Post-Graduate Degree Programme
(CBCS)
in
ZOOLOGY
(M.Sc. Programme)

SEMESTER-III

Cell and Development Biology

ZDSE(MJ)T-302

Self-Learning Material

DIRECTORATE OF OPEN AND DISTANCE LEARNING
UNIVERSITY OF KALYANI
Kalyani, Nadia
West Bengal, India

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Acknowledgements:

The author thankfully acknowledges all the faculty members of Department of Zoology, University of Kalyani for their academic contribution and valuable suggestions regarding the preparation of Self Learning Material.

MAY 2023

Directorate of Open and Distance Learning, University of Kalyani.

Published by the Directorate of Open and Distance Learning,
University of Kalyani, Kalyani-741235, West Bengal.

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-Chancellor has 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.) Manas Kumar Sanyal, 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.

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Theory (Discipline Specific Elective – Major II) - [ZDSE(MJ)T-302]

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	X	Differentiation: Cell aggregation and differentiation in *Dictyostelium*		
	XI	FTIR based identification of early lineage commitment in differentiating cells		
	XII	Reversibility of differentiated state, criteria for dedifferentiation, metaplasia and transdifferentiation, modulation		
	XIII	Neural crest cell migration based differentiation		
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	Total counseling session 12hrs.			

UNIT I

Morphogenesis: Morphogenetic processes, cell size and shape, Cell fusion

Objective: In this unit we will discuss about Morphogenetic processes, cell size and shape, Cell fusion.

Introduction

Morphogenesis, the shaping of an organism by embryological processes of differentiation of cells, tissues, and organs and the development of organ systems according to the genetic "blueprint" of the potential organism and environmental conditions.

Plant morphogenesis is brought about chiefly through differential growth. Permanent embryonic tissue results in a morphogenetic potential that varies greatly with the environment and continues to produce new organs throughout the life of the plant. Animal morphogenesis is accomplished by growth and by cell movement. A fixed pattern is established early; the organism is determined as to shape, size, and organ complement. Once organs are formed, no new ones (with few exceptions) are produced.

Morphogenetic process

I. Morphogenesis by differential growth

After their initiation, the various organs and regions of an organism may increase in size at different rates. Such processes of differential growth will change the overall shape of the body in which they occur. Processes of this kind take place very commonly in animals, particularly in the later stages of development. They are of major importance in the morphogenesis of plants, where the overall shape of the plant, the shape of individual leaves, and so on, depends primarily on the rates of growth of such component elements as the stems, the lateral shoots, and the vein and intervein material in leaves. In both animals and plants, such growth processes are greatly influenced by a variety of hormones. It is probable that factors internal to individual cells also always play a role.

Although differential growth may produce striking alterations in the general shape of organisms, these effects should probably be considered as somewhat superficial, since they only modify a basic pattern laid down by other processes. In a plant, for instance, the fundamental pattern is determined by the arrangement of the lateral buds around

the central growing stem; whether these buds then grow fast or slowly relative to the stem is a secondary matter, however striking its results may be.

II. Morphogenetic fields

Many fundamental processes of pattern formation (*e.g.,* the arrangement of lateral buds in growing plants) occur within areas or three-dimensional masses of tissue that show no obvious indications of where the various elements in the pattern will arise until they actually appear. Such masses of tissue, in which a pattern appears, have been spoken of as "fields." This word was originally used in the early years of the 20th century by German authors who suggested an analogy between biological morphogenetic fields and such physical entities as magnetic or electromagnetic fields. The biological field is a description, but not an explanation, of the way in which the developing system behaves. The system develops as though each cell or subunit within it possessed "positional information" that specifies its location within the field and a set of instructions that lays down the developmental behaviour appropriate to each position.

There have been several attempts to account for the nature of the positional information and of the corresponding instructions. The oldest and best known of these is the gradient hypothesis. In many fields there is some region that is in some way "dominant," so that the field appears as though organized around it. It is suggested that this region has a high concentration of some substance or activity, which falls off in a graded way throughout the rest of the field. The main deficiency of the hypothesis is that no one has yet succeeded in identifying satisfactorily the variables distributed in the gradients. Attempts to suppose that they are gradients of metabolic activity have, on investigation, always run into difficulties that can only be solved by defining metabolic activity in terms that reduce the hypothesis to a circular one in which metabolic activity is defined as that which is distributed in the gradient.

Recently, a new suggestion has been advanced concerning position information. Most processes within cells normally involve negative feedback control systems. These systems have a tendency to oscillate, or fluctuate regularly. In fact, any aspect of cell metabolism may be basically oscillatory in character; the cycle of cell growth and division may be only one example of a much more widespread phenomenon. The substances involved in these oscillations are likely to include diffusible molecules capable of influencing the behaviour of nearby cells. It is easy to envisage the possibility that there might be localized regions with oscillations of higher frequency or greater amplitude that act as centres from which trains of waves are radiated in all directions. It has been suggested that positional information is specified in terms of differences in phase between two or more such trains of transmitted oscillations.

Certain types of field phenomenon may involve an amplification of stochastic (random) variations. In systems containing a number of substances, with certain suitable rates of reaction and diffusion, chance variation on either side of an initial condition of equilibrium may become amplified both in amplitude and in the area involved. In this

way, the processes may give rise to a pattern of differentiated areas, distributed in arrangements that depend on the boundary conditions.

III. Morphogenesis by the self-assembly of units

Complex structures may arise from the interaction between units that have characteristics such that they can fit together in a certain way. This is particularly appropriate for morphogenesis at the simple level of molecules or cells. Units such as the atoms of carbon, hydrogen, oxygen, nitrogen, and so on, can assemble themselves into orderly molecular structures, and larger molecules, such as those of tropocollagen, or protein subunits in general, can assemble themselves into complexes whose structure is dependent on localized and directional intermolecular forces. It seems that such comparatively large entities as the units that come together to form the head structures of bacteriophages or bacterial flagella are capable of orderly self-assembly, but the chemical forces that give rise to the interunit bonds are still little understood.

Processes that fall into the same general category as self-assembly may occur within aggregates of cells. The units that self-assemble are the cells themselves. Interaction and aggregation may be allowed to occur in assemblages of cells of one or more different kinds. In such cases it is commonly found that the originally isolated cells tend to adhere to one another, at first more or less at random and independently of their character, but later they become rearranged into a number of regions consisting of cells of a single kind. When the cells in the initial collection differ in two different characteristics, for instance in species and organ of origin, the assortment in some cases brings together cells from the same organ, in other cases cells from the same species. Mixtures of chick and mouse cells, for instance, reassort themselves into groups derived from the same organ, whereas cells from two different species of amphibia sort out into groups from the same species more or less independently of organ type.

This morphogenetic process probably has only a restricted application to the formation of structures in normal development, in which only in a few tissues (*e.g.,* the connective system) do cells ever pass through a free stage in which they are not in intimate contact with other cells, and cells of different origin do not normally become intermingled so as to call for processes of reassortment. To explain normal morphogenetic processes of plants and animals one must look to the results that can be produced by the differential behaviour of cells that remain in constant close contact with one another. Several authors have shown how striking morphogenetic changes could be produced within a mass of cells that remain in contact, but that undergo changes in the intensity of adhesion between neighbouring cells, in the area of surface in the proportion to cell volume, and so on.

IV. Differentiation

Differentiation is simply the process of becoming different. If, in connection with biological development, morphogenesis is set aside as a component for separate consideration, there are two distinct types of differentiation. In the first type, a part of a developing system will change in character as time passes; for instance, a part of the mesoderm, starting as embryonic cells with little internal features, gradually develops striated myofilaments, and with a lapse of time develops into a fully formed muscle fibre. In the second type, space rather than time is involved; for instance, other cells within the same mass of embryonic mesoderm may start to lay down an external matrix around them and eventually develop into cartilage. In development, differentiation in time involves the production of the characteristic features of the adult tissues, and is referred to as histogenesis. Differentiation in space involves an initially similar (homogeneous) mass of tissue becoming separated into different regions and is referred to as regionalization.

Histogenesis involves the synthesis of a number of new protein species according to an appropriate timetable. The most easily characterized are those proteins formed in a relatively late stage of histogenesis, such as myosin and actin in muscle cells. The synthesis of proteins is under the control of genes, and the problem of histogenesis essentially reduces to that of the genetic mechanisms that direct protein synthesis.

Regionalization is concerned with the appearance of differences between various parts of what is at first a homogeneous, or nearly homogeneous, mass. It is a prelude to histogenesis, which then proceeds in various directions in the different regions so demarcated. The processes by which the different regions acquire distinct contrasting characteristics must be related to some of the processes discussed under morphogenesis. Unlike morphogenesis, regionalization need not involve any change in the overall spatial shape of the tissues undergoing it. Regionalization falls rather into the type of process for which field theories have been invoked.

Cell Shape:

The variety of cell shapes seen in prokaryotes and eukaryotes reflects the functions that each cell has, confirming the structure-function relationship seen throughout biology. Each cell type has evolved a shape that is best related to its function. For example, the neuron in Figure below has long, thin extensions (axons and dendrites) that reach out to other nerve cells. The extensions help the neuron pass chemical and electrical messages quickly through the body. The shapes of the red blood cells (erythrocytes) enable these cells to easily move through capillaries. The spikes on the pollen grain help it stick to a pollinating insect or animal so that it can be transferred to and pollinate another flower. The long whip-like flagella (tails) of the algae *Chlamydomonas* help it swim in water. Different cells within a single organism can come in a variety of sizes and shapes. They may not be very big, but their shapes can be very different from each

other. However, these cells all have common abilities, such as obtaining and using food energy, responding to the external environment, and reproducing. In part, a cell's shape determines its function.

Cell Size:

If cells are the main structural and functional unit of an organism, why are they so small? And why are there no organisms with huge cells? The answers to these questions lie in a cell's need for fast, easy food. The need to be able to pass nutrients and gases into and out of the cell sets a limit on how big cells can be. The larger a cell gets, the more difficult it is for nutrients and gases to move in and out of the cell.

As a cell grows, its volume increases more quickly than its surface area. If a cell was to get very large, the small surface area would not allow enough nutrients to enter the cell quickly enough for the cell's needs. However, large cells have a way of dealing with some size challenges. Big cells, such as some white blood cells, often grow more nuclei so that they can supply enough proteins and RNA for the cell's requirements. Large, metabolically active cells often have lots of cell protrusions, resulting in many folds throughout the membrane. These folds increase the surface area available for transport of materials into or out of the cell. Such cell types are found lining your small intestine, where they absorb nutrients from your food through protrusions called microvilli**.**

Scale of Measurements

- ✓ 1 centimeter (cm) = 10 millimeters (mm) = 10^{-2} meters (m)
- ✓ 1 mm = 1000 micrometers (μm) = 10^{-3} m
- ✓ 1 μm = 1000 nanometers (nm) = 10^{-6} m
- ✓ 1 nm = 10^{-3} μm

An increased surface area to volume ratio means increased exposure to the environment. This means that nutrients and gases can move in and out of a small cell more easily than in and out of a larger cell.

The cells you have learned about so far are much smaller than the period at the end of this sentence, so they are normally measured on a very small scale. The smallest prokaryotic cell currently known has a diameter of only 400 nm. Eukaryotic cells normally range between 1– 100μm in diameter. The mouse cells in Figure above are about 10 μm in diameter. One exception, however, is eggs. Eggs contain the largest known single cell, and the ostrich egg is the largest of them all. The ostrich egg in Figure above is over 10,000 times larger than the mouse cell.

Cell Fusion

Cell-cell fusion refers to the process by which two or more cells combine their plasma membranes to become a single hybrid cell containing DNA from each parent cell [1]. This fundamental biological process has been well documented in many organisms, including plants [2], yeast [3], *C. elegans* [4], *D. melanogaster* [5], and higher eukaryotes [6]. The functional consequence of cell-cell fusion is the formation of a hybrid cell that can maintain genotypic and phenotypic properties of both parent cells. In this sense, cell-cell fusion is a robust mediator of cellular reprogramming that can lead to the creation of cells with novel properties.

There are two different types of cell fusion that can occur. These two types include homotypic and heterotypic cell fusion.

- **Homotypic cell fusion** occurs between cells of the same type. An example of this would be osteoclasts or myofibers being fusing together with their respective type of cells. Whenever the two nuclei merge a synkaryon is produced. Cell fusion normally occurs with nuclear fusion, but in the absence of nuclear fusion, the cell would be described as a binucleated heterokaryon. A heterokaryon is the melding of two or more cells into one and it may reproduce itself for several generations. If two of the same types of cells fuse, but their nuclei do not fuse, then the resulting cell is called a syncytium.

- **Heterotypic cell fusion** occurs between cells of different types, making it the exact opposite of homotypic cell fusion. The result of this fusion is also a synkaryon produced by the merging of the nuclei, and a binucleated heterokaryon in the absence of nuclear fusion. An example of this would be Bone Marrow Derived Cells (BMDCs) being fused with parenchymatous organs.

Cell fusion method

There are four methods that cell biologists and biophysicists use to fuse cells. These four ways include electrical cell fusion, polyethylene glycol cell fusion, and sendai virus induced cell fusion and a newly developed method termed optically controlled thermoplasmonics.

1. **Electrical cell fusion** is an essential step in some of the most innovative methods in modern biology. This method begins when two cells are brought into contact by dielectrophoresis. Dielectrophoresis uses a high frequency alternating current, unlike electrophoresis in which a direct current is applied. Once the cells are brought together, a pulsed voltage is applied. The pulse voltage causes the cell membrane to permeate and subsequent combining of the membranes and the cells then fuse. After this, alternative voltage is applied for a brief period of time to stabilize the process. The result of this is that the cytoplasm has mixed together and

the cell membrane has completely fused. All that remains separate is the nuclei, which will fuse at a later time within the cell, making the result a heterokaryon cell.

2. **Polyethylene glycol cell fusion** is the simplest, but most toxic, way to fuse cells. In this type of cell fusion polyethylene glycol, PEG, acts as a dehydrating agent and fuses not only plasma membranes but also intracellular membranes. This leads to cell fusion since PEG induces cell agglutination and cell-to-cell contact. Though this type of cell fusion is the most widely used, it still has downfalls. Oftentimes PEG can cause uncontrollable fusion of multiple cells, leading to the appearance of giant polykaryons. Also, standard PEG cell fusion is poorly reproducible and different types of cells have various fusion susceptibilities. This type of cell fusion is widely used for the production of somatic cell hybrids and for nuclear transfer in mammalian cloning.

3. **Sendai virus induced cell fusion** occurs in four different temperature stages. During the first stage, which lasts no longer than 10 minutes, viral adsorption takes place and the adsorbed virus can be inhibited by viral antibodies. The second stage, which is 20 minutes, is pH dependent and an addition of viral antiserum can still inhibit ultimate fusion. In the third, antibody-refractory stage, viral envelope constituents remain detectable on the surface of cells. During the fourth stage, cell fusion becomes evident and HA neuraminidase and fusion factor begin to disappear. The first and second stages are the only two that are pH dependent.

4. **Thermoplasmonics induced cell fusion** Thermoplasmonics is based on a near infrared (NIR) laser and a plasmonic nanoparticle. The laser which typically acts as an optical trap, is used to heat the nanoscopic plasmonic particle to very high and extremely locally elevated temperatures. Optical trapping of such a nanoheater at the interface between two membrane vesicles, or two cells, leads to immediate fusion of the two verified by both content and lipid mixing. Advantages include full flexibility of which cells to fuse and fusion can be performed in any buffer condition unlike electroformation which is affected by salt.

Importance of cell fusion

i. In human therapy
ii. To study the control of cell division and gene expression.
iii. To investigate malignant transformations.
iv. To obtain viral replication.
v. For gene and chromosome mapping.
vi. For production of monoclonal antibodies by producing hybridoma.
vii. For production of Induced stem cells.
viii. To assess protein shuttling in what is known as a *heterokaryon fusion assay*

Probable questions:

1. What do you mean by morphogenesis?
2. Discuss the process of morphogenesis.
3. What is differentiation?
4. Write short notes on cell shape.
5. What is cell fusion?
6. What do you mean by Homotypic cell fusion?
7. What are the advantages of cell fusion?
8. Discuss any two methods of cell fusion?

Suggested readings:

1. Chen EH, Olson EN. Unveiling the mechanisms of cell-cell fusion. *Science.* 2005; 308:369–73.
2. Ohgawara T, Kobayashi S, Ohgawara E, Uchimiya H, Ishii S. Somatic hybrid plants obtained by protoplast fusion between Citrus sinensis and Poncirus trifoliata. *Theoretical and Applied Genetics.* 1985; 71:1–4.
3. Trueheart J, Boeke JD, Fink GR. Two genes required for cell fusion during yeast conjugation: evidence for a pheromone-induced surface protein. *Molecular and cellular biology.* 1987; 7:2316–28.
4. Shemer G, Suissa M, Kolotuev I, Nguyen KC, Hall DH, Podbilewicz B. EFF-1 is sufficient to initiate and execute tissue-specific cell fusion in C. elegans. *Current Biology.* 2004; 14:1587–91.
5. Bate MI. The embryonic development of larval muscles in Drosophila. *Development.* 1990; 110:791–804.
6. Rao PN, Johnson RT. Mammalian cell fusion: studies on the regulation of DNA synthesis and mitosis. *Nature.* 1970; 225:159–64.
7. Ogle BM, Cascalho M, Platt JL. Biological implications of cell fusion. *Nature reviews Molecular cell biology.* 2005; 6:567–75.
8. Gilbert S.F. 2010. Developmental Biology, IX Edition, Sinauer Associates, Inc., Publishers,

UNIT II

Morphogenesis: Cell death

Objective: In this unit we will discuss about cell death process in development.

Introduction to Apoptosis:

Every normal living cell of animals, plants and even bacteria are mortal. I.e., they must die after some time. Cell death is a finely tuned programme inherent in the cells genetic machinery. This normal cell death which is the part of normal development and maintenance of homeostasis is called apoptosis or programmed cell death (PCD).

This phenomenon is very much different from death of a cell due to pathological cause or necrosis. This process is highly regulated and any defect in apoptotic machinery will lead to extended survival of cells which may result in neoplastic cell expansion, leading to genetic instability and accumulation of mutations.

Why do cells die?

Cell death is an important process in the body. It removes cells in situations including:

- When cells are not needed, such as during certain stages of development.
- To create a structure in the body, for example, the outer layer of the skin is made of dead cells.
- To remove excess cells, such as white blood cells after an infection has been cleared.
- If cells are damaged, such as by radiation or toxins.
- When cells are infected by viruses.

How do cells die?

Cells can die because they are damaged, but most cells die by killing themselves.

There are several distinct ways in which a cell can die. Some occur by an organised, 'programmed' process. Some cell death processes leave no trace of the dead cell, whereas others activate the immune system with substances from the dead cell.

1. Apoptosis: is a form of cell death that prevents immune activation. Apoptotic cells have a particular microscopic appearance. The cell activates proteins called caspases that are normally dormant. These caspases dismantle the cell from within. The apoptotic cell breaks into small packages that can be engulfed by other cells. This

prevents the cell contents leaking out of the dying cell and allows the components to be recycled.

2. **Necrosis**: occurs when a cell dies due to lack of a blood supply, or due to a toxin. The cells' contents can leak out and damage neighbouring cells, and may also trigger inflammation.

3. **Necroptosis**: is similar in appearance to necrosis, in that the dying cell's contents can leak out. However, like apoptosis, necroptosis is a programmed suicide process triggered by specific proteins in the dying cell.

4. **Pyroptosis**: is a form of cell death that occurs in some cells infected with certain viruses or bacteria. A cell dying by pyroptosis releases molecules, called cytokines that alert neighbouring cells to the infection. This triggers inflammation, a protective response that restricts the spread of the viruses and bacteria.

5. **Cell death proteins:** Many proteins have been discovered that control whether a cell dies by the processes of apoptosis, necroptosis or pyroptosis. Some key cell death control proteins include:

i. **Caspases**: these enzymes are switched on in apoptotic cells, and digest other proteins to bring about cell death. Some caspases have roles in processes other than cell death.

ii. **Bcl-2 family proteins**: these proteins interact with each other to determine whether a cell undergoes apoptosis or stays alive. Some Bcl-2 family proteins promote survival, and block apoptosis. Others are 'pro-death', and trigger apoptosis.

iii. **Death receptors**: these are proteins on the surface of the cell. When they are bound by certain cytokines (hormone-like signalling proteins), they cause changes in the cell that can lead to cell death.

iv. **RIP kinases**: two proteins called 'RIP1 kinase' and 'RIP3 kinase' trigger necroptosis.

v. **IAPs**: or 'inhibitor of apoptosis proteins' can prevent cell death. They can do this by blocking several cell death proteins including caspases and RIP1 kinase.

vi. **SMAC/Diablo**: is an inhibitor of IAPs. In healthy cells, SMAC is stored away from IAPs, in parts of the cell called mitochondria. When cell death is triggered, SMAC can leak out and block IAPs function. Thus, the release of SMAC out of mitochondria can promote cell death.

- **Process of Apoptosis**

Apoptosis, the programmed cell death is characterized by chromatin condensation and cell shrinkage in the early stage and then the nucleus and cytoplasm fragment, forming membrane-bound apoptotic bodies which can be engulfed by phagocytes. In contrast, cells undergo another form of cell death, necrosis, swell and rupture. The released intracellular contents can damage surrounding cells and often cause inflammation. Apoptosis is an important process during normal development. It also involved in aging and various diseases such as cancer, AIDS, Alzheimer's disease and Parkinson's disease.

Programmed cell death, or apoptosis, is mediated by proteolytic enzymes called caspases, which are synthesized in the precursor forms as procaspases. When activated by various signals, caspases function to cause cell death in most organisms, ranging from *C. elegans* to human beings. Apoptosis provides a means deciding the shapes of body parts in the course of development and a means of eliminating cells producing anti-self antibodies or infected with pathogens as well as cells containing large amounts of damaged DNA. Cytotoxic T cells initiate apoptosis in cells to which they bind through T-cell receptor-class I MHC-peptide interactions aided by interactions with the coreceptor molecule CD8.

Under some circumstances, such as when DNA damage is extensive, p53 also activates expression of genes that lead to apoptosis, the process of programmed cell death that normally occurs in specific cells during the development of multicellular animals. In vertebrates, the p53 response evolved to induce apoptosis in the face of extensive DNA damage, presumably to prevent the accumulation of multiple mutations that might convert a normal cell into a cancer cell.

During apoptosis, the cell is digested by a class of proteases called caspases. More than 10 caspases have been identified. Some of them (e.g., caspase 8 and 10) are involved in the initiation of apoptosis, others (caspase 3, 6, and 7) execute the death order by destroying essential proteins in the cell (Fig. 1).

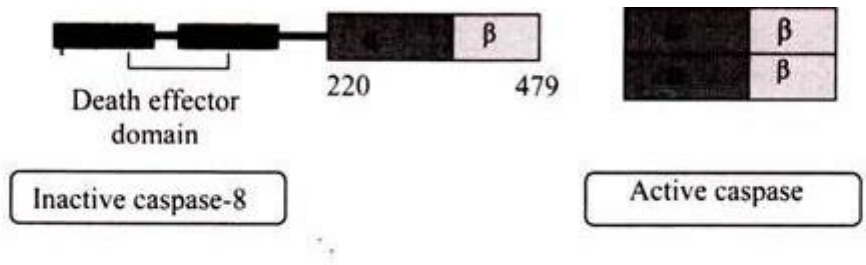


Fig 1: Comparison between active and inactive forms of Caspases. Newly produced caspases are in active. Specifically cleaved caspases will dimerize and become active

The apoptotic process can be summarized as follows:

i. Activation of initiating caspases by specific signals.

ii. Activation of executing caspases by the initiating caspases which can cleave inactive caspases at specific sites.

iii. Degradation of essential cellular proteins by the executing caspases with their protease

iv. Death receptors- Fas/CD95, DR4/DR5, DR3, and TNFR (Tumor Necrosis Factor Receptor).

v. Adaptors- FADD (Fas-associated death domain protein) and TRADD (TNFR-associated death domain protein).

vi. Activation- Binding of death ligands (FasL/CD95L, TRAIL/APO-2L, APO-3L and TNF) induces trimerization of their receptors, which then recruit adaptors and subsequently activate the caspases (Fig. 2).

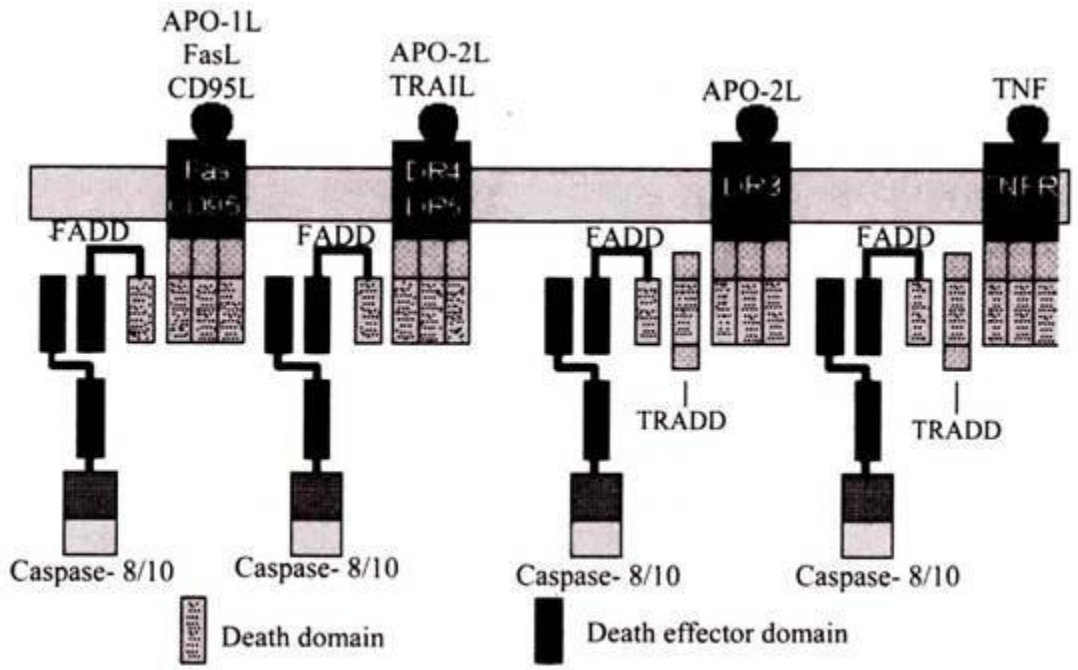


Fig 2: Coupling of caspase 8 or 10 death receptor

- **How does cell death impact health?**

Many diseases are associated with abnormal cell death. Some examples of this are:

Cancer	Cancer cells often resist cell death, even after anti-cancer treatment.
Autoimmunity e.g. Lupus, type 1 diabetes	Immune cells that attack the body's own tissues normally die. If this cell death does not occur it can cause diseases such as lupus or type 1 diabetes.
Viral infection	Viruses need to keep a cell alive in order to reproduce. Cell death can therefore prevent viral replication.
Heart attack	Many cells, including those in the heart and brain, trigger their apoptosis machinery when they lose their blood supply.

- **Significance of Apoptosis**

Apoptosis is significant for the following reasons:

1. It helps to maintain homeostasis in the multicellular organisms.
2. Proper size of the body is maintained by apoptosis.
3. Apoptosis maintains the constancy of cell number in an organism.
4. The unwanted cells are eliminated from the body by apoptosis.
5. The dangerous T-lymphocytes are eliminated by apoptosis.
6. Programmed cell death is crucial for cell development.

- **Role of Apoptosis**

Apoptosis plays an important role in the body of an organism. Following are a few such roles performed by the process:

1. The separation of the fingers during the development of the foetus is due to apoptosis.
2. It results in the closure of the neural tube in the dorsal part.
3. Programmed cell death results in the removal of vestigial remnants such as pronephros.
4. During the determination of sex of the foetus, the Wolffian ducts are removed by cell death.

5. In the urachus, apoptosis allows the removal of redundant tissues between the bladder and umbilicus.

- **Relationship Between Apoptosis and Cancer**

Cancer is the uncontrolled division of cells that leads to the development of tumour. If the apoptotic signalling works properly, these unwanted cells can be removed from the body. The main reason for cancer is that they have the ability to prevent apoptosis and therefore multiply uncontrollably.

Probable questions:

1. What do you mean by programmed cell death?
2. How is apoptosis important?
3. Discuss the Process of Apoptosis with diagram.
4. How does cell death impact health?
5. What is the Significance of Apoptosis?

Suggested readings:

1. Carlson BM. 2014. Human Embryology and Developmental Biology. 5th Edn. Elsevier.
2. Das N. 2012. Fundamental Concept of Developmental Biology. New Central Book Agency
3. Gardner DK. 2006. In Vitro Fertilization: a Practical Approach. CRC Press.
4. Gilbert S.F. 2010. Developmental Biology, IX Edition, Sinauer Associates, Inc., Publishers,
5. Schoenwolf GC, Bleyl SB, Brauer PR, Francis-West PH. 2009. Ladesn's Human Embryology. Elsevier
6. https://www.wehi.edu.au/research/research-fields/cell-death

UNIT III

Cell adhesion, cell sorting, morphogenetic field, and regionalization

Objective: In this unit we will learn about Cell adhesion, cell sorting, morphogenetic field, and regionalization.

Cell adhesion

Cell adhesion is the binding of a cell to another cell (or a cell to an extracellular matrix component). Cell binding is essential in organ formation during embryonic development and in conferring structural framework and tissue maintenance. Without cell adhesion, the cells will be fluid as it is in certain diseases such as metastatic cancer and osteoporosis. Cell adhesion is, therefore, essential in tissue formation, angiogenesis, cell communication, and cell regulation.

The adhesion of a cell to another cell or a component of the extracellular matrix is necessary for organ creation during embryogenesis and for providing a basic structure and tissue maintenance.

- ***What is cell adhesion and why is it important?***

Cell adhesion is such a mechanism by which cells adhere to themselves and with other cells with the help of cell surface proteins. The contact can either be direct like in cell junction or it can be indirect in which cells are connected by extracellular matrix adhesion. It is a gel-like material that consists of different chemicals released by the cell. Adhesion is required for cell communication and regulation, as well as for the development and maintenance of tissues. Adhesion is also required for the formation of new tissues. *Cell adhesion is defined as a cell's ability to adhere to another cell or an extracellular matrix (ECM).* (Khalili & Ahmad, 2015).

- **What is the function of cell adhesion molecules?**

Cell adhesion molecules (CAMs) are a collection of proteins on the cell surface that are necessary for the process by which cells adhere to the extracellular matrix. *Adhesion cell molecules* are also known as *cell adhesion proteins*. Cell adhesion molecules (CAMs) are molecules that assist cells to adhere to one another and their surroundings.

Types of Cell Adhesion

Let us now know cell adhesion types. *Homophilic adhesion* is where cell adhesion is facilitated by the binding of similar adhesion molecules. *Heterophilic adhesion* – where cell adhesion is facilitated by the binding of unlike adhesion molecules.

It may also be by adhesion molecules or cells involved. Examples:

- Cadherins (cadherin cell adhesion) and nectins (nectin cell adhesion) are two well-characterized cell-cell adhesion molecules that play a role in cell adhesion regulation. (Rikitake, Mandai, & Takai, 2012)
- Epithelial cell adhesion molecule is a transmembrane glycoprotein that promotes Calcium ions homotypic cell-cell adhesion in epithelia.
- Laminate cell adhesion molecule: Laminins are a critical and physiologically active component of the basal lamina, exerting influence on proliferation and differentiation, movement, and attachment.
- Gelatin cell adhesion: Gelatin contains collagen's RGD sequences, which makes it extremely efficient in cell-matrix adhesion.
- The neural cell adhesion molecule (NCAM) is a neuronal surface glycoprotein with immunoglobulin-like properties that interact with a range of other cell adhesion proteins to regulate adhesion, direction, and segmentation throughout neuronal growth. (Weledji & Assob, 2014)
- Fibroblast adhesion is where the adhesion of cells to the tissue matrix by molecules released by fibroblasts, e.g., fibronectin and SAM (substrate-attached material). (

Four main families of cellular adhesion molecules are *selectins, immunoglobulin superfamily, integrins,* and *cadherins.*

Cadherins and immunoglobulin superfamily are homophilic CAMs, meaning they attach to the same type of CAMs on the same cell, whereas selectins and integrins bind to various types of CAMs as they are *heterophilic*. Each of the adhesion molecules has a distinct function and binds with various ligands. Defects in CAM expression are frequently the cause of cell adhesion problems.

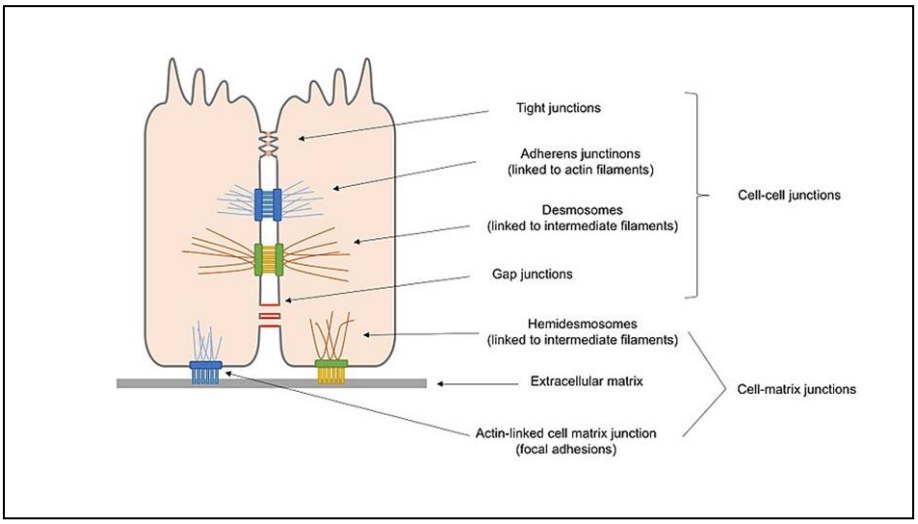


Figure 1: Overview diagram of different types of cell junctions present in epithelial cells, including cell-cell junctions and cell-matrix junctions. Image Credit: Wikimedia.

Cell junctions are formed when bindings across CAMs allow cells of multicellular organisms to adhere to one another, generating structures known as cell junctions. A second way of categorizing cell junctions is to divide them into two broad categories based on their mode of interconnecting with the cell: the first category is *cell-cell junctions*, which are primarily mediated by cadherins, and the second category is *cell-matrix junctions*, which are primarily controlled by integrins.

Following are the types of cell junctions based on their functionality.

1. **Anchoring junctions** (desmosomes, hemidesmosomes, and adherens junctions) keep cells together as one and improve cell-to-cell interaction.
2. **Tight junctions** (occluding junctions) are diffusion-impermeable barriers that are formed when cells come into contact.
3. **Gap junctions** (channel-forming junctions) join nearby cells' cytoplasms, enabling molecules to pass across.
4. **Signal-transmitting junctions** can function similarly to synapses in the nervous system.

Cell-cell junctions

Cell-cell interactions have a variety of types. Cadherins are the most abundant CAMs identified in adherens junctions and desmosomes. This class of CAMs consists of membrane proteins with extracellular domains that promote cell-cell adhesion and operate properly when extracellular calcium ions are present. Cadherins form homophilic cell adhesion bonds between cells of the same kind, leading them to stick together. This will progress toward selective cell adhesion, which enables vertebrate cells to assemble themselves into well-organized tissues. Cadherins are proteins found in multicellular animals that are necessary for cell-cell adhesion and communication.

There are two types of cadherins:

1. classical cadherins
2. non-classical cadherins

➢ *Adherens junctions*

Adherens junctions are largely responsible for maintaining the shape of tissues and retaining cells together. Cadherins connect cells via their cytoplasmic domain, which shares a fundamental calcium-sensitive region. When the extracellular domains of cadherins come into touch with calcium ions for homophilic interaction, they undergo a structural transition from an inactive flexible shape to a more rigid form. Cadherin intracellular domains are also highly conserved since they form complexes with catenin-like proteins.

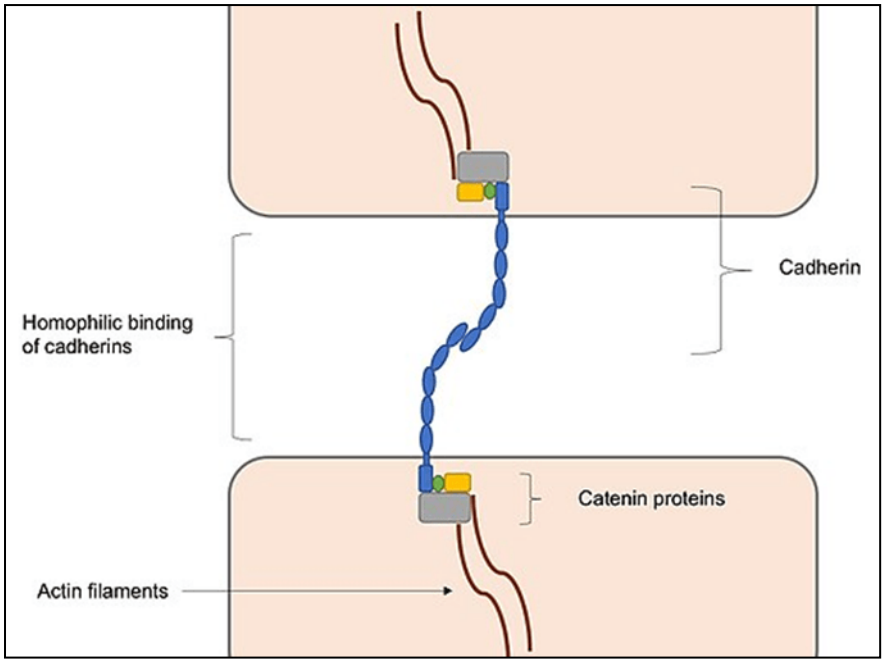


Figure 2: Adherens junction showing homophilic binding between cadherins. Image Credit: Wikimedia.

Between actin and cadherin filaments, these protein complexes behave as connective tissue. This connection with *actin* filaments is necessary for the development of adherens junctions, which are necessary for intracellular adhesion. Interactions with actin filaments, which are involved in the formation of adherens junctions and the creation of adhesions, may facilitate cadherin clustering. This is largely because cadherin clusters promote actin filament polymerization, which aids in the development of adherens junctions by connecting to cadherin–catenin complexes formed at the junction, hence increasing adhesion formation.

- *Desmosomes*

Desmosomes are made up of different components for adherens junction but have an indistinguishable structure to adherens junction. Adhesion molecules include non-classical cadherins such as desmocollins and desmogleins. Rather than actin filaments, they are coupled to intermediate filaments. Because desmosomal cadherins lack catenin, their intracellular domains are associated with desmosomal plaque proteins. These proteins are involved in the formation of thick cytoplasmic adhesion plaques in desmosomes. It also attaches cadherins with the intermediate filaments. Desmosomes give rigidity and dynamic stress resistance to cells by allowing them to be released onto flexible yet elastic intermediate filaments, which is not achievable with stiff actin filaments.

Desmosomes are essential for tissues that tolerate and experience high mechanical stress. Examples of these muscles are epithelia and heart muscles, which explains their abundance in these tissues.

- *Tight junctions*

A tight junction is a type of connection that is present in epithelial and endothelial tissues. Tight junctions serve as barriers, closing gaps and controlling the passage of molecules and extracellular fluids between cells. Tight junctions are formed when transmembrane proteins like tricellulins, occludins, and claudins bind homophilically to one another on adjacent membranes, resulting in the formation of tight junctions. To maintain the structure of the tight junction, scaffold proteins bind to the intracellular domains of these tight junction proteins, which are clustered and connected to actin filaments in a pattern similar to anchoring junctions. In addition to being essential for the creation of tight junctions, claudins also create paracellular holes that allow for the selective pathway of certain ions across tight junctions, allowing for the penetration of the membrane barrier.

- *Gap junctions*

Connexons, which are channels, are used to construct gap junctions. Known as connexins, these are transmembrane proteins that are organized in six-membered groups on the surface of the cell membrane. Continuity channels can emerge when connexons from neighboring cells join together and align with one another. These channels not only serve to keep cells together and preserve structural stability, but they also facilitate the transport of ions and tiny molecules throughout the cytoplasms of neighboring cells.

Gap junction channels are formed as a result of the connexins that create the connexons, and they are permeable to particular ions. This allows gap junctions to participate in cell signaling by controlling the flow of molecules across signaling pathways.

Gap junctions are capable of responding in a variety of ways when subjected to a wide range of stimuli and are dynamically controlled, either through fast processes such as voltage gating or through slow mechanisms such as modifying the number of channels available in the junction.

Clinical Implications

Let's take a look at the clinical implications of cell adhesion.

I. Adhesion in vascular injury

Thrombocyte adhesion is the initial and essential step as a result of vascular injury. In this, the platelets attach to the extracellular matrix and specific membrane receptors of the vessel and lead toward the formation of the clot. (Ruggeri & Mendolicchio, 2007)

II. Adhesion in inflammation

Molecules of cellular adhesion also play part in the inflammatory response. The adhesion receptors integrins, selectins, and immunoglobulin (Ig) gene superfamily regulate the several phases of white blood cells migration from the blood circulation to sites of inflammation. (González-Amaro, Diaz-González, & Sánchez-Madrid, 1998)

Additionally, there are various human genetic illnesses associated with an inability to express certain adhesion molecules. One such condition is leukocyte adhesion deficiency-I (LAD-I), which results in decreased or absent expression of the 2 integrin subunit. This results in decreased production of 2 integrin heterodimers, which are essential for white blood cells to adhere firmly to the endothelium wall in inflammatory areas to combat infections. Such patients have severe infections that can be fatal.

Platelet endothelial cell adhesion molecule, also referred to as a collection of differentiation 31 (CD31), is a protein encoded by the PECAM1 gene on chromosome 17q23.3.PECAM-1 and is involved in the removal of old neutrophils from the body. Intercellular adhesion appears to be required for neutrophil migration at sites of inflammation.

The autoimmune disease named Pemphigus is also caused by insufficient cell adhesion since it is caused by autoantibodies attacking the desmosomal cadherins of a person. It causes epidermal cells to divide and blister the skin.

To infect and cause illness, pathogenic microbes such as bacteria, viruses, and protozoans must first attach to host cells. Anti-adhesion treatment can help prevent infection. It works by attacking the adhesion molecules of the host cell or pathogen. In addition to changing adhesion molecule synthesis, competitive inhibitors that attach to adhesion molecules and hinder cell-cell interaction can be utilized as anti-adhesive medications.

III. Adhesion in metastasis and cancer cell

What is cell adhesion in cancer?

The inter and extracellular matrix of cancer is characterized by the presence of several diverse cell adhesion molecules. It takes several steps for cancer to develop, and adhesion molecules are essential for the production of invasive, recurrent, and distant metastasis. Evidence suggests that deviations in the adhesion properties of cancerous cells play an integral part not only in the development but also in the spread of cancer. Because intercellular adhesion loss and cell shedding from the underlying lamina propria enable the tumor tissue to depart its origin site, damage the extracellular matrix, develop a more invasive phenotype, and finally penetrate and disseminate throughout the body. (Okegawa, Pong, Li, & Hsieh, 2004)

During the process of cancer metastasis, cell adhesion failure occurs. Tumor cells that have lost their ability to adhere to one another might depart their original site of origin and multiply through the vascular system due to the breakdown of cell-cell adhesion.

Cadherins are one type of CAM that is mutated in patients with cancer. They are rendered inactive either via genetic modification or through the action of other oncogenic signaling molecules. It promotes cancer cells to spread and become more metastatic as a result of the treatment. Other CAMs, such as selectins and integrins, may aid in the progression of metastasis by promoting cell-cell interaction between metastatic tumor cells that are migrating across the circulatory system. These molecules may be beneficial as cancer therapeutic targets as a result of the link between CAMs and tumor metastasis, according to the findings.

Cell sorting

Cell sorting

Cell sorting is a method used to separate cells isolated from an organism's tissues according to their type. Cells are mostly commonly separated relying on differences in cell size, shape (morphology), and surface protein expression. The resulting homogenous populations of cells have important applications in research and as therapeutics.

Methods

Currently there are several methods for cell sorting. Some are primitive and do not require special equipment whereas others rely on sophisticated electronic appliances. Three major types of cell sorting are fluorescent activated cell sorting, magnetic cell selection and single cell sorting.

- **Single cell sorting**

Single cell sorting provides a method for sorting a heterogeneous mixture of cells based upon intracellular and extracellular properties. There are several methods for sorting single cells:

1. The IsoRaft array provides a rapid, cost-effective method for isolating cells, analyzing cells over time, and generating clonal populations with the unique ability to monitor all intra- and extracellular properties. This system is ideal for both adherent and non-adherent cell types.

2. The DEPArray lab-on-a-chip technology platform is designed to individually identify, manipulate and sort specific cells within a heterogeneous population based on intra- and extracellular properties, not including morphology. The DEPArray cell-sorting and isolation technology, followed by NGS analysis, can reveal comprehensive genomic information from any FFPE sample, regardless of sample cellularity and size of the specimen. Moreover, the methodology informs a new model for conducting clinical biopsies of tumors, as well as for performing translational cancer research and the way new cancer drugs are developed and biomarkers discovered.

- **Fluorescent activated cell sorting**

Fluorescent Activated Cell Sorting, or FACS, utilizes Flow cytometry to provide a fast, objective and quantitative measurement of intra- and extracellular properties, not including morphology, for sorting a heterogeneous mixture of cells.

- **Magnetic cell sorting**

Magnetic cell sorting provides a method for enriching a heterogeneous mixture of cells based upon extracellular properties, typically cell-surface proteins (antigens). There are several types of magnetic cell sorting:

1. Magnetic-activated cell sorting (MACS) is a column based separation technique where labeled cells are passed through a magnetic column.
2. SEP system provides a column-free cell separation technique in which a tube of labeled cells is placed inside a magnetic field. Positively selected cells are retained in the tube while negatively selected cells are in the liquid suspension.

- **Buoyancy activated cell sorting**

Buoyancy activated cell sorting (BACS), developed by Akadeum Life Sciences, is a separation technique in which microbubbles bind to cells through antibodies binding to the surface of cells. The targeted cells are then removed from a biological sample through flotation.

Morphogenetic field

In the developmental biology of the early twentieth century, a morphogenetic field is a group of cells able to respond to discrete, localized biochemical signals leading to the development of specific morphological structures or organs. The spatial and temporal extents of the embryonic field are dynamic, and within the field is a collection of interacting cells out of which a particular organ is formed. As a group, the cells within a given morphogenetic field are constrained: thus, cells in a limb field will become a limb tissue; those in a cardiac field will become heart tissue. So it can be said that a morphogenetic field is a region of an embryo that forms a discrete structure, such as a limb or heart. However, specific cellular programming of individual cells in a field is flexible: an individual cell in a cardiac field can be redirected via cell-to-cell signaling to replace specific damaged or missing cells. Imaginal discs in insect larvae are examples of morphogenetic fields.

Regionalization

In the field of developmental biology, regionalization is the process by which different areas are identified in the development of the early embryo. The process by which the cells become specified differs between organisms.

In terms of developmental commitment, a cell can either be specified or it can be determined. Specification is the first stage in differentiation. A cell that is specified can have its commitment reversed while the determined state is irreversible. There are two main types of specification: autonomous and conditional. A cell specified autonomously will develop into a specific fate based upon cytoplasmic determinants with no regard to the environment the cell is in. A cell specified conditionally will develop into a specific fate based upon other surrounding cells or morphogen gradients. Another type of specification is syncytial specification, characteristic of most insect classes.

Specification in sea urchins uses both autonomous and conditional mechanisms to determine the anterior/posterior axis. The anterior/posterior axis lies along the animal/vegetal axis set up during cleavage. The micromeres induce the nearby tissue to become endoderm while the animal cells are specified to become ectoderm. The animal cells are not determined because the micromeres can induce the animal cells to also take on mesodermal and endodermal fates. It was observed that β-catenin was present in the nuclei at the vegetal pole of the blastula. Through a series of experiments, one study confirmed the role of β-catenin in the cell-autonomous specification of vegetal cell fates and the micromeres inducing ability. Treatments of LiCl sufficient to vegetalize the embryo resulted in increases in nuclearly localized b-catenin. Reduction of expression of β-catenin in the nucleus correlated with loss of vegetal cell fates. Transplants of micromeres lacking nuclear accumulation of β-catenin were unable to induce a second axis.

For the molecular mechanism of β-catenin and the micromeres, it was observed that Notch was present uniformly on the apical surface of the early blastula but was lost in the secondary mesenchyme cells (SMCs) during late blastula and enriched in the presumptive endodermal cells in late blastula. Notch is both necessary and sufficient for determination of the SMCs. The micromeres express the ligand for Notch, Delta, on their surface to induce the formation of SMCs.

The high nuclear levels of b-catenin results from the high accumulation of the disheveled protein at the vegetal pole of the egg. disheveled inactivates GSK-3 and prevents the phosphorylation of β-catenin. This allows β-catenin to escape degradation and enter the nucleus. The only important role of β-catenin is to activate the transcription of the gene Pmar1. This gene represses a repressor to allow micromere genes to be expressed.

The aboral/oral axis (analogous to the dorsal/ventral axes in other animals) is specified by a nodal homolog. This nodal was localized on the future oral side of the embryo. Experiments confirmed that nodal is both necessary and sufficient to promote development of the oral fate. Nodal also has a role in left/right axis formation.

Probable questions:

1. What is cell adhesion and why is it important?
2. Name cell adhesion molecules and state what the function of cell adhesion molecules is?
3. What do you mean by tight junction?
4. Write short notes on Cadherin.
5. What are the clinical implications of cell adhesion?
6. What is cell sorting? Write the methods of cell sorting.
7. Write short notes on Morphogenetic field.
8. Write short notes on regionalization.

Suggested readings:

1. Carlson BM. 2014. Human Embryology and Developmental Biology. 5th Edn. Elsevier.
2. Das N. 2012. Fundamental Concept of Developmental Biology. New Central Book Agency
3. Gardner DK. 2006. In Vitro Fertilization: a Practical Approach. CRC Press.
4. Gilbert S.F. 2010. Developmental Biology, IX Edition, Sinauer Associates, Inc., Publishers,
5. Schoenwolf GC, Bleyl SB, Brauer PR, Francis-West PH. 2009. Ladesn's Human Embryology. Elsevier
6. González-Amaro, R., Diaz-González, F., & Sánchez-Madrid, F. (1998). Adhesion molecules in inflammatory diseases. Drugs, 56(6), 977-988.
7. Khalili, A. A., & Ahmad, M. R. (2015). A review of cell adhesion studies for biomedical and biological applications. International journal of molecular sciences, 16(8), 18149-18184.
8. Harper, P. A., & Juliano, R. L. (1981). Two distinct mechanisms of fibroblast adhesion. *Nature*, *290*(5802), 136–138. https://doi.org/10.1038/290136a0

UNIT IV

Morphogenetic movements

Objective: In this unit we will discuss about Morphogenetic movements.

Introduction

Morphogenetic movement is the movement of cells to form different tissues or the organs in the organisms. It is caused by the dynamic movement of embryonic epithelial cells. In this movement, the embryonic cells get rearranged.

The following general types of morphogenetic movements have been recognized:

a. Individual cells move by:

i. MIGRATION -movement of individual cells over other cells or matrix.

ii. INGRESSION -movement of individual cells or small groups from an epithelium into a cavity.

b. Groups of cells move by:

i. INVAGINATION -local inward buckling of an epithelium

ii. INVOLUTION -inward movement of a cell layer around a point or edge

iii. EPIBOLY -spread of an outside cell layer to envelop a yolk mass or deeper layer

iv. DELAMINATION -splitting 1 cell sheet into 2 or more parallel sheets.

v. CONVERGENT EXTENSION -elongation of a cell layer in one dimension with shortening in another

- ✓ **Invagination**

  During invagination, an epithelial sheet bends inward to form an inpocketing. One way to think of this in three dimensions is to imagine that you are poking a partially deflated beach ball inward with your finger. The resulting bulge or tube is an invagination. If the apical side of the epithelium forms the lumen (central empty space) of the tube, then the movement is termed invagination. If the lumen is formed by basal surfaces, then the movement is termed an evagination.

- ✓ **Ingression**

  During ingression, cells leave an epithellial sheet by transforming from well-behaved epithellial cells into freely migrating mesenchyme cells. To do so, they must presumably alter their cellular architecture, alter their program of motility,

and alter their adhesive relationship(s) to the surrounding cells. Primary mesenchyme cells are an example of a mesenchymal cell type that emigrates out of an epithelium.

- ✓ **Involution**

  During involution, a tissue sheet rolls inward to form an underlying layer via bulk movement of tissue. One helpful image here is of a tank tread or conveyor belt. As material moves in from the edges of the sheet, material originally at the sites of inward rolling (shown in blue here) is free to move further up underneath the exterior tissue.

- ✓ **Epiboly**

  During epiboly, a sheet of cells spreads by thinning. i.e., the sheet thins, while its overall surface area increases in the other two directions. Epiboly can involve a monolayer (i.e. a sheet of cells one cell layer thick), in which case the individual cells must undergo a change in shape. In other cases, however, a sheet that has several cell layer can thin by changes in position of its cells. In this case, epiboly occurs via intercalation, one of the other movements described on this page.

- ✓ **Intercalation**

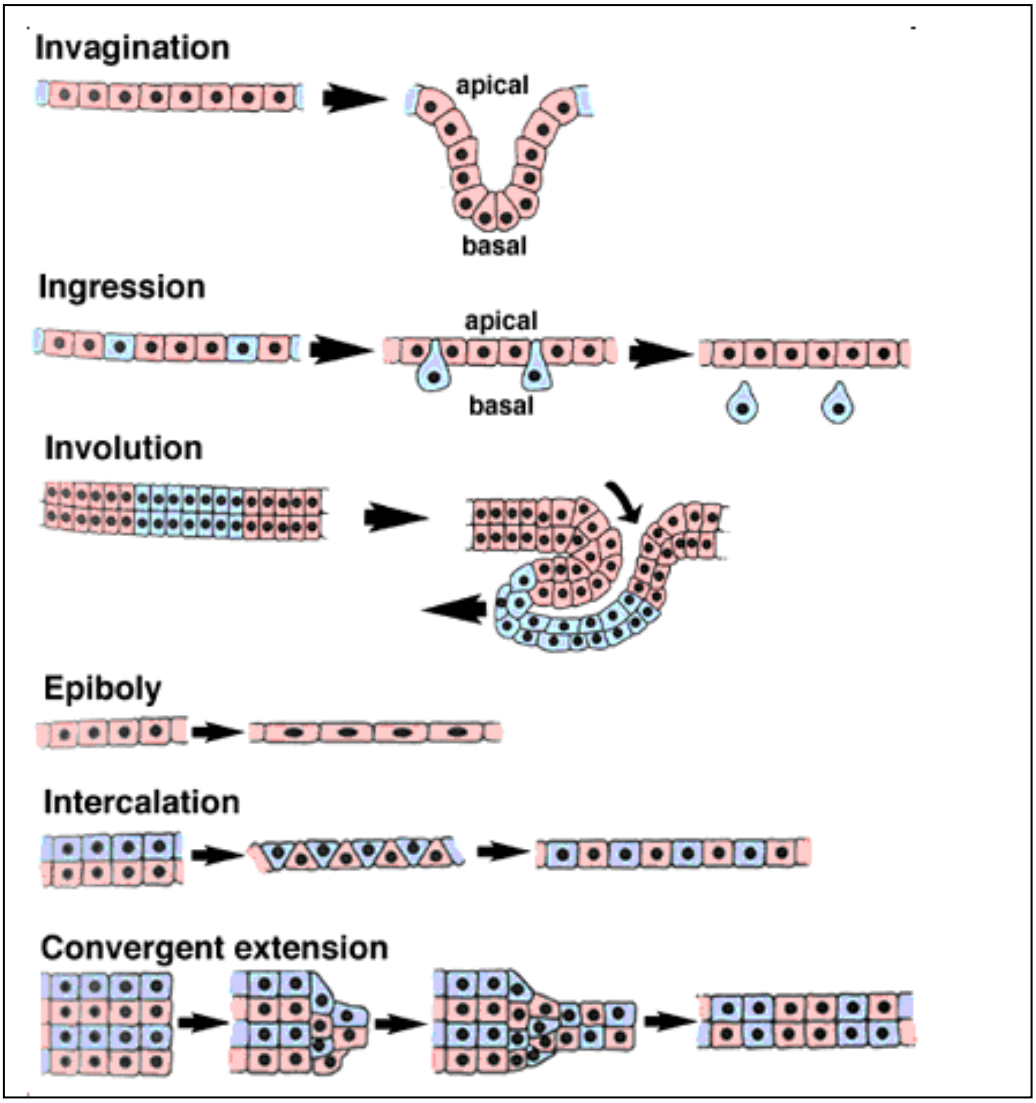
  During intercalation, two or more rows of cells move between one another, creating an array of cells that is longer (in one or more dimensions) but thinner. The overall change in shape of the tissue results from cell rearrangement. Intercalation can be a powerful means of expanding a tissue sheet. A specialized form of intercalation is convergent extension, which is described on this page.

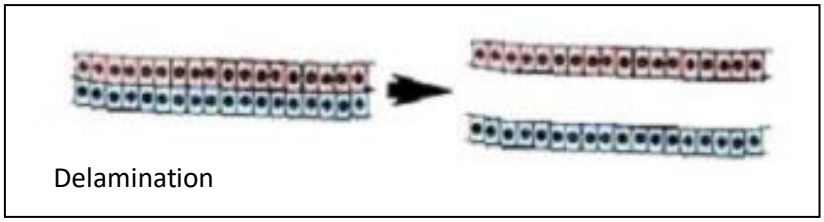
- ✓ **Convergent Extension**

  During convergent extension, two or more rows of cells intercalate, but the intercalation is highly directional. Cells converge by intercalating perpendicular to the axis of extension, resulting in the overall extension of the tissue in a preferred direction. If we had a way to label cells from rows on either side of the axis of extension, they would be found to mix with one another as a result of these oriented intercalation events.

- ✓ **Delamination**

  The word delamination means mass separation of groups of cells from other cell groups. The separation of endodermal, mesodermal and notochordal cells from each other in teleost fishes is a good example for delamination. According to a widely accepted view the endoderm formation in birds takesplace by delamination





Delamination

Probable questions:

1. What do you mean by morphogenetic movement?
2. Discuss different types of morphogenetic movements with diagram.
3. What is involution?
4. What is epiboly?
5. What is delamination?

Suggested readings:

1. Carlson BM. 2014. Human Embryology and Developmental Biology. 5th Edn. Elsevier.
2. Das N. 2012. Fundamental Concept of Developmental Biology. New Central Book Agency
3. Gardner DK. 2006. In Vitro Fertilization: a Practical Approach. CRC Press.
4. Gilbert S.F. 2010. Developmental Biology, IX Edition, Sinauer Associates, Inc., Publishers,
5. Schoenwolf GC, Bleyl SB, Brauer PR, Francis-West PH. 2009. Ladesn's Human Embryology. Elsevier

UNIT V

Teratogenesis: Genetic teratology, Environmental teratology, Developmental mechanism

Objective: In this unit we will discuss about Teratogenesis: Genetic teratology, Environmental teratology, Developmental mechanism

Introduction:

Teratology is the study of abnormal development in embryos and the causes of congenital malformations or birth defects. These anatomical or structural abnormalities are present at birth although they may not be diagnosed until later in life. They may be visible on the surface of the body or internal to the viscera. Congenital malformations account for approximately 20% of deaths in the perinatal period. Approximately 3% of newborn infants will have major malformations and another 3% will have malformations detected later in life.

There are a variety of causes of congenital malformations including: 1) genetic factors (chromosomal abnormalities as well as single gene defects); 2) environmental factors (drugs, toxins, infectious etiologies, mechanical forces); and 3) multifactorial etiologies including a combination of environmental and genetic factors. The graph below (Fig. 23-1) divides these etiologies by percentages.

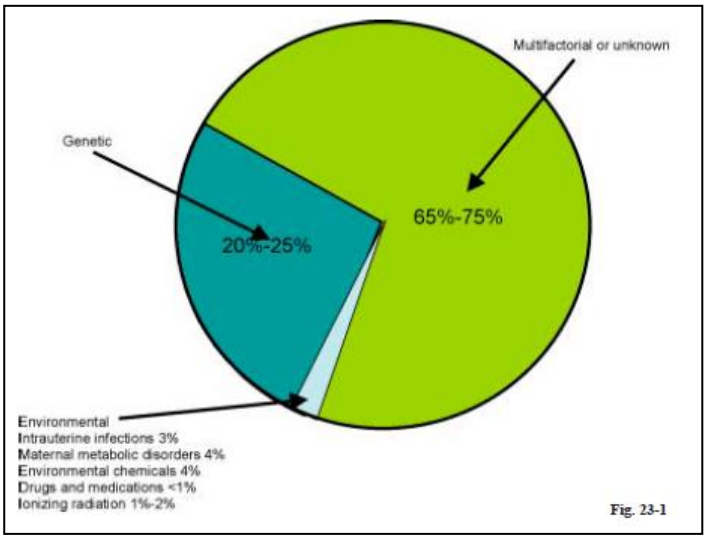


Figure: Factors responsible for teratogenesis.

Malformations may be single or multiple and have major or minor clinical significance. Single minor malformations are observed in approximately 14% of newborns. These malformations are usually of no clinical consequence and may include features such a simian crease or ear tags. Specific minor malformations suggest the possibility of an

associated major malformation. For instance, the finding of a single umbilical artery should suggest the possibility of associated congenital heart problems. The greater the number of minor malformations, the greater the likelihood of an associated major malformation. The more severe and the greater the number of major malformations, the greater the likelihood of a spontaneous miscarriage or shortened life span.

Genetic etiologies of malformations

Genetic factors are the most common causes of congenital malformations and account for approximately one fourth of all congenital malformations. Chromosomal abnormalities including numerical and structural abnormalities are a common cause of congenital malformations. Specific genetic syndromes are associated with the most common of these chromosomal defects. Trisomy 21 is referred to as Down syndrome and has associated characteristic facial features, congenital heart disease, growth retardation, and mental retardation. Monosomy of the X-chromosome is referred to as Turner syndrome and is associated with webbing of the neck, lymphedema of the hands and feet, and later in life short stature and infertility. Trisomy 13 is associated with midline defects including cleft lip and cleft palate, central nervous system malformations, microphthalmