell Ribosome:

Plant cell do have ribosomes and they are composed of proteins and ribosomal RNA. The ribosomes in a plant cell are found in the cytoplasm, the surface of the rough endoplasmic reticulum, the mitochondria and on chloroplasts. There are two types of ribosomes - free ribosomes and attached ribosomes. The attached ribosomes are bound to the surface of the endoplasmic reticulum and they are the site for protein synthesis. Synthesis of proteins also occurs in the free ribosomes.

Animal Cell Ribosome:

Ribosomes are protein builders of the cell. They are found in many places in the cytoplasm. They might be seen freely floating in the cytoplasm and they are seen attached to the endoplasmic reticulum. Ribosomes of animal cells are also made of two subunits large (60S) and small (40S. When there is need for proteins in a cell, the mRNA is produced in the nucleus and is sent to the cell and the ribosomes. The two subunits of the ribosomes come together at the time of protein formation and combine with the mRNA molecule and hence the proteins are synthesized.

10 **Microtubules and microtubular organelles:** These are seen in the cytoplasm of all eukaryotes and their length ranges upto infinity with diameter of about 24nm. Cross sectional view reveals their internal structure. They have a dense wall of 6nm thickness and a very light or a hollow centre. The wall of microtubule is composed of 13 globular subunits called protofilaments, of about 4 to 5 nm diameter. Chemically microtubules are composed of two kinds of proteins: alpha tubulin (Tubulin A) and beta tubulin (Tubulin B) subunits each having a molecular weight of 50,000 daltons. Among the 13 dimers of protofilaments each is composed of dimer of alpha and beta tubulins which run parallel to the long axis of the tubule. The repeating unit is an αβ heterodimer which is arranged head to tail within the microtubule as αβ → αβ→ αβ. As the microtubules two ends are not the same, hence they are polar (figure 3.29).

Microtubules undergo reversible assembly and disassembly based on the necessity of the cell. This is regulated by Microtubule Associated Proteins (MAP). Assembly involves preferential addition of subunits to one end of the tubule called as the A end and the other end is called as the D end. This involved hydrolysis of GTP and their assembly is specifically oriented, as in centrioles, basal bodies and centromeres. Calcium and calmodulin along with few other factors regulate invivo polymerization of tubulin.

The cells organelles which are derived from the assembly of microtubules are- cilia, flagella,basal bodies and centrioles.

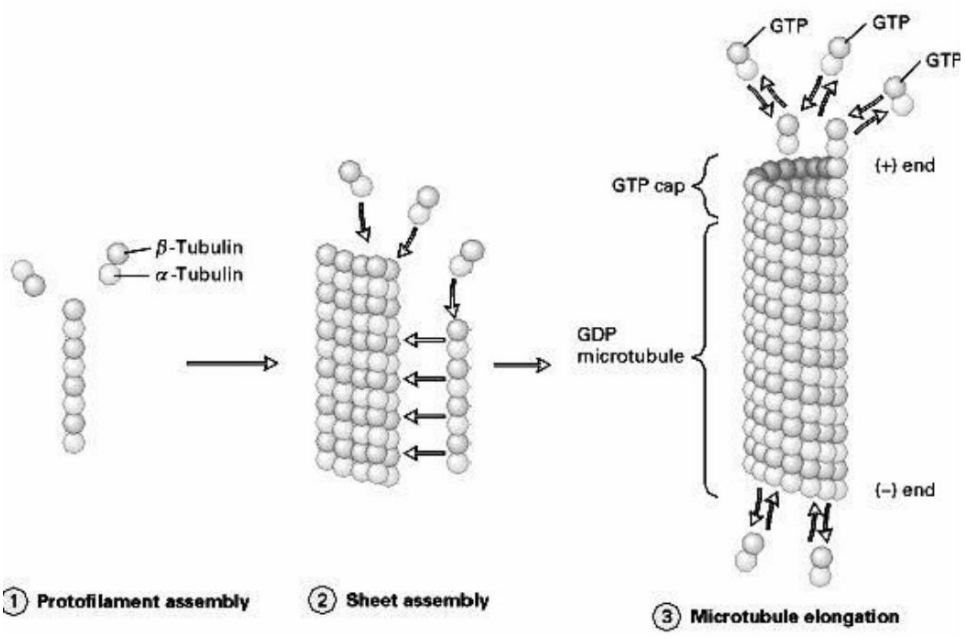


Figure 3.29: Assembly of Microtubules

(i) **Cilia and Flagella:** Cilia are shorter and smaller than flagella while both are used for locomotion by few cells .in protozoans like Paramecium, cilia are used for locomotion, while flagella are required for locomotion of spermatozoons and even in Euglena. Both cilia and flagella have a common architecture: A common bundle of microtubules called Axoneme surrounded by plasma membrane exists outside the cell, while the other end is connected to a intra cellular basal granule which originates from a centrioles. Each axoneme is filled with ciliary in which are embedded two central singlet microtubules each with13 protofilaments and nine outer pairs of microtubules called doublets. This recurring motif is called 9+2 array. The doublets comprise of A subfibre and a B subfibre. Subfibre A has two dynein arms which are oriented in clock wise direction. Doublets are linked together by nexin links. Each sub fibre A is also connected to the central microtubule by radial spokes terminating in fork-like structures called Spoke-knobs or heads (Figure 3.30).

Propulsion of both cilia and flagella is caused by bending at their base. Ciliary movement is associated with whip like stroke powered by ATP followed by a recovery stroke.

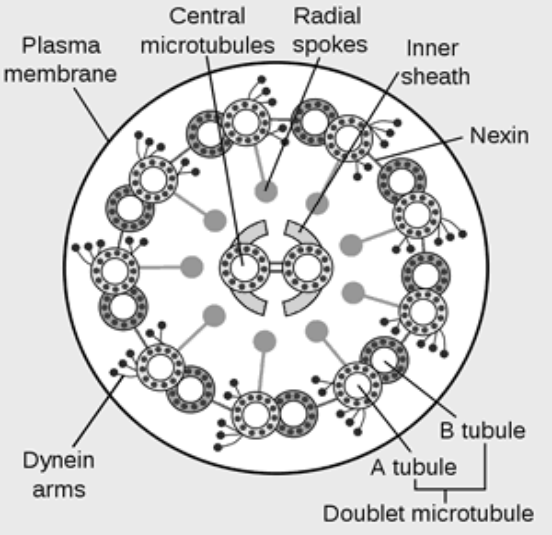


Figure 3.31: Internal structure of flagellum showing 9+2 arrangement

(ii) **Basal body and Centrioles:** Both are similar in structure and function. They have a nucleated centre from which microtubules grow. Centrioles are cylindrical and are open at both the ends usually, unless they carry

a cilium or flagellum. The wall of centrioles has nine groups of microtubules arranged in a circle and each group is called as a blade which is a triplet of A, B and C tubules. Tubule A has 13 protofilaments, while tubules B and C have 10 protofilaments (figure 3.31). During cell division, centrioles divide and until then a single centrioles exists attached to nuclear envelop.

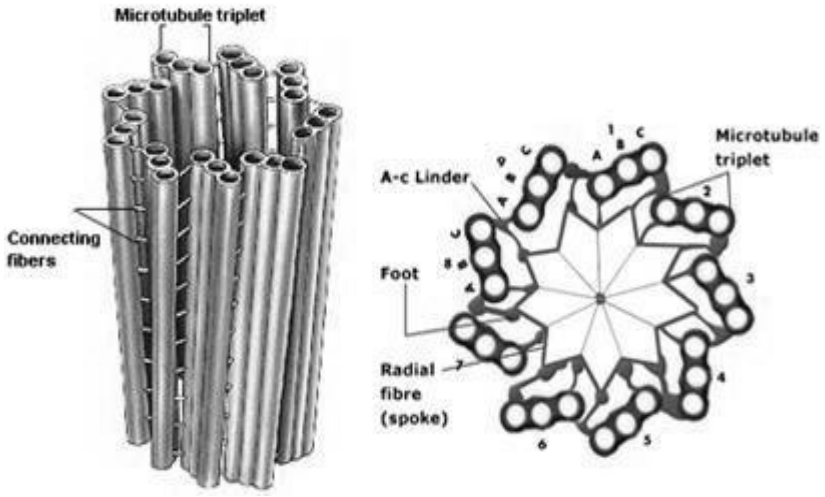


Figure3.31: Structure of centrioles

C. Nucleus: Chromatin, nuclear envelop and nucleolus

(i) Nucleus : Nucleus is the centrally located spherical component of a cell that regulates all activities of the cell and carried the hereditary material in it. It is the most prominent organelle of the cell. The number of nuclei may vary, they may be uni-nucleate (single nucleus), bi-nucleate (two nuclei) or even multi-nucleate. Nucleus is present in all eukaryotic cells, they may be absent in few cells like the mammalian RBCs. It was first cell organelle to be discovered. Antonie von Leeuvenhoek (1632 - 1723) observed lumen (nucleus) in the red blood cells of salmon. Nucleus was also described by Franz Bauer in 1804 and by Robert Brown in 1831. Robert Brown in 1833 named and discovered nucleus in plant cells.

The word nucleus is derived from a *Latin* word *nucleus* or *nuculeus* which means 'kernel'. Nucleus a double-membrane bound cell organelle present in eukaryotic cells. It constitutes most of the genetic material of the cell - the DNA and maintains the integrity of the genes which regulate the gene expression, in-turn regulating the activities of the cell. Therefore, the nucleus is known as the control center of the cell. The

nucleus is not just a storage compartment for DNA. It's the site of some essential cellular processes. First, DNA can be duplicated in the nucleus.

This process is called replication and creates an identical copy of DNA. Second, the nucleus is the site of transcription DNA is copied into RNA, which is then turned is translated into protein in the cytoplasm.

Nucleus Structure:

It occupies about 10% of the total volume of the cell. In mammalian cells the average diameter of the nucleus is approximately 6 micrometers. A semi-fluid matrix nucleoplasm is seen inside the nucleus which is a viscous fluid and is similar to the composition of the cytoplasm (figure 3.32).

Nuclear Envelope:

The nuclear envelope is also known as the nuclear membrane which comprises of two membranes the outer membrane and the inner membrane. The outer membrane of the nucleus is continuous with the membrane of the rough endoplasmic reticulum. The space between these layers is known as the perinuclear space. The nuclear envelope encloses the nucleus and separates the genetic material of the cell from the cytoplasm of the cell. It also serves as a barrier to prevent passage of macro-molecules freely between the nucleoplasm and the cytoplasm.

Nuclear Pore:

The nuclear envelope is perforated with numerous pores called nuclear pores which are composed of many proteins known as nucleoproteins. The nuclear pores regulate the passage of the molecules between the nucleus and cytoplasm. They allow the passage of molecules of only about 9nm wide. The larger molecules are transferred through active transport. Molecules like of DNA and RNA are allowed into the nucleus. But energy molecules (ATP), water and ions are permitted freely. There may be 60to300 pores per nucleus.

(ii) Chromatin: Chromosomes in a normal eukaryotic cell exist as chromatin network (during interphase) (figure 3.32). The chromatin has two forms:

1. Euchromatin- which is well dispersed form of chromatin which takes lighter DNA stain and is genetically active (involved in gene duplication and transcription) and phenogenesis (Phenotypic expression).
2. Heterochromatin: This is highly condensed from of chromatin that takes dark stain and is genetically inert. This is seen in the chromatin associated with regions of centromere and in sex chromosomes.

The chromatin contains DNA molecule, five types of histone proteins and some RNA molecules. The chromatin has a unit structure in the form of nucleosome. The chromatin binds strongly to the inner part of nuclear lamina, which is fibrous and think and located on the inner side. Nuclear lamina is inturn made up of three types of proteins A, B and C and is required during the interphase.

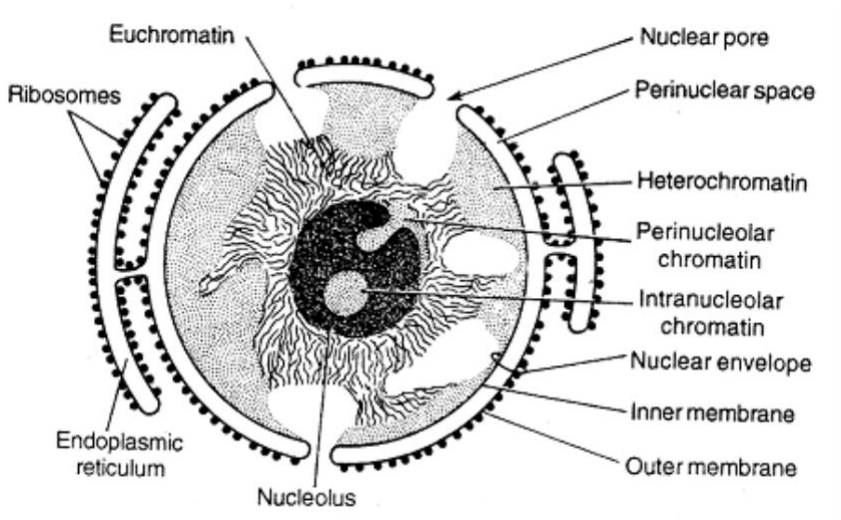


Figure 3.32: Structure of Nucleus and its components

(iii) Nucleolus: nucleolus is the conspicuous, darkly stained circular sub organelle in the nucleus. It has no limiting membrane and is formed during interphase by the ribosomal DNA of nuclear organizer. It is the site where ribosomes are manufactures. The ribosomal DNA transcribes into rRNA molecules and these undergo processing before their step-wise addition to 70types of ribosomal proteins takes place to form the ribosomal subunits.

Differences between Prokaryotic and Eukaryotic cells

S.No	Feature	Prokaryotes	Eukaryotes
1.	Occurrence	Prokaryotic cells are the characteristics of bacteria and blue green algae (cyanobacteria)	These are cells are found in all, animals and plants, except bluegreen algae and bacteria.
2.	Size	Mostly 1-10 μm	Mostly 10-100 μm
3.	Multicellular forms	Rare	Common, with extensive tissue formation
4.	Cell wall	Present in most but not in all cells. In Bacteria, cell wall is made up of murein, poly saccharides, lipids and proteins. Read more	The animal cells lack cell wall, but plants cell wall is made up of cellulose and chitinous cell wall is present in fungi. *Read more*
5.	Plasma membrane	Present	Present
6.	Nucleus	Absent. Nucleoid region is present where genetic material resides.	Present
7.	Nuclear Membranes	Absent	Present
8.	Chromatin with histones	Absent	Present
9.	Number of chromosomes	Each cell has only one chromosome.	Number of chromosomes per cell depends upon the type of organism.
10.	Chromosome	The chromosome is circular ring lacking a centromere.	Each chromosome is linear having a centromere with two kinetochores meant for attachment to the spindle fibres during cell division.
11.	Genetic material	Circular or linear, double stranded DNA: only exons are present	Linear double stranded DNA: genes frequently interrupted by intron sequences, especially in higher eukaryotes (called as split genes).

12.	Nucleoliand Mitotic apparatus	Absent	Present
13.	Nucleolus	Absent	Present (for the synthesis and organisation of ribosomes)
14.	Plasmids	Commonly present	Rare
15.	Mesosomes	Mesosomes perform the function of golgibodies and mitochondria, and also help in the separation of chromosomes during cell division.	Absent
Cell organelles			
16.	Mitochondria	Absent	Present
	Endoplasmic reticulum	Absent	Present
	Lysosomes	Absent	Present
	Vacuoles	Absent	Present
	Chloroplasts	Absent	Present in plants
	Centrioles	Absent	Present in higher plants
	Ribosomes	Only 70S type which are present free in the cytoplasm or are engaged in proteins synthesis	The cytoplasm has 80S ribososmes, while plastids and mitochondria have 70S ribosomes.
	Microtubules	Absent	Present
	Flagella	Simple structure composed of protein flagellin	Complex andhas9+2 structure of tubulin and proteins
17.	Respiration	Many are strict anaerobes	All are aerobic, while few are anaerobes by secondary modification
18.	Photosynthetic Enzymes	Bound to plasma membrane as composite chromatophores	Enzymes are packed in plastids bound by a membrane

19.	Metabolic patterns	Great variations	All share cytochrome electron transport chains, Krebs cycle oxidation, Embden – Meyer glucose metabolism of Glycolysis
20.	Sexual system	Rarely seen. If present one way (and usually forming partial diploids or merozygotes), transfer of DNA from donor to recipient occurs via conjugation. Gene transfer takes place by transformation and transduction also.	Both sexes are involved and entire geneome is transferred. Alternation of haploid and diploid generation's is also evident.
21.	Cyclosis	There are no streaming movements of cytoplasm	Cytoplasm shows streaming movement
22.	Protein synthesis	Transcription and translation movements of cytoplasm	Transcription occurs in nucleus and translation takes place in cytoplasm
23.	Duration of cell cycle	Cell cycle is short and takes place in minutes	Cell cycle is long and takes 12-24 hours to complete.

Difference between Animal Cell and Plant cell

Animal cell	Plant Cell
Animal cells are usually smaller is size.	Plant cells are usually larger in size.
Cell wall is completely absent.	Presence of cell wall is a characteristic feature of plant cell.
Cellulose in any form is not present.	Cell wall is made up of cellulose.
Cytoplasm of animal cells is dense, more granular and it occupies most of the space in the cell.	In a plant cell, cytoplasm is pushed to the periphery of the cell. The cytoplasm forms a thin lining against the cell wall.
Vacuoles are absent usually. If present, they are small organelles, they are temporary and they serve as organelles for excretion or secretion.	Vacuoles are prominent and large organelles in the plant cell. One or more vacuoles may be present. The central space in the cell may be occupied by a large single vacuole.
Plastids are absent.	Plastids are present. They may of three types chromoplasts, chloroplasts and leucoplasts.
Centrosome is present.	Centrosome is absent in plant cells. Instead of centrosome, there are two small clear areas called polar caps are present.
Golgi complex is prominent and highly complex, it is present near the nucleus of the cell.	Golgi apparatus is present in form subunits called dictyosomes.

PRACTICE QUESTIONS

1. Which among the following is an example for streptobacilli?
(A) Streptococcus pneumonia
(B) Vibrio cholera
(C) Bacillus anthracis
(D) Streptobacilli pyrogenes

2. The basic difference between gram positive and gram negative bacterium is-
(A) Gram positive have high lipid that retain grams stain
(B) Gram negative have low lipid content
(C) Gram negative high lipid contents looses dye when treated with alcohol, gram positive have low lipid hence retain color and do not loose dye
(D) Gram negative high lipid contents looses dye when treated with alcohol, gram positive have low lipid and thick peptidoglycan layer that prevents penetration of solvent and hence dye is retained.

3. RNA polymerase in bacteria is responsible for synthesis of –
(A) mRNA and t-RNA (B) m RNA. t-RNA and r-RNA
(C) t-RNA and r-RNA (D) m-RNA and r-RNA

4. The rotor of the flagellar motor is driven by _____.
(A) Proton gradient (B) ATP (C) GTP (D) Creatine phosphate

5. Which among the following statements is false regarding chromatin?
(A) It is of two types- euchromatin and heterochromatin
(B) Euchromatin is involved in phenotypic expression
(C) Chromatin takes dark stain and is inert
(D) Heterochromatin is involved in phenogenesis

6. Centrioles have microtubules and they have triplets of Tubules A, B and C which are made up of____ and ____ protofilaments in them.
(A) 13 in tubule A and 10 in tubules B and C
(B) 13 in tubule B and 10 in tubules A and C
(C) 13 in tubules A, C and 10 in tubule B
(D) 10 in tubule A and 13 in tubules B and C

7. Match the nutritional type in microorganisms with representative microorganism

a.	Photolithotroph	1.	Nitrifying bacteria
b.	Photoorganotroph	2.	Purple non sulfur bacteria
c.	Chemolithotrophs	3.	Few pathogenic protozoan's
d.	Chemoorganotroph	4.	Cyanobacteria

(A) a - 1 , b - 2 , c - 3 , d- 4 (B) a - 4 , b - 3 , c - 2 , d- 1
(C) a - 3 , b - 2 , c - 1 , d- 4 (D) a - 4 , b - 2 , c - 1 , d-3

8. The following statements are referring to which term among the following.
(a) stimulate ethylene synthesis;
(b) induce phytoalexin synthesis;
(c) induce chitinase and other enzymes;
(d) increase cytoplasmic calcium levels and
(e) cause an "oxidative burst".

(A) Antibodies (B) Cell signaling (C) Cell wall (D) Interferons

9. The plasma membrane is made up of lipid and proteins. It is made up of three classes of lipids- phospholipids, glycolipids and sterols.
The phospholipids and glycolipids have double bonds which are in _____ configuration.
(A) Cis only (B) Cis and Trans (C) Trans only (D) No double bonds exist

10. The four major phospholipids associated with plasma membrane are-
(A) Phosphatidylinositol, phosphatidylethanolamine, phosphatidylserine, and sphingomyelin
(B) Phosphatidylcholine, phosphatidylamine, phosphatidylserine, and sphingomyelin
(C) Phosphatidylcholine, phosphatidylethanolamine, phosphatidylthreonine, and sphingomyelin
(D) Phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, and sphingomyelin

ANSWER KEYS

1	C	**2**	D	**3**	B	**4**	A	**5**	D	**6**	A	**7**	D
8	C	**9**	A	**10**	D								

EXPLANATIONS

1. Bacillus anthracis is an example for chain of bacilli

2. The difference in both the cell type is due to the lipid content. High lipid content in Gram negative bacterial cell wall gets dissolved due to alcohol and causes leaching of stain. Gram positive bacteria have a peptidoglycan layer that prevents penetration of solvent inside the cell

3. RNA polymerase present in cytosol forms mRNA, tRNA and rRNA. mRNA formed by transcription from nuclear material is directly ready for translation by ribosomes of cytosol.

4. The flagellar motor is driven by energy stored in the transmembrane proton gradient.

5. The chromatin has two forms: 1. Euchromatin- which is well dispersed form of chromatin which takes lighter DNA stain and is genetically active (involved in gene duplication and transcription) and phenogenesis (Phenotypic expression). 2. Heterochromatin: This is highly condensed from of chromatin that takes dark stain and is genetically inert.

6. Centrioles are cylindrical and are open at both the ends usually, unless they carry a cilium or flagellum. The wall of centrioles has nine groups of microtubules arranged in a circle and each group is called as a blade which is a triplet of A, B and C tubules. Tubule A has 13 protofilaments, while tubules B and C have 10 protofilaments

7.
(A) Photolithotroph - Cyanobacteria
(B) Photoorganotroph - Purple non sulfur bacteria
(C) Chemolithotrophs - Nitrifying bacteria
(D) Chemoorganotroph - Few pathogenic protozoan's

8. Cell wall serve a variety of functions including:
(a) stimulate ethylene synthesis;
(b) induce phytoalexin (defense chemicals produced in response to a fungal/bacterial infection) synthesis;
(c) induce chitinase and other enzymes;
(d) increase cytoplasmic calcium levels and

(e) cause an "oxidative burst". This burst produces hydrogen peroxide, superoxide and other active oxygen species that attack the pathogen directly or cause increased cross-links in the wall making the wall harder to penetrate.

9. The phospholipids and glycolipids have fatty acids containing even number of carbon atoms (16 to 20) and are saturated or unsaturated fatty acids. The configuration of double bonds is always "cis". The length of the fatty acid and the degree of unsaturation affects the membrane fluidity. The plasma membranes of animal cellscontain four major phospholipids (phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, and sphingomyelin), which together account for more than half of the lipid in most membranes.

4. CELL CYCLE AND ITS REGULATION

CELL CYCLE AND ITS REGULATION

" Where a cell arises, there must be a previous cell, just as animals can only arise from animals and plants from plants." This is the cell doctrine, proposed by the German Pathologist Rudolf Virchow in 1858. This is achieved by way of cell division. All living organisms, right from bacteria to multicellular animals are products of cell growth and division whose beginning extends back to 3 billion year ago. Cells reproduce by initially duplicating their content and then dividing into two. This cycle of duplication and division is called cell cycle.in unicellular organisms, cell cycle results in the production of a complete new organism, while in multicellular organisms, it is highly complex and is essential for functioning of the organism. Millions of cells are to be synthesized in us to replace dead cells for our survival.

Certain characteristics of cell cycle are however universal, i.e., to pass the genetic information to the next generation of cells. For this the DNA in each chromosome is first replicated to produce identical copies, and then these duplicated copies of chromosomes are to be segregated equally to the daughter cells (Figure 4.1). However eukaryotes have evolved a complex system comprising of many proteins to regulate the cell cycle which is known as the "Cell-cycle control system". It comprises of biochemical switches that control main events of cell cycle from signals both from inside as well as outside the cells. It involved controlling progression inside the cells and at times delays progression into next phase until the previous events are completed. If this cell-cycle control system fails, it can result in cancer.

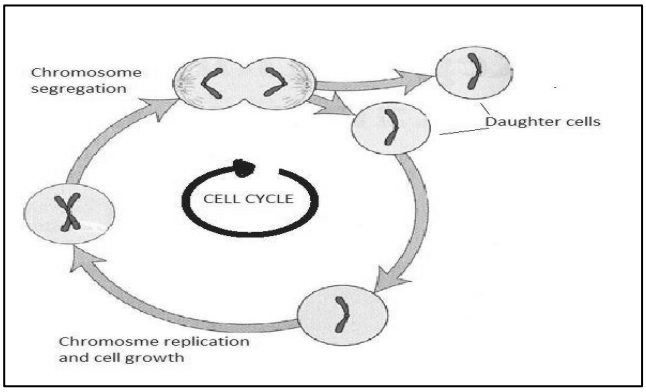


Figure 4.1: The cell cycle

An overview of Cell Cycle:

The basic function of Cell cycle is to duplicate DNA in the chromosome accurately and its segregation into two genetically identical daughter cells. Hence cell cycle has two important phases: S-phase (synthetic phase) during which DNA duplication occurs and it occupies about half of the cell cycle in a typical mammalian cell. After S-phase, chromosome segregation and cell division occurs in M-phase (M for Mitosis) which requires much less time.

M-phase involves packaging of duplicated DNA into chromosomes, their condensation, and break down of nuclear envelope and replicated copes of chromosomes appear each consisting of a pair of sister chromatids. These are found attached to mitotic spindle. As mitosis proceeds during metaphase, chromosomes get aligned at the equator of the mitotic spindle, poised for segregation. In Anaphase sudden separation of sister chromatids takes place, where they move to opposite poles. This is followed by decondensation of chromosomes and reformation of Nuclei. The cells are now pinches to undergo division of cytoplasm (cytokinesis) which completes cell division (Figure: 4.2).

Before duplication of DNA, a much longer gap exists for growth and accumulation of proteins which is called as gap phase. This includes G_1 phase and G_2phase, where in G_1 is between M phase and S phase and G_2 is between S phase and mitosis (Figure 4.3). Thus eukaryotic cell cycle comprises of four sequence of phases: G_1, S, G_2 and M phases. The G_1, S and G_2 together are called the interphase. A typical human cell cycle, if it occurs in 24 hours, interphase occupies 23 hours and mitosis occurs in 1 hour. If extracellular conditions are unfavorable, the cell cycle is delayed and cells enter into a specialized resting state known as G_o phase which can be for few hours, weeks to years, until the onset of favorable conditions

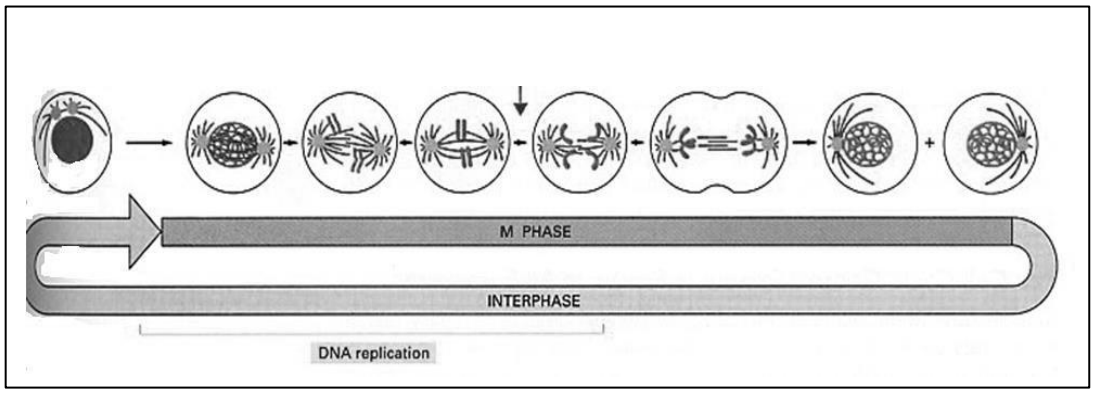


Figure 4.2: Events of Eukaryotic cell division

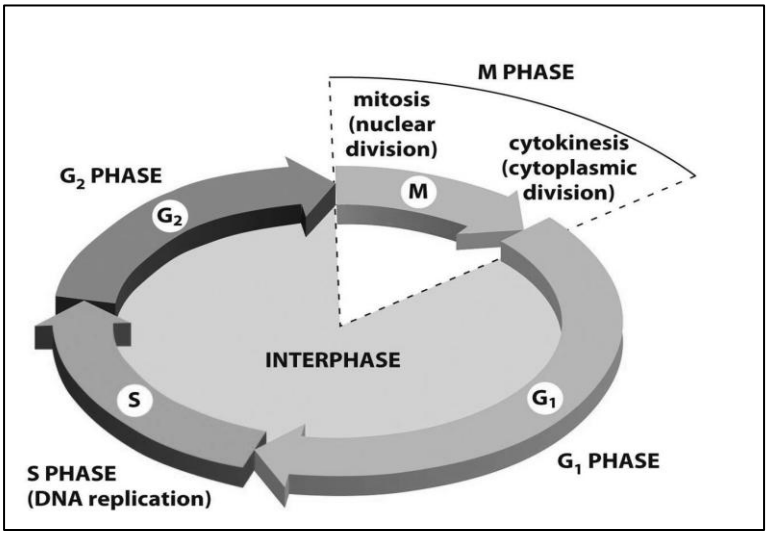


Figure 4.3: Phases of Cell Cycle

Components of Cell Cycle control system:

The cell cycle control system operates much like the control system of a washing machine. This control system in cells must have the following features.

- A clock that turns on each event at a specific timer this providing a fixed amount of time for the completion of each event
- A mechanization for initialing events in the correct order i.e., entry into mitotic phase after DNA replication is over.
- Mechanization to ensure that each stage is triggered only once for cycle.
- Binary (on/ off) switches thattrigger events in a complete, irreversible fashion.
- Robustness. Backup mechanism to ensure that the cell cycle works properly even when parts of the system mall function.
- Adaptability so that the system behavior can be modified to suit specific cell types or environmental conditions.
- Hence a cell cycle has several control systems to monitor its smooth preogression.

1. **Check Point: T**hese are the points at which cycle can be arrested if previous events have not been completed. The two prominent check points are the G_1check point, the G_2 check point and
   metaphase check point (Figure 4.4).

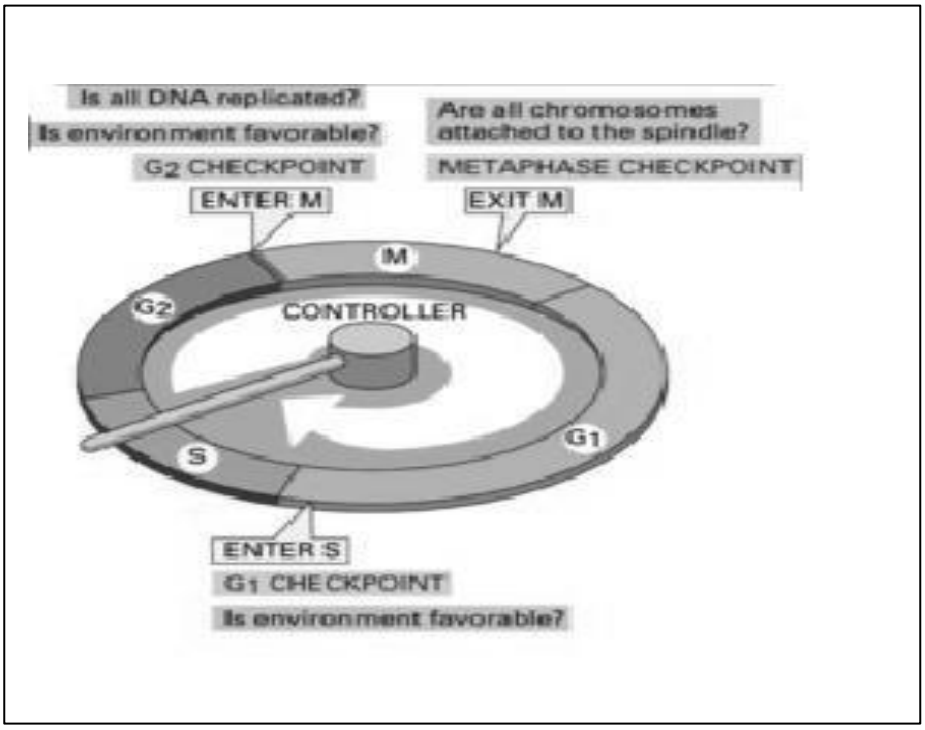


Figure 4.4: The control of the Cell cycle

Progression into M phase is delayed at G_1 and G_2 check point if DNA is damaged by radiation or chemicals. At this stage of delay DNA is repaired and then released for next phase for progression. Metaphase check point operates through next intracellular signals. It maintains attachment mitotic spindle to chromosomes. A cell has many chromosomes, and if chromosome sends a positive signal to cell cycle control system after attachment of mitotic spindle is attached. It becomes difficult to identify the fraction of positive signal to be sent by a chromosome left out with spindle unattached. On the other hand, if unattached chromosome send a negative signal to inhibit progression of cell cycle, it will be easily detected by the cell cycle and its progression stops.

2. **Cyclically activated Protein Kinase:** The heart of cell cycle is the family of protein kinases known as Cyclin-dependent kinases (Cdks). Activity of these rises and falls as the cell progresses through the cell cycle, by their phosphorylation. They mainly regulate DNA replication, mitosis and cyt okinesis.

   Eg: Increased phosphorylation of Cdks leads to chromosome condensation, nuclear envelope break down and spindle assembly before mitosis.

Cyclical changes in Cdk activity are controlled by a complex array of enzymes and other proteins. Most important of Cdk regulators are cyclins. These are named so because they undergo a cycle of synthesis and degradation in each cell cycle while Cdk levels remain constant. Cyclical changes in cyclin levels result in the cyclic assembly and activation of cyclin-Cdkcomplexes and this activation triggers Cell-cycle events (Figure: 4.5).

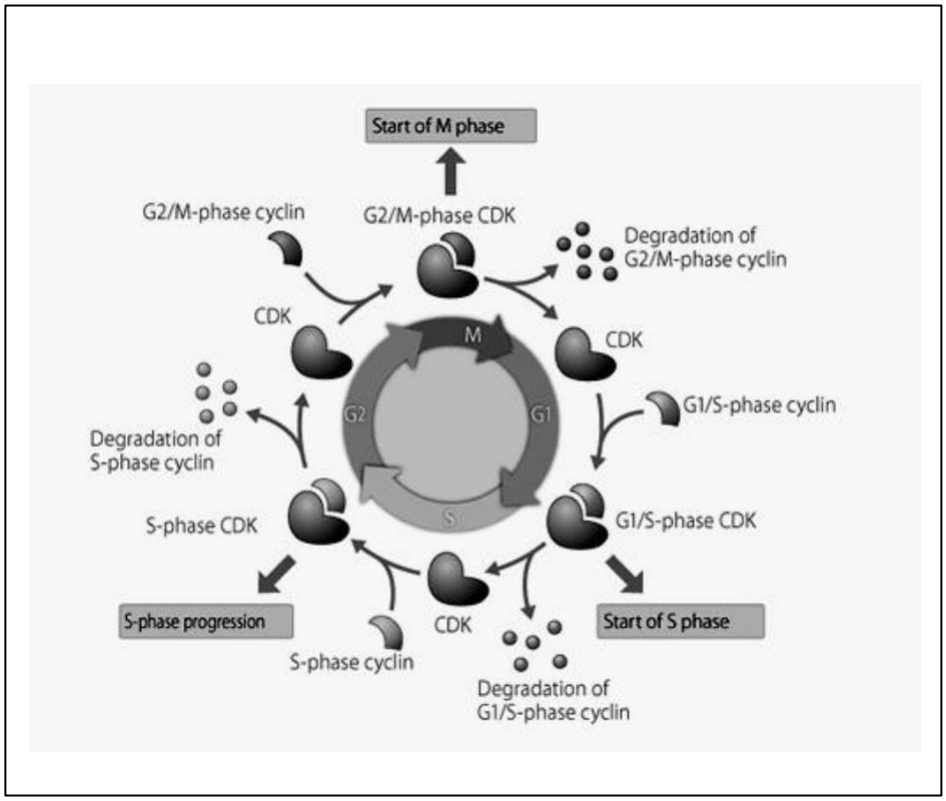


Figure 4.5: Simplified view of cell cycle control system by Cdks

There are four classes of cyclins each defined by the stage of the cell cycle which they bind Cdks and function. Three of these classes are required in all eukaryotic cells:

1. **G_1/S – cyclins:** They bind Cdks at the end of G_1 and commit the cell to DNA replication.
2. **S- cyclins:** they bind Cdks during S phase and are required for the initiation of DNA replication.
3. **M-cyclins:** they promote the events of mitosis.

   The fourth class of cyclins, the G_1 – cyclins, helps promote passage through the start or the restriction point in late G_1 phase. In yeast cells, a single

Cdk protein binds all classes of cyclins, while in vertebrates there are four Cdks. Two interact with G_1-cyclins, one with G_1/S and S-cyclins and one with M-cyclin (Table -1).

Table.1 Table indicating cyclins in vertebrates

Cyclin- dependent Kinases	Vertebrates	
	Cyclins	**CDK partner**
G_1-Cdk	Cyclin D	Cdk4, Cdk6
G_1/S Cdk	Cyclin E	Cdk2
S Cdk	CyclinA	Cdk2
M Cdk	Cyclin B	Cdk 1

The activity of Cdk in association with cyclins is revealed by studying its 3d structure. In this absence of Cdk, cyclins exist in an inactive state and the active site region is blocked by a region of protein (Eg: T-loop in Cdk2). Binding of cyclin causes T-loop to move out of the active site, resulting in its partial activation. While phosphorylation by Cdk- activating kinase (CAK) at a threonine residue near the entrance of Cdk active site improves the ability of Cdk to bind to its target site(figure 4.6).

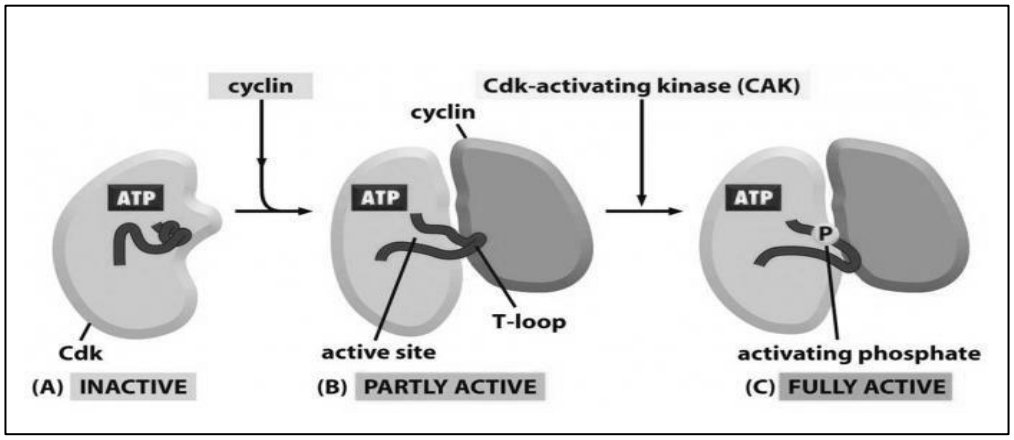


Figure 4.6: Structural basis of Cdk activation

Suppression of Cdk activity:

Additional mechanism exists to regulate Cdk activity during cell cycle. The activity of cyclin-Cdk complex can be inhibited by phosphorylation at a pair of amino acids in the roof of its active sire by a protein kinase known as Wee 1. While dephosphorylation of the same by a Phosphatase known as Cdc25

increases Cdk activity (Figure 4.7). the cyclin-Cdk complex activity can also be regulated by the binding of Cdk inhibitor proteins (CKIs). These are primarily employed in control of G_1 and S phase and they dramatically rearrange the structure of Cdk active site (figure 4.8).

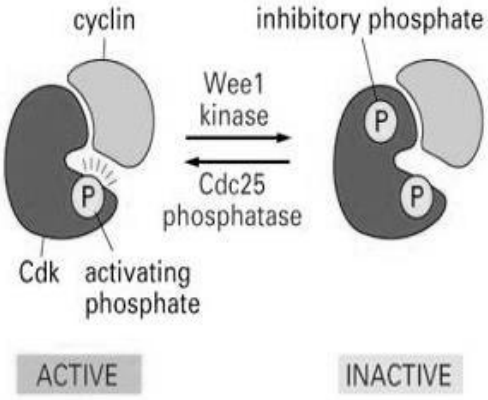


Figure 4.7: Regulation of Cdk activity of cell activity by inhibitory phosphorylation

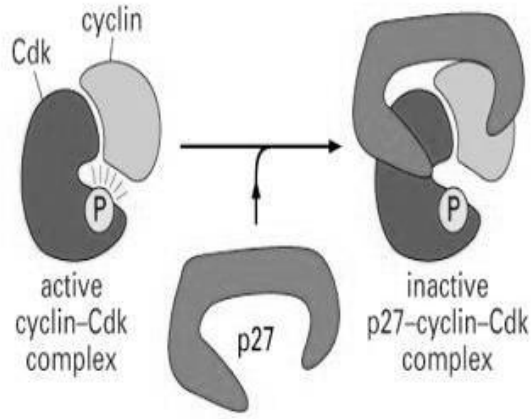


Figure 4.8: Inhibition of cyclin-Cdk complex by CKI

3. **Cyclical Proteolysis:** Cell cycle control depends on atleast two distinct enzyme complexes that act at different times in the cell cycle to cause proteolysis of key proteins and there by inactivating them. The cyclins are proteolyzed by a Ubiquitin-dependent mechanism, like that involved in the proteolysis of many other intracellular proteins. An activated enzyme complex recognizes specific Amino acid sequences on the cyclin and attaches multiple copies of Ubiquitin to it, thereby marking the protein for complete destruction in

Proteosomes. The rate- limiting step in cyclin destruction is the final Ubiquitin-transfer reaction catalyzed by enzymes known as Ubiquitin ligases (figure 4.9).

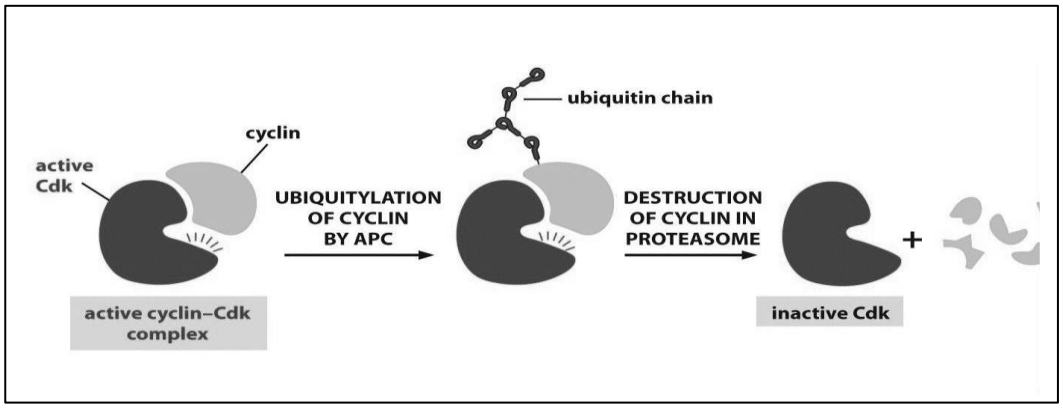
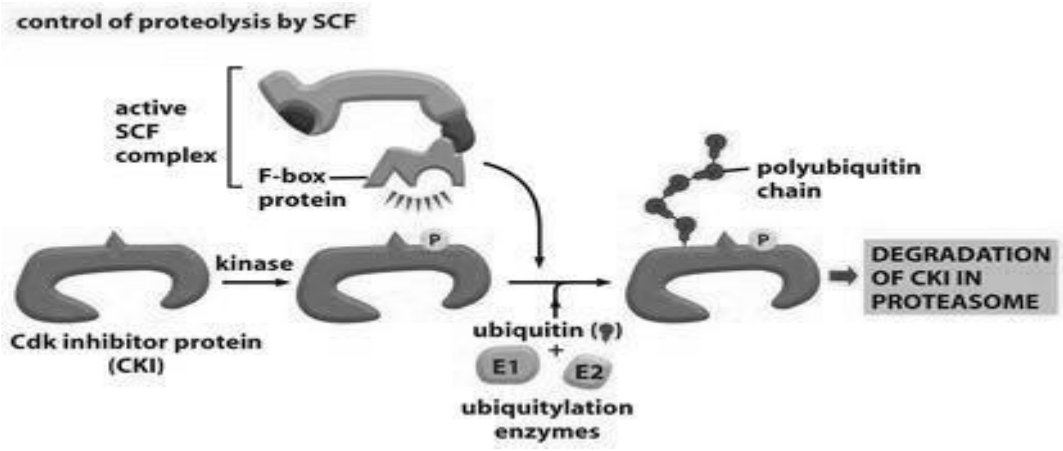


Figure 4.9: Ubiquitin based proteolysis of cyclins

Two Ubiquitin ligases are important in the destruction of cyclins and other cell regulations. In $G_1$and S phase, an enzyme complex called SCF is responsible for ubiquitylation and destruction if G_1/S-cyclins and certain CKI proteins that control S – phase initiation. In M- phase, the anaphase-promoting complex (APC) is responsible for Ubiquitylation and proteolysis of M-cyclins and other regulators of histones (Figure 4.10). APC has two large multi subunit complexes, containing same related components as SFC, but they are regulated in different ways. SCF activity is constant during the cell cycle. Ubiquitylation by SCF is controlled by changes in the phosphorylation state of its target proteins. Only specific phosphorylated proteins are recognized, Ubiquitylated and destroyed by APC activity, by contrast changes at different stages in the cell cycle. APC is turned on mainly by the addition of activating subunits to the complex.



SCF along with E1 and E2 serves as Ubiquitin ligase APC activated by Cdc20 related proteins along with E1 and E2 acts as ubiquitin ligase

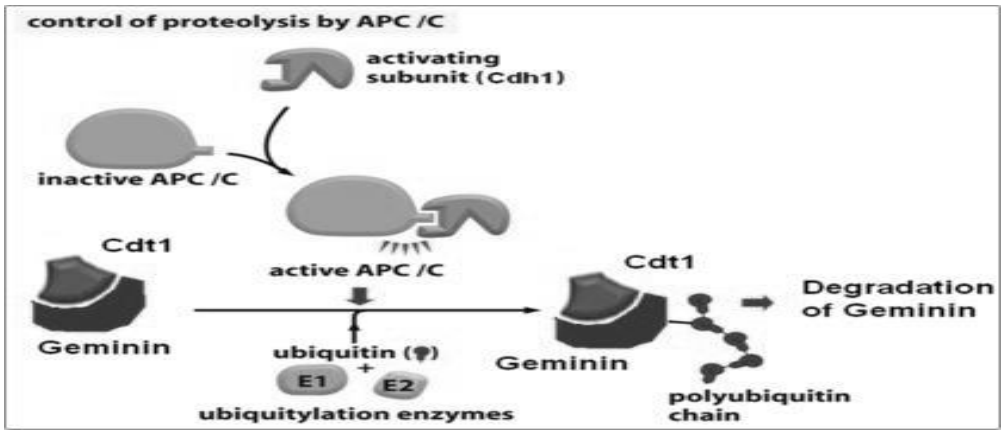


Figure 4.10: Control of Proteolysis by SCF and APC during cell cycle

INTRACELLULAR CONTROL OF CELL – CYCLE EVENTS

Each of the different Cyclin- Cdk complexes serves as a molecular switch that triggers specific cell cycle event.

