Post-Graduate Degree Programme (CBCS)

in

ZOOLOGY

SEMESTER-IV

ELECTIVE THEORY PAPER

CELL AND DEVELOPMENTAL BIOLOGY

ZDSE(MJ)T-404

SELF LEARNING MATERIAL

DIRECTORATE OF OPEN AND DISTANCE LEARNING
UNIVERSITY OF KALYANI
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Director's Message

Satisfying the varied needs of distance learners, overcoming the obstacle of distance and reaching the unreached students are the threefold functions catered by Open and Distance Learning (ODL) systems. The onus lies on writers, editors, production professionals and other personnel involved in the process to overcome the challenges inherent to curriculum design and production of relevant Self Learning Materials (SLMs). At the University of Kalyani a dedicated team under the able guidance of the Hon'ble Vice-Chancellorhas invested its best efforts, professionally and in keeping with the demands of Post Graduate CBCS Programmes in Distance Mode to devise a self-sufficient curriculum for each course offered by the Directorate of Open and Distance Learning (DODL), University of Kalyani.

Development of printed SLMs for students admitted to the DODL within a limited time to cater to the academic requirements of the Course as per standards set by Distance Education Bureau of the University Grants Commission, New Delhi, India under Open and Distance Mode UGC Regulations, 2020 had been our endeavour. We are happy to have achieved our goal.

Utmost care and precision have been ensured in the development of the SLMs, making them useful to the learners, besides avoiding errors as far as practicable. Further suggestions from the stakeholders in this would be welcome.

During the production-process of the SLMs, the team continuously received positive stimulations and feedback from Professor (Dr.) Amalendu Bhunia, Hon'ble Vice- Chancellor, University of Kalyani, who kindly accorded directions, encouragements and suggestions, offered constructive criticism to develop it within proper requirements. We gracefully, acknowledge his inspiration and guidance.

Sincere gratitude is due to the respective chairpersons as well as each and every member of PGBOS (DODL), University of Kalyani. Heartfelt thanks are also due to the Course Writers-faculty members at the DODL, subject-experts serving at University Post Graduate departments and also to the authors and academicians whose academic contributions have enriched the SLMs. We humbly acknowledge their valuable academic contributions. I would especially like to convey gratitude to all other University dignitaries and personnel involved either at the conceptual or operational level of the DODL of University of Kalyani.

Their persistent and coordinated efforts have resulted in the compilation of comprehensive, learner-friendly, flexible texts that meet the curriculum requirements of the Post Graduate Programme through Distance Mode.

All the Self Learning Materials are self-writing and collected from e-book, journals and websites.

Director
Directorate of Open and Distance Learning
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ELECTIVE THEORY PAPER [ZDSE(MJ)T – 404]

CELL AND DEVELOPMENTAL BIOLOGY

Unit-I - Cell Synchronization				
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	VI	Intracellular receptors, steroid hormone signaling pathways		
	VII	Characteristics of tumor cells; detection of tumor using CAT scan, MRI scan and fMRI scan		
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	XIII	Emergence of central nervous system, neural tube polarity, program cell death during neuronal development		
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Unit I

Physiology of cell division: Cell Cycle, synchrony in cell division, inhibition of cell division, source of energy

Objective: In this unit you will know about physiology of cell division which includes cell cycle, synchrony in cell division, inhibition of cell division and the source of energy for cell division.

Introduction

All living organisms of the biological world start life as one cell, i.e., unicellular zygote, the product of the union of gametes - a sperm and an egg. Of course, unicellular organisms live their entire lives as one cell. But in a multicellular organism, the unicellular zygote undergoes countless divisions and produces many cells.

These cells ultimately build the organism to a level of cellular complexity and organisation. The process by which any cell produces its own replica is known as cell division. Thus, simply by cell division a zygote enables an organism to grow. During this period of growth, many cells undergo a course of specialisation that commits them to perform specific functions.

Some cells function in cell division—either they divide to produce gametes for sexual reproduction or they divide to make new cells for growth or to replace old and damaged cells. Thus, cell division is at the core of life itself. It helps organisms to grow, reproduce and repair damaged and worn tissue—three fundamental activities of life.

New cells originate only from other living cells. In 1850, Virchow enunciated that every cell originates through division of pre-existing cells. The cell that undergoes division is termed a mother cell, while the cells derived from the division of a mother cell are known as daughter cells. The mother cell transmits copies of its hereditary material in the form of DNA or chromosome to its daughter cells, the next cell generation of cells.

For hereditary information to be transmitted from generation to generation, DNA must be replicated before the cells divide so that each new daughter cell receives a complete copy of hereditary instruction. Since DNA is a part of a eukaryotic cell's chromosome, the chromosomes duplicate as well. After chromosomal duplication, the rest of the division activities proceed in a way that ensures each daughter cell receives the same share of genetic information as well as almost equal proportion of the cell's cytoplasm and organelles. Therefore, in order to divide, a cell must double its mass and increase in shape and size. Cells generally divide when they attain the maximum size.

Phases of Cell Cycle

Most cells divide one or more times during their life time. When they do, they pass through an ordered sequence of events that collectively forms the cell cycle. The duration of the cell cycle varies greatly from one cell to another. The shortest cell cycle occurs in early embryo and can last as little as 8 minutes. The cell cycle of growing eukaryotic cell lasts from 90 minutes to more than 24 hours, its duration varying considerably within a population of cells.

The cell cycle of the eukaryotic cell is divided into two fundamental parts:

i. *Interphase*, and

ii. *Mitosis* (including Cytokinesis)

Interphase is the period of non-apparent division whereas mitosis is the period of division. Actually, for many years cell biologists were concerned with the period of division in which changes visible under the compound microscope could be observed—whereas during interphase no visible changes under compound microscope were seen.

Even chromosomes were not visible in the interphase because the refractive index of the nuclear sap and that of the chromosome present in re-condensed, hydrated and dispraised state become identical. The whole nucleus appears as idle. So, interphase was mistakenly considered as resting stage. During interphase of nucleus several changes take place at the molecular level that are not visible microscopically. Interphase is a period of intense biosynthetic activity in which the cell doubles in size and duplicates precisely its chromosome complement. So, this phase is also known as metabolic phase and the nucleus is known as the metabolic nucleus.

i) Interphase

The time from the end of one mitosis to the start of the next mitosis is called interphase. It is the phase between the end of last telophase and subsequent prophase. This is the longest period in cell division. Feulgen staining of metabolic nucleus followed by a cytophotometric quantitative assay first suggested that doubling of DNA takes place during interphase.

Auto-radiographic studies with labelled thymine demonstrated that doubling of DNA—i.e., replication or synthesis of DNA— did not take place throughout the entire interphase. It occurs only in a restricted portion of the interphase—the so-called S phase, i.e., synthetic period. This period is preceded and followed by two gap periods of interphase (G_1 and G_2) in which there is no DNA synthesis. Thus, the interphase can be subdivided into three successive sub-phases G_1, S and G_2 and it normally comprises 90% or more of the total cell cycle (Fig. 1).

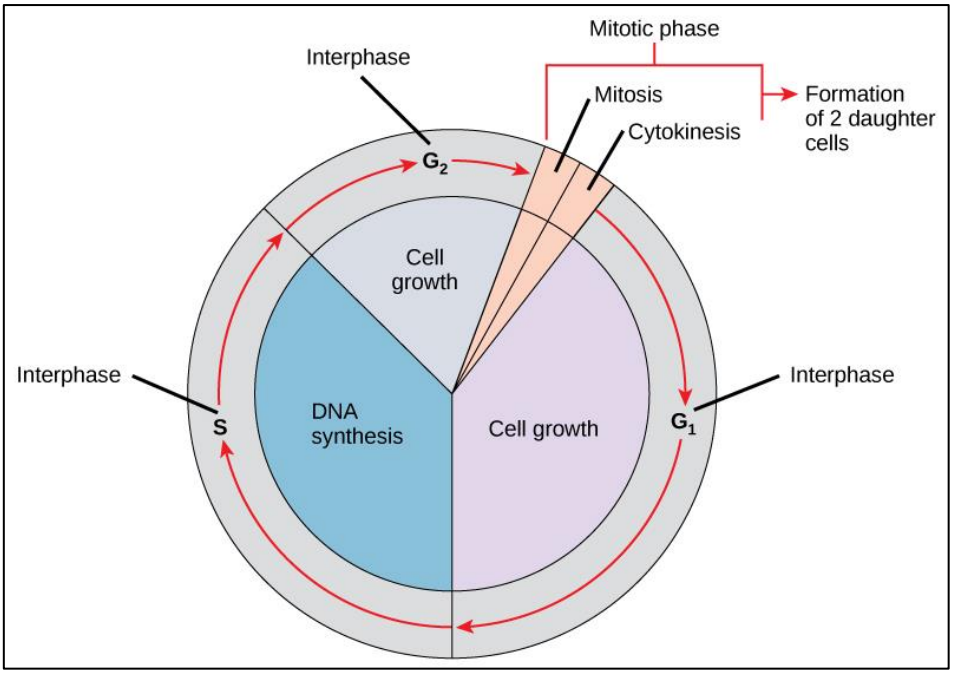

Fig 1: The cell cycle consists of interphase and the mitotic phase. During interphase, the cell grows and the nuclear DNA is duplicated. Interphase is followed by the mitotic phase. During the mitotic phase, the duplicated chromosomes are segregated and distributed into daughter nuclei. The cytoplasm is usually divided as well, resulting in two daughter cells.

ii) G_1 Phase (first gap)

The first stage of interphase is called the G_1 phase, or first gap, because little change is visible. However, during the G_1 stage, the cell is quite active at the biochemical level. The cell is accumulating the building blocks of chromosomal DNA and the associated proteins, as well as accumulating enough energy reserves to complete the task of replicating each chromosome in the nucleus.

iii) S Phase (DNA Synthesis)

Throughout interphase, nuclear DNA remains in a semi-condensed chromatin configuration. In the S phase (synthesis phase), **DNA replication** results in the formation of two identical copies of each chromosome—sister chromatids—that are firmly attached at the centromere region. At this stage, each chromosome is made of two sister chromatids and is a duplicated chromosome. The centrosome is duplicated during the S phase. The two centrosomes will give rise to the mitotic spindle, the apparatus that orchestrates the movement of chromosomes during mitosis. The centrosome consists of a pair of rod-like centrioles at right angles to each other. Centrioles help organize cell division. Centrioles are not present in the centrosomes of many eukaryotic species, such as plants and most fungi.

iv) G_2 Phase (2^{nd} gap)

In the G_2 phase, or second gap, the cell replenishes its energy stores and synthesizes the proteins necessary for chromosome manipulation. Some cell organelles are duplicated, and the cytoskeleton is dismantled to provide resources for the mitotic spindle. There may be additional cell growth during G_2. The final preparations for the mitotic phase must be completed before the cell is able to enter the first stage of mitosis.

The Mitotic Phase

To make two daughter cells, the contents of the nucleus and the cytoplasm must be divided. The mitotic phase is a multistep process during which the duplicated chromosomes are aligned, separated, and moved to opposite poles of the cell, and then the cell is divided into **two new identical daughter cells**. The first portion of the mitotic phase, mitosis, is composed of five stages, which accomplish nuclear division. The second portion of the mitotic phase, called cytokinesis, is the physical separation of the cytoplasmic components into two daughter cells.

Mitosis

Mitosis is divided into a series of phases—prophase, prometaphase, metaphase, anaphase, and telophase—that result in the division of the cell nucleus.

- ***Division of the Nucleus of a Cell (Karyokinesis)***:

The mitosis is a part of somatic cell division which includes the division of the nucleus (called mitosis or karyokinesis) and the division of the cytoplasm (called cytokinesis).

In mitosis, the metabolic nucleus passes through a complicated system of changes in the form of four different stages, viz., prophase, metaphase, anaphase and telophase. Some important aspects of all these stages are discussed below.

i) Prophase

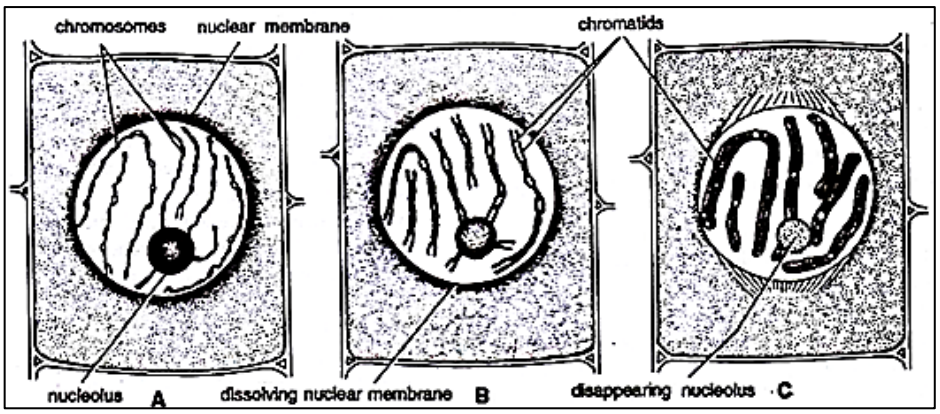

Fig 3: A-B Early prophase stage; C – Late prophase stage

1. It is the first and the longest phase in the mitotic cell division.

2. Chromosomes become visible in the nucleus as short, thick, helically coiled threads (Fig. 3A).

3. Each chromosome splits into two chromatids (Fig. 3B, C) joined at the centromere.

4. Nuclear membrane starts dissolving.

5. Nucleolus also starts dissolving and disappearing.

6. Prophase changes into next stage called metaphase.

4. Chromosomes become shorter and thicker.

5. Chromosomes arrange themselves in the centre or on the equator of spindle.

6. At the end of metaphase, two chromatids of each chromosome also start separating.

ii) Metaphase

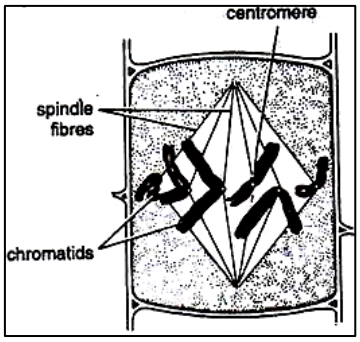

Fig 4: Metaphase stage

1. Nuclear membrane disintegrates and disappears completely (Fig 4).

2. Nucleolus disintegrates and disappears completely.

3. Spindle fibres start appearing and these fibres get attached to chromosomes at centromeres.

4. Chromosomes become shorter and thicker.

5. Chromosomes arrange themselves in the centre or on the equator of spindle.

6. At the end of metaphase, two chromatids of each chromosome also start separating.

7. Metaphase changes into the next stage called anaphase.

iii) Anaphase

1. Chromatids separate from each other at centromere and called daughter chromosomes (Fig 5).

2. Daughter chromosomes move to the opposite poles of the spindle.

3. Daughter chromosomes appear 'V', 'U' or J-shaped during their movement towards poles.

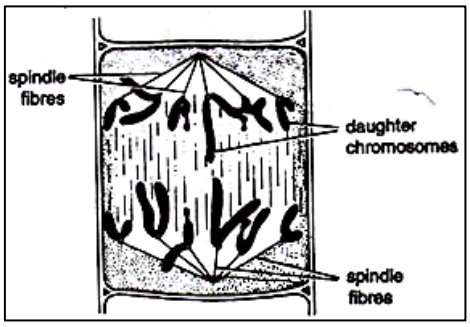

Fig 5: Anaphase stage

4. Anaphase changes into the next stage called telophase.

iv) Telophase

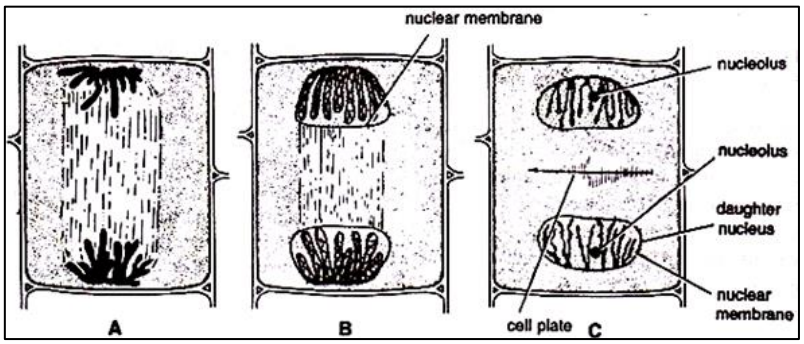

Fig 6: A-C various stages of telophase

1. Daughter chromosomes are now at the end of the spindle, i.e., presentontwo opposite poles (Fig 6A).

2. Nuclear membrane reforms around each group of daughter chromosomes (Fig 6B).

3. Nucleolus reforms (Fig 6C).

4. Two nuclei are thus organised, one at each pole of the parent cell.

5. Chromosomes begin to lose their compact structure.

6. Spindle fibres disappear gradually.

7. Thus formed two daughter nuclei are exactly similar to the parent nucleus.

Cytokinesis:

Cytokinesis is the second part of the mitotic phase during which cell division is completed by the **physical separation of the cytoplasmic components** into two daughter cells. Although the stages of mitosis are similar for most eukaryotes, the process of cytokinesis is quite different for eukaryotes that have cell walls, such as plant cells.

In cells such as animal cells that lack cell walls, cytokinesis begins following the onset of anaphase. A contractile ring composed of actin filaments forms just inside the plasma membrane at the former metaphase plate. The actin filaments pull the equator of the cell inward, forming a fissure. This fissure, or "crack," is called the cleavage furrow. The furrow deepens as the actin ring contracts, and eventually the membrane and cell are cleaved in two (Figure 7).

In plant cells, a cleavage furrow is not possible because of the rigid cell walls surrounding the plasma membrane. A new cell wall must form between the daughter cells. During interphase, the Golgi apparatus accumulates enzymes, structural proteins, and glucose molecules prior to breaking up into vesicles and dispersing throughout the dividing cell. During telophase, these Golgi vesicles move on microtubules to collect at the metaphase plate. There, the vesicles fuse from the center toward the cell walls; this structure is called a cell plate. As more vesicles fuse, the cell plate enlarges until it merges with the cell wall at the periphery of the cell. Enzymes use the glucose that has accumulated between the membrane layers to build a new cell wall of cellulose. The Golgi membranes become the plasma membrane on either side of the new cell wall (Figure 7).

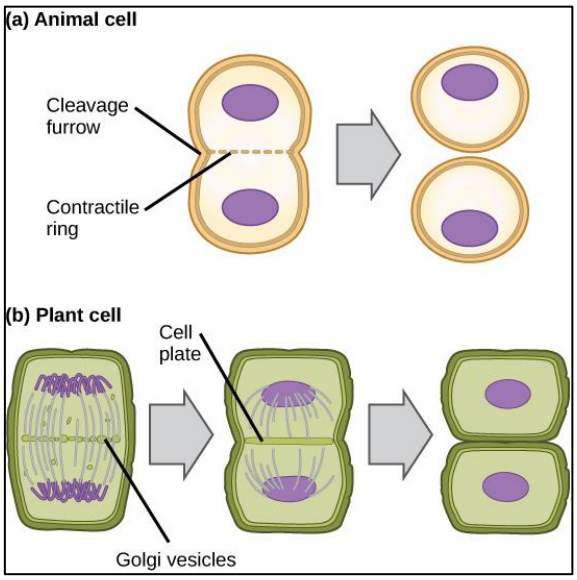

Fig 7: I*n part (a), a cleavage furrow forms at the former metaphase plate in the animal cell. The plasma membrane is drawn in by a ring of actin fibers contracting just inside the membrane. The cleavage furrow deepens until the cells are pinched in two. In part (b), Golgi vesicles coalesce at the former metaphase plate in a plant cell. The vesicles fuse and form the cell plate. The cell plate grows from the center toward the cell walls. New cell walls are made from the vesicle contents.*

G_0 Phase

Not all cells adhere to the classic cell-cycle pattern in which a newly formed daughter cell immediately enters interphase, closely followed by the mitotic phase. Cells in the G_0 phase are **not actively preparing to divide**. The cell is in a quiescent (inactive)

stage, having exited the cell cycle. Some cells enter G_0 temporarily until an external signal trigger the onset of G_1. Other cells that never or rarely divide, such as mature cardiac muscle and nerve cells, remain in G_0 permanently (Figure 8).

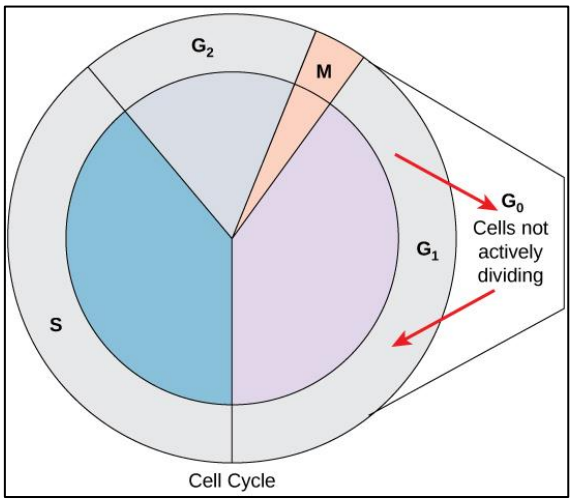

Figure 8: Cells that are not actively preparing to divide enter an alternate phase called G0. In some cases, this is a temporary condition until triggered to enter G1. In other cases, the cell will remain in G_0 permanently.

- **Significance of Mitosis**

1. Mitosis results in the formation of two daughter cells identical with that of the parental cell.

2. By this process, DNA, the main component of chromosomes, is distributed equally among the two newly formed nuclei.

3. Both the daughter cells formed after mitosis are identical and have the same genetic constitution, qualitatively as well as quantitatively, as the parent cell.

4. The number of chromosomes remains the same from one generation to another generation.

5. Resulted daughter cells have the same characters as were present in the parent cell.

6. The characters of the plants grown by vegetative reproduction may be preserved for a long period.

- **Control of cell cycle**

The length of the cell cycle is highly variable even within the cells of an individual organism. In humans, the frequency of cell turnover ranges from a few hours in early embryonic development to an average of two to five days for epithelial cells, or to an entire human lifetime spent in G_0 by specialized cells such as cortical neurons or cardiac muscle cells. There is also variation in the time that a cell spends in each phase of the cell cycle. When fast-dividing mammalian cells are grown in culture (outside the body under optimal growing conditions), the length of the cycle is approximately 24 hours. In rapidly dividing human cells with a 24-hour cell cycle, the G_1 phase lasts approximately 11 hours.

The timing of events in the cell cycle is controlled by mechanisms that are both internal and external to the cell.

- **Regulation at Internal Checkpoints**

It is essential that daughter cells be exact duplicates of the parent cell. Mistakes in the duplication or distribution of the chromosomes lead to mutations that may be passed forward to every new cell produced from the abnormal cell. To prevent a compromised cell from continuing to divide, there are internal control mechanisms that operate at three main cell cycle checkpoints at which the cell cycle can be stopped until conditions are favorable. These checkpoints occur near the end of G_1, at the G_2–M transition, and during metaphase (Figure 9).

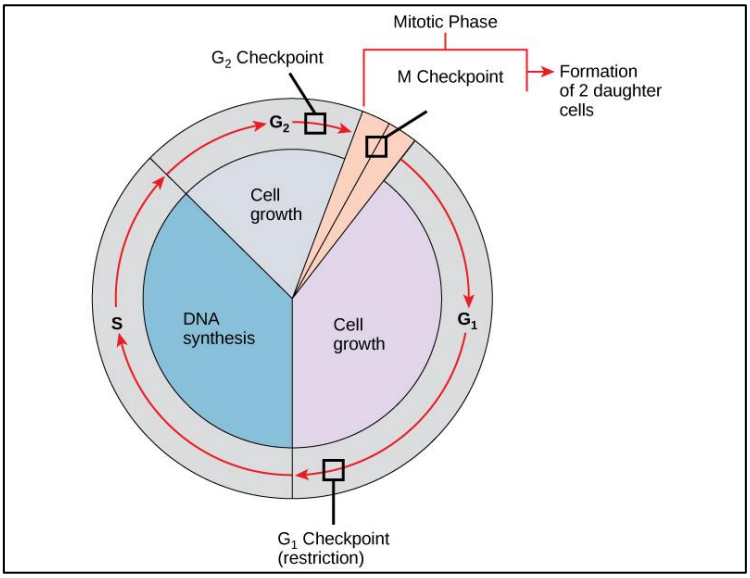

Figure 9: The cell cycle is controlled at three checkpoints. Integrity of the DNA is assessed at the G1 checkpoint. Proper chromosome duplication is assessed at the G2 checkpoint. Attachment of each kinetochore to a spindle fiber is assessed at the M checkpoint.

The G_1 Checkpoint

The G_1 checkpoint determines whether all conditions are favorable for cell division to proceed. The G_1 checkpoint, also called the restriction point, is the point at which the cell irreversibly commits to the cell-division process. In addition to adequate reserves and cell size, there is a check for damage to the genomic DNA at the G_1 checkpoint. A cell that does not meet all the requirements will not be released into the S phase.

The G_2 Checkpoint

The G_2 checkpoint bars the entry to the mitotic phase if certain conditions are not met. As in the G_1 checkpoint, cell size and protein reserves are assessed. However, the most

important role of the G_2 checkpoint is to ensure that all of the chromosomes have been replicated and that the replicated DNA is not damaged.

The M Checkpoint

The M checkpoint occurs near the end of the metaphase stage of mitosis. The M checkpoint is also known as the spindle checkpoint because it determines if all the sister chromatids are correctly attached to the spindle microtubules. Because the separation of the sister chromatids during anaphase is an irreversible step, the cycle will not proceed until the kinetochores of each pair of sister chromatids are firmly anchored to spindle fibres arising from opposite poles of the cell.

• Synchrony in cell division

Cell synchronization is a process by which cells in a culture at different stages of the cell cycle are brought to the same phase. Cell synchrony is a vital process in the study of cells progressing through the cell cycle as it allows population-wide data to be collected rather than relying solely on single-cell experiments. The types of synchronization are broadly categorized into two groups;

i) physical fractionization, and

ii) chemical blockade

For many of the studies of cell division it has been found desirable to have all of a population of cells in a culture divide at one time. Such synchronised cell division makes possible more effective analysis of the various components of the process.

Eggs of marine animals have been favourite objects for such studies since practically all the eggs divide at the same time in a suspension of healthy and normal cells. This synchrony in dividing marine eggs implies that some event has occurred to achieve the synchrony, e.g., the almost simultaneous entry of sperm in all eggs starts a train of events that takes about the same amount of time in each of the cells.

Cleavage in the embryo continues in synchrony for several generations unless cells are separated from one another. When the eggs have been affected by unfavourable conditions, e.g., temperature, radiations, pH, division may be delayed, and when it starts, some eggs are found to cleave before others.

The susceptibility of the cells to the unfavourable conditions varies about a mean for the population and a characteristic distribution curve is obtained for onset of division in the population. In a culture of bacteria, protozoa or tissue culture cells the number of cells in division at any one time is limited, being from 5 to 10 per cent. The ratio of the number of cells in division to the total number of cells is called the mitotic index.

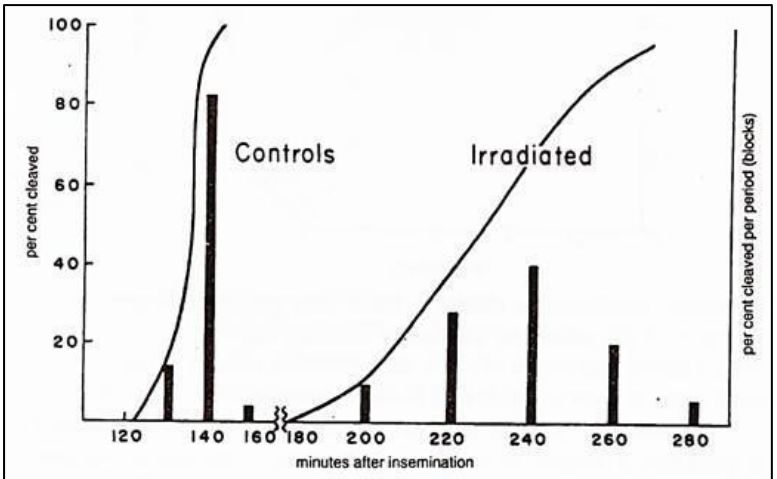

Figure 9.2: High degree of synchronyu in division of normal sea urchin eggs (inseminated simultaneously) as shown in the curve for "controls".

The mitotic index appears to be a function of the generation time for a given species under the conditions provided and the actual length of time a cell of this species remains in mitosis. The mitotic index may be increased by selecting a single cell from such an asynchronous culture and using it as the progenitor of a culture.

For a few generations thereafter, synchrony is obtained, but it also gradually disappears. A variation of this technique consists of filtering bacteria of a given size from a mixed population of cells, thus obtaining cells in essentially the same stage of growth which will divide at approximately the same time.The failure to achieve synchrony of division in a culture of cells is probably a result, in part, of differential exposure to various environmental conditions. In a syncytium (multinucleate cell) in which mixing of all materials occurs and the conditions of the environment are similar, the division of nuclei is usually perfectly synchronised. Also, in the syncytial insect egg nuclear division continues synchronously for as many as eleven generations.

The same is true for the nuclei in the syncytial endosperm of plant embryos. It is thought likely that nuclei in division may secrete division- stimulating materials. To explain the low mitotic index in most cell cultures it has been suggested by some that the division-stimulating material from the few cells in division does not affect many other cells, either because they are distant or because the membranes of such cells are relatively impervious to the hypothetical division-controlling substances.

This concept of control of synchrony by secretion of materials from nuclei has some experimental backing. For example, grafts can be made between two multinucleate amoebas (*Chaos chaos*), each with nuclei synchronously dividing but out of phase with one another. After one cycle the division of all nuclei from both amoebas now present in one cell is synchronous, the larger piece imposing its time upon the smaller.

There are many examples of synchronised cell division which can be found in cells of higher plants and animals. Cells in synchronous division are found, for example, in spermatogenic cells lining the lumen of a germinative tubule, which are connected to one another by cytoplasmic bridges. Such bridges insure entry of the division-stimulating material from one cell to another.It is important at this time to emphasize that a

suspension of cells, whether they are cells in tissue culture, protozoans, yeast or bacteria, are fundamentally different from a suspension of marine eggs. Marine eggs have undergone a period of growth in the ovary and they have self- contained supplies which will serve for many cell divisions before intake of nutrients need take place.Cells other than eggs, however, must incorporate nutrients and grow before they can divide. Perhaps not all cells are able to incorporate the same nutrients at the same rate.

Since it is not possible to achieve synchrony of cell division in cell suspensions other than marine eggs, attempts have been made to shock cells into synchrony.

Two methods have been used:

i. Chemical shock and

ii. Physical shock.

Chemical shock consists of withholding or limiting the supply of some nutrient necessary for division and then supplying it to the culture at one time in a large quantity, inducing in this manner a high level of simultaneous metabolic activity. The *physical shock* is one that is unfavourable for the act of cell division yet favourable to other metabolic activities preceding division, thus allowing the cells in the pre-division stages of the division cycle to, catch up with those in the later stages of the division cycle.

Only a few experiments using shocks for synchronisation of cell divisions will be considered here. Cell division and DNA synthesis in *Escherichia coli* T-15, a thymine-requiring mutant (thymineless), are blocked immediately upon transfer of the organism to a thymine-free medium.However, RNA and protein synthesis continue, apparently at the original rate. When thymine is added 30 minutes later, DNA synthesis is resumed and nearly all the cells are found to divide simultaneously after a lag of 35 to 40 minutes. Similarly, in *Lactobacillus acidophilus*, synchrony can be induced by the addition of thymidine to a thymidine-starved culture.

Yeast cells starved in succinate buffer until some of the reserves are gone will divide synchronously after return to complete nutrient medium, including carbohydrate and nitrogen sources. Similar results were obtained with a variety of cells needing some particular metabolite.For example, synchronous division in the cells of the epidermis of the insect *Rhodnius* follows its periodic ingestion of blood. In *Chlorella* and various algae, all photosynthetic, the daily periodic lighting regimen makes possible synchronisation of cell division, presumably by periodic accumulation of food reserves during the period of illumination. Lighting, too, may be considered a physical shock, much like temperature; in fact, all the arguments used for temperature apply to light in light- sensitive cells. Environmental changes produce oscillations in growth of many types of cells.

Use of temperature as a physical shock to obtain synchronised division stems from the notion that the processes that occur during the division cycle are differentially sensitive to temperature. If some reaction in the pre-division period is more sensitive to heat than are the reactions in the synthetic period, then a high temperature might prevent division without stopping syntheses.

Thus, cells lagging behind in preparations for division might be given a chance to catch up with the others. As expected, a single temperature shock synchronises only a small fraction of the cell population because it allows only a small proportion of the cells to accumulate in pre-division stages.

Cold shocks have also been used to induce synchronisation of cell division in a number of protozoans and bacteria. Nutritional deficiency, coupled with temperature shocks, has also been very effective. X-rays have also been shown to induce synchrony in division, again presumably by holding back the cells in the radiation-sensitive pre-division stages and causing accumulation of the cells which will ultimately divide at nearly the same time.

The specific protein is presumably "**division protein**" thought to be required for structuring the cell for division. It has been extracted and characterised. Interestingly, DNA synthesis, as measured by ^{3}H- thymidine incorporation is not synchronised with cell division in heat- shocked cultures. It would be of interest to determine the DNA polymerase activity at various times during the cell division cycle.

Synchronised cultures of various cells in suspensions are now being widely used in biochemical and cytochemical research. It is important to note, however, that changes in size and composition of the cells after synchronisation must be taken into consideration. For example, resistance of such cells to ultraviolet radiations is markedly altered.

- ## Source of Energy for Cell Division

Cell division, is a fundamental activity of the living cell. During cell division the cell does work at the expense of energy derived from nutrients, as witness the case just cited of synchronised cell division in yeast obtained whenever the cells are supplied with adequate food. It has also been shown that addition of glucose to isolated epidermal tissue culture results in synchronous division of many cells. Presumably, cell division had previously been blocked by lack of nutrient since such cells store little glycogen. Even if glucose is supplied, division fails if oxygen is not available.

Glucose may be replaced by lactate, glutamate, fumarate or citrate. All the experiments suggest that operation of the Krebs cycle supplies the energy for division of the cells. As expected, mitosis is inhibited by Krebs' cycle poisons such as malonate, cyanide and carbon monoxide, and by phlorhizin, a phosphorylation inhibitor.

The latter finding suggests that **high energy phosphate bonds are an energy source** or are involved in building this source. Marine eggs and protozoans require oxygen for division, but frog eggs and many embryonic tissue cells do not, presumably supplying their energy for cell division by glycolysis. Cells which require oxygen for division have a lower rate of glycolysis compared to their rate of respiration.

Cells which divide in absence of oxygen have rates of glycolysis much higher than that of respiration, sometimes several fold greater. As might be expected, in cells which can supply their energy needs for division by glycolysis cell division is very sensitive to glycolytic inhibitors such as iodoacetic acid. In order to have an effect on cell division the energy sources must be supplied early in the cell division cycle during what Bullough calls the antephase.

Once a critical concentration of the **energy-rich substances has been built up and the prophase begins,** he found that nothing short of killing the cell will stop it from dividing. This has been noticed not only for cells deprived of oxygen or metabolically poisoned, but also for those damaged by radiations or subjected to other injuries; such cells divide and soon thereafter cytolyse. Initiation of mitosis appears to begin a series of concatenated and irreversible reactions which stop only when the cells have divided.

Once the energy has siphoned out it carries the cell through mitosis and cleavage; if at this time the cell is poisoned for a period by monoxide and energy is no longer being accumulated in the reservoir, cleavage will nevertheless continue to completion. However, the next cell division is delayed for an interval equal to the period of application of the poison. Lack of oxygen and various other poisons that affect the aerobic enzymes would also affect cell division in a similar manner.

By **varying the carbon monoxide concentration**, and thus obtaining varying degrees of inhibition of ATP production, Epel demonstrated that the mitotic rate paralleled the ATP level in cells. He also found that division could be blocked at any stage of mitosis if the inhibitor was applied at the appropriate time. When phosphorylation is uncoupled by dinitrophenol (DNP), **ATP fails to accumulate and cell division is also blocked**. Such a concentration of DNP, which uncouples phosphorylation, causes an increased rate of respiration, but the respiration is now useless and idling.

This point again at **ATP as the likely source of energy for cell division**. It has become increasingly evident that specific protein (division protein) probably plays an important role in cell division in *Tetrahymena*. It is thought that a critical concentration of this particular protein is needed for cell division. It has also been shown that ATP, GTP and RNA accumulate in the period just preceding cytoplasmic division and that the changes are reversed at mitosis. However, such an accumulation of free energy sources is interpretable on the basis of protein synthesis, because protein synthesis decreases for a period beginning just about two thirds of the time to the next cell division. At this time the protein formed on the ribosomes is less readily released than it was previously. The call for ATP is then less than its concentration in the cell, along with that of GTP, and RNA therefore increases. This change in concentration thus appears to be incidental to the slowing or cessation of protein synthesis rather than due to accumulation of an energy reservoir. The high energy phosphates then play a part in reversing the binding of the protein to the ribosomes.

Probable questions:

1. Describe the interphase stage of cell cycle in details.
2. Discuss in details about the cell cycle check points.
3. Describe the stages of mitosis cell division with suitable diagram.
4. Discuss the role of cyclins in cell cycle regulation.
5. How DNA damage halts the cell cycle in G_1?
6. Discuss the role of p^{53} in cell cycle regulation.
7. Discuss the synchrony of cell division.
8. Discuss about the energy source in cell cycle event.

Suggested readings:

1. Albert Bruce, Bray Dennis, LevisJulian, Raff Martin, Roberts Keith and Watson James (2008). Molecular Biology of the Cell, V Edition, Garland publishing Inc., New York and London.
2. Cooper, G.M. and Hausman, R.E. (2009). The Cell: AMolecularApproach.5thEdition. ASM Press and Sunderland, Washington, D.C.; Sinauer Associates, MA.
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Unit II

Intracellular Signaling and Cell surface receptor Signaling: a) G-proteins, G-protein-coupled receptors and their effectors

Objective: In this unit we will discuss about Intracellular Signaling and Cell surface receptor Signaling emphasized on G-proteins, G-protein-coupled receptors and their effectors.

Introduction

Cell signalling is the process of cells communicating with other cells within the body, or with the external environment. As a process, cell signalling refers to a vast network of communication between, and within, each cell of our body. With cell signalling, cells are able to coordinate within large, multicellular organisms.

Cell signalling can occur through a number of different pathways, but the overall theme is that the actions of one cell influence the function of another. Cell signalling is needed by multicellular organisms to coordinate a wide variety of functions. Nerve cells must communicate with muscle cells to create movement, immune cells must avoid destroying cells of the body, and cells must organize during the development of a baby.

Most cell signals are chemical in nature. For example, prokaryotic organisms have sensors that detect nutrients and help them navigate toward food sources. In multicellular organisms, growth factors, hormones, neurotransmitters, and extracellular matrix components are some of the many types of chemical signals cells use. These substances can exert their effects locally, or they might travel over long distances. For instance, neurotransmitters are a class of short-range signalling molecules that travel across the tiny spaces between adjacent neurons or between neurons and muscle cells. Other signalling molecules must move much farther to reach their targets. One example is follicle-stimulating hormone, which travels from the mammalian brain to the ovary, where it triggers egg release.

Some cells also respond to mechanical stimuli. For example, sensory cells in the skin respond to the pressure of touch, whereas similar cells in the ear react to the movement of sound waves. In addition, specialized cells in the human vascular system detect changes in blood pressure — information that the body uses to maintain a consistent cardiac load.

Some forms of cell signalling are intracellular, while others are intercellular. Intracellular signals are produced by the same cell that receives the signal. On the other hand, intercellular signals can travel all throughout the body. This allows certain glands within the body to produce signals which take action on many different tissues across the body. Cell signalling is how a tiny gland within the brain can react to external stimuli and

coordinate a response. In response to stimuli like light, odours, or touch, the gland can, in turn, release a hormone which activates responses in diverse body systems to coordinate a response to a threat or opportunity.

- **Cell surface receptors**

Cells have proteins called receptors that bind to signalling molecules and initiate a physiological response. Different receptors are specific for different molecules. Dopamine receptors bind dopamine, insulin receptors bind insulin, nerve growth factor receptors bind nerve growth factor, and so on. In fact, there are hundreds of receptor types found in cells, and varying cell types have different populations of receptors. Receptors can also respond directly to light or pressure, which makes cells sensitive to events in the atmosphere.

Receptors are generally transmembrane proteins, which bind to signalling molecules outside the cell and subsequently transmit the signal through a sequence of molecular switches to internal signalling pathways.

There are two types of receptors:

1. Internal receptors (intracellular or cytoplasmic receptors) and

2. cell-surface receptors.

1. Internal receptors

Internal receptors, also known as intracellular or cytoplasmic receptors, are found in the cytoplasm of the cell and respond to hydrophobic ligand molecules that are able to travel across the plasma membrane (Fig 4). Once inside the cell, many of these molecules bind to proteins that act as regulators of mRNA synthesis to mediate gene expression. Gene expression is the cellular process of transforming the information in a cell's DNA into a sequence of amino acids that ultimately forms a protein. When the ligand binds to the internal receptor, a conformational change exposes a DNA-binding site on the protein. The ligand-receptor complex moves into the nucleus, binds to specific regulatory regions of the chromosomal DNA, and promotes the initiation of transcription. Internal receptors can directly influence gene expression without having to pass the signal on to other receptors or messengers.

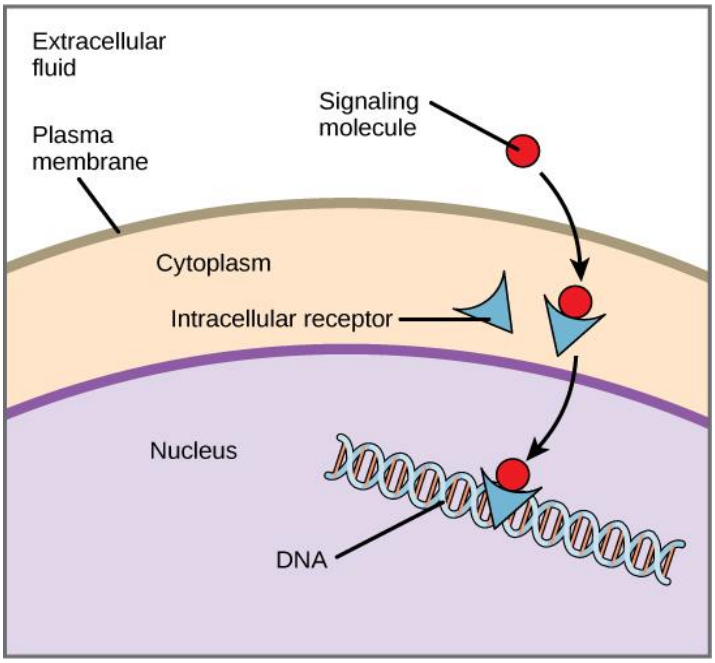

Fig 4: Intracellular Receptors: Hydrophobic signalling molecules typically diffuse across the plasma membrane and interact with intracellular receptors in the cytoplasm. Many intracellular receptors are transcription factors that interact with DNA in the nucleus and regulate gene expression.

2. Cell-Surface Receptors

Cell-surface receptors, also known as transmembrane receptors, are cell surface, membrane-anchored, or integral proteins that bind to external ligand molecules. This type of receptor spans the plasma membrane and performs signal transduction, converting an extracellular signal into an intracellular signal. Ligands that interact with cell-surface receptors do not have to enter the cell that they affect. Cell-surface receptors are also called cell-specific proteins or markers because they are specific to individual cell types.

Each cell-surface receptor has three main components: an external ligand-binding domain (extracellular domain), a hydrophobic membrane-spanning region, and an intracellular domain inside the cell. The size and extent of each of these domains vary widely, depending on the type of receptor.

Cell-surface receptors are involved in most of the signalling in multicellular organisms. There are three general categories of cell-surface receptors

I. G-protein-coupled receptors,

II. ion channel receptors, and

III. Enzyme-linked receptors.

I. G-protein-coupled receptors

G-Protein coupled receptors (GPCRs) are a group of seven transmembrane proteins which bind signal molecules outside the cell, transduce the signal into the cell and finally cause a cellular response (Fig 5). The GPCRs work with the help of a G-Protein which binds to the energy rich GTP.

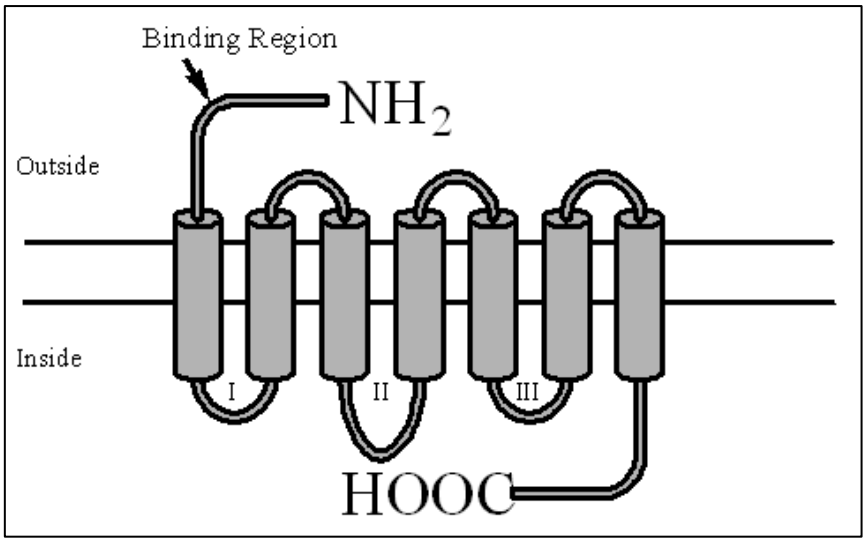

Fig 5: Seven transmembrane domain of G-protein coupled receptor

It is also known as heptahelical receptors, serpentine receptors, and G protein-linked receptors. These proteins make up transmembrane receptors whose purpose is to find molecules on the outside of the cell and initiate the signal transduction pathways. The signal transduction pathways are the processes by which a cell changes the form of one signal into the stimulus or a signal of another. These processes are carried out by enzymes. As the number of proteins and molecules increases, the size of the signal cascade increases rapidly, allowing for a large response, to a relatively small initiation factor.

G protein linked receptors are activated by ligands in the form of hormones, proteins, or other signalling molecule. This in turn leads to the activation of an intracellular G-protein by way of a certain interaction with the receptor. The G proteins act like relay batons to pass messages from circulating hormones into cells and transmit the signal throughout the cell with the ultimate goal of amplifying the signal in order to produce a cell response. Firstly, a hormone such as an epinephrine encounters a receptor in the membrane of a cell then a G protein is activated as it makes contact with the receptor to which the hormone is attached. Lastly, the G protein passes the message of a hormone to the cell by switching on a cell enzyme that triggers a response (Medicines by Design 46).

In addition to signalling, they have other physiological roles: -Sense of smell-the olfactory epithelium receptors bind odorants and pheromones -Mood regulation-receptors in the brain bind neurotransmitters (dopamine) -Immune system regulation- deals with inflammation and response to foreign bodies -Nervous system transmission-proteins control blood pressure, heart rate, and digestive processes -Cell density sensing.

- **G-Protein Coupled Receptor Structure**

The structure of most G-Protein Coupled Receptors is not very well known. The typical method of determining protein structure is by x-ray crystallography of the protein once it has been crystallized. However, due to the membrane environment, flexibility, and dynamic shifting of GPCRs, it is difficult to form crystals of them. Some have been crystallized by mutating certain amino acids to stabilize the structure, but there is no universal way to study them all.

Although it is tough to find exact information about the structure there are a few known traits of the proteins. G-proteins represent the level of middle management in the cellular organisation and are able to communicate between the receptors and the effector enzymes or ion-channels. They were called G-proteins because of their interaction with the guanine nucleotides, GTP and GDP.

The G proteins are bound to the cytoplasmic surface of the plasma membrane. They are heterotrimeric molecules consisting of 3 subunits α, β and γ. Their classification as stimulatory or inhibitory is based on the identity of their distinct α subunit. The β and γ subunits remain associated as β γ complex with the cytoplasmic surface of the membrane when the system is inactive or in resting state, GDP is bound to the α subunit.

- **Conformational change**

The receptor molecule exists in equilibrium between the active and inactive states (Fig 6). The ligand binding pushes the equilibrium towards the active sites. There are three types of ligands that bind to the g-proteins. The first are agonists, ligands that shift the equilibrium towards the active states. Inverse agonist shifts the equilibrium towards the inactive states, and neutral antagonists are ligands that do not change the equilibrium.

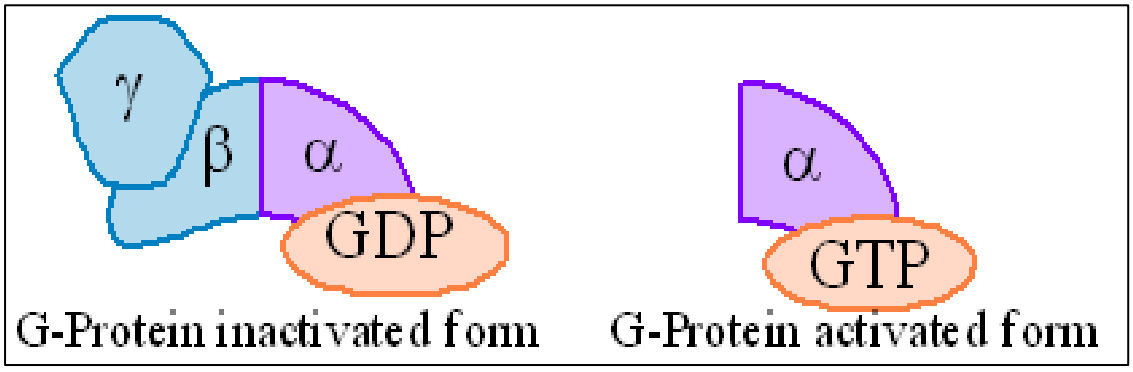

Fig 6: Active and inactive state of G-protein coupled receptor

- **Mechanism of G-protein coupled receptor**

Cell signalling using G-protein-linked receptors occurs as a cyclic series of events (Fig 7). Before the ligand binds, the inactive G-protein can bind to a newly revealed site on the receptor specific for its binding. Once the G-protein binds to the receptor, the resultant shape change activates the G-protein, which releases GDP and picks up GTP.

The subunits of the G-protein then split into the α subunit and the βγ subunit. One or both of these G-protein fragments may be able to activate other proteins as a result. After a while, the GTP on the active α subunit of the G-protein is hydrolysed to GDP and the βγ subunit is deactivated. The subunits reassociate to form the inactive G-protein and the cycle begins a new.

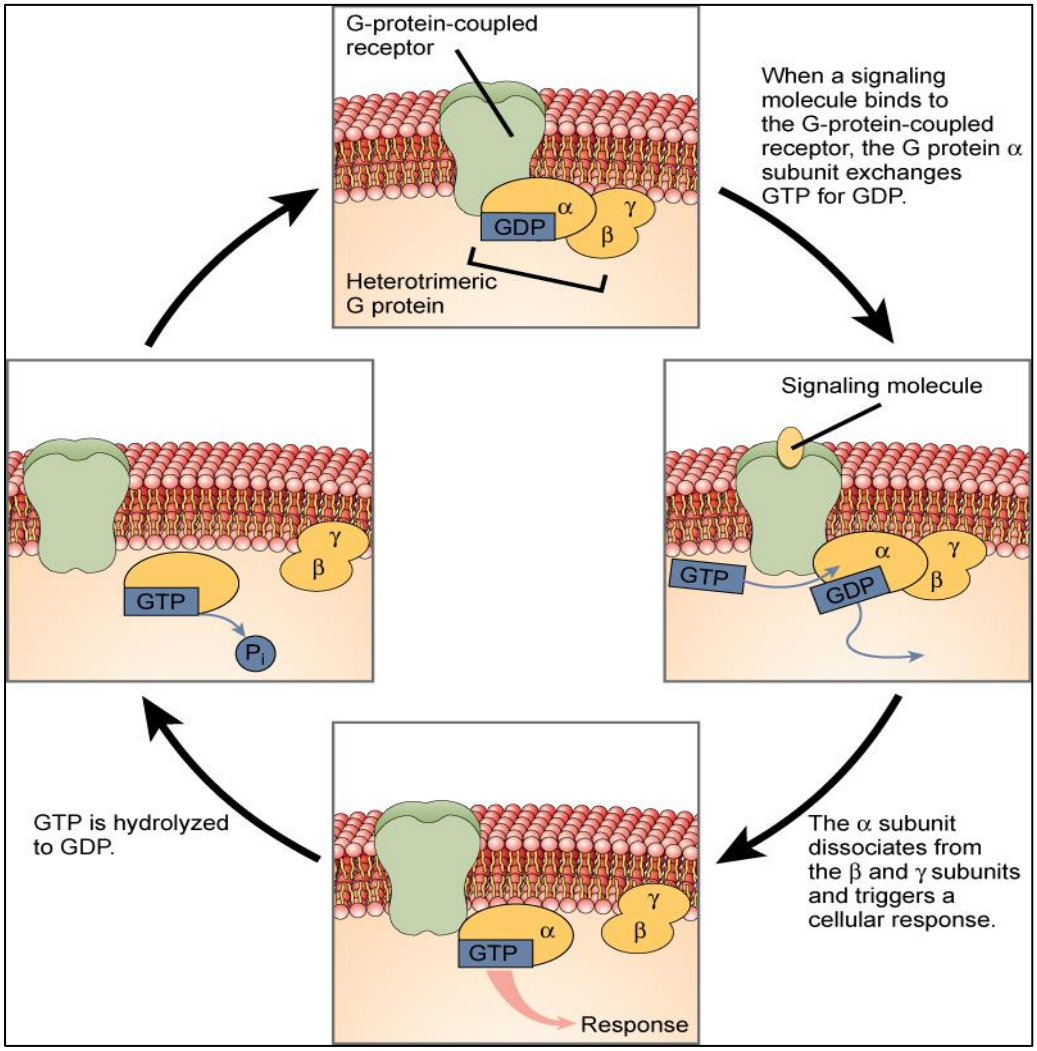

Fig 7: Heterotrimeric G proteins have three subunits: α, β, and γ. When a signalling molecule binds to a G-protein-coupled receptor in the plasma membrane, a GDP molecule associated with the α subunit is exchanged for GTP. The β and γ subunits dissociate from the α subunit, and a cellular response is triggered either by the α subunit or the dissociated $\beta\gamma$ pair. Hydrolysis of GTP to GDP terminates the signal.

- **Uses of G-Protein Coupled Receptors**

G-Protein Coupled Receptors are widespread in their use. In the eyes, Opsins, a GPCRs, translate electromagnetic radiation into cellular signals thus allowing visual perception. In the nose, olfactory epithelium binds odorants and pheromones which allow for the sense of smell. However, there are problems associated with GPCRs. Many human

diseases, including bacterial infections, involve the GPCRs where bacteria produce toxins which interfere with the function of the G-Protein. Examples of such diseases include cholera, whooping cough, botulism, etc.

Here is a closer view of how altered G-protein affects cholera and whooping cough. When there is a β subunit binding to $G_{\alpha s}$ gangliosides and a catalytic subunit entering the cell, then choleragen, a toxin resulted from cholera, forms. The catalytic subunit alters the $G_{\alpha s}$ ganglioside in which α part of the protein is adjusted via attaching ADP-ribose to arginine. As a result of this alteration, $G_{\alpha s}$ ganglioside become stable, meaning that now $G_{\alpha s}$ ganglioside is in its active form. The active $G_{\alpha s}$ ganglioside then does its job to activate protein kinase A, denoted PKA. The chloride channel is by PKA, PKA enters and thus there is no more absorption of Na^{+}. This is saying that there is a huge loss of NaCl and water in the body as seen in the symptoms of cholera. In treating cholera, the most effective way is to rehydrate the body using glucose-electrolyte solution.

Whooping cough, on the other hand, is different from cholera in which the ADP ribose moiety is added by the toxin. In this case, Ca^{2+} channel is closed whereas the K^{+} channel is opened by $G_{\alpha s}$ ganglioside. The result is that the $G_{\alpha s}$ ganglioside is in its inactive form and ultimately ending up with uncontrollable coughs.

In addition, Pharmacologists estimate that 60% of all medicines achieve their effect by acting on G Protein pathways. Since G protein is a switch molecule which passes the message inward (like relay baton), it can be turned on only when needed, then shut off. Some illnesses like cholera, occur when a G protein is errantly left on. Discovery about Gprotein switches and its structure which is made into 3 subunits (α, β, γ) will help us understand how we can inhibit the transmission or increase with some other ligand. G-protein coupled receptors are trans-membrane receptor proteins that when activated by ligands (hormones, proteins or other signalling molecules), they lead to the activation of an intracellular G-protein through a specific interaction with the receptor. The G-protein in turn transmits the signal to other proteins within the cell to ultimately amplify the signal and produce a cellular response. Understanding the structure and dynamics of the receptor could clarify the specific interactions the receptor makes with the ligand on the outside and the G-protein on the intracellular side, thereby leading to the understanding of how the receptor works. Consequently, drugs (agonists or antagonists) can be designed to bind the receptor and control its response, whether it's to transmit the signal to the G-protein or inhibit the transduction. Moreover, monitoring the effects of such signals can help in understanding the type of induced cellular responses and potentially uncover diseases that are proliferated in this manner.

Probable questions:

1. Define cell signalling.
2. Write down the structure of G-protein-linked receptors.
3. What is the mechanism of action of GPCR?
4. What is the main function of G protein coupled receptors?
5. State where the G-protein-linked receptors is used.

Suggested readings:

1. Albert Bruce, Bray Dennis, LevisJulian, Raff Martin, Roberts Keith and Watson James (2008). Molecular Biology of the Cell, V Edition, Garland publishing Inc., New York and London.

2. Cooper, G.M. and Hausman, R.E. (2009). The Cell: AMolecularApproach.5th Edition. ASM Press and Sunderland, Washington, D.C.; Sinauer Associates, MA.

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Unit III

Intracellular Signaling and Cell surface receptor Signaling: Receptor Tyrosine kinases (RTKs), Auto-phosphorylation of RTKs

Objective: In this unit we will discuss about Intracellular Signaling and Cell surface receptor Signaling emphasized on Receptor Tyrosine kinases (RTKs), Auto-phosphorylation of RTKs.

Cell-Surface Receptors

Cell-surface receptors, also known as transmembrane receptors, are cell surface, membrane-anchored, or integral proteins that bind to external ligand molecules. This type of receptor spans the plasma membrane and performs signal transduction, converting an extracellular signal into an intracellular signal. Ligands that interact with cell-surface receptors do not have to enter the cell that they affect. Cell-surface receptors are also called cell-specific proteins or markers because they are specific to individual cell types.

Each cell-surface receptor has three main components: an external ligand-binding domain (extracellular domain), a hydrophobic membrane-spanning region, and an intracellular domain inside the cell. The size and extent of each of these domains vary widely, depending on the type of receptor.

Cell-surface receptors are involved in most of the signalling in multicellular organisms. There are three general categories of cell-surface receptors

IV. G-protein-coupled receptors,
V. ion channel receptors, and
VI. Enzyme-linked receptors.

- ## Enzyme-linked receptors

Enzyme-linked receptors are cell-surface receptors with intracellular domains that are associated with an enzyme. In some cases, the intracellular domain of the receptor actually *is* an enzyme that can catalyze a reaction. Other enzyme-linked receptors have an intracellular domain that interacts with an enzyme. 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.

Classification

There are five main types of enzyme-linked receptors:

1. Receptor Tyrosine Kinase (RTK): Contains intrinsic tyrosine kinase activity (EGFR, VEGFR)
2. Receptor Serine/Threonine Kinase: Contains intrinsic serine/threonine kinase activity (TGF-βR)
3. Receptor Guanylyl Cyclases: Contain intrinsic cyclase activity (ANP)
4. Tyrosine-Kinase Associated Receptors: Receptors that associate with proteins that have tyrosine kinase activity (Cytokine Receptors)
5. Receptor Tyrosine Phosphatases
6. Receptor tyrosine kinases (RTKs) are a class of enzyme-linked receptors found in humans and many other species. A kinase is just a name for an enzyme that transfers phosphate groups to a protein or other target, and a receptor tyrosine kinase transfers phosphate groups specifically to the amino acid tyrosine.

- ## Mechanism of Receptor tyrosine kinases (RTKs) activity

Receptor tyrosine kinases (RTKs) are a diverse group of transmembrane proteins that act as receptors for cytokines, growth factors, hormones and other signaling molecules. RTKs are part of the larger family of protein tyrosine kinase. RTK consists of epidermal growth factor receptor family (EGFRs), platelet-derived growth factor receptor family (PDGFRs), macrophage colony stimulating factor receptor family (MCSFRs), insulin-like growth factor-1 receptor family (IGF1Rs), insulin receptor family (INSR), nerve growth factor receptors family (NGFRs), fibroblast growth factor receptor family (FGFRs), vascular endothelial growth factor receptor family (VEGFRs) and hepatocyte growth factor receptor family (HGFRs).

The RTK usually contains a transmembrane domain. RTKs are activated by the ligands that bind to their extracellular domain. Ligands can induce the receptor dimerization, which are extracellular signal molecules. The C-terminal region in the cell has the highest level of conservation and contains catalytic domains. These catalytic domains are responsible for the kinase activity of these receptors, which catalyse receptor autophosphorylation and tyrosine substrate phosphorylation.

Receptor tyrosine kinases have been shown to play critical roles in a variety of cellular processes including growth, differentiation and angiogenesis, and in the development and progression of many types of cancer. It seems that Inhibition of receptor tyrosine kinases are effective measures in cancer treatment.

The majority of growth factor receptors are composed of extracellular, transmembrane, and cytoplasmic tyrosine kinase (TK) domains. Receptor tyrosine kinase (RTK) activation regulates many key processes including cell growth and cell survival. However, dysregulation of receptor tyrosine kinase has been found in a wide

range of cancers, and it has been shown to correlate with the development and progression of numerous cancers. Therefore, receptor tyrosine kinase has become an attractive therapeutic target. One way to effectively block signaling from receptor tyrosine kinase is inhibition of its catalytic activity with small-molecule inhibitors. Low-molecular-weight TK inhibitors (TKIs), such as imatinib, targeting tumors with mutant c-Kit, and gefitinib, targeting non-small cell lung cancer with mutant epidermal growth factor receptor (EGFR), have received marketing approval in Japan. MET, fibroblast growth factor receptor (FGFR), and insulin-like growth factor-I receptor (IGF-IR) are frequently genetically altered in advanced cancers. TKIs of these receptors have not yet appeared on the market, but many anticancer drug candidates are currently undergoing clinical trials. Most of these TKIs were designed to compete with ATP at the ATP-binding site within the TK domain.

Signalling molecules first bind to the extracellular domains of two nearby receptor tyrosine kinases (Fig 1).

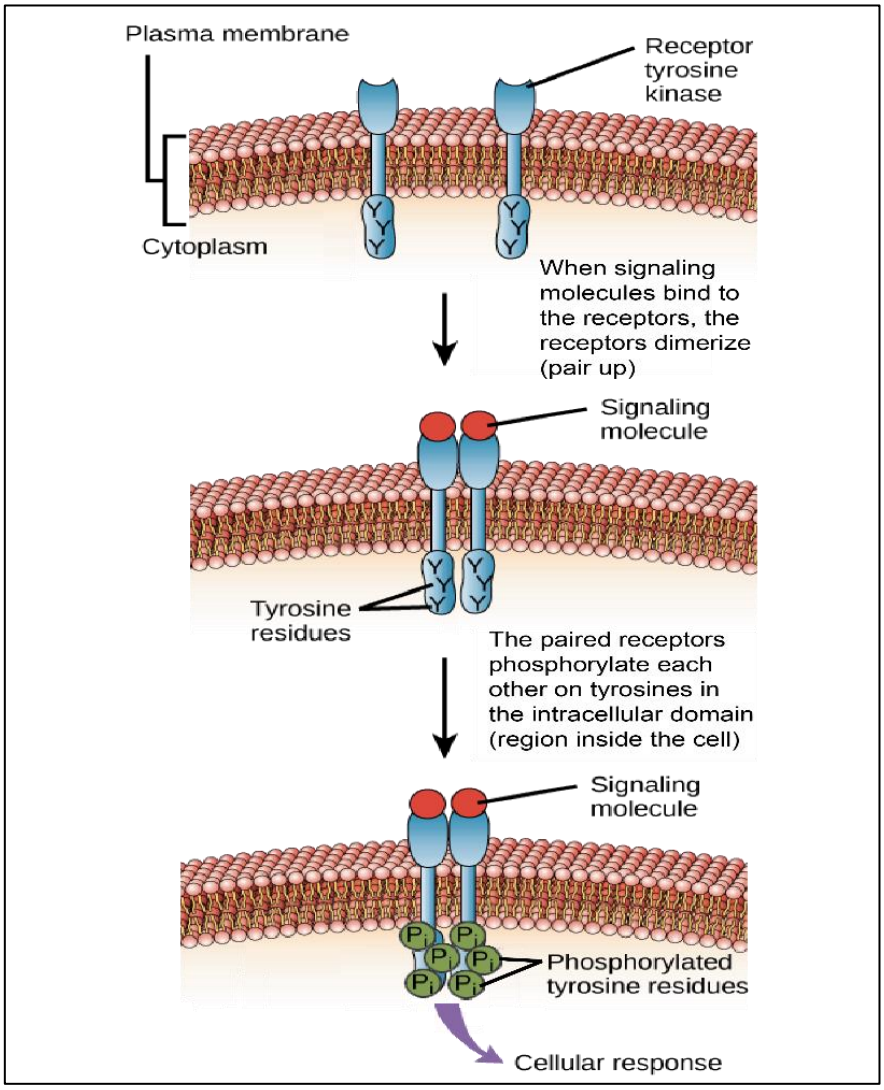

Fig 1: A receptor tyrosine kinase is an enzyme-linked receptor with a single transmembrane region, and extracellular and intracellular domains. Binding of a signalling molecule to the extracellular domain causes the receptor to dimerize. Tyrosine residues on the intracellular domain are then auto phosphorylated, triggering a downstream cellular response. The signal is terminated by a phosphatase that removes the phosphates from the phosphotyrosine residues.

The two neighbouring receptors then come together, or dimerize. The receptors then attach phosphates to tyrosines in each other's' intracellular domains. The phosphorylated tyrosine can transmit the signal to other molecules in the cell.

In many cases, the phosphorylated receptors serve as a docking platform for other proteins that contain special types of binding domains. A variety of proteins contain these domains, and when one of these proteins binds, it can initiate a downstream signalling cascade that leads to a cellular response. Receptor tyrosine kinases are crucial to many signalling processes in humans. For instance, they bind to growth factors, signalling molecules that promote cell division and survival. Growth factors include platelet-derived growth factor (PDGF), which participates in wound healing, and nerve growth factor (NGF), which must be continually supplied to certain types of neurons to keep them alive. Because of their role in growth factor signalling, receptor tyrosine kinases are essential in the body, but their activity must be kept in balance: overactive growth factor receptors are associated with some types of cancers.

- **Uses of Enzyme-linked receptors**

Kinase is a large family of enzymes that are responsible for catalysing the transfer of a phosphoryl group from a nucleoside triphosphate donor, such as ATP, to an acceptor molecule. Tyrosine kinases catalyse the phosphorylation of tyrosine residues in proteins. The phosphorylation of tyrosine residues in turn causes a change in the function of the protein that they are contained in.

Phosphorylation at tyrosine residues controls a wide range of properties in proteins such as enzyme activity, subcellular localization, and interaction between molecules. Furthermore, tyrosine kinases function in many signal transduction cascades wherein extracellular signals are transmitted through the cell membrane to the cytoplasm and often to the nucleus, where gene expression may be modified. Finally, mutations can cause some tyrosine kinases to become constitutively active, a nonstop functional state that may contribute to initiation or progression of cancer.

The receptor tyrosine kinases function in transmembrane signalling, whereas tyrosine kinases within the cell function in signal transduction to the nucleus. Tyrosine kinase activity in the nucleus involves cell-cycle control and properties of transcription factors.

Probable questions:

1. Define cell signalling.
2. Write down the structure of Receptor tyrosine kinases (RTKs).
3. What is the mechanism of action of RTKs?
4. What is the main function of Receptor tyrosine kinases?
5. State where the Receptor tyrosine kinases is used.

Suggested readings:

1. Albert Bruce, Bray Dennis, LevisJulian, Raff Martin, Roberts Keith and Watson James (2008). Molecular Biology of the Cell, V Edition, Garland publishing Inc., New York and London.
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Unit IV

Initiation of MAP kinase signaling

Objective: In this unit we will discuss about Initiation of MAP kinase signaling.

Introduction

Mitogen-activated protein kinase (MAPK) signalling. This is a classic example of a protein phosphorylation cascade that often begins with Ras and consists of a number of parallel pathways that function to control many cellular processes and particularly those related to cell proliferation, cell stress and apoptosis. Mitogen-activated protein (MAP) kinases are serine/threonine-specific protein kinases that respond to extracellular stimuli (mitogens, osmotic stress, heat shock and pro-inflammatory cytokines) and regulate various cellular activities, such as gene expression, mitosis, differentiation, proliferation, and cell survival/apoptosis. MAPK pathways are activated within the protein kinase cascades called "MAPK cascade". Each one consists of three enzymes, MAP kinase, MAP kinase kinase (MKK, MEKK, or MAP2K) and MAP kinase kinase kinase (MKKK or MAP3K) that are activated in series. A MAP3K that is activated by extracellular stimuli, which phosphorylates a MAP2K on its serine and threonine residues and this MAP2K activates a MAP kinase through phosphorylation on its serine and tyrosine residues.

Mitogen-activated protein kinase (MAPK) signalling

The multifunctional mitogen-activated protein kinase (MAPK) signalling system consists of separate pathways that function to control a number of different cellular processes such as gene transcription, metabolism, motility, cell proliferation, apoptosis, synaptic plasticity and long-term memory. These different downstream effectors are activated by the final MAPK components associated with the three main signalling pathways:

- Extracellular-signal-regulated kinase (ERK) pathway
- c-Jun N-terminal kinase (JNK) pathway
- p38 pathway

These different pathways are assembled by combining components from an extensive mitogen-activated protein kinase (MAPK) signalling toolkit. The mitogen-activated protein kinase (MAPK) signalling properties such as their spatio-temporal control mechanisms help to explain how they operate to regulate so many cellular processes. The activity of the MAPK signalling pathway is reversed by the mitogen-activated protein kinase (MAPK) phosphatases.

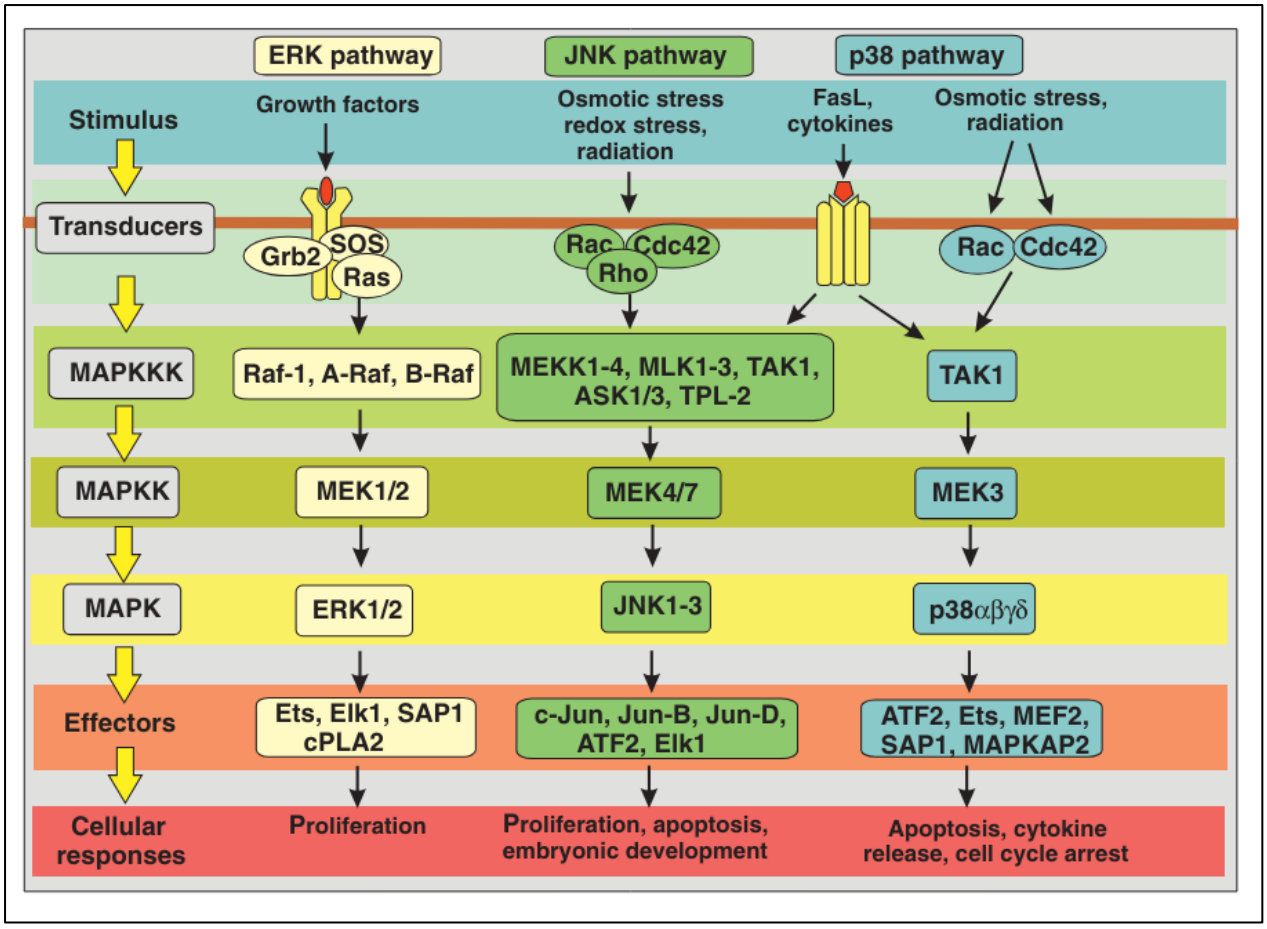

Fig 1: mitogen-activated protein kinase (MAPK) signalling: Organization of the main mitogen-activated protein kinase (MAPK) signalling pathways. The generic pathway on the left summarizes the sequential organization of the mitogen-activated protein kinase (MAPK) signalling system. External stimuli act through a variety of transducers to stimulate the first element of the signalling pathway, which is one of the 14 different MAPK kinase kinases (MAPKKKs). These MAPKKKs then phosphorylate the next element, which is one of the seven MAPK kinases (MAPKKs). This MAPKK then phosphorylates one of the 12 MAPKs, the names of which define the different signalling pathways. The three main pathways are the ERK pathway, the JNK pathway and the p38 pathway.

I. Extracellular-signal-regulated kinase (ERK) pathway

The extracellular-signal-regulated kinase (ERK) pathway is one of the major signalling cassettes of the mitogenactivated protein kinase (MAPK) signalling pathway (Fig 1). It performs a number of important signalling functions, including the control of cell proliferation and the synaptic plasticity responsible for learning and memory.

The main MAPK/ERK kinase kinase (MEKK) components are the Raf family members Raf-1, A-Raf and B-Raf that phosphorylate two serine residues on the MAPK/ERK kinase (MEK) components MEK1/2. The latter are dual-specificity protein kinases that phosphorylate the tyrosine and threonine residues of the characteristic MAPK

components ERK1/2 that are responsible for stimulating the downstream effectors, many of which are transcription factors (Fig 2). There is thus a linear transfer of information through a phospho-relay system based on a sequential series of phosphorylation events.

An important feature of this ERK pathway, which can be activated by both protein tyrosine kinase-linked receptors (PTKRs) and by G protein-coupled receptors (GPCRs), is its spatial organization (Fig 2).

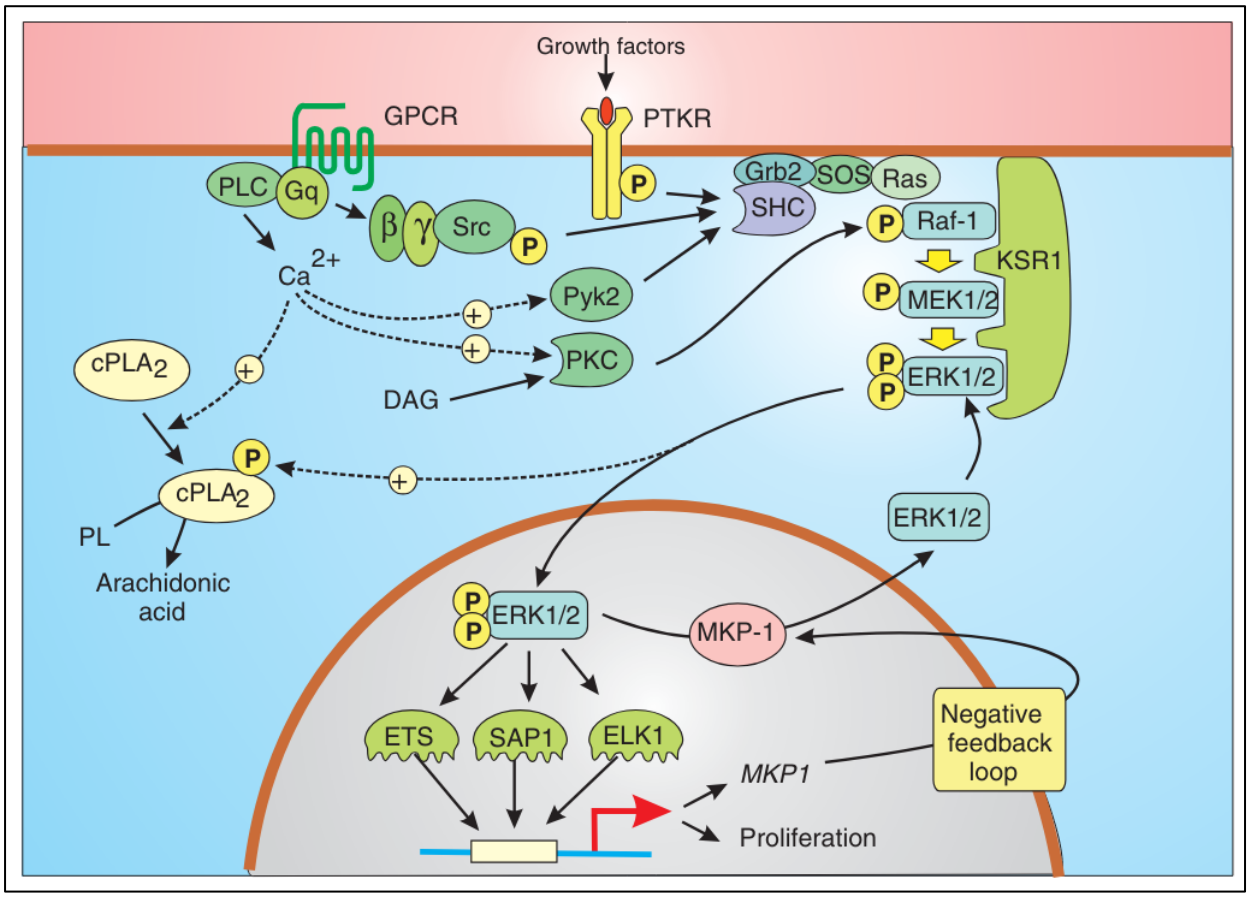

Fig 2: Extracellular-signal-regulated kinase (ERK) pathway. The extracellular-signal-regulated kinase (ERK) pathway, which can be made up from different components (Module 2: Figure MAPK signalling), is represented here by Raf-1, mitogen-activated protein kinase (MAPK)/ERK kinase 1/2 (MEK1/2) and ERK1/2, which can be activated by either protein tyrosine kinase-linked receptors (PTKRs) or by G protein-coupled receptors (GPCRs).

II. c-Jun N-terminal kinase (JNK) pathway

The c-Jun N-terminal kinase (JNK) pathway is one of the major signalling cassettes of the mitogen-activated protein kinase (MAPK) signalling pathway. It functions in the control of a number of cellular processes, including proliferation, embryonic

development and apoptosis. The pathway takes its name from the c-Jun N-terminal kinases 1–3 (JNK1–JNK3), which are the MAPKs that interact with the final effectors (Fig 1). They contain the dual phosphorylation motif Thr-Pro-Tyr, which is phosphorylated following activation of the upstream phosphorylation cascade. The JNK pathway is activated by a bewildering number of mechanisms. This complexity is evident by the fact that there are 13 MAPK kinase kinases (MAPKKKs) responsible for feeding information into the JNK pathway. The apoptosis signal-regulating kinase 1 (ASK1) is an example of such a kinase that initiates the signalling cascade that leads to JNK activation. One way of trying to cope with this complexity is to examine specific examples such as the activation of JNK by the interleukin-1 receptor (Module 2: Figure JNK signalling). The JNK pathway can also be activated through G protein-coupled receptors (GPCRs) using G proteins such as G12/13 and Gγq/11.

This JNK pathway contributes to the control of a large number of cellular processes:

- Phosphorylation of the transcription factor p53.
- The JNK pathway has been implicated in the mitogen-activated protein kinase (MAPK) signalling in cardiac hypertrophy.

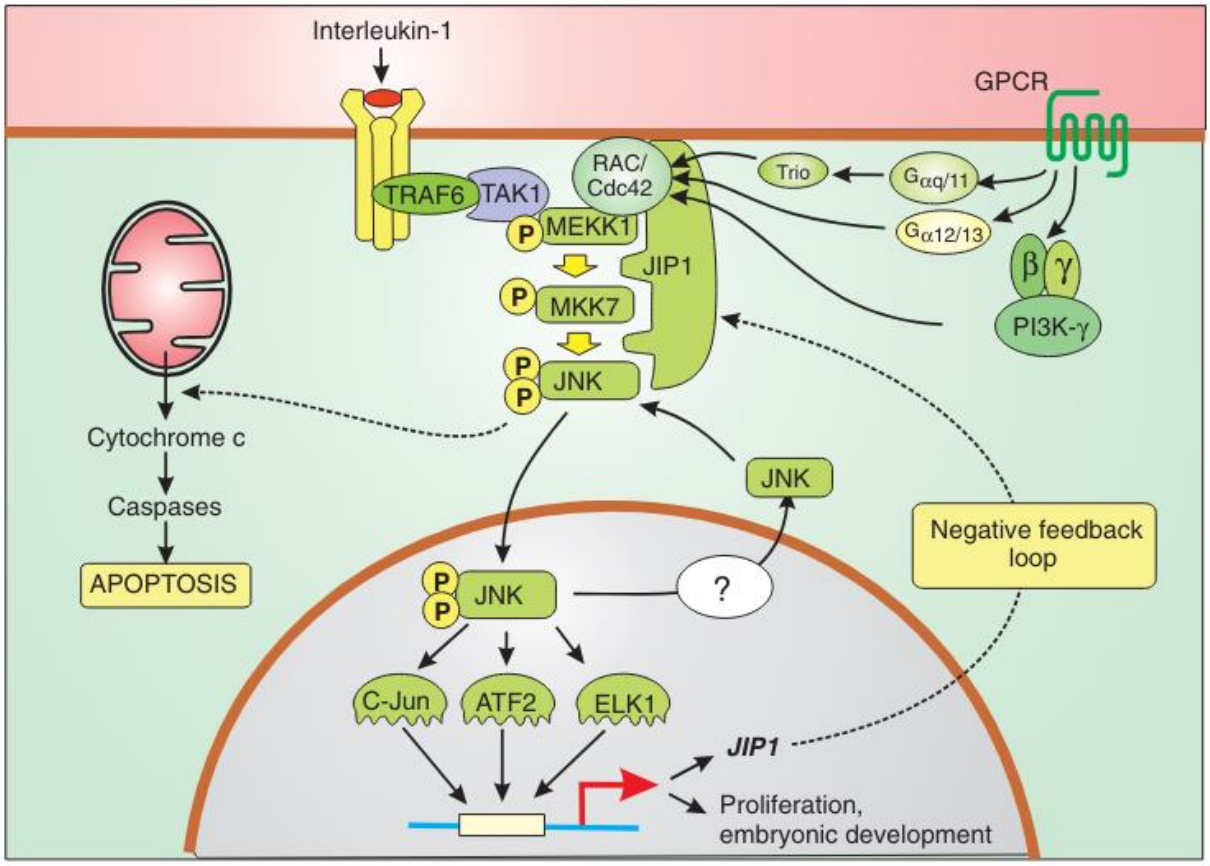

Fig 3: The c-Jun N-terminal kinase (JNK) pathway: The c-Jun N-terminal kinase (JNK) pathway can be activated in many ways, including via different receptor mechanisms and by various environmental stresses such as osmotic, redox and radiation stress. These

different inputs are transduced by separate mechanisms that all feed into the JNK signalling cascade. With regard to receptor activation, the JNK pathway can be activated by various cytokines such as interleukin-1 as illustrated here. The interleukin-1 receptor (IL-1R) is composed of two receptor-binding domains that interact with interleukin-1 and a non-binding accessory protein. Once activated by interleukin-1, the IL-1R recruits the adaptor protein tumour-necrosis-factor-receptor-associated factor 6 (TRAF6), which then recruits the mitogen-activated protein kinase kinase kinase (MAPKKK) called transforming growth factor β-activated kinase 1 (TAK1) responsible for initiating the phosphorylation cascade by phosphorylating MAPK/extracellular-signal-regulated kinase (ERK) kinase kinase 1 (MEKK1). The MEKK1 then phosphorylates the dual-specificity protein kinase MAPK kinase 7 (MKK7) responsible for phosphorylating the tyrosine and threonine residues on JNK. This activation cascade occurs in the vicinity of the plasma membrane, where it is organized by the scaffolding protein JNK-interacting protein 1 (JIP1). Once JNK is phosphorylated, it leaves the multimolecular activation complex and then diffuses into the nucleus, where it activates transcription factors responsible for controlling processes such as proliferation, apoptosis and embryonic development

III. p38 pathway

The p38 pathway is one of the major signalling cassettes of the mitogen-activated protein kinase (MAPK) signalling pathway. It functions in the control of apoptosis and the release of cytokines by macrophages and neutrophils. The pathway takes its name from the family of p38 kinases, which are the MAPKs that interact with the final effectors (Fig 1). The p38 pathway can be activated either by different receptor mechanisms or by various environmental stresses such as osmotic, redox or radiation stress. For example, one of the targets of the p38 pathway that is activated by UV irradiation is one of the Cdc25 enzymes that control cell cycle progression. Phosphorylation of Ser-323 on Cdc25B results in the binding of 14-3-3 protein, which then prevents this enzyme from initiating entry into mitosis.

Probable questions:

1. What is mitogen-activated protein kinase enzymes?
2. What is the mechanism of the MAPK pathway?
3. What is the function of mitogen-activated protein kinase MAPK?
4. Describe c-Jun N-terminal kinase (JNK) pathway.

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1. Albert Bruce, Bray Dennis, LevisJulian, Raff Martin, Roberts Keith and Watson James (2008). Molecular Biology of the Cell, V Edition, Garland publishing Inc., New York and London.
2. Cooper, G.M. and Hausman, R.E. (2009). The Cell: AMolecularApproach.5th Edition. ASM Press and Sunderland, Washington, D.C.; Sinauer Associates, MA.
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7. Cell Signalling Biology Michael J. Berridge Module 2 Cell Signalling Pathways

Unit V

Intracellular Signaling and Cell surface receptor Signaling: JAK- STAT Signaling pathway, TGFβ Signaling pathway

Objective: In this unit we will discuss in details about JAK- STAT Signaling pathway, TGFβ Signaling pathway.

Introduction

The Janus kinase/signal transducers and activators of transcription (JAK/STAT) pathway is a chain of interactions between proteins in a cell.

JAK- STAT Signaling pathway

- **JAK**

Many tyrosine kinases are cell membrane receptors, collectively referred to as RTKs, whereas JAKs are a class of non-transmembrane tyrosine kinases that have only catalytic domains but no Src homology 2 (SH2) domain. JAK is an acronym for Janus kinase. In Roman mythology, Janus is the god in charge of the beginning and the end. It is called a Janus kinase because JAK phosphorylates both the cytokine receptors and multiple signaling molecules containing SH2 domain.

The JAK protein family consists of four members: JAK1, JAK2, JAK3 and Tyk2, which have seven JAK homology (JH) domains. The JH1 domain is a kinase domain and the JH2 domain is a pseudo-kinase domain, JH6 and JH7 are receptor binding regions (Figure 2). For example, in JAK3, JH1 is the C-terminal tyrosine kinase domain. Adjacent to the kinase domain is the JH2, or pseudo-kinase domain, which itself lacks catalytic activity but is essential for regulating normal kinase activity. The amino terminus of the JAKs (JH5–JH7) has homology to the band four-point-one, ezrin, radixin, moesin (FERM)-domain containing proteins. This region of the JAKs mediates binding to cytokine receptors and also regulates catalytic activity. Mutations have been identified in all of these domains; most have considerable effects on the protein expression of JAKs, but some missense mutations or small in-frame deletions allow for some protein expression. These mutations affect kinase activity, receptor binding and intracellular transport.

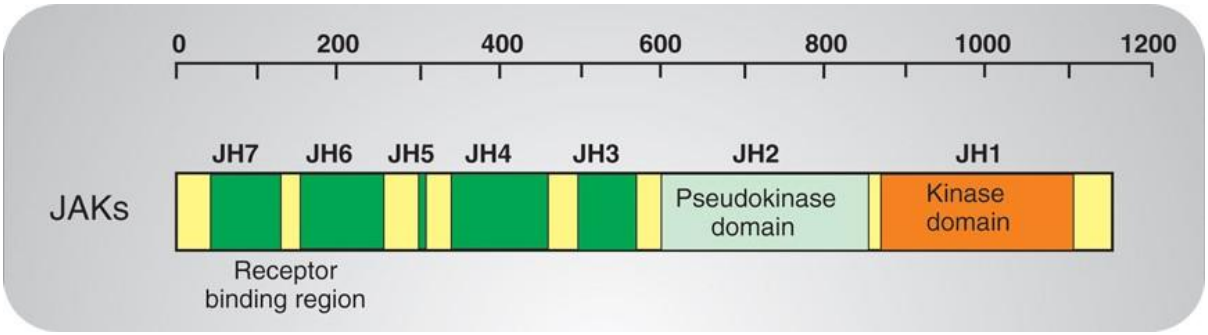

Figure 2. Structure of JAKs

- **STAT**

STAT is called "signal transducer and activator of transcription." As its name implies, STATs play a key role in signal transduction and transcriptional activation. Since the first STAT protein STAT1 was purified in 1991, other members of the STAT protein family have also been cloned. Currently, six members of the STAT family have been found, STAT1, STAT2, STAT3, STAT4, STAT5, and STAT6. STAT proteins are structurally divided into the following functional segments: N-terminal conserved sequence, DNA binding domain, SH3 domain, SH2 domain and C-terminal transcriptional activation domain (Figure 3). Among them, the most conserved and functionally most significant sequence is the SH2 domain, which has the exact same core sequence "GTFLLRFSS" as the SH2 domain.

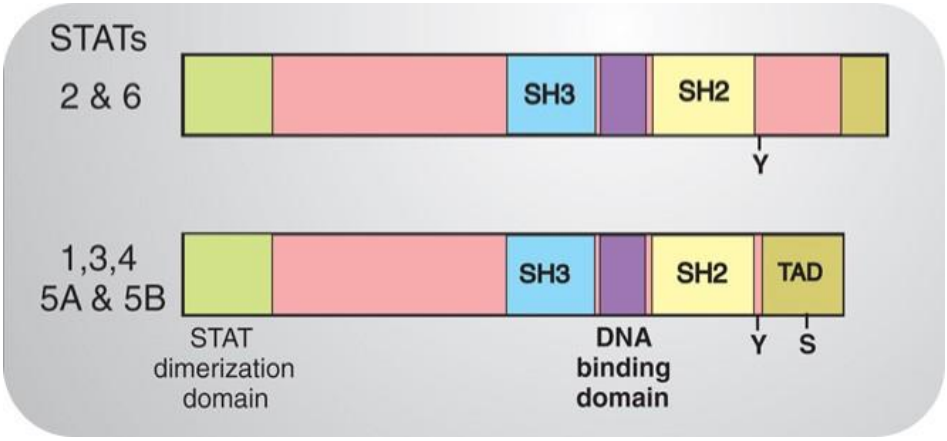

Figure 3. Structure of STATs

- **JAK-STAT signaling**

Compared with other signaling, JAK-STAT signal pathway is relatively simple. The signaling process is as follows: The binding of cytokines to the corresponding receptors results in the dimerization of the receptor, which brings the JAKs that are coupled to the receptors close to each other and are activated by interacting tyrosine

phosphorylation. The tyrosine residues on the catalytic receptor undergo the phosphorylation modification after JAK activation. Then, these phosphorylated tyrosine sites form a "docking site" with the surrounding amino acid sequence. At the same time, the STAT protein containing SH2 domain is recruited to this "docking site". Finally, JAK catalyzes the phosphorylation of the STAT that binds to the receptor. The activated STAT protein enters the nucleus as a dimer and binds to the target gene, regulating gene transcription. It is worth mentioning that a JAK can participate in the signal transduction process of a variety of cytokines. One cytokine signaling pathway can also activate multiple JAKs, but cytokines have a certain choice of activated STATs. For example, IL-4 activates STAT6, whereas IL-12 specifically activates STAT4.

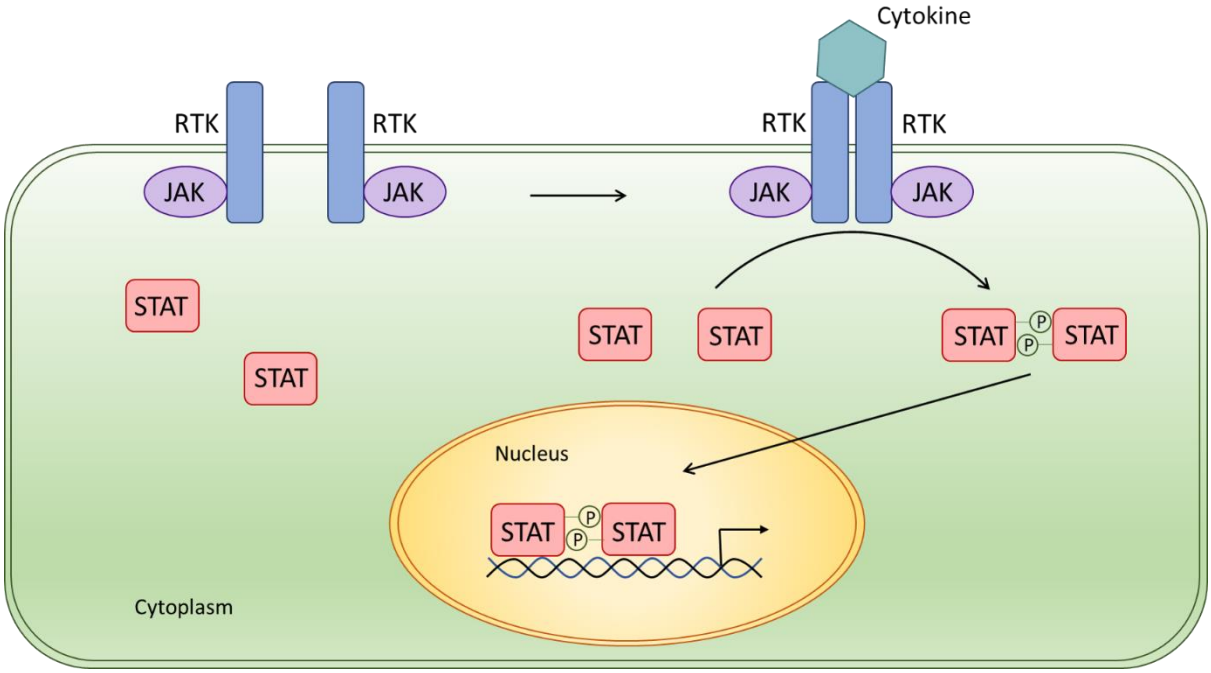

Figure 4. Schematic JAK-STAT signal pathway.

- **The Function of JAK-STAT Signaling Pathway**

The JAK-STAT signaling pathway transmits information from extracellular chemical signals to the nucleus resulting in DNA transcription and expression of genes involved in immunity, proliferation, differentiation, apoptosis and oncogenesis. This pathway plays a prominent role in mediating signal transduction for many cytokines.

- **Diseases and drug targets**

JAK-STAT signal pathway mediates the growth, survival and apoptosis of cardiomyocytes and participates in the regulation of angiogenesis, playing an important role in the pathogenesis of heart diseases. Myocardial hypertrophy caused by stress load, heart failure, myocardial protection induced by ischemic preconditioning, and cardiac dysfunction caused by ischemia-reperfusion are all closely related to this

signaling. In recent years, studies have shown that JAK-STAT signal pathway also plays an important role in myocardial protection mechanism induced by ischemic preconditioning. Myocardial ischemic preconditioning rapidly induced phosphorylation of JAK2 and STAT3, but activation of JAK2 and STAT3 was still detected after preconditioning for 30 min and reperfusion for 2 h.

STAT is involved in the regulation of cell growth, differentiation and apoptosis. In normal physiological conditions, the activation of STATs is transient and tightly regulated. However, the persistent activation of STATs is found in many human tumors. The abnormal activation of STAT3 is found in almost all head and neck tumors. STAT3 also continues to be activated in various myelomas. Leukemia is a malignant disease of hematopoietic tissue, also known as "blood cancer." In leukemic cells, JAK-STAT signaling pathway is constantly activated, therefore, STAT protein has become one of the key detection indicators. For example, STAT1, STAT3 and STAT5 are the most common and persistent activators of leukemia. Different types of leukemia cells may manifest abnormal activation of one or more STAT proteins. For example, lymphocytic leukemia and monocystic leukemia cells are commonly activated by STAT5, while myeloid leukemia cells are activated continuously by STAT3.

Altogether, these findings suggest that targeting the aberrant JAK/STAT signaling in carcinoma may hold great potential as a novel therapeutic intervention for the treatment of patients with cancer; and that the drugs/inhibitors of JAK/STAT cascade currently in clinical trials for solid tumors and hematopoietic malignancies (Figure 5) should also be tested for their efficacy as therapeutics in carcinoma.

TGF-beta (TGF- β) signaling pathway

Transforming growth factor beta (TGF-beta) signaling pathway is a series of signal transduction processes mediated by transforming growth factor. TGF-β signaling pathway plays a key role in cell proliferation, interstitial production, differentiation, apoptosis, embryonic development, organ formation, immune function and inflammatory response.

- **TGF-beta superfamily**

Transforming growth factor β (TGF-β) superfamily is composed of many genes which coding secretory protein, mainly includes TGF-βs, the growth and differentiation factor (GDF) subfamily, an extensive bone morphogenetic protein (BMP) subfamily, activin, inhibin, anti-muller hormone, follistatin (FS), mullerian inhibitor substance (MIS), etc, as well as several additional members such as myostatin (Mstn) cloned from mouse skeletal muscle. More details>>

- **The process of TGF-β signaling pathway**

Transforming growth factor beta (TGF-β) is a cytokine that participates in both physiological and pathological processes. During tumor progression, TGF-β signaling

regulates immune/inflammatory response and tumor microenvironment. The TGF-β superfamily consists of at least 40 structurally and functionally related cytokines. TGF-β family proteins are classified into several subtypes, including TGF-βs, activins/inhibins, and bone morphogenetic proteins (BMPs)/growth differentiation factors according to structural characteristics. TGF-β signals through TGF-β receptors (TGF-βRs, TβRs) I and II to activate downstream signaling pathways. The TGF-βRs are single-pass transmembrane proteins with serine/threonine kinase activity. In the absence of ligand, TGF-βRI and TGF-βRII exist as monomers, homodimers, or heterodimers on the cell surface. Ligand binding promotes formation of a tetrameric complex between TGF-βRII dimers and two TGF-βRIs. TGF-βRIs and TGF-βRIIs have N-terminal extracellular ligand binding domains, transmembrane segments, and C-terminal cytosolicserine/threonine kinase domains. TGF-β binds specifically to the constitutively active TGF-βRII, which activates TGF-βRI by phosphorylating the glycine/serine-rich domain. Activated TGF-βRI then phosphorylates downstream effectors to induce signal transduction. TGF-βR activity is regulated by beta glycan, a type III TGF-βR, and endoglin.

Canonical TGF-β signaling is dependent upon Smad family proteins. Active TGF-βI at the cell surface phosphorylates receptor-activated Smads (R-Smads). There are two sub-classes of R-Smads in which Smad2 and Smad3 mediate the TGF-β/activin pathway. Smad4 acts as a co-factor that binds to activated R-Smads to form a complex that translocates to the nucleus and regulates transcription. TβRs can also activate non-Smad-dependent signaling pathways, including the mitogen-activated protein kinase (MAPK) pathway mediated by p38, c-Jun amino terminal kinase (JNK), extracellular signal-regulated kinases (ERK), nuclear factor-κB (NF-κB), Rho, and phosphatidylinositol 3-kinase (PI3K)-Akt. TGF-β pathway is a very complex signaling network, and its role in cellular homeostasis varies with different genetic profiles of cancer cells. On the one hand, TGF-β signaling can induce cell differentiation and act as a tumor suppressor in non-malignant tumor, on the other hand, it is also capable of promoting angiogenesis and EMT in cancer cells. Once signal transduction is initiated, TGF-β is involved in cell differentiation and can also act as a tumor suppressor in cell cycle control. Furthermore, TGF-β induces p53-independent expression of p21 and inhibits oncogene expression, while TGF-β receptors also cross-talk with oncogenic signaling pathways. TGF-β pathway has dual anti- and pro-tumoral roles at the cancer cell level, depending on tumor stage and genetic alteration background, with mechanistic differences between cancer models.

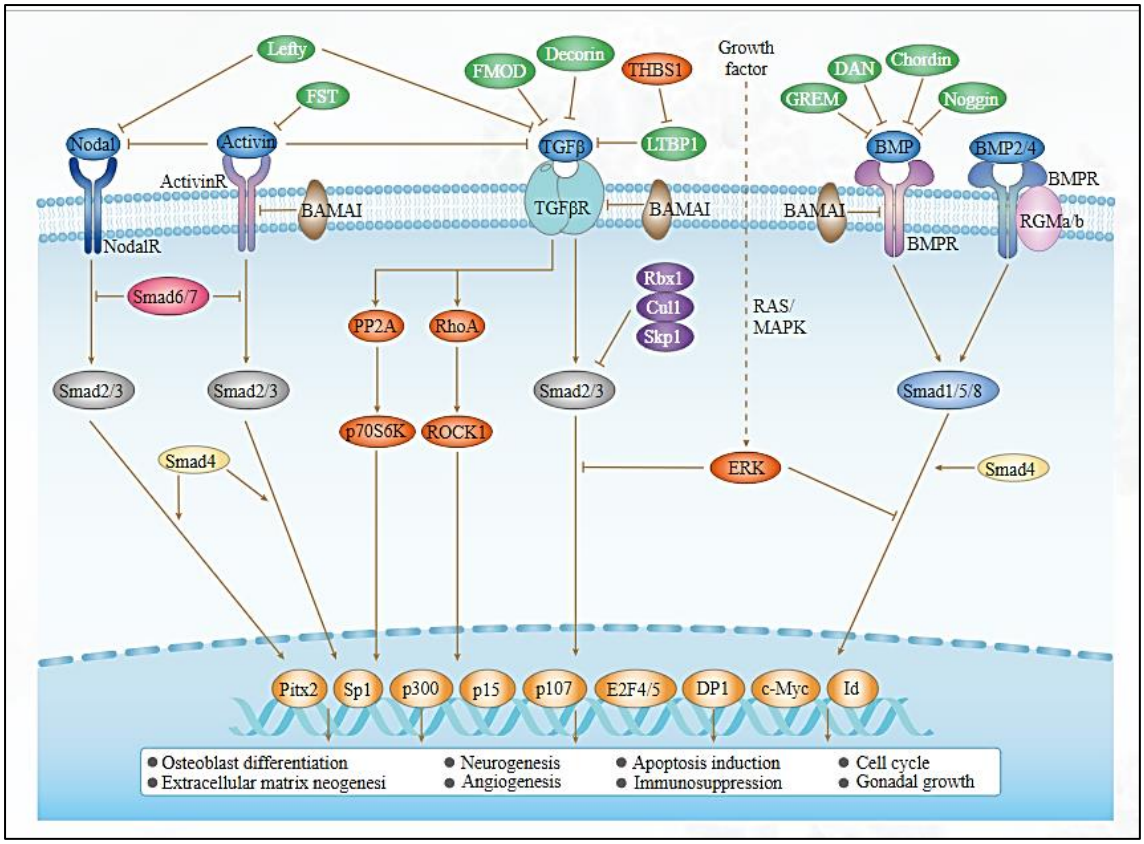

Fig: TGF-β signaling pathway

Probable questions:

1. What is the JAK and stat signaling pathway?
2. What is the significance of the JAK-STAT Signalling pathways?
3. Describe the steps of JAK-STAT Signalling pathways.
4. State the relation between JAK-STAT Signalling pathways with diseases and drug targets.
5. Mention the steps of TGF-β signaling pathway.

Suggested readings:

1. Albert Bruce, Bray Dennis, LevisJulian, Raff Martin, Roberts Keith and Watson James (2008). Molecular Biology of the Cell, V Edition, Garland publishing Inc., New York and London.
2. Cooper, G.M. and Hausman, R.E. (2009). The Cell: AMolecularApproach.5th Edition. ASM Press and Sunderland, Washington, D.C.; Sinauer Associates, MA.
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Unit VI

Intracellular receptors, steroid hormone signaling pathways

Objective: In this unit we will discuss about different types of Intracellular receptors and steroid hormone signaling pathways.

Internal receptors

Internal receptors, also known as intracellular or cytoplasmic receptors, are found in the cytoplasm of the cell and respond to hydrophobic ligand molecules that are able to travel across the plasma membrane (Fig 1). Once inside the cell, many of these molecules bind to proteins that act as regulators of mRNA synthesis to mediate gene expression. Gene expression is the cellular process of transforming the information in a cell's DNA into a sequence of amino acids that ultimately forms a protein. When the ligand binds to the internal receptor, a conformational change exposes a DNA-binding site on the protein. The ligand-receptor complex moves into the nucleus, binds to specific regulatory regions of the chromosomal DNA, and promotes the initiation of transcription. Internal receptors can directly influence gene expression without having to pass the signal on to other receptors or messengers.

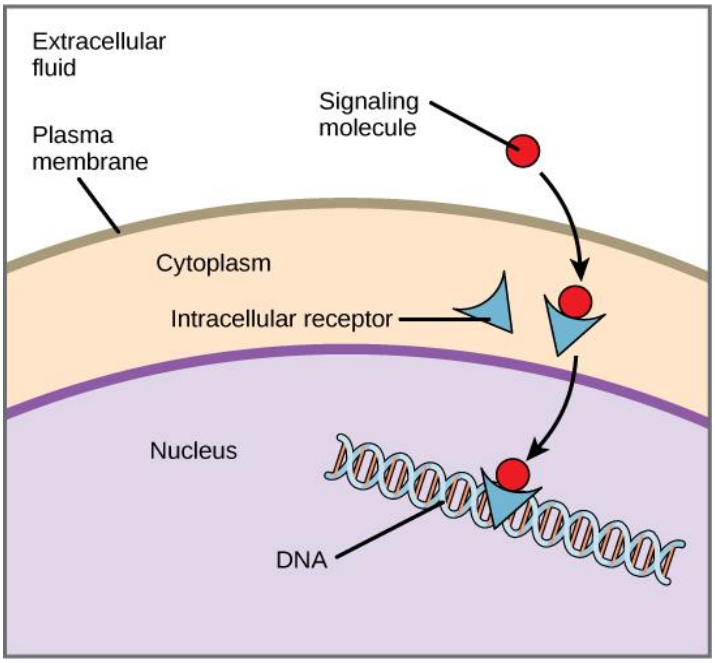

Fig 1: Intracellular Receptors: Hydrophobic signalling molecules typically diffuse across the plasma membrane and interact with intracellular receptors in the cytoplasm. Many intracellular receptors are transcription factors that interact with DNA in the nucleus and regulate gene expression.

- **What is steroid?**

Steroids are a man-made version of chemicals, known as hormones, that are made naturally in the human body. Steroids are designed to act like these hormones to reduce inflammation.

They're also known as corticosteroids, and are different to anabolic steroids used by bodybuilders and athletes.

Steroids won't cure your condition, but they're very good at reducing inflammation and will ease symptoms such as swelling, pain and stiffness.

Usually, inflammation is the body's natural reaction to infection or bacteria. Your immune system produces extra fluid to fight infections or bacteria, which causes swelling, redness and heat in the affected area. You might have noticed this if you have had a cut or wound on your skin.

In some conditions, such as rheumatoid arthritis, the immune system produces inflammation in the joints or other parts of the body by mistake, which can cause permanent damage if left untreated. Steroids can be used to reduce this immune reaction.

- **Steroid hormone and its types**

Steroid hormone, any of a group of hormones that belong to the class of chemical compounds known as steroids; they are secreted by three "steroid glands"—the adrenal cortex, testes, and ovaries—and during pregnancy by the placenta. All steroid hormones are derived from cholesterol. They are transported through the bloodstream to the cells of various target organs where they carry out the regulation of a wide range of physiological functions.

These hormones often are classified according to the organs that synthesize them: the adrenal steroids are so called because they are secreted by the adrenal cortex, and the sex hormones are those produced by the ovaries and testes. This distinction is not exclusive, however, because the adrenal cortex also secretes sex hormones, albeit to a lesser extent than do the gonads, and the ovaries under abnormal conditions may produce adrenal steroids. The adrenal cortex produces the adrenocortical hormones, which consist of the glucocorticoids and the mineralocorticoids. Glucocorticoids such as cortisol control or influence many metabolic processes, including the formation of glucose from amino acids and fatty acids and the deposition of glycogen in the liver. Glucocorticoids also help to maintain normal blood pressure, and their anti-inflammatory and immunosuppressive actions have rendered them useful in treating rheumatoid arthritis and preventing the rejection of transplanted organs. Mineralocorticoids such as aldosterone help maintain the balance between water and salts in the body, predominantly exerting their effects within the kidney.

The androgens are the male sex hormones. The principal androgen, testosterone, is produced primarily by the testes and in lesser amounts by the adrenal cortex and (in

women) by the ovaries. Androgens are primarily responsible for the development and maintenance of reproductive function and stimulation of the secondary sex characteristics in the male. Androgens also have an anabolic (synthesizing and constructive, rather than degradative) function in stimulating the production of skeletal muscles and bone as well as red blood cells. To enhance the anabolic activity of androgens without increasing their masculinizing ability, anabolic steroids were developed. Though originally intended to combat diseases marked by wasting, these synthetic hormones have been abused by individuals desiring to increase their muscle mass, such as athletes seeking to gain a competitive advantage. Overdosing has been linked to serious side effects, including infertility and coronary heart disease.

Estrogens are one of the two types of female sex hormones. They are secreted mainly by the ovaries and in smaller amounts by the adrenal glands and (in men) by the testes. Estradiol is the most potent of the estrogens. Functioning similarly to androgens, the estrogens promote the development of the primary and secondary female sex characteristics; they also stimulate linear growth and skeletal maturation. In other mammals these hormones have been shown to precipitate estrus (heat). The ovarian production of estrogen plummets during menopause.

Progestins, the most important of which is progesterone, are the other type of female sex hormone and are named for their role in maintaining pregnancy (pro-gestation).

Steroid hormone signaling pathways

The steroid hormone cell signaling functions in transcriptional activation and gene expression. The steroid hormone signaling pathway may be activated by steroid hormones, such as estrogen and progesterone, which bind to a steroid binding protein (SBP) (Fig 2).

Steroid hormones (e.g., estrogen, androgen, progesterone) travel through the bloodstream from an endocrine gland bound by a steroid binding protein (SBP). The steroid is released from the SBP and is transported across the extracellular membrane and into the cell where is binds a nuclear receptor (e.g., ESR1, AR, PGR). The steroid also binds a heat shock protein (HSP90), a chaperone protein that aids in protein folding. HSP90 dissociates, and the steroid and nuclear receptor cross the nuclear membrane. The steroid and nuclear receptor homodimerize and bind to the hormone response element (HRE) within the promoter of a gene, which activates gene transcription and promotes cell growth and survival. Specific nodes in the pathway that are therapeutically actionable are noted.

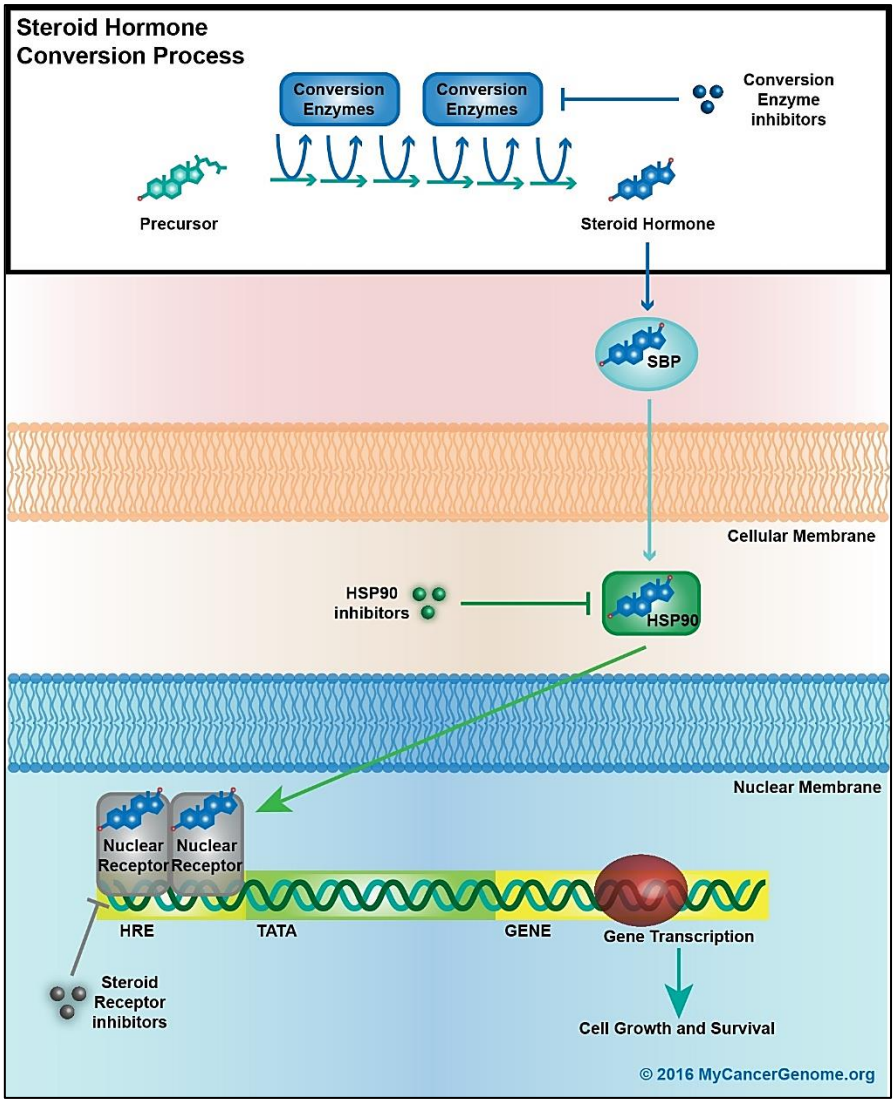

Figure 2. Steroid hormone signaling pathways

Probable questions:

1. What is steroid?
2. What is steroid hormone?
3. Name two steroid hormones and mention their function.
4. What are the steps required for a steroid hormone signaling pathway?

Suggested readings:

1. Cooper, G.M. and Hausman, R.E. (2009). The Cell: AMolecularApproach.5th Edition. ASM Press and Sunderland, Washington, D.C.; Sinauer Associates, MA.
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Unit VII

Characteristics of tumor cells; detection of tumor using CAT scan, MRI scan and fMRI scan

Objective: In this unit we will learn characteristics of tumor cells; detection of tumor using CAT scan, MRI scan and fMRI scan.

Introduction

In multicellular organisms, cell division is a normal process. Cells divide for growth, for the development of organs, for healing of wounds and also for the replacement of older and damaged cells. Cell division is a very complex process which is controlled by a regulatory mechanism at both molecular and cellular level. In higher multicellular organism, each and every cell belongs to a particular type of tissue like epithelial tissue, connective tissue muscular tissue etc. Hence, when a cell of a specific tissue divides, it normally produces its own kinds of cell of the tissue to which it belongs. It never produces the cells of other tissues. Therefore, the process by which cells achieve this specification and specialisation is known as cellular differentiation. Differentiation of cell begins during embryonic gastrulation stage and continues through tissue formation. Actually, differentiation has a genetic basis and the process results from the interaction of the nucleus and the cytoplasm. After the cells become well differentiated, they cannot go back normally to the undifferentiated stage unless disturbed internally or externally.

Therefore, in multicellular organism, the cell division, differentiation and survival of individual cells are carefully regulated to meet the needs of the organism as a whole. When this regulation is lost due to any reason, the cells behave unusually and defy their control mechanism. Then the cells grow and divide in an uncontrolled manner ultimately spreading throughout the body and interfering with the functions of normal tissues and organs. As a whole, this condition leads to cancer. Cancer develops from defects in fundamental regulatory mechanisms of the cell.

Cancer is a non-infectious disease. It starts at the molecular level of the cell and, ultimately affects the cellular behaviour. Generally, it can be defined as uncontrolled proliferation of cells without any differentiation. Cancer cells are different from normal cells in some aspects. They do not remain confined to one part of the body.They penetrate and infiltrate into the adjoining tissues and dislocate their functions. Some of the cancer cells get detached from the main site of origin and travel by blood and lymph to sites distant from the original tumour and form fresh colonies, called metastasis or secondary growth.

- ## Characteristics of tumour cells

All types of cancer can result from uncontrolled cell growth and division of any of the different kinds of cells in the body. The uncontrolled cell growth produces a mass of cells which are called tumours or neoplasm tumours may be benign or malignant.

Types of Tumours

There are two types of tumours: *benign* and *malignant*.

(i) Benign Tumour (= Non-malignant Tumour):

A benign tumour remains confined to its original location. They do not invade the surrounding normal tissues. They do not spread to distant body sites. It causes limited damage to the body. It is non-cancerous.

The most common example of tumour is the skin wart. A benign tumour consists of closely resembles normal cells and may function like normal cells. Generally benign tumours are harmless and can usually be removed surgically. However, these tumours may sometimes become quite harmful if they are located in organs like brain and liver.

(ii) Malignant Tumour (= Cancerous Tumour):

A malignant tumour does not remain confined to its original location.It first grows slowly. No symptoms are noticed. This stage is called the latent stage. The tumour later grows quickly. The cancer cells go beyond adjacent tissue and enter the blood and lymph. Once this happens, they migrate too many other sites in the body where the cancer cells continue to divide. A phenomenon in which cancer cells spread to distant sites through body fluids to develop secondary tumour is called metastasis. Only malignant tumours are properly designated as cancer.

- ## Properties of Cancer Cells

i) Uncontrolled proliferative ability,

ii) Extracellular growth factors are not required,

iii) Overgrowth and ability to invade new sites (metastasis),

iv) Nucleus becomes irregular with abundant granules,

v) There is increase in number of lysosomes, reduction in mitochondrial cristae, more melanin and debris in cytoplasm,

vi) Cancer cells resist induction of cell death which promotes development of tumours.

- ## Characteristics of Cancer Cells/ Malignant tumour cells

The uncontrolled growth of cancer cells results from accumulated abnormalities affecting many of the cell regulatory mechanisms. The process of cell changes in which a normal cell loses its ability to control its rate of division and thus becomes a tumour cell is called cell transformation.

Cancer cells shows some typical characteristic properties that are absent in normal cells. Sometimes cancer cell properties are just opposite to the properties of normal cell. Cancer cells in vivo differ from their normal counterparts in several respects. Some characteristic properties of cancer cells can also be demonstrated by cell culture in vitro.

(i) Immortalization:

Normal cell culture does not survive indefinitely for example, human cell culture die after about 50 generations. On the other hand, transformed cell cultures can go on indefinitely and remain immortal if the nutrition is provided and overcrowding avoided.

(ii) Loss of Contact Inhibition:

Normal cells growing in tissue culture tend to make cell contacts by adhesion to neighbouring cells. At the points of adhesion some kind of electron-dense plaque is formed in both contacting cells. At the same time there is a slowing down of the amoeboid process which results in contact inhibition of movement. In contrast, cancer cells are unable to form adhesive junctions and do not show this type of contact inhibition.

Experimentally, it has been observed that when normal cells have become completely surrounded by other cells, their mobility stops and they form a monolayer. At the same time there is inhibition of growth and the number of cells in the petridish remains practically constant. On the other hand, cancer cells continue to multiply and pile up forming irregular masses several layers deep. Cancerous cells undergo a change in property of their cell membranes and cell coat such as disappearance of gap junction, loss of coupling changes in glycolipid and glycoprotein and a reduction in gangliosides.

In the cell coat fibronectin, a large glycoprotein found in footprints of moving cultured cells is reduced in cancerous cells. These changes enable the cells to dissociate from neighbouring cells and show loss of contact inhibition.

(iii) Reduced Cellular Adhesion:

Most cancer cells are less adhesive than the normal cells due to reduced expression of cell surface adhesive molecules. When normal cells are transformed into cancer cells, then a change of stickiness of their cell membrane results. Normal cells show stickiness or adhesiveness.

If normal cells are grown in a liquid nutrient medium kept in a glass vessel, the cells stick to glass wall rather than float in the medium. But when cancer cells are allowed to grow in nutrient medium, they stick to each other less than do normal cells.

Adhesiveness shows considerable specificity. For example, a liver cell tends to stick with another liver cell and not to other types of cell such as kidney cell. Cancerous cells do not show this property. They are able to mix and stick to any type of normal cell. For example, a malignant liver cell can mix and stick to normal kidney cell. Hence this unusual behaviour of cancer cell explains that cancer cells can invade several normal organs.

(iv) Invasiveness:

One of the most important characteristics of cancer cells is their invasiveness. It is the ability to invade other tissues. Malignant cells generally secrete proteases that digest extracellular matrix components, allowing the cancer cells to invade adjacent normal tissues. For example, secretion of collagenase by the cancer cells helps to digest and penetrate through basal laminae to invade the underlying connective tissue. Cancer cells also secrete growth factors that promote the formation of new blood vessels. This is known as angiogenesis. Angiogenesis is necessary to support the growth of tumour beyond the size of about a million cells at which point new blood vessels are needed to supply oxygen and nutrients to the multiplying tumour cells.Actually the growth factor secreted by the tumour cells stimulates the endothelial cells present in the wall of capillaries.

As a result, new outgrowth of the capillaries is formed into the tumour. These outgrowths of capillaries are also helpful for metastasis of malignant cells. Therefore, angiogenic stimulation induces the growth of new blood capillaries which penetrate easily in the tumour tissue and provide the opportunity for the cancer cells to enter the circulatory system. As a result, metastasis process begins.

(v) Failure to Differentiate:

Another general characteristic of most of the cancer cells is that they fail to differentiate. This property is closely related with the abnormal proliferation. Normal cells are fully differentiated. In most fully differentiated cells, cell division ceases. In case of cancer-cells, normal differentiation program is blocked at the early stages of differentiation. The relationship between defective differentiation and rapid proliferation is clearly noted in case of leukaemia.

All of the different types of blood cells develop from a common pluripotent stem cell in the bone marrow. Some of the descended cells develop erythrocytes but others differentiate to form lymphocytes, granulocytes and macrophages. Cells of each of these types become round as they differentiate but once they become fully differentiated cell division ceases But leukaemia cells fail to undergo terminal differentiation. Instead, they become blocked at early stage of maturation at which they retain their capacity for proliferation and continue to divide.

(vi) Auto stimulation of Cell Division:

Cancer cells produce growth factor that stimulates their own cell division. Such abnormal production of a growth factor by the cancer cell leads to continuous auto stimulation of cell division. This is known as autocrine growth stimulation. Hence the cancer cells are

less dependent on general growth factor produced within the body physiologically from normal source for inducing growth of all normal cells. It is also noted that the reduced growth factor dependence of cancer cell results from abnormalities in intracellular signalling system.

(vii) Apoptosis:

For every cell, there is a fixed span of life, i.e., time to live and time to die. This cell death is a very orderly process and so it is called Programmed Cell Death or PCD or Apoptosis. Apoptosis is a mechanism of programmed cell death or cell suicide which is essential for the survival of the organism, for the normal development of the organism as the programmed destruction of the organism as the programmed destruction of cells is found during embryo-genesis. It also protects the organism by removing damaged cells which may be due to viral infection or due to exposure to radiations. It also inhibits the tumour development and so any defect in the control of apoptosis may lead to cancer.

- **Difference between cancer cells and normal cells**

	Normal Cell	***Cancer Cell***
Cell shape	Uniform	Irregular
Nucleus	Spheroid shape, single nucleus	Irregular shape, multi-nucleation common
Chromatin	Fine, evenly distributed	Coarse, aggregated
Nucleolus	Single, inconspicuous nucleolus	Multiple, enlarged nucleoli
Cytoplasm	Large cytoplasmic volume	Small cytoplasmic volume
Growth	Controlled	Uncontrolled
Maturation	Mature into specialized cells	Remain immature and undifferentiated
Blood supply	Normal angiogenesis (occurs during development/ healing)	Tumour-induced angiogenesis
Oxygen	Favoured (for aerobic respiration) but will undergo anaerobic respiration if required	Not required (thrive in hypoxic conditions), favour anaerobic respiration
Location	Remain in their intended location	Can spread to different locations in the body (metastasis)

Detection of tumor using CAT scan, MRI scan and fMRI scan

- **Computed tomography**

Computed tomography (CT) is a noninvasive imaging procedure that uses special x-ray equipment to create detailed pictures, or scans, of areas inside the body.

Each picture created during a CT procedure shows the organs, bones, and other tissues in a thin "slice" of the body. The entire series of pictures produced in CT is like a loaf of sliced bread—you can look at each slice individually (2-dimensional pictures), or you can look at the whole loaf (a 3-dimensional picture). Computer programs are used to create both types of pictures.

Modern CT machines take continuous pictures in a helical (or spiral) fashion rather than taking a series of pictures of individual slices of the body, as the original CT machines did. Helical CT (also called spiral CT) has several advantages over older CT techniques: it is faster and produces better quality 3-D pictures of areas inside the body, which may improve detection of small abnormalities.

CT has many uses in the diagnosis, treatment, and monitoring of cancer, including

i. screening for cancer
ii. helping diagnose the presence of a tumor
iii. providing information about the stage of a cancer
iv. determining exactly where to perform (i.e., guide) a biopsy procedure
v. guiding certain local treatments, such as cryotherapy, radiofrequency ablation, and the implantation of radioactive seeds for brachytherapy
vi. helping plan external-beam radiation therapy or surgery
vii. determining whether a cancer is responding to treatment
viii. detecting recurrence of a tumor

In addition, CT is widely used to help diagnose circulatory (blood) system diseases and conditions, such as coronary artery disease (atherosclerosis), blood vessel aneurysms, and blood clots; spinal conditions; kidney and bladder stones; abscesses; inflammatory diseases, such as ulcerative colitis and sinusitis; and injuries to the head, skeletal system, and internal organs. CT imaging is also used to detect trauma, brain bleeds, and abnormal brain function or deposits in adult patients with cognitive impairment who are being evaluated for Alzheimer's disease and other causes of cognitive decline.

What can a person expect during a CT procedure?

During a CT procedure, the person lies very still on a table, and the table passes slowly through the center of a large donut-shaped x-ray machine. The person might hear whirring sounds during the procedure. At times during a CT procedure, the person may be asked to hold their breath to prevent blurring of the images.

Sometimes, people having CT are given a contrast (imaging) agent, or dye, before the procedure. The contrast dye highlights specific areas inside the body, resulting in clearer pictures. The dye may be given by mouth, injected into a vein, or given by enema. Iodine and barium are two dyes commonly used in CT.

In very rare cases, the contrast agents used in CT can cause allergic reactions. Some people experience mild itching or hives (small bumps on the skin). Symptoms of a more serious allergic reaction include shortness of breath and swelling of the throat or other parts of the body. People should tell the technologist immediately if they experience any of these symptoms so they can be treated promptly. Very rarely, the contrast agents used in CT can cause kidney problems for certain patients, such as those with impaired kidney function. Kidney function can be checked with a simple blood test before the contrast agent is injected.

CT is a noninvasive procedure and does not cause any pain. However, lying in one position during the procedure may be slightly uncomfortable. The length of a CT procedure depends on the size of the area being scanned, but it usually lasts only a few minutes to half an hour. For most people, CT is performed on an outpatient basis at a hospital or a radiology center, without an overnight hospital stay.

Some people are concerned about experiencing claustrophobia during a CT procedure. However, most CT scanners surround only portions of the body, not the whole body. Therefore, people are not enclosed in a machine and are unlikely to feel claustrophobic.

People should let their health care provider and the technologist know if there is any possibility that they are pregnant. Depending on the part of the body to be scanned, the provider may reduce the radiation dose or use an alternative imaging method. However, the level of radiation exposure in a CT scan is believed to be too low to harm a growing fetus.

How does a CT scan work?

A CT scan takes pictures of the inside of the body using x-rays taken from many angles. A computer combines these pictures into a detailed, 3-dimensional image. This image will show abnormal areas and any tumors.

Some people receive a special dye called a contrast medium before the scan. When injected in your vein, the contrast dye travels through the bloodstream and helps create a clearer picture of specific parts of your body. It may also be given as a liquid to swallow, depending on what part of your body needs to be scanned.

Areas commonly scanned for cancer include the head, neck, chest, abdomen, pelvis, or limbs. A "total body" CT scan generally includes at least the chest, abdomen, and pelvis. Doctors often use this is for cancer staging.

The benefits of having a CT scan usually outweigh the risks. During a CT scan, you will be exposed to a small amount of radiation. This low dose of radiation has not been shown to cause harm. For children or for people who need multiple CT scans and x-rays, there may be a small potential increased risk of cancer in the future. In many cases, doctors will use low-dose CT scans for children or limit the area that needs to be scanned. If you are having multiple CT scans and x-rays, ask your doctor about tests that lessen your exposure to radiation.

➢ Magnetic Resonance Imaging (MRI)

An **MRI** produces detailed images of the inside of the body using magnetic fields, not x-rays. MRI can be used to measure the tumor's size. A special dye called a contrast medium is given before the scan to create a clearer picture. This dye can be injected into a patient's vein. MRIs create more detailed pictures than computed tomography (CT) scans are the preferred way to diagnose a brain tumor. The MRI may be of the brain, spinal cord, or both, depending on the type of tumor suspected and the likelihood that it will spread in the CNS. There are different types of MRI. The results of a neuro-examination, done by the internist or neurologist, helps determine which type of MRI to use.

Intravenous (IV) gadolinium-enhanced MRI is typically used to help create a clearer picture of a brain tumor. This is when a patient first has a regular MRI and afterwards is given a special type of contrast medium called gadolinium through an IV. Then, a second MRI is done to get another series of pictures using the dye.

An MRI technique called "diffusion weighted imaging" helps show the cellular structure of the brain. Another technique called "perfusion imaging" shows how much blood is reaching the tumor. These methods may help doctors predict how well treatment will work.

A spinal MRI may be used to diagnose a tumor on or near the spine.

A **functional MRI (fMRI)** provides information about the location of specific areas of the brain that are responsible for muscle movement and speech. During the fMRI examination, the patient is asked to do certain tasks that cause changes in the brain and can be seen on the fMRI image. This test is used to help plan surgery, so the surgeon can avoid damaging the functional parts of the brain while removing the tumor.

Magnetic resonance spectroscopy (MRS) is a test using an MRI that provides information on the chemical composition of the brain. It can help tell the difference between any dead tissue caused by previous radiation treatments and new tumor cells in the brain.

Why would a CT scan be used instead of an MRI?

In many cases, a brain tumor diagnosis begins with a neurological examination followed by a magnetic resonance imaging (MRI) scan of the head. Because an MRI produces high-quality images of soft tissues and blood vessels, it can be useful for diagnosing a brain

tumor. However, a CT scan can provide more detailed images of the bone structures near a brain tumor, such as the skull or spine. A CT scan may also be used to diagnose a brain tumor if the patient has a pacemaker and cannot have an MRI, which involves the use of powerful magnetic fields that can interfere with a pacemaker's function.

Probable questions:

1. Write down the differences between benign and malignant tumour.
2. Write down the characteristics of cancer cells.
3. What is MRI scan used for?
4. How does a CT scan work?
5. What is difference between MRI and CT scan?

Suggested readings:

1. Hardin, J. Bertoni, G and Klein smith, J. L. (2012). Becker's World of the Cell. 8th Edn, Pearson Benjamin Cummings, San Francisco.
2. Pal, A. (2011). Textbook of Cell and Molecular Biology 3rd Edn, Bokks and Allied, Kolkata.
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Unit VIII

Oncogenes and their proteins, classification and characteristics of chemical carcinogen

Objective: In this unit we will discuss about Oncogenes and their proteins, classification and characteristics of chemical carcinogen.

Oncogenes:

Oncogenes are genes capable of causing cancer. These were first recognised as unique genes of tumour-causing viruses that are responsible for the process of transformation (viral oncogenes).

There are two classes of oncogenes — one is viral oncogene present in viruses that causes the transformation of target cells. The counterpart of which stays within the host cell involved in normal cellular functions are called cellular oncogenes. The cellular sequences themselves are not oncogenic, are described as proto-oncogenes, whose capture by retrovirus and subsequent modification may create an oncogene.

Oncogenic potential of tumour virus resides in a single function or a group of related functions that are active early in the viral lytic cycle. Tumour viruses carry genes (v-one) which confer on them the ability to convert host cell into tumorigenic state. About 100 viral oncogenes have been identified so far.

When transformation occurs, the relevant genes are integrated into the genomes of transformed cells and expressed constitutively. Oncogenes of DNA tumour viruses do not have cellular counterparts. In case of non- defective RNA viruses, tumorigenicity does not rely upon an individual viral oncogene, but upon the ability of the virus to activate a cellular proto- oncogene.

Acute transforming retroviruses capture cellular oncogene (absent in ancestral virus) by means of a transduction event during an infective cycle. At least 25 c-onc genes have been identified by their representation in retroviruses. Viral infection is not really necessary for tumour formation as evidenced by transfection assay.

Oncogenes of Rous Sarcoma Virus:

(i) The genome of this retrovirus contains four genes named gag, pol, env, and src (Fig 1).

(ii) The gag gene codes for group-specific antigens of the virus, pol for the reverse transcriptase that characterizes retroviruses, and env for certain glycoproteins of the viral envelope. A protein-tyrosine kinase was shown to be the product of src (i.e., the sarcoma-causing gene) that is responsible for transformation.

(iii) Certain glycolytic enzymes become target proteins for the src protein-tyrosine kinase. This shows that transformed cells often show increased rates of glycolysis. The product of src may also catalyze phosphorylation of phosphatidylinositol-to-phosphatidylinositol mono- and bi-phosphate.

(iv) When phosphatidylinositol 4, 5-bi-phosphate is hydrolyzed by the action of phospholipase C, 2 second messengers are released: inositol triphosphate and diacylglycerol. The first compound mediates release of Ca^{++} from intracellular sites of storage (e.g., the endoplasmic reticulum).

(v) Diacylglycerol stimulates the activity of the plasma membrane-bound proteins kinase C which in turn phosphorylase a number of proteins, some of which may be components of iron pumps.

(vi) Mild alkalinization of the cell brought about by activation of a Na^{+}/H^{+} anti-port system can play a role in stimulating mitosis.

The product of src may, therefore, affect a large number of cellular processes by its ability to phosphorylate various target proteins and enzymes and by stimulating the pathway of synthesis of the polyphosphoinositides.

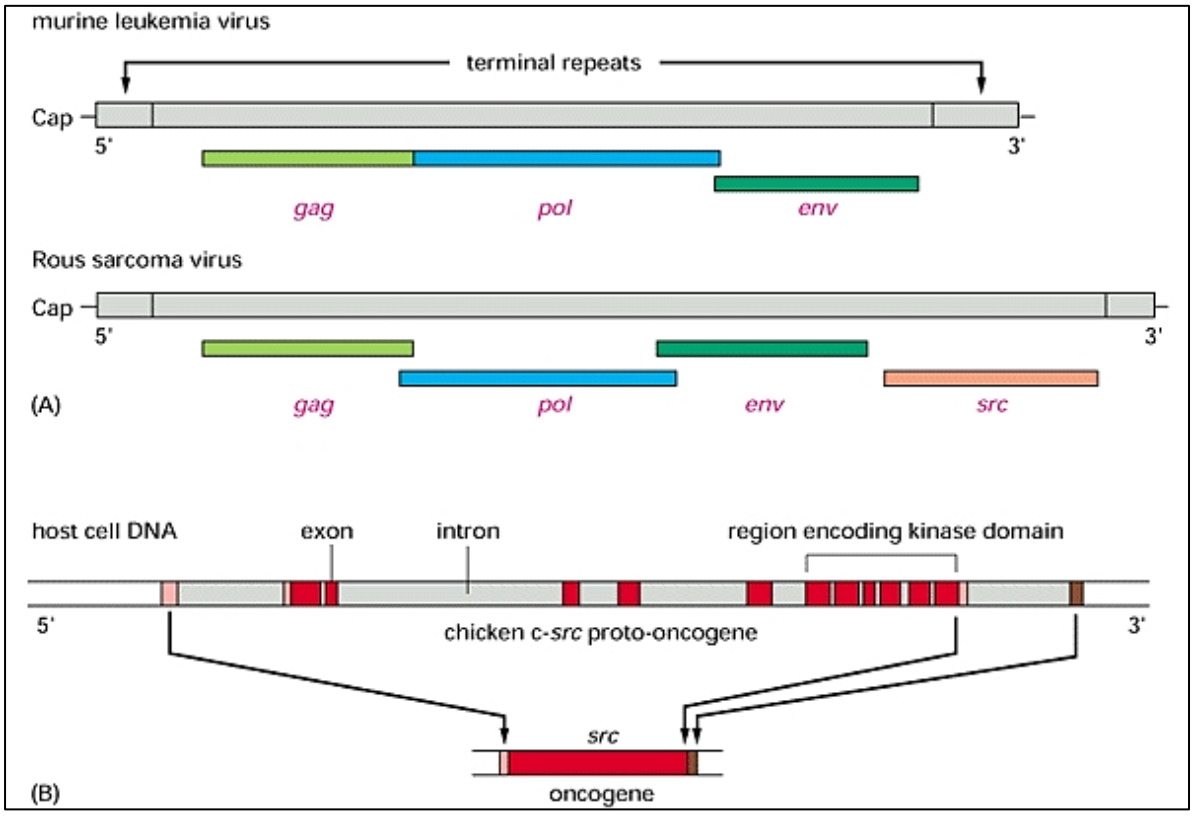

Fig 1: Oncogene of Rous sarcoma virus and its incorporation into host genome

- **Oncoproteins**

Oncoproteins are the product (the proteins) that are coded for by oncogenes and are produced when the gene is transcribed and translated (the process of "writing down the code" on RNA and manufacturing the proteins).

There are many types of oncoproteins depending on the specific oncogene present, but most work to stimulate cell growth and division, inhibit cell death (apoptosis), or inhibit cellular differentiation (the process by which cells become unique). These proteins can also play a role in the progression and aggressiveness of a tumour that is already present.

Types and Examples

Different types of oncogenes have different effects on growth (mechanisms of action), and to understand these it's helpful to look at what is involved in normal cell proliferation (the normal growth and division of cells). Most oncogenes regulate the proliferation of cells, but some inhibit differentiation (the process of cells becoming unique types of cells) or promote survival of cells (inhibit programmed death or apoptosis). Recent research also suggests that proteins produced by some oncogenes work to suppress the immune system, reducing the chance that abnormal cells will be recognized and eliminated by immune cells such as T-cells.

i) Growth Factors

Some cells with oncogenes become self-sufficient by making (synthesizing) the growth factors to which they respond. The increase in growth factors alone doesn't lead to cancer but can cause rapid growth of cells that raises the chance of mutations.

An example includes the proto-oncogene SIS, that when mutated results in the overproduction of platelet-derived growth factor (PDGF). Increased PDGF is present in many cancers, particularly bone cancer (osteosarcoma) and one type of brain tumor.

ii) Growth Factor Receptors

Oncogenes may activate or increase growth factor receptors on the surface of cells (to which growth factors bind).

One example includes the HER2 oncogene that results in a significantly increased number of HER2 proteins on the surface of breast cancer cells. In roughly 25% of breast cancers, HER2 receptors are found in numbers 40 times to 100 times higher than in normal breast cells. Another example is the epidermal growth factor receptor (EGFR), found in around 15% of non-small cell lung cancers.

iii) Signal Transduction Proteins

Other oncogenes affect proteins involved in transmitting signals from the receptor of the cell to the nucleus. Of these oncogenes, the ras family is most common (KRAS, HRAS, and

NRAS) found in roughly 20% of cancers overall. BRAF in melanoma is also in this category.

iv) Non-Receptor Protein Kinases

Non-receptor protein kinases are also included in the cascade that carries the signal to grow from the receptor to the nucleus.

A well-known oncogene involved in chronic myelogenous leukemia is the Bcr-Abl gene (the Philadelphia chromosome) caused by a translocation of segments of chromosome 9 and chromosome 22. When the protein produced by this gene, a tyrosine kinase, is continually produced it results in a continuous signal for the cell to grow and divide.

v) Transcription Factors

Transcription factors are proteins that regulate when cells enter, and how they progress through the cell cycle.

An example is the Myc gene that is overly active in cancers such as some leukemias and lymphomas.

vi) Cell Cycle Control Proteins

Cell cycle control proteins are products of oncogenes that can affect the cell cycle in a number of different ways.

Some, such as cyclin D1 and cyclin E1 work to progress through specific stages of the cell cycle, such as the G1/S checkpoint.

vii) Regulators of Apoptosis

Oncogenes may also produce oncoproteins that reduce apoptosis (programmed cell death) and lead to prolonged survival of the cells.

An example is Bcl-2, an oncogene that produces a protein associated with the cell membrane that prevents cell death (apoptosis)

• Classification and characteristics of chemical carcinogen

The carcinogenic chemicals that act as initiating agent are capable to bind with DNA. Hence, they interfere with the normal function of DNA and induce somatic mutation and, consequently, bring about stable, inheritable changes in the cell's properties.

I. On the basis of action of chemical carcinogens on DNA, there are two broad categories of carcinogens—direct acting and indirect acting (Fig 2).

Direct acting carcinogens are highly electrophilic compounds that react with DNA.Indirect acting carcinogens are converted to ultimate carcinogens by introduction of electrophilic centres. In other words, indirect acting carcinogens must be metabolised before they can react with DNA.

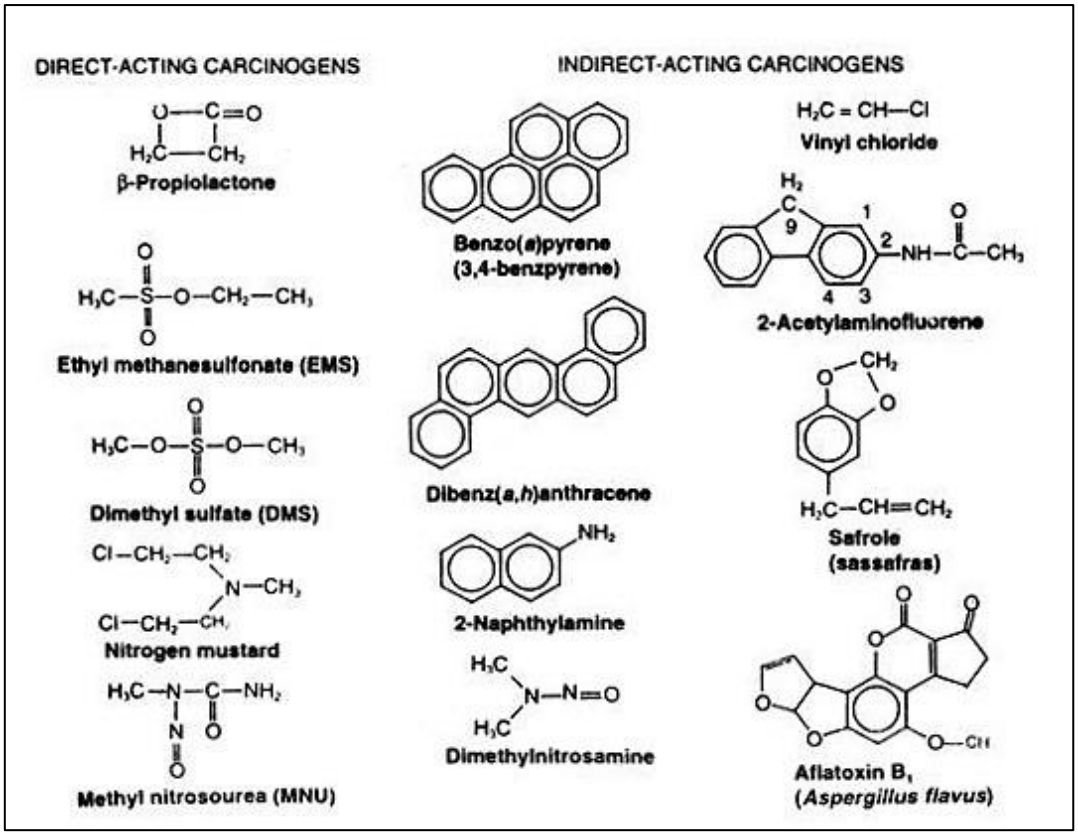

Fig 2: Structure of some direct acting and indirect acting chemical carcinogens

II. The vast majority fall into one of the following five categories depending in the carbon-containing compounds.

They range from small organic molecules containing only a few carbon atoms to large, complex molecules constructed from multiple carbon-containing rings (Fig 2).

1. Carcinogenic polycyclic aromatic hydrocarbons (or simply polycyclic hydrocarbons) are a diverse group of compounds constructed from multiple, fused benzene rings. Polycyclic hydrocarbons are natural components of coal tars, soots, and oils, and are also produced during the incomplete combustion of coal, oil, tobacco, meat, and just about any other organic material that can be burned.

The carcinogenic potency of polycyclic hydrocarbons varies widely, from weak or noncarcinogenic molecules to very potent carcinogens. The polycyclic hydrocarbons benzopyrene and dibenzoanthracene, isolated from coal tar in the 1930s, were the first purified chemical carcinogens of any kind to be identified.

2. Carcinogenic aromatic amines are organic molecules that possess an amino group (—NH_2) attached to a carbon backbone containing one or more benzene rings. Some aromatic amines are aminoazo compounds, which means that they contain an azo group (N=N) as well as an amino group. Among the carcinogens in these categories are the aromatic amines benzidine, 2-naphthylamine, 2-acetylaminofluorene, and 4-

aminobiphenyl, and the aminoazo dyes 4- dimethylaminoazobenzene and o-aminoazotoluene.

Many of these compounds were once employed in the manufacturing of dyes, although most are no longer used in significant quantities because of their toxicity. Some aromatic amines, such as 2-naphthylamine and 4-aminobiphenyl, are components of tobacco smoke. As in the case of polycyclic hydrocarbons, the carcinogenic potency of aromatic amines and aminoazo dyes varies from substances that are strongly carcinogenic to substances that are not carcinogenic at all.

3. Carcinogenic N-nitroso compounds are organic chemicals that contain a nitroso group (N=O) joined to a nitrogen atom. Members of this group include the nitrosamines and nitrosoureas, which are potent carcinogens when tested in animals. Most of these compounds are industrial or research chemicals encountered mainly in the workplace, although a few are present in cigarette smoke.

Nitrates and nitrites used in the curing of meats, which are not carcinogenic in themselves, can be converted in the stomach into nitrosamines, but no consistent relationship between these compounds and human cancer has been established.

4. Carcinogenic alkylating agents are molecules that readily undergo reactions in which they attach a carbon-containing chemical group to some other molecule. Unlike the three preceding groups of carcinogens, which are defined by their chemical structures (i.e., the presence of multiple benzene rings, amino groups, or nitroso groups), alkylating agents are defined not by their structural features but by their chemical reactivity—that is, their ability to join a chemical group to another molecule. The N-nitroso compounds, discussed in the preceding paragraph, are examples of carcinogens that function as alkylating agents.

Other examples include vinyl chloride (used in the production of plastics) and ethylene oxide (used in the production of antifreeze and other chemicals). Vinyl chloride and ethylene oxide are among the highest-volume chemicals produced in the United States. Other carcinogenic alkylating agents include sulfur mustard (a chemical warfare agent) and several drugs used in cancer chemotherapy.

5. Carcinogenic natural products are a structurally diverse group of cancer-causing molecules produced by biological organisms, mainly microorganisms and plants. Included in this category is aflatoxin, a carcinogenic chemical made by the mold Aspergillus. One of the most potent carcinogens known, aflatoxin sometimes contaminates grains and nuts that have been stored under humid conditions. Other carcinogenic natural products include plant-derived molecules such as safrole, a major component of sassafras root bark, and pyrrolizidine alkaloids, produced by a variety of different plants.

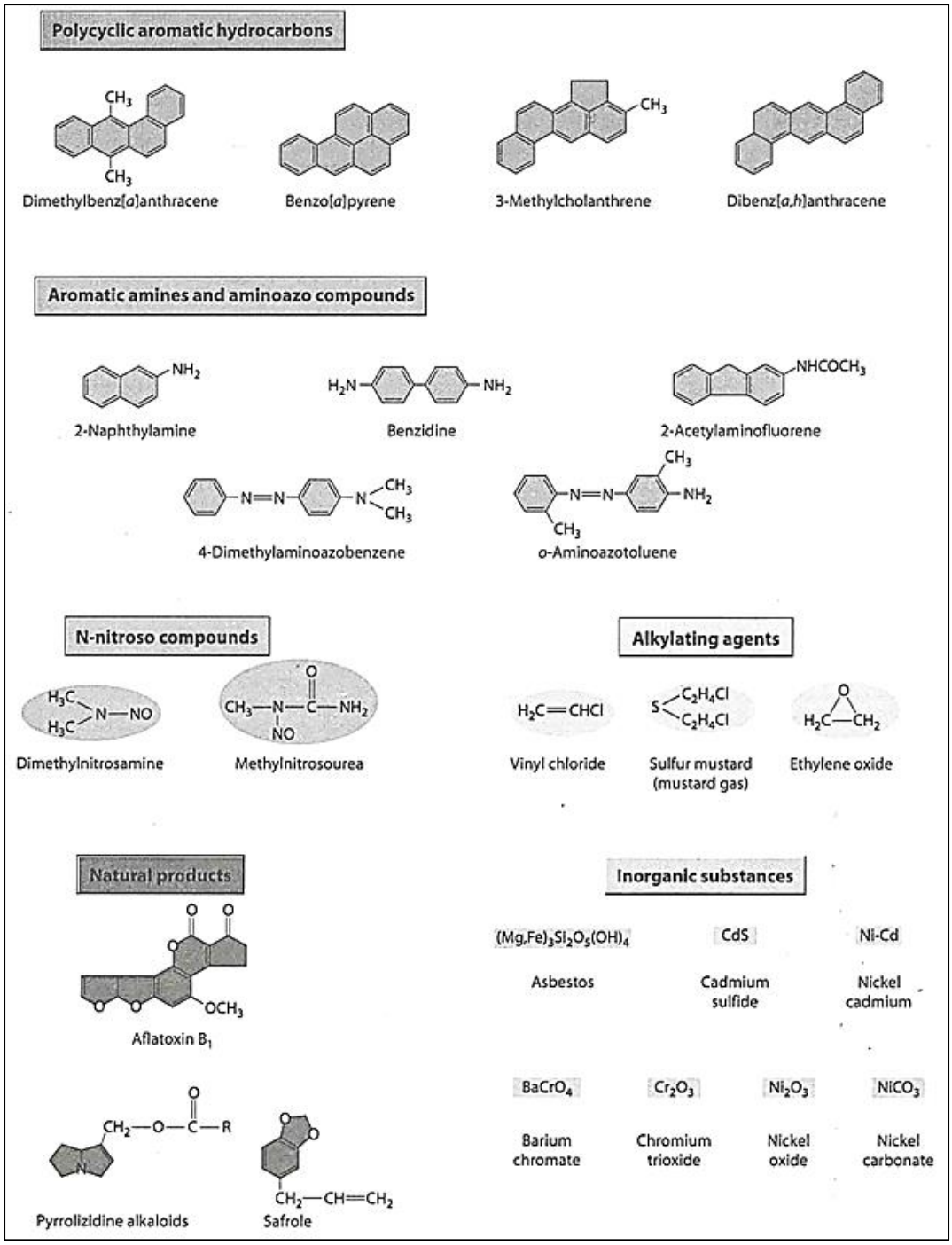

Fig 3: Main classes of carcinogenic chemicals

In addition to the preceding five classes of organic molecules, a small number of *inorganic substances (compounds without carbon and hydrogen) are carcinogenic*. Included in this group are compounds containing the metals cadmium, chromium, and nickel.

Some inorganic substances appear to be carcinogenic in the absence of chemical reactivity. For example, **asbestos** is a mineral composed of silicon, oxygen, magnesium, and iron, but its ability to cause cancer is related to the crystal structure and size of the microscopic fibers it forms rather than their precise chemical makeup.

Characteristics of chemical carcinogens

	Characteristic	Examples of relevant evidence
1.	**Is electrophilic or can be metabolically activated**	Parent compound or metabolite with an electrophilic structure (e.g., epoxide, quinone), formation of DNA and protein adducts
2.	**Is genotoxic**	DNA damage (DNA strand breaks, DNA–protein cross-links, unscheduled DNA synthesis), intercalation, gene mutations, cytogenetic changes (e.g., chromosome aberrations, micronuclei)
3.	**Alters DNA repair or causes genomic instability**	Alterations of DNA replication or repair (e.g., topoisomerase II, base-excision or double-strand break repair)
4.	**Induces epigenetic alterations**	DNA methylation, histone modification, microRNA expression
5.	**Induces oxidative stress**	Oxygen radicals, oxidative stress, oxidative damage to macromolecules (e.g., DNA, lipids)
6.	**Induces chronic inflammation**	Elevated white blood cells, myeloperoxidase activity, altered cytokine and/or chemokine production
7.	**Is immunosuppressive**	Decreased immunosurveillance, immune system dysfunction
8.	**Modulates receptor-mediated effects**	Receptor in/activation (e.g., ER, PPAR, AhR) or modulation of endogenous ligands (including hormones)
9.	**Causes immortalization**	Inhibition of senescence, cell transformation
10.	**Alters cell proliferation, cell death or nutrient supply**	Increased proliferation, decreased apoptosis, changes in growth factors, energetics and signaling pathways related to cellular replication or cell cycle control, angiogenesis

*Abbreviations:*AhR, aryl hydrocarbon receptor; ER, estrogen receptor; PPAR, peroxisome proliferator–activated receptor. Any of the 10 characteristics in this table could interact with any other (e.g., oxidative stress, DNA damage, and chronic inflammation), which when combined provides stronger evidence for a cancer mechanism than would oxidative stress alone.

Probable questions:

1. What is oncoproteins? Give examples
2. Define chemical carcinogen with examples
3. Classify chemical carcinogens with examples.
4. Briefly discuss characteristics of chemical carcinogen.

Suggested readings:

1. Hardin, J. Bertoni, G and Klein smith, J. L. (2012). Becker's World of the Cell. 8th Edn, Pearson Benjamin Cummings, San Francisco.

2. Pal, A. (2011). Textbook of Cell and Molecular Biology 3rd Edn, Bokks and Allied, Kolkata.

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Unit IX

Role of radiation and DNA repair in carcinogenesis and importance of nano-medicine in cancer therapy

Objective: In this unit we will discuss about Role of radiation and DNA repair in carcinogenesis. Importance of nano-medicine in cancer therapy.

Role of radiation and DNA repair in carcinogenesis

Carcinogenesis, also called oncogenesis or tumorigenesis, is the formation of a cancer, whereby normal cells are transformed into cancer cells. The process is characterized by changes at the cellular, genetic, and epigenetic levels and abnormal cell division.

Radiation carcinogenesis is a biological phenomenon whereby living normal cells are damaged by ionizing radiations, which starts a progressive process causing the surviving cells to change their phenotype such that normal controls of cell death and apoptosis are lost and uncontrolled cancerous growth is initiated.

Several types of radiation trigger the development of cancer. Like carcinogenic chemicals, radiation is another source of cancer risk routinely encountered in the environment. Radiation is defined simply as energy traveling through space. There are many different types of radiation, each defined by its wavelength and energy content. Natural sources of radiation include ultraviolet radiation from the sun, cosmic rays from outer space, and emissions from naturally occurring radioactive elements.

Medical, industrial, and military activities have created additional sources of radiation, mainly in the form of X-rays and radioactivity. Among the various types of radiation, two main classes have been clearly identified as causes of cancer- ultraviolet radiation and ionizing radiation.

The ability of **ultraviolet radiation** to cause cancer was first deduced from the observation that skin cancer is most prevalent in people who spend long hours in the sun and is more frequent in geographical areas where the sunlight is especially intense. Because ultraviolet radiation is absorbed by normal skin pigments, dark-skinned individuals have lower rates of skin cancer than do fair-skinned individuals.

Exposure to sunlight rarely causes any type of malignancy other than skin cancer because ultraviolet radiation is too weak to pass through the skin and into the interior of the body. Fortunately, the most common types of skin cancer rarely metastasize, and their superficial location makes these cancers relatively easy to remove surgically.

During the 1780s, the British House of Commons decided to deal with overcrowding in British jails by banishing criminals to the (then) remote island of

Australia. Within a few decades, the east coast of Australia came to be inhabited by light-skinned British men and women whose descendants now represent a large part of the Australian population (Fig 3). The white skin and fair complexion of these people makes them particularly vulnerable to the intense Australian sunlight, and as a result, the white population of Australia has the highest skin cancer rate of any people in the world. Such high rates cannot be explained by hereditary factors because in England, where the sun is weaker and often covered by clouds, this same group of people had low skin cancer rates.

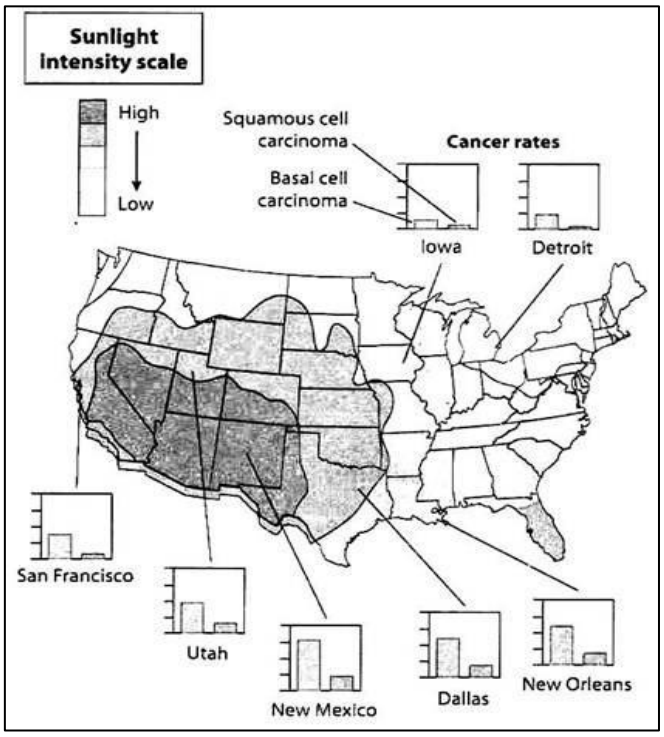

Fig 3: Relationship between skin cancer and sunlight exposure

Ionizing radiation poses a more serious cancer hazard because it is strong enough to penetrate through the skin and reach internal organs. The first type of ionizing radiation found to be a cancer hazard was X-rays, which were discovered in 1895 by Wilhelm Roentgen. Shortly thereafter, people working with X-rays began to develop cancer in unexpectedly high numbers. Another form of ionizing radiation, called **nuclear radiation**, is emitted by radioactive elements.

Sunlight Contains Several Classes of UV Radiation:

To explain how sunlight causes cancer, we need to describe the types of radiation given off by the sun. The sunlight that reaches the earth contains several forms of electromagnetic radiation, which is defined as waves of electric and magnetic fields that are propagated through space at the speed of light. Electromagnetic radiation occurs in a variety of forms that differ in wavelength and energy content (Fig 4).

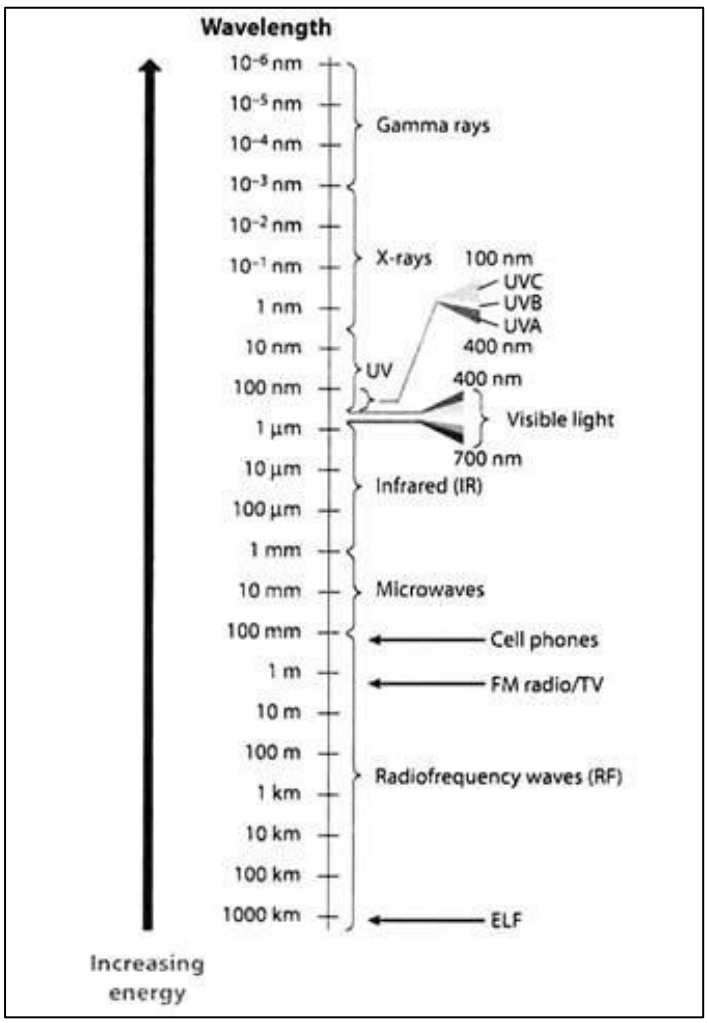

Fig 4: Electromagnetic spectrum

Wavelength and energy are inversely related to each other—that is, radiation of shorter wavelength possesses more energy than radiation of longer wavelength. The longest-wavelength component of sunlight is infrared radiation, which creates the warmth we feel from the sun.

Next comes *visible light*, which is of shorter wavelength than infrared radiation and provides the illumination that allows us to see colors. Finally, ultraviolet radiation (UV) is the shortest-wavelength component of sunlight and possesses the greatest energy, making it capable of inflicting damage on human tissues.The*ultraviolet radiation* in sunlight is in turn subdivided into three classes—A, B, and C—in order of decreasing wavelength (Table 1). UVA has the longest wavelength and the least energy. Defined as the portion of the UV spectrum whose wavelength falls between 315 and 400 nanometers (nm), UVA is the predominant type of ultraviolet radiation to reach the earth because it is not filtered out by the earth's atmosphere.

Table 1 Types of Ultraviolet Radiation in Sunlight

Type	Wavelength	Properties
UVA	315–400 nm	• Not filtered by ozone layer • Causes skin aging • Stimulates cell proliferation
UVB	280–315 nm	• Partially filtered by ozone layer • Causes sunburn, tanning, skin cancer
UVC	100–280 nm	• Filtered out by ozone layer • Artificial sources cause skin burns and skin cancer

UVA was once thought to be harmless because of its lower energy content, but long-term exposure to UVA is now known to cause aging of the skin and to act as a promoting agent for skin cancer by stimulating cell proliferation.

UVB radiation is of higher energy than UVA, falling in the wavelength range of 280 to 315 nm. Animal studies have shown the UVB is largely responsible for the carcinogenic properties of sunlight. More than 90% of the UVB radiation emitted by the sun is absorbed by ozone molecules present in the upper atmosphere, but enough UVB passes through to the earth's surface to cause sunburn, tanning, aging of the skin, and skin cancer.

Finally, UVC falls in the wavelength range of 100 to 280 nm and is the most energetic type of UV radiation emitted by the sun. This high-energy, short-wavelength form of UV radiation can cause severe burns, but it is completely absorbed by the upper layers of the atmos-phere before reaching the earth. UVC radiation is generally encountered only from artificial light sources, such as the germicidal lamps that use UVC to destroy bac-teria when sterilizing medical and scientific equipment.

- **UVB Radiation Creates Pyrimidine Dimers in DNA**

UVB is the highest-energy component of sunlight to reach the earth, but its energy level is still relatively low and thus it cannot penetrate very far into the body. Instead, UVB is absorbed by cells located in the outer layers of the skin, which explains why sunlight rarely causes any type of malignancy other than skin cancer. The damaging effects of UVB on skin cells often precede the development of cancer by many years.

For example, consider what happens to people who move from England, with its weak sunlight and cloudy skies, to the intensely sunny climate of Australia. Those who move to Australia when they are young develop skin cancer at high rates when they reach middle age, whereas those who move to Australia later in life retain the low skin cancer rates that are typical of people who remain in England. Such observations suggest that skin cancers observed later in life are the result of sunlight damage that occurred many years earlier.

Such a pattern is reminiscent of the initiation phase of chemical carcinogenesis, in which carcinogens trigger DNA mutations that persist for many years, passed from one cell generation to the next as genetically damaged cells proliferate and give rise to tumours. By analogy, researchers have looked to see whether sunlight causes skin cell mutations early in life that can be linked to the later development of cancer.

This is a complicated task because even if mutations are discovered in skin cancer cells, how can you be certain that sunlight caused them? A useful clue has come from studying the interactions of UVB—the main carcinogenic component of sunlight— with different kinds of cells and viruses. The shorter wavelengths of UVB (near 280 nm) are absorbed by the DNA bases, imparting enough energy to alter chemical bonds.

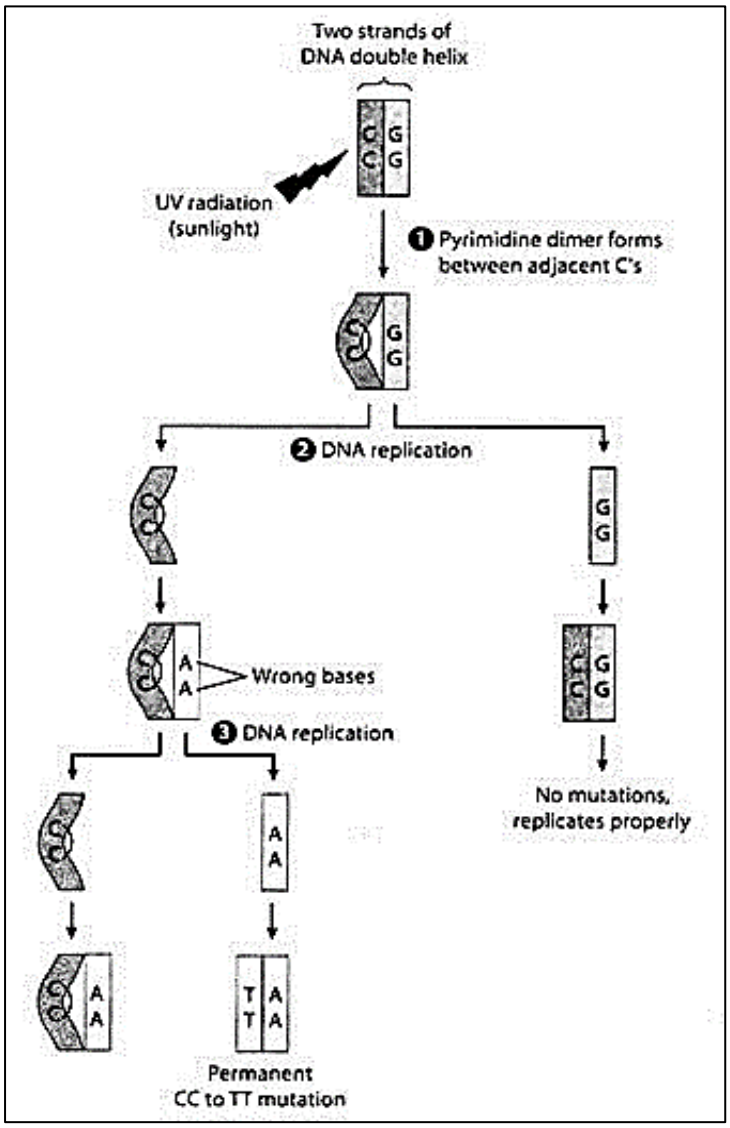

Fig 5: Conversion of a Pyrimidine Dimer into a permanent mutation

The most common reaction occurs in regions containing the bases cytosine (C) and thymine (T), a class of single-ring bases known as pyrimidines. In loca-tions where two of these pyrimidine bases lie next to each other, absorption of UVB radiation triggers the formation of covalent bonds between the adjacent bases, creating a unique type of mutation called a pyrimidine dimer. All four combinations of two adja-cent pyrimidines—that is, CC, CT, TC, and TT—are frequently converted into covalently linked dimers by UVB radiation.

Although cells can repair pyrimidine dimers, repair needs to occur before DNA replication creates a perma-nent, non-correctable mutation. Figure 4 illustrates how such a permanent mutation could arise, using a CC dimer as an example. During DNA replication, the DNA strand in the region of the CC dimer is distorted and therefore tends to pair improperly with bases in the newly forming DNA strand.

Instead of pairing correctly with its comple-mentary base G, the base C in a CC dimer often pairs incorrectly with the base A (Fig 5 step②). During the next round of DNA replication, the incorrectly inserted A will then form a base pair with its normal complementary base, T, creating an AT base pair Figure 4, step ③). This AT base pair now looks normal to the cellular DNA repair machinery and so will continue to be replicated as if no error had been introduced.

Because the base T resides where the base C had been located in the original DNA molecule, the preceding type of mutation is called a C→T substitution. In some cases both C's of the dimer are replaced by the same mecha-nism, creating a CC→TT mutation.

At this point the initial CC dimer in the original DNA strand could be repaired, but the C→T or CC →TT substitution in the newly replicated DNA will be permanent. Such base

sub-stitution patterns involving adjacent pyrimidines are unique to UV radiation and are therefore used as a dis-tinctive "signature" to identify mutations caused by sunlight.

- **Mutations in the p^{53} Gene Triggered by UVB Radiation can lead to Skin Cancer**

After scientists discovered that UV radiation selectively induces the formation of pyrimidine dimers, the next task was to determine whether these mutations are associated with skin cancer. Among the first genes to be examined for the presence of pyrimidine dimers was the p^{53} gene, a gene chosen for study because it is known to be mutated in many kinds of human cancer.

When skin cancer cells are examined for the presence of p^{53} mutations, nonmelanoma skin cancers are routinely found to exhibit p^{53} mutations with the distinctive UV **"signature"**—that is, C →T or CC → TT substitutions at dipyrimidine sites. In contrast, the p^{53} mutations arising in cancers of internal body organs do not generally exhibit this UV-specific pattern (Figure 6).

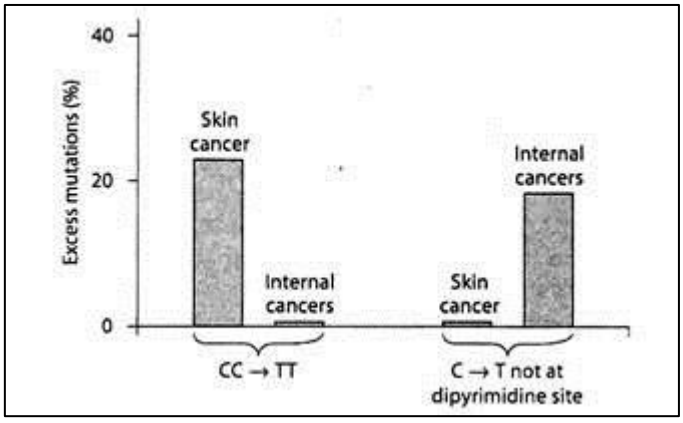

Fig 6: Incidence of two types if p^{53} mutations in skin cancer and internal cancer

The preceding observations indicate that the p^{53} mutations seen in nonmelanoma skin cancer cells are triggered by sunlight, but do these mutations actually cause cancer to arise, or are they simply an irrelevant by-product of long-term exposure to sunlight? This question can be resolved by looking at the precise location of UV-induced mutations within the p^{53} gene. The DNA base sequence of most genes is arranged in a series of three-base units called codons, each of which specifies a particular amino acid in the protein encoded by the gene. Typically, the first two bases of a codon are more important in determining the amino acid than is the third. For example, the codons GAA and GAG both specify the same amino acid (glutamine), so changing the third base from A to G in this codon does not change the amino acid. A similar principle applies to the codons for many other amino acids.

If the p^{53} mutations seen in non-melanoma skin cancers were simply a random by-product of sunlight exposure, mutations in a codons third base (which do not change an amino acid) should be as frequent as mutations in the first or second base (which do

change an amino acid). In fact, DNA sequencing has revealed that p^{53} mutations are not randomly distributed but instead involve base changes that alter amino acids.

In other words, the p^{53} mutations seen in non-melanoma skin cancers alter the amino acid sequence of the protein encoded by the p^{53} gene, as would be expected if these mutations are involved in the mechanism by which sunlight causes cancer. The p^{53} gene is not, however, the only mutant gene to be involved in non-melanoma skin cancers, nor is it as frequently mutated in melanomas (Fig 7).

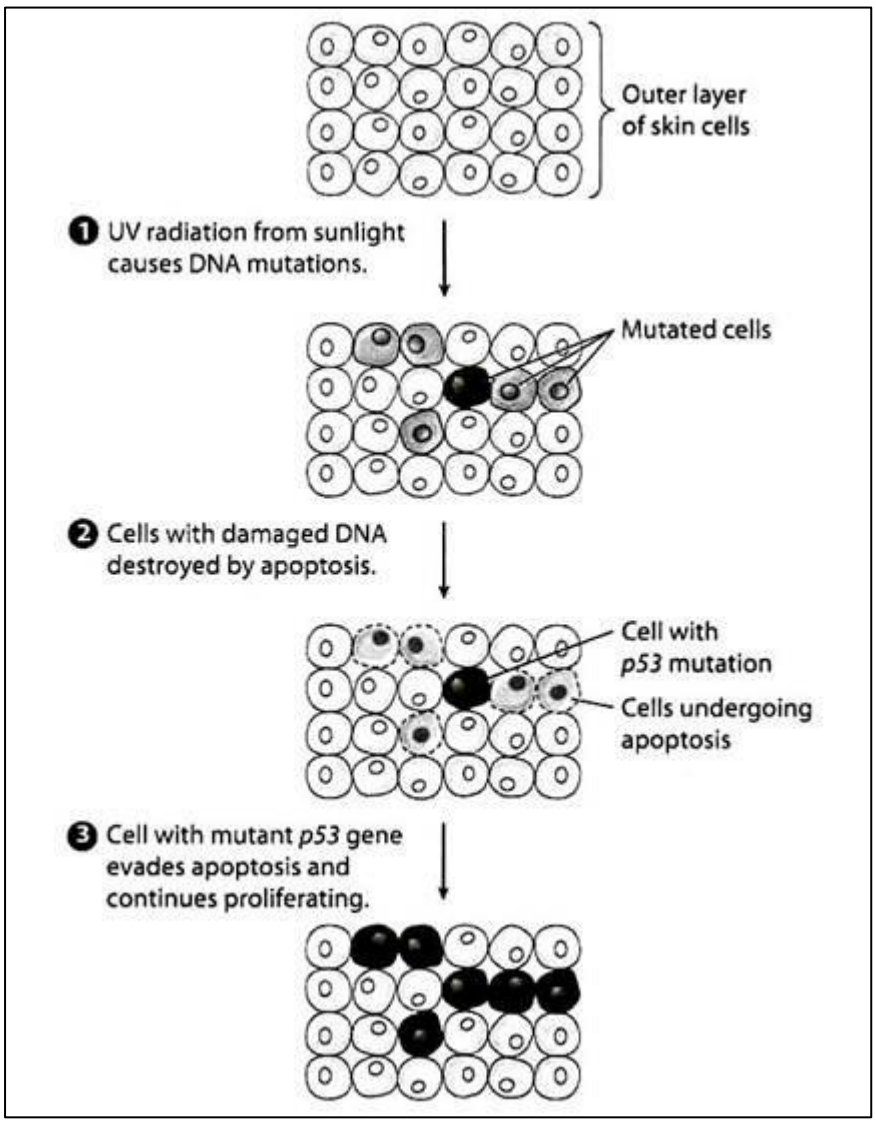

Fig 7: Sunlight-induced p^{53} mutations and the development of skin cancer

Sunlight-induced mutation of the p53 gene is thus comparable to the ini-tiation stage of chemical carcinogenesis, in which an initial mutation creates a precancerous cell that is later converted into a tumour by a promotion phase involving sustained cell proliferation.

- **Ionizing Radiation and Cancer**

Although UV radiation is responsible for more cases of cancer than all other carcinogens combined, its inability to penetrate very far into the body means that it only causes skin cancer, which is often easy to cure.

We now turn our attention to higher-energy forms of radiation that penetrate into the body and can therefore cause cancer to arise in internal organs. This type of radiation is called ionizing radiation because it removes electrons from biological molecules, thereby generating highly reactive ions that damage DNA in various ways.

Ionizing Radiation Initiates Carcinogenesis by Causing DNA Damage

As was the case for carcinogenic chemicals and UV radiation, DNA damage lies at the heart of the mechanism by which ionizing radiation causes cancer. The ability of ionizing radiation to trigger mutations was first described in the 1920s by Hermann Muller in studies involving fruit flies. When the mutation rate is plotted against the dose of ionizing radiation, the dose-response curve appears to be linear over a wide range of radiation doses.

In contrast to UV radiation, which creates a distinctive type of DNA mutation (pyrimidine dimers), ionizing radiation damages DNA in a variety of ways (Figure 8). By definition, ionizing radiation strips away electrons from molecules, generating highly unstable ions that rapidly undergo chemical changes and break chemical bonds. Because roughly 80% of the mass of a typical cell is accounted for by water molecules, many of the bonds broken by ionizing radiation reside in water.

The disruption of water molecules produces highly reactive fragments called free radicals, a general term that refers to any atom or molecule containing an unpaired electron. The presence of an unpaired electron makes free radicals extremely reactive.One of the free radicals produced when ionizing radiation interacts with water is the hydroxyl radical (OH), which readily attaches itself to DNAbases.

The presence of these added hydroxyl groups alters the base-pairing properties of the bases during DNA replication, leading to various mutations.In addition to generating water-derived free radicals, ionizing radiation also attacks DNA directly, stripping away electrons and breaking bonds. Such reactions cleave the bonds that join bases to the DNA backbone, thereby causing individual bases to be lost; ionizing radiation also attacks the DNA backbone itself, creating single- or double-strand breaks in the DNA double helix.

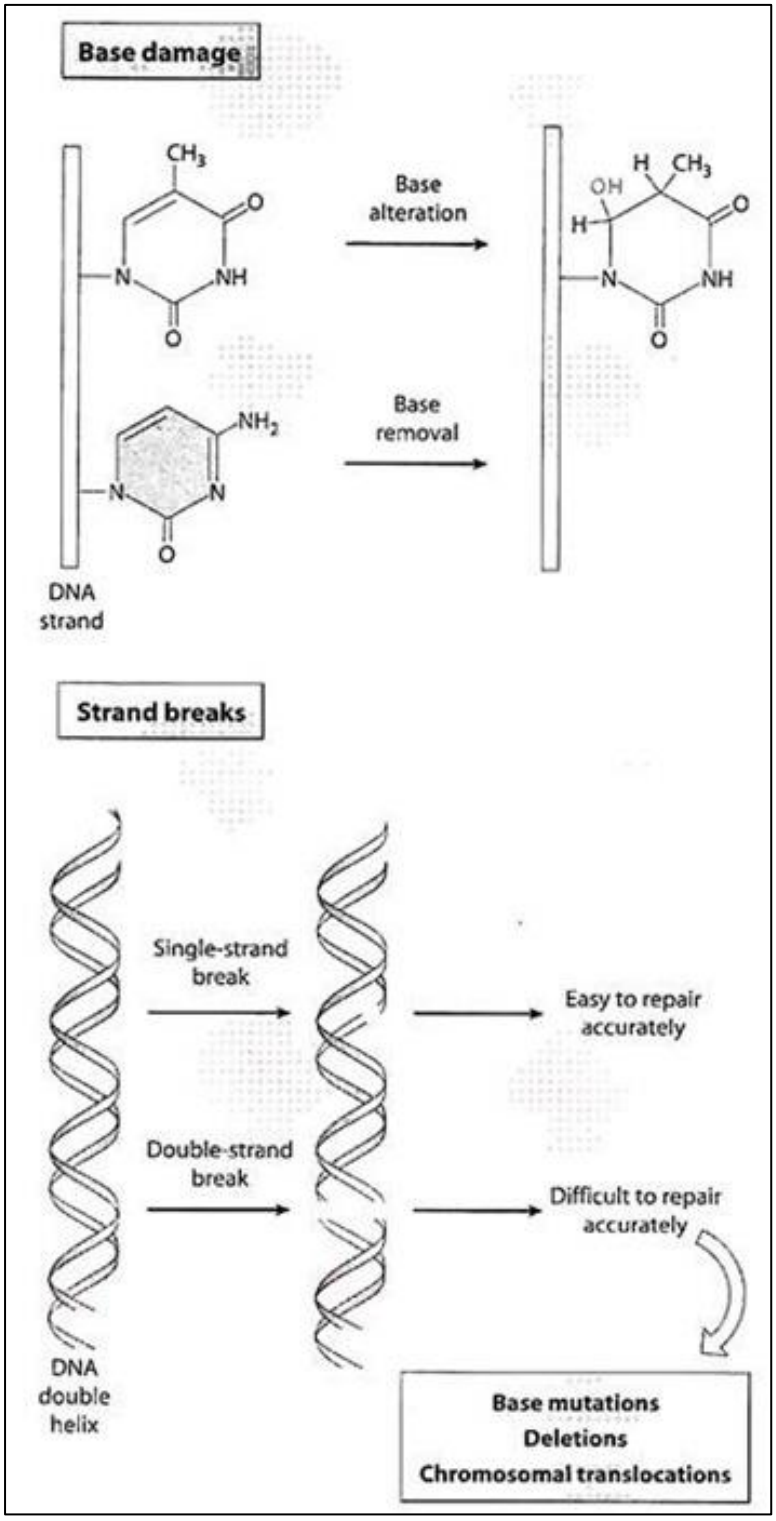

Fig 8: types of DNA damage caused by ionizing radiation

Fortunately, it is relatively easy to repair single-strand breaks or the loss of individual bases because the opposite DNA strand of the double helix remains intact and serves as a template for fixing the defective strand by normal repair mechanisms.

Double-strand breaks are more difficult to fix, and imperfect attempts at repair may create localized mutations in the region of the break or larger-scale alterations, such as major deletions or sequence rearrangements. If double-strand breaks occur in more than one chromosome, DNA derived from two different chromosomes may be mistakenly joined together. The result is a chromosomal translocation in which a segment of one chromosome is physically joined to another chromosome.

It usually takes many years for cancer to arise following radiation-induced DNA damage. Radiation is thus acting in the initiation phase of carcinogenesis, playing a role comparable to that of mutagenic chemicals in the initiation of chemical carcinogenesis. As would be expected, treating radiation-exposed cells with promoting agents, such as phorbol esters, increases the rate at which tumors appear. Cells that have been initiated by exposure to ionizing radiation often exhibit a persistent elevation in the rate at which new mutations and chromosomal abnormalities arise. This

condition, called genetic instability, creates conditions favourable for accumulation of the subsequent mutations that are required in the stepwise progression toward malignancy.

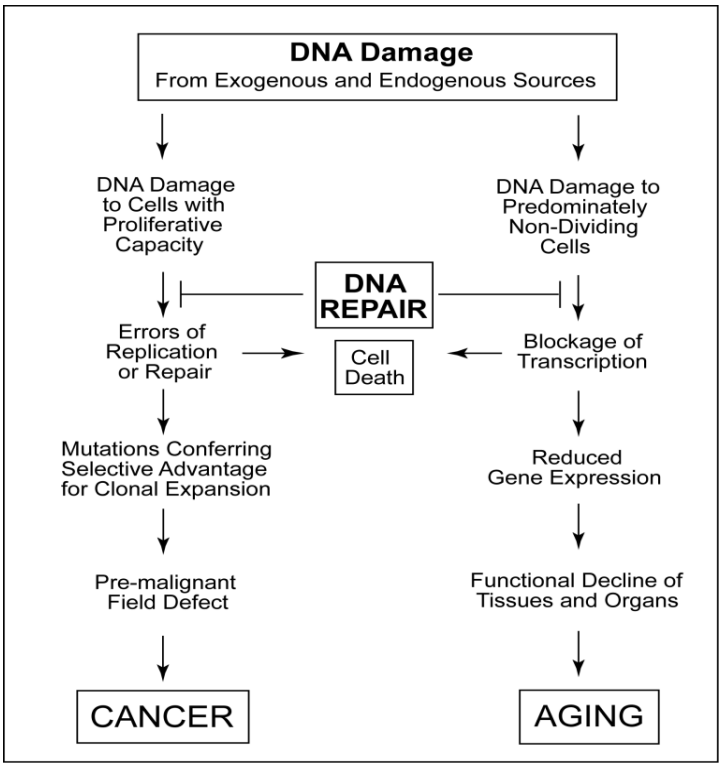

Fig 9: Role of DNA repair in carcinogenesis

The DNA sequence can be changed as the result of copying errors introduced by DNA polymerases during replication and by environmental agents such as mutagenic chemicals and certain types of radiation. If DNA sequence changes, whatever their cause, are left uncorrected, both growing and nongrowing somatic cells might accumulate so many mutations that they could no longer function. In addition, the DNA in germ cells might incur too many mutations for viable offspring to be formed. Thus the correction of DNA sequence errors in all types of cells is important for survival.

The relevance of DNA damage and repair to the generation of cancer (carcinogenesis) became evident when it was recognized that all agents that cause cancer (carcinogens) also cause a change in the DNA sequence and thus are mutagens.

Importance of nano-medicine in cancer therapy

- **Nano-Medicine in Cancer Therapy**

Nanomedicine in cancer therapy represents an innovative frontier in medical science. It involves using nanoparticles, which are tiny particles, often on the scale of atoms and molecules, to diagnose, treat, and prevent cancer. These nanoparticles can be engineered to carry drugs, probes, or both directly to cancerous cells, thus improving the efficacy of

treatment and reducing side effects. The principle behind nano-medicine in cancer therapy is to exploit the unique properties of materials at the nanoscale, which behave differently compared to their larger-scale counterparts. This approach has opened up new avenues for targeted therapy, where nanoparticles can be designed to recognize and bind to specific cancer cells, delivering therapeutic agents precisely where they are most needed. This precision medicine approach marks a significant shift from traditional cancer treatments, offering hope for more effective and less toxic therapies.

- **Latest Advancements in Nano-Medicine for Cancer Therapy**

The field of nano-medicine in cancer therapy has seen remarkable advancements in recent years. One of the most significant developments is the use of targeted drug delivery systems. These systems use nanoparticles as carriers for anticancer drugs, ensuring that the medication reaches the tumor site in high concentrations while sparing healthy tissues. Another breakthrough is the development of nanotheranostics, a technology that combines diagnosis and therapy. This approach uses nanoparticles that can both detect cancerous cells and deliver therapeutic agents, offering a two-pronged attack on cancer. Additionally, the use of nanotechnology in immunotherapy, where nanoparticles are used to boost the body's immune response against cancer cells, represents a novel and promising approach. These advancements have not only enhanced the effectiveness of cancer treatments but also significantly reduced the adverse side effects often associated with conventional cancer therapies.

- **Targeted Drug Delivery**

In the context of nano-medicine in cancer therapy, targeted drug delivery is a game-changer. It involves designing nanoparticles that are programmed to seek out and bind to specific cancer cells. These nanoparticles are typically conjugated with ligands or antibodies that recognize and attach to receptors on the surface of cancer cells. Once bound, the nanoparticles release the drug directly into the tumor, maximizing its therapeutic effect while minimizing systemic toxicity. This targeted approach is particularly beneficial in treating cancers that are difficult to access or those that do not respond well to traditional chemotherapy, revolutionizing the paradigm of cancer treatment.

- **Achievements in Nano-Medicine for Cancer Therapy**

The achievements in nano-medicine for cancer therapy are substantial and diverse. One notable accomplishment is the development of nanoparticle-based chemotherapy agents that have received FDA approval. These agents demonstrate significantly reduced side effects compared to traditional chemotherapy due to their targeted delivery capabilities. Additionally, significant strides have been made in using nanoparticles for photothermal and photoacoustic therapy, where nanoparticles are used to destroy cancer cells with

heat or sound waves. Another achievement is the successful application of nano-medicine in overcoming multi-drug resistance in cancer cells, a major challenge in oncology. Furthermore, nanoparticles have been instrumental in the development of personalized cancer vaccines, which are tailored to an individual's unique tumor profile, enhancing the effectiveness of immunotherapy. These achievements not only highlight the potential of nano-medicine in revolutionizing cancer therapy but also underscore its role in ushering in a new era of personalized medicine.

- **Overcoming Drug Resistance**

A significant achievement of nano-medicine in cancer therapy is its ability to overcome drug resistance, a major hurdle in effective cancer treatment. Nanoparticles can be engineered to bypass the mechanisms that cancer cells use to resist drugs, such as efflux pumps that expel the drug from the cell. By encapsulating drugs within nanoparticles, they can be protected from these resistance mechanisms and delivered directly to the cancer cells. This approach has shown promising results in treating cancers that have developed resistance to conventional therapies, offering new hope to patients who have limited treatment options.

- Advantages of Nano-Medicine in Cancer Therapy

Nanomedicine in cancer therapy offers a plethora of advantages over traditional cancer treatments. Firstly, the precision targeting of nanoparticles minimizes the impact on healthy cells, reducing the side effects and improving the quality of life for patients. Secondly, nano-medicine allows for the controlled release of drugs, ensuring a more constant therapeutic effect. This precision reduces the need for frequent dosing, enhancing patient compliance. Thirdly, the small size of nanoparticles enables them to penetrate tumors more effectively, addressing the challenge of drug delivery in solid tumors. Additionally, the versatility of nano-medicine allows for the combination of multiple therapeutic agents within a single nanoparticle, offering a synergistic approach to treatment. Finally, the integration of diagnostic and therapeutic functions in nanotheranostics facilitates real-time monitoring of treatment, enabling personalized cancer therapy tailored to individual patient needs.

- **Reduced Side Effects and Improved Patient Quality of Life**

One of the most significant advantages of nano-medicine in cancer therapy is the reduction of side effects and the consequent improvement in patient quality of life. Traditional cancer treatments, like chemotherapy, often have severe side effects due to the non-specific targeting of both cancerous and healthy cells. Nano-medicine circumvents this issue by delivering drugs directly to the tumor site, thereby sparing healthy tissues. This targeted approach results in fewer side effects, such as nausea, hair

loss, and fatigue. Consequently, patients experience a better quality of life during treatment, which is crucial in cancer care.

- ## Enhanced Drug Delivery and Penetration

Enhanced drug delivery and penetration are key advantages of nano-medicine in cancer therapy. The nano-scale size of these particles allows them to traverse biological barriers and access hard-to-reach tumor sites, a feat often challenging for conventional drug formulations. This capability is particularly important in treating solid tumors, where poor drug penetration is a significant barrier. Nanoparticles can also be designed to release their payload in response to specific stimuli within the tumor environment, ensuring that the drug is released precisely where and when it is needed. This targeted delivery not only improves the efficacy of the treatment but also reduces the risk of systemic toxicity.

- ## The Future of Nano-Medicine in Cancer Therapy

The future of nano-medicine in cancer therapy is incredibly promising, with ongoing research paving the way for more advanced and effective treatments. Future developments are expected to focus on multifunctional nanoparticles that can perform a variety of tasks, from targeting and imaging to drug delivery and monitoring treatment response. There is also a growing interest in using nano-medicine for personalized treatment strategies, where therapies are tailored to the genetic and molecular profiles of individual tumors. Additionally, the integration of artificial intelligence and machine learning with nano-medicine is anticipated to enhance the precision and effectiveness of cancer treatments. These advancements will likely lead to more efficient, less invasive, and highly personalized cancer therapies, significantly improving patient outcomes and potentially transforming the landscape of cancer treatment.

Probable questions:

1. Discuss the role of radiation in carcinogenesis.
2. Discuss the role of DNA damage repair in carcinogenesis.
3. State the role of ultraviolet radiation in carcinogenesis.
4. How mutations in the p53 Gene triggered by UVB Radiation lead to Skin Cancer?
5. How ionizing radiation initiates carcinogenesis by causing DNA damage?
6. State the Advantages of Nano-Medicine in Cancer Therapy
7. Discuss the Future of Nano-Medicine in Cancer Therapy

Suggested readings:

1. Hardin, J. Bertoni, G and Klein smith, J. L. (2012). Becker's World of the Cell. 8th Edn, Pearson Benjamin Cummings, San Francisco.

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6. Cooper, G.M. and Hausman, R.E. (2009). The Cell: AMolecularApproach.5thEdition. ASM Press and Sunderland, Washington, D.C.; Sinauer Associates, MA.

7. https://opalbiopharma.com/nano-medicine-in-cancer-therapy/

Unit X

Cell -cell adhesion: types of cell binding, adhesive proteins, their role in cell-cell interaction, gap junctions, extracellular matrix, integrins differentiation movement of leucocytes into tissues

Objective: In this unit we will learn about Cell -cell adhesion: types of cell binding, adhesive proteins, their role in cell-cell interaction, gap junctions, extracellular matrix, integrins differentiation movement of leucocytes into tissues.

Introduction

Cell adhesion is the process by which cells interact and attach to neighbouring cells through specialised molecules of the cell surface. Adhesion of cells is a primary feature of the architecture of many tissues.

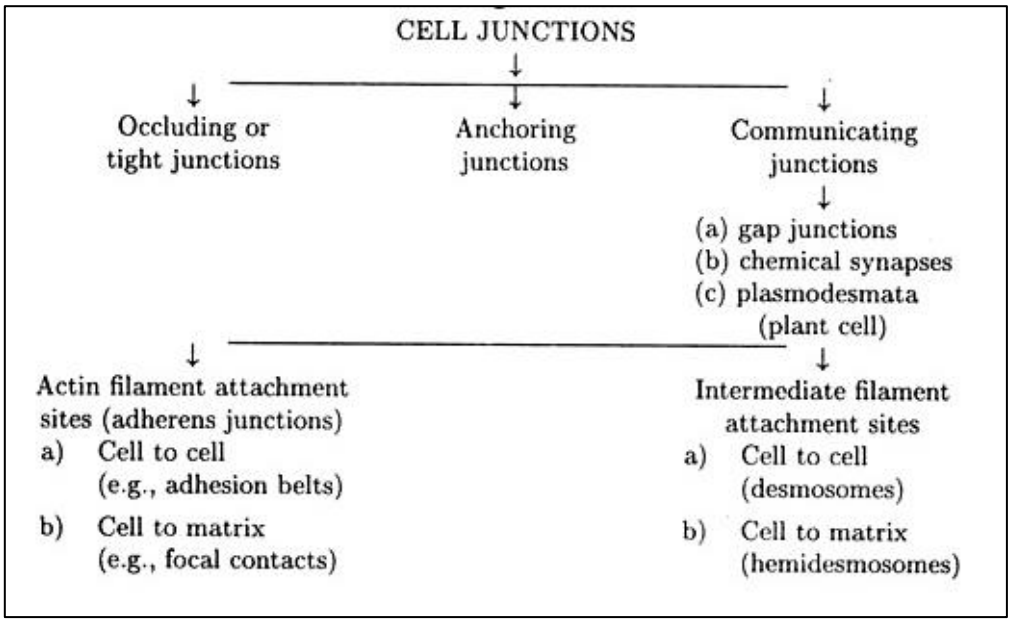
Cell junctions fall into three function groups:

1. Occluding or tight junction;

2. Anchoring junction;

3. Communicating junction.

The classification of all types of cell junctions is shown in details in the chart below.



Communicating Junctions:

It is a type of cell junction that mediates the passage of chemical or electrical signals from one interacting cell to its partner.

1. Gap junction

Definition: Gap junctions are a type of cell junction in which adjacent cells are connected through protein channels. These channels connect the cytoplasm of each cell and allow molecules, ions, and electrical signals to pass between them. Gap junctions are found in between the vast majority of cells within the body because they are found between all cells that are directly touching other cells. Exceptions include cells that move around and do not usually come into close contact with other cells, such as sperm cells and red blood cells. Gap junctions are only found in animal cells; plant cells are connected by channels called plasmodesmata instead.

Structure: In vertebrate cells, gap junctions are made up of connexin proteins (The cells of invertebrates have gap junctions that are composed of innexin proteins, which are not related to connexin proteins but perform a similar function.) (Fig 2). Groups of six connexins form a connexon, and two connexons are put together to form a channel that molecules can pass through. Other channels in gap junctions are made up of pannexin proteins. Relatively less is known about pannexins; they were originally thought only to form channels within a cell, not between cells. Hundreds of channels are found together at the site of a gap junction in what is known as a gap junction plaque. A plaque is a mass of proteins.

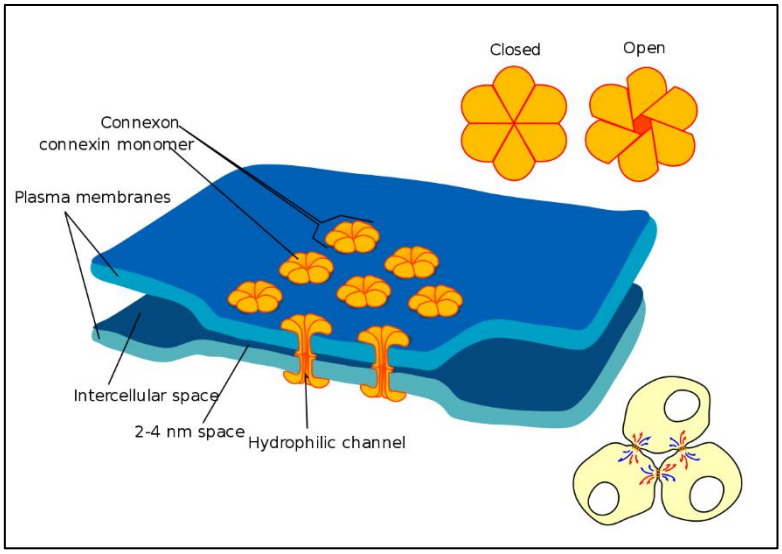


Fig 2: Structure of gap junction

Function: The main function of gap junctions is to connect cells together so that molecules may pass from one cell to the other. This allows for cell-to-cell communication, and makes it so that molecules can directly enter neighbouring cells without having to go through the extracellular fluid surrounding the cells. Gap junctions are especially important during embryonic development, a time when neighbouring cells must communicate with each other in order for them to develop in the right place at the right time. If gap junctions are blocked, embryos cannot develop normally.

Gap junctions make cells chemically or electrically coupled. This means that the cells are linked together and can transfer molecules to each other for use in reactions. Electrical

coupling occurs in the heart, where cells receive the signal to contract the heart muscle at the same time through gap junctions. It also occurs in neurons, which can be connected to each other by electrical synapses in addition to the well-known chemical synapses that neurotransmitters are released from.

When a cell starts to die from disease or injury, it sends out signals through its gap junctions. These signals can cause nearby cells to die even if they are not diseased or injured. This is called the "bystander effect", since the nearby cells are innocent bystanders that become victims. However, sometimes groups of adjacent cells need to die during development, so gap junctions facilitate this process. In addition, cells can also send therapeutic compounds to each other through gap junctions, and gap junctions are being researched as a method of therapeutic drug delivery.

Examples:

- The action potential in heart (cardiac) muscle flows from cell to cell through the heart providing the rhythmic contraction of the heartbeat.
- At some so-called electrical synapses in the brain, gap junctions permit the arrival of an action potential at the synaptic terminals to be transmitted across to the postsynaptic cell without the delay needed for release of a neurotransmitter.
- As the time of birth approaches, gap junctions between the smooth muscle cells of the uterus enable coordinated, powerful contractions to begin.
- Several inherited disorders of humans such as
- certain congenital heart defects and
- certain cases of congenital deafnesshave been found to be caused by mutant genes encoding connexins.

Adhesive proteins, their role in cell-cell interaction

Cell-cell adhesion is controlled by cell adhesion molecules (CAMs) which recognize different ligands at cell junctions. Cell adhesion molecules (CAMs) are a subset of cell adhesion proteins located on the cell surface involved in binding with other cells or with the extracellular matrix (ECM) in the process called cell adhesion. In essence, cell adhesion molecules help cells stick to each other and to their surroundings. CAMs are uniformly distributed along the regions of plasma membranes that contact other cells, and the cytosol-facing domains of these proteins are usually connected to elements of the cytoskeleton.Eukaryotes, prokaryotes and viruses have disparate cell adhesions molecules. For mammalian cells there are four main classes of cell adhesion molecule (Fig 5):

1. Cadherins (calcium dependent glycoproteins)
2. Integrins (transmembrane receptor proteins not dependent on calcium)

3. Immunoglobin superfamily members (molecules involved in cell adhesion with an immunoglobin domain that are not dependent on calcium)
4. Selectins (single-chained glyoproteins that are calcium dependent)
5. Connexins

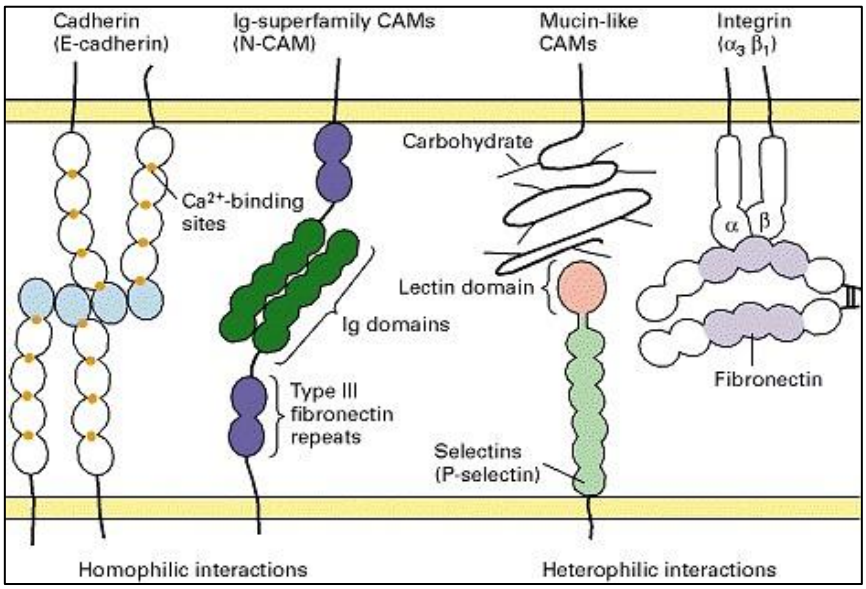


Fig 5: Major families of cell-adhesion molecules (CAMs)

Integral membrane proteins are built of multiple domains. Cadherin and the immunoglobulin (Ig) superfamily of CAMs mediate homophilic cell-cell adhesion. In a heterophilic interaction, the lectin domain of selectins binds carbohydrate chains in mucin-like CAMs on adjacent cells in the presence of Ca^{2+}.

Types of Adhesion molecules

1. Cadherins

- The cadherin superfamily comprises classical and non-classical cadherins
- Present in all multicellular animals
- Mediate calcium ion (Ca^{2+}) dependent cell-cell adhesions
- more than 180 members in humans
- *Classical cadherins*
- (e.g.: E-cadherin, N-cadherin and P-cadherin) contain 5 cadherin repeats
- *Require calcium ions to bind*
- Homophilic binding through end element
- Functional unit a dimer

Structure: A cadherin generally has a cytoplasmic domain, a transmembrane domain, and an extracellular domain (Fig 6). The latter is comprised of five subdomains held together by calcium ions. Removal of these ions will result in the collapse of the extracellular domain, thus disrupts homophilic adhesion. This is why cadherins are described as calcium-dependent cell adhesion molecules.

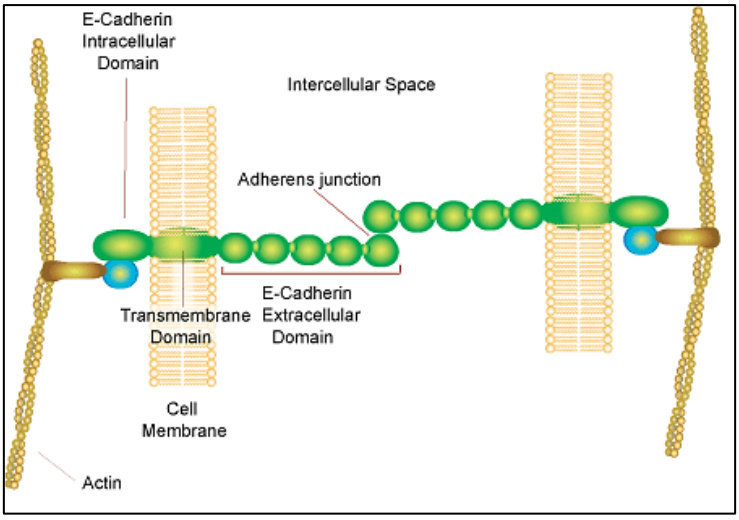


Fig 6: Structure of cadherin protein

2. Immunoglobulin Superfamily

- Vertebrates have 100+
- In addition to adhesion, they also have role in immune system
- Contain varying number of Ig-related domains
- G. Edelman - Nobel Prize in Physiology or Medicine in 1972
- "For their discoveries concerning the chemical structure of antibodies"
- studying the nervous system

3. Selectins

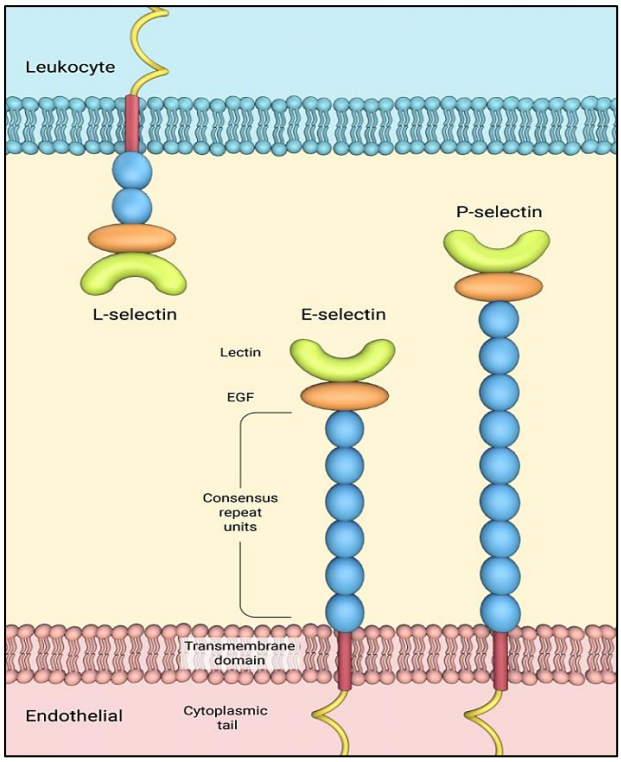


Fig 7: Selectin structure

- Cell Surface carbohydrate-binding proteins

- Vertebrates have only in circulatory system
- Role in inflammatory response: adhesion of leukocytes (blood cells) to endothelium (vessel wall)
- Cooperate with integrins and Ig-SF receptors
- Selectins 2 Heterophilic interactions
- Bind counterreceptors
- L-selectin on white blood cells (Fig 7)
- P-selectin on blood platelets and on endothelial cells that have been locally activated
- E-selectin on activated endothelial cells

4. **Integrins** (Fig 8)
   - Mammals have genes for 18 alpha and 8 beta integrins
   - Role in cell adhesion to extracellular matrix (ECM) basement membranes
   - Induction of cell polarization by adhesion
   - Glycosylated proteins
   - Bind through C terminal lectin domain of selectin
   - Comprising sandwich of beta sheets
   - Held together by hydrophobic interactions
   - Mainly receptors for ECM proteins
   - Fibronectin, laminin, collagen
   - Some heterotypic binding Ig superfamily
   - Interact with cell cytoskeleton

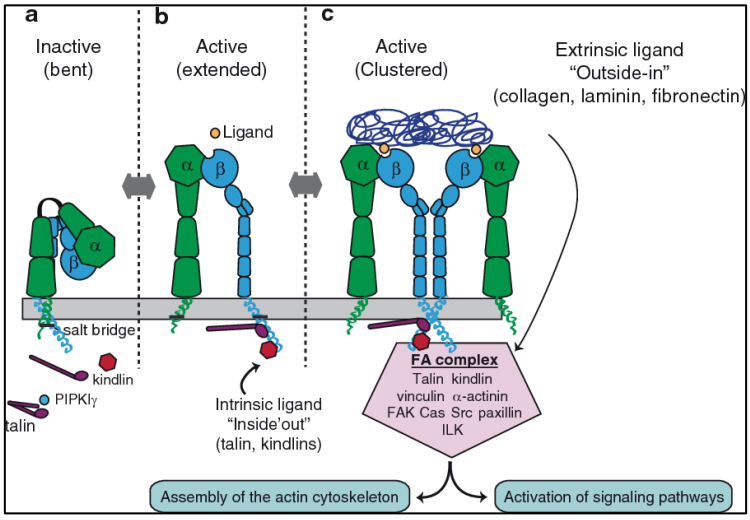


Fig 8: Structure of integrin

5. Connexins

Connexins are tetratransmembrane proteins that assemble into hexameric pore-forming structures known as connexons or hemichannels. Connexons typically dock with their

counterparts in adjacent cells to form intercellular gap junction channels, but may remain unpaired as cell surface hemichannels.

Structure

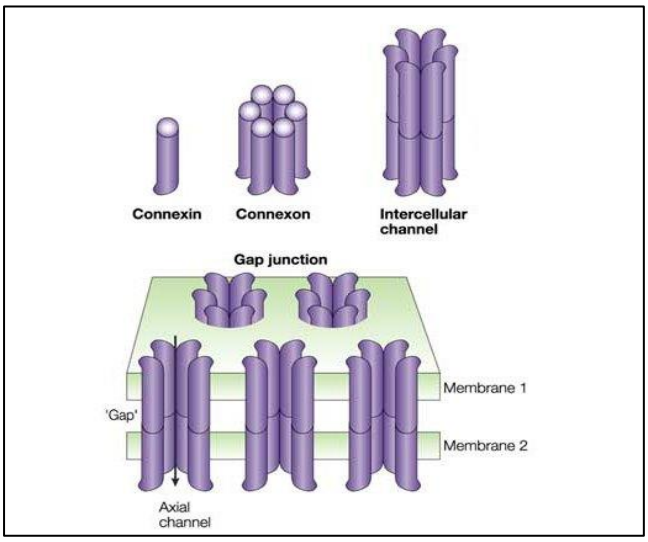


Fig 9: Structure of connexins

Connexins contain four highly ordered transmembrane segments (TMSs), primarily unstructured C and N cytoplasmic termini, a cytoplasmic loop (CL) and two extra-cellular loops, (EL-1) and (EL-2). Connexins are assembled in groups of six to form hemichannels, or connexons, and two hemichannels then combine to form a gap junction.

Function:

Connexin gap junctions are found only in vertebrates, while a functionally analogous (but genetically unrelated) group of proteins, the innexins, are responsible for gap junctions in invertebrate species. Innexin orthologs have also been identified in Chordates, but they are no longer capable of forming gap junctions. Instead, the channels formed by these proteins (called pannexins) act as very large transmembrane pores that connect the intra- and extracellular compartments.

Within the CNS, gap junctions provide electrical coupling between progenitor cells, neurons, and glial cells. By using specific connexin knockout mice, studies revealed that cell coupling is essential for visual signaling. In the retina, ambient light levels influence cell coupling provided by gap junction channels, adapting the visual function for various lighting conditions. Cell coupling is governed by several mechanisms, including connexin expression

Extracellular matrix

The ECM is able to hold water, provide appropriate hydration of the tissue and form part of a selective barrier to the external environment. The ECM also initiates crucial biochemical and biomechanical cues for tissue morphogenesis, differentiation and homeostasis.

In human, the main components of the extracellular matrix are:

1. Fibrous elements (e.g. collagen, elastin, reticulin),

2. Link proteins (e.g. fibronectin, laminin), and
3. Space filling molecules (e.g. proteoglycans, glycosaminoglycans)

- ECM COMPOSITION

The ECM is a complex mixture of proteins and glycosaminoglycans (a class of negatively charged polysaccharides). It is composed of three categories of materials:

1. Glycosaminoglycans and their proteoglycansthat resist compressive forces
2. Adhesive glycoproteins (laminin, fibronectin, tenascin, nidogen)
3. Fibrous proteinsthat provide tensile strength (collagens, elastin)

GLYCOSAMINOGLYCANS (GAGS)

GAGs were originally primarily known for being "space fillers" in the ECM. More recently they have been shown to be active signaling molecules whose roles in a variety of cellular processes (including cytokine production, leukocyte recruitment and inflammatory response) are important for controlling cell fate.

Glycosaminoglycans (GAGs) are linear polysaccharides composed of two basic saccharides: an amino sugar and a uronic acid. The amino sugar is typically either N-acetyl-D-glucosamine (D-GlcNAc) or N-acetyl-D-galactosamine (D-GalNAc). The uronic acid is either D-glucuronic acid (D-GlcA) or L-iduronic acid (L-IdoA). These basic components are further varied by epimerization, sulfation, and deacetylation. The order of the carbohydrate chain and the other chemical modifications determine their specificity and functionality.

Hyaluronan is the simplest GAG since it is non-sulfated, doesn't undergo epimerization, and is composed of an unmodified disaccharide repeat. It is also not typically covalently linked to any proteins. The other GAGs are chondroitin sulfate (CS), dermatan sulfate (DS), keratan sulfate (KS), and heparan sulfate (HS). These four GAGs are typically covalently attached to proteins to form proteoglycans. Chondroitin and heparan sulfate are extensively modified by sulfation.

PROTEOGLYCANS

A proteoglycan is composed of a core protein with one or more covalently attached GAGs. They are stored in secretory granules, inserted into the plasma membrane or secreted into the ECM.

GLYCOPROTEINS

I. Laminin

Currently there are 15 known heterotrimeric laminins[6]. **Laminin** is composed of α, β, and γ chains of which there are 5 α, 4 β, and 6 γ chains[2]. It has a cross-like structure with 3

short arms and 1 long arm. The α chains possess a large globular domain known as the G domain at the C-termini. This domain is composed of 3 LG domains (LG1-LG3) connected by a linker region to two more LG domains (LG4-LG5). Integrins bind to LG1-3 and dystroglycan to LG4-LG5. Heparan sulfate has been shown to bind to LG4 of the α1 chain6. Laminin is a component of the basement matrix; however, the isoform of laminin present varies with the tissue2. The laminin-1 form is the most prominent in early development and is comprised of α1, β1, and γ1 chains.

II. Fibronectin

Fibronectin is a dimer with a molecular weight of ~270 kDa. It exists as a soluble form in blood and body fluids and in fibrils in the ECM. It binds collagen, heparin, other fibronectin proteins, and cell surface integrins. Fibronectin binds integrins through the tri-peptide motif of arginine, glycine, and aspartic acid (RGD).

FIBROUS PROTEINS

III. Collagen

Collagens are the major structural component of the ECM[1]. They are the most prevalent protein in the skin and bone, making up 25% of the total protein mass[2]. Collagens provide scaffolding for the attachment of laminin, proteoglycans and cell surface receptors. Twenty-eight types of collagens (I–XXVIII) have been identified so far in vertebrates. Collagens are triple helical proteins that are formed from either homotrimers or heterotrimers of polypeptide chains, referred to as α-chains. α-chains have a three amino acid repeat of Gly-X-Y, where X is typically proline and Y is 4-hydroxyproline (post-translationally modified proline).

IV. Elastin

Elastin, as its name suggests, provides elasticity to the ECM. It is produced as tropoelastin, a 72 kDa precursor protein and is secreted from the cell. In the extracellular space, it crosslinks with other elastin molecules to form sheets and fibers. Elastin is the primary ECM protein present in arteries where it composes ~50% of their dry weight

• Various Roles of ECM

The role of the ECM depends on its nature and composition. eg, the matrix may be mineralised in bone to resist compression or dominated by tension resisting collagen fibres in tendons. Below various examples are given.

i. Tendon

The ECM of tendon is composed predominantly of collagen, which accounts for ~ 60–85% of the dry weight of the tissue. Approximately 95% of the collagen is type I. The collagen provides excellent mechanical strength to the tendon along one axis! allowing them to be able to withstand tension.

ii. Cartilage

Chondrocytes synthesise the cartilage ECM and makes it both stiff and elastic.

1. Mainly composed of type II collagen, up to 25 % of dry weight, with types IX and XI collagens present in lower proportions.
2. The second most abundant molecules are glycosaminoglycans, such as hyaluronan and aggrecan, which join together to form macromolecular complexes.

Collagen counteracts tensile loads and glycosaminoglycans decrease the mechanical pressures.

iii. Bone

Osteoblasts synthesise bone ECM. Consists of:

1. Type I collagen mixed with a matrix of calcium phosphate crystal (which is up to 70 % of the dry weight). Collagen allows bone to be elastic enough to avoid bone fragility and not to be easily broken whilst calcium phosphate crystal provides stiffness and hardness.
2. Proteoglcans and glycoproteins, which are less abundant, but vital for the organization of collagen fibers, mineralization and resorption of bone. Chondroitin sulfate accounts for 67-97 % of the bone glycosaminoglycans.[5]

iv. Skin

1. The ECM of skin is composed of various polysaccharides, water and collagen proteins, giving the skin its unique properties, ie great tensile strength of plus substantial elasticity and compressibility. The ECM molecules, which are secreted by fibroblasts and epidermal cells, are importantly.
2. Fibrous structural proteins, eg collagens, elastin and laminin, which give the ECM strength and resilience
3. Proteoglycans, eg dermatan sulfate and hyaluronan, which are large, highly hydrated molecules that help cushion cells in the ECM.

v. Blood

The extracellular matrix of blood is called plasma, making blood unique among connective tissues because it is fluid. This fluid, which is mostly water, suspends the formed elements and enables them to circulate throughout the body within the cardiovascular system.

vi. Nervous Tissue

Macroglia provide most of the the ECM in the nervous system, with the ECM playing an important role in supporting and signaling. ECM components are key mediators of glial activation and have the capacity to evoke both regenerative and degenerative effects on glia and neurons. The production of ECM components changes drastically

during reactive gliosis, a reaction that happens in response to nervous tissue damage. There is only a small amount of extracellular matrix in the nervous tissue.

Movement of leukocytes into tissue

Diapedesis of leukocytes is a major event in the migration of leukocytes from the blood circulation to sites of inflammation or tissue injury and in the recirculation of lymphocytes from the blood to the lymphatic compartment. Diapedesis of leukocytes is often confused with the terms 'leukocyte transmigration' or 'leukocyte extravasation'. These terms define the entire process of leukocyte migration from the blood circulation to the extravascular connective tissue, across the vessel wall endothelium. Diapedesis is regarded as the final event in the transmigration, i.e. the actual penetration of the vascular endothelium and the sub endothelial matrix.

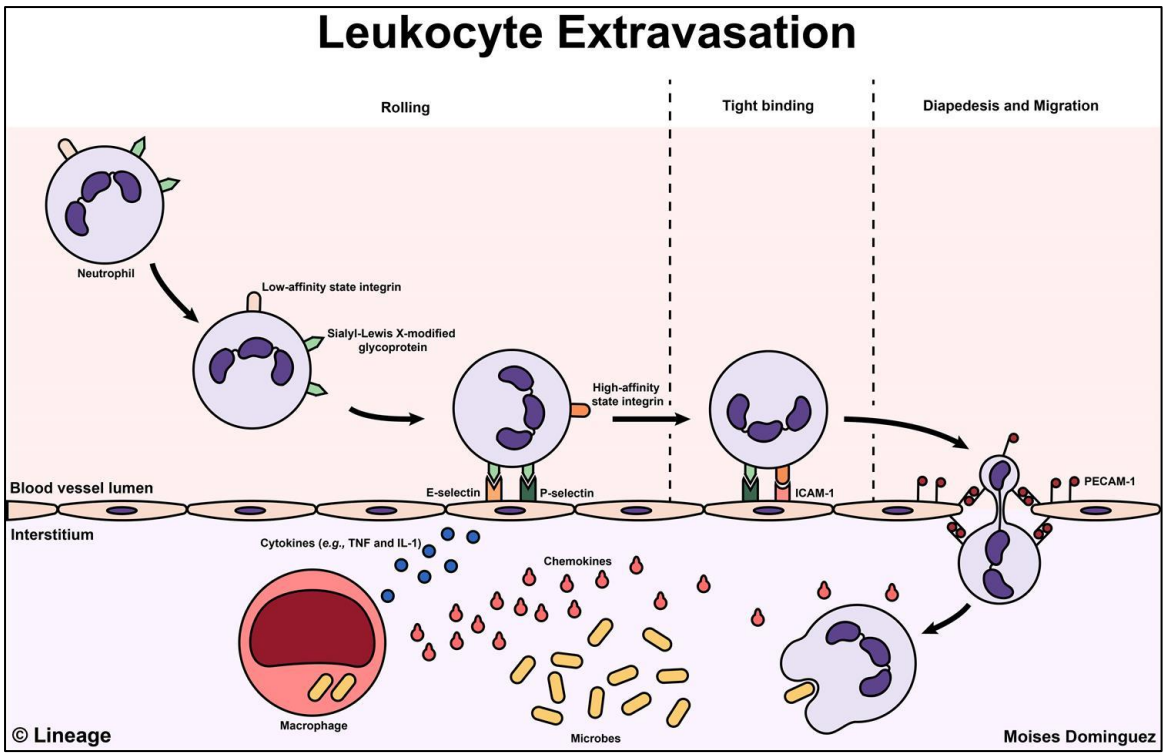
The inflammatory or leukocytic infiltrate consists of white blood cells which leave the blood and enter (infiltrate) the inflamed connective tissue.

Cells of the inflammatory infiltrate include neutrophils, lymphocytes and monocytes. Immigration of these cells into peripheral tissues is one of the principal purposes for inflammation, bringing to a site of injury the immune-system cells which can combat infection and clean up damaged tissue.

Neutrophils (neutrophilic leukocytes) are the first white blood cells to enter the tissue during acute inflammation. Neutrophils are anti-bacterial cells which lyse (break down) bacterial cells by releasing lysosomal enzymes. Neutrophils recognize bacteria as foreign by the antibody molecules which have attached to the bacterial surface. Antibody molecules (molecules which bind to one specific antigen or foreign substance which the body has previously encountered) are found in blood plasma and interstitial fluid.

Lymphocytes accumulate somewhat later during the inflammatory process. Their presence in large numbers indicates the continuing presence of antigen and thus may suggest an established infection. Lymphocytes produce the multitude of diverse antibody molecules (one specific type of antibody per lymphocyte) which provide the mechanism for chemical recognition of foreign materials (distinguishing between self and non-self) and so for mediating and regulating immune responses.

Monocytes are phagocytic cells which circulate in the blood. An equivalent cell type, called the *macrophage*, is a resident cell in connective tissue. Monocytes/macrophages engulf and digest foreign microorganisms, dead or worn-out cells, and other tissue debris. They interact closely with lymphocytes to recognize and destroy foreign substances.



- **Leukocytes play an important role in eliminating offending agents**
  - ✓ neutrophils and macrophages are capable of destroying microbes, necrotic tissue, and foreign substances
  - ✓ The process of leukocytes migrating from the blood vessel to tissue involves multiple steps and are mediated by adhesion molecules and chemokines

1. Margination

leukocytes become redistributed closer to the vessel wall

2. rolling
   - ✓ leukocytes transiently attach to endothelium and then detach
   - ✓ these cells therefore "roll" on the vessel wall
   - ✓ the endothelium possesses E-selectin and P-selectin
   - ✓ cytokines from the inflamed tissue regulate selectin expression
   - ✓ Sialyl-Lewis X protein binds to E-selectin and P-selectin (found on leukocytes)

3. firm endothelial adhesion

✓ mediated by integrin proteins
✓ vascular cell adhesion molecule 1 (VCAM1) and intercellular adhesion molecule-1 (ICAM-1) are found on the endothelium
✓ low affinity integrin are found on leukocytes
✓ migration through the vessel wall

4. migration to tissue

Probable questions:

1. Discuss the structure and function of gap junction.
2. What do you mean by adhesive protein? Give example.
3. Write short notes on cadherin.
4. Draw the structure of selectin protein.
5. What is the function of connecxin?
6. Describe the role of adhesive proteins in details.
7. Discuss the movement of leucocytes into tissues emphasising on leukocyte extravasation

Suggested readings:

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Unit XI

Developmental Neurobiology: General organization of nerve fibers

Objective: This section of Developmental Biology, particularly neurobiology, covers the overall arrangement of nerve fibers. By the end of this topic, you will gain an understanding of nerve fiber structure and the primary components of nerves.

General organization of nerve fibers

A bundle, but more often several bundles, of nerve fibers enclosed in connective tissue sheaths make up a grossly visible anatomical structure, the peripheral nerve. A peripheral nerve is composed of:

- Axons (nerve fibers)
- Schwann cells
- Supporting connective tissue with blood vessels.

The smallest functional unit of a peripheral nerve is the axon, the so-called nerve fiber. Except for a few very thin nerves made up of nonmyelinated axons, nerves have a whitish, glassy appearance because of their myelin and collagen content. In myelinated fibers, the area that a Schwann cell occupies is defined as an internode, and the interval between internodes is the Ranvier's node. The myelin sheaths on both sides of a node terminate in paranodal bulbs, which often show an asymmetry related to growth.

Nerve fibers of peripheral nerves are surrounded by connective tissue sheaths that are organized in distinct patterns. There are three layers of supporting connective tissue in a peripheral nerve: the endoneurium, the perineurium, and the epineurium (**Fig.1**).

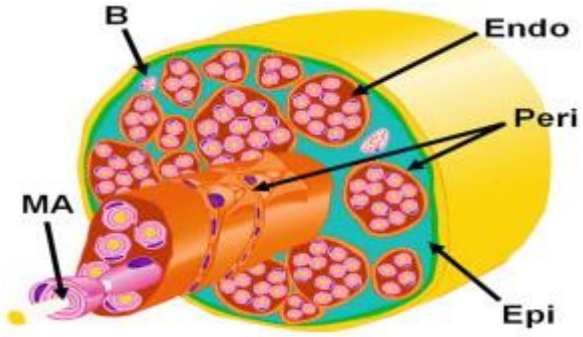

Fig.1. *Diagram showing the arrangement of connective tissue in a peripheral nerve. Individual myelinated (MA) and also nonmyelinated axons and their associated Schwann cells are sheathed by endoneurium (Endo) and bound into fascicles by perineurium (Peri) made up from flattened epithelia-like cells. The dense connective tissue epineurium (Epi) binds individual fascicles into a peripheral nerve and may contain numerous blood vessels (B).*

Loose connective tissue that surrounds individual nerve fiber is called **endoneurium** (endoneurial connective tissue or intrafascicular connective tissue). The endoneurium surrounds the axon and its associated Schwann cells, as well as capillaries. Like all the connective tissue in the human body, endoneurial connective tissue is composed of fixed and migrant cells, collagen and reticular fibers, and amorphous substance. The endoneurial extracellular matrix contains fibers mainly of collagen origin that run along the nerve axis in variable densities and are aggregated in bundles. So, in the endoneurium, the collagen fibers are closely packed around each nerve fiber to form the supporting walls of the endoneurial tubes.

The **perineurium** is a dense and mechanically strong sheath that surrounds groups of axons called fascicles. The perineurium is composed of concentric layers of flattened epithelia-like cells (*pars epitheloidea*) separated by layers of mainly longitudinally oriented collagen fibers (*pars fibrosa*). About seven to ten layers are usually present. Occasional elastic fibers are present, too. A striking feature of the perineurium is the close cohesion between cells placed on a single basal lamina. These cells are also joined by special cell junctions.

The whole peripheral nerve must be surrounded by connective tissue with different features than the spiderweb-like and delicate endoneurium. This relatively thick layer of irregular dense connective tissue with some areas of adipose tissue is called the **epineurium**, which is an external protective and supporting coat. The epineurium normally constitutes 30-70% of the total cross-sectional area of a nerve bundle and carries the main supply channels of the intraneural vascular system, the *vasa nervorum*, relatively large vessels that enter through the epineurium up to small capillaries found in the endoneurium.

A Nerve Fiber

A nerve fiber is a threadlike extension of a nerve cell (the neuron) and consists of an axon and myelin sheath (if present). A neuron is formed by its perikaryon or the cell body and cytoplasmic processes. As known, two types of processes are discernible: axon and dendrite (or dendrites). A**xon** can be defined as a single process of the neuron that is specialized to conduct electrical impulses away from the perikaryon (neuron body) or from the receptor portion of that same neuron. It is enclosed by the axolemma. Cytoplasm within the axon is termed axoplasm. The main functional characteristic of the axon is that electrical impulses are conducted in a nondecremental manner. Axons are of uniform caliber, can be branched, and as in a peripheral nerve, they can be both myelinated and nonmyelinated. Various axons (or nerve fibers) form the peripheral nerve as its main component.

Myelinated nerve fibers are enveloped by a specific glial cell population, the Schwann cell; better said, by numerous concentric layers of the Schwann cell's plasma membrane with very little cytoplasm and intracellular components.

Nonmyelinated nerve fibers are generally much thinner, partially owing to missing myelin sheath, and several of them are embedded (but not enwrapped) within simple folds of the Schwann cell. So, axons inside the peripheral nerves are never "naked."

They always have some form of coating and covering, which is the product of the activities of the Schwann cell

Axoplasm of both myelinated and nonmyelinated nerve fibers contains microfilaments; neurofilaments (intermediate filaments specific to neurons); neurotubules composed of alpha-tubulin, beta-tubulin, and microtubule-associated proteins (collectively referred to as neurofibrils); and abundant mitochondria and secretory vesicles. A complex network of neurofibrils (the common term for neurotubules and neurofilaments visible at the light microscopic level) is seen running through the cytoplasm and processes of neurons.

As mentioned above, **the myelin sheath** is built up by a spiraling growth around the axon of the Schwann cell surface membrane. The myelin sheath, a lipoprotein complex whose abundant lipid component is partially removed during histological procedures, is constituted of tightly packed myelin sheath lamellae that wrap spirally around the axon in regular layers. At the end of the myelination process, the axon is left without communication with the external environment and is thus completely insulated during that particular myelin sheath.

The **Schwann cell** (or neurolemmocyte) belongs to the group of peripheral neuroglial cells. They contain nuclei in which heterochromatin and euchromatin are regularly alternated and are well visible under the light microscope. The most abundant cells of connective tissue, fibroblasts (but not their inactive counterparts, fibrocytes), in contrast to Schwann cells, possess larger and more rounded-off nuclei, and they occupy a larger area than Schwann cells. The shape of a fibroblast is irregular, and starlike, with various long cytoplasmic processes.

At the electron microscopic level, Schwann cells are identifiable by their relation to myelinated and nonmyelinated axons. In addition, they possess a basement membrane, and their cytoplasm is filled by mitochondria, Golgi apparatus, and other cell organelles as well as by secretory granules.

Probable questions;

1. What is a nerve fiber?
2. What is the difference between nerve fiber and neuron?
3. Give structural organization of a nerve fiber.

Suggested reading:

1. Randall, D. and Warren Burggren. Eckert Animal Physiology 4th edition. W.H. Freeman.

2. Sembulingam and Sembulingam (2012) Essentials of Medical Physiology. 6th Edn. Jaypee Pub, New Delhi

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Unit XII

Axon ultrastructure, neurotubules and neurofilaments, neural induction and neurogenesis

Objective: This unit provides a comprehensive explanation of the ultrastructure of axons, neurotubules, and neurofilaments, along with the topics of neural induction and neurogenesis. By the end of this unit, you will have a thorough understanding of the anatomy of axons, as well as the process of neural induction and neurogenesis. Moreover, you will also acquire knowledge about neurotubules and neurofilaments.

Axon

In 1860, the German anatomist Otto Friedrich Karl Deiters (1834-1863) described the basic structure of the nerve cell and identified two different protoplasmatic protrusions of the cell body that he termed "axis cylinder," and "protoplasmatic processes," respectively axons and dendrites. Axons are the elongated portion of the neuron located in the center of the cell between the soma and axon terminals. In size, the axon may represent over 95% of the total volume of the neuron.

Axons, the threadlike part of a nerve cell that conducts impulses, are both flexible and strong. Recent studies have shown that under the axonal membrane, rings composed of actin filaments (**Fig.1**) give the structure its flexibility. However, those studies had not been able to define the precise architecture of these rings. They are formed of long braided actin filaments.

Histological observation of the axon shows a cylindrical structure, but recent 3D electron microscopy studies demonstrated that it probably does not have the shape of a perfect cylinder. The diameter is variable as it ranges between 1 and 25 micrometers.

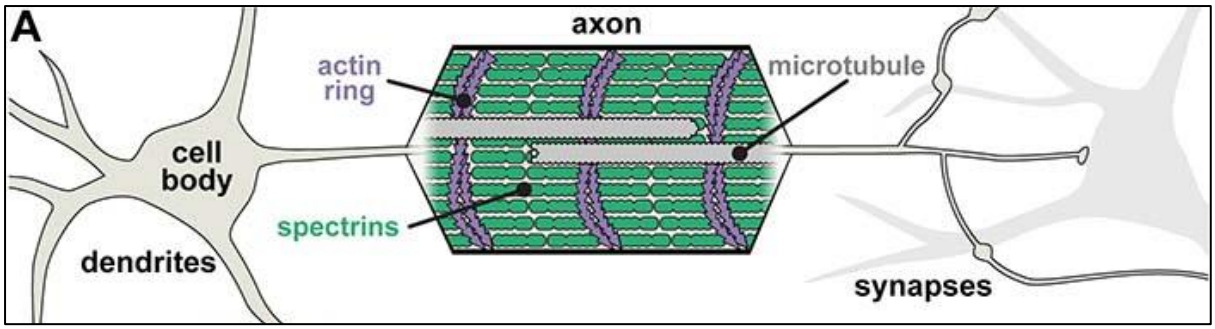

Fig.1 *Microscopic structure of an Axon*

The axon is devoid of a rough endoplasmic reticulum. As a result, traditional cellular stains such as the Nissl staining method, which identifies the so-called Nissl bodies (granules of the rough endoplasmic reticulum) are only able to stain the soma and dendrites but not the axon or axon hillock. The axon connects to the soma at a cone-like part of the structure known as the axon hillock. This part of the axon has considerable

functional importance since action potential originates here. In other words, this region of the neurolemma processes the incoming signals from other neurons.

Structural term

In structural terms, the axon hillock may contain fragments of Nissl substance. The axon hillock continues with the initial segment of the axone, located about 30 to 40 micrometers from the perikaryon and close to the first myelinated element. Structurally, in this axonal segment, the various axoplasmic elements begin to align longitudinally. Neurofilaments and mitochondria are present. Microtubules are also present, arranged into fascicles interconnected by sidearms. Furthermore, residual Nissl substance can persist.

Of note, the axolemma of the axonal region where action potential originates and starts its run shows a dense granular layer similar to that demonstrated at the nodes of Ranvier.

Axons are both structurally supportive of a neuron and the facilitators of communication both intra- and interneuronally. The diameter of a single axon is incredibly small and, therefore, commonly measured in micrometers with the average diameter of an axon being about 1 micrometer. A cluster of axons together forms a nerve. The axonal membrane is a phospholipid bilayer that has proteins embedded inside it. These voltage-gated ion channels facilitate the movement of ions in and out of the membrane and are critical to neuronal transmission.

The cytoskeleton elements (neurotubules and neurofilaments)

The axons have evolved a unique organisation of the cytoskeleton. It has an axi-symmetric structure with a central core of aligned microtubules arranged in a polar fashion and cross-linked by associated proteins (Fig.2). This core is surrounded by neurofilaments and a membrane-associated cortex of cross-linked actin filaments.

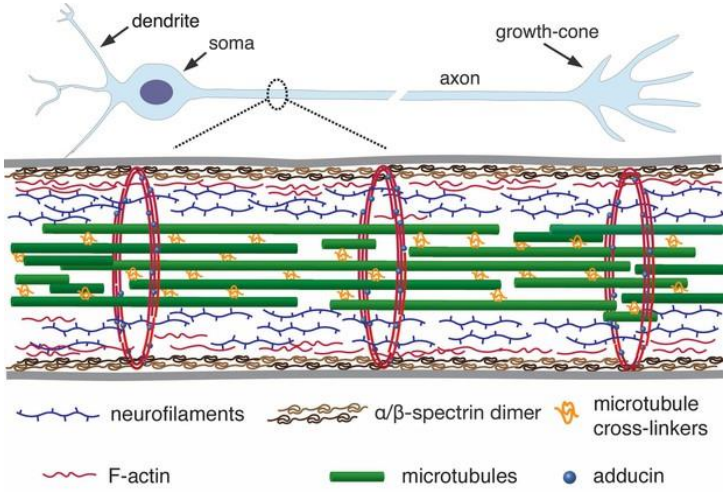

Fig.2 *A simplified schematic of the axonal cytoskeleton.*

Typically, the axonal core has a bundle of microtubules that are cross-linked by a variety of microtubule-associated proteins including tau (in some cases, a looser organization of microtubules interdispersed with neurofilaments is seen). This core is

surrounded by neurofilaments. The outermost scaffold has an array of periodically spaced rings composed of F-actin filaments. The actin rings are interconnected by α/β spectrin tetramers, which are aligned along the axonal axis (only tetramers in a cross-section are shown for clarity). Other cortical F-actin structures also exist. A myriad of proteins, including motor proteins (not shown), interconnect the various filaments, and also the membrane (grey lines) to the inner skeleton.

Difference in structure with dendrites

An important axonal structural element is the architecture of the cytoskeleton. Interestingly, this architecture is partly different in composition and organization from that of the dendrite. Although the cytoskeletal proteins are mainly synthesized in the cell body and then transported to their various cellular sites, the dendritic microtubule-associated protein 2 (MAP2) is synthesized directly in the dendritic compartment. On the contrary, due to the lack of a protein synthetic apparatus, the axonal microtubules are synthesized and organized in the soma (by the microtubule-organizing center, MTOC) and then in the axon compartment. Furthermore, MAP2 is not present in the axonal compartment. Another difference concerns the microtubule polarity. While dendritic microtubules show a mixed polarity with both plus and minus ends distal to the cell body, the axon microtubules, have the plus end distal to the cell body. Again, research has demonstrated that axonal and dendritic microtubules differ in the different quantities of tau proteins and the different degrees of phosphorylation. Finally, although neurofilaments are abundant in the axons, they are scarce in the dendritic site.

Neural induction and neurogenesis

The increasingly sophisticated effort to understand exactly how neural inductive signals work has therefore focused on molecules that can modify patterns of gene expression. An instructive example is retinoic acid, a derivative of vitamin A and a member of the steroid/thyroid superfamily of hormones. Retinoic acid activates a unique class of transcription factors—the retinoid receptors—that modulate the expression of several target genes. Peptide hormones provide another class of inductive signals, including those that belong to the fibroblast growth factor (**FGF**) and transforming growth factor (**TGF**) families. Another peptide hormone essential for neural induction is the **sonic hedgehog** (**shh**). These molecules, like retinoic acid, are produced by a variety of embryonic tissues including the notochord, the floorplate, and the neural ectoderm itself; they bind to cell surface receptors, many of which are protein kinases. Some of these molecular signals have been implicated in determining the fates of specific classes of cells in the developing nervous system **(Fig.3**). For example, shh is essential for the differentiation of motor neurons in the ventral spinal cord, whereas a TGF family molecule called *dorsalin* is important for the establishment of dorsal cells in the spinal cord—including the neural crest. Signaling via these peptide hormones activates a cascade of subsequent gene expression in ectodermal cells. In general, if the signaling mediated by any of these molecules is disrupted, the early development of the nervous system is compromised.

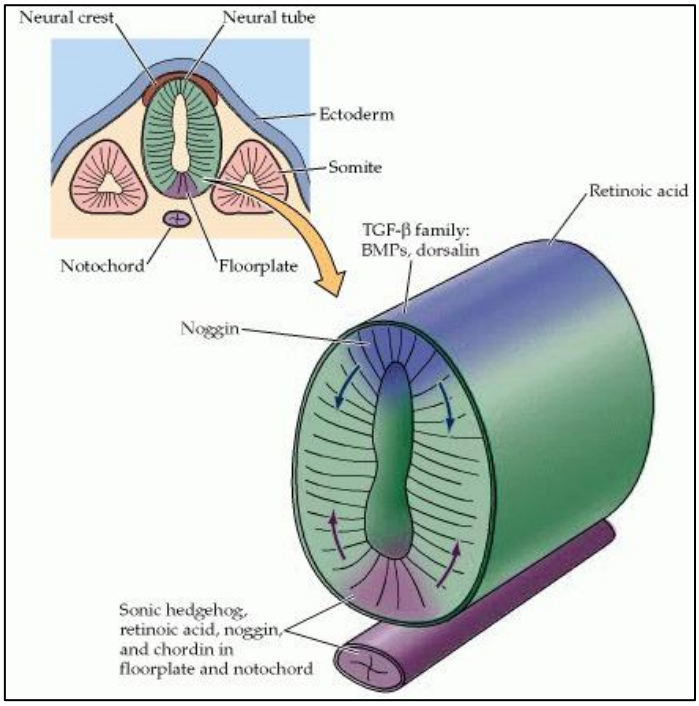

Fig.3. Location of some inductive signals in the developing neural tube. (*Inductive signals are provided by either the notochord, the floorplate, the roofplate and dorsal ectoderm, or the somites. These signals act locally on either the ventral or dorsal neuroepithelium of the developing spinal cord and hindbrain to elicit distinct patterns of gene expression and, ultimately, differentiation of specific classes of neurons. The peptide hormone sonic hedgehog (shh) is the most important ventral signal and is produced by both the notochord and floorplate. In addition, noggin, chordin, and retinoic acid are produced either by the notochord or floorplate. In contrast, a variety of signals including dorsalin and other members of the TGF family as well as noggin and retinoic acid are provided by the roofplate and dorsal ectoderm. These signals influence the differentiation of several dorsal cell types including the neural crest.*)

A particularly intriguing aspect of molecular signals that influence neural induction is the mechanism by which one class of inductive signals—the bone morphogenetic proteins, or BMPs—cause neural differentiation. As the name suggests, these peptide hormones, which are members of the TGF-β family, elicit osteogenesis from mesodermal cells. If ectodermal cells are exposed to BMP, they assume an epidermal fate. How then does the ectoderm manage to become neuralized, especially since BMPs are produced by the notochord, floorplate, and somites? All of these structures are in position to signal to the neuroectoderm, and therefore to convert it to epidermis. This epidermal fate is evidently avoided in the neural plate by the local activity of other inductive signaling molecules called noggin and chordin. Both of these molecules bind directly to the BMPs and thus prevent their binding to BMP receptors. In this way, the neuroectoderm is “rescued” from becoming epidermis. Such negative regulation has led

to the conclusion that becoming a neuron is the “default” fate for embryonic ectodermal cells.

Probable questions:

1. Give detail structure of neuron with suitable diagram.
2. What is cell body?
3. Discuss the ultra-structure of axon.
4. Write short notes on neurotubules.
5. Write short notes on neurofilaments.
6. What are the cytoskeletal elements of the axons?
7. How does neural induction take place? Name different genes involved in this phenomenon.

Suggested reading:

1. Randall, D. and Warren Burggren. Eckert Animal Physiology 4th edition. W.H. Freeman.

2. Sembulingam and Sembulingam (2012) Essentials of Medical Physiology. 6th Edn. Jaypee Pub, New Delhi

3. Ganong’s Review of Medical Physiology; McGraw Hill

1. Guyton, A.C. and Hall, J.E.; 2011. Textbook of Medical Physiology, XII Edition, Saunders Company

2. Hall JE. 2015. Guyton and Hall Textbook of Medical Physiology. Saunders publication.

Unit XIII

Emergence of central nervous system, neural tube polarity, program cell death during neuronal development

Objective: This unit is dedicated to the study of the central nervous system (CNS) and its emergence. We will be exploring the polarity of the neural tube and the process of programmed cell death in neural development. By delving into this topic, readers will gain a better understanding of how the CNS develops, how the neural tube's polarity is determined, and the role of programmed cell death in neural development.

Central Nervous System (CNS)

The CNS system involves 3 germinal layers: ectoderm, mesoderm, and endoderm.

1. The ectoderm is the only key initiating player in the embryogenesis of the CNS. The ectoderm is further sub-specialized as the (1) surface ectoderm, which differentiates into the epidermis, nails, and hair. The ectoderm is also sub-specialized to form the (2) neural ectoderm, which gives rise to the neural tube and neural crest, which subsequently gives rise to the brain, spinal cord, and peripheral nerves.
2. The endoderm and the mesoderm gives rise other organs of the body.

Development

Beginning with the trilaminar germ disc, which refers to the epiblast and hypoblast, the epiblast cells undergo an epithelial-mesenchymal transition that replaces the hypoblast. They also proliferate in the middle layer to form the mesoderm where it will remain mesenchymal to form connective tissue. The primitive streak then starts to appear superiorly from the thickened region of the ectoderm. It grows caudal to the cranial and induces notochord formation. The ectoderm then invaginates as cells migrate to form the primitive node and primitive pit where the notochordal process is formed.

1. The *primitive pit* is a depression at the center of the primitive node, which is an opening in the notochordal canal.
2. *Neurulation* refers to the folding of the neural plate. The neural plate folds, via induction from the notochord, into the neural tube, which then becomes the neuroectoderm, which finally forms the CNS, namely the brain and spinal cord; the brain from cranial two-thirds of the segment and spinal cord from caudal one-third of the segment)
3. *Neural Crest cells* form dorsal root ganglia and connective tissue in the head and neck. The CNS is derived from the neuroectoderm: notochord induces the formation of the neural plate (thickening of the ectodermal layer), which further differentiates to form neural folds with a neural groove in between, leading to the formation of the neural tube (via neurulation).

Spinal Cord

The spinal cord is formed from the neural plate, which now contains 3 layers:

1. Ventricular layer that lines the central canal
2. Mantle layer that contains neuronal bodies, which will eventually form the gray matter
3. Marginal layer that contains axons, and will eventually form the white matter

Three membranous layers cover the whole CNS:

1. Dura mater: derived from surrounding mesenchyme and is tough and durable.
2. Arachnoid mater: derived from neural crest; forms as a single layer with Pia mater.
3. Pia mater: derived from neural crest; intimately covers the CNS.

Brain

During brain formation, 3 primary brain vesicles differentiate into 5 secondary brain vesicles. (**Fig.1**)

1. Prosencephalon, which becomes the forebrain: This later develops into the cerebral hemispheres which contain structures underneath such as the epithalamus, thalamus, and hypothalamus. This section of the brain is responsible for consciousness, sensorimotor transformation, and sensory integration.
2. Mesencephalon, which becomes the midbrain: This part of the brain undergoes little structure reorganization compared to the spinal cord and other brain vesicles.
3. Rhombencephalon, which becomes the hindbrain: This part can be further divided into 3 segments:
   i. Metencephalon: The dorsal growth of the cerebellum (integrates sensory information to fine-tune output)
   ii. Caudal myelencephalon: Similar to the structure of the spinal cord with a "closed" central canal of the Medulla
   iii. Rostral myelencephalon: "Open part" of medulla; cerebrospinal fluid (CNF) is produced via choroid plexus and leaks into the subarachnoid space.

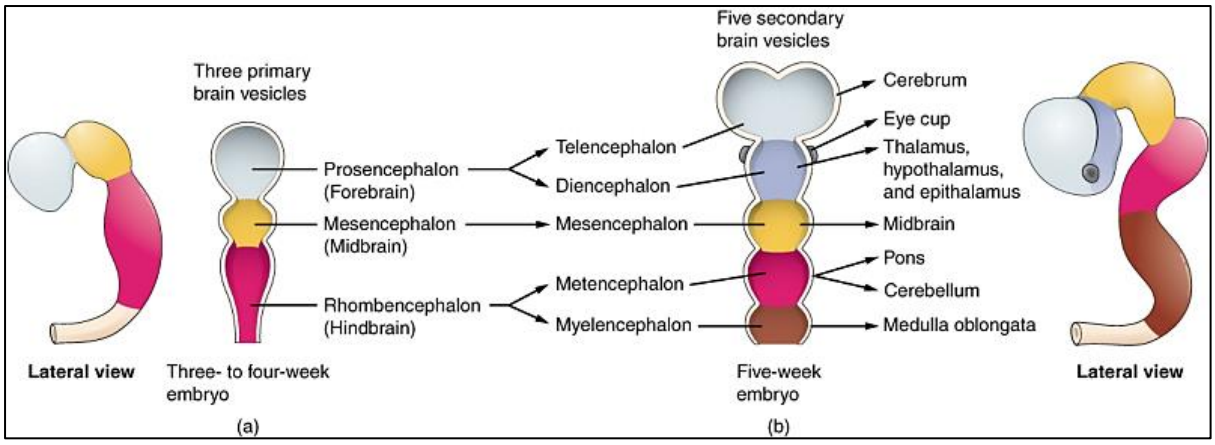

Fig.1. *Brain vesicles*

During this time in development, certain **genes** become vital in ensuring accurate structural layout of the CNS: Sonic hedgehog (Shh), the Pax genes, bone morphogenic proteins, and a transforming growth factor (TGF-B) called dorsalin. These components are all influential in the appropriate dorsoventral patterning of the developing neural tube.

Polarity

The central nervous system of vertebrates consists of a hollow neural tube. In higher vertebrates, the neural tube emerges from two distinct types of events called primary and secondary neurulation. Primary neurulation or neural tube closure (NTC) involves the transformation of a flat neural plate into a hollow tube and forms the brain and much of spinal cord. Secondary neurulation hollows a solid nerve cord to form the caudal-most portion of the spinal cord.

Primary neurulation involves a series of shape changes in the neural plate, beginning with apicobasal thickening or cell elongation along the apicobasal axis of the neural plate (**Fig. 2A);** This is followed by the formation of a wedge-shaped tissue at the future ventral midline, the median hinge point (MHP; **Fig. 2B**). Here, polarized cell behaviors and MHP's association with the subjacent notochord, jointly generate forces, which buckle the ventral midline and elevate the neural folds on either side.

A pair of dorsolateral hinge points (DLHP) forms at some axial levels of the neural plate (**Fig. 2C**) Cell behaviors similar to MHP occur at the DLHP and help bend the neural folds so that they pivot toward each other. Like the MHP, the DLHP also associates with adjacent tissues, in this case, the surface ectoderm (**Fig. 2C**). The intrinsic forces generated by polarized cell behaviors at the DLHP, and the extrinsic forces applied by the surface ectoderm, together help oppose the neural folds so that dorsal midline fusion can be accomplished (**Fig. 2D**).

The final steps in NTC involve the dorsal midline fusion of the neural folds and the surface ectoderm (**Fig. 2D**).

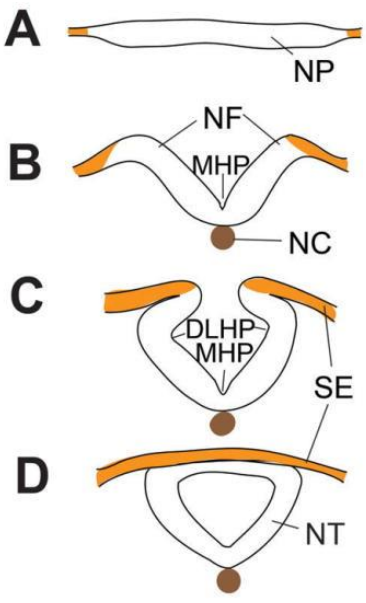

Fig 2 (A-D) **Neural tube closure**

(A–D) Neural tube closure events shown in cross-sectional view. **(A)** Apicobasal thickening of the neural plate prior to neural tube closure (NTC). **(B)** Median hinge point (MHP) formation at the ventral midline of the neural plate. Note the association of the notochord (NC) with the MHP and the elevation of the neural folds (NF) above the ventral midline. **(C)** Formation of the dorsolateral hinge point (DLHP) and the association of the NP with the surface ectoderm (SE). **(D)** Dorsal midline fusion of the neural plate and SE.

Programme cell death

During nervous system development, about one-and-a-half times the adult number of neurons are created. These "extra" neurons are then destroyed or commit suicide. This process of programmed cell death occurs through a series of events termed apoptosis and is an appropriate and essential event during brain development.

1. Apoptosis by the extrinsic/death receptor pathway

Kerr et al.(1972) first described apoptosis in terms of morphological changes which include chromatin condensation, nuclear membrane breakdown, cell shrinkage, and formation of small vesicular bodies near the cell surface, named apoptotic bodies.

Apoptosis is triggered by two principal pathways: the intrinsic (or mitochondrial) pathway and the extrinsic (or death receptor) pathway (**Fig.3**). The extrinsic apoptosis pathway is triggered by the ligation of tumor necrosis factor (TNF)-family death receptors at the cell surface. Receptor ligation can result in the recruitment of Fas-associated death domain protein (FADD), which in turn binds pro-caspase-8 molecules, allowing autoproteolytic processing and activation of caspase-8 to occur. Once activated, caspase-8 may in turn activate downstream effector caspases by direct proteolytic cleavage or indirectly by cleavage of the BH3-only protein Bid to produce tBid which translocates to mitochondria to induce Bax activation and mitochondrial outer membrane permeabilization (MOMP). TNF-α and Fas ligand can induce apoptosis of some neurons during inflammatio, and a Fas-dependent apoptotic pathway was described for motor neurons, involving p38, nitric oxide (NO), and from thence classical caspase-dependent apoptosis.

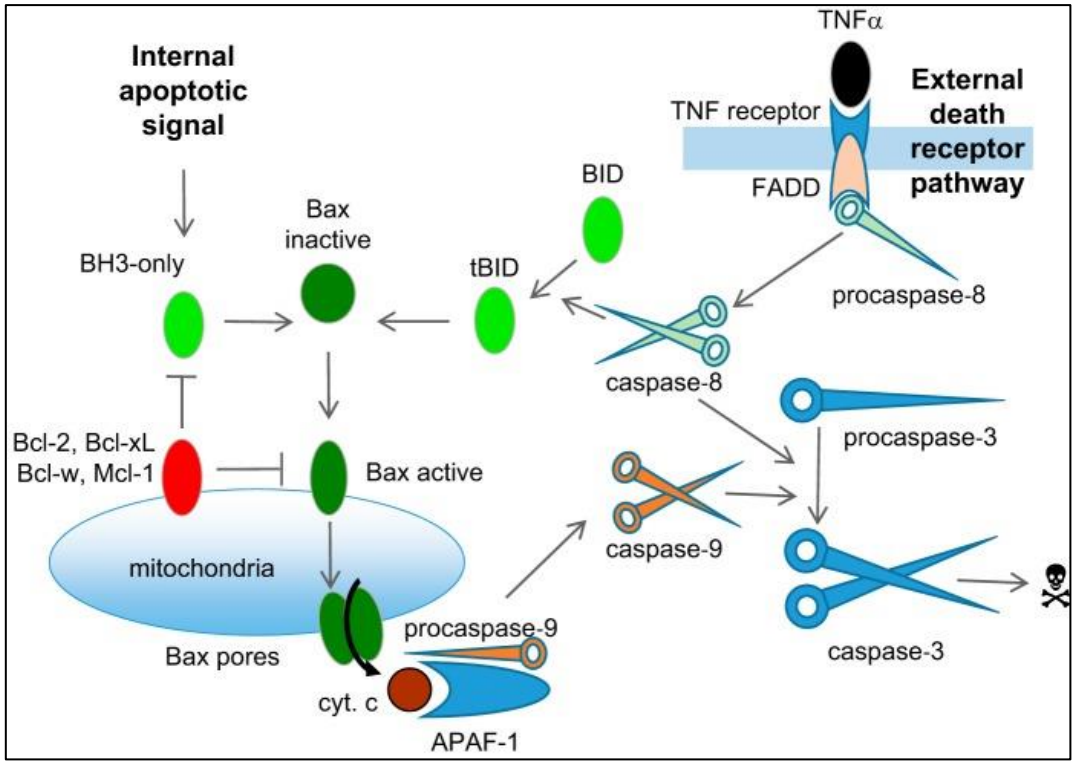

Fig.3 Overview of apoptosis. *The internal (mitochondrial) pathway of apoptosis is triggered within the cell, causing expression or activation of BH3-only proteins that activate Bax (and/or Bak in some cells) to form pores in the outer mitochondrial membrane, releasing cytochrome c to bind APAF-1, activating caspase-9 to cleave and activate downstream caspases, which degrades cellular proteins. The external (death receptor) pathway starts outside the cell with death ligands activating death receptors to activate caspase-8, which either cleaves downstream caspases or cleaves and activates the BH3-only protein Bid. Anti-apoptotic proteins, such as Bcl-2, hold inactive Bax or BH3-ony proteins.*

Apoptosis by the intrinsic/mitochondrial pathway

The intrinsic apoptosis pathway centers on the regulation of MOMP by the Bcl-2 family proteins (**Fig.3 and 4**). Members of the Bcl-2 family share homology within at least one of up to four Bcl-2 homology (BH) domains which are required for the homo- and heterotypic interactions that determine the decision to undergo MOMP. The pro-apoptotic members Bax and Bak contain BH1–3 and are thought to be almost entirely essential for the execution of apoptosis via the intrinsic pathway, although a novel but as yet unknown Bax/Bak-independent intrinsic apoptotic pathway has recently been reported. Neurons are unique because Bak is expressed as an alternately transcribed product (N-Bak) that is translationally repressed and does not participate in apoptosis. Thus the induction of intrinsic apoptosis in neurons is entirely dependent on Bax expression and activation, and indeed deletion and inhibition of Bax prevent aberrant neuronal cell death in several in vitro and in vivo models of neurodegeneration.

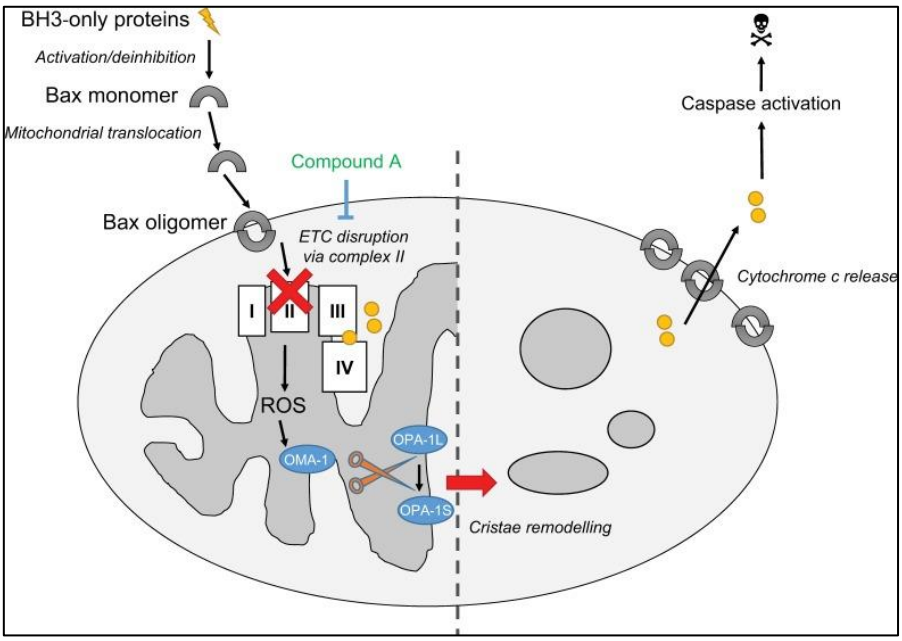

Fig.4 *Bax signaling at the mitochondria. BH3-only proteins activate Bax to oligomerize and form pores in the outer mitochondrial membrane, causing cytochrome c release and inhibition of complex II, inhibition of respiration and ROS production, activating the protease OMA-1 to remodel the inner mitochondrial membrane, which enables greater cytochrome c release, which triggers caspase activation and apoptosis.*

Of note, Bok, a Bcl-2 family member, was recently described to induce MOMP and apoptosis in non-neuronal cells following disruption of proteasome or the endoplasmic reticulum-associated degradation pathway. Unlike Bax and Bak, Bok appears unresponsive to signals from other Bcl-2 family proteins, is constitutively active, and is regulated primarily through degradation. The role of Bok in neurons is unclear. While it is highly expressed in the mouse brain, its expression doesn't seem to be necessary even in the absence of Bax expression, for proteasome- or excitotoxicity-induced neuronal cell death,

Probable questions:

1. How central nervous system develops?
2. State determination of neural tube polarity.
3. Express through a schematic diagram neural tube apoptosis.
4. Explain pathways of neural cell apoptosis.

Suggested reading:

1. Randall, D. and Warren Burggren. Eckert Animal Physiology 4th edition. W.H. Freeman.

2. Sembulingam and Sembulingam (2012) Essentials of Medical Physiology. 6th Edn. Jaypee Pub, New Delhi

3. Ganong's Review of Medical Physiology; McGraw Hill

3. Guyton, A.C. and Hall, J.E.; 2011. Textbook of Medical Physiology, XII Edition, Saunders Company

4. Hall JE. 2015. Guyton and Hall Textbook of Medical Physiology. Saunders publication.

Unit XIV

Common neuronal disorders

Objective: In this unit we will discuss about Common neuronal disorders.

Introduction

According to the World Health Organisation (WHO), neurological disorders are any diseases affecting the entirety of the nervous system. These are conditions affecting the Central Nervous System's (CNS) neurons or tracts in the Spinal Cord or in the whole brain or one of its constituents e.g. Cerebrum (Cortex), Basal Ganglia, Diencephalon, Brain stem (Midbrain, Pons and Medulla Oblongata), Cerebellum. In addition to conditions affecting the Peripheral Nervous System involving Cranial nerves or their nuclei, Spinal plexuses, peripheral nerves, nerve roots, autonomic nervous system, neuromuscular junction and muscles

Common Neurological Disorders and Their Symptom

some common neurological disorders and their symptoms. Migraines are intense headaches often accompanied by nausea and sensitivity to light and sound. Various factors, including stress, certain foods, or hormonal changes, can trigger them.

1. **Migraines**

Migraines are intense headaches often accompanied by nausea and sensitivity to light and sound. Various factors, including stress, certain foods, or hormonal changes, can trigger them.

Symptoms:

- Throbbing head pain, usually on one side
- Nausea and vomiting
- Visual disturbances (called aura)
- Sensitivity to light and sound

2. **Epilepsy**

Recurrent seizures, which can range in severity from mild to severe, are characteristic of the illness known as epilepsy. Seizures happen due to abnormal electrical activity in the brain.

Symptoms:

- Uncontrolled shaking or convulsions

- Loss of consciousness
- Confusion after the seizure

3. Alzheimer's Disease

Alzheimer's disease is a progressive brain disorder that affects memory, thinking, and behaviour. It is a type of dementia.

Symptoms:

- Memory loss, especially recent events
- Difficulty in problem-solving
- Personality changes
- Confusion

4. Parkinson's Disease

Parkinson's disease affects movement and can lead to tremors, stiffness, and difficulty with balance and coordination.

Symptoms:

- Tremors (shaking)
- Stiffness in muscles
- Slowed movements
- Balance problems

5. Multiple Sclerosis (MS)

Multiple sclerosis is an autoimmune disorder that damages the protective covering of nerve fibres in the brain and spinal cord.

Symptoms:

- Fatigue
- Muscle weakness
- Problems with coordination and balance
- Vision problems

If you are undergoing these kinds of neurological disorders symptoms, it is essential to get a consultation from the best neuro doctor in Delhi.

Causes of Neurological Disorders

1. **Genetics:** Our parents may have passed on some neurological conditions to us. These disorders are more likely to occur if specific genes are defective or mutated. Muscular dystrophy and Huntington's disease are two examples of such illnesses.
2. **Infections:** Infections like meningitis or encephalitis can harm and inflame the nervous system. Bacteria or viruses can bring on these infections and, if not treated right away, could result in long-term neurological issues.
3. **Trauma:** Head injuries or other physical trauma can damage the brain and nerves, which can cause neurological problems. Even a moderate concussion can negatively impact brain function.
4. **Toxins:** Exposure to certain chemicals, heavy metals, or drugs can damage the nervous system over time. Lead poisoning and drug abuse are examples of toxin-related causes of **neurological disorders**.
5. **Autoimmune Disorders:** Sometimes, the immune system mistakenly attacks the nervous system. Conditions like multiple sclerosis fall into this category.
6. **Degenerative Diseases:** These are conditions where the nervous system deteriorates over time. Alzheimer's disease and Parkinson's disease are common examples.

Probable questions:

1. What are some common early signs of neurological disorders?
2. What are the symptoms of Perkinson's disease.

Suggested readings:

7. Karp, G. (2008). Cell and Molecular biology: Concepts and Application. 5th Edn, John Wiley.
8. Lodish, Berk, Matsudaira, Kaiser, Bretscher, Ploegh, Amon, and Martin (2016) Molecular Cell Biology. 8th Edn. W.H. Freeman

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