1. S-phase cyclin-Cdk complexes (S-Cdks) initiate DNA replication once per cycle:

Studies in yeast have revealed that DNA replication begins at Origins of replication. A large, multiprotein complex known as The Origin Recognition Complex (ORC), which binds to Origin of replication and served as landing pad for several other regulatory proteins was identified.

ORC remains associated with the replication origin throughout the cell cycle. In early G phase, Cdc associates with ORC. Aided by Cdc6, Mcm ring complexes then assemble on the adjacent DNA resulting in the formation of the pre-replicative complex. The S-Cdk triggers origin firing, assembling DNA polymerase and other replications proteins and activating the MCM protein rings to migrate along DNA strands as DNA helicase. The S-Cdk also blocks re-replication causing the deassociation of Cdc 6 from origins, its degradation, and the export of all excess MCM out of nucleus. Cdc 6 and MCM cannot return to reset an ORC- containing origin of another round of DNA replication until M-Cdk has been inactivated towards the end of mitosis (Figure 4.11).

Thus several cyclin- Cdk complexes cooperate to restrain pre-RC assembly and prevent DNA replication.

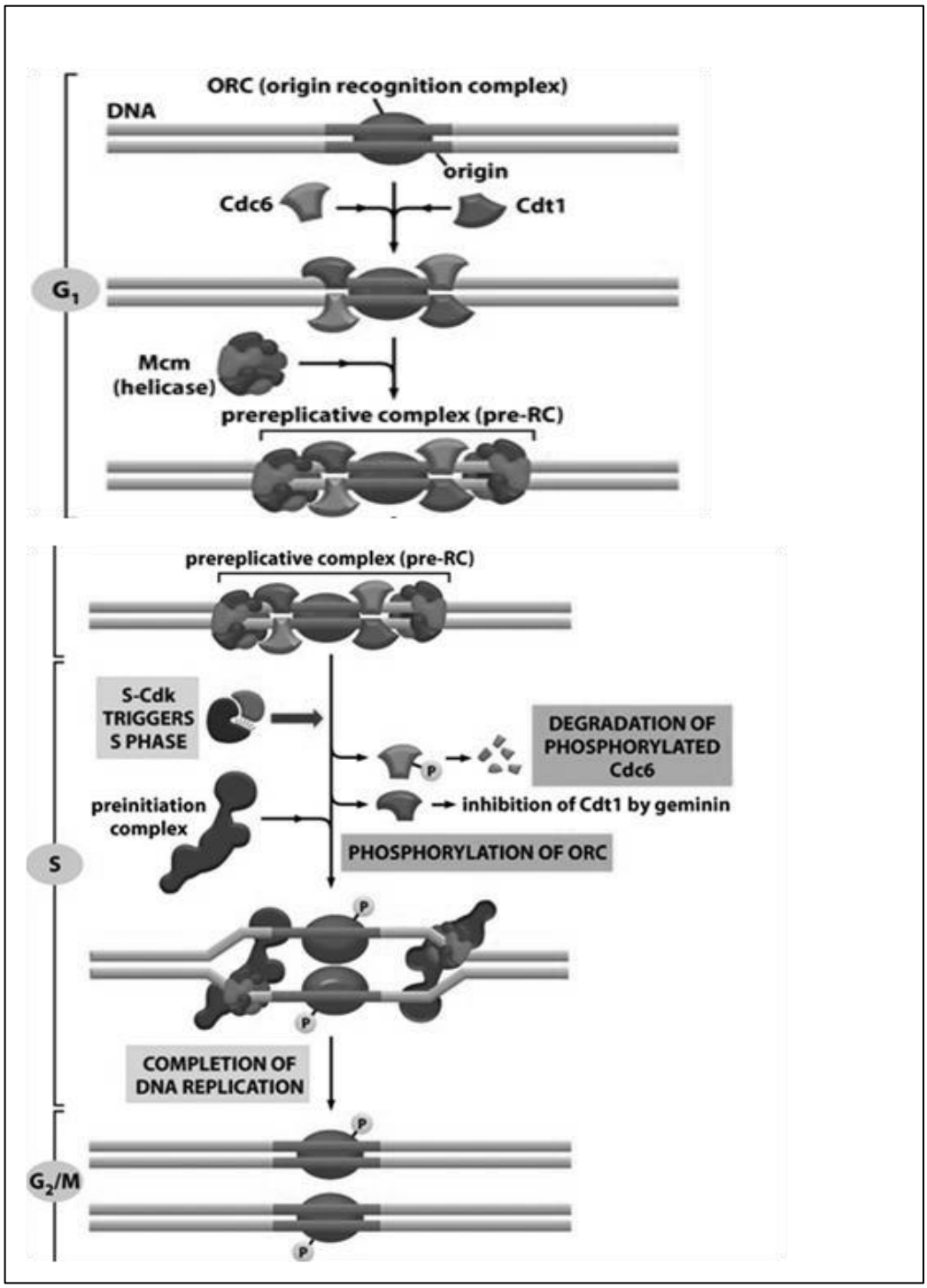


Figure 4.11: The initiation of DNA replication once per cell cycle by Cdc6 and Mcm rings

2. The Activation of the M-phase Cyclin-Cdk complexes (M-Cdks) triggers entry into matrix:

The events of mitosis are triggered by M-Cdks, which are activated after S-phase is completed. Activation of M-Cdk begins with the accumulation of M-Cyclin after its synthesis during G_2 phase. Thus accumulation leads to gradual accumulation of M-Cdk as the cell approaches mitosis. This inactive M-Cdk stock pile is activated in late G_2 phase by protein phosphatase Cdc25, which removes inhibitory phosphates from inactive M-Cdks. At the same time activity of inhibitory kinase Wee1 activity is suppressed ensuring M-Cdk to gain activity. Two protein kinases activate Cdc25:

1. Polokinase that phosphorylates Cdc25 at the end of the sites, and
2. Activating kinase of M-Cdk itself, which phosphorylates a different set of sites of Cdc 25. M-Cdk also phosphorylates and inhibits Wee 1 (figure 4.12).

The ability of M-Cdk to activate its own activation (Cdc25) and inhibit its own inhibitor (Wee1) indicates that M-Cdk activation in mitosis involves a positive feedback loop.

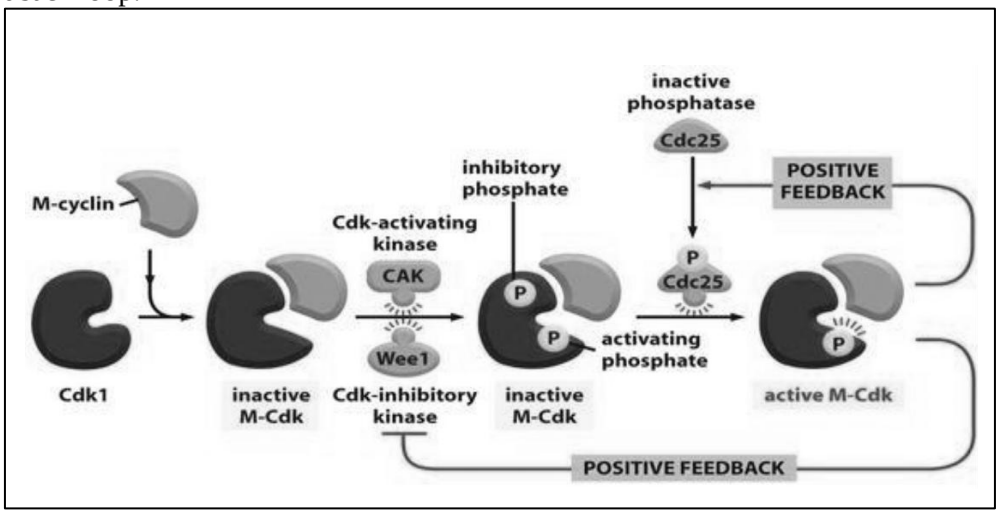


Figure 4.12: Activation of M-Cdk

3. The DNA replication check Point:

Entry into mitotic phase is blocked by incomplete DNA replication, else it would be a disaster leading to inheritance of incomplete sets of chromosomes by the daughter cells. Sensor mechanisms of unknown molecular nature, detect either the unreplicated DNA or the corresponding unfinished replication forks and send a negative signal to the cell cycle control system, blocking the activation of M-Cdk.

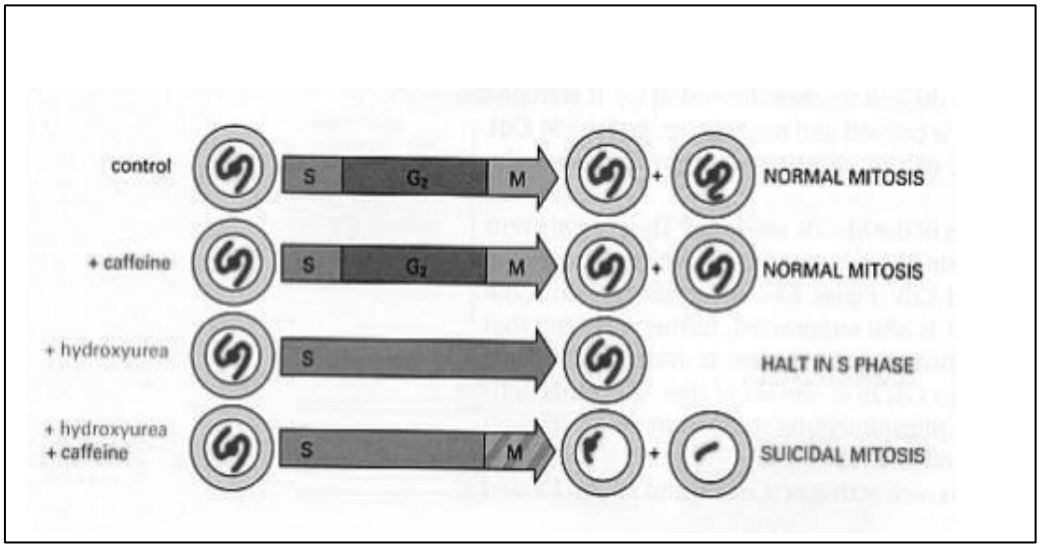


Figure 4.13: DNA replication in cells treated with caffeine and Hydroxyl urea indicating S phase check point

This figure shows studies of mammalian cells treated with caffeine and Hydroxylurea either alone or in combination. This block activities of Hydroxy urea (stops DNA synthesis) activities. Check point mechanism that arrests cells in S phase delaying mitosis. But if caffeine is added with hydroxyl urea, check point mechanism fails, cells proceed into mitosis and results in incomplete DNA replication. The final targets of the negative check point signals are the enzymes that control M-Cdk activation. The negative signal activates a protein kinase that inhibits Cdc25 protein phosphatase. As a result, M-Cdk remains phosphorylated and inactive until DNA replication is completed.

4. M-Cdk prepares the Duplicated chromosomes for separation:

M-Cdks bring about diverse functions and complex arrangements that occur in the early stages of mitosis. M-Cdk must induce the assembly of the mitotic spindle and ensure that replicated chromosomes attach to the spindle. In many organisms they trigger chromosome condensation, nuclear envelope breakdown, actin cytoskeleton rearrangement and the reorganization of Golgi apparatus and Endoplasmic reticulum. In each of these events M-Cdks phosphorylates the respective regulatory proteins.

Eg 1: Breaking down nuclear envelope: Phosphorylation of the polymerized lamin filament (That makes up nuclear envelope and makes it rigid) by M-Cdk results in their depolymerization which is the first step in breakdown of nuclear envelop.

Eg 2: Condensation of chromosomes in Xenopus, requires a complex of fine proteins. Phosphorylation of this condensing by M-Cdk promotes coiling of DNA molecules.

Eg 3: Spindle formation requires assembly of microtubules. Phosphorylation of large number of proteins that regulate microtubule behavior is by M-Cdk.

5. Sister chromatid separation is triggered by Proteolysis:

After M-Cdk has triggered the complex rearrangements that occur in early mitosis, the cell cycle reaches a stage where sister chromatids separation has to take place during the transition called Metaphase – to- Anaphase transition. This is by Anaphase promoting complex (APC).

The sister chromatids are bound tightly by complex of proteins- The Cohesion complex that deposits along the chromosomes in S phase. anaphase must involve disruption of this cohesion such that they move to opposite poles of the spindle. This process is initiated by a remarkable cascade of signaling events. It requires action of APC enzyme complex whose target is the protein Securin. Securin binds to protease Separase and prevents cleavage of Cohesion complex binding the sister Chromatids.

Events of APC activation require protein Cdc 20. As such Cdc20 concentration increases, as cell approaches mitosis. Thus phosphorylation of APC binds Cdc 20 to APC. This Cdc20-APC complex causes proteolysis of Securin, releasing Separase which helps in separation of sister chromatids (Figure 4.14).

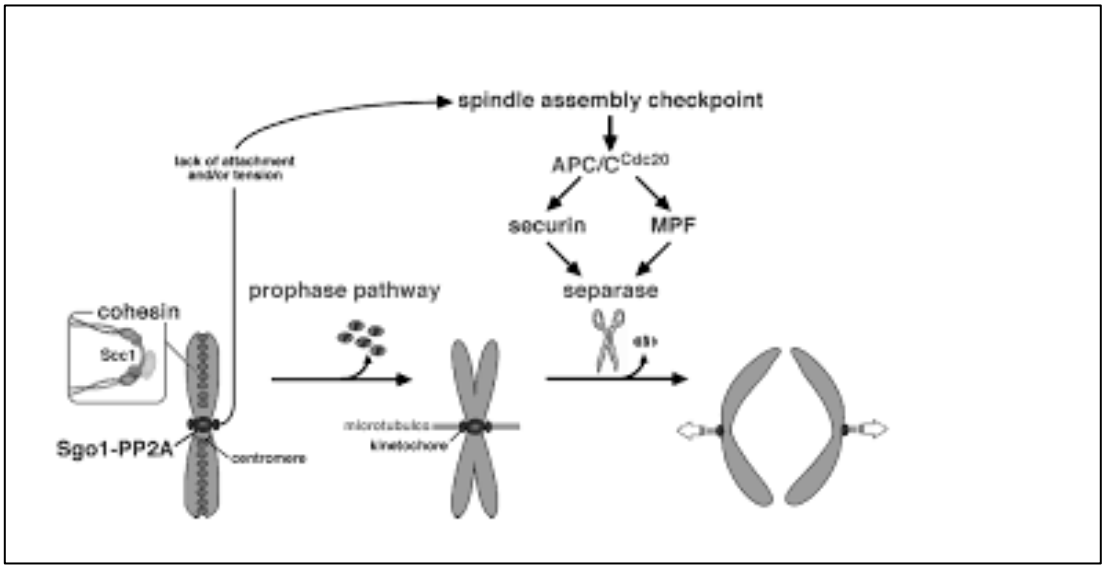


Figure 4.14: Separation of sister chromatids triggered by proteolysis

6. The spindle Attachment checkpoint:

In most cells, this check point operates to ensure that all chromosomes are properly attached to the spindle before the sister chromatids separate. The sensor mechanism monitors the state of the Kinetochore, to which microtubules or spindle attaches. Single kinetochores recruit Mad2 and allow their binding. Binding of spindle releases Mad2, and if any kinetochore is still left withMad2 it inhibits Cdc20 –APC activity and destruction of Securin.

7. Exit from Mitosis requires the inactivation of M-Cdk:

Mitosis exit must involve disassembly of spindle, decondensation of chromosomes and reformation of nuclear envelop. These are the reverse of events triggered by M-Cdk. Hence inactivation of M-Cdk has to take place which is mainly by Ubiquitin- dependent proteolysis (figure 4.15). Ubiquitylation of cyclins is usually triggered by Cdc20 – APC complex that promotes destruction of Securin, promotion from metaphase to Anaphase and also inactivation of M-Cdk.

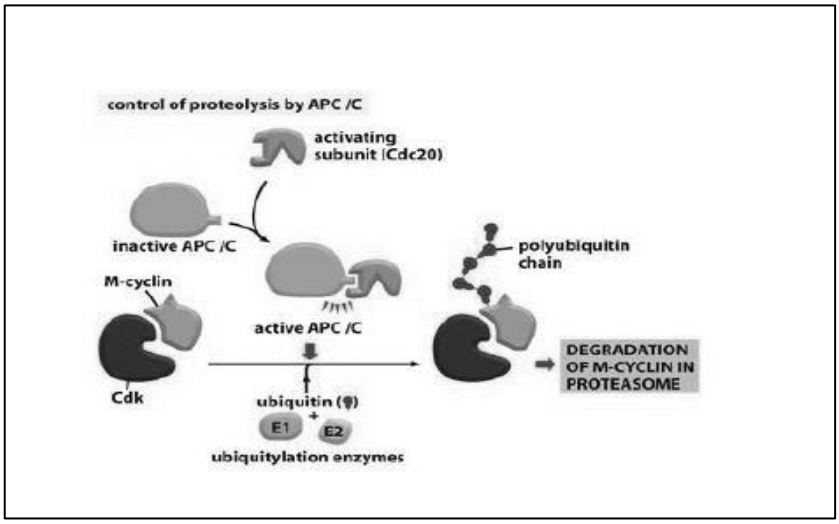


Figure 4.15: Degradation of M- cyclins

8. M-Cdk activity inhibition promotes cells into G_1 phase:

Cells adopt several mechanisms to ensure that Cdk reactivation is prevented after mitosis. One mechanism makes use of another APC-activating protein called Hct 1, a close relative of Cdc 20. The Hct 1- APC complex is inhibited by M-Cdk, which directly phosphorylates Hct1. As a result of this, Hct 1-APC activity increases in late mitosis after the Cdc20-APC complex has initiated destruction of M-Cyclins. Therefore M-cyclin destruction continues after mitosis, although Cdc-20- APC activity has declined, Hct 1-APC activity is high (figure 4.16).

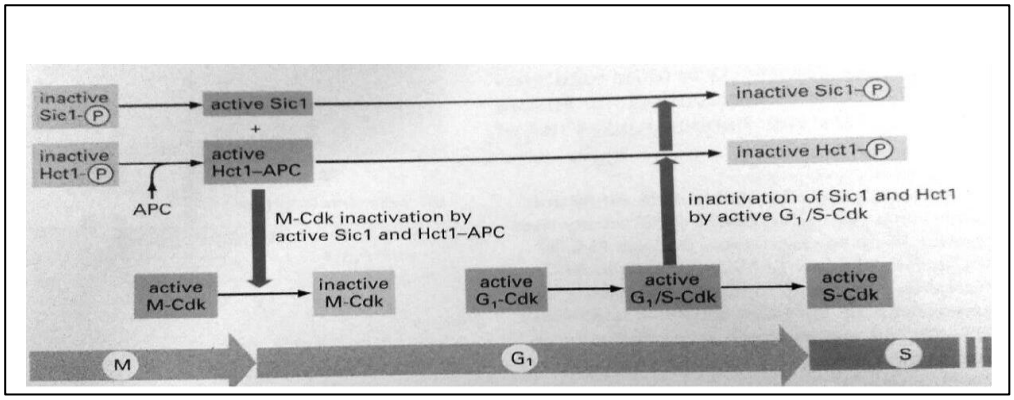


Figure 4.16: The control of G1 progression by Cdk activity

Second mechanism is that which suppresses Cdk activity in G_1 phase, which depends on increased production of CKI s. The Cdk inhibitory protein, like Hct1, Sic 1 is inhibited by M- Cdk which phosphorylates Sic 1 during mitosis. M-Cdk also phosphorylates and inhibits a gene regulatory protein required for Sic1 synthesis. This Sic1 and M-Cdk, like Hct 1 and M-Cdk mutually inhibit each other. Late mitosis triggers rapid accumulation of Sic 1 protein, and the CKI helps to ensure that M-Cdk activity is stably inhibited.

Then How do cells enter S Phase?? Hct 1- APC activation, CKI accumulation and decrease cyclin production ensure cell progression into G_1 phase and Cdk activity is suppressed.

Cells start accumulation of G_1 – cyclins which is stimulated by the extracellular signals that promote cell proliferation. Accumulation of G_1 cyclins leads to an increase in G_1- Cdk activity. In yeast, G_1- Cdk activity triggers transcription of G_1/S – cyclin genes leading to increased synthesis of G_1/S – Cdl complexes. This initiates events that commit to enter S-phase. it stimulates transcription of S-cyclin genes, resulting in synthesis of S-cyclins and formation of S- Cdk complexes. These complexes are inhibited by Sic 1, but G_1/S- Cdk phosphorylates and inactivates sic 1, thereby counting S-Cdk activation. G_1/S- Cdk and S-Cdk also phosphorylates and inactivates Hct 1 – APC. Thus the same feedback loops that trigger rapid M-Cdk inactivation in late mitosis now work in reverse at the end of G_1 phase to ensure the rapid and complete activation of S-Cdk activity (figure 4.16).

In mammalian cells: The transition from G_1 to S phase in mammals is mediated by a gene regulatory protein called E2F. E2F binds to specific DNA sequences in the promoter regions of genes that encode for S-phase entry including

G_1/S cyclins and S- cyclin. Function of E2F is controlled by Retinoblastoma protein (Rb), an inhibitor of cell cycle progression.

In G_1 phase, Rb binds to E2F blocking the transcription of S-phase genes. Accumulation of active G_1- Cdk due to stimulus by external factors, it phosphorylates Rb, reducing affinity for E2F. E2F release activates gene expression of S-phase genes (Figure 4.17).

Loss of Rb gene leads to excessive cell proliferation in the immature retina (eye cancer) known as Retinoblastoma.

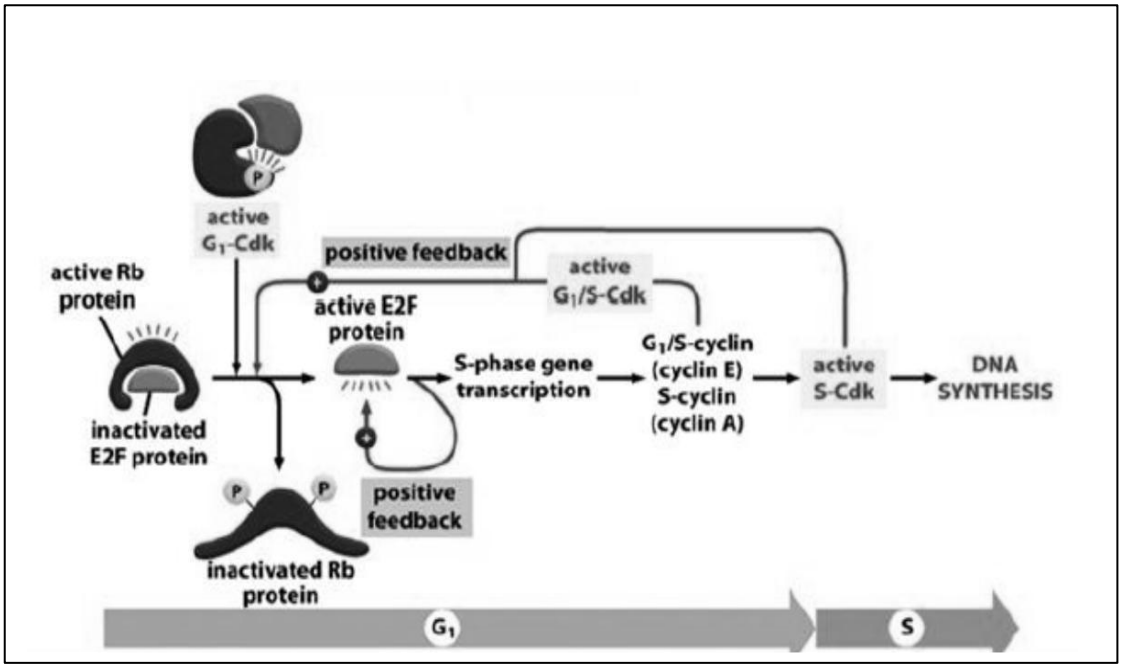


Figure 4.17: Mechanism controlling S-phase initiation in animalcells.

Cell cycle Progression and Cell Growth:

Cell growth depends on nutrients and growth signals from environment. The length of time depends on nutrients availability. This coordination of cell cycle is based on nutrient availability and is attributed to the total amount of G_1 cyclins called Cln3. Increase in Cln3 stimulates cell division, even if cells are small. As cells grow, the total number of Cln3 molecules increase in parallel with total cell protein. When cells are small a Cln3 binding protein inactivates its activity. As cell grows and reaches its threshold size, the number of Cln3 increase along with Cln3 binding protein. When cell exceeds its size, free Cln3 exist, which can bind to Cdk and initiate a new cell cycle (figure 4.18).

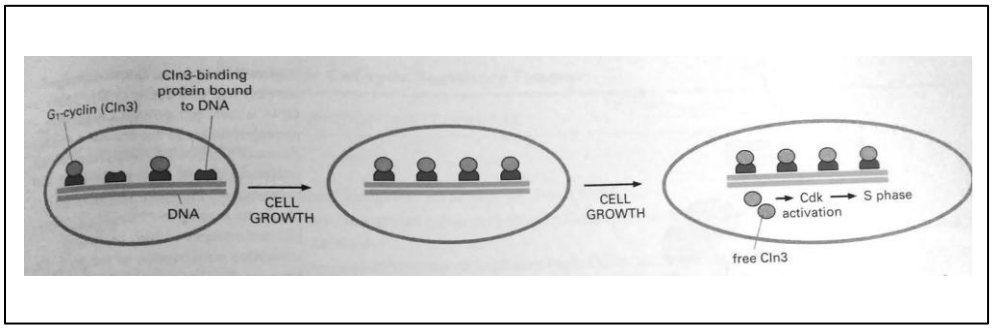


Figure 4.18: Regulation of cell cycle progression and cell growth by Cln3

Blocking of Cell cycle progression by damaged DNA and p53:

This is DNA damage check point. There are 2 such check points – one at late G_1 phase which prevents entry into S phase and the other at late G_2, which prevents entry into mitosis. A G_1 check point, DNA damage leads to activation of the gene regulatory protein p53, which stimulates transcription of many genes, of which CKI protein called p21, that binds to G_1/S – Cdk and S-Cdk and inhibits cell activities thereby blocking entry into S phase.

p53 operates by indirect mechanism. In undamaged cells, p53 is highly unstable and is present in low concentrations. It interacts with Mdm2, that acts as a Ubiquitin ligase and targets p53 for destruction by proteosomes. DNA damage activates protein kinase which phosphorylates p53 that reduces binding to Mdm 2. Increase in p53, stimulates gene transcription (figure 4.19) of p21 that prevents entry into S phase.

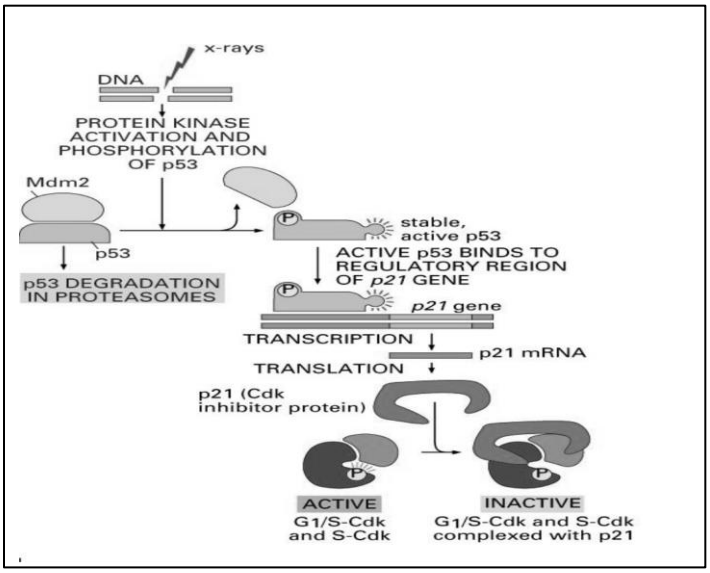
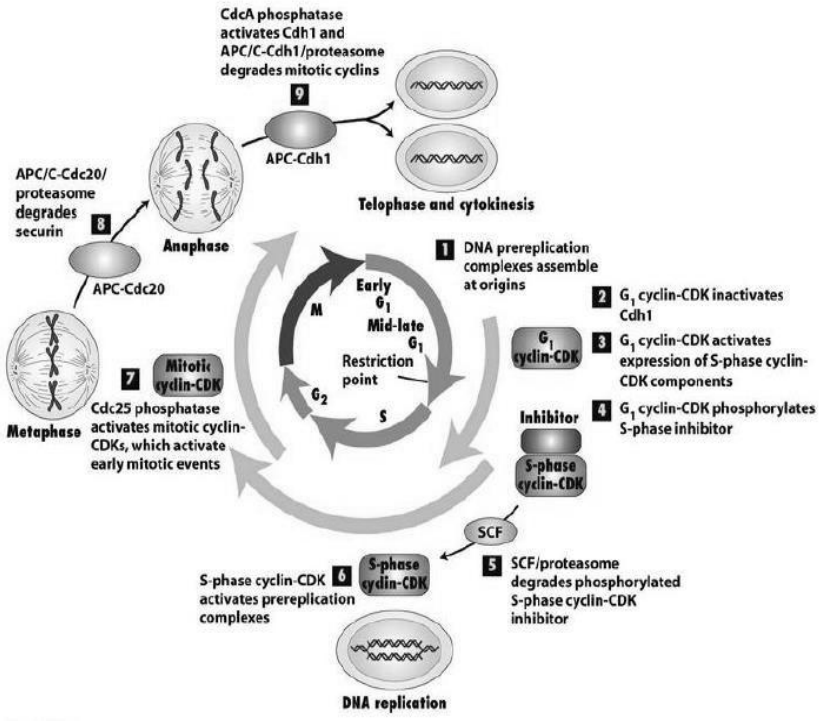


Figure 4.19: Blocking cell cycle by damaged DNA and p53

Summary of cell cycle and its control system:



PRACTICE QUESTIONS

1. Which among the following statements defines, Cell Cycle?

   (A) It comprises of switches that control main events of cell cycle from signals from inside the cells. It involves controlling progression inside the cells and generally advances progression into next phase despite the stage of previous event.

   (B) It comprises of biochemical switches that control main events of cell cycle from signals both from inside as well as outside the cells. It involved controlling progression inside the cells and at times delays progression into next phase until the previous events are completed.

   (C) It comprises of DNA switches that control main events of cell cycle from signals both from inside as well as outside the cells. It control only first event and wait for its completion.

   (D) It comprises of feed back units that control main events of cell cycle from signals outside the cells alone. It has no involvement in controlling progression of phases, but operates by unknown mechanisms.

2. The sequence of events in Eukaryotic cell cycle are

(A) G_1, S, M, G_2 phases
(B) G_0, G_1, S, M, G_2 phases
(C) G_1, G_2, S, M phases
(D) G_1, S, G_2 , M phases

3. The Metaphase check point operation is as follows-

(A) Waits for chromosomes to unwind
(B) Operates on negative feed back signal given by chromosomes that do not have spindle fibre attached to their centromere
(C) Operates on positive signal given by chromosomes that do not have spindle fibre attached to them.
(D) Waits till chromosomes are placed along the equatorial plate

4. What is the role of S-Cyclins?

(A) Bind Cdk and help synthesis of proteins
(B) Bind Cdks during S phase and are required for the initiation of DNA replication
(C) Bind Cdk and regulate cell growth
(D) None of the above

5. Mechanism by which p53 regulates cell cycle -

(A) Bind to DNA and inhibits DNA replication
(B) Binds to all damaging agents that damage DNA
(C) its increase stimulates proteosomes and prevents cell cycle progression
(D) Increase in p53, stimulates gene transcription of p21 that prevents entry into S phase

6. Match Cyclins and their CDK partners

Cyclins	CDK partner
a. Cyclin D	**1**. Cdk 1
b. Cyclin E	**2.** Cdk 2
c. Cyclin A	**3.** Cdk 4
d. Cyclin B	**4.** Cdk 6

(A) a-3, 4 , b - 2 , c - 2, d – 1 (B) a- 4, b - 3, c -2 , d - 1
(C) a- 3, b -4 , c – 1, 2, d - 3 (D) a- 4, b - 1, c - 2, d – 3

7. The G_1/S cyclins and M-cyclins are ubiquitylated by _____ and _____ respectively.
(A) CKI and SCF (B) SCF, CKI and APC
(C) SCF and APC (D) APC and CKI

8. Which among the following statements are true?
(a) S-Cdks can initiate DNA replication based on circumstances any number of times
(b) S-Cdks initiate DNA replication once per cell cycle
(c) Aided by Cdc6, Mcm ring complexes forms pre-replicative complex
(d) Pre-replicative complex formation is at ORC
(e) In early G phase Cdc associates with ORC
(f) In late Gphase Cdc associates with ORC
(A) b, c, d, e (B) a, c, d, f (C) b, c, d,e (D) a, c, d, e

9. Role of M-Cdks is-
(a) Induces assembly of mitotic spindle
(b) Ensures that replicated chromosomes are attached to spindle
(c) Triggers Chromosome condensation
(d) Contributes to break down of Nuclear envelop
(A) a, b, d (B) a, b (C) b,c, d (D) All of the above

10. E2F is a gene regulatory protein that binds to specific DNA sequences in promoter regions of genes that encode for _____ phase.
(A) G_1 Phase (B) S-phase (C) G_2 phase (D) M phase

11. The function of E2F is controlled by _____, an inhibitor of cell cycle progression
(A) p53 (B) CKI (C) RIST protein (D) Retinoblastoma protein

ANSWER KEYS

1	B	**2**	D	**3**	B	**4**	B	**5**	D	**6**	A	**7**	B
8	C	**9**	D	**10**	B	**11**	D						

EXPLANATIONS

1. Cell cycle - It comprises of biochemical switches that control main events of cell cycle from signals both from inside as well as outside the cells. It involved controlling progression inside the cells and at times delays progression into next phase until the previous events are completed.

2. The sequence of four phases in a eukaryotic cell cycle are- G_1, S, $G_2$and M phase.

3. M phase check point operates based on negative feed back signal given by chromosomes that do not have spindle attached them. Hence the progression into M phase is delayed in G_1 phase itself.

4. **S- cyclins:** they bind Cdks during S phase and are required for the initiation of DNA replication.

5. p53 operates by indirect mechanism. In undamaged cells, p53 is highly unstable and is present in low concentrations. It interacts with Mdm2, that acts as a Ubiquitin ligase and targets p53 for destruction by proteosomes. DNA damage activates protein kinase which phosphorylates p53 that reduces binding to Mdm 2. Increase in p53, stimulates gene transcription of p21 that prevents entry into S phase.

6.

Cyclins	**CDK partner**
A. Cyclin D	**3,4.** Cdk 4, Cdk 6
B. Cyclin E	**2.** Cdk 2
C. Cyclin A	**3.** Cdk 2
D. Cyclin B	**4.** Cdk 1

7. In G1and S phase, an enzyme complex called SCF is responsible for ubiquitylation and destruction if G1/S-cyclins and certain CKI proteins that control S – phase initiation. In M-phase, the anaphase-promoting complex (AP(C) is responsible for Ubiquitylation and proteolysis of M-cyclins and other regulators of histones.

8. S-phase cyclin-Cdk complexes (S-Cdks) initiate DNA replication once per cycle.

Studies in yeast have revealed that DNA replication begins at Origins of replication. A large, multiprotein complex known as The Origin Recognition Complex (OR(C), which binds to Origin of replication and served as landing pad for several other regulatory proteins was identified.
ORC remains associated with the replication origin throughout the cell cycle. In early G phase, Cdc associates with ORC. Aided by Cdc6, Mcm ring complexes then assemble on the adjacent DNA resulting in the formation of the pre-replicative complex. The S-Cdk triggers origin firing, assembling DNA polymerase and other replications proteins and activating the MCM protein rings to migrate along DNA strands as DNA helicase. The S-Cdk also blocks re-replication causing the deassociation of Cdc 6 from origins, its degradation, and the export of all excess MCM out of nucleus. Cdc 6 and MCM cannot return to reset an ORC-containing origin of another round of DNA replication until M-Cdk has been inactivated towards the end of mitosis

9. M-Cdk is multifunctional and does all of the above

10. The transition from G1 to S phase in mammals is mediated by a gene regulatory protein called E2F. E2F binds to specific DNA sequences in the promoter regions of genes that encode for S-phase entry including G1/S cyclins and S- cyclin. Function of E2F is controlled by Retinoblastoma protein (R(B), an inhibitor of cell cycle progression. Loss of Rb gene leads to excessive cell proliferation in the immature retina (eye cancer) known as Retinoblastoma.

5. CELL - CELL COMMUNICATION

CELL- CELL COMMUNICATION

Cell communication is required for governing the activities of a multicellular organism. These communication mechanisms depend on the extracellular signal molecules produced by the neighboring cells. Organisms have developed specific signals to communicate to the cells to act in a specified way. These are proteins which include intracellular or extracellular signal molecules and cell surface receptors. Among the intracellular signaling proteins, which bind the signal molecules that distribute the signal to appropriate parts of the cell. The intracellular signaling proteins include kinases, Phosphatases, GTP binding proteins and many other proteins with which they interact and alter pathways. Depending on the signals effect, the target proteins can be gene regulatory proteins, Ion channels, components of a metabolic pathway, part of a cytoskeleton etc (figure 5.1).

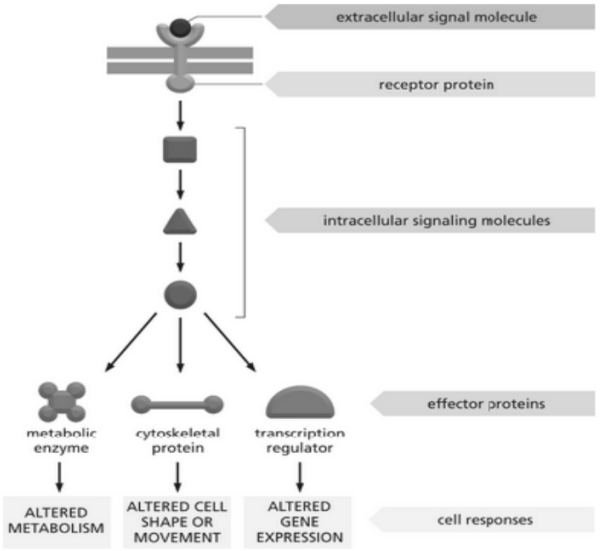


Figure 5.1: Overview of Intracellular signaling pathways

General Principles of Cell Communication

1. **Extra cellular signal molecules bind to specific receptors:** Higher animal communicate by means of hundreds of signal molecules. These include proteins, small peptides, amino acids, nucleotides, steroids, retinoids, fatty acid derivatives, dissolved gases like nitric oxide and carbon monoxide. These are secreted by signaling cells into extracellular space byexocytosis. Most

signal molecules are hydrophilic and hence they cannot cross membrane by diffusion, while few small ones do cross by diffusion.

Regardless of the nature of signal molecules, target cells respond by expressing specific binding proteins called receptors. These signals are produced at very low concentrations of ~10^{-6}M, with a high affinity constant $K_\alpha \geq 10^8$ liters per mole. Binding of signal to receptor generates a cascade of intracellular signals that alter the behavior of cells. Few signals are to penetrate the cells and bind to intracellular receptors; such signals are usually small, hydrophobic and can cross plasma membrane (figure 5.2).

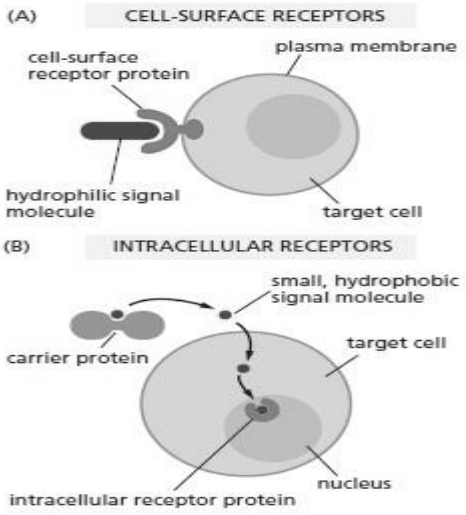


Figure 5.2: Extra cellular molecules and types of receptors

2. **Forms of intracellular signals:** There are four different types of intracellular signaling (Figure 5.3):
   - **(a) Contact dependent signaling:** Important during development and in immune responses. The secreted signals molecules are carried far afield to act on distant targets.
   - **(b) Paracrine signaling:** These affect the cells in the immediate environment and act as local mediators. These act on cells around, i.e., onto their proper target cells and must not diffuse too far. These are taken up immediately by cells, and are also destroyed by extracellular enzymes or enzymes present in the extracellular matrix.
   - **(c) Synaptic signaling:** These are short range signals and play specific role in cell communication. These are seen in specialized cells and have evolved

to communicate with parts of body. Eg: nerve impulse transmission by the neurotransmitter acetylcholine across synaptic space.

(d) Endocrine signals: These are specialized signals that control behavior of the organism. These signals are called hormones or endocrine secretions which travel through blood stream to reach target cells.

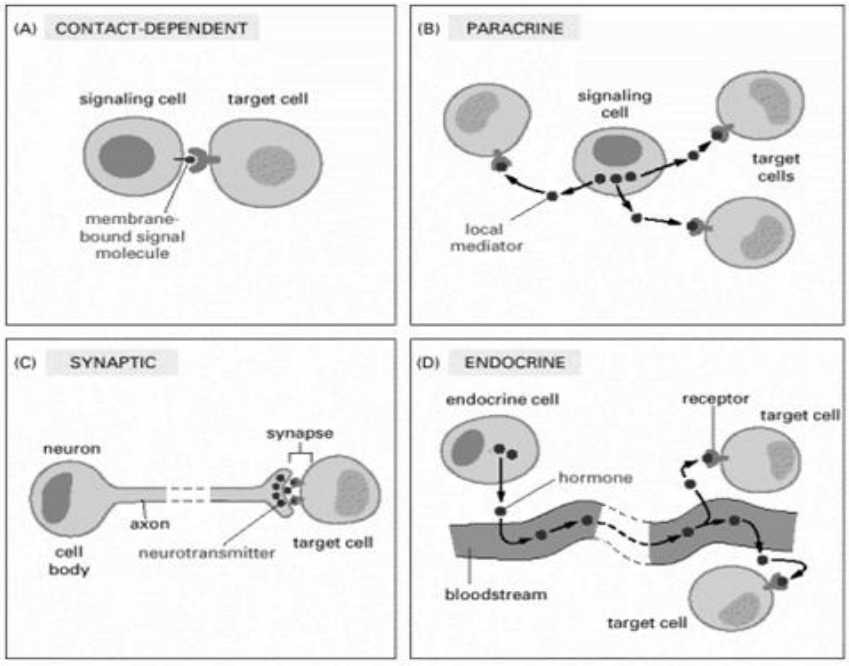


Figure 5.3: Forms of Intracellular signaling

3. Autocrine signaling: Signals sent by cells onto cells of same type and also for themselves are called autocrine signals. Eg: The cells during development if they have to take up a pathway for differentiation the cells secrete autocrine signals to reinforce themselves for developmental activities. Autocrine signals also stimulate cells of similar type (identical cells) which can respond to differentiation inducing signals (figure 5.4).

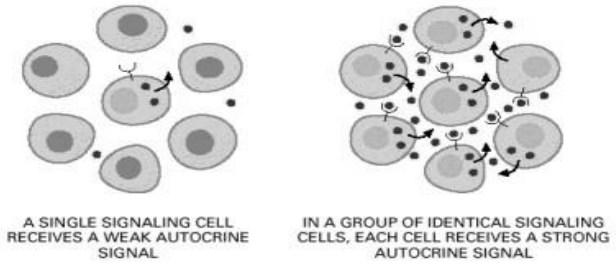


Figure 5.4: Autocrine signaling

4. **Role of gap junctions:** These are specialized cell-cell junctions that are formed between closely apposed plasma membranes. These are water filled channels that connect adjacent cells and allow exchange of small intracellular signals Ca^{2+} and cyclic AMP to pass through (Figure 5.5).

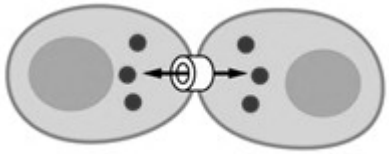


Figure 5.5: Signaling via gap junctions

5. **Cells respond to several signals:** Cells in a multicellular organism are exposed to different signals. They have the ability to respond to them differently and every response is programmed, leading to execution of specialized functions (figure 5.6). They have set of receptors that bind to signal molecules. Invitro, if cells are deprived of these signals, they undergo apoptosis.

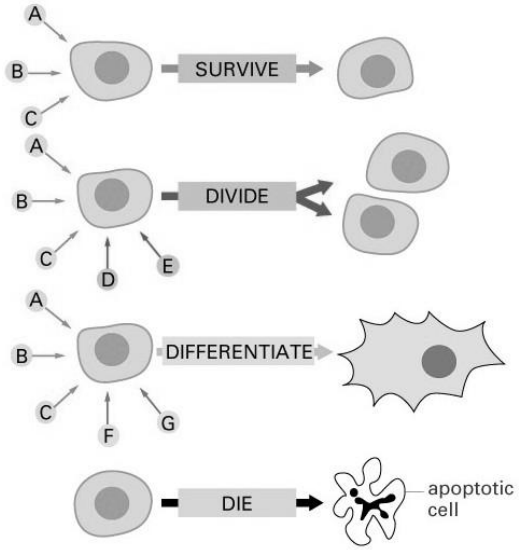


Figure 5.6: Animal cell dependence on multiple signals for survival

6. Concentration and life span of signals: These two depend upon the necessity of their persistence within the cells. Few signal molecules presence is necessary for long duration as in the case of cells in the stages of development. In an adult tissue, responses to fade and signals have to cease after certain span. There is rapid turnover of signal molecules, as in case of nerve impulse transmission, acetylcholine is destroyed by acetylcholine esterase and fresh

signal at axon causes release of new molecules of acetylcholine to transmit the signal to muscle cells, if not the muscle would be left in a continuous state of contraction. They are released in micro to nanomolar concentrations and destroyed every time. Few proteins (intracellular) serve for long duration ($\leq$ 10 minutes) as they have key regulatory roles.

7. **Some signals directly bind to target proteins:** Nitric oxide gas signal binds to guanylyl cyclase. Acetylcholine released by nerve ending activates NO synthase in the endothelial cells of blood vessels to produce NO. Nitric oxide diffuse out of the endothelial cells into the underlying smooth muscles, binds to guanylyl cyclase to produce cyclic GMP. This triggers relaxation of blood vessels and enhances flow of blood through them (figure 5.7). The life span of NO is very short and it is converted into nitrates and nitrites by oxygen and water. Even Carbon Monoxide is another gas signal that is used as intercellular signal to stimulate guanylyl cyclase.

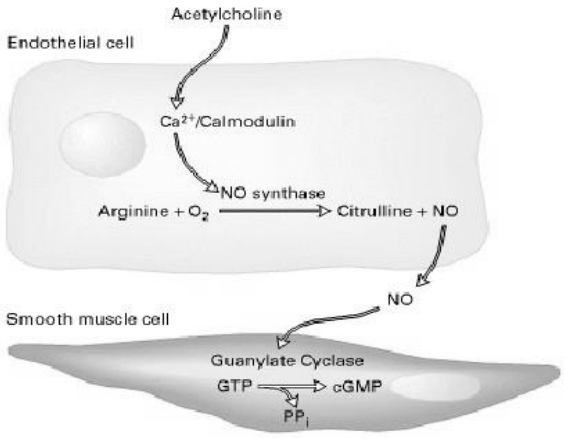


Figure 5.7: Responses induced by acetyl choline via NO resulting in smooth muscle contraction

8. **Signals that bind to nuclear receptors:** Some signal molecules like steroid hormones, thyroid hormones, retinoids and vitamin D, bind to receptors which are part of Nuclear Receptor Super Family. They bind to receptor proteins, activate them to bind to DNA to regulate transcription of specific genes.

   Eg 1: Steroid hormones – cortisol(figure5.8): It is produced by cortex of adrenal gland and influences metabolism in various ways. It is located in cytosol and enters nucleus after binding to nuclear hormone receptor protein.

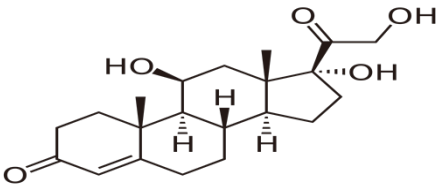


Figure 5.8: Structure of cortisol

These DNA binding proteins are either homodimers or heterodimers. Binding of ligand to the inactive receptor changes its conformation, this involves dissociation of inhibitory complexes from them. Ligand binding promotes binding of receptor to coactivator protein, then binding to Receptor's transcription activating domain, which increases gene transcription (figure 5.8).

This response induced by activation of Nuclear hormone receptors occurs in two phases- Early primary response and delayed secondary response. Primary response lasts for around 30 minutes and results in protein products synthesis, which in turn activates other genes to produce their products (secondary response) (figure 5.9).

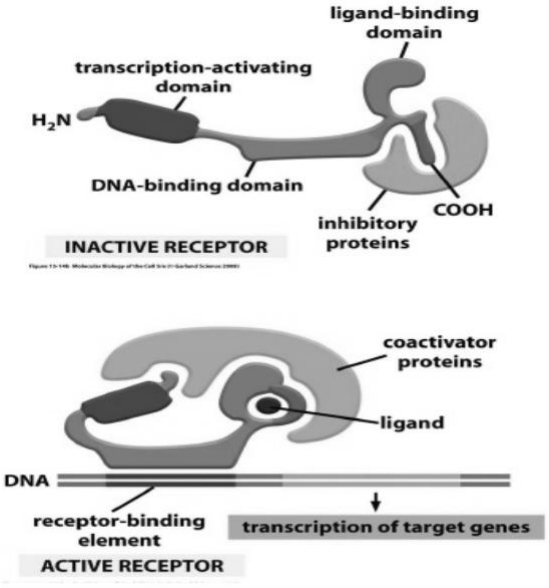


Figure 5.9: Nuclear Receptor Binding protein stages of activity

9. Classes of cell surface receptors:

There are three major classes of cell surface receptors (figure 5.10):

(i) Ion – channel – linked receptors

(ii) G- Protein linked receptors

(iii) Enzyme linked receptors

(i) **Ion-channel linked receptor:** These are known as Transmitter-gated ion channels/ Ionotrophic receptors. They are involved in rapid synaptic signaling between electrically excitable cells. This is mediated by few neurotransmitters that transiently open/close ion channels formed by proteins to which they bind.

(ii) **G-Protein linked receptors:** These regulate the activity of separate plasma membrane bound target proteins, which can be enzymes or Ion channels. The interaction between receptor and target proteins is mediated by a trimeric GTP-binding protein(G-protein).

(iii) **Enzyme –linked receptors:** These when activated , function either directly as enzymes or are directly associated with enzymes that they activate.

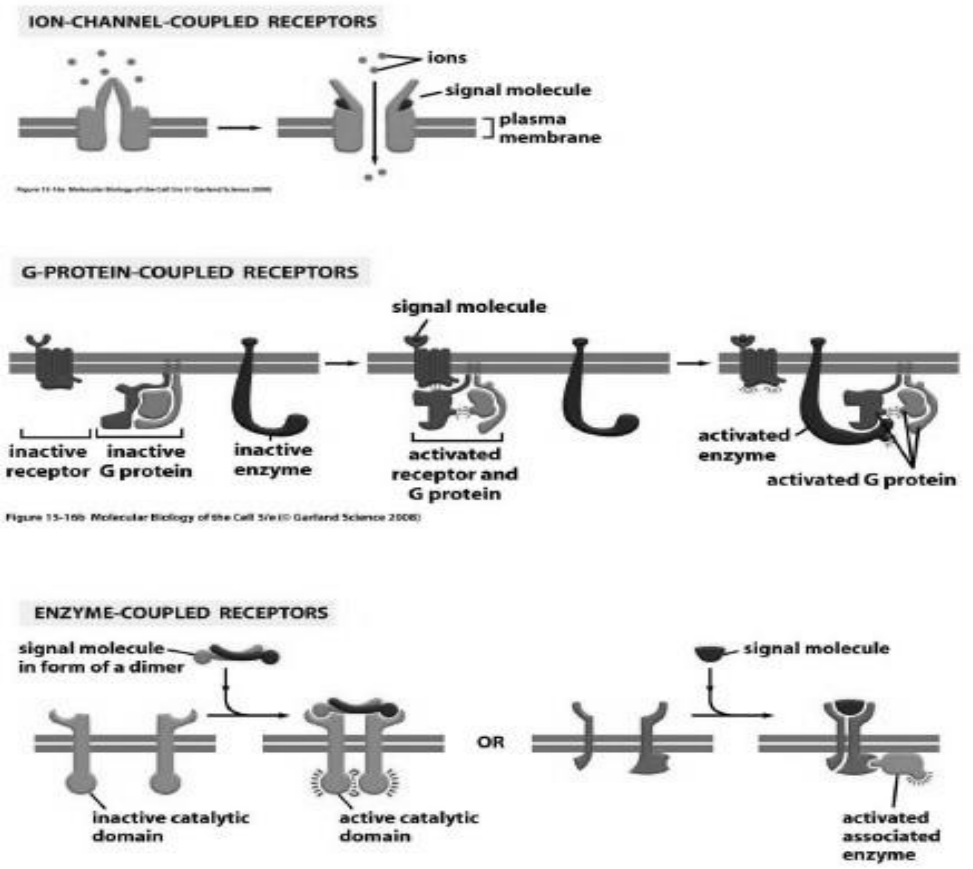


Figure 5.10: Three major classes of cell surface receptors

10. **Intra cellular Signaling Proteins:** The intracellular mediators or secondary messengers are produced by the binding of G-protein linked or Enzyme- linked receptor binding to their receptors. The secondary messengers are generated in

large numbers in response to receptor activation and they rapidly diffuse from source. Eg: Cyclic AMP and Ca^{2+}, which are water soluble and diacyl glycerol which is lipid soluble and diffuses even along plasma membrane.

Intra cellular signaling proteins act in a cascade manner. A series of signaling proteins and small intracellular mediators relay the extracellular signal into the cell causing a change in gene expression (figure 5.11).

Proteins involved in intracellular signaling are categorized as follows:

1. **Relay Proteins:** They simply pass the message to the next signaling component in the chain.
2. **Messenger Proteins:** Carry signal from one part of the cell to another, such as from cytosol to the nucleus.
3. **Adaptor Proteins:** Link one signaling protein to another, without themselves conveying a signal.
4. **Amplifier Proteins:** These are usually enzymes or ion channels, that amplify the signal they receive either by producing large amounts of small intracellular mediators or by activating large number of downstream intracellular signaling proteins. When there are multiple amplification steps in a relay chain it is referred to as Signaling cascade.
5. **Transducer Proteins:** they convert the signal into different form. The enzyme that makes cyclic-AMP- it converts the signal and amplifies it (Transducer + amplifier).
6. **Bifurcation Proteins:** Spread the signal from one signaling pathway to another.
7. **Integrator Proteins:** Receive signals from two or more signaling pathways and integrate them before relaying a signal onward.
8. **Latent gene Regulatory Proteins:** they are activated at the cell surface by activated receptors and then migrate to the nucleus to stimulate gene transcription.

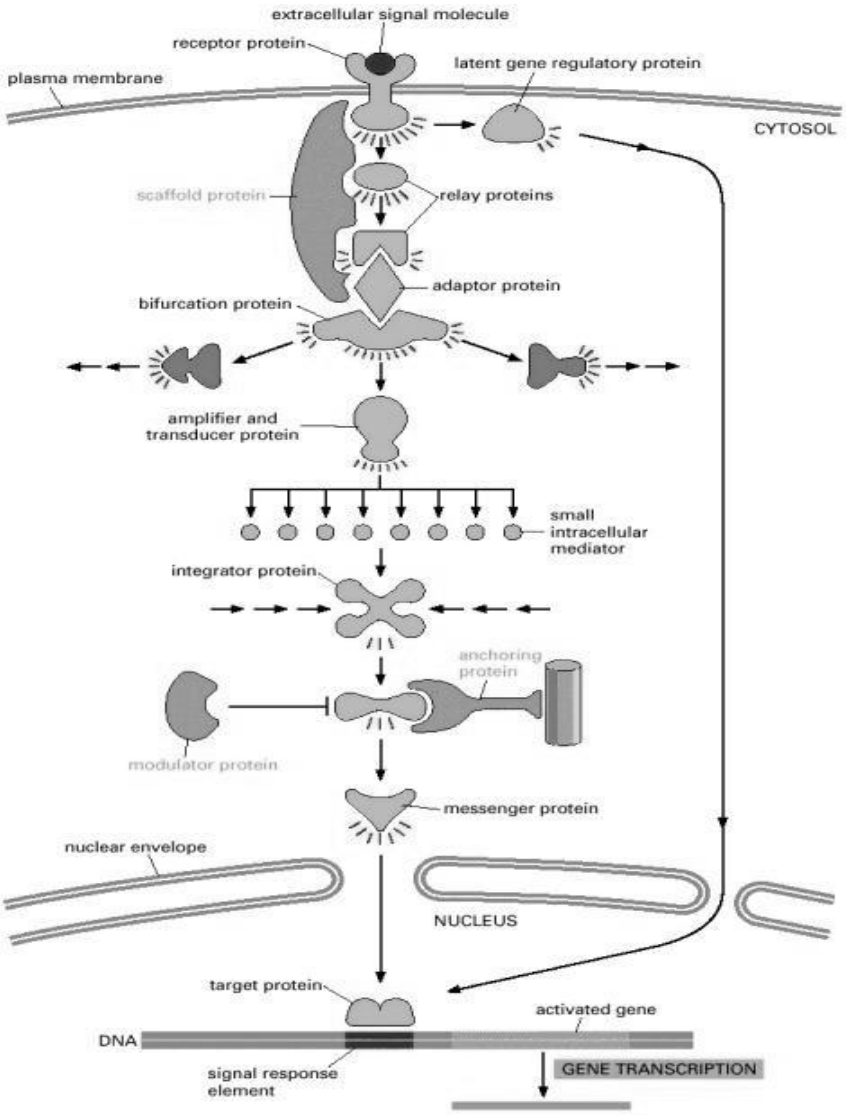


Figure 5.11: Different kinds of Intracellular signaling proteins

11. Molecular switches: Upon receipt of a signal they switch from inactive state to active state, until another process switches them off. Molecular switches fall into two main classes, that operate in different ways. Both classes of proteins involve activation and inactivation of phosphate groups. For one class phosphate is added by protein kinase using ATP as phosphate source. In the other class the signaling protein is induced to exchange its bound GDP with GTP (figure5.12).Many of the signaling proteins controlled by phosphorylation are protein kinases and are often organized into phosphorylation cascades. The two main protein kinases are Serine/Threonine kinase, which phosphorylates Serine and Threonine, and the other is Tyrosine kinase which phosphorylates tyrosine

residues in the proteins. GTP- binding protein alters between active state with GTP bound to them and the other inactive state when GDP is bound. The two major types of GTP binding proteins are – A large trimeric GTP- binding protein and a small monomer GTPase.

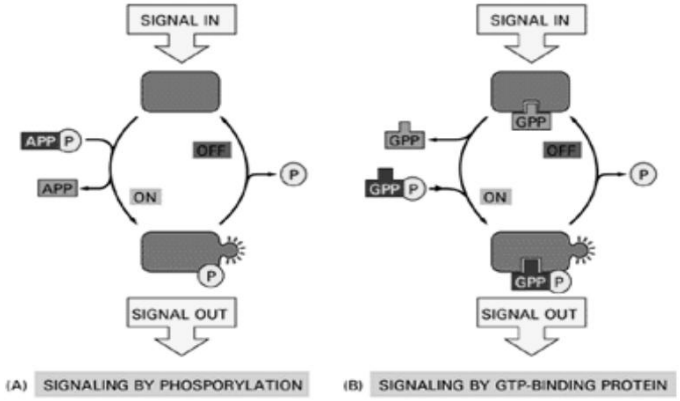


Figure 5.12: Types of intracellular proteins that act as Molecular switches

12. **Intracellular signaling complexes:** These are extracellular signals acting through a single type of G-protein linked or Enzyme-linked receptor usually activates multiple parallel signaling pathway and can thereby influence multiple aspects of cell behavior such as shape, movement , metabolism and gene expression. Cells use Scaffold proteins, which organize groups of interacting signaling proteins into signaling complexes. Scaffold guides the interactions between the successive components in such a complex, the signal can be relayed with precision, speed and efficiency; moreover, unwanted cross talk between signaling pathways is avoided. These complexes form transiently, as and when signaling proteins assemble around a receptor after an extracellular signal molecule attaches to it. The cytoplasmic tail of activated protein gets activated by Phosphorylation and this acts as docking site for intracellular signaling proteins (figure 5.13).

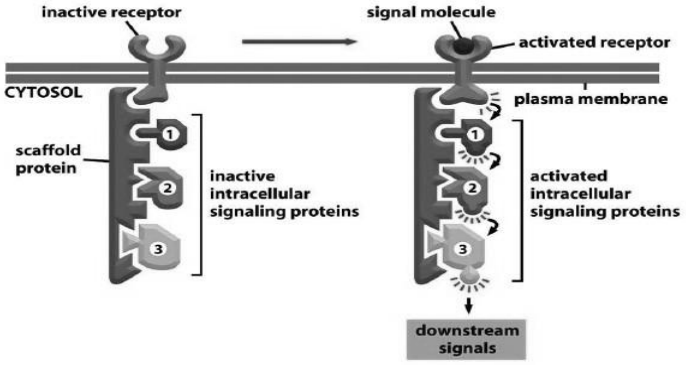


Figure 5.13: Preformed signaling proteins complex on scaffold

13. **Modular Binding Domains:** These are compact protein modules that bind to a particular motif in the protein or lipid, with which the signaling protein interacts. They bind to signaling proteins forming the route of the signaling pathways.

Eg: Src homology 2 (SH2) domains and phosphotyrosine –binding (PTB) domains (figure 5.14). It is signaling protein I that contains three different binding domains and a catalytic protein kinase domain. It moves to the plasma membrane when extra cellular signals lead to the creation of various phosphorylated docking sites on the cytosolic face of the membrane. Its SH2 domain binds to phosphorylated tyrosines on the receptor protein and its PH domain binds to phosphorylated inositol phospholipids in the inner leaflet of the lipid bilayer.

Protein I phosphorylates signaling protein 2 on tyrosines, which allows protein2 to bind to the PTB domain on protein1 and to the SH2 domain on an adopter protein that links protein2 and protein 3, causes phosphorylation of protein 3 by protein 2.

The adapter protein shown consists of 2 binding domains, one SH2 domain, which binds to a phosphotyrosine on protein 2 and an SH3 domain, which binds to a protein rich motif on protein 3.

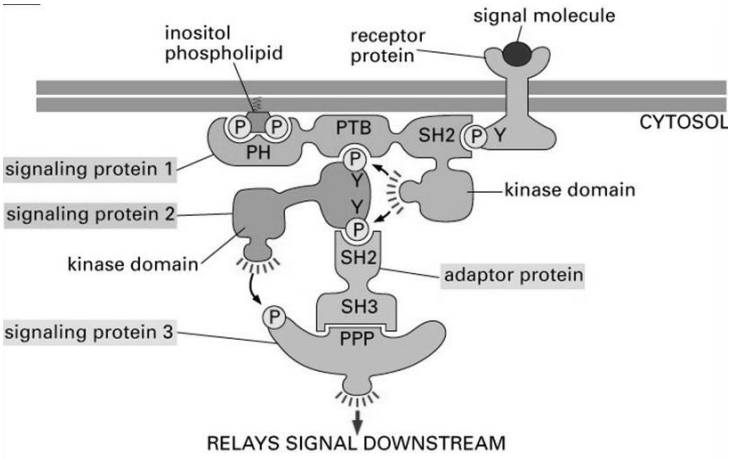


Figure 5.14 Src homology 2 (SH2) domains and phosphotyrosine –binding (PTB) domains

14. **Desensitization of Cell Signaling:** The target cells of cell signaling undergo a reversible process of adaptation or sensitization where by a prolonged exposure to a stimulus decreases the cells response to that level of exposure. There are five ways of desensitization of signal molecules (figure 5.15). Ligand binding to cell surface receptors, may induce their endocytosis and temporary sequestration in endosomes. Such Ligand- induced

receptor endocytosis can lead to the destruction of the receptors in lysosomes, a process referred to as receptor down-regulation. In other cases, desensitization occurs due to rapid inactivation of receptor due to its phosphorylation. Production of an inhibitor that blocks transduction process can also be a reason for desensitization.

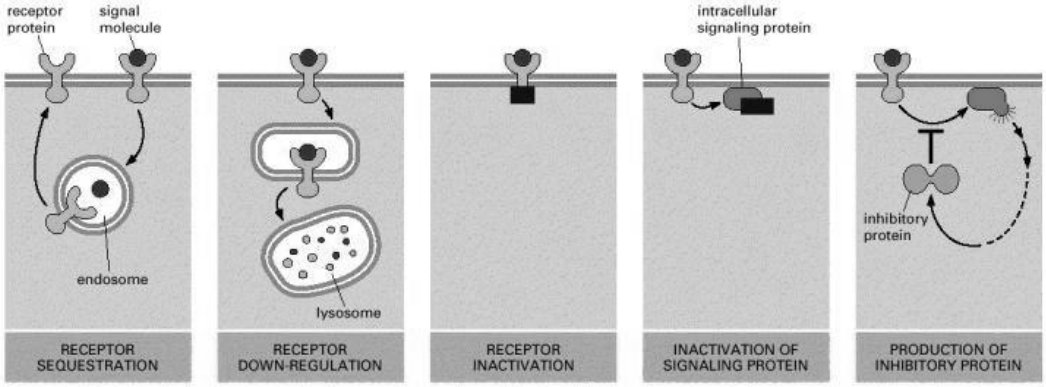


Figure 5.15: Five ways of desensitization to signal molecules

CLASSES OF CELL SURFACE RECEPTORS:

1. Ion Channel Linked Receptors:

Most of the membrane transport proteins on the plasma membrane are concerned with transport of inorganic ions like Na^+, K^+, Ca^{2+} or Cl^- and are referred to as Ion channels. These ions diffuse rapidly sown their concentration gradient through the channel across the membrane and control many cell functions. Few important channel families are listed below:

Family	**Representative Subfamilies**	
Voltage gated cation channels	Voltage gated Na^+ channels	
	Voltage gated K^+ channels	
	Voltage gated Ca^{2+} channels	
Transmitter gated ion channels	Acetylcholine gated cation channels	Excitatory
	Glutamate gated Ca^{2+} channels	Excitatory
	Serotonin gated cation channels	Excitatory
	GABA gated Cl^- channels	Inhibitory
	Glycine gated Cl^- channels	Inhibitory

Few Characteristics of these Channels:

(a) Fluctuation between open and closed state: as the ions channels are selective they allow movement of ions, based on the concentration gradient. Their opening and closing is associated with conformation changes. When pores open, it is associated with lining up of polar groups along the pore, while hydrophobic amino acids interact with lipid bilayer. This opening allows penetration of ions that have shed most of their water molecules to pass though the nearest channel called as

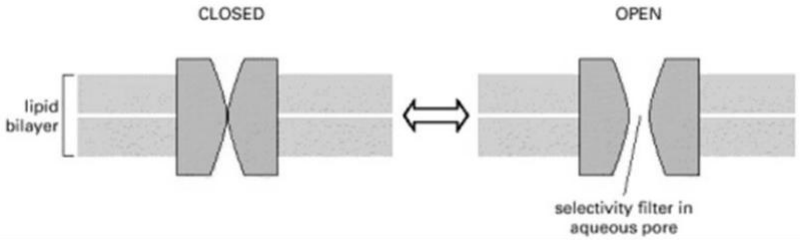


Figure 5.16: Opening and closing of Ion channels

(b) Gating of Ion Channels: few channels open due to some stimulus and these are called gated channels. The types of stimuli that can open these gated channels are negative voltage across the membrane (Voltage gated channels), a mechanical stress (mechanically gated channels), or binding of a ligand (Ligand- gated channels). The Ligand can be either extracellular mediator (as in transmitter gated channels) or a intracellular mediator (Ion-gated channels)or a nucleotide (nucleotide gated channel) (figure 5.17).

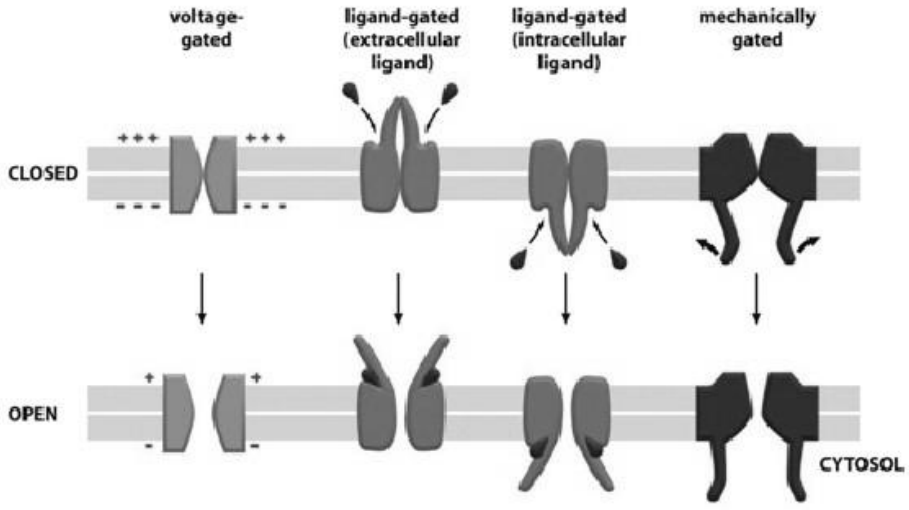


Figure 5.17: Types of Gated Ion channels

(c) **Membrane Potential role in Na^+- K^+ Pumps:** Membrane potential arises due to differences in the electrical charge across the membrane. Usually animal cells have low Na^+ inside them while K^+ ions are more in the cytosol. The balancing if Na^+ charge gradient is done by K^+ ions. K^+ ions can freely move through K^+ leaky channels across the membrane thereby balancing the concentration of these ions. A state when there is no net flow of ions is called resting potential and the cell is called a polarized cell. A stimulus can disturb this cell and cause penetration of Na^+ ions into the cells and this is called Avalanche effect, which continues until equilibrium is established. At one point Na^+ inflow stops and this cell is called a depolarized cell, the potential of cell at this state is called action potential. The cell again has to restore back to polarized state and this is by pumping out of Na^+ ions and in flow of K^+ through Na^+ - K^+ pumps. Cells start pumping our 3 Na^+ ions for every 2 K^+ ions until equilibrium is reached (figure 5.18). In animal cells, the potential difference varies from – 20 to 200mV depending on the type of cell.

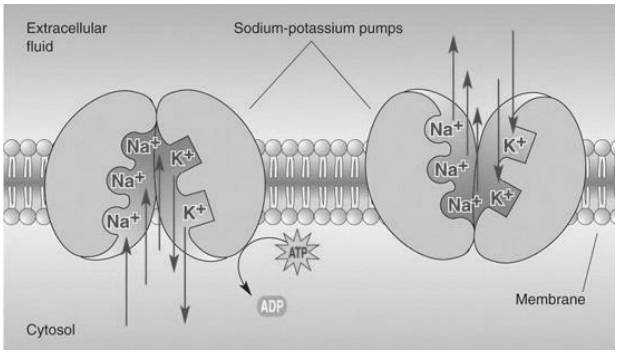


Figure 5.18: Functioning of Na^+ - K^+ pumps

(d) **Functioning of Neuron by Voltage gated Channels:** Aneuron can receive input from other neurons via a chemical called a neurotransmitter. If this input is strong enough, the neuron will pass the signal to downstream neurons. Transmission of a signal within a neuron (in one direction only, from dendrite to axon terminal) is carried out by the opening and closing of voltage-gated ion channels, which cause a brief reversal of the resting membrane potential to create an action potential . As an action potential travels down the axon, the polarity changes across the membrane. Once the signal reaches the axon terminal, it stimulates other neurons.

Depolarization and the Action Potential

When neurotransmitter molecules bind to receptors located on a neuron's dendrites, voltage- gated ion channels open. At excitatory synapses, positive ions flood the interior of the neuron and depolarize the membrane, decreasing the difference in voltage between the inside and outside of the neuron. A stimulus from a sensory cell or another neuron depolarizes the target neuron to its threshold potential (-55 mV), and Na+ channels in the axon hillock open, starting an action potential. Once the sodium channels open, the neuron completely depolarizes to a membrane potential of about +40 mV (figure 5.19). The action potential travels down the neuron as Na+ channels open.

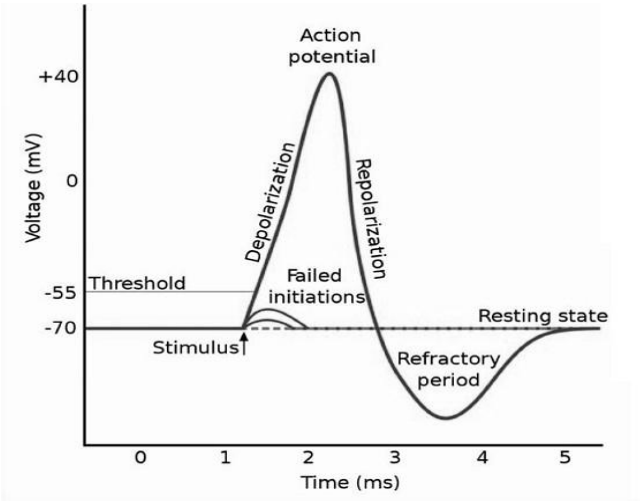


Figure 5.19: Action potential curve of neuron

Hyperpolarization and Return to Resting Potential

Action potentials are considered an "all-or nothing" event. Once the threshold potential is reached, the neuron completely depolarizes. As soon as depolarization is complete, the cell "resets" its membrane voltage back to the resting potential. The Na_+ channels close, beginning the neuron's refractory period. At the same time, voltage-gated K_+ channels open, allowing K_+ to leave the cell. As K_+ ions leave the cell, the membrane potential once again becomes negative. The diffusion of K_+ out of the cell hyperpolarizes the cell, making the membrane potential morenegative than the cell's normal resting potential(figure 5.20). At this point, the sodium channelsreturn to their resting state, ready to open again if the membrane potential again exceeds the threshold otential. Eventually, the extra K^+ ions diffuse out of the cell through the potassium leakage channels, bringing the cell from its hyperpolarized state back to its resting membrane potential.

Myelin and Propagation of the Action Potential

For an action potential to communicate information to another neuron, it must travel along the axon and reach the axon terminals where it can initiate neurotransmitter release. The speed of conduction of an action potential along an axon is influenced by both the diameter of the axon and the axon's resistance to current leak. Myelin potential conduction. Diseases like multiple sclerosis cause degeneration of the myelin, which slows action potential conduction because axon areas are no longer insulated so the current leaks.acts as an insulator that prevents current from leaving the axon, increasing the speed of action

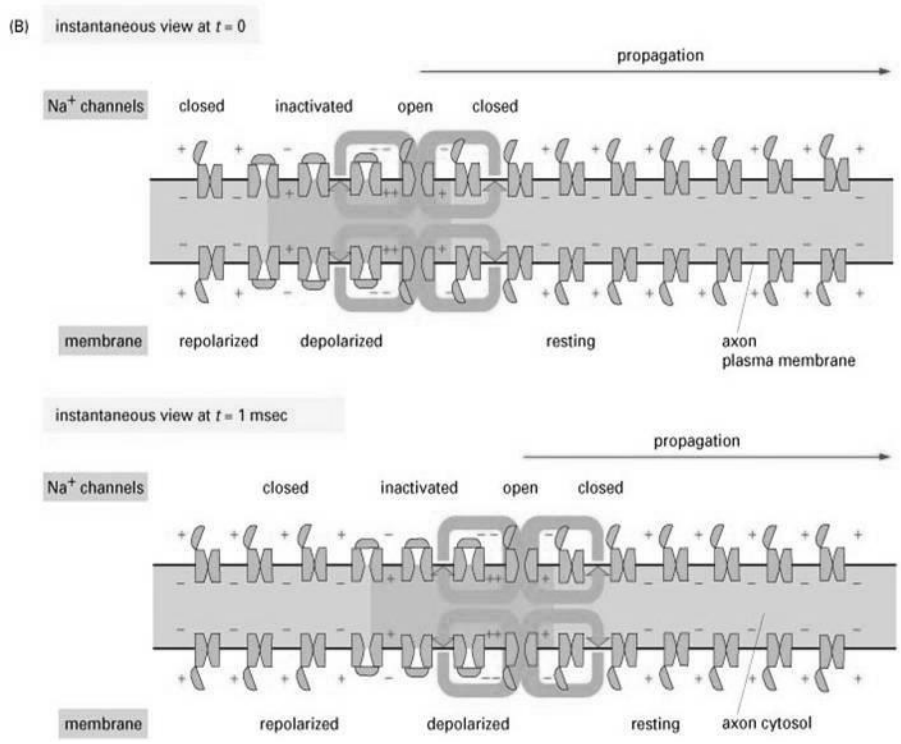


Figure 5.20: Propagation of action potential along axon of neuron

Node of Ranvier: A node of Ranvier is a natural gap in the myelin sheath along the axon. These unmyelinated spaces are about one micrometer long and contain voltage gated Na^+ and K^+ channels. The flow of ions through these channels, particularly the Na^+ channels, regenerates the action potential over and over again along the axon. Action potential "jumps" from one node to the next in saltatory conduction (figure 5.21). If nodes of Ranvier were not present along an axon, the action potential would propagate very slowly; Na^+

and K^+ channels would have to continuously regenerate action potentials at every point along the axon. Nodes of Ranvier also save energy for the neuron since the channels only need to be present at the nodes and not along the entire axon.

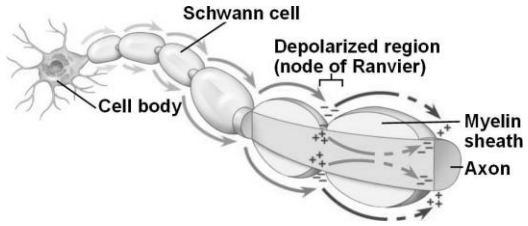


Figure 5.21 Nodes of Ranvier and salutatory conduction of nerve impulse

Synapse usually occurs between the axon of a pre-synaptic neuron and a dendrite or cell body of a post-synaptic neuron (Figure 5.22). At a synapse, the end of the axon is swollen and referred to as the end bulb or synaptic knob. Within the end bulb are found many synaptic vesicles that have the neurotransmitter and mitochondria that provide ATP. Between the end bulb and the dendrite of the post – synaptic neuron or onto the surface of skeletal muscle membrane, the gap is referred to as synaptic cleft. The impulse has to be transmitted through this space.

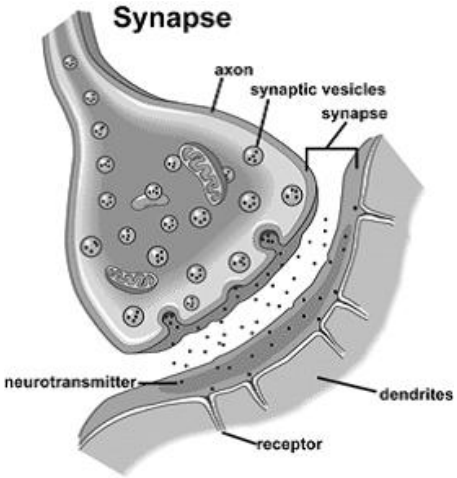


Figure 5.22: Synapse

Mechanism of Action

Synaptic transmission at the neuromuscular junction begins when an action potential reaches the presynaptic terminal of a motor neuron, which activates

voltage-dependent calcium channels to allow calcium ions to enter the neuron. Calcium ions bind to sensor proteins (synaptotagmin) on synaptic vesicles, triggering vesicle fusion with the cell membrane and subsequent neurotransmitter release from the motor neuron into the synaptic cleft (figure 5.23). In vertebrates, motor neurons release acetylcholine (ACh), a small molecule neurotransmitter, which diffuses across the synaptic cleft and binds to nicotinic acetylcholine receptors (nAChRs) on the cell membrane of the muscle fiber, also known as the sarcolemma. nAChRs are ionotropic receptors, meaning they serve as ligand-gated ion channels. The binding of ACh to the receptor can depolarize the muscle fiber, causing a cascade that eventually results in muscle contraction.

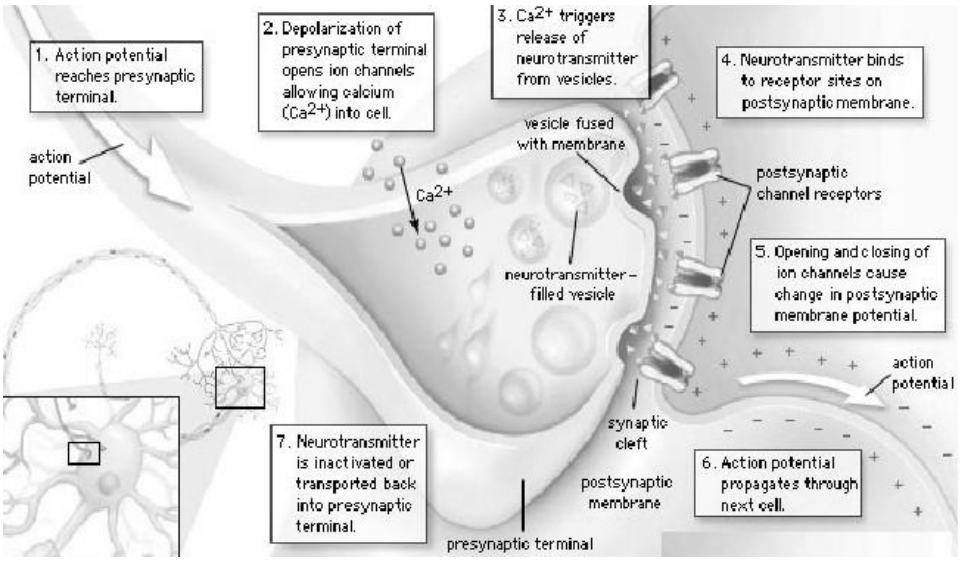


Figure 5.23: Events at Motor end plate

Acetylcholine Receptors At The Neuromuscular Junction

1. Ion channel linked receptor
2. Ions
3. Ligand (such as acetylcholine)

When ligands bind to the receptor, the ion channel portion of the receptor opens, allowing ions to pass across the cell membrane.

Acetylcholine is a neurotransmitter synthesized from dietary choline and acetyl-CoA (ACoA), and is involved in the stimulation of muscle tissue in vertebrates. The acetylcholine receptor subtype that is found at the neuromuscular junction of skeletal muscles is the nicotinic acetylcholine receptor (nAChR), which is a ligand-gated ion channel. Each subunit of

this receptor has a characteristic "cys-loop," which is composed of a cysteine residue followed by 13 amino acid residues and another cysteine residue. The two cysteine residues form a disulfide linkage which results in the "cys-loop" receptor that is capable of binding acetylcholine and other ligands.

AChRs at the skeletal neuromuscular junction form heteropentamers composed of two α, one β, one ε, and one δ subunits. When a single ACh ligand binds to one of the α subunits of the ACh receptor it induces a conformational change at the interface with the second AChR α subunit. This conformational change results in the increased affinity of the second α subunit for a second ACh ligand. AChRs therefore exhibit a sigmoidal dissociation curve due to this cooperative binding . The presence of the inactive, intermediate receptor structure with a single-bound ligand keeps ACh in the synapse that might otherwise be lost by cholinesterase hydrolysis or diffusion. The persistence of these ACh ligands in the synapse can cause a prolonged post-synaptic response.

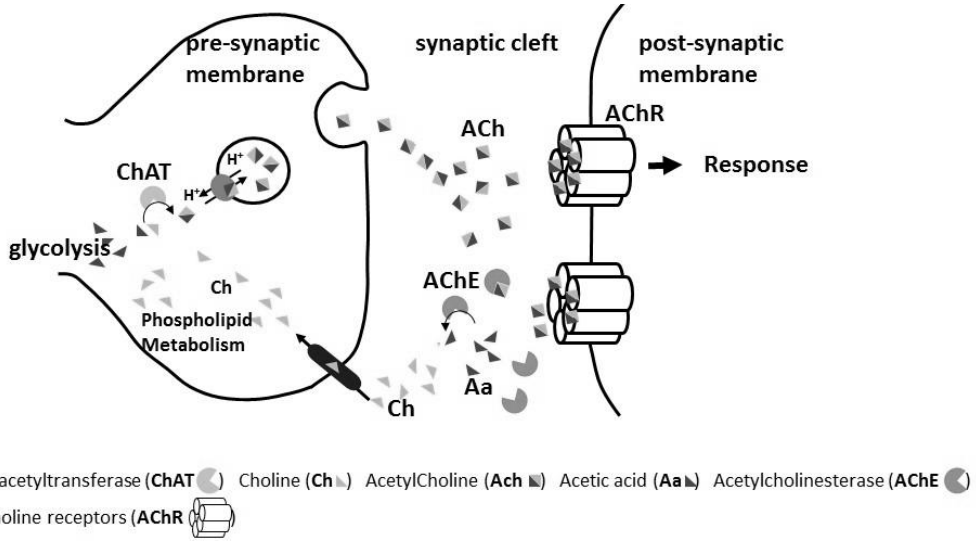


Figure 5.24: Events at synaptic cleft involving Acetylcholine and its receptor, followed by acetylcholine hydrolysis by acetylcholine esterase

2. G-protein linked cell surface receptors:

G-protein-linked receptors form the largest family of cell-surface receptors and are found in all eukaryotes. These receptors mediate the responses to an enormous diversity of signal molecules, including hormones, neurotransmitters, and local mediators. These signal molecules that activate them are as varied in structure as they are in function: the list includes proteins and small peptides, as well as derivatives of amino acids and fatty acids. The same ligand can activate many differentreceptor family members; at least 9 distinct G-protein-linked receptors are activated by adrenaline, for example,

another 5 or more by acetylcholine, and at least 15 by the neurotransmitter serotonin.

Despite the chemical and functional diversity of the signal molecules that bind to them, all G- protein-linked receptors have a similar structure. They consist of a single polypeptide chain that threads back and forth across the lipid bilayer seven times and are therefore sometimes called serpentine receptors. In addition to their characteristic orientation in the plasma membrane, they have the same functional relationship to the G proteins they use to signal the cell interior that an extracellular ligand is present.

G-protein:

Trimeric GTP-binding proteins (G proteins) are attached to the cytoplasmic face of the plasma membrane, where they serve as relay molecules, functionally coupling the receptors to enzymes or ion channels in this membrane. There are various types of G proteins, each specific for a particular set of serpentine receptors and for a particular set of downstream target proteins in the plasma membrane. All have a similar structure, however, and they operate in a similar way.

G proteins are composed of three protein subunits—α, β, and γ. In the unstimulated state, the α subunit has GDP bound and the G protein is inactive (Figure 15-27). When stimulated by an activated receptor, the α subunit releases its bound GDP, allowing GTP to bind in its place. This exchange causes the trimer to dissociate into two activated components—an α subunit and a βγ complex (figure 5.25).

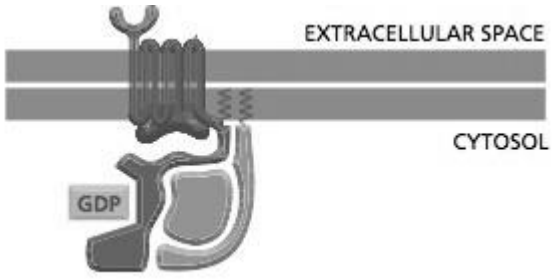


Figure 5.25: GTP binding protein (G protein)

Activation of G Protein

The dissociation of the trimeric G protein activates its two components in different ways. GTP binding causes a conformational change that affects the surface of the α subunit that associates with the βγ complex in the trimer. This change causes the release of the βγ complex, but it also causes and the α subunit to adopt a new shape that allows it to interact with its target proteins. The βγ complex does not change its conformation, but the surface previously masked by the α subunit is now available to interact with a second set of target proteins. The targets of the dissociated components of the G protein are either enzymes or ion channels in the plasma membrane, and they relay the signal onward.

The α subunit is a GTPase, and once it hydrolyzes its bound GTP to GDP, it reassociates with a βγ complex to re-form an inactive G protein, reversing the activation process (Figure 15-29). The time during which the α subunit and βγ complex remain apart and active is usually short, and it depends on how quickly the α subunit hydrolyzes its bound GTP. An isolated α subunit is an inefficient GTPase, and, left to its own devices, the subunit would inactivate only after several minutes. Its activation is usually reversed much faster than this, however, because the GTPase activity of the α subunit is greatly enhanced by the binding of a second protein, which can be either its target protein or a specific modulator known as a regulator of G protein signaling (RGS). RGS proteins act as α-subunit-specific GTPase activating proteins (GAPs), and they are thought to have a crucial role in shutting off G-protein-mediated responses in all eukaryotes. There are about 25 RGS proteins encoded in the human genome, each of which is thought to interact with a particular set of G proteins.

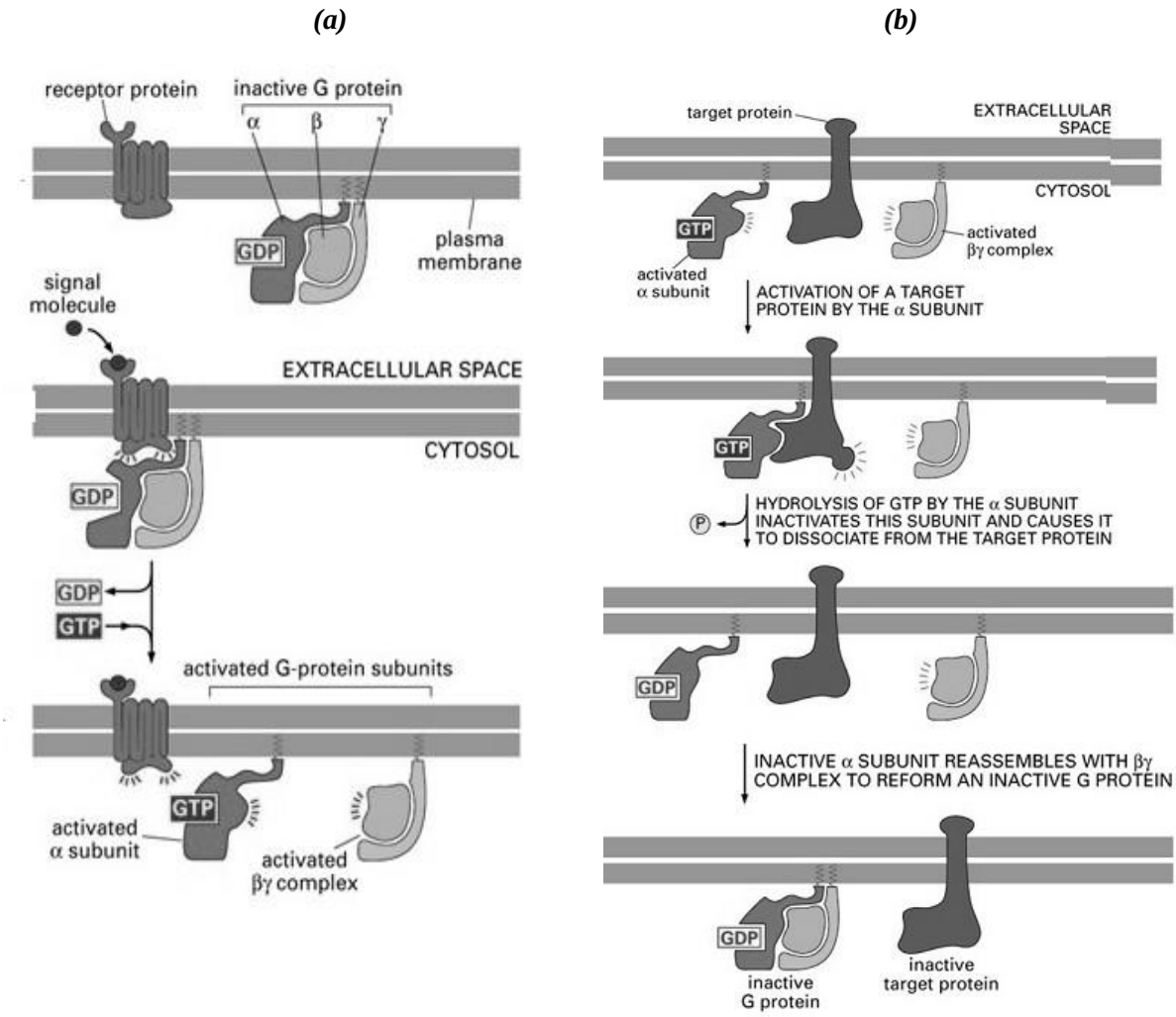


Figure 5.26: a)Disassembly and activation of GTP binding protein b) Switching off of G-protein by hydrolysis of ATP

Some G proteins signal by regulating the production of cyclic AMP

Cyclic AMP (cAMP) was first identified as a small intracellular mediator in the 1950s. It has since been found to act in this role in all prokaryotic and animal cells that have been studied. The normal concentration of cyclic AMP inside the cell is about 10^{-7} M, but an extracellular signal can cause cyclic AMP levels to change by more than twentyfold in seconds (Figure 15-30). As explained earlier (see Figure 15-10), such a rapid response requires that a rapid synthesis of the molecule be balanced by its rapid breakdown or removal. In fact, cyclic AMP is synthesized from ATP by a plasma-membrane-bound enzyme adenylyl cyclase, and it is rapidly and

continuously destroyed by one or more cyclic AMP phosphodiesterases that hydrolyze cyclic AMP to adenosine 5′-monophosphate (5′-AMP) (Figure 5.27).

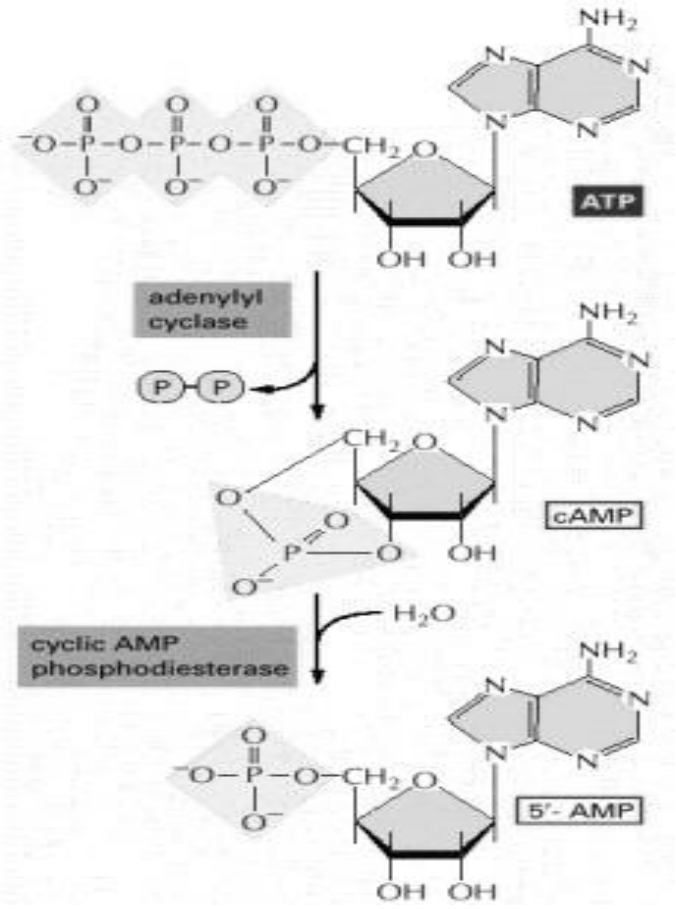


Figure5.27: Synthesis and degradation of cyclic AMP

Cyclic-Amp-Dependent Protein Kinase(Pka): This enzyme catalyzes the transfer of the terminal phosphate group from ATP to specific serines or threonines of selected target proteins, thereby regulating their activity. In the inactive state, PKA consists of a complex of two catalytic subunits and two regulatory subunits. The binding of cyclic AMP to the regulatory subunits alters their conformation, causing them to dissociate from the complex. The released catalytic subunits are thereby activated to phosphorylate specific substrate protein molecules (Figure 5.28). The regulatory subunits of PKA also are important for localizing the kinase inside the cell: special PKA anchoring proteins bind both to the regulatory subunits and to a membrane or a component of the cytoskeleton, thereby tying up the enzyme complex to a particular subcellular compartment. Some of these anchoring proteins also bind other kinases and some phosphatases, creating a signaling complex.

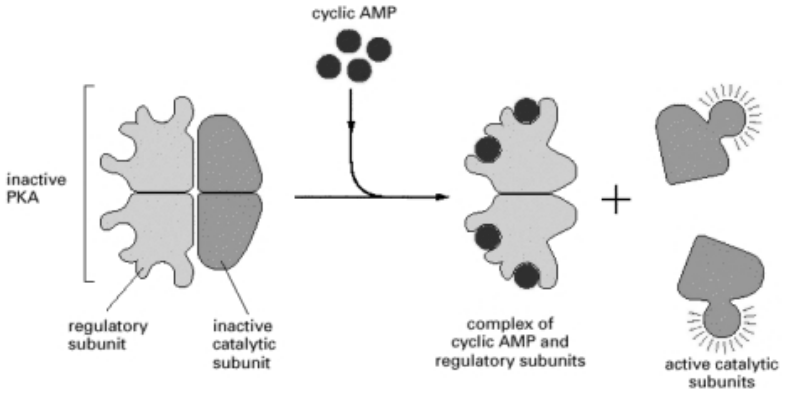


Figure 5.28 Activation of PKA

Cyclic AMP role in activating genes:

In cells that secrete the peptide hormone somatostatin, for example, cyclic AMP activates the gene that encodes this hormone. The regulatory region of the somatostatin gene contains a short DNA sequence, called the cyclic AMP response element (CRE) that is also found in the regulatory region of many other genes activated by cyclic AMP. A specific gene regulatory protein called CRE-binding (CREB) protein recognizes this sequence. When CREB is phosphorylated by PKA on a single serine, it recruits a transcriptional coactivator called CREB- binding protein (CBP), which stimulates the transcription of these genes (Figure 5-29).

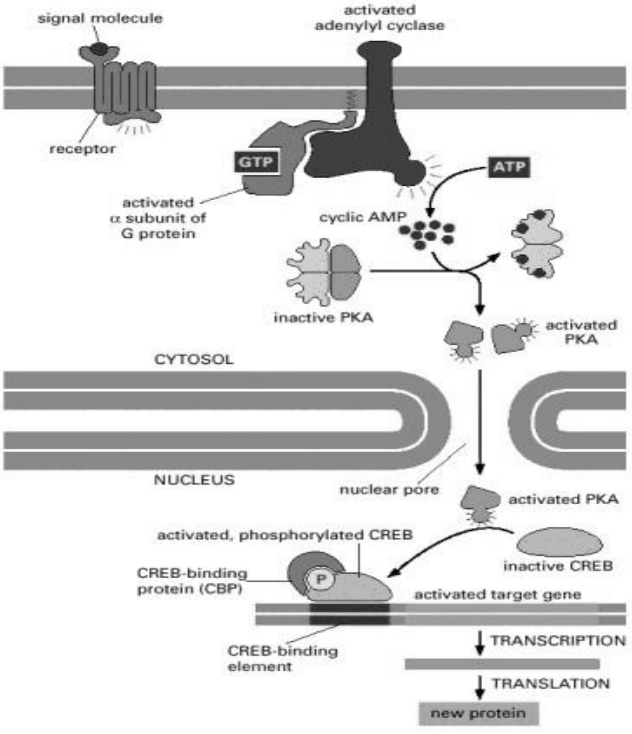


Figure 5.29: Gene transcription activation by cyclic AMP

Dephosphorylation of PKA activated proteins by Phosphatases:

Since the effects of cyclic AMP are usually transient, cells must be able to dephosphorylate the proteins that have been phosphorylated by PKA. In general, the dephosphorylation of phosphorylated serines and threonines is catalyzed by four types of serine/threonine phosphoprotein phosphatases—protein phosphatases I, IIA, IIB, and IIC. Except for protein phosphatase-IIC (which is a minor phosphatase, unrelated to the others), all of these phosphatases are composed of a homologous catalytic subunit complexed with one or more of a large set of regulatory subunits; the regulatory subunits help to control the phosphatase activity and enable the enzyme to select specific targets. Protein phosphatase I is responsible for dephosphorylating many of the proteins phosphorylated by PKA. It inactivates CREB, for example, by removing its activating phosphate, thereby turning off the transcriptional response caused by a rise in cyclic AMP concentration. Protein phosphatase IIA has a broad specificity and seems to be the main phosphatase responsible for reversing many of the phosphorylations catalyzed by serine/threonine kinases. Protein phosphatase IIB, also called calcineurin, is activated by Ca^{2+} and is especially abundant in the brain.

Inositol Phospholipid Signaling Pathway activation by G Protein

Many G-protein-linked receptors exert their effects mainly via G proteins that activate the plasma-membrane-bound enzyme phospholipaseC-β. The phospholipase acts on an inositol phospholipid called phosphatidylinositol 4,5-bisphosphate [$PI(4,5)P_2$], which is present in small amounts in the inner half of the plasma membrane lipid bilayer. Receptors that operate through this inositol phospholipid signaling pathway mainly activate a G protein called G_q, which in turn activates phospholipase C-β, in much the same way that G_s activates adenylyl cyclase. The activated phospholipase cleaves $PI(4,5)P_2$ to generate two products: inositol 1,4,5- trisphosphate and diacylglycerol (Figure 5-30), which diffuses through the cytosol and releases Ca^{2+} from the ER, and diacylglycerol, which remains in the membrane and helps to activate the enzyme protein kinase C.

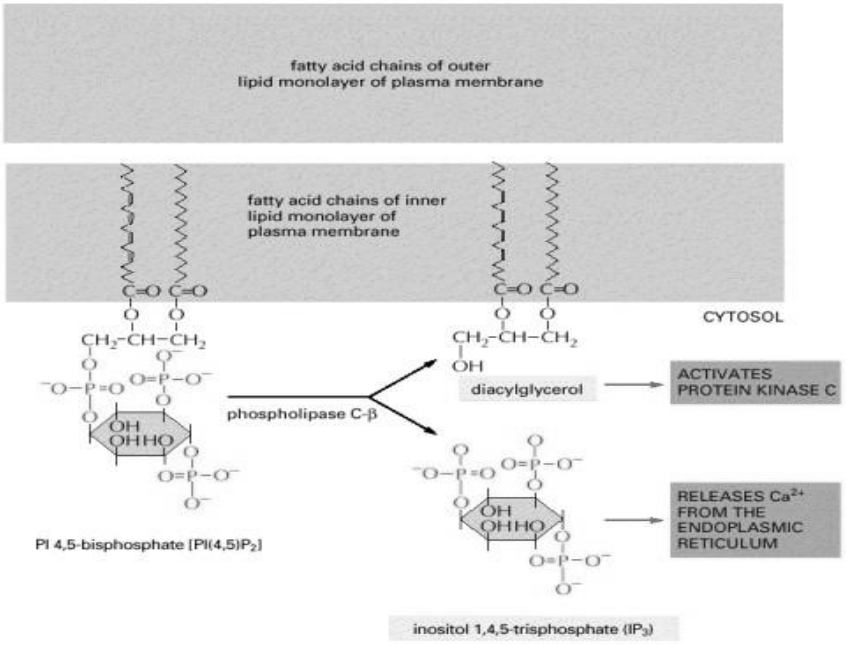


Figure 5.30: Hydrolysis of PI(4,5)P_2 by phospholipase C-β

Calcium as a Ubiquitous Messenger:

Calcium ions play several roles in animal cells. In muscle cells, Ca^{2+} triggers contraction, and in many secretory cells, including nerve cells, it triggers secretion. Ca^{2+} can be used as a signal in this way because its concentration in the cytosol is normally kept very low (~10^{-7} M), whereas its concentration in the extracellular fluid (~10^{-3} M) and in the ER lumen is high. Thus, there is a large gradient tending to drive Ca^{2+} into the cytosol across both the plasma membrane and the ER membrane. When a signal transiently opens Ca^{2+} channels in either of these membranes, Ca^{2+} rushes into the cytosol, increasing the local Ca^{2+} concentration by 10–20-fold and triggering Ca^{2+}-responsive proteins in the cell.

Three main types of Ca^{2+} channels can mediate this Ca^{2+} signaling:

1. Voltage-dependent Ca^{2+} channels in the plasma membrane open in response to membrane depolarization and allow, for example, Ca^{2+} to enter activated nerve terminals and trigger neurotransmitter secretion.
2. IP_3-gated Ca^{2+}-release channels allow Ca^{2+} to escape from the ER when the inositol phospholipid signaling pathway is activated, as just discussed.

3. Ryanodine receptors (so called because they are sensitive to the plant alkaloid ryanodine) react to a change in plasma membrane potential to release Ca^{2+} from the sarcoplasmic reticulum and thereby stimulate the contraction of muscle cells; they are also present in the ER of many nonmuscle cells, including neurons, where they can contribute to Ca^{2+} signaling.

Families of Trimeric G-proteins and their function: They are summarized in the table below:

FAMILY	SOME FAMILY MEMBERS	ACTION MEDIATED BY	FUNCTIONS
I	G_s	α	activates adenylyl cyclase; activates Ca^{2+} channels
	G_{olf}	α	activates adenylyl cyclase in olfactory sensory neurons
II	G_i	α	inhibits adenylyl cyclase
		βγ	activates K^+ channels
	G_o	βγ	activates K^+ channels; inactivates Ca^{2+} channels
		α and βγ	activates phospholipase C-β
	G_t (transducin)	α	activates cyclic GMP phosphodiesterase in vertebrate rod photoreceptors
III	G_q	α	activates phospholipase C-β

Desensitization of G-protein linked receptor:Target cells use a variety of mechanisms to desensitize, or adapt, when they are exposed to a high concentration of stimulating ligand for a prolonged period. Few mechanisms that involve an alteration in G-protein-linked receptors themselves.

These receptors can desensitize in three general ways:

1. They can become altered so that they can no longer interact with G proteins (receptor inactivation).
2. They can be temporarily moved to the interior of the cell (internalized) so that they no longer have access to their ligand (receptor sequestration).
3. They can be destroyed in lysosomes after internalization (receptor down-regulation).

In each case, the desensitization process depends on phosphorylation of the receptor, by PKA, PKC, or a member of the family of G-protein-linked receptor kinases (GRKs). The GRKs phosphorylate multiple serine and threonines on a receptor, but they do so only after the receptor has been activated by ligand binding. The binding of an arrestin to the phosphorylated receptor prevents the receptor from binding to its G protein and can direct its endocytosis (Figure 5.31).

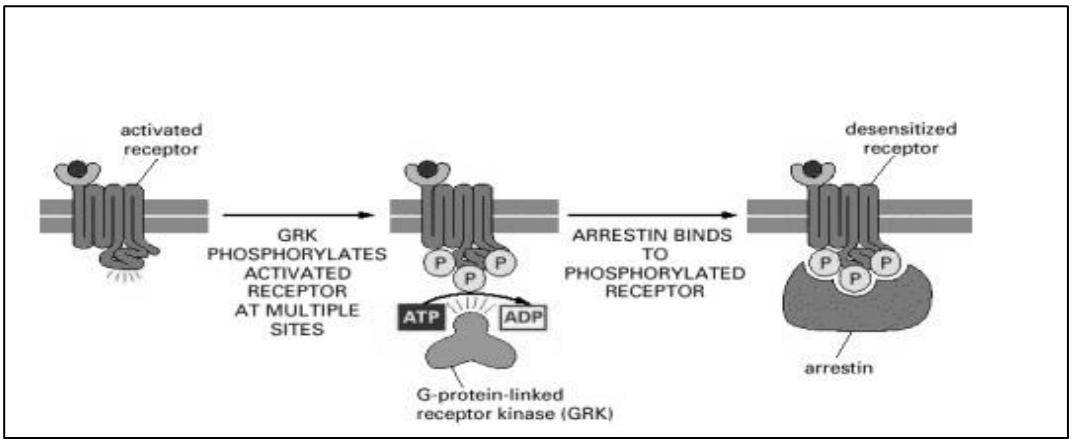


Figure 5.31: The roles of G-protein-linked receptor kinases (GRKs) and arrestins in receptordesensitization

3. Signaling through Enzyme linked Cell Surface Receptors:

Enzyme-linked receptors are a second major type of cell-surface receptor. They were recognized initially through their role in responses to extracellular signal proteins that promote the growth, proliferation, differentiation, or survival of cells in animal tissues. These signal proteins are often collectively called growth factors, and they usually act as local mediators at very low concentrations (about 10^{-9}-10^{-11} M). The responses to them are typically slow (on the order of hours) and usually require many intracellular signaling steps that eventually lead to changes in gene expression. Enzyme-linked receptors have since been found also to mediate direct, rapid effects on the cytoskeleton, controlling the way a cell moves and changes its shape. Disorders of cell proliferation, differentiation, survival, and migration are fundamental events that can give rise to cancer, and abnormalities of signaling through enzyme-linked receptors have major roles in this class of disease.

These are transmembrane proteins with their ligand-binding domainon the outer surface of the plasma membrane and cytosolic domain either has an intrinsic enzyme activity or associates directly with an enzyme.

Six classes of enzyme-linked receptors include:

1. Receptor tyrosine kinases: They phosphorylate specific tyrosines on a small set of intracellular signaling proteins.
2. Tyrosine-kinase-associated receptors – They associate with intracellular proteins that have tyrosine kinase activity.
3. Receptor like tyrosine Phosphatases: They remove phosphate groups from tyrosines of specific intracellular signaling proteins.

4. Receptor serine/threonine kinases – They phosphorylate specific serines or threonines on associated latent gene regulatory proteins.
5. Receptor guanylyl cyclases- they directly catalyze the production of cyclic GMP in the cytosol.
6. Histidine-kinase-associated receptors activate a "two-component" signaling pathway in which the kinase phosphorylates itself on histidine and then immediately transfers the phosphate to a second intracellular signaling protein.

1. **Tyrosine receptor Kinases:** Receptor tyrosine kinases consist of a large variety of secreted growth factors and hormones. Many cell-surface-bound signal proteins also act through these receptors. Few of these include: epidermal growth factor (EGF),platelet-derived growth factor (PDGF), fibroblast growth factors (FGFs), hepatocyte growth factor (HGF), insulin, insulinlike growth factor-1 (IGF-1), vascular endothelial growth factor (VEGF), macrophage-colony- stimulating factor(M-CSF), and all the neurotrophins, including nerve growth factor (NGF).

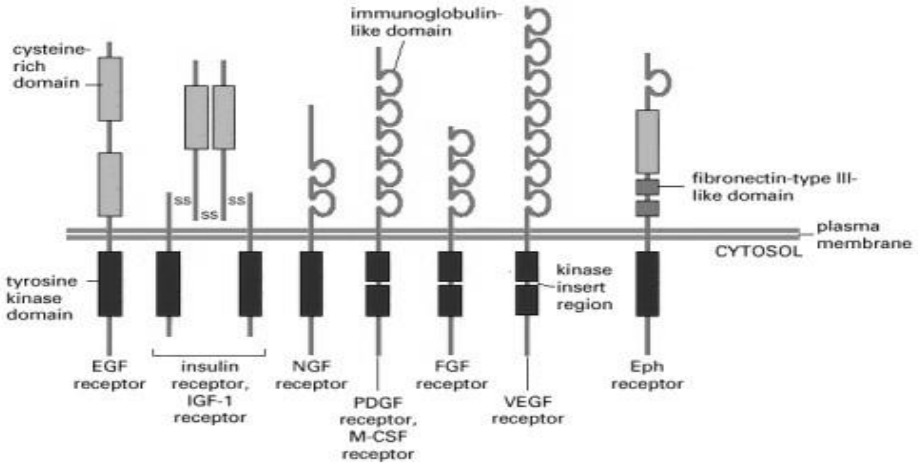


Figure 5.32: Seven subfamilies of Receptor Tyrosine kinases

The largest class of these membrane-bound ligands is the ephrins, which regulate the cell adhesion and repulsion responses that guide the migration of cells and axons along specific pathways during animal development. The receptors for ephrins, called Eph receptors, are also the most numerous receptor tyrosine kinases. The ephrins and Eph receptors can simultaneously act as both ligand and receptor: on binding to an Eph receptor, some ephrins not only activate the Eph receptor but also become activated themselves to transmit signals into the interior of the ephrin-expressing cell. In this way, an interaction between an ephrin protein on one cell and an Eph protein on another cell can lead to bidirectional reciprocal signaling that changes the behavior of both cells.

Receptor tyrosine kinases can be classified into more than 16 structural subfamilies, each dedicated to its complementary family of protein ligands. Once activated, the kinase domain transfers a phosphate group from ATP to selected tyrosine side chains, both on the receptor proteins themselves and on intracellular signaling proteins that subsequently bind to the phosphorylated receptors.

When the receptor chains are cross-linked, the kinase domains of adjacent receptors cross-phosphorylate each other, stimulating the kinase activity of the receptor and creating docking sites for intracellular proteins. (A) Platelet-derived growth factor (PDGF) is a covalently linked dimer with two receptor-binding sites, so it can directly cross-link adjacent receptors to initiate the intracellular signaling process. The PDGF dimers are composed of A and B chains in different combinations, and they can activate different combinations of two types of PDGF receptor chains (α and β), which have somewhat different signaling properties. (B) Some monomeric ligands, such as fibroblast growth factors (FGFs), bind in clusters to proteoglycans, enabling the ligands to cross-link their receptors. The proteoglycans can be in the extracellular matrix or, as shown here, on the cell surface. There are more than 20 types of FGFs and more than 4 types of FGF receptors. (C) Membrane-bound signaling proteins, such as ephrins, can cross-link their receptors even though they are monomeric, because they cluster in the plasma membrane of the signaling cell. Some ephrins (B-type) are transmembrane proteins (as shown), whereas others (A-type) are linked to the membrane by a glycosylphosphatidylinositol (GPI) anchor (Figure 5.33). For the most part, A-type ephrins activate A-type Eph receptors, and B- type ephrins activate B-type Eph receptors.

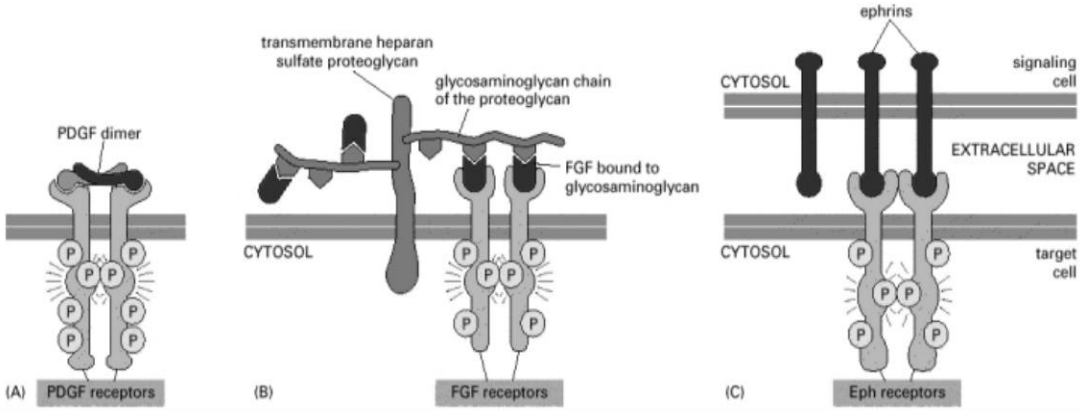


Figure 5.33: Three ways in which signaling proteins can cross-link receptor chains

Phosphorylated Tyrosine serves as docking site for proteins:

Autophosphorylation of the cytosolic tail of receptor tyrosine kinases contributes to the activation process in two ways. First, phosphorylation of tyrosines within the kinase domain increases the kinase activity of the enzyme. Second, phosphorylation of tyrosines outside the kinase domain creates high-affinity docking sites for the binding of a number of intracellular signaling proteins in the target cell. Each type of signaling protein binds to a different phosphorylated site on the activated receptor because it contains a specific phosphotyrosine-binding domain that recognizes surrounding features of the polypeptide chain in addition to the phosphotyrosine. Once bound to the activated kinase, the signaling protein may itself become phosphorylated on tyrosines and thereby activated; alternatively, the binding alone may be sufficient to activate the docked signaling protein. In summary, autophosphorylation serves as a switch to trigger the transient assembly of a large intracellular signaling complex, which then broadcasts signals along multiple routes to many destinations in the cell.

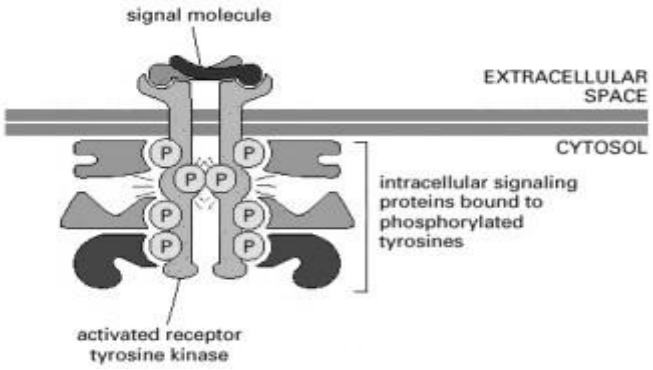


Figure 5.34: Docking of intracellular signaling proteins on an activated receptor tyrosine kinase

Phosphorylated Tyrosines acting as Docking sites:

Although the intracellular signaling proteins that bind to phosphotyrosines on activated receptor tyrosine kinases and docking proteins have varied structures and functions. These can be either SH2 domains or, less commonly, PTB domains (phosphotyrosine-binding). By recognizing specific phosphorylated tyrosines, these small domains serve as modules that enable the proteins that contain them to bind to activated receptor tyrosine kinases, as well as to many other intracellular signaling proteins that have been transiently phosphorylated on tyrosines. Many signaling proteins also contain other protein modules that allow them to interact specifically with other proteins as part of the signaling process. These include the SH3 domain,

which binds to proline-rich motifs in intracellular proteins. Some signaling proteins are composed almost entirely of SH2 and SH3 domains and function as adaptors to couple tyrosine- phosphorylated proteins to other proteins that do not have their own SH2 domains. Such adaptor proteins help to couple activated receptors to the important downstream signaling protein Ras.

Ras is activated by Guanine Nucleotide Exchange factor:

Ras proteins belong to the large Ras superfamily of monomeric GTPases, involved in relaying signals from cell-surface receptors to the actin cytoskeleton and the Rab family, involved in regulating the traffic of intracellular transport vesicles. The Ras proteins contain a covalently attached lipid group that helps to anchor the protein to the plasma membrane where the protein functions. There are multiple Ras proteins, and different ones act in different cell types.

Ras helps to broadcast signals from the cell surface to other parts of the cell. It is often required, for example, when receptor tyrosine kinases signal to the nucleus to stimulate cell proliferation or differentiation by altering gene expression. Ras functions as a switch, cycling between two distinct conformational states—active when GTP is bound and inactive when GDP is bound. Two classes of signaling proteins regulate Ras activity by influencing its transition between active and inactive states. Guanine nucleotide exchange factors (GEFs) promote the exchange of bound nucleotide by stimulating the dissociation of GDP and the subsequent uptake of GTP from the cytosol, thereby activating Ras. GTPase-activating proteins (GAPs) increase the rate of hydrolysis of bound GTP by Ras, thereby inactivating Ras (Figure 5-35). Mutation of Ras leads to cancer.

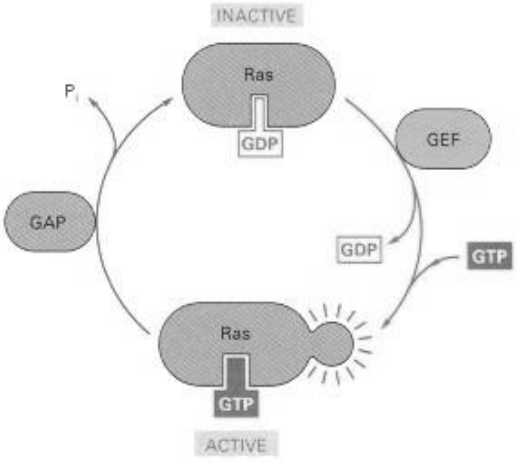


Figure 5.35 Regulation of Ras activity

Once activated, Ras in turn activates various other signaling proteins to relay the signal downstream along several pathways. One of the signaling pathways Ras activates is a serine/threonine phosphorylation cascade that is highly conserved in eukaryotic cells from yeasts to humans. A crucial component in this cascade is a novel type of protein kinase called MAP- kinase. Once activated, Ras in turn activates various other signaling proteins to relay the signal downstream along severalpathways. One of the pathways is Serine/Threonine phosphorylation cascade. Crucial components of this cascade is Protein kinase MAP Kinase (Mitotic Activated Protein Kinase) (Figure 5.36).

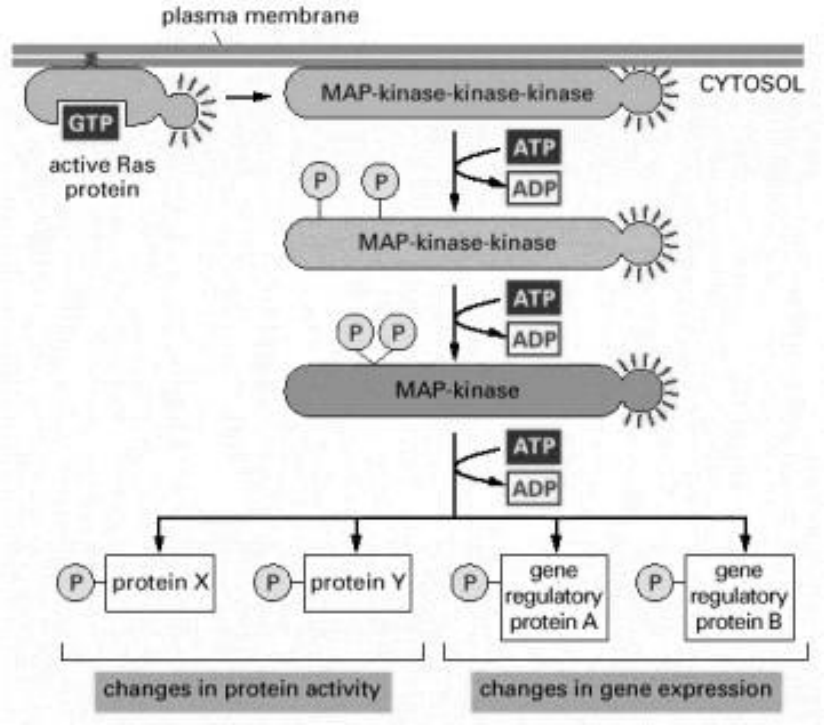


Figure 5.36: MAP-kinase serine-threonine phosphorylation pathway activated by Ras

P13-Kinase produces Inositol Phospholipid Socking Sites

One of the major intracellular signaling pathways leading to cell growth involves phosphatidylinositol 3-kinase (PI 3-kinase). This kinase principally phosphorylates inositol phospholipids rather than proteins; it can be activated by receptor tyrosine kinases, as well as by many other types of cell-surface receptors, including some that are G-protein-linked. Phosphatidylinositol (PI) is unique among membrane lipids because it can undergo reversible phosphorylation at multiple sites to generate a variety of distinct inositol phospholipids. When activated, PI 3-kinase

catalyzes the phosphorylation of inositol phospholipids at the 3 position of the inositol ring to generate lipids called $PI(3,4)P_2$ or$PI(3,4,5)P_3$ (Figure 5.37). The $PI(3,4)P_2$ and $PI(3,4,5)P_3$ then serve as docking sites for intracellular signaling proteins, bringing these proteins together into signaling complexes, which relay the signal into the cell from the cytosolic face of the plasma membrane. The generation of inositol phospholipid docking sites by PI 3-kinase.

They remain in the plasma membrane until they are dephosphorylated by specific inositol phospholipid phosphatases that remove phosphate from the 3 position of the inositol ring. There are various types of PI 3-kinases. The one that is activated by receptor tyrosine kinases consists of a catalytic and regulatory subunit. The regulatory subunit is an adaptor protein that binds to phosphotyrosines on activated receptor tyrosine kinases through its SH2 domains. Another PI 3-kinase has a different regulatory subunit and is activated by the βγ complex of a trimeric G protein when G-protein-linked receptors are activated by their extracellular ligand. The catalytic subunit, which is similar in both cases, also has a binding site for activated Ras, which allows Ras to directly stimulate PI 3-kinases.

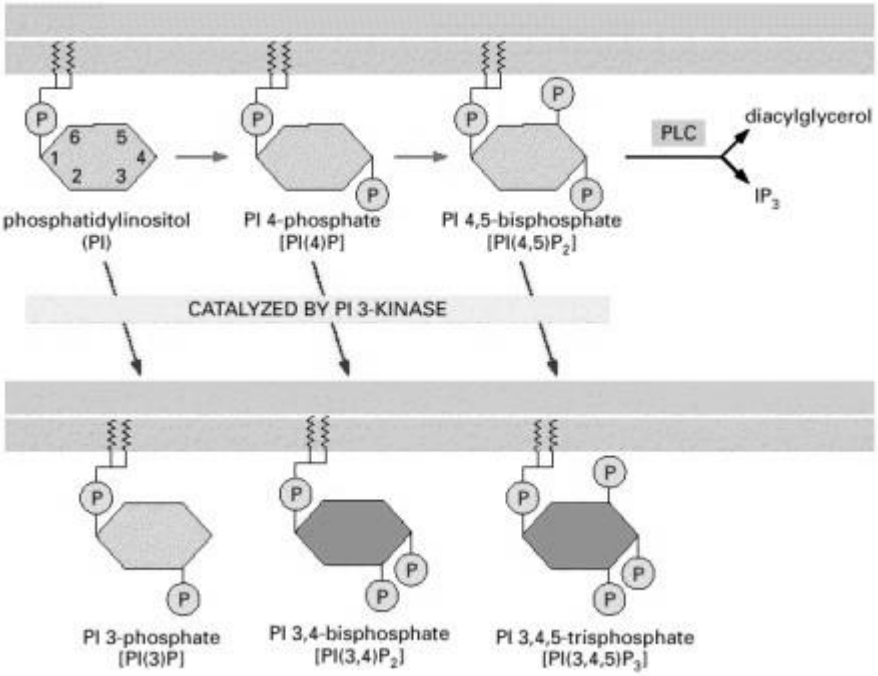


Figure 5.37: Generation of Inositol Phospholipid docking sites by PI 3- Kinases

PI 3-kinase promotes cell survival

An extracellular survival signal activates a receptor tyrosine kinase, which recruits and activates PI 3-kinase (figure 5.38). The PI 3-kinase produces $PI(3,4,5)P_3$ and $PI(3,4)P_2$ (not shown), both of which serve as docking sites for two serine/threonine kinases with PH domains—protein kinase B (PKB) and the phosphoinositol-dependent kinase PDK1. The binding of PKB to the inositol lipids alters its conformation so that the protein can be phosphorylated and activated by PDK1. The activated PKB now dissociates from the plasma membrane and phosphorylates the BAD protein, which, when unphosphorylated, holds one or more death-inhibitory proteins in an inactive state. Once phosphorylated, BAD releases the inhibitory proteins, which now can block programmed cell death (apoptosis) and thereby promote cell survival.

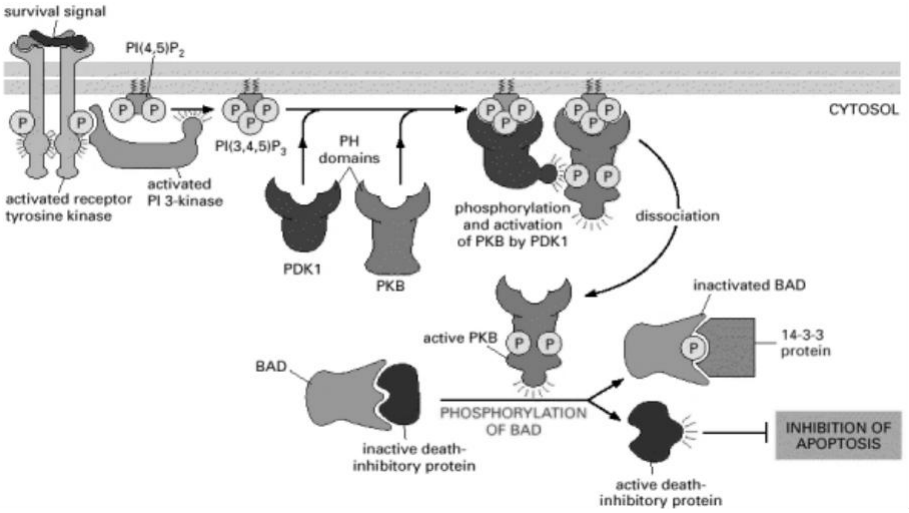


Figure 5.38: One way in which signaling thorugh PI 3 kinase promotes cell survival

Cytokine Receptor and Jak-STAT pathway:

When activated, interferon receptors activate a novel class of cytoplasmic tyrosine kinases called Janus kinases (Jaks) (after the two-faced Roman god). The Jaks then phosphorylate and activate a set of latent gene regulatory proteins called STATs (signal transducers and activators oftranscription), which move into the nucleus and stimulate the transcription of specific genes. More than 30 cytokines and hormones activate the Jak-STAT pathway by binding to cytokine receptors. All STATs also have an SH2 domain that enables them to dock onto specific phosphotyrosines on some activatedreceptor tyrosine kinase receptors. These receptors can directly activate the bound STAT, independently of Jaks.

Cytokine receptors are composed of two or more polypeptide chains. All cytokine receptors, however, are associated with one or more Jaks. There are four known Jaks—Jak1, Jak2, Jak3, and Tyk2—and each is associated with particular cytokine receptors. The receptors for α- interferon, for example, are associated with Jak1 and Tyk2, whereas the receptors for γ- interferon are associated with Jak1 and Jak2. The receptor for the hormone erythropoietin, which stimulates erythrocyte precursor cells to survive, proliferate, and differentiate, is associated with only Jak2.

There are seven known STATs, each with an SH2 domain that performs two functions. First, it mediates the binding of the STAT protein to a phosphotyrosine docking site on an activated cytokine receptor ; once bound, the Jaks phosphorylate the STAT on tyrosines, causing it to dissociate from the receptor. Second, the SH2 domain on the released STAT now mediates its binding to a phosphotyrosine on another STAT molecule, forming either a STAT homodimer or heterodimer which moves into the nucleus, where, in combination with other gene regulatory proteins, it binds to a specific DNA response element in various genes and stimulates their transcription (Figure 5.39).

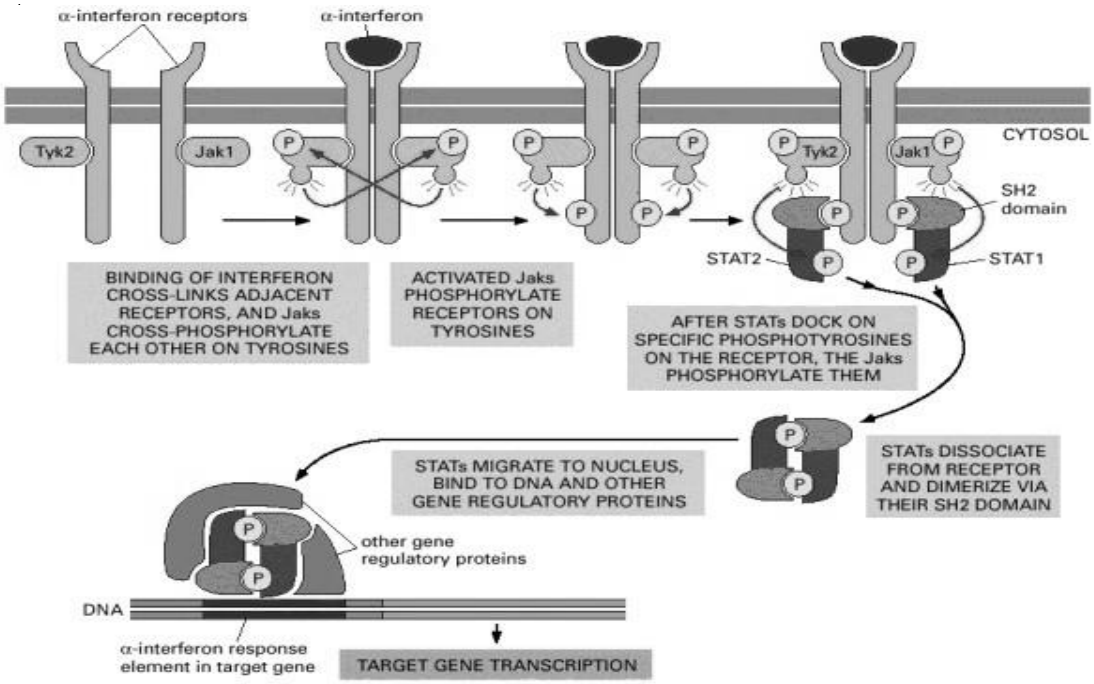


Figure 5.39: The Jak-STAT signaling pathway activated by Alpha Interferon

Protein Tyrosine Phosphatases as cell surface receptors:

ProteinTyrosine Phosphatases (PTPs) are involved in removal of phosphate groups from selected phosphotyrosines on a subset of tyrosine-phosphorylated proteins. There are two tyrosine Phosphatases in vertebrates, having SH2 domains and these are SHP-1 and SHP-2. SHP-1helps to terminate some cytokine responses in blood cells by dephosphorylating activated Jacks, while both SHP-1 and SHP-2 help to terminate responses mediated by some receptor tyrosine kinases. Some receptor like tyrosine phosphatases display features of cell-adhesion proteins and can even mediate homophilic cell-cell binding in cell adhesion assays. Transmembrane tyrosine phosphatases can also serve as signaling ligands that activate receptors on a neighboring cell. Signal proteins of TGF-□ superfamily

The transforming growth factor -□□□uperfamily consists of large number of structurally related, secreted, dimeric proteins, which either act as hormones or local mediators. During development they regulate pattern formation and influence various cell behaviors, including proliferation, differentiation, extracellular matrix production and cell death.

There are two classes of these receptorserine/threonine kinases — type I and type II—which are structurally similar. Each member of the TGF-β superfamilybinds to a characteristic combination of type-I and type-II receptors, both of which are required for signaling. Typically, the ligand first binds to and activates a type-II receptor homodimer, which recruits, phosphorylates, and activates a type-I receptor homodimer, forming an active tetrameric receptor complex. The type-I receptor directly binds and phosphorylates a latent gene regulatory protein of the Smad family. Activated TGF-β receptors and activin receptors phosphorylate Smad2 or Smad3, while activated BMP receptors phosphorylate Smad1, Smad5, or Smad8. Once one of these Smads has been phosphorylated, it dissociates from the receptor and binds to Smad4, which can form a complex with any of the above five receptor-activated Smads. The Smad complex then moves into the nucleus, where it associates with other gene regulatory proteins, binds to specific sites in DNA, and activates a particular set of target genes.

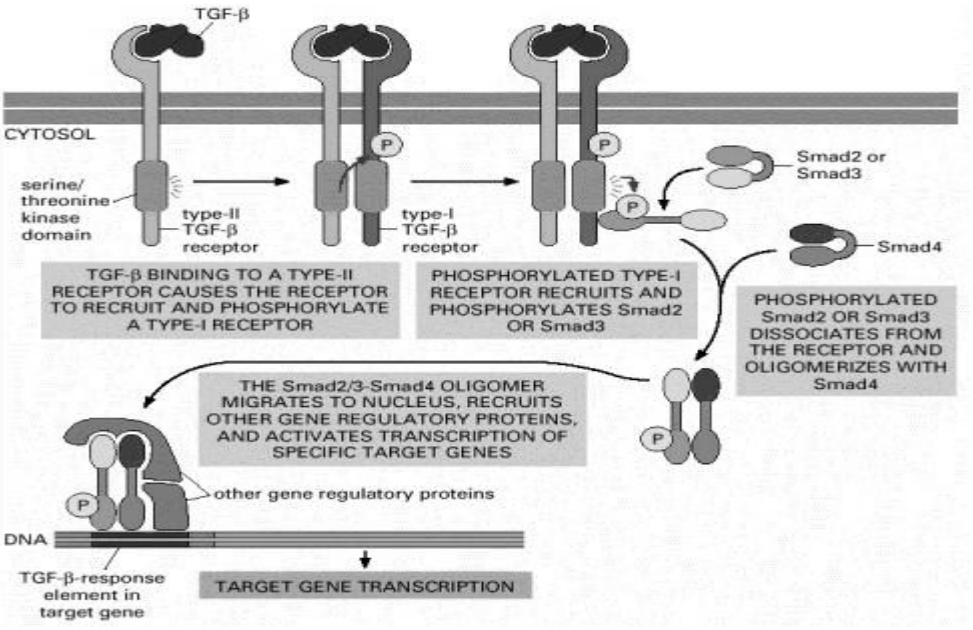


Figure 5.40: A smad dependent signaling pathway activated by TGF-β

Receptor guanylyl cyclases: These are single-pass transmembrane proteins with an extracellular binding site for a signal molecule and an intracellular guanylyl cyclase catalytic domain. The binding of the signal molecule activates the cyclase domain to produce cyclic GMP, which in turn binds to and activates a cyclic GMP-dependent protein kinase (PKG),which phosphorylates specific proteins on serine or threonine. Thus, receptor guanylyl cyclases use cyclic GMP as an intracellular mediator in the same way that some G-protein-linked receptors use cyclic AMP, except that the linkage between ligand binding and cyclase activity is a direct one.

Among the signal molecules that use receptor guanylyl cyclase receptors are the natriuretic peptides (NPs), a family of structurally related secreted signal peptides that regulate salt and water balance and dilate blood vessels. There are several types of NPs, including atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP). Muscle cells in the atrium of the heart secrete ANP when blood pressure rises. The ANP stimulates the kidneys to secrete Na^+ and water and induces the smooth muscle cells in blood vessels walls to relax. Both of these effects tend to lower the blood pressure.

Histidine- kinase associated Receptors:

Bacterial movements are mediated by histidine-kinase-associated chemotaxis receptors, which typically are dimeric transmembrane proteins that bind specific attractants and repellents on the outside of the plasma membrane. The cytoplasmic tails of the receptors are stably associated with an adaptor protein CheW and a histidine kinase CheA, which help to couple the receptors to the flagellar motor. Repellent binding activates the receptors, whereas attractant binding inactivates them; a single receptor can bind either type of molecule, with opposite consequences. The binding of a repellent to the receptor activates CheA, which phosphorylates itself on a histidine and almost immediately transfers the phosphate to an aspartic acid on a messenger protein CheY. The phosphorylated CheY dissociates from the receptor, diffuses through the cytosol, binds to the flagellar motor, and causes the motor to rotate clockwise, so that the bacterium tumbles. CheY has intrinsic phosphatase activity and dephosphorylates itself in a process that is greatly accelerated by the CheZ protein (Figure 5.41).

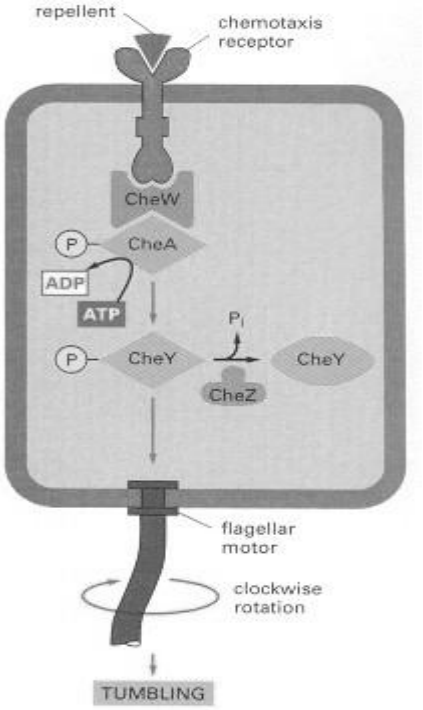


Figure 5.41: Two component signal pathway that enables chemotactic receptors to control bacterial chemotaxis

5. CELL - CELL COMMUNICATION

CELL- CELL COMMUNICATION

Cell communication is required for governing the activities of a multicellular organism. These communication mechanisms depend on the extracellular signal molecules produced by the neighboring cells. Organisms have developed specific signals to communicate to the cells to act in a specified way. These are proteins which include intracellular or extracellular signal molecules and cell surface receptors. Among the intracellular signaling proteins, which bind the signal molecules that distribute the signal to appropriate parts of the cell. The intracellular signaling proteins include kinases, Phosphatases, GTP binding proteins and many other proteins with which they interact and alter pathways. Depending on the signals effect, the target proteins can be gene regulatory proteins, Ion channels, components of a metabolic pathway, part of a cytoskeleton etc (figure 5.1).

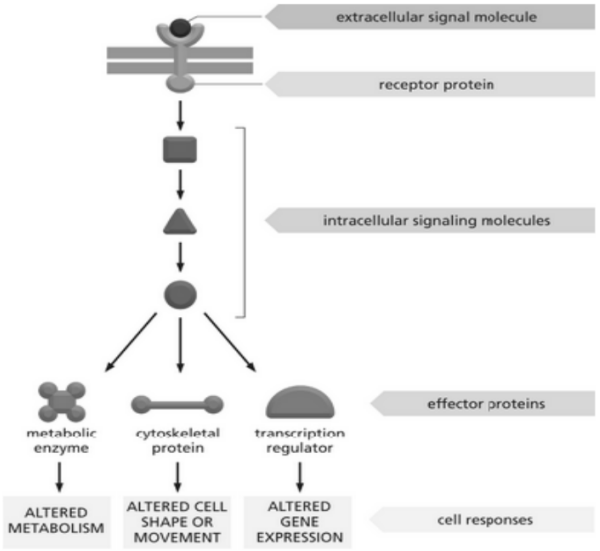


Figure 5.1: Overview of Intracellular signaling pathways

General Principles of Cell Communication

1. **Extra cellular signal molecules bind to specific receptors:** Higher animal communicate by means of hundreds of signal molecules. These include proteins, small peptides, amino acids, nucleotides, steroids, retinoids, fatty acid derivatives, dissolved gases like nitric oxide and carbon monoxide. These are secreted by signaling cells into extracellular space byexocytosis. Most

signal molecules are hydrophilic and hence they cannot cross membrane by diffusion, while few small ones do cross by diffusion.

Regardless of the nature of signal molecules, target cells respond by expressing specific binding proteins called receptors. These signals are produced at very low concentrations of ~10^{-6}M, with a high affinity constant $K_\alpha \geq 10^8$ liters per mole. Binding of signal to receptor generates a cascade of intracellular signals that alter the behavior of cells. Few signals are to penetrate the cells and bind to intracellular receptors; such signals are usually small, hydrophobic and can cross plasma membrane (figure 5.2).

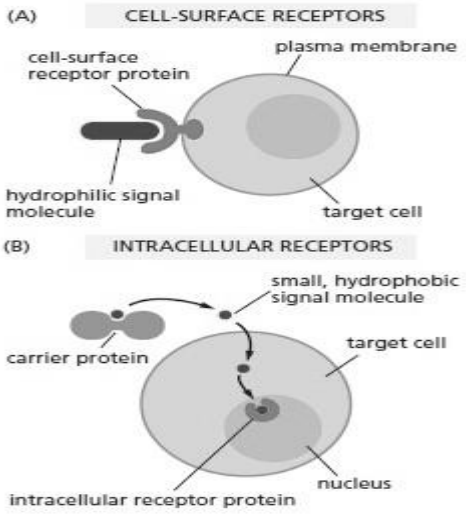


Figure 5.2: Extra cellular molecules and types of receptors

2. **Forms of intracellular signals:** There are four different types of intracellular signaling (Figure 5.3):
   - **(a) Contact dependent signaling:** Important during development and in immune responses. The secreted signals molecules are carried far afield to act on distant targets.
   - **(b) Paracrine signaling:** These affect the cells in the immediate environment and act as local mediators. These act on cells around, i.e., onto their proper target cells and must not diffuse too far. These are taken up immediately by cells, and are also destroyed by extracellular enzymes or enzymes present in the extracellular matrix.
   - **(c) Synaptic signaling:** These are short range signals and play specific role in cell communication. These are seen in specialized cells and have evolved

to communicate with parts of body. Eg: nerve impulse transmission by the neurotransmitter acetylcholine across synaptic space.

(d) Endocrine signals: These are specialized signals that control behavior of the organism. These signals are called hormones or endocrine secretions which travel through blood stream to reach target cells.

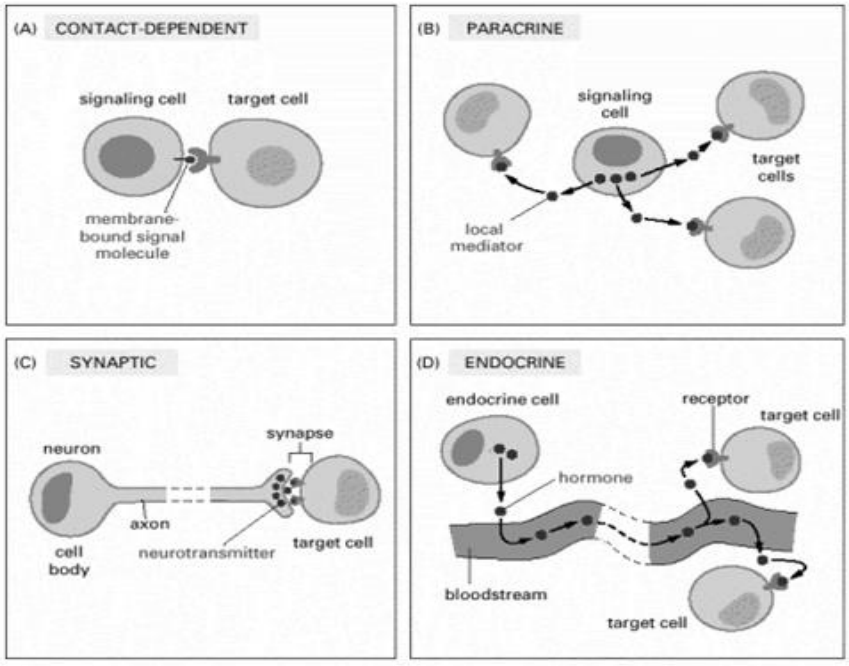


Figure 5.3: Forms of Intracellular signaling

3. Autocrine signaling: Signals sent by cells onto cells of same type and also for themselves are called autocrine signals. Eg: The cells during development if they have to take up a pathway for differentiation the cells secrete autocrine signals to reinforce themselves for developmental activities. Autocrine signals also stimulate cells of similar type (identical cells) which can respond to differentiation inducing signals (figure 5.4).

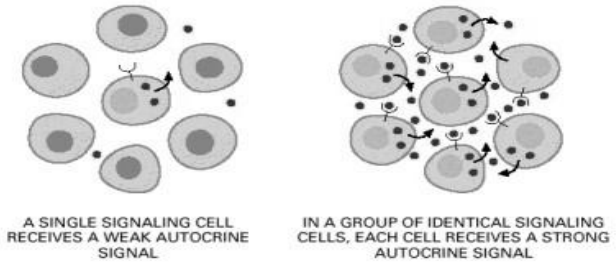


Figure 5.4: Autocrine signaling

4. **Role of gap junctions:** These are specialized cell-cell junctions that are formed between closely apposed plasma membranes. These are water filled channels that connect adjacent cells and allow exchange of small intracellular signals Ca^{2+} and cyclic AMP to pass through (Figure 5.5).

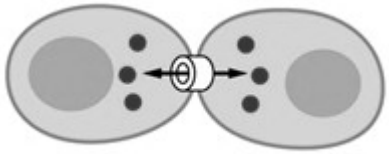


Figure 5.5: Signaling via gap junctions

5. **Cells respond to several signals:** Cells in a multicellular organism are exposed to different signals. They have the ability to respond to them differently and every response is programmed, leading to execution of specialized functions (figure 5.6). They have set of receptors that bind to signal molecules. Invitro, if cells are deprived of these signals, they undergo apoptosis.

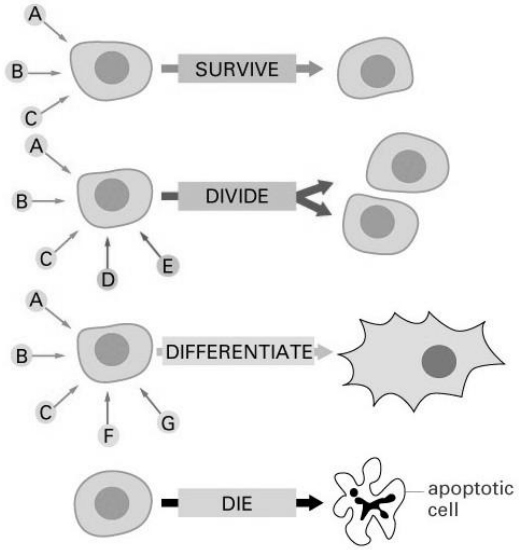


Figure 5.6: Animal cell dependence on multiple signals for survival

6. Concentration and life span of signals: These two depend upon the necessity of their persistence within the cells. Few signal molecules presence is necessary for long duration as in the case of cells in the stages of development. In an adult tissue, responses to fade and signals have to cease after certain span. There is rapid turnover of signal molecules, as in case of nerve impulse transmission, acetylcholine is destroyed by acetylcholine esterase and fresh

signal at axon causes release of new molecules of acetylcholine to transmit the signal to muscle cells, if not the muscle would be left in a continuous state of contraction. They are released in micro to nanomolar concentrations and destroyed every time. Few proteins (intracellular) serve for long duration ($\leq$ 10 minutes) as they have key regulatory roles.

7. **Some signals directly bind to target proteins:** Nitric oxide gas signal binds to guanylyl cyclase. Acetylcholine released by nerve ending activates NO synthase in the endothelial cells of blood vessels to produce NO. Nitric oxide diffuse out of the endothelial cells into the underlying smooth muscles, binds to guanylyl cyclase to produce cyclic GMP. This triggers relaxation of blood vessels and enhances flow of blood through them (figure 5.7). The life span of NO is very short and it is converted into nitrates and nitrites by oxygen and water. Even Carbon Monoxide is another gas signal that is used as intercellular signal to stimulate guanylyl cyclase.

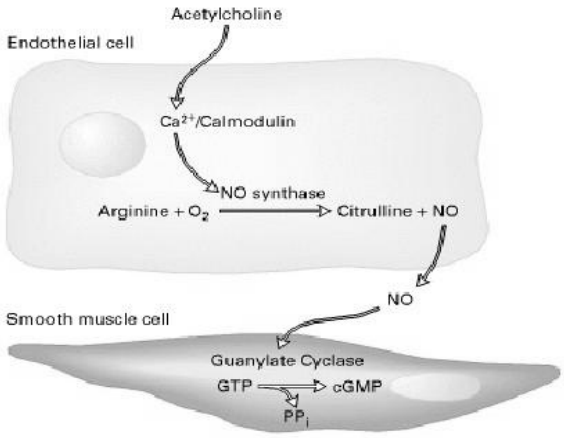


Figure 5.7: Responses induced by acetyl choline via NO resulting in smooth muscle contraction

8. **Signals that bind to nuclear receptors:** Some signal molecules like steroid hormones, thyroid hormones, retinoids and vitamin D, bind to receptors which are part of Nuclear Receptor Super Family. They bind to receptor proteins, activate them to bind to DNA to regulate transcription of specific genes.

   Eg 1: Steroid hormones – cortisol(figure5.8): It is produced by cortex of adrenal gland and influences metabolism in various ways. It is located in cytosol and enters nucleus after binding to nuclear hormone receptor protein.

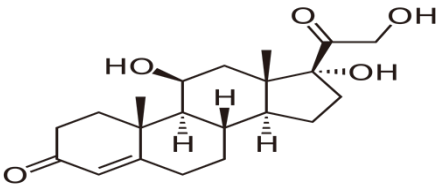


Figure 5.8: Structure of cortisol

These DNA binding proteins are either homodimers or heterodimers. Binding of ligand to the inactive receptor changes its conformation, this involves dissociation of inhibitory complexes from them. Ligand binding promotes binding of receptor to coactivator protein, then binding to Receptor's transcription activating domain, which increases gene transcription (figure 5.8).

This response induced by activation of Nuclear hormone receptors occurs in two phases- Early primary response and delayed secondary response. Primary response lasts for around 30 minutes and results in protein products synthesis, which in turn activates other genes to produce their products (secondary response) (figure 5.9).

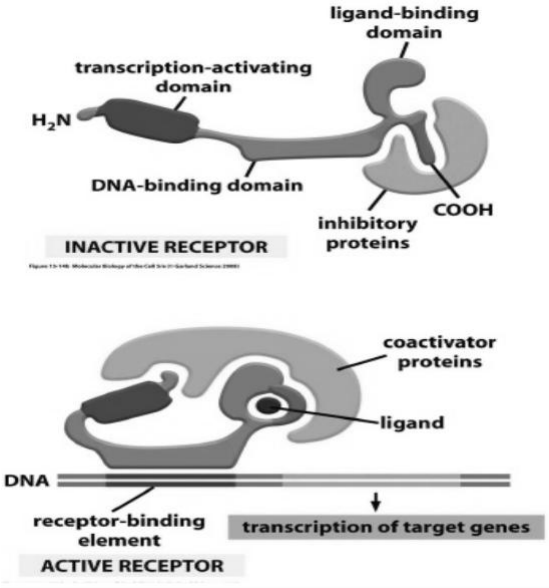


Figure 5.9: Nuclear Receptor Binding protein stages of activity

9. Classes of cell surface receptors:

There are three major classes of cell surface receptors (figure 5.10):

(i) Ion – channel – linked receptors

(ii) G- Protein linked receptors

(iii) Enzyme linked receptors

(i) **Ion-channel linked receptor:** These are known as Transmitter-gated ion channels/ Ionotrophic receptors. They are involved in rapid synaptic signaling between electrically excitable cells. This is mediated by few neurotransmitters that transiently open/close ion channels formed by proteins to which they bind.

(ii) **G-Protein linked receptors:** These regulate the activity of separate plasma membrane bound target proteins, which can be enzymes or Ion channels. The interaction between receptor and target proteins is mediated by a trimeric GTP-binding protein(G-protein).

(iii) **Enzyme –linked receptors:** These when activated , function either directly as enzymes or are directly associated with enzymes that they activate.

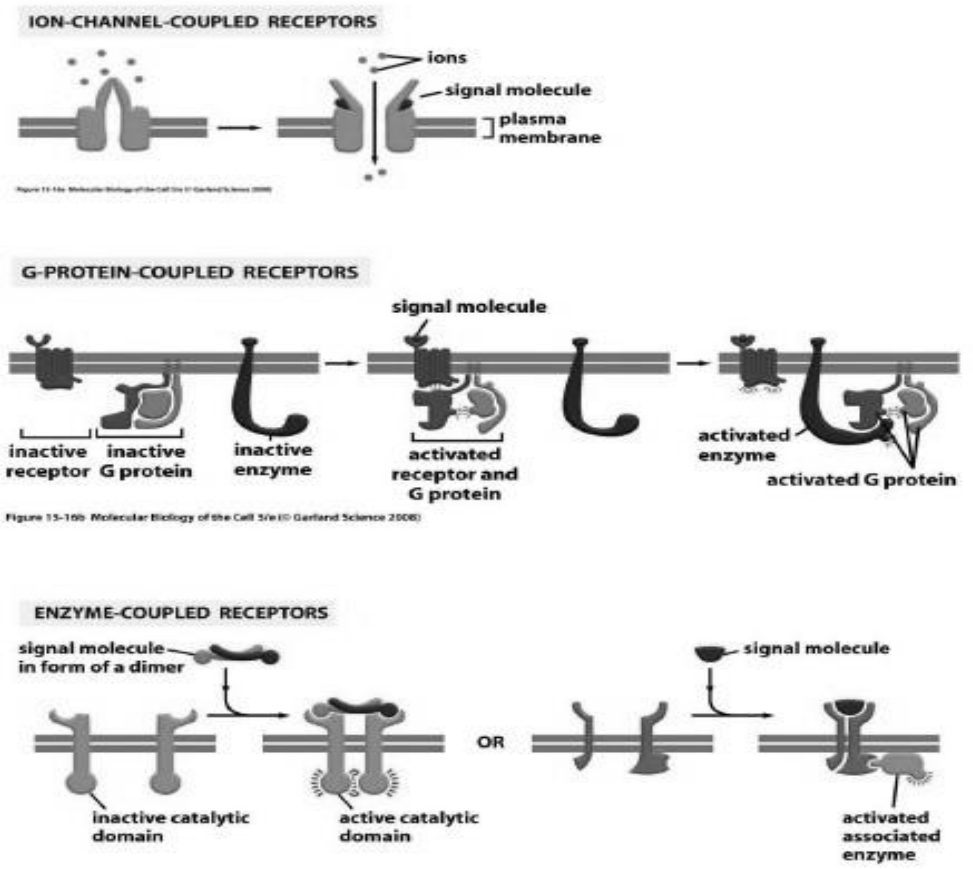


Figure 5.10: Three major classes of cell surface receptors

10. **Intra cellular Signaling Proteins:** The intracellular mediators or secondary messengers are produced by the binding of G-protein linked or Enzyme- linked receptor binding to their receptors. The secondary messengers are generated in

large numbers in response to receptor activation and they rapidly diffuse from source. Eg: Cyclic AMP and Ca^{2+}, which are water soluble and diacyl glycerol which is lipid soluble and diffuses even along plasma membrane.

Intra cellular signaling proteins act in a cascade manner. A series of signaling proteins and small intracellular mediators relay the extracellular signal into the cell causing a change in gene expression (figure 5.11).

Proteins involved in intracellular signaling are categorized as follows:

1. **Relay Proteins:** They simply pass the message to the next signaling component in the chain.
2. **Messenger Proteins:** Carry signal from one part of the cell to another, such as from cytosol to the nucleus.
3. **Adaptor Proteins:** Link one signaling protein to another, without themselves conveying a signal.
4. **Amplifier Proteins:** These are usually enzymes or ion channels, that amplify the signal they receive either by producing large amounts of small intracellular mediators or by activating large number of downstream intracellular signaling proteins. When there are multiple amplification steps in a relay chain it is referred to as Signaling cascade.
5. **Transducer Proteins:** they convert the signal into different form. The enzyme that makes cyclic-AMP- it converts the signal and amplifies it (Transducer + amplifier).
6. **Bifurcation Proteins:** Spread the signal from one signaling pathway to another.
7. **Integrator Proteins:** Receive signals from two or more signaling pathways and integrate them before relaying a signal onward.
8. **Latent gene Regulatory Proteins:** they are activated at the cell surface by activated receptors and then migrate to the nucleus to stimulate gene transcription.

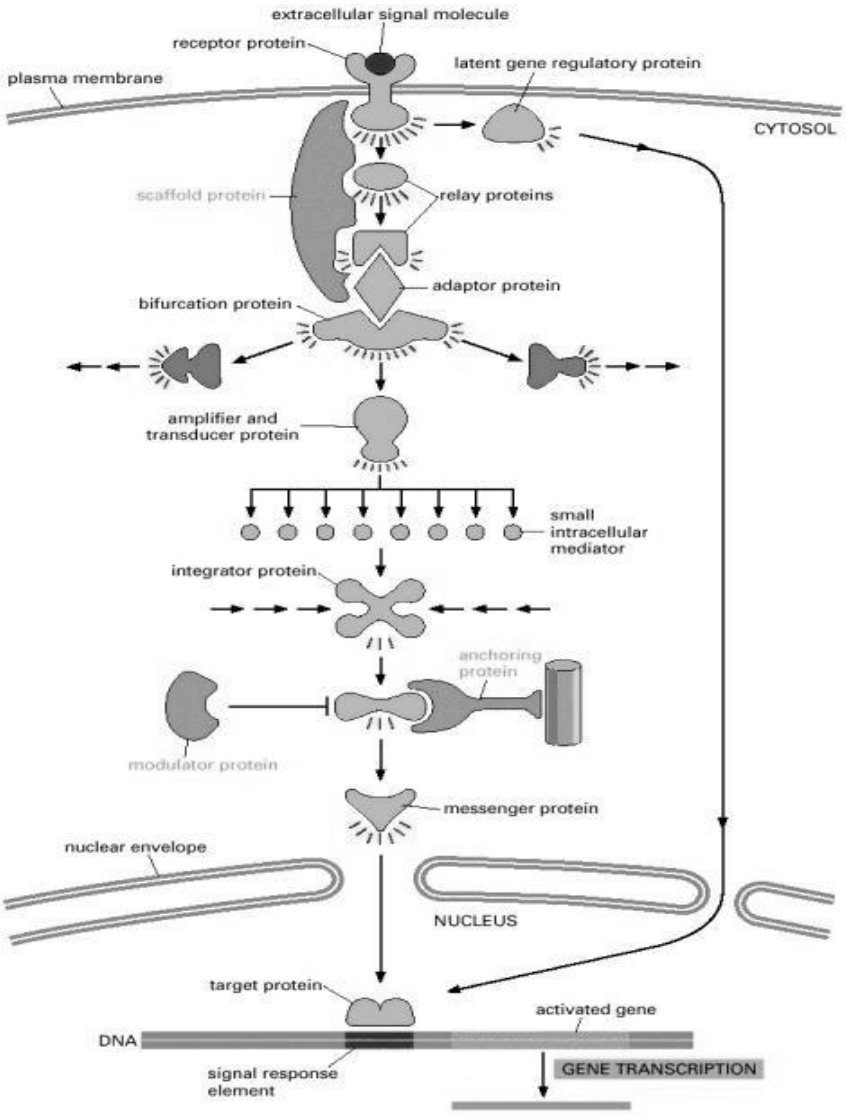


Figure 5.11: Different kinds of Intracellular signaling proteins

11. Molecular switches: Upon receipt of a signal they switch from inactive state to active state, until another process switches them off. Molecular switches fall into two main classes, that operate in different ways. Both classes of proteins involve activation and inactivation of phosphate groups. For one class phosphate is added by protein kinase using ATP as phosphate source. In the other class the signaling protein is induced to exchange its bound GDP with GTP (figure5.12).Many of the signaling proteins controlled by phosphorylation are protein kinases and are often organized into phosphorylation cascades. The two main protein kinases are Serine/Threonine kinase, which phosphorylates Serine and Threonine, and the other is Tyrosine kinase which phosphorylates tyrosine

residues in the proteins. GTP- binding protein alters between active state with GTP bound to them and the other inactive state when GDP is bound. The two major types of GTP binding proteins are – A large trimeric GTP- binding protein and a small monomer GTPase.

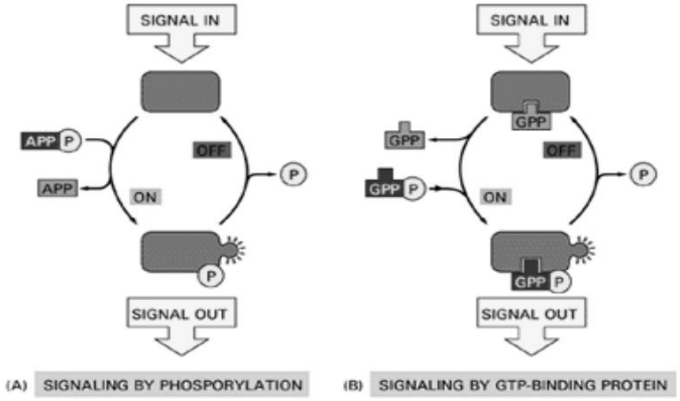


Figure 5.12: Types of intracellular proteins that act as Molecular switches

12. **Intracellular signaling complexes:** These are extracellular signals acting through a single type of G-protein linked or Enzyme-linked receptor usually activates multiple parallel signaling pathway and can thereby influence multiple aspects of cell behavior such as shape, movement , metabolism and gene expression. Cells use Scaffold proteins, which organize groups of interacting signaling proteins into signaling complexes. Scaffold guides the interactions between the successive components in such a complex, the signal can be relayed with precision, speed and efficiency; moreover, unwanted cross talk between signaling pathways is avoided. These complexes form transiently, as and when signaling proteins assemble around a receptor after an extracellular signal molecule attaches to it. The cytoplasmic tail of activated protein gets activated by Phosphorylation and this acts as docking site for intracellular signaling proteins (figure 5.13).

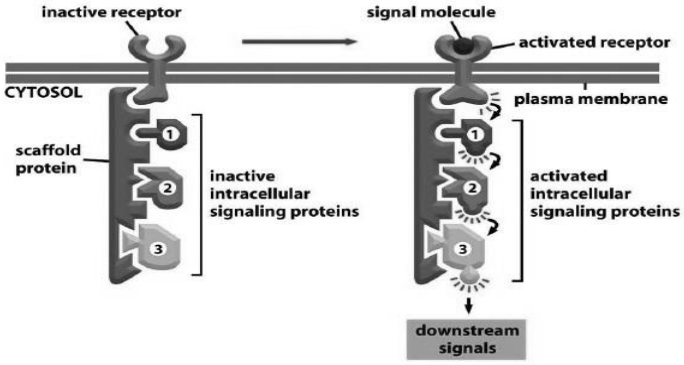


Figure 5.13: Preformed signaling proteins complex on scaffold

13. **Modular Binding Domains:** These are compact protein modules that bind to a particular motif in the protein or lipid, with which the signaling protein interacts. They bind to signaling proteins forming the route of the signaling pathways.

Eg: Src homology 2 (SH2) domains and phosphotyrosine –binding (PTB) domains (figure 5.14). It is signaling protein I that contains three different binding domains and a catalytic protein kinase domain. It moves to the plasma membrane when extra cellular signals lead to the creation of various phosphorylated docking sites on the cytosolic face of the membrane. Its SH2 domain binds to phosphorylated tyrosines on the receptor protein and its PH domain binds to phosphorylated inositol phospholipids in the inner leaflet of the lipid bilayer.

Protein I phosphorylates signaling protein 2 on tyrosines, which allows protein2 to bind to the PTB domain on protein1 and to the SH2 domain on an adopter protein that links protein2 and protein 3, causes phosphorylation of protein 3 by protein 2.

The adapter protein shown consists of 2 binding domains, one SH2 domain, which binds to a phosphotyrosine on protein 2 and an SH3 domain, which binds to a protein rich motif on protein 3.

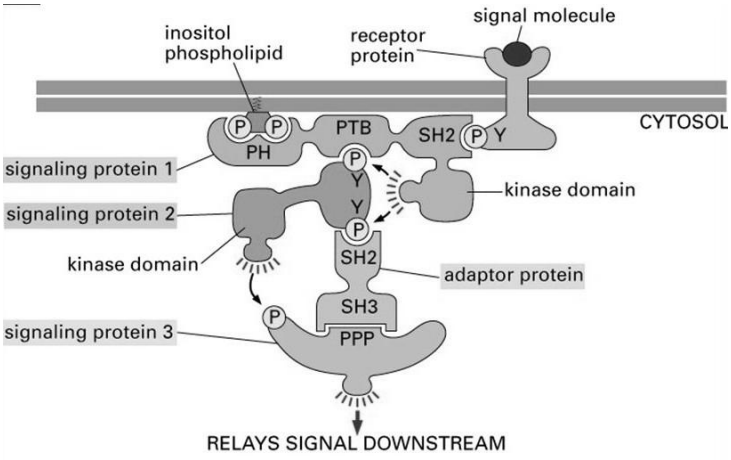


Figure 5.14 Src homology 2 (SH2) domains and phosphotyrosine –binding (PTB) domains

14. **Desensitization of Cell Signaling:** The target cells of cell signaling undergo a reversible process of adaptation or sensitization where by a prolonged exposure to a stimulus decreases the cells response to that level of exposure. There are five ways of desensitization of signal molecules (figure 5.15). Ligand binding to cell surface receptors, may induce their endocytosis and temporary sequestration in endosomes. Such Ligand- induced

receptor endocytosis can lead to the destruction of the receptors in lysosomes, a process referred to as receptor down-regulation. In other cases, desensitization occurs due to rapid inactivation of receptor due to its phosphorylation. Production of an inhibitor that blocks transduction process can also be a reason for desensitization.

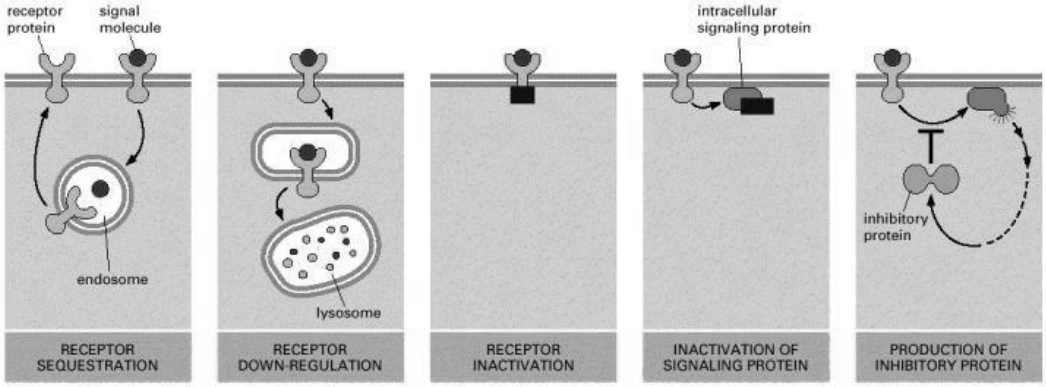


Figure 5.15: Five ways of desensitization to signal molecules

CLASSES OF CELL SURFACE RECEPTORS:

1. Ion Channel Linked Receptors:

Most of the membrane transport proteins on the plasma membrane are concerned with transport of inorganic ions like Na^+, K^+, Ca^{2+} or Cl^- and are referred to as Ion channels. These ions diffuse rapidly sown their concentration gradient through the channel across the membrane and control many cell functions. Few important channel families are listed below:

Family	**Representative Subfamilies**	
Voltage gated cation channels	Voltage gated Na^+ channels	
	Voltage gated K^+ channels	
	Voltage gated Ca^{2+} channels	
Transmitter gated ion channels	Acetylcholine gated cation channels	Excitatory
	Glutamate gated Ca^{2+} channels	Excitatory
	Serotonin gated cation channels	Excitatory
	GABA gated Cl^- channels	Inhibitory
	Glycine gated Cl^- channels	Inhibitory

Few Characteristics of these Channels:

(a) Fluctuation between open and closed state: as the ions channels are selective they allow movement of ions, based on the concentration gradient. Their opening and closing is associated with conformation changes. When pores open, it is associated with lining up of polar groups along the pore, while hydrophobic amino acids interact with lipid bilayer. This opening allows penetration of ions that have shed most of their water molecules to pass though the nearest channel called as

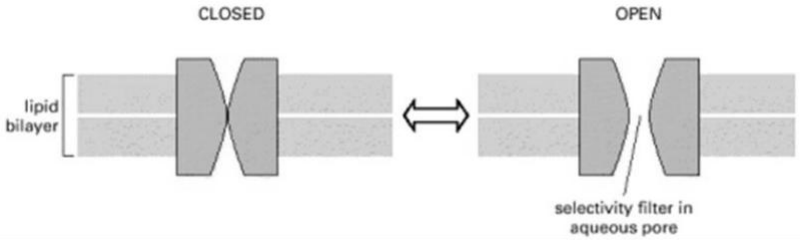


Figure 5.16: Opening and closing of Ion channels

(b) Gating of Ion Channels: few channels open due to some stimulus and these are called gated channels. The types of stimuli that can open these gated channels are negative voltage across the membrane (Voltage gated channels), a mechanical stress (mechanically gated channels), or binding of a ligand (Ligand- gated channels). The Ligand can be either extracellular mediator (as in transmitter gated channels) or a intracellular mediator (Ion-gated channels)or a nucleotide (nucleotide gated channel) (figure 5.17).

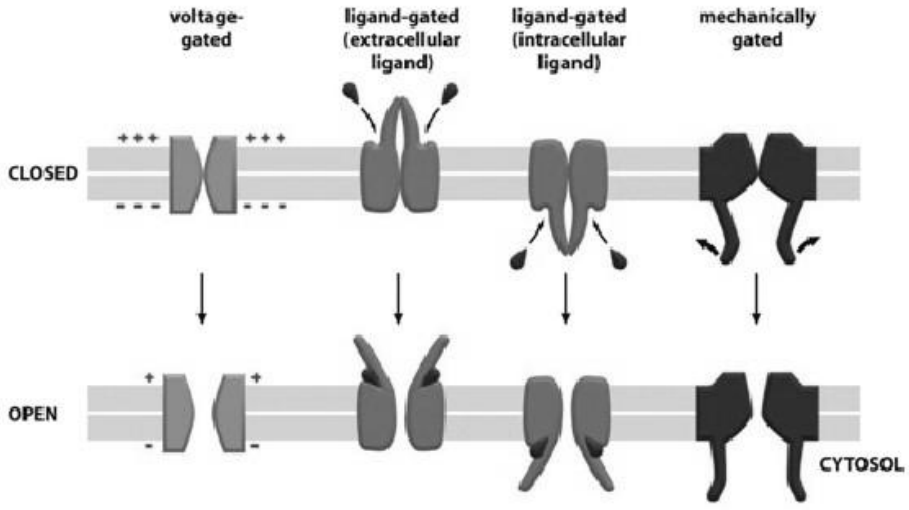


Figure 5.17: Types of Gated Ion channels

(c) **Membrane Potential role in Na^+- K^+ Pumps:** Membrane potential arises due to differences in the electrical charge across the membrane. Usually animal cells have low Na^+ inside them while K^+ ions are more in the cytosol. The balancing if Na^+ charge gradient is done by K^+ ions. K^+ ions can freely move through K^+ leaky channels across the membrane thereby balancing the concentration of these ions. A state when there is no net flow of ions is called resting potential and the cell is called a polarized cell. A stimulus can disturb this cell and cause penetration of Na^+ ions into the cells and this is called Avalanche effect, which continues until equilibrium is established. At one point Na^+ inflow stops and this cell is called a depolarized cell, the potential of cell at this state is called action potential. The cell again has to restore back to polarized state and this is by pumping out of Na^+ ions and in flow of K^+ through Na^+ - K^+ pumps. Cells start pumping our 3 Na^+ ions for every 2 K^+ ions until equilibrium is reached (figure 5.18). In animal cells, the potential difference varies from – 20 to 200mV depending on the type of cell.

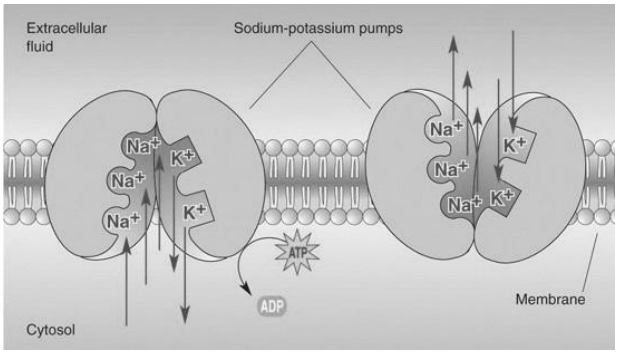


Figure 5.18: Functioning of Na^+ - K^+ pumps

(d) **Functioning of Neuron by Voltage gated Channels:** Aneuron can receive input from other neurons via a chemical called a neurotransmitter. If this input is strong enough, the neuron will pass the signal to downstream neurons. Transmission of a signal within a neuron (in one direction only, from dendrite to axon terminal) is carried out by the opening and closing of voltage-gated ion channels, which cause a brief reversal of the resting membrane potential to create an action potential . As an action potential travels down the axon, the polarity changes across the membrane. Once the signal reaches the axon terminal, it stimulates other neurons.

Depolarization and the Action Potential

When neurotransmitter molecules bind to receptors located on a neuron's dendrites, voltage- gated ion channels open. At excitatory synapses, positive ions flood the interior of the neuron and depolarize the membrane, decreasing the difference in voltage between the inside and outside of the neuron. A stimulus from a sensory cell or another neuron depolarizes the target neuron to its threshold potential (-55 mV), and Na+ channels in the axon hillock open, starting an action potential. Once the sodium channels open, the neuron completely depolarizes to a membrane potential of about +40 mV (figure 5.19). The action potential travels down the neuron as Na+ channels open.

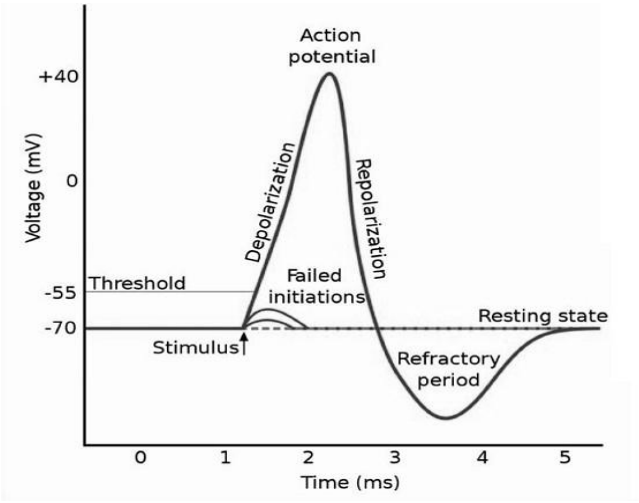


Figure 5.19: Action potential curve of neuron

Hyperpolarization and Return to Resting Potential

Action potentials are considered an "all-or nothing" event. Once the threshold potential is reached, the neuron completely depolarizes. As soon as depolarization is complete, the cell "resets" its membrane voltage back to the resting potential. The Na^+ channels close, beginning the neuron's refractory period. At the same time, voltage-gated K^+ channels open, allowing K^+ to leave the cell. As K^+ ions leave the cell, the membrane potential once again becomes negative. The diffusion of K^+ out of the cell hyperpolarizes the cell, making the membrane potential morenegative than the cell's normal resting potential(figure 5.20). At this point, the sodium channelsreturn to their resting state, ready to open again if the membrane potential again exceeds the threshold otential. Eventually, the extra K^+ ions diffuse out of the cell through the potassium leakage channels, bringing the cell from its hyperpolarized state back to its resting membrane potential.

Myelin and Propagation of the Action Potential

For an action potential to communicate information to another neuron, it must travel along the axon and reach the axon terminals where it can initiate neurotransmitter release. The speed of conduction of an action potential along an axon is influenced by both the diameter of the axon and the axon's resistance to current leak. Myelin potential conduction. Diseases like multiple sclerosis cause degeneration of the myelin, which slows action potential conduction because axon areas are no longer insulated so the current leaks.acts as an insulator that prevents current from leaving the axon, increasing the speed of action

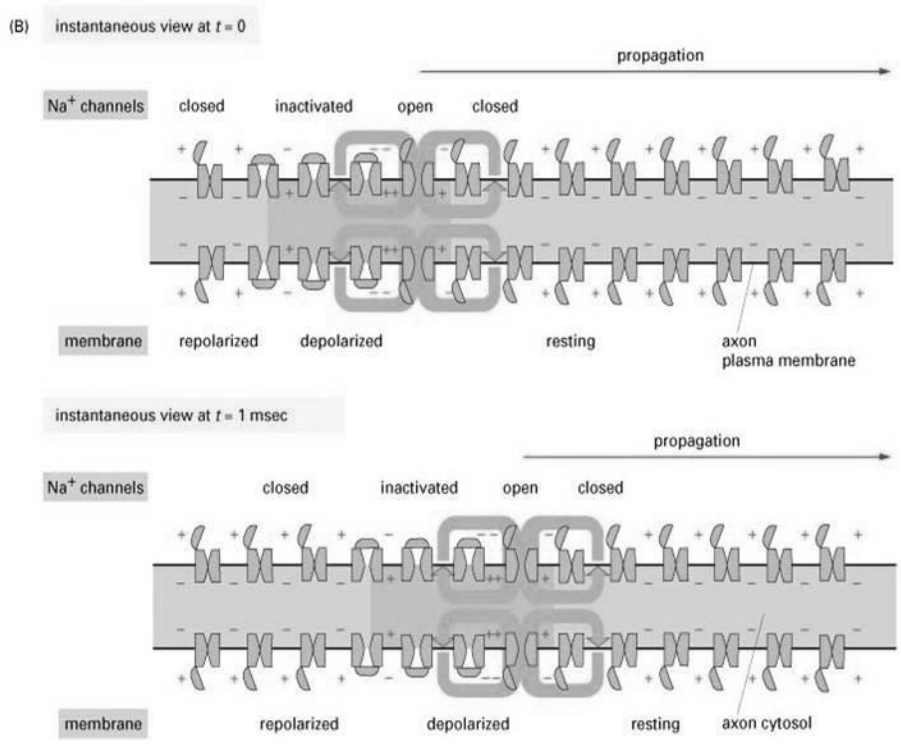


Figure 5.20: Propagation of action potential along axon of neuron

Node of Ranvier: A node of Ranvier is a natural gap in the myelin sheath along the axon. These unmyelinated spaces are about one micrometer long and contain voltage gated Na^+ and K^+ channels. The flow of ions through these channels, particularly the Na^+ channels, regenerates the action potential over and over again along the axon. Action potential "jumps" from one node to the next in saltatory conduction (figure 5.21). If nodes of Ranvier were not present along an axon, the action potential would propagate very slowly; Na^+

and K^+ channels would have to continuously regenerate action potentials at every point along the axon. Nodes of Ranvier also save energy for the neuron since the channels only need to be present at the nodes and not along the entire axon.

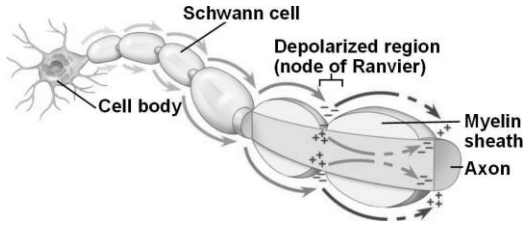


Figure 5.21 Nodes of Ranvier and salutatory conduction of nerve impulse

Synapse usually occurs between the axon of a pre-synaptic neuron and a dendrite or cell body of a post-synaptic neuron (Figure 5.22). At a synapse, the end of the axon is swollen and referred to as the end bulb or synaptic knob. Within the end bulb are found many synaptic vesicles that have the neurotransmitter and mitochondria that provide ATP. Between the end bulb and the dendrite of the post – synaptic neuron or onto the surface of skeletal muscle membrane, the gap is referred to as synaptic cleft. The impulse has to be transmitted through this space.

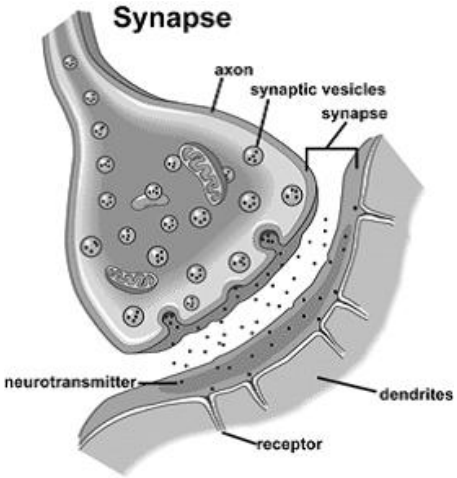


Figure 5.22: Synapse

Mechanism of Action

Synaptic transmission at the neuromuscular junction begins when an action potential reaches the presynaptic terminal of a motor neuron, which activates

voltage-dependent calcium channels to allow calcium ions to enter the neuron. Calcium ions bind to sensor proteins (synaptotagmin) on synaptic vesicles, triggering vesicle fusion with the cell membrane and subsequent neurotransmitter release from the motor neuron into the synaptic cleft (figure 5.23). In vertebrates, motor neurons release acetylcholine (ACh), a small molecule neurotransmitter, which diffuses across the synaptic cleft and binds to nicotinic acetylcholine receptors (nAChRs) on the cell membrane of the muscle fiber, also known as the sarcolemma. nAChRs are ionotropic receptors, meaning they serve as ligand-gated ion channels. The binding of ACh to the receptor can depolarize the muscle fiber, causing a cascade that eventually results in muscle contraction.

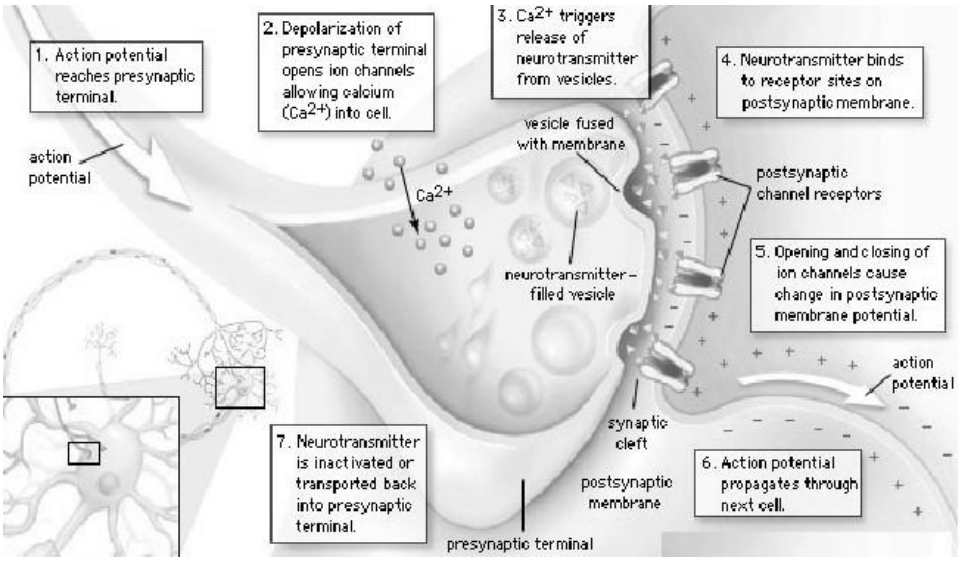


Figure 5.23: Events at Motor end plate

Acetylcholine Receptors At The Neuromuscular Junction

1. Ion channel linked receptor
2. Ions
3. Ligand (such as acetylcholine)

When ligands bind to the receptor, the ion channel portion of the receptor opens, allowing ions to pass across the cell membrane.

Acetylcholine is a neurotransmitter synthesized from dietary choline and acetyl-CoA (ACoA), and is involved in the stimulation of muscle tissue in vertebrates. The acetylcholine receptor subtype that is found at the neuromuscular junction of skeletal muscles is the nicotinic acetylcholine receptor (nAChR), which is a ligand-gated ion channel. Each subunit of

this receptor has a characteristic "cys-loop," which is composed of a cysteine residue followed by 13 amino acid residues and another cysteine residue. The two cysteine residues form a disulfide linkage which results in the "cys-loop" receptor that is capable of binding acetylcholine and other ligands.

AChRs at the skeletal neuromuscular junction form heteropentamers composed of two α, one β, one ε, and one δ subunits. When a single ACh ligand binds to one of the α subunits of the ACh receptor it induces a conformational change at the interface with the second AChR α subunit. This conformational change results in the increased affinity of the second α subunit for a second ACh ligand. AChRs therefore exhibit a sigmoidal dissociation curve due to this cooperative binding . The presence of the inactive, intermediate receptor structure with a single-bound ligand keeps ACh in the synapse that might otherwise be lost by cholinesterase hydrolysis or diffusion. The persistence of these ACh ligands in the synapse can cause a prolonged post-synaptic response.

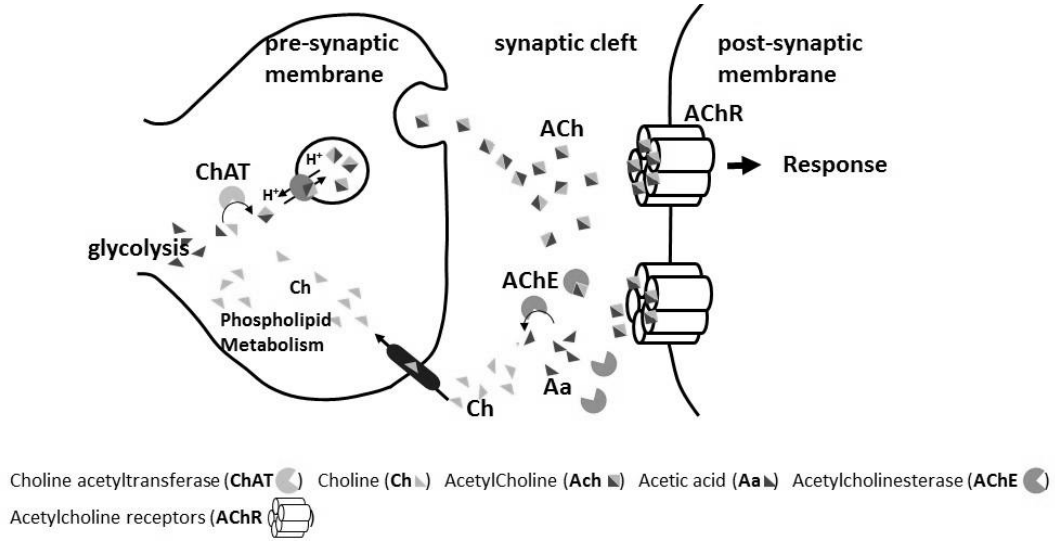


Figure 5.24: Events at synaptic cleft involving Acetylcholine and its receptor, followed by acetylcholine hydrolysis by acetylcholine esterase

2. G-protein linked cell surface receptors:

G-protein-linked receptors form the largest family of cell-surface receptors and are found in all eukaryotes. These receptors mediate the responses to an enormous diversity of signal molecules, including hormones, neurotransmitters, and local mediators. These signal molecules that activate them are as varied in structure as they are in function: the list includes proteins and small peptides, as well as derivatives of amino acids and fatty acids. The same ligand can activate many differentreceptor family members; at least 9 distinct G-protein-linked receptors are activated by adrenaline, for example,

another 5 or more by acetylcholine, and at least 15 by the neurotransmitter serotonin.

Despite the chemical and functional diversity of the signal molecules that bind to them, all G- protein-linked receptors have a similar structure. They consist of a single polypeptide chain that threads back and forth across the lipid bilayer seven times and are therefore sometimes called serpentine receptors. In addition to their characteristic orientation in the plasma membrane, they have the same functional relationship to the G proteins they use to signal the cell interior that an extracellular ligand is present.

G-protein:

Trimeric GTP-binding proteins (G proteins) are attached to the cytoplasmic face of the plasma membrane, where they serve as relay molecules, functionally coupling the receptors to enzymes or ion channels in this membrane. There are various types of G proteins, each specific for a particular set of serpentine receptors and for a particular set of downstream target proteins in the plasma membrane. All have a similar structure, however, and they operate in a similar way.

G proteins are composed of three protein subunits—α, β, and γ. In the unstimulated state, the α subunit has GDP bound and the G protein is inactive (Figure 15-27). When stimulated by an activated receptor, the α subunit releases its bound GDP, allowing GTP to bind in its place. This exchange causes the trimer to dissociate into two activated components—an α subunit and a βγ complex (figure 5.25).

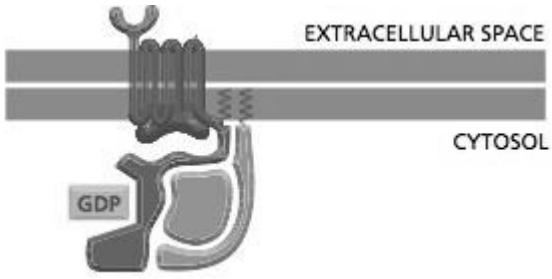


Figure 5.25: GTP binding protein (G protein)

Activation of G Protein

The dissociation of the trimeric G protein activates its two components in different ways. GTP binding causes a conformational change that affects the surface of the α subunit that associates with the βγ complex in the trimer. This change causes the release of the βγ complex, but it also causes and the α subunit to adopt a new shape that allows it to interact with its target proteins. The βγ complex does not change its conformation, but the surface previously masked by the α subunit is now available to interact with a second set of target proteins. The targets of the dissociated components of the G protein are either enzymes or ion channels in the plasma membrane, and they relay the signal onward.

The α subunit is a GTPase, and once it hydrolyzes its bound GTP to GDP, it reassociates with a βγ complex to re-form an inactive G protein, reversing the activation process (Figure 15-29). The time during which the α subunit and βγ complex remain apart and active is usually short, and it depends on how quickly the α subunit hydrolyzes its bound GTP. An isolated α subunit is an inefficient GTPase, and, left to its own devices, the subunit would inactivate only after several minutes. Its activation is usually reversed much faster than this, however, because the GTPase activity of the α subunit is greatly enhanced by the binding of a second protein, which can be either its target protein or a specific modulator known as a regulator of G protein signaling (RGS). RGS proteins act as α-subunit-specific GTPase activating proteins (GAPs), and they are thought to have a crucial role in shutting off G-protein-mediated responses in all eukaryotes. There are about 25 RGS proteins encoded in the human genome, each of which is thought to interact with a particular set of G proteins.

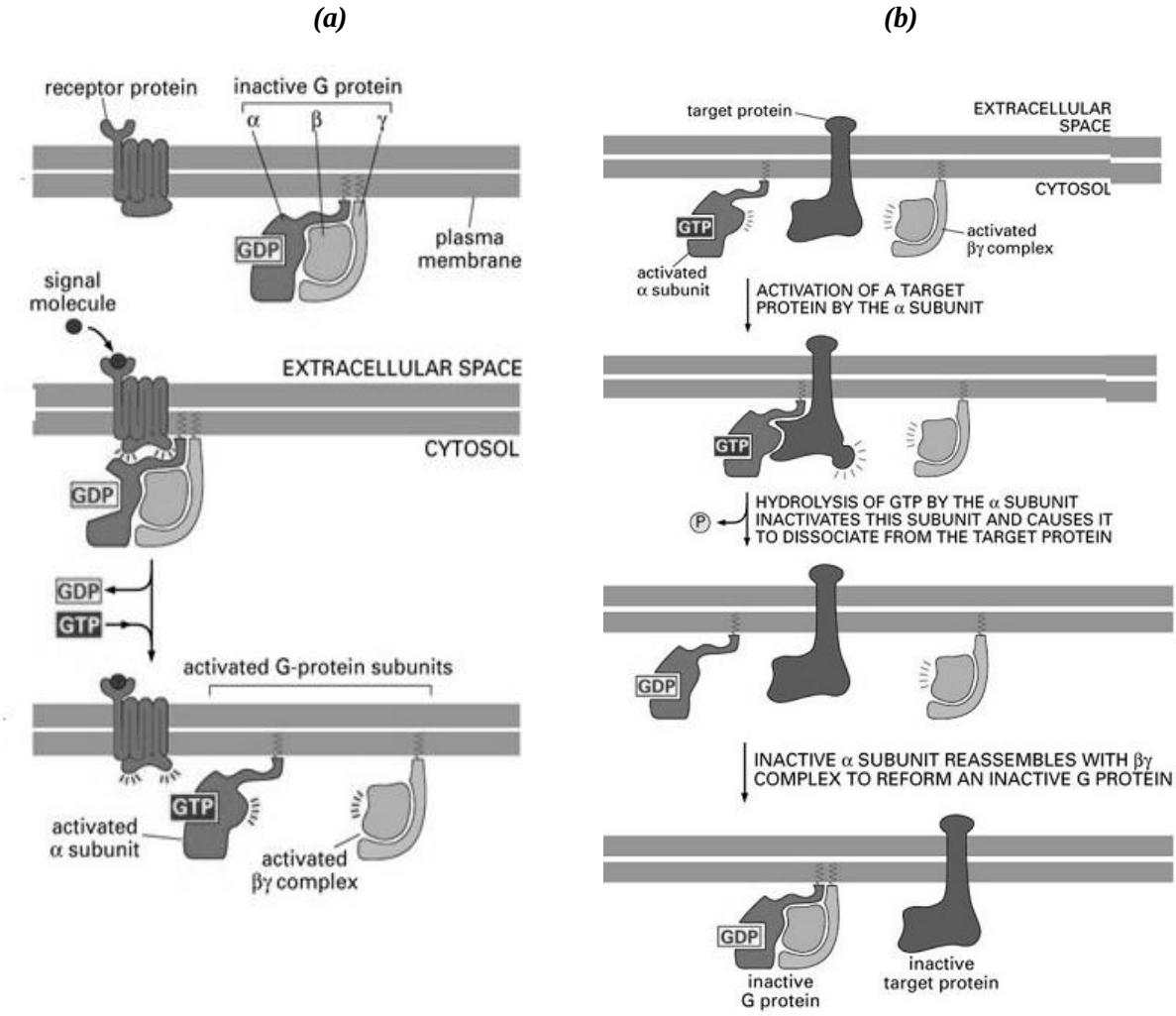


Figure 5.26: a)Disassembly and activation of GTP binding protein b) Switching off of G-protein by hydrolysis of ATP

Some G proteins signal by regulating the production of cyclic AMP

Cyclic AMP (cAMP) was first identified as a small intracellular mediator in the 1950s. It has since been found to act in this role in all prokaryotic and animal cells that have been studied. The normal concentration of cyclic AMP inside the cell is about 10^{-7} M, but an extracellular signal can cause cyclic AMP levels to change by more than twentyfold in seconds (Figure 15-30). As explained earlier (see Figure 15-10), such a rapid response requires that a rapid synthesis of the molecule be balanced by its rapid breakdown or removal. In fact, cyclic AMP is synthesized from ATP by a plasma-membrane-bound enzyme adenylyl cyclase, and it is rapidly and

continuously destroyed by one or more cyclic AMP phosphodiesterases that hydrolyze cyclic AMP to adenosine 5′-monophosphate (5′-AMP) (Figure 5.27).

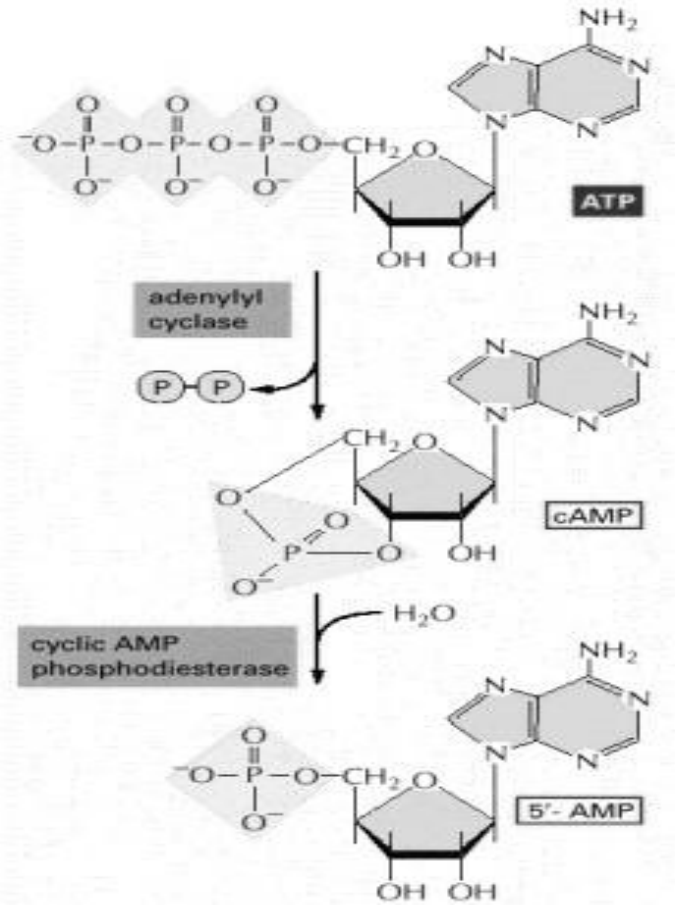


Figure5.27: Synthesis and degradation of cyclic AMP

Cyclic-Amp-Dependent Protein Kinase(Pka): This enzyme catalyzes the transfer of the terminal phosphate group from ATP to specific serines or threonines of selected target proteins, thereby regulating their activity. In the inactive state, PKA consists of a complex of two catalytic subunits and two regulatory subunits. The binding of cyclic AMP to the regulatory subunits alters their conformation, causing them to dissociate from the complex. The released catalytic subunits are thereby activated to phosphorylate specific substrate protein molecules (Figure 5.28). The regulatory subunits of PKA also are important for localizing the kinase inside the cell: special PKA anchoring proteins bind both to the regulatory subunits and to a membrane or a component of the cytoskeleton, thereby tying up the enzyme complex to a particular subcellular compartment. Some of these anchoring proteins also bind other kinases and some phosphatases, creating a signaling complex.

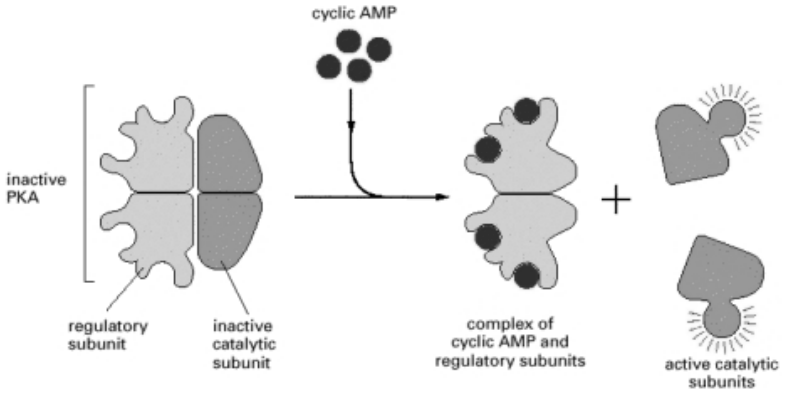


Figure 5.28 Activation of PKA

Cyclic AMP role in activating genes:

In cells that secrete the peptide hormone somatostatin, for example, cyclic AMP activates the gene that encodes this hormone. The regulatory region of the somatostatin gene contains a short DNA sequence, called the cyclic AMP response element (CRE) that is also found in the regulatory region of many other genes activated by cyclic AMP. A specific gene regulatory protein called CRE-binding (CREB) protein recognizes this sequence. When CREB is phosphorylated by PKA on a single serine, it recruits a transcriptional coactivator called CREB- binding protein (CBP), which stimulates the transcription of these genes (Figure 5-29).

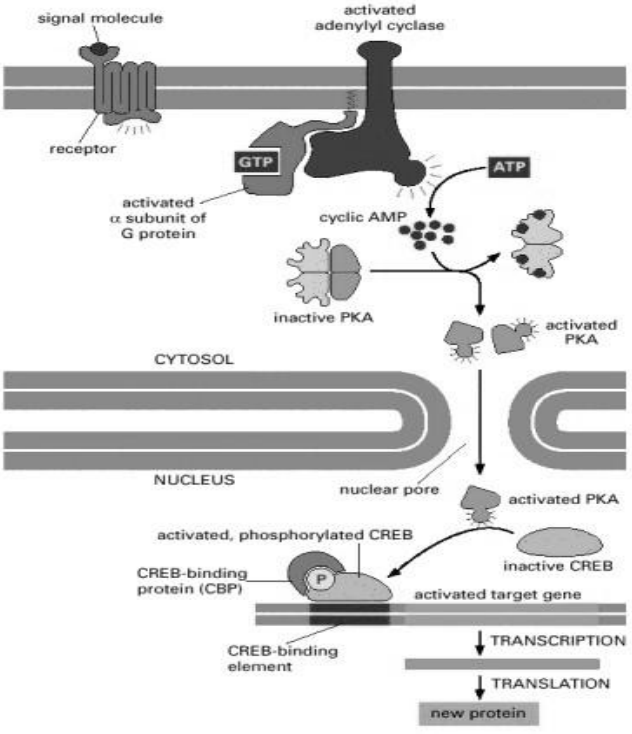


Figure 5.29: Gene transcription activation by cyclic AMP

Dephosphorylation of PKA activated proteins by Phosphatases:

Since the effects of cyclic AMP are usually transient, cells must be able to dephosphorylate the proteins that have been phosphorylated by PKA. In general, the dephosphorylation of phosphorylated serines and threonines is catalyzed by four types of serine/threonine phosphoprotein phosphatases—protein phosphatases I, IIA, IIB, and IIC. Except for protein phosphatase-IIC (which is a minor phosphatase, unrelated to the others), all of these phosphatases are composed of a homologous catalytic subunit complexed with one or more of a large set of regulatory subunits; the regulatory subunits help to control the phosphatase activity and enable the enzyme to select specific targets. Protein phosphatase I is responsible for dephosphorylating many of the proteins phosphorylated by PKA. It inactivates CREB, for example, by removing its activating phosphate, thereby turning off the transcriptional response caused by a rise in cyclic AMP concentration. Protein phosphatase IIA has a broad specificity and seems to be the main phosphatase responsible for reversing many of the phosphorylations catalyzed by serine/threonine kinases. Protein phosphatase IIB, also called calcineurin, is activated by Ca^{2+} and is especially abundant in the brain.

Inositol Phospholipid Signaling Pathway activation by G Protein

Many G-protein-linked receptors exert their effects mainly via G proteins that activate the plasma-membrane-bound enzyme phospholipaseC-β. The phospholipase acts on an inositol phospholipid called phosphatidylinositol 4,5-bisphosphate [$PI(4,5)P_2$], which is present in small amounts in the inner half of the plasma membrane lipid bilayer. Receptors that operate through this inositol phospholipid signaling pathway mainly activate a G protein called G_q, which in turn activates phospholipase C-β, in much the same way that G_s activates adenylyl cyclase. The activated phospholipase cleaves $PI(4,5)P_2$ to generate two products: inositol 1,4,5- trisphosphate and diacylglycerol (Figure 5-30), which diffuses through the cytosol and releases Ca^{2+} from the ER, and diacylglycerol, which remains in the membrane and helps to activate the enzyme protein kinase C.

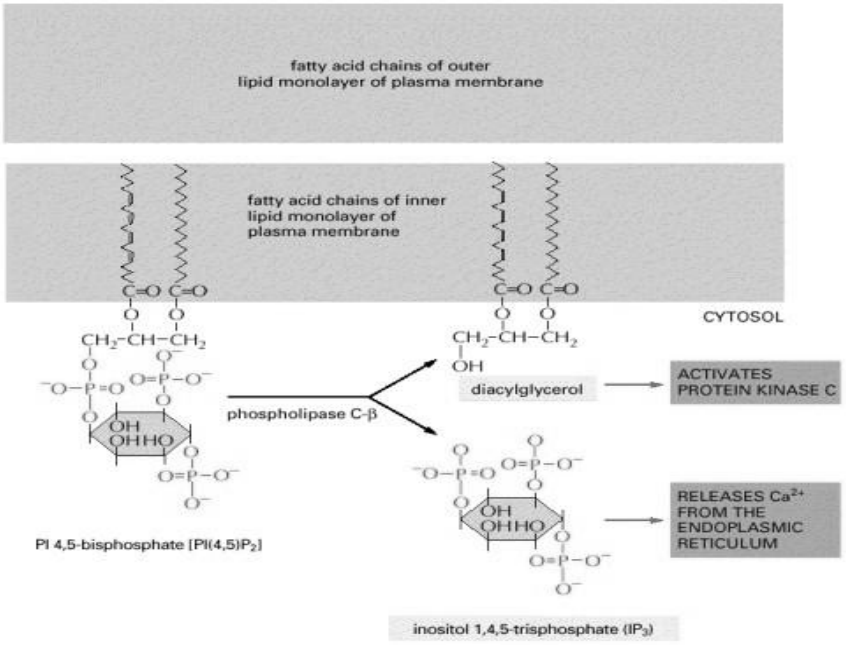


Figure 5.30: Hydrolysis of PI(4,5)P_2 by phospholipase C-β

Calcium as a Ubiquitous Messenger:

Calcium ions play several roles in animal cells. In muscle cells, Ca^{2+} triggers contraction, and in many secretory cells, including nerve cells, it triggers secretion. Ca^{2+} can be used as a signal in this way because its concentration in the cytosol is normally kept very low (~10^{-7} M), whereas its concentration in the extracellular fluid (~10^{-3} M) and in the ER lumen is high. Thus, there is a large gradient tending to drive Ca^{2+} into the cytosol across both the plasma membrane and the ER membrane. When a signal transiently opens Ca^{2+} channels in either of these membranes, Ca^{2+} rushes into the cytosol, increasing the local Ca^{2+} concentration by 10–20-fold and triggering Ca^{2+}-responsive proteins in the cell.

Three main types of Ca^{2+} channels can mediate this Ca^{2+} signaling:

1. Voltage-dependent Ca^{2+} channels in the plasma membrane open in response to membrane depolarization and allow, for example, Ca^{2+} to enter activated nerve terminals and trigger neurotransmitter secretion.
2. IP_3-gated Ca^{2+}-release channels allow Ca^{2+} to escape from the ER when the inositol phospholipid signaling pathway is activated, as just discussed.

3. Ryanodine receptors (so called because they are sensitive to the plant alkaloid ryanodine) react to a change in plasma membrane potential to release Ca^{2+} from the sarcoplasmic reticulum and thereby stimulate the contraction of muscle cells; they are also present in the ER of many nonmuscle cells, including neurons, where they can contribute to Ca^{2+} signaling.

Families of Trimeric G-proteins and their function: They are summarized in the table below:

FAMILY	SOME FAMILY MEMBERS	ACTION MEDIATED BY	FUNCTIONS
I	G_s	α	activates adenylyl cyclase; activates Ca^{2+} channels
	G_{olf}	α	activates adenylyl cyclase in olfactory sensory neurons
II	G_i	α	inhibits adenylyl cyclase
		βγ	activates K^+ channels
	G_o	βγ	activates K^+ channels; inactivates Ca^{2+} channels
		α and βγ	activates phospholipase C-β
	G_t (transducin)	α	activates cyclic GMP phosphodiesterase in vertebrate rod photoreceptors
III	G_q	α	activates phospholipase C-β

Desensitization of G-protein linked receptor:Target cells use a variety of mechanisms to desensitize, or adapt, when they are exposed to a high concentration of stimulating ligand for a prolonged period. Few mechanisms that involve an alteration in G-protein-linked receptors themselves.

These receptors can desensitize in three general ways:

1. They can become altered so that they can no longer interact with G proteins (receptor inactivation).
2. They can be temporarily moved to the interior of the cell (internalized) so that they no longer have access to their ligand (receptor sequestration).
3. They can be destroyed in lysosomes after internalization (receptor down-regulation).

In each case, the desensitization process depends on phosphorylation of the receptor, by PKA, PKC, or a member of the family of G-protein-linked receptor kinases (GRKs). The GRKs phosphorylate multiple serine and threonines on a receptor, but they do so only after the receptor has been activated by ligand binding. The binding of an arrestin to the phosphorylated receptor prevents the receptor from binding to its G protein and can direct its endocytosis (Figure 5.31).

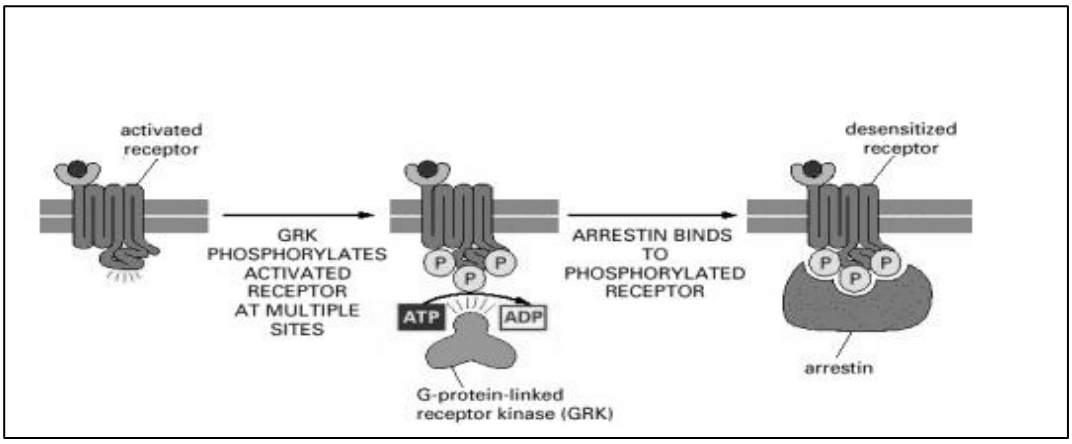


Figure 5.31: The roles of G-protein-linked receptor kinases (GRKs) and arrestins in receptordesensitization

3. Signaling through Enzyme linked Cell Surface Receptors:

Enzyme-linked receptors are a second major type of cell-surface receptor. They were recognized initially through their role in responses to extracellular signal proteins that promote the growth, proliferation, differentiation, or survival of cells in animal tissues. These signal proteins are often collectively called growth factors, and they usually act as local mediators at very low concentrations (about 10^{-9}-10^{-11} M). The responses to them are typically slow (on the order of hours) and usually require many intracellular signaling steps that eventually lead to changes in gene expression. Enzyme-linked receptors have since been found also to mediate direct, rapid effects on the cytoskeleton, controlling the way a cell moves and changes its shape. Disorders of cell proliferation, differentiation, survival, and migration are fundamental events that can give rise to cancer, and abnormalities of signaling through enzyme-linked receptors have major roles in this class of disease.

These are transmembrane proteins with their ligand-binding domainon the outer surface of the plasma membrane and cytosolic domain either has an intrinsic enzyme activity or associates directly with an enzyme.

Six classes of enzyme-linked receptors include:

1. Receptor tyrosine kinases: They phosphorylate specific tyrosines on a small set of intracellular signaling proteins.
2. Tyrosine-kinase-associated receptors – They associate with intracellular proteins that have tyrosine kinase activity.
3. Receptor like tyrosine Phosphatases: They remove phosphate groups from tyrosines of specific intracellular signaling proteins.

4. Receptor serine/threonine kinases – They phosphorylate specific serines or threonines on associated latent gene regulatory proteins.
5. Receptor guanylyl cyclases- they directly catalyze the production of cyclic GMP in the cytosol.
6. Histidine-kinase-associated receptors activate a "two-component" signaling pathway in which the kinase phosphorylates itself on histidine and then immediately transfers the phosphate to a second intracellular signaling protein.

1. **Tyrosine receptor Kinases:** Receptor tyrosine kinases consist of a large variety of secreted growth factors and hormones. Many cell-surface-bound signal proteins also act through these receptors. Few of these include: epidermal growth factor (EGF),platelet-derived growth factor (PDGF), fibroblast growth factors (FGFs), hepatocyte growth factor (HGF), insulin, insulinlike growth factor-1 (IGF-1), vascular endothelial growth factor (VEGF), macrophage-colony- stimulating factor(M-CSF), and all the neurotrophins, including nerve growth factor (NGF).

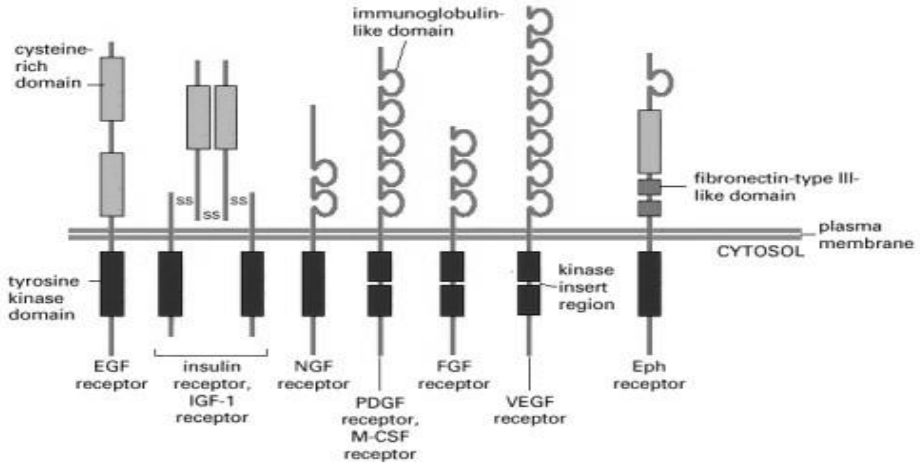


Figure 5.32: Seven subfamilies of Receptor Tyrosine kinases

The largest class of these membrane-bound ligands is the ephrins, which regulate the cell adhesion and repulsion responses that guide the migration of cells and axons along specific pathways during animal development. The receptors for ephrins, called Eph receptors, are also the most numerous receptor tyrosine kinases. The ephrins and Eph receptors can simultaneously act as both ligand and receptor: on binding to an Eph receptor, some ephrins not only activate the Eph receptor but also become activated themselves to transmit signals into the interior of the ephrin-expressing cell. In this way, an interaction between an ephrin protein on one cell and an Eph protein on another cell can lead to bidirectional reciprocal signaling that changes the behavior of both cells.

Receptor tyrosine kinases can be classified into more than 16 structural subfamilies, each dedicated to its complementary family of protein ligands. Once activated, the kinase domain transfers a phosphate group from ATP to selected tyrosine side chains, both on the receptor proteins themselves and on intracellular signaling proteins that subsequently bind to the phosphorylated receptors.

When the receptor chains are cross-linked, the kinase domains of adjacent receptors cross-phosphorylate each other, stimulating the kinase activity of the receptor and creating docking sites for intracellular proteins. (A) Platelet-derived growth factor (PDGF) is a covalently linked dimer with two receptor-binding sites, so it can directly cross-link adjacent receptors to initiate the intracellular signaling process. The PDGF dimers are composed of A and B chains in different combinations, and they can activate different combinations of two types of PDGF receptor chains (α and β), which have somewhat different signaling properties. (B) Some monomeric ligands, such as fibroblast growth factors (FGFs), bind in clusters to proteoglycans, enabling the ligands to cross-link their receptors. The proteoglycans can be in the extracellular matrix or, as shown here, on the cell surface. There are more than 20 types of FGFs and more than 4 types of FGF receptors. (C) Membrane-bound signaling proteins, such as ephrins, can cross-link their receptors even though they are monomeric, because they cluster in the plasma membrane of the signaling cell. Some ephrins (B-type) are transmembrane proteins (as shown), whereas others (A-type) are linked to the membrane by a glycosylphosphatidylinositol (GPI) anchor (Figure 5.33). For the most part, A-type ephrins activate A-type Eph receptors, and B- type ephrins activate B-type Eph receptors.

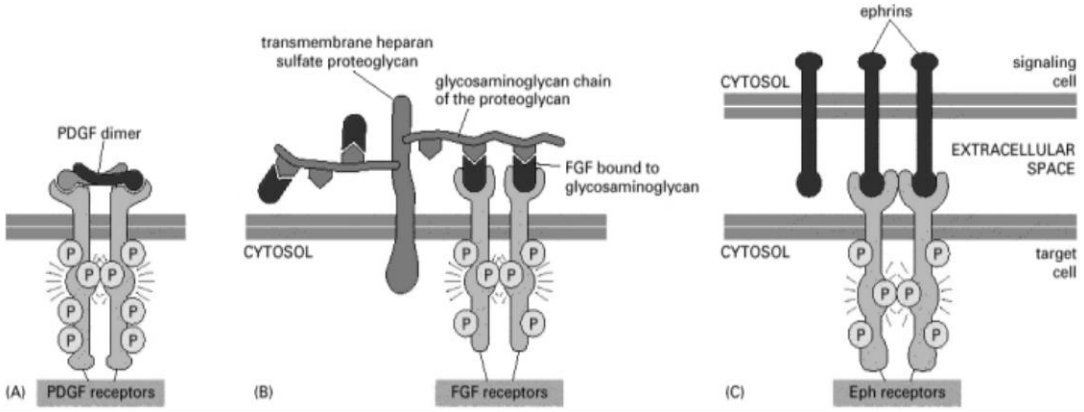


Figure 5.33: Three ways in which signaling proteins can cross-link receptor chains

Phosphorylated Tyrosine serves as docking site for proteins:

Autophosphorylation of the cytosolic tail of receptor tyrosine kinases contributes to the activation process in two ways. First, phosphorylation of tyrosines within the kinase domain increases the kinase activity of the enzyme. Second, phosphorylation of tyrosines outside the kinase domain creates high-affinity docking sites for the binding of a number of intracellular signaling proteins in the target cell. Each type of signaling protein binds to a different phosphorylated site on the activated receptor because it contains a specific phosphotyrosine-binding domain that recognizes surrounding features of the polypeptide chain in addition to the phosphotyrosine. Once bound to the activated kinase, the signaling protein may itself become phosphorylated on tyrosines and thereby activated; alternatively, the binding alone may be sufficient to activate the docked signaling protein. In summary, autophosphorylation serves as a switch to trigger the transient assembly of a large intracellular signaling complex, which then broadcasts signals along multiple routes to many destinations in the cell.

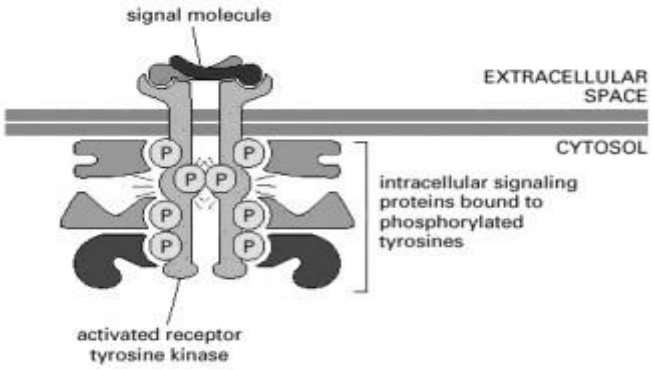


Figure 5.34: Docking of intracellular signaling proteins on an activated receptor tyrosine kinase

Phosphorylated Tyrosines acting as Docking sites:

Although the intracellular signaling proteins that bind to phosphotyrosines on activated receptor tyrosine kinases and docking proteins have varied structures and functions. These can be either SH2 domains or, less commonly, PTB domains (phosphotyrosine-binding). By recognizing specific phosphorylated tyrosines, these small domains serve as modules that enable the proteins that contain them to bind to activated receptor tyrosine kinases, as well as to many other intracellular signaling proteins that have been transiently phosphorylated on tyrosines. Many signaling proteins also contain other protein modules that allow them to interact specifically with other proteins as part of the signaling process. These include the SH3 domain,

which binds to proline-rich motifs in intracellular proteins. Some signaling proteins are composed almost entirely of SH2 and SH3 domains and function as adaptors to couple tyrosine- phosphorylated proteins to other proteins that do not have their own SH2 domains. Such adaptor proteins help to couple activated receptors to the important downstream signaling protein Ras.

Ras is activated by Guanine Nucleotide Exchange factor:

Ras proteins belong to the large Ras superfamily of monomeric GTPases, involved in relaying signals from cell-surface receptors to the actin cytoskeleton and the Rab family, involved in regulating the traffic of intracellular transport vesicles. The Ras proteins contain a covalently attached lipid group that helps to anchor the protein to the plasma membrane where the protein functions. There are multiple Ras proteins, and different ones act in different cell types.

Ras helps to broadcast signals from the cell surface to other parts of the cell. It is often required, for example, when receptor tyrosine kinases signal to the nucleus to stimulate cell proliferation or differentiation by altering gene expression. Ras functions as a switch, cycling between two distinct conformational states—active when GTP is bound and inactive when GDP is bound. Two classes of signaling proteins regulate Ras activity by influencing its transition between active and inactive states. Guanine nucleotide exchange factors (GEFs) promote the exchange of bound nucleotide by stimulating the dissociation of GDP and the subsequent uptake of GTP from the cytosol, thereby activating Ras. GTPase-activating proteins (GAPs) increase the rate of hydrolysis of bound GTP by Ras, thereby inactivating Ras (Figure 5-35). Mutation of Ras leads to cancer.

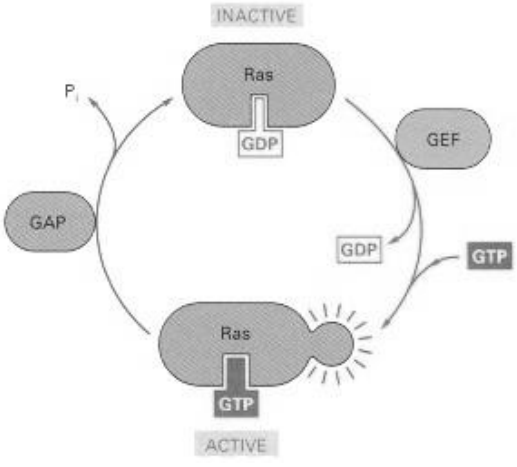


Figure 5.35 Regulation of Ras activity

Once activated, Ras in turn activates various other signaling proteins to relay the signal downstream along several pathways. One of the signaling pathways Ras activates is a serine/threonine phosphorylation cascade that is highly conserved in eukaryotic cells from yeasts to humans. A crucial component in this cascade is a novel type of protein kinase called MAP- kinase. Once activated, Ras in turn activates various other signaling proteins to relay the signal downstream along severalpathways. One of the pathways is Serine/Threonine phosphorylation cascade. Crucial components of this cascade is Protein kinase MAP Kinase (Mitotic Activated Protein Kinase) (Figure 5.36).

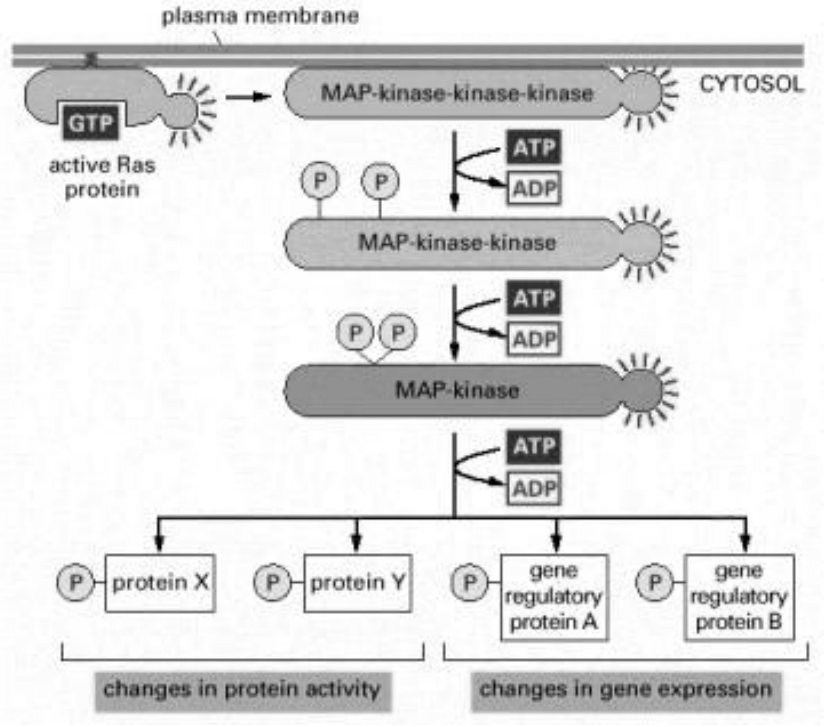


Figure 5.36: MAP-kinase serine-threonine phosphorylation pathway activated by Ras

P13-Kinase produces Inositol Phospholipid Socking Sites

One of the major intracellular signaling pathways leading to cell growth involves phosphatidylinositol 3-kinase (PI 3-kinase). This kinase principally phosphorylates inositol phospholipids rather than proteins; it can be activated by receptor tyrosine kinases, as well as by many other types of cell-surface receptors, including some that are G-protein-linked. Phosphatidylinositol (PI) is unique among membrane lipids because it can undergo reversible phosphorylation at multiple sites to generate a variety of distinct inositol phospholipids. When activated, PI 3-kinase

catalyzes the phosphorylation of inositol phospholipids at the 3 position of the inositol ring to generate lipids called $PI(3,4)P_2$ or$PI(3,4,5)P_3$ (Figure 5.37). The $PI(3,4)P_2$ and $PI(3,4,5)P_3$ then serve as docking sites for intracellular signaling proteins, bringing these proteins together into signaling complexes, which relay the signal into the cell from the cytosolic face of the plasma membrane. The generation of inositol phospholipid docking sites by PI 3-kinase.

They remain in the plasma membrane until they are dephosphorylated by specific inositol phospholipid phosphatases that remove phosphate from the 3 position of the inositol ring. There are various types of PI 3-kinases. The one that is activated by receptor tyrosine kinases consists of a catalytic and regulatory subunit. The regulatory subunit is an adaptor protein that binds to phosphotyrosines on activated receptor tyrosine kinases through its SH2 domains. Another PI 3-kinase has a different regulatory subunit and is activated by the βγ complex of a trimeric G protein when G-protein-linked receptors are activated by their extracellular ligand. The catalytic subunit, which is similar in both cases, also has a binding site for activated Ras, which allows Ras to directly stimulate PI 3-kinases.

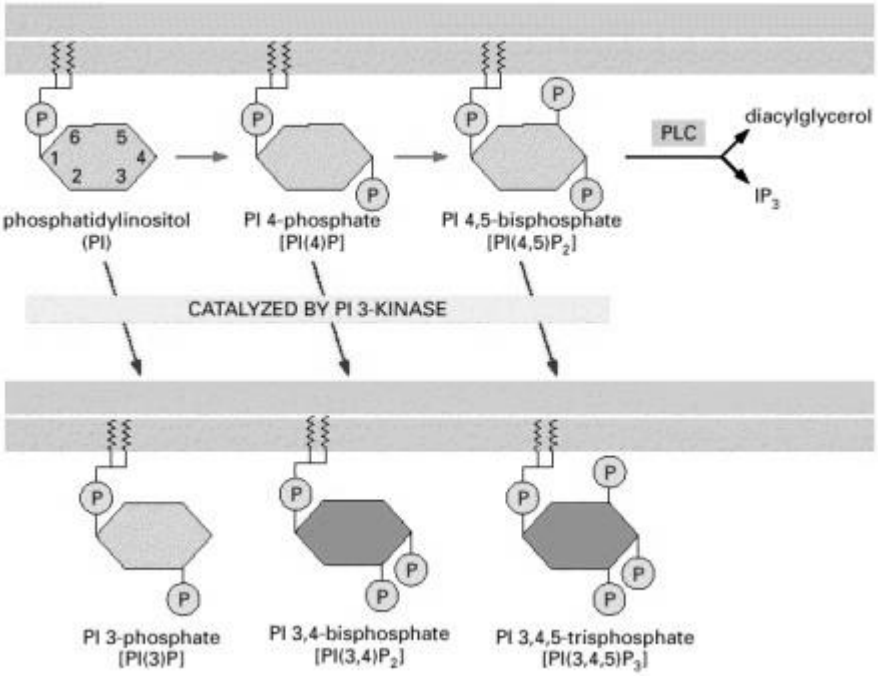


Figure 5.37: Generation of Inositol Phospholipid docking sites by PI 3- Kinases

PI 3-kinase promotes cell survival

An extracellular survival signal activates a receptor tyrosine kinase, which recruits and activates PI 3-kinase (figure 5.38). The PI 3-kinase produces $PI(3,4,5)P_3$ and $PI(3,4)P_2$ (not shown), both of which serve as docking sites for two serine/threonine kinases with PH domains—protein kinase B (PKB) and the phosphoinositol-dependent kinase PDK1. The binding of PKB to the inositol lipids alters its conformation so that the protein can be phosphorylated and activated by PDK1. The activated PKB now dissociates from the plasma membrane and phosphorylates the BAD protein, which, when unphosphorylated, holds one or more death-inhibitory proteins in an inactive state. Once phosphorylated, BAD releases the inhibitory proteins, which now can block programmed cell death (apoptosis) and thereby promote cell survival.

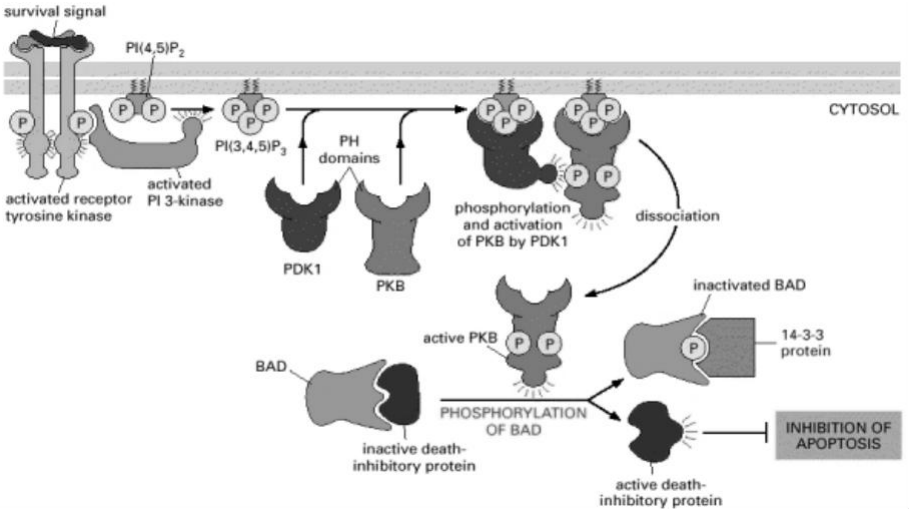


Figure 5.38: One way in which signaling thorugh PI 3 kinase promotes cell survival

Cytokine Receptor and Jak-STAT pathway:

When activated, interferon receptors activate a novel class of cytoplasmic tyrosine kinases called Janus kinases (Jaks) (after the two-faced Roman god). The Jaks then phosphorylate and activate a set of latent gene regulatory proteins called STATs (signal transducers and activators oftranscription), which move into the nucleus and stimulate the transcription of specific genes. More than 30 cytokines and hormones activate the Jak-STAT pathway by binding to cytokine receptors. All STATs also have an SH2 domain that enables them to dock onto specific phosphotyrosines on some activatedreceptor tyrosine kinase receptors. These receptors can directly activate the bound STAT, independently of Jaks.

Cytokine receptors are composed of two or more polypeptide chains. All cytokine receptors, however, are associated with one or more Jaks. There are four known Jaks—Jak1, Jak2, Jak3, and Tyk2—and each is associated with particular cytokine receptors. The receptors for α- interferon, for example, are associated with Jak1 and Tyk2, whereas the receptors for γ- interferon are associated with Jak1 and Jak2. The receptor for the hormone erythropoietin, which stimulates erythrocyte precursor cells to survive, proliferate, and differentiate, is associated with only Jak2.

There are seven known STATs, each with an SH2 domain that performs two functions. First, it mediates the binding of the STAT protein to a phosphotyrosine docking site on an activated cytokine receptor ; once bound, the Jaks phosphorylate the STAT on tyrosines, causing it to dissociate from the receptor. Second, the SH2 domain on the released STAT now mediates its binding to a phosphotyrosine on another STAT molecule, forming either a STAT homodimer or heterodimer which moves into the nucleus, where, in combination with other gene regulatory proteins, it binds to a specific DNA response element in various genes and stimulates their transcription (Figure 5.39).

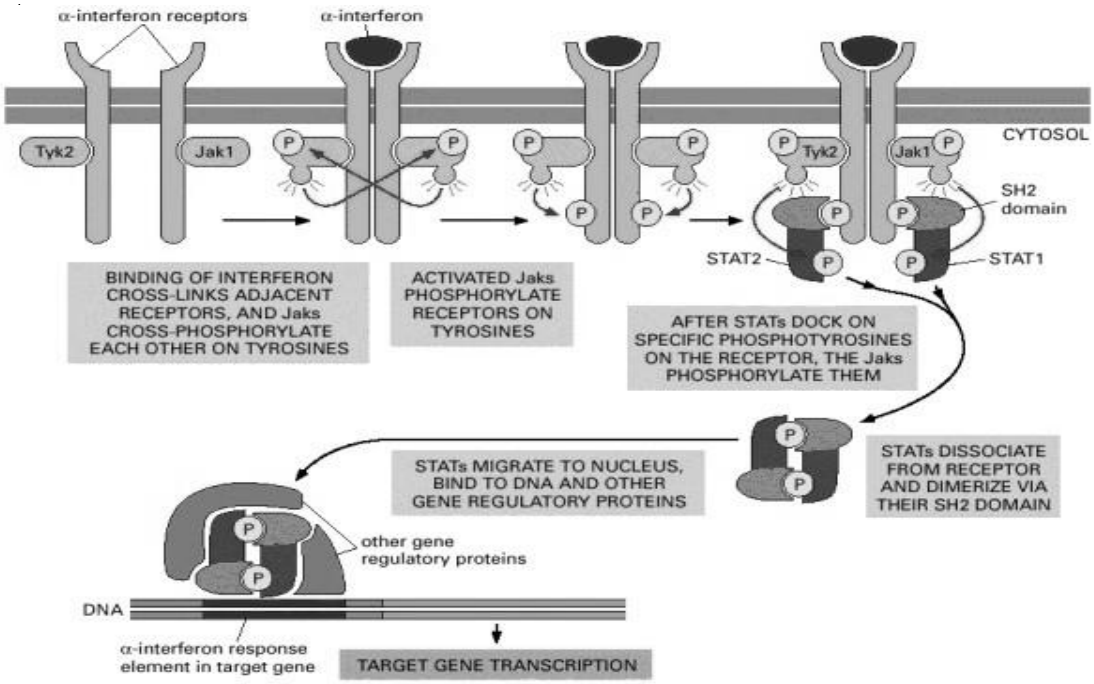


Figure 5.39: The Jak-STAT signaling pathway activated by Alpha Interferon

Protein Tyrosine Phosphatases as cell surface receptors:

ProteinTyrosine Phosphatases (PTPs) are involved in removal of phosphate groups from selected phosphotyrosines on a subset of tyrosine-phosphorylated proteins. There are two tyrosine Phosphatases in vertebrates, having SH2 domains and these are SHP-1 and SHP-2. SHP-1helps to terminate some cytokine responses in blood cells by dephosphorylating activated Jacks, while both SHP-1 and SHP-2 help to terminate responses mediated by some receptor tyrosine kinases. Some receptor like tyrosine phosphatases display features of cell-adhesion proteins and can even mediate homophilic cell-cell binding in cell adhesion assays. Transmembrane tyrosine phosphatases can also serve as signaling ligands that activate receptors on a neighboring cell. Signal proteins of TGF-□ superfamily

The transforming growth factor -□□□uperfamily consists of large number of structurally related, secreted, dimeric proteins, which either act as hormones or local mediators. During development they regulate pattern formation and influence various cell behaviors, including proliferation, differentiation, extracellular matrix production and cell death.

There are two classes of these receptorserine/threonine kinases — type I and type II—which are structurally similar. Each member of the TGF-β superfamilybinds to a characteristic combination of type-I and type-II receptors, both of which are required for signaling. Typically, the ligand first binds to and activates a type-II receptor homodimer, which recruits, phosphorylates, and activates a type-I receptor homodimer, forming an active tetrameric receptor complex. The type-I receptor directly binds and phosphorylates a latent gene regulatory protein of the Smad family. Activated TGF-β receptors and activin receptors phosphorylate Smad2 or Smad3, while activated BMP receptors phosphorylate Smad1, Smad5, or Smad8. Once one of these Smads has been phosphorylated, it dissociates from the receptor and binds to Smad4, which can form a complex with any of the above five receptor-activated Smads. The Smad complex then moves into the nucleus, where it associates with other gene regulatory proteins, binds to specific sites in DNA, and activates a particular set of target genes.

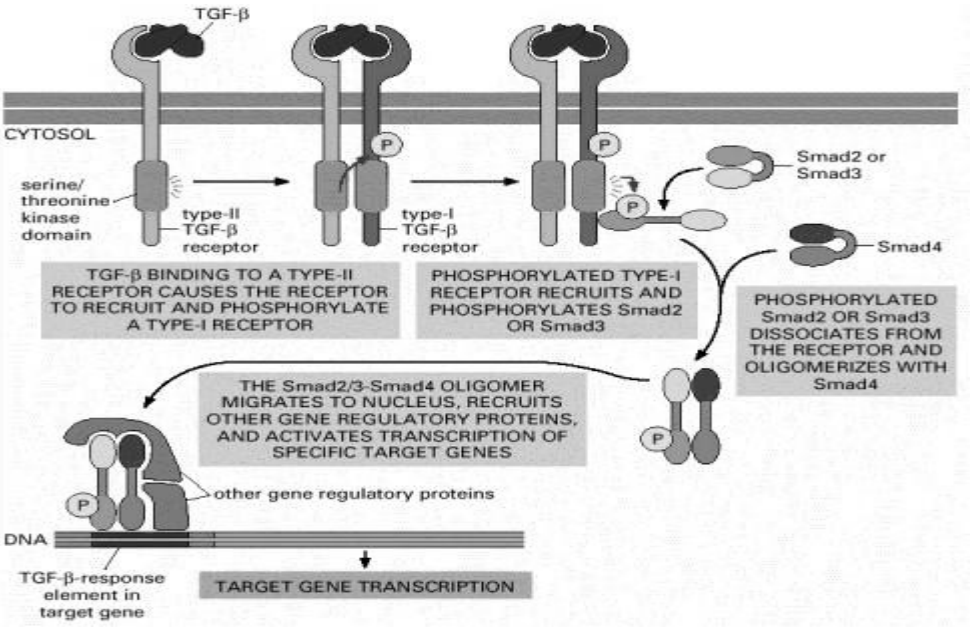


Figure 5.40: A smad dependent signaling pathway activated by TGF-β

Receptor guanylyl cyclases: These are single-pass transmembrane proteins with an extracellular binding site for a signal molecule and an intracellular guanylyl cyclase catalytic domain. The binding of the signal molecule activates the cyclase domain to produce cyclic GMP, which in turn binds to and activates a cyclic GMP-dependent protein kinase (PKG),which phosphorylates specific proteins on serine or threonine. Thus, receptor guanylyl cyclases use cyclic GMP as an intracellular mediator in the same way that some G-protein-linked receptors use cyclic AMP, except that the linkage between ligand binding and cyclase activity is a direct one.

Among the signal molecules that use receptor guanylyl cyclase receptors are the natriuretic peptides (NPs), a family of structurally related secreted signal peptides that regulate salt and water balance and dilate blood vessels. There are several types of NPs, including atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP). Muscle cells in the atrium of the heart secrete ANP when blood pressure rises. The ANP stimulates the kidneys to secrete Na^+ and water and induces the smooth muscle cells in blood vessels walls to relax. Both of these effects tend to lower the blood pressure.

Histidine- kinase associated Receptors:

Bacterial movements are mediated by histidine-kinase-associated chemotaxis receptors, which typically are dimeric transmembrane proteins that bind specific attractants and repellents on the outside of the plasma membrane. The cytoplasmic tails of the receptors are stably associated with an adaptor protein CheW and a histidine kinase CheA, which help to couple the receptors to the flagellar motor. Repellent binding activates the receptors, whereas attractant binding inactivates them; a single receptor can bind either type of molecule, with opposite consequences. The binding of a repellent to the receptor activates CheA, which phosphorylates itself on a histidine and almost immediately transfers the phosphate to an aspartic acid on a messenger protein CheY. The phosphorylated CheY dissociates from the receptor, diffuses through the cytosol, binds to the flagellar motor, and causes the motor to rotate clockwise, so that the bacterium tumbles. CheY has intrinsic phosphatase activity and dephosphorylates itself in a process that is greatly accelerated by the CheZ protein (Figure 5.41).

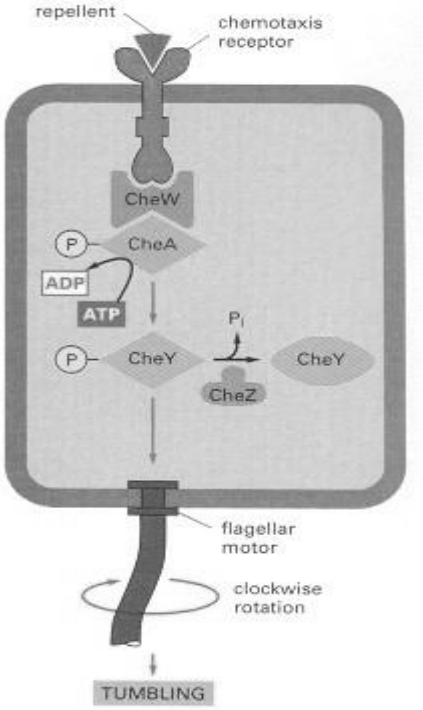


Figure 5.41: Two component signal pathway that enables chemotactic receptors to control bacterial chemotaxis

ANSWER KEYS

1	D	**2**	A	**3**	B	**4**	B	**5**	C	**6**	A	**7**	C
8	B	**9**	D	**10**	A								

EXPLANATIONS

1. Regardless of the nature of signal molecules, target cells respond by expressing specific binding proteins called receptors. These signals are produced at very low concentrations of ~10- 6M, with a high affinity constant Kα > 108 liters per mole. Binding of signal to receptor generates a cascade of intracellular signals that alter the behavior of cells. Few signals are to penetrate the cells and bind to intracellular receptors; such signals are usually small, hydrophobic and can cross plasma membrane

2. **Autocrine Signaling:** Signals sent by cells onto cells of same type and also for themselves are called autocrine signals.
Eg: The cells during development if they have to take up a pathway for differentiation the cells secrete autocrine signals to reinforce themselves for developmental activities. Autocrine signals also stimulate cells of similar type (identical cells) which can respond to differentiation inducing signals.

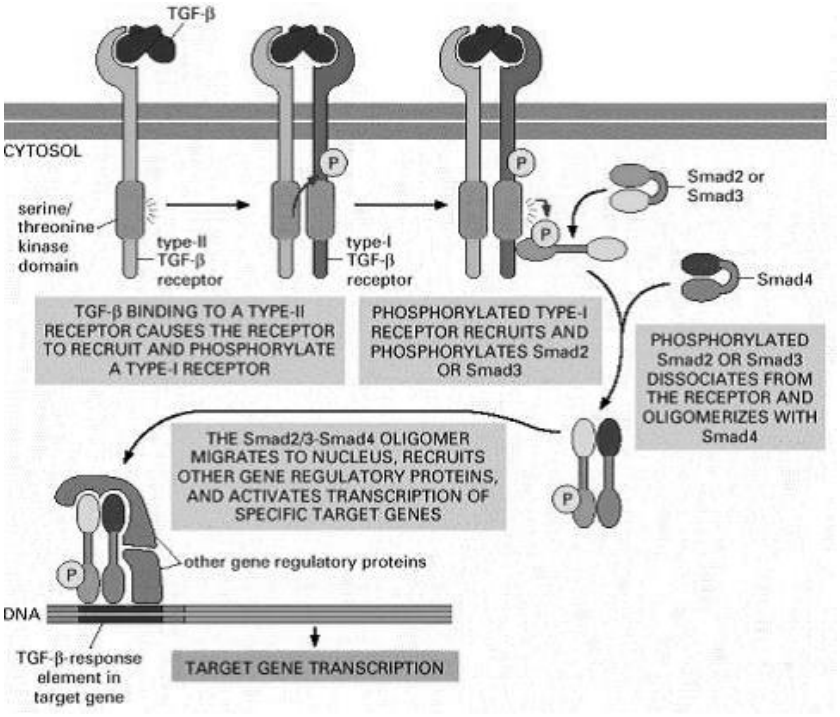
3. The cell signals turn over rate is very high
As soon as their role is over they are catabolized and are also synthesized in very low concentrations.

4. There are three major classes of cell surface receptors are
(i) Ion – channel – linked receptors
(ii) G- Protein linked receptors
(iii) Enzyme linked receptors

5. Natriuretic peptides (NPs), a family of structurally related secreted signal peptides that regulate salt and water balance and dilate blood vessels. There are several types of NPs, including atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP).

6. Nitric oxide gas signal binds to guanylyl cyclase. Acetylcholine released by nerve ending activates NO synthase in the endothelial cells of blood vessels to produce NO. Nitric oxide diffuse out of the endothelial cells into the underlying smooth muscles, binds to guanylyl cyclase to produce cyclic GMP. This triggers relaxation of blood vessels and enhances flow of blood through them.

7.



The sequence of events in smad dependent signaling pathway activated by TGF-β are represented in the following figure

TGF-β binding to Type II receptor causes the receptor to recruit and phosphorylate a Type –I receptor

Phosphorylated Type –I receptor recruits and phosphorylates Smd2 or Smad 4.

Phosphorylated Smad 2 or Smad 3 dissociates from receptor and oligomerizs with Smad 4

The Smad 2/3-Smad-4 oligomer migrates to nucleus, recruits other gene regulatory proteins and activates transcription of specific target genes

8. Two classes of signaling proteins regulate Ras activity by influencing its transition between active and inactive states. Guanine nucleotide exchange factors (GEFs) promote the exchange of bound nucleotide by stimulating the dissociation of GDP and the subsequent uptake of GTP from the cytosol, thereby activating Ras. GTPase-activating proteins (GAPs) increase the rate of hydrolysis of bound GTP by Ras, thereby inactivating Ras. Mutation of Ras leads to cancer.

9. The four types of Jaks are-

(A) Jak1, Jak2, Jak3 and Tyk2 (B) Jak1, Jak2, Jak3 and Jak4

(C) Jak1, Jak2, Jak3 and Jak 5 (D) Jak2, Jak3, Jak4 and Tyk2

10. When activated, interferon receptors activate a novel class of cytoplasmic tyrosine kinases called Janus kinases (Jaks) (after the two-faced Roman go(D). The Jaks then phosphorylate and activate a set of latent gene regulatory proteins called STATs (signal transducers and activators of transcription), which move into the nucleus and stimulate the transcription of specific genes. More than 30 cytokines and hormones activate the Jak-STAT pathway by binding to cytokine receptors.
Cytokine receptors are composed of two or more polypeptide chains. All cytokine receptors, however, are associated with one or more Jaks. There are four known Jaks—Jak1, Jak2, Jak3, and Tyk2—and each is associated with particular cytokine receptors.

5. CELL - CELL COMMUNICATION

CELL SIGNALING

Cell signaling includes, binding of these to appropriate receptors, initiating series of intracellular reactions that regulate various aspects of cell behavior, including metabolism, movement, proliferation, survival and differentiation.

Modes of Cell – Cell signaling: Cell signaling can result either the direct interaction of a cell with the neighboring cell or from the action of secreted signaling molecules (Figure 6.1). The signaling system involving secretion of several molecules are categorized into three- Endocrine signaling, Paracrine signaling and Autocrine signaling.

In direct cell-cell signaling or contact dependent cell signaling. In this the cells exchange signals with neighboring cell by way of integrins and cadherins or via cell adhesion molecules. This is required for cell involved in development.

Endocrine signaling involves release of hormones that are carried by circulation to target cells. Animals produce more than 50 different types of signals by various endocrine glands. In Paracrine signaling, a molecule released by one cell, acts on neighboring target cells, Eg:nerve impulse transmission across neurons via acetylcholine. Autocrine signaling is the release of signal molecules that bind to the same cell. Eg:TH cells secrete IL-2 to stimulate themselves.

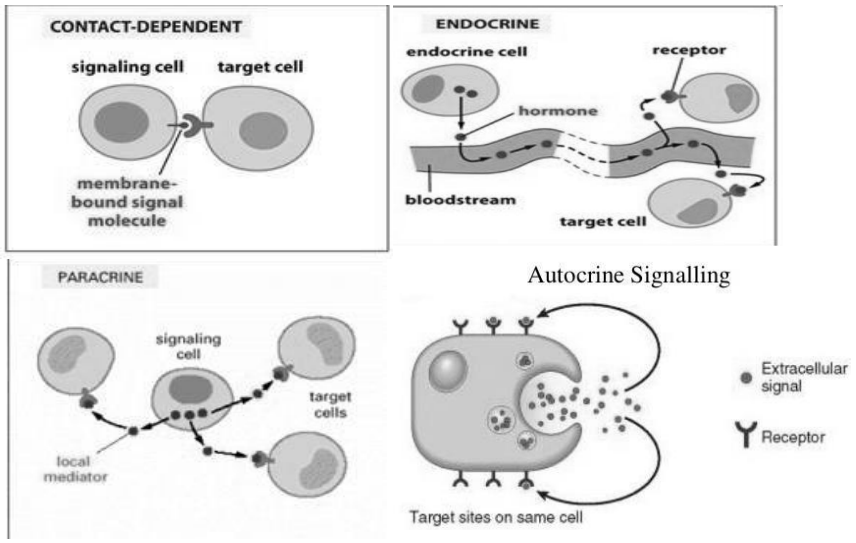


Figure 6.1: Modes of Cell- cell signaling

I. CELL SIGNALING MOLECULES

Various cell signaling molecules are categorized as follows:

1. Steroid hormones
2. Nitric oxide and carbon monoxide
3. Neurotransmitters
4. Peptide hormones and growth hormones
5. Eicosnoids
6. Plant hormones

1. Steroid Hormones: These include hormones that are synthesized from cholesterol – testosterone, estrogen, progesterone, the corticosteroids and ecdysone, thyroid hormones, Vitamin D3 and retinoic acid. Testosterone, estrogen and progesterone are the sex steroids which are produced by the gonads and Corticosteroids are produced by the adrenal gland. Corticosteroids include Glucocorticoids that stimulate production of glucose and the mineralocorticoids which act on kidney to regulate salt and water balance. Ecdysone is a insect hormone that triggers metamorphosis of larvae to adults. Brassinosteroids are plant specific steroid hormones that control developmental processes, including cell growth and differentiation. Thyroid hormones are synthesized from tyrosine in the thyroid gland which plays important role in regulation of metabolism. Vitamin D3 regulates Ca^{2+} metabolism and bone growth while retinoic acid synthesized from vitamin A plays important role in vertebrate development.

All the bind to a family of receptors known as Nuclear receptor superfamily, which can enter the cells by diffusion through the plasma membrane, bind to intracellular receptors, which inturn bind to specific sites on DNA leading to gene regulation (Activation/ suppression) (figure 6.2).

2. Nitric Oxide and Carbon Monoxide: Nitric oxide is a major Paracrine signaling molecule in the nervous, immune and circulatory systems. It can directly diffuse across plasma membrane of its target cells. NO is synthesized from amino acid arginine by the enzyme nitric oxide synthase and can affect cells nearby too. But its half life is few seconds and is eliminated. The major intracellular target for NO is guanylyl cyclase. NO is produced by endothelial cells of blood vessels when stimulated by aetylcholine. This can diffuse down to the smoot h muscles present below the blood vessel, binds to this enzyme guanylyl cyclase resulting in the synthesis of cyclic

GMP that that induces smooth muscle relaxation and increases blood flow to the heart (figure 6.3).

Carbon monoxide (CO) functions as a signal molecule in the nervous system. Synthesis of CO in brain cells can stimulate guanylyl cyclase which finally leads relaxation of smooth muscles.

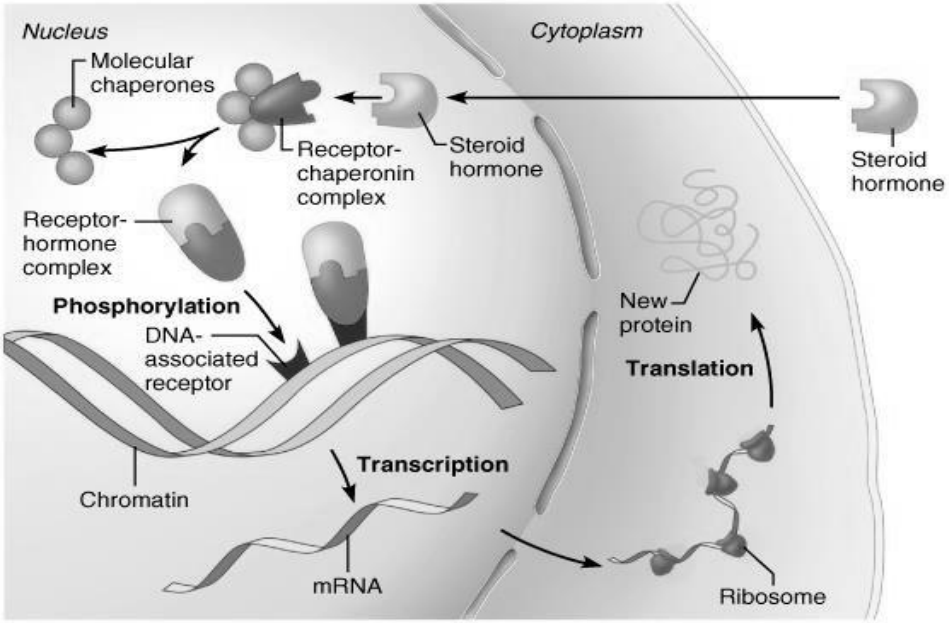


Figure 6.2: Steroid hormone and a nuclear binding receptor mediated gene regualtion

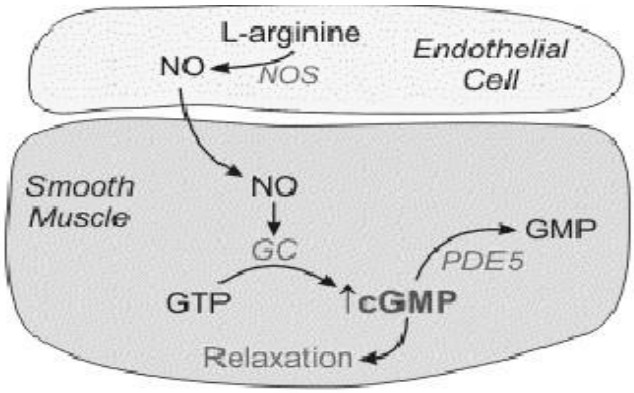


Figure 6.3: NO signaling smooth muscle contraction

3. **Neurotransmitters:** They carry signals between neurons or between neurons and other target cells. These hydrophilic molecules include- acetylcholine, dopamine, epinephrine, serotonin, histamine, glutamate, glycine and gama-aminobutyric acid (GABA). These bind to cell curface receptors there by inducing conformational changes, that open ion channels directly resulting in

changes in ion flux in the target cell. While few of these bind to G-protein receptors and regulate cell activities.

4. **Peptide Hormones and Growth Factors:** This group includes peptide hormones, neuropeptides, and a diverse array of polypeptide growth factors and are listed table below. Peptide hormones are produced by pituitarygland, neuropeptides are secreted by neurons. Among these enkephalins and endorphins decrease pain responses in central nervous system. Polypeptide growth hormones include variety of signals that control animal cell growth and differentiation. Platelet derived growth factor (PDGF) is produced in wound healing and is stored in Blood platelets.

Signaling Molecules	Activities
Peptide Hormones	
Insulin	Regulation of glucose uptake; Stimulation of cell proliferation
Glucagon	Stimulation of glucose synthesis
Growth hormone	General stimulation of growth
Follicle- stimulating hormone (FSH)	Stimulation of the growth of oocytes and ovarian follicles
Prolactin	Stimulation of milk production
Neuropeptides and neurohormones	
Substance P	Sensory synaptic transmission
Oxytocin	Stimulation of smooth muscle contraction
Vasopressin	Stimulation of water reabsorption in the
Enkephalin	Analgesic
β – Endorphin	Analgesic
Growth factors	
Nerve growth factor (NGF)	Differentiation and survival of neurons
Epidermal growth factor (EGF)	Proliferation of many types of cells
Platelet-derived growth factor (PDGF)	Proliferation of fibroblasts and other types of cells.
Interleukin – 2	Proliferation of T lymphocytes
Erythropoietin	Development of RBC

5. **Eicasanoids:** These are lipids which serve as signaling molecules and act by binding to cell surface receptors. These include- prostaglandins, prostacyclin, thromoxanes and leukotrienes. These are a part of autocrine and paracrine signaling pathways. They are involved in variety of responses like blood platelet aggregation, inflammation and smooth muscle contraction.

   These are synthesized from arachidonic acid which is formed from phospholipids. The first step in the pathway pathway leading to synthesis of either prostaglandins or thromboxanes is the conversion of arachidonic acid to

prostaglandin H2. The sme enzyme cyclooxygenase is the target of aspirin and other nonsteroidal anit-inflammatory drugs (NSAIDs). Reduction in the synthesis of prostaglandins by aspirin reduces inflammation and pain. By inhibiting synthesis of thromboxanes, aspirin also reduced platelet aggregation and blood clotting. Because of this activity, small daily doses of aspirin are frequently prescribed for prevention of strokes. In addition, aspirin and NSAIDs have been found to reduce the frequency of colon cancer in human by inhibiting synthesis of prostaglandins that actually stimulate cell proliferation and promote cancer development.

6. **Plant Hormones:** Plant growth and development is regulated by these, and their levels are modified by environmental factors like sun light, infection etc. These are divided into five major groups- auxins, gibberellins, cytokinins, abscisic acid and ethylene. Auxins induce plant cell proliferation by weakening the cell wall. They regulate cell division and differentiation. Gibberlins are responsible for stem elongation; ethylene is involved in fruit ripening, cytokinins in cell division and abscisic acid onset of dormancy.

II. CELL SURFACE RECEPTORS

These are the sites to which cell signaling molecules bind. The receptors under study include:

1. G-protein coupled receptors
2. Receptor Protein –Tyrosine kinase
3. Cytokine receptors and Nonreceptor Protein- Tyrosine Kinases
4. Receptors linked to other enzymatic activities.

1. **G-protein Coupled Receptors:** The largest family of cell surface receptors are the G-protein coupled receptors (GPCRs). There are hundreds of different GPCR proteins, and they are the targets of many drugs. A diverse set of ligands bind to this type of receptors including peptide hormones, neurotransmitters, neuropeptides and eicosonoids. These receptors all have a similar structure with seven transmembrane domains. On the basis of their seven transmembrane domain structure, many GPCRs have been identified in the human genome. These are known as orphan receptors, because their ligands have not been identified.

   GPCRs are associated with heterotrimeric G-proteins i.e., G-proteins composed of three different subunits: alpha, beta, and gamma. The subunits are tethered at the membrane surface by covalently attached lipid molecules.

   When a ligand binds, the receptor activates the attached G-protein by causing the exchange of GTP for GDP. The activated G-protein then dissociates into an alpha (G-alpha) and a beta- gamma complex. G-alpha bound to GTP is active,

and can diffuse along the membrane surface to activate target proteins, often enzymes that generate second messengers. Likewise, the beta- gamma complex is also able to diffuse along the inner membrane surface and activate proteins, typically affecting ion channels.

Inactivation occurs because G-alpha has intrinsic GTPase activity. After GTP hydrolysis, G- alpha bound to GDP will reassociate with a beta-gamma complex to form an inactive G-protein that can again associate with a receptor. The GTPase activity of the G-alpha can be made faster by other proteins which include the target protein or by a separate regulatory protein.

Different G-alpha proteins activate different second messenger pathways (Figure 6.4)

There are several different classes of heterotrimeric G-proteins that are defined by their different G-alpha subunits. One type of G-alpha activates the enzyme adenylyl cyclase, which catalyzes the formation of the second messenger cyclic AMP (cAMP). Because an activated adenylyl cyclase can generate many molecules of cAMP it will result in signal amplification. cAMP can have several effects, but a major effect is to bind to and activate protein kinase A (PKA; also known as cAMP-dependent kinase). PKA then phosphorylates target proteins in the cell. cAMP is rapidly broken down by phosphodiesterases, limiting the length of the signal.

Another type of G-alpha activates the enzyme phospholipase C. Phospholipase C cleavesPI P2, a membrane phospholipid, to generate two second messengers, IP3 and diacylglycerol (DAG). IP3 is water soluble, diffusing through the cytosol to bind to and open a ligand-gated Ca++ channel in the endoplasmic reticulum (or sarcoplasmic reticulum in muscle cells). Thus, stimulation of a receptor linked to this G-alpha is a way to increase Ca++ inside the cytosol.

Ca++ in the cytosol exerts its effects by binding to Ca++-binding proteins such as calmodulin.

DAG is lipid soluble and stays in the membrane. It activates protein kinase C (PKC), which, like PKA phosphorylates particular target proteins.

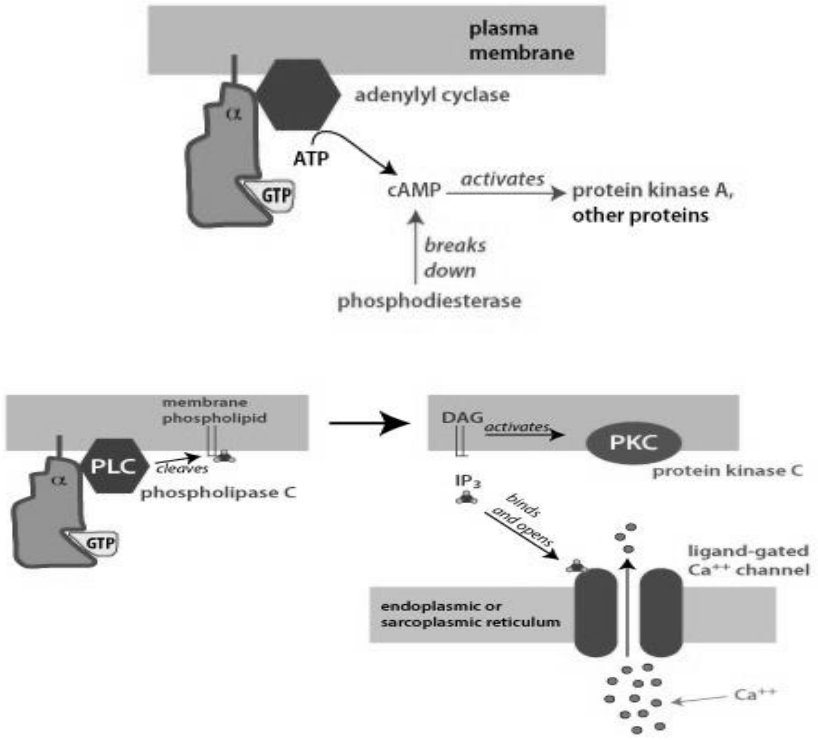


Figure 6.4 G-Protein activities and the two secondary messenger pathways

Desensitization

In the continuing presence of ligand, many GPCRs show desensitization. The mechanism is shown in the figure. A protein known as a G-proteinReceptor Kinase (GRK) phosphorylates the receptor on particular residues. This increases its affinity for a protein called beta-arrestin, that binds to the receptor and reduces signaling by preventing the association with the G-protein. Beta arrestin can target the receptor for endocytosis, leading to receptor down regulation.

2. **Receptor Protein- Tyrosine Kinases: T**hese phosphorylate their substrate proteins on tyrosine residues. Human genome encodes 59 receptor protein – tyrosine kinases, including receptors for EGF, NGF, PDGF, Insulin and many other growth factors. All these receptors have a common structural organization: an N-terminal extracellular Ligand-binding domain, a single transmembrane alpha helix, and a cytosolic C-terminal domain with protein- tyrosine kinase activity. First step in signaling is, dimerization of receptor which is Ligand induced. Some factors lead to dimerization by binding to a different site on the dimer promoting binding of different receptor molecules, while few induce conformational changes. These dimerized polypeptides cross phosphorylate

each other, which is autophosphorylation of the receptor(Figure 6.5). Fist phosphorylation increases catalytic activity while second one at catalytic domain creates specific binding sites for additional proteins that transmit intracellular signals downstream of the activated receptor. The association of these downstream signaling molecules with receptor protein – tyrosine kinases is mediated by protein domains that bind to specific phosphotyrosine- containing peptides. 1) SH2 domains (Src homology 2) and 2) PTB domains.

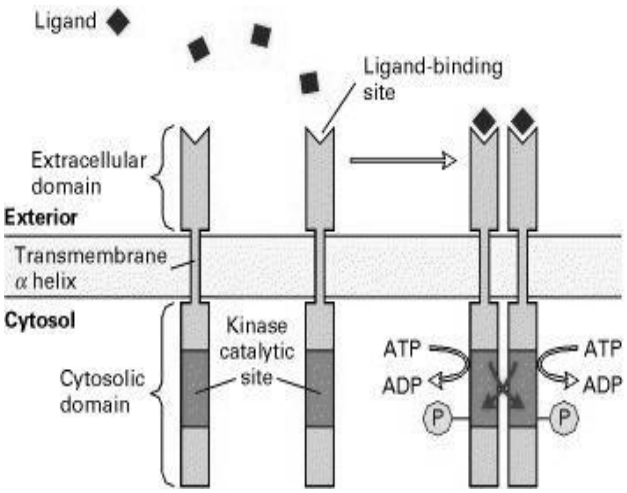


Figure 6.5: General structure and activation of Receptor Tyrosine kinases

3. **Cytokine receptors and Nonreceptor Protein- Tyrosine Kinases:** These include receptors for cytokines like Interleukin- 2, erythropoietin etc and even few polypeptide hormones like growth hormone. Cytokine receptors contain N-terminal extracellular Ligand-binding domains, single transmembrane alpha helix, and C-terminal cytoplasmic domains. These function in association with nonreceptor protein-tyrosine kinases, which are activated as a result of ligand binding. First step in their activation is Ligand induced dimerization and cross phosphorylation of the associated non-receptor protein tyrosine kinases(figure 6.6). These activated kinases then phosphorylate the receptor, providing phosphorylation binding sites for the recruitment of downstream signaling molecules that contain SH-2 domains.

Kinases associated with cytokine receptors belong to the family of Janus kinases (JAK) family. These are required universally for signaling from cytokine receptors.

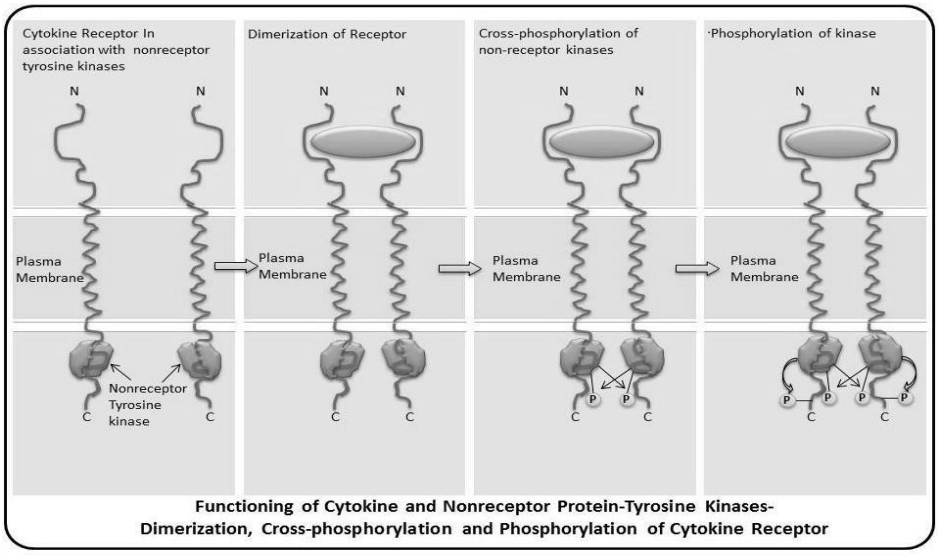


Figure 6.6: Signaling from cytokine receptors

4. **Receptors linked to other Enzymatic activities:** Though most of the enzyme-linked receptors stimulate protein-tyrosine phosphorylation, some receptors are associated with other enzymatic activities. These receptors include Protein-tyrosine Phosphatases, Protein-serine/Threonine kinases and guanylyl cyclases. Protein Tyrosine Phosphatases remove phosphate groups from phosphotyrosine residues, and this activity plays a role of counterbalancing the effect of protein tyrosine kinases. There are certain Phosphatases which are cell surface receptors. There are 21 such receptor protein-tyrosine Phosphatases encoded in the human genome.

Eg: CD45 dephosphorylase, transforming growth factor-□ etc.

III. Pathways of Intracellular Signal Transduction

These are a chain of reactions transmitted from cell surface t o a variety of intracellular targets and this phenomenon is called Intracellular signal transduction. The targets of such signaling pathways frequently include transcription factors that function to regulate gene expression. Intracellular signaling pathways thus connect the cell surface to the nucleus, leading to changes in gene expression in response to extracellular stimuli. These pathways include:

1. The cAMP pathway: secondary messengers and protein phosphorylation
2. Cyclic GMP
3. Phospholipids and Ca2+
4. The PI 3-kinase/Akt and mTOR pathways

5. MAP kinase pathways
6. The JK/STAT and TGFβ / Smad Pathways
7. NF- κB signaling
8. The Hedgehog, Wnt and Notch pathways

1. The cAMP pathway: secondary messengers and protein phosphorylation:

Intracellular signaling was first elucidated by studies of the action of hormones such as epinephrine, which signals the breakdown of glycogen to glucose in anticipation of muscular activity. Epinephrine mediates this by an increase in the intracellular concentration of cyclic AMP (cAMP), leading to the concept that cAMP is a second messenger in hormonal signaling (the first messenger being the hormone itself). Cyclic AMP is formed from ATP by the action of adenylyl cyclase and degraded to AMP by cAMP phosphodiesterase (Figure 6.7). The epinephrine receptor is coupled to adenylyl cyclase via a G protein that stimulates enzymatic activity, thereby increasing the intracellular concentration of cAMP (Figure 6.8).

Most other effects of cAMP in animal cells are mediated by the action of cAMP-dependent protein kinase, or protein kinase A. 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 the regulation of glycogen metabolism, protein kinase A phosphorylates two key target enzymes (Figure 6.9). The first is another protein kinase, phosphorylase kinase, which is phosphorylated and activated by protein kinase A. Phosphorylase kinase in turn phosphorylates and activates glycogen phosphorylase, which catalyzes the breakdown of glycogen to glucose-1-phosphate. In addition, protein kinase A phosphorylates the enzyme glycogen synthase, which catalyzes glycogen synthesis. In this case, however, phosphorylation inhibits enzymatic activity. Elevation of cAMP and activation of protein kinase A thus blocks further glycogen synthesis at the same time as it stimulates glycogen breakdown (figure 6.10).

In many animal cells, increases in cAMP activate the transcription of specific target genes that contain a regulatory sequence called the cAMP response element, or CRE (Figure 6.11). In this case, the signal is carried from the cytoplasm to the nucleus by the catalytic subunit of protein kinase A, which is able to enter the nucleus following its release from the regulatory subunit. Within the nucleus, protein kinase A phosphorylates a transcription factor called CREB (for CRE-binding protein), leading to the activation of cAMP-inducible genes. Such regulation of gene expression by cAMP plays important

roles in controlling the proliferation, survival, and differentiation of a wide variety of animal cells.

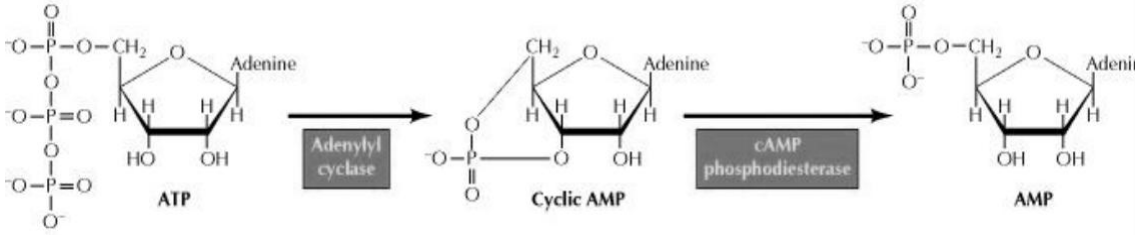


Figure 6.7: Synthesis and degradation of cAMP

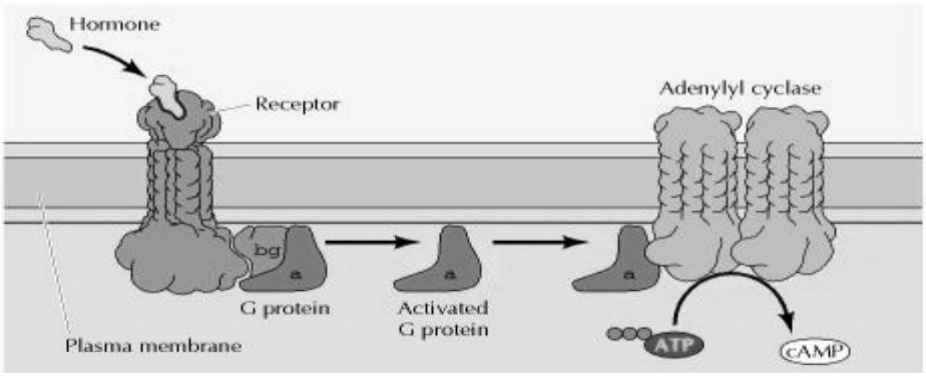


Figure 6.8: G Protein mediated induction of Adenylyl cyclase increase camp

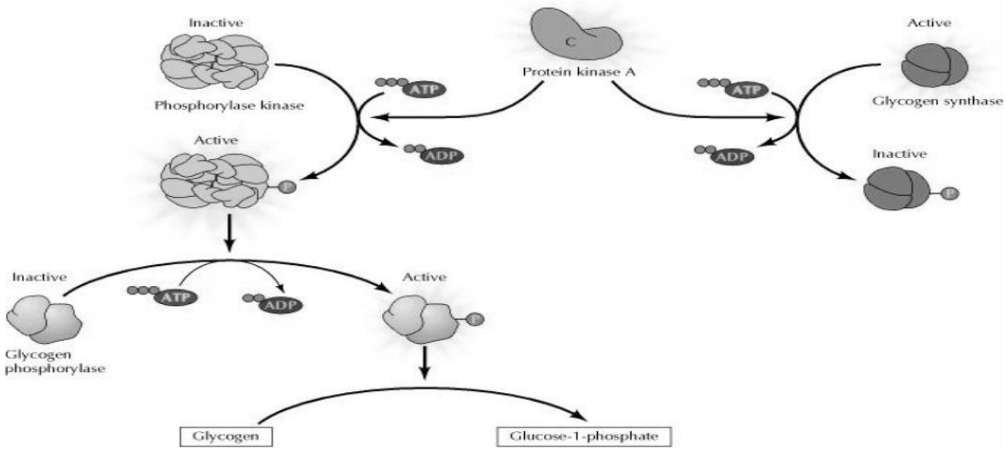


Figure 6.10: Regulation of Glycogen metabolism by Protein Kinase A

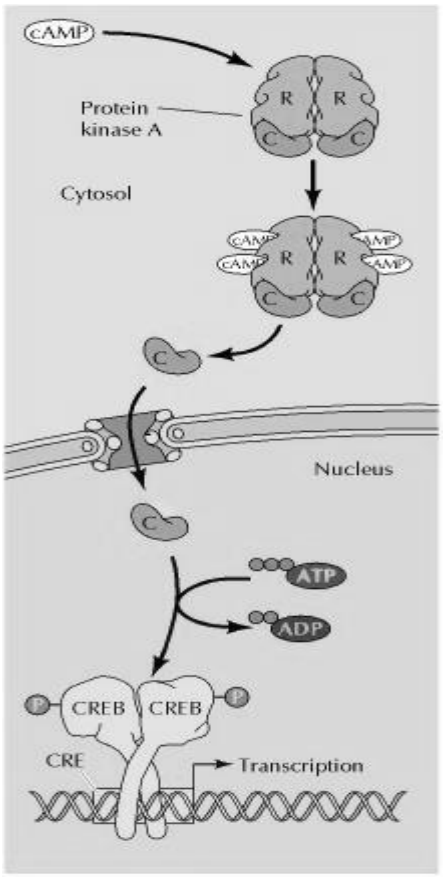


Figure 6.11: CyclicAMP induced gene expression

2. Cyclic GMP

Cyclic GMP is formed from GTP by guanylyl cyclases and degraded to GMP by a phosphodiesterase. Different types of guanylyl cyclases are activated by both nitric oxide and peptide ligands. 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 a cGMP-dependent protein kinase, although cGMP can also act to regulate other targets, including ion channels.

The best-characterized role of cGMP is in the vertebrate eye, where it serves as the second messenger responsible for converting the visual signals received as light to nerve impulses. The photoreceptor in rod cells of the retina is a G protein-coupled receptor called rhodopsin (Figure 6.12). Rhodopsin is activated as a result of the absorption of light by the associated small molecule 11-cis-retinal, which then isomerizes to all-trans-retinal, inducing a conformational

change in the rhodopsin protein. Rhodopsin then activates the G protein transducin, and the α subunit of transducin stimulates the activity of cGMP phosphodiesterase, leading to a decrease in the intracellular level of cGMP. This change in cGMP level in retinal rod cells is translated to a nerve impulse by a direct effect of cGMP on ion channels in the plasma membrane, similar to the action of cAMP in sensing smells.

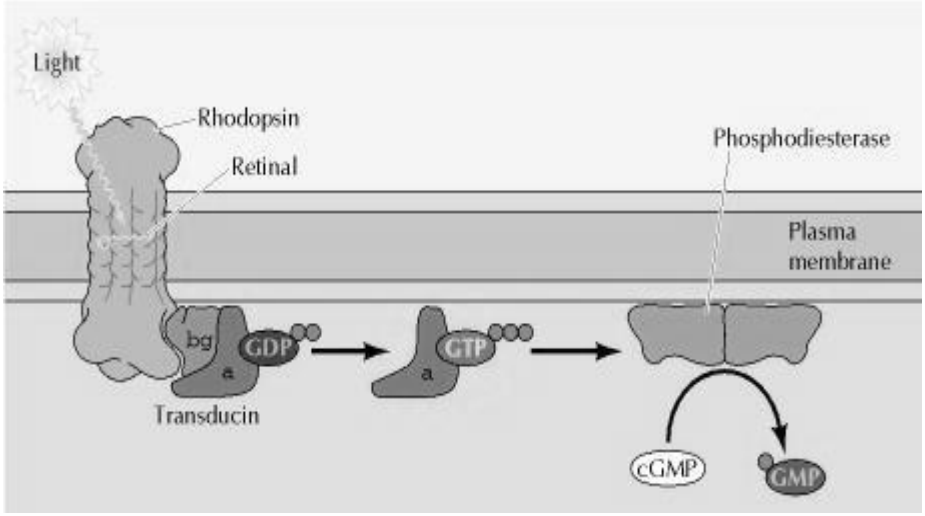


Figure 6.12: Role of cGMP in Photoreception

3. Phospholipids and Ca2+:

Many pathways of intracellular signaling is based on the use of second messengers derived from the membrane phospholipid phosphatidylinositol 4,5-bisphosphate (PIP2). PIP2 is a minor component of the plasma membrane, localized to the inner leaflet of the phospholipid bilayer. A variety of hormones and growth factors stimulate the hydrolysis of PIP2 by phospholipase C—a reaction that produces two distinct second messengers, diacylglycerol and inositol 1,4,5- trisphosphate (IP3). Diacylglycerol and IP3 stimulate distinct downstream signaling pathways (protein kinase C and Ca2+ mobilization, respectively), so PIP2 hydrolysis triggers a two-armed cascade of intracellular signaling.

Hydrolysis of PIP2 is activated downstream of both G protein-coupled receptors and protein- tyrosine kinases. This occurs because one form of phospholipase C (PLC-β) is stimulated by G proteins, whereas a second (PLC-γ) contains SH2 domains that mediate its association with activated receptor protein-tyrosine kinases. This interaction localizes PLC-γ to the plasma membrane as well as leading to its tyrosine phosphorylation, which increases its catalytic activity (figure 6.13).

The diacylglycerol produced by hydrolysis of PIP2 activates protein-serine/threonine kinases belonging to the protein kinase C family, many of which play important roles in the control of cell growth and differentiation. Whereas diacylglycerol remains associated with the plasma membrane, the other second messenger produced by PIP2cleavage, IP3, is a small polar molecule that is released into the cytosol, where it acts to signal the release of Ca2+ from intracellular stores. IP3 acts to release Ca2+ from the endoplasmic reticulum (where it is present bound to calmodulin) by binding to receptors that are ligand-gated Ca2+channels. As a result, cytosolic Ca2+ levels increases, which affects the activities of a variety of targetproteins, including protein kinases and phosphatases. For example, some members of the protein kinase C family require Ca2+ as well as diacylglycerol for their activation, so these protein kinases are regulated jointly by both arms of the PIP2signaling pathway (figure 6.14).

Many effects of Ca2+ are mediated by the Ca2+-binding protein calmodulin, which is activated by Ca2+ binding when the concentration of cytosolic Ca2+ increases to about 0.5 μM. Ca2+/calmodulin then binds to a variety of target proteins, including protein kinases. One example of such a Ca2+/calmodulin-dependent protein kinaseis myosin light-chain kinase, which signals actin-myosin contraction by phosphorylating one of the myosin light chains (Figure 6.15). Other protein kinases that are activated by Ca2+/calmodulin include members of the CaM kinase family, which phosphorylate a number of different proteins, including metabolic enzymes, ion channels, and transcription factors. One form of CaM kinase is particularly abundant in the nervous system, where it regulates the synthesis and release of neurotransmitters. In addition, CaM kinases can regulate gene expression by phosphorylating transcription factors. Interestingly, one of the transcription factors phosphorylated by CaM kinase is CREB, which is phosphorylated at the same site by protein kinase A. This phosphorylation of CREB illustrates one of many intersections between the Ca2+ and cAMP signaling pathways. Other examples include the regulation of adenylyl cyclases and phosphodiesterases by Ca2+/calmodulin, the regulation of Ca2+ channels by cAMP, and the phosphorylation of a number of target proteins by both protein kinase A and Ca2+/calmodulin-dependent protein kinases. The cAMP and Ca2+ signaling pathways thus function coordinately to regulate many cellular responses.

4. The PI 3-Kinase/Akt and mTOR Pathway

PIP2 not only serves as the source of diacylglycerol and IP3, but is also the starting point of a distinct second messenger pathway that plays a key role in

regulating cell survival. In this pathway, PIP2 is phosphorylated on the 3 position of inositol by the enzyme phosphatidylinositide (PI) 3-kinase (Figure 6.16). Like phospholipase C, one form of PI 3-kinase is activated by G proteins, while a second has SH2 domains and is activated by association with receptor protein-tyrosine kinases. Phosphorylation of PIP2 yields phosphatidylinositol 3,4,5-trisphosphate (PIP3), which functions as a distinct second messenger. A key target of PIP3, which is critical for signaling cell survival, is a protein-serine/threonine kinase called Akt. PIP3 binds to a domain of Akt known as the pleckstrin homology domain (figure 6.17). This interaction recruits Akt to the inner face of the plasma membrane, where it is phosphorylated and activated by other protein kinases (called PDKs) that also contain pleckstrin homology domains and bind PIP3. The formation of PIP3 thus results in the association of both Akt and PDKs with the plasma membrane, leading to phosphorylation and activation of Akt. Once activated, Akt phosphorylates a number of target proteins, including proteins that are direct regulators of cell survival, transcription factors, and other protein kinases that regulate cell metabolism and protein synthesis.

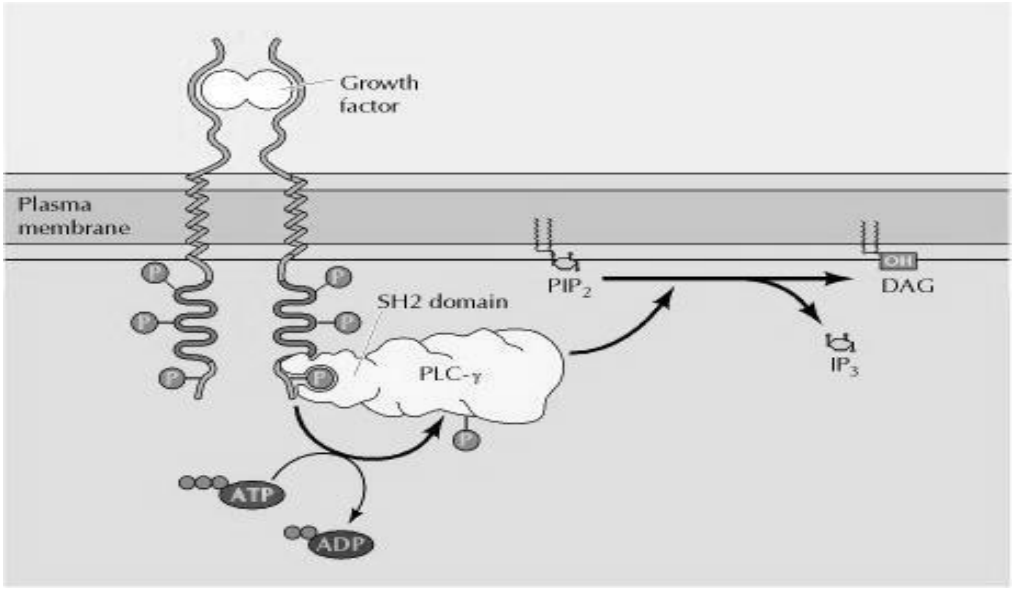


Figure 6.13: Activation of Phospholipase C by Protein-Tyrosine Kianses

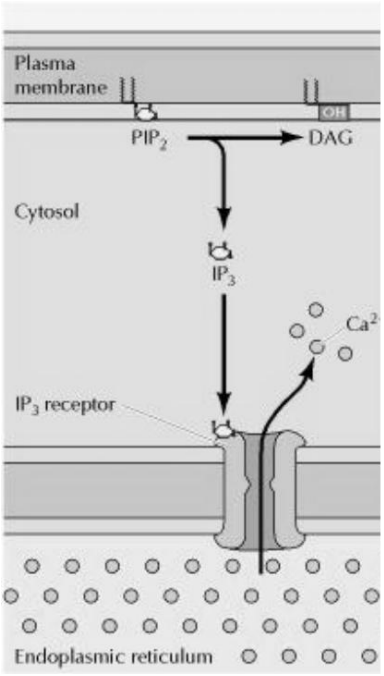


Figure 6.14: Ca2+ mobilization by IP3

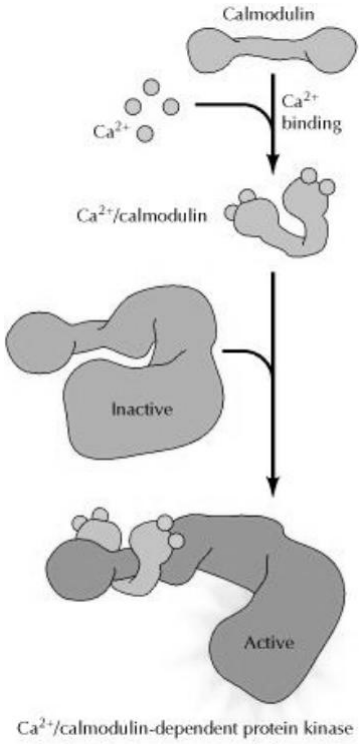


Figure 6.15: Function of calmodulin

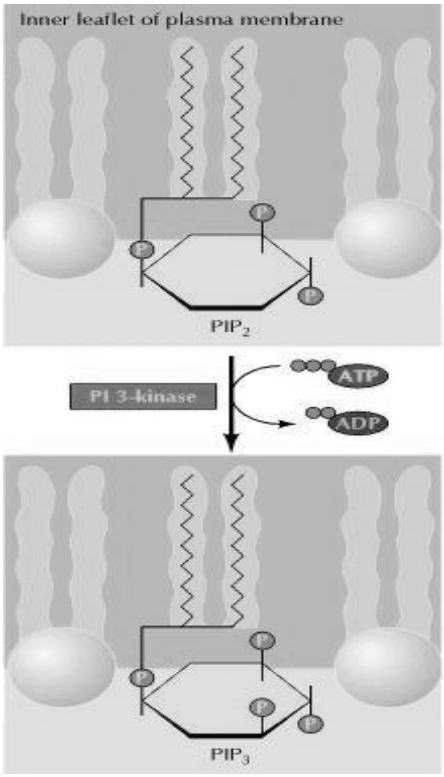


Figure 6.16:ctivity of PI 3 Kinase

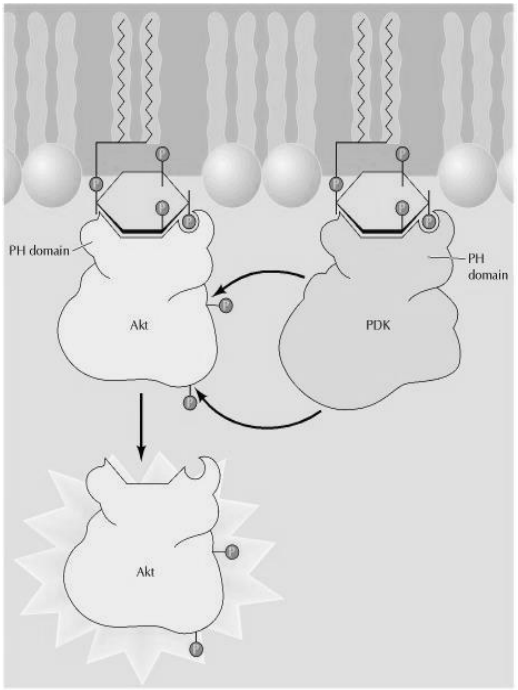


Figure 6.17: Activation of Akt Protein Kinase

5. MAP kinase Pathway:

The MAP kinase pathway refers to a cascade of protein kinases that are highly conserved in evolution and play central roles in signal transduction in all eukaryotic cells, ranging from yeasts to humans. The central elements in the pathway are a family of protein- serine/threonine kinases called the MAP kinases (for mitogen-activated protein kinases) that are activated in response to a variety of growth factors and other signaling molecules. In yeasts, MAP kinase pathways control a variety of cellular responses, includ ing mating, cell shape, and sporulation. In higher eukaryotes (including C.elegans, Drosophila, frogs, and mammals), MAP kinases are ubiquitous regulators of cell growth and differentiation.

The best-characterized forms of MAP kinase in mammalian cells belong to the ERK (extracellular signal-regulatedkinase) family that plays a central role in signaling cell proliferation induced by growth factors that act through either protein-tyrosine kinase or G protein-coupled receptors.

Activation of ERK is mediated by two upstream protein kinases, which are coupled to growth factor receptors by a GTP-binding protein called Ras (figure 6.18). Activation of Ras leads to activation of the Raf protein-serine/threonine kinase, which phosphorylates and activates a second protein kinase called MEK (for MAP kinase/ERK kinase). MEK is a dual-specificity protein kinase that activates members of the ERK family by phosphorylation of both threonine and tyrosine residues separated by one amino acid. Once activated, ERK phosphorylates a variety of targets, including other protein kinases and transcription factors.

The Ras proteins are guanine nucleotide-binding proteins that function analogously to the α subunits of G proteins, alternating between inactive GDP-bound and active GTP-bound forms (Figure 6.19). In contrast to the G protein α subunits, however, Ras functions as a monomer rather than in association with βγ subunits. Ras activation is mediated by guanine nucleotide exchange factors that stimulate the release of bound GDP and its exchange for GTP. Activity of the Ras-GTP complex is then terminated by GTP hydrolysis, which is stimulated by the interaction of Ras-GTP with GTPase-activating proteins. It is interesting to note that the mutations of ras genes in human cancers have the effect of inhibiting GTP hydrolysis by the Ras proteins. These mutated Ras proteins therefore remain continuously in the active GTP-bound form, driving the unregulated proliferation of cancer cells even in the absence of growth factor stimulation.

The Ras proteins are prototypes of a large family of approximately 50 related proteins, frequently called small GTP-binding proteins because Ras and its relatives are about half the size of G protein α subunits. While the Ras proteins regulate cell

growth and differentiation, other subfamilies of small GTP-binding proteins control distinct cellular activities. The best understood mode of Ras activation is that mediated by receptor protein-tyrosine kinases. Autophosphorylation of these receptors results in their association with Ras guanine nucleotide exchange factors as a result of SH2-mediated protein interactions. One well-characterized example is provided by the guanine nucleotide exchange factor Sos, which is bound to the SH2-containing protein Grb2 in the cytosol of unstimulated cells. Tyrosine phosphorylation of receptors (or of other receptor-associated proteins) creates a binding site for the Grb2 SH2 domains. Association of Grb2 with activated receptors localizes Sos to the plasma membrane, where it is able to interact with Ras proteins, which are anchored to the inner leaflet of the plasma membrane by lipids attached to the Ras C terminus. Sos then stimulates guanine nucleotide exchange, resulting in formation of the active Ras-GTP complex. In its active GTP- bound form, Ras interacts with a number of effector proteins, including the Raf protein- serine/threonine kinase. This interaction with Ras recruits Raf from the cytosol to the plasma membrane, where it is activated as a result of phosphorylation by both protein-tyrosine and protein-serine/threonine kinases.

Activation of Raf initiates a protein kinase cascade leading to ERK activation. ERK then phosphorylates a variety of target proteins, including other protein kinases. Importantly, a fraction of activated ERK translocates to the nucleus, where it regulates transcription factors by phosphorylation (Figure 6.20). In this regard, it is notable that a primary response to growth factor stimulation is the rapid transcriptional induction of a family of approximately 100 genes called immediate-early genes. The induction of a number of immediate-early genes is mediated by a regulatory sequence called the serum response element (SRE), which is recognized by a complex of transcription factors including the serum response factor (SRF) and Elk-1. ERK phosphorylates and activates Elk-1, providing a direct link between the ERK family of MAP kinases and immediate-early gene induction. Many immediate-early genes themselves encode transcription factors, so their induction in response to growth factor stimulation leads to altered expression of a battery of other downstream genes, thereby establishing new programs of gene expression.

Both yeasts and mammalian cells have multiple MAP kinase pathways that control distinct cellular responses. Each cascade consists of three protein kinases: a terminal MAP kinase and two upstream kinases (analogous to Raf and MEK) that regulate its activity. In the yeast S. cerevisiae, five different MAP kinase cascades regulate mating, sporulation, filamentation, cell wall remodeling, and response to high osmolarity. In mammalian cells, at least five MAP kinases have been identified. In addition to members of the ERK family, these include the JNK and p38 MAP kinases, which are

preferentially activated in response to inflammatory cytokines and cellular stress (e.g., ultraviolet irradiation) (Figure 6.21). Whereas ERK signaling principally leads to cell proliferation, survival, and differentiation, the JNK and p38 MAP kinase pathways often lead to inflammation and cell death. Like ERK, the JNK and p38 MAP kinases can translocate to the nucleus and phosphorylate transcription factors that regulate gene expression. Multiple MAP kinase pathways thus function in all types of eukaryotic cells to control cellular responses to diverse environmental signals.

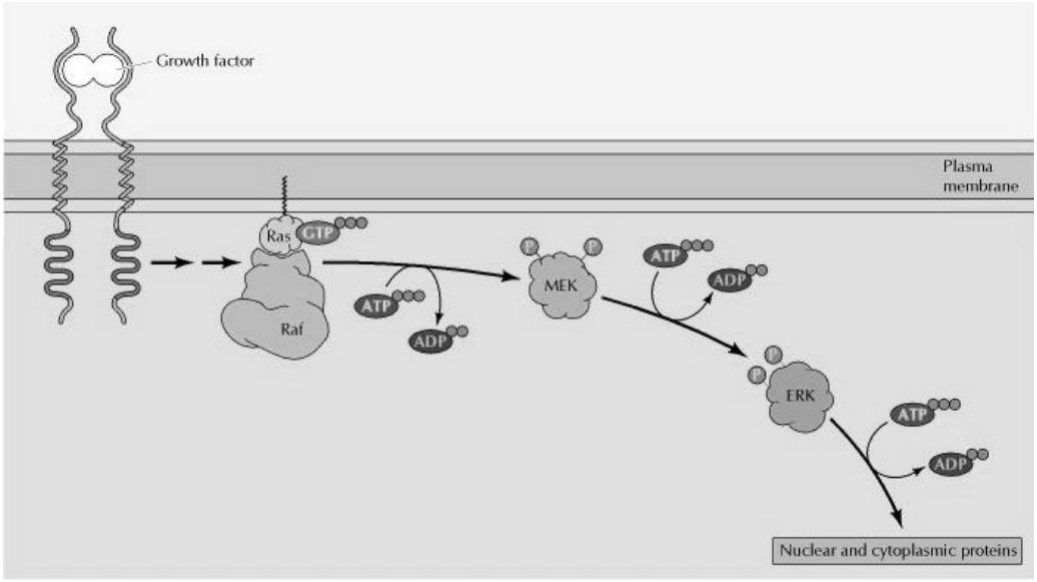


Figure 6.18: Activation of ERK MAP Kinase

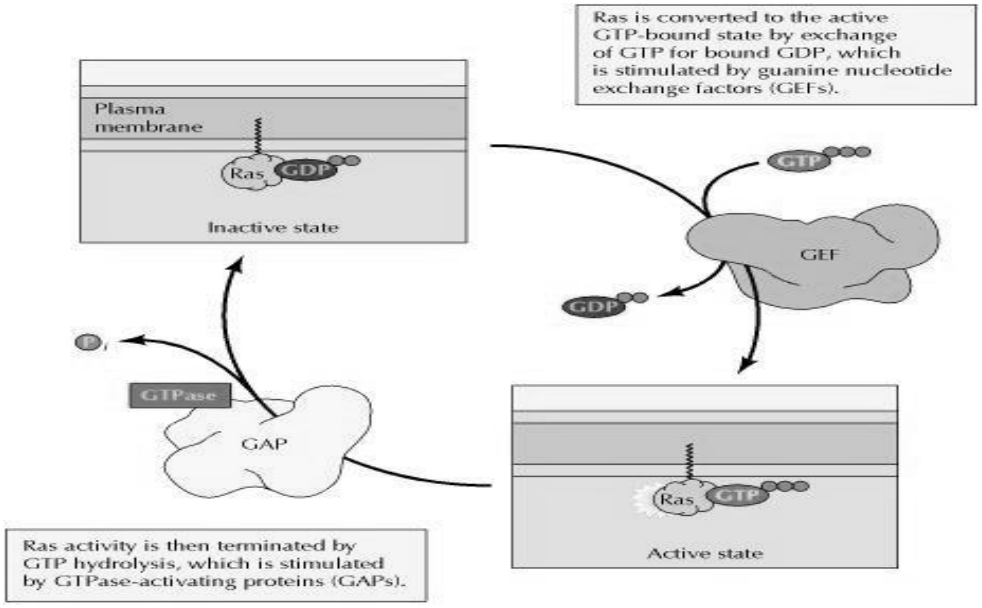
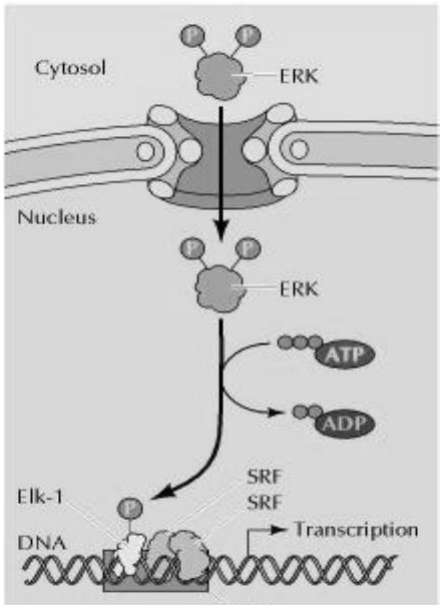


Figure 6.19: Regulation of Ras proteinFigure



6.20 Induction of Immediate earlygenes by ERK

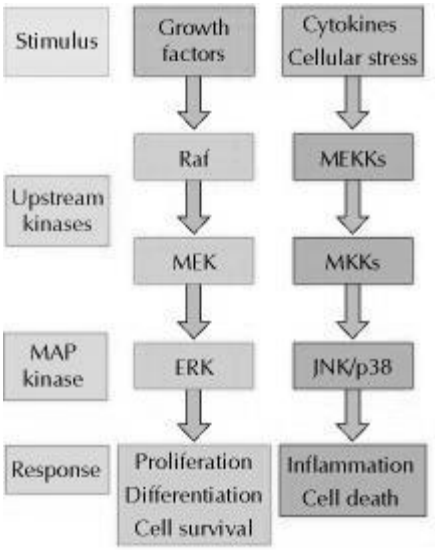


Figure 6.21: Pathways of MAP kinase activation in Mammals

6. The JK/STAT and TGFβ / Smad Pathways

An alternative pathway, known as the JAK/STAT pathway, provides a much more immediate connection between protein-tyrosine kinases and transcription factors apart from MAP kinases. In this pathway protein-tyrosine phosphorylation directly affects transcription factor localization and function (figure 6.22). The key elements in this pathway are the STAT proteins (signal transducers and activators of transcription), are a family of transcription factors that contain SH2 domains. They are inactive in unstimulated cells, where they are localized to the cytoplasm. Stimulation of cytokine receptors leads to recruitment of STAT proteins, which bind via their SH2 domains to phosphotyrosine-containing sequences in the cytoplasmic domains of receptor polypeptides. Following ther association with activated receptors, the STAT proteins are phosphorylated by members of the JAK family of nonreceptor protein-tyrosine kinases, which are associated with cytokine receptors. Tyrosine phosphorylation promotes the dimerization of STAT proteins, which then translocate to the nucleus, where they stimulate transcription of their target genes.

Further studies have shown that STAT proteins are also activated downstream of receptor protein-tyrosine kinases, where their phosphorylation may be catalyzed either by the receptors themselves or by associated

nonreceptor kinases. The STAT transcription factors thus serve as direct links between both cytokine and growth factor receptors on the cell surface and regulation of gene expression in the nucleus.

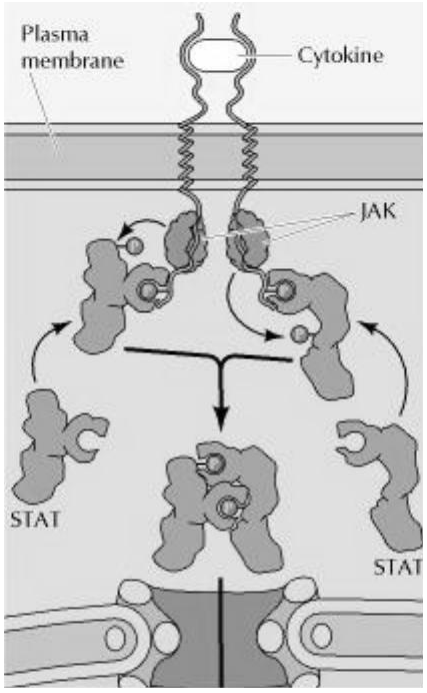


Figure 6.22: JAK/STAT patway

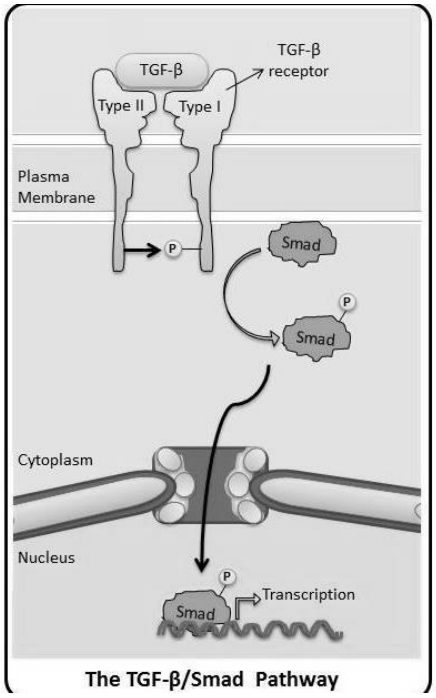


Figure 6.23: Signaling from TGF-□ receptors

The receptors for members of the nases, which directly phosphoryla TGF-□ family of growth factors are protein-serine/Threonine ki te transcription factors of the smad family (figure 6.23). The receptors are composed of Type I and Type II polypeptides, which become associated following Ligand binding. The Type II receptors then phosphorylates the type I receptor, which in turn phosphorylates a Smad protein. The phosphorylated Smads then translocate to the nucleus and regulate gene expression. Human genome codes for 42 different members of TGF-□ , which elicit different responses in their target cells. This is accomplished by comabnitorial interactionof seven different type I receptors and five type II receptors, which lead to activation of different members of Smad family. Smad family members can also be phosphorylated by ERK, and this intersection between the TGF□ / Smad pathway and ERK plays a critical role in embryonic development.

7. NF- κB signaling

1 NF- κB signaling is another example of a signaling pathway that directly targets a specific family of transcription factors. The NF- κB family consists of

five transcription factors that play key roles in the immune system and in inflammation as well as in regulation of proliferation and survival of many types of animal cells. Members of this transcription factor family are activated in response to a variety of stimuli, including cytokines, growth factors, viral infections and DNA damage. In unstimulated cells NF- κB proteins are bound to inhibitory 1κB proteins that maintain NF- κB in an inactive state in the cytosol (figure 6.24). Activation of NF- □B results from signals that activate the 1□B kinase, which phosphorylates 1κB. this phosphorylation targets 1κB fir ubiquitination and egradation by the proteosome, freeing NF- κB to translocate to the nucleus and induce expression of its target genes.

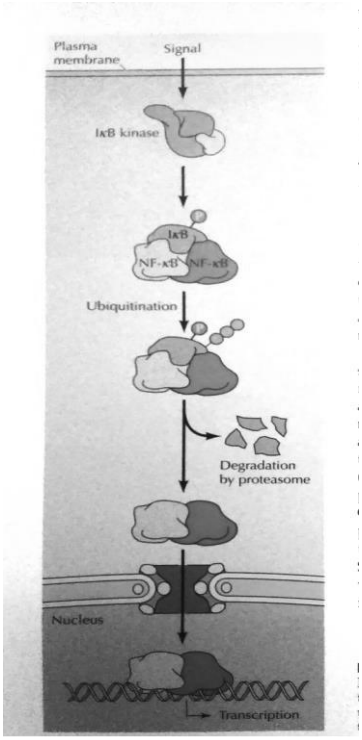


Figure 6.24 NF- □B signaling

8. The Hedgehog, Wnt and Notch pathways

The Hedgehog and Wnt pathways are closely related and play important role during embryonic development. The Hedgehog genes encode secreted proteins that are modified by the addition of lipids. The functional receptor for Hedgehog consists of two transmembrane proteins, patched and smoothed. Hedgehog binds to Patched, which acts as a negative regulator for smoothed (figure 6.25). This binding to Patched allows Smoothened to propagate an intracellular signal, leading to the activation of a transcriptional factor called Gli in mammals and Ci in Drosophila, which otherwise is found bound to a protein that anchors the complex of microtubules. Hedgehog signaling promotes interaction of

Smoothened with this protein, to release Ci, which enables to translocate to the nucleus and activate transcription of target genes.

The Wnt proteins are a family of secreted growth factors that bind to receptors of the LRP and Frizzled family, which are related to activation of cytoskeletal protein called Dishevelled, and inhibition of a complex of the proteins axin, APC and the protein kinase GSK-3□. Within the complex GSK-3□ phosphorylates □-catenin, leading to ubiquitination and degradation, there by Wnt signaling results in increase of □-catenin levels (figure 6.26).

The Notch pathway is another highly conserved signaling pathway that control cell fate during animal development. Notch is a large protein with a single transmembrane domain that serves as a receptor of signaling by transmembrane proteins. Ligand binding leads to proteolytic cleavage of Notch, and an intracellular domain of Notch is then translocated to nucleus that interacts with transcriptional factor that converts it from repressor state to an activator state of its target gene (figure 6.27).

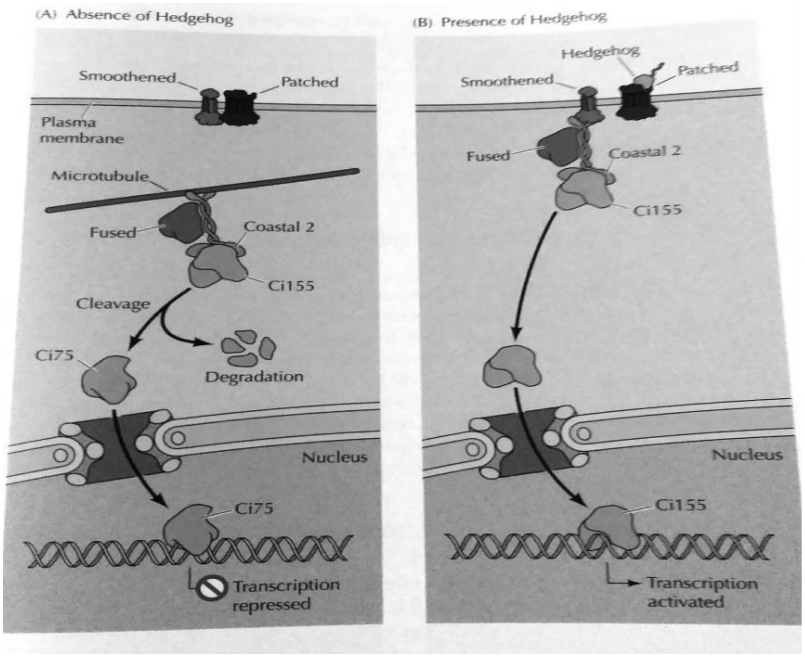


Figure6.25: Hedgehog signaling

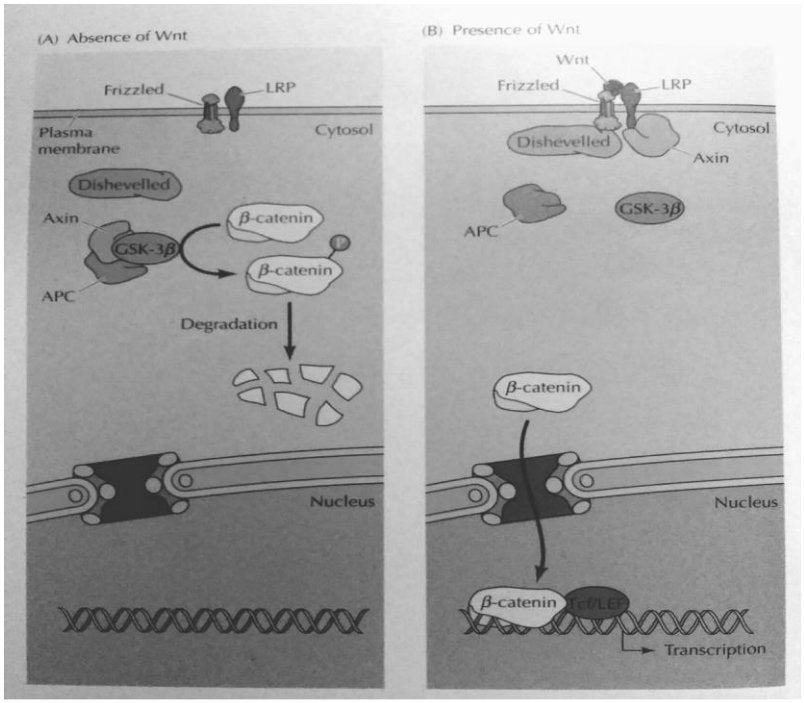


Figure 6.26: Wnt Pathway

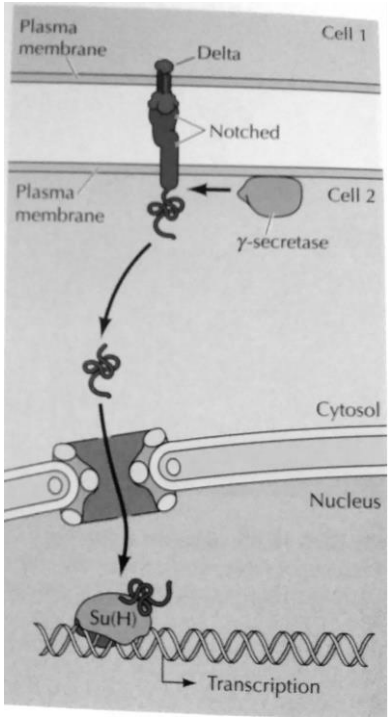


Figure 6.27: Notch Signaling

IV. Signal Transduction and the Cytoskeleton

The functions of most cells are also directly affected by cell adhesion and the organization of the cytoskeleton. The receptors responsible for cell adhesion thus act to initiate intracellular signaling pathways that regulate other aspects of cell behavior, including gene expression. Conversely, growth factors frequently act to induce cytoskeletal alterations resulting in cell movement or changes in cell shape. Components of the cytoskeleton thus act as both receptors and targets in cell signaling pathways, integrating cell shape and movement with other cellular responses.

1. Integrins and Signal Transduction:

The integrins are the major receptors responsible for the attachment of cells to the extracellular matrix. At two types of cell-matrix junctions, the integrins also interact with components of the cytoskeleton to provide a stable linkage between the extracellular matrix and adherent cells. In addition to this structural role, the integrins serve as receptors that activate intracellular signaling pathways, thereby controlling gene expression and other aspects of cell behavior in response to adhesive interactions.

Like members of the cytokine receptor superfamily, the integrins have short cytoplasmic tails that lack any intrinsic enzymatic activity. However, protein-tyrosine phosphorylation is an early response to the interaction of integrins with extracellular matrix components, suggesting that the integrins are linked to nonreceptor protein-tyrosine kinases. In particular, a nonreceptor protein-tyrosine kinase called FAK (focal adhesion kinase) plays a key role in integrin signaling (Figure 6.27). FAK is localized to focal adhesions and rapidly becomes tyrosine-phosphorylated following the binding of integrin to extracellular matrix components, such as fibronectin. Like other protein-tyrosine kinases, the activation of FAK is thought to involve autophosphorylation induced by the clustering of integrins bound to the extracellular matrix.

In addition to FAK, members of the Src family of nonreceptor protein-tyrosine kinases also associate with focal adhesions and are involved in integrin signaling. Interestingly, Src and FAK appear to function in association with each other as a result of the binding of the Src SH2 domain to an autophosphorylation site of FAK. Src then phosphorylates additional sites on FAK, so these two nonreceptor protein-tyrosine kinases act jointly in signaling from integrin receptors.

Tyrosine phosphorylation of FAK creates binding sites for the SH2domains of other downstream signaling molecules, including PI 3-kinase and the Grb2-Sos complex. Recruitment of the Sos guanine nucleotide exchange factor

leads to activation of Ras, which in turn couples integrins to activation of the ERK MAP kinase signaling pathway. Integrin activation of the FAK and Src nonreceptor protein-tyrosine kinases thus links cell adhesion to changes in gene expression and cell behavior that are analogous to those induced by the binding ofgrowth factors to their cell surface receptors.

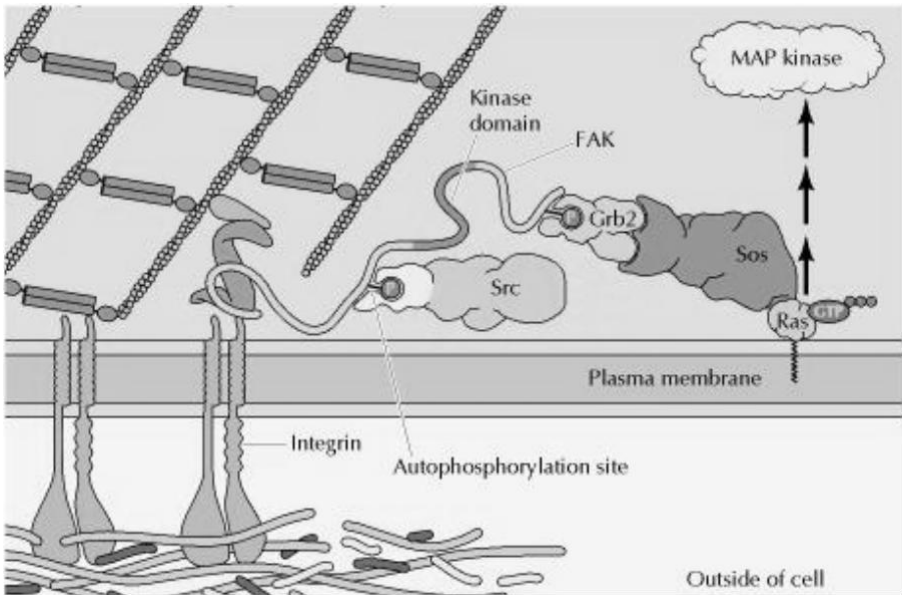


Figure 6.27: Model for signaling from the FAK Protein -Tyrosine kinase

2. Regulation of Actin cytoskeleton:

Cellular responses to extracellular signals, including growth factors, frequently include changes in cell movement and cell shape and these aspects of cell behavior are governed principally by the actin cytoskeleton. In particular, many types of cell movement are based on the dynamic assembly and disassembly of actin filaments underlying the plasma membrane. Remodeling of the actin cytoskeleton therefore represents a key element of the response of many cells to growth factors and other extracellular stimuli.

Members of the Rho subfamily of small GTP-binding proteins (including Rho, Rac, and Cdc42) play central roles in regulating the organization of the actin cytoskeleton and thus control a variety of cell processes, including cell motility, cell adhesion, and cytokinesis. The role of Rho family members in regulating different aspects of actin remodeling was first elucidated by studies of the response of fibroblasts to growth factor stimulation (Figure 6.28). The cytoskeletal alterations resulting from growth factor stimulation include the

production of cell surface protrusions (filopodia, lamellipodia, and membrane ruffles) as well as the formation of focal adhesions and stress fibers.

Further Rho family members play similar roles in regulating the actin cytoskeleton in all types of eukaryotic cells. For example, Rho is required for cytokinesis (cell division following mitosis), which is mediated by an actin-myosin contractile ring (figure 6.29). In neurons, Rac, Cdc42 and Rho regulate the extension and retraction of axons during development of the nervous system. In smooth muscle cells, Rho contributes to regulation of contraction. In epithelial cells, Rho family members regulate the formation of adherens junctions, which involve the linkage of cadherins to the actin cytoskeleton (figure 6.30). Members of the Rho family thus serve as universal regulators of the actin cytoskeleton, linking extracellular signals to changes in cell shape and movement. In addition, Rho family members can activate MAP kinase signaling pathways, so these small GTP-binding proteins function as dual regulators of cytoskeletal remodeling and gene expression.

A large number of proteins have been identified as potential targets of Rho, Rac, and Cdc42, and the cellular roles of many of these candidate target proteins remain to be elucidated. However, recent experiments have clarified some of the pathways by which Rho family members regulate cytoskeletal alterations. A key target of Rho appears to be a protein-serine/threonine kinase called Rho kinase. Activation of Rho kinase increases the phosphorylation of the light chain of myosin II by two mechanisms: Rho not only directly phosphorylates the myosin light chain but also phosphorylates and inhibits myosin light chain phosphatase. The resulting increase in myosin light chain phosphorylation activates myosin and leads to the assembly of actin- myosin filaments, resulting in cytoskeletal alterations such as the formation of stress fibers and focal adhesions, adherens junctions, and cytokinesis. In addition to affecting phosphorylation of myosin light chain, Rho kinase phosphorylates another protein kinase (LIM-kinase), which in turn phosphorylates the actin-binding protein cofilin. Cofilin is a key regulator of actin filament disassembly, so its phosphorylation by LIM-kinase directly links Rho to regulation of actin filament dynamics.

A key target of both Rac and Cdc42 is the protein-serine/threonine kinase called PAK. Like Rho kinase, PAK also affects phosphorylation of the myosin light chain, but in the opposite direction. PAK phosphorylates and inhibits myosin light chain kinase, leading to a decrease in myosin light chain phosphorylation. This results in inactivation of myosin II and a decrease in actin-myosin

interaction, consistent with the ability of Rac and Cdc42 to stimulate the formation of cell surface protrusions (filopodia and lamellipodia) rather than stress fibers and focal adhesions. Activation of Rac also leads to stimulation of LIM-kinase and phosphorylation of cofilin, linking Rac to remodeling of the actin cytoskeleton. Other targets of Rac and Cdc42 also participate in the formation of filopodia and lamellopodia, as well as in the regulation of cell-cell interactions at adherens junctions.

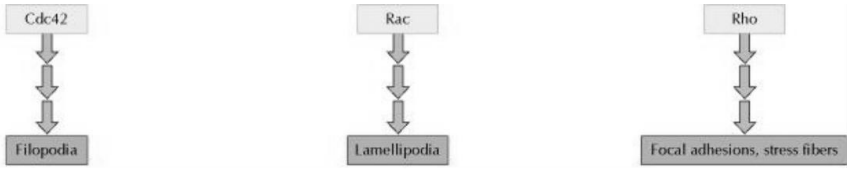


Figure 6.28: Regualtion of actin remodeling by Rho family proteins

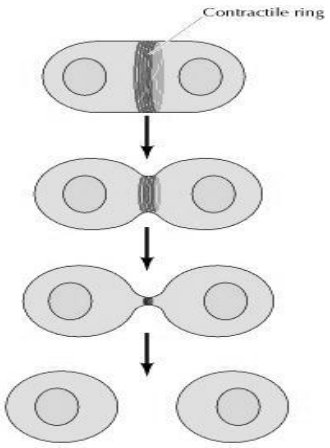


Figure 6.29 Contractile ring consisting of actin filaments

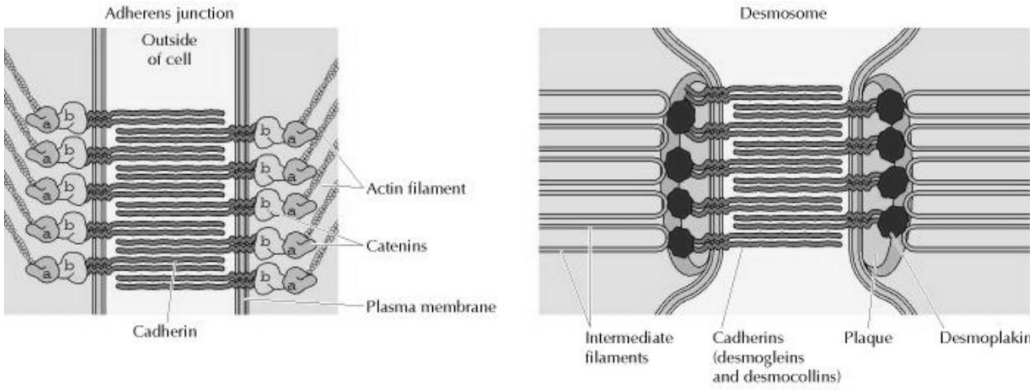


Figure 6.30: Cell – cell junctions mediated by Cadherins

V. Signaling networks:

Signaling pathways are rarely linear. The activities of individual pathways are regulated by feedback loops that control the extent and duration of signaling activity. Signaling pathways do not work in isolation , rather there is frequent cross talk between the pathways, and hence intracellular signal transduction is to be understood as a network of connected pathways.

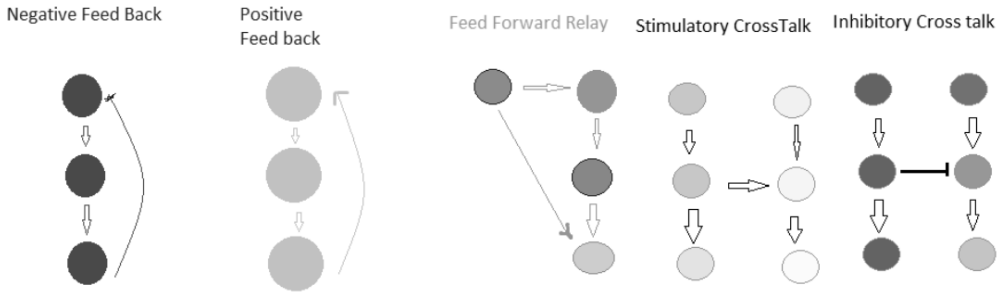
1. Feedback and Crosstalk: The activity of signaling pathways is controlled by feedback loops, which are similar in principal to feedback regulation of metabolic pathways.

Eg: NF- κB is activated by signals that lead to proteolysis of the inhibitor 1κB, allowing NF- κB to translocate to the nucleus and induce expression of its target genes. One of the target genes induced by NF- κB encodes 1κB, so NF- κB signaling leads to the synthesis of new 1κB which inhibits continued NF- κB activity.

Cross talk refers to the interaction of one signaling pathway with another. Examples for cross talk between pathways are- Ca2+- cAMP signaling, between cAMP and ERK pathways, between ERK and TGFκ/Smad pathways, and between integrins signaling and receptor protein-tyrosine kinase.

2. Networks of cellular Signal Transduction:

The extensive crosstalk between individual signal transduction pathways mean that multiple pathways interact with one another to form signaling networks within the cell. Some of the pathways can connect within a networks as shown in figure 6.31.



6.31: Elements of signaling networks

In feedback loops a downstream element of a pathway either inhibits (negative feed back) or stimulates (Positive feedback) an upstream element. In feed forward relays, an upstream element of a pathway stimulates both its immediate

targets and another element further downstream. Cross talk occurs when an element of one pathway either stimulates or inhibits an element of a second pathway.

A full understanding of cell signaling will require the development of network models that predict the dynamic behavior of the interconnected signaling pathways that ultimately result in a biological response. It is an integrated system and includes several signaling pathways involving receptors that stimulate cascade of protein kinases that ultimately effect one gene expression b y regulating transcription factors. The human genome encodes approximately 1500 different receptors, 700 protein kinases and Phosphatases and nearly 2000 transcription factors, indicating the potential for cross regulation among various pathways. The figure 6.32 represents an imaginative model for signaling in a mammalian neuron. Understanding mammalian signaling network is a tough task yet it is important to understand it in quantitative terms for the better understanding of cell biology.

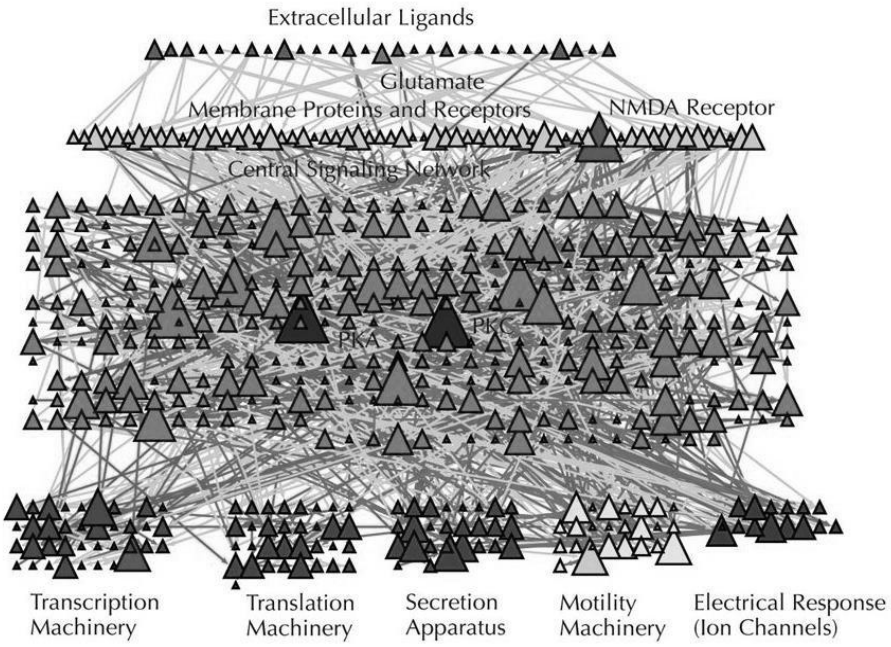


Figure 6.32: A mammalian Signaling network

The network depicts connection between 545 signaling elements in a mammalian neuron. Green arrows are stimulatory, red arrows are inhibitory and blue arrows are neutral links between elements.

PRACTICE QUESTIONS

1. Which among the following are examples of neurotransmitters?
(A) GABA (B) Glutamate (C) Epinephrine (D) All of the above

2. Among the listed cell surface receptors, which one is called as Orphan receptor.
(A) G-protein coupled receptors
(B) Receptor Protein –Tyrosine kinase
(C) Cytokine receptors and Nonreceptor Protein- Tyrosine Kinases
(D) Receptors linked to other enzymatic activities.

3. cAMP activate activates target genes that contain regulatory sequences which are called _____.
(A) ORI (B) Promoter
(C) cAMP response elements (D) cAMP binding sequences

4. Full form of MAP kinase is
(A) Mitotic activating Phosphokinase (B) Mitotic activating Protein kinase
(C) Mitogen activated Proline kinase (D) Mitogen activated protein kinase

5. _____ an d _____ are the two nonreceptor protein tyrosinekinases which act jointly insignaling from integrin receptor.
(A) PI 3 kinase and FSH (B) FAK and FSH
(C) FAK and ERK (D) Src and FAK

6. In _____, an upstream element of a pathway stimulates both its immediate targets and another element further downstream.
(A) Positive feedback loop (B) Negative feedback loop
(C) Stimulatory cross talk (D) Feed forward relay

7. Match Signaling molecules with their activities

A.	FSH	1.	Proliferation of T lymphocytes
B.	Substance P	2.	Stimulation of the growth of oocytes and ovarian follicles
C.	Enkephalin	3.	Sensory Synaptic transmission
D.	Interleukin – 2	4.	Analgesic

(A) A - 2 , B - 4 , C - 1 , D - 3 (B) A -2 , B - 3 , C - 4 , D - 1
(C) A - 2 , B - 4 , C - 3 , D - 1 (D) A - 4 , B - 2 , C - 1 , D - 3

8. _____ play key roles in the immune system and in inflammation as well as in regulation of proliferation and survival of many types of animal cells.

(A) MAP kinase pathways

(B) The JK/STAT and TGFβ / Smad Pathways

(C) NF- κB signaling

(D) The Hedgehog, Wnt and Notch pathways

9. G-protein is heteromeric. G-alpha activates the enzyme phospholipase C.

Phospholipase C cleaves P1 P2 to generate the two secondary messengers _____ and _____.

(A) IP_3 and Diacyl glycerol

(B) Cyclic GMP and IP_3

(C) Diacyl glycerol and cAMP

(D) cAMP and PKA

10. Role of the water soluble messenger among the two is _____

(A) Activation of proteins by phosphorylation

(B) Opens sodium and potassium channels

(C) Opens Ligand-gated Calcium channels

(D) Opens ligated gated sodium channels

ANSWER KEYS

1	D	**2**	A	**3**	C	**4**	D	**5**	D	**6**	D	**7**	B
8	C	**9**	A	**10**	C								

EXPLANATIONS

1. **Neurotransmitters:** They carry signals between neurons or between neurons and other target cells. These hydrophilic molecules include- acetylcholine, dopamine, epinephrine, serotonin, histamine, glutamate, glycine and gama- aminobutyric acid (GAB(A).

2. G-protein coupled receptors are called as orphan receptors because their ligands are still under investigation.

3. In many animal cells, increases in cAMP activate the transcription of specific target genes thatcontain a regulatory sequence called the cAMP response element, or CRE

4. Full form of MAP kinase is Mitogen activated protein kinase

5. Src and FAK appear to function in association with each other, the two nonreceptor protein-tyrosine kinases act jointly in signaling from integrin receptors.

6. In feedback loops a downstream element of a pathway either inhibits (negative feed back) or stimulates (Positive feedback) an upstream element. In feed forward relays, an upstream element of a pathway stimulates both its immediate targets and another element further downstream. Cross talk occurs when an element of one pathway either stimulates or inhibits an element of a second pathway.

7.

a. FSH	**1.** Stimulation of the growth of oocytes and ovarian follicles
b. Substance P	**2.** Sensory Synaptic transmission
c. Enkephalin	**3.** Analgesic
d. Interleukin – 2	**4.** Proliferation of T lymphocytes

8. The NF- κB family consists of five transcription factors that play key roles in the immune system and in inflammation as well as in regulation of proliferation and survival of many types of animal cells.

10. Phospholipase C cleaves PI P2, a membrane phospholipid, to generate two second messengers, IP3 and diacylglycerol (DAG). IP3 is water soluble, diffusing through the cytosol to bind to and open a ligand-gated Ca^{++} channel in the endoplasmic reticulum (or sarcoplasmic reticulum in muscle cells).

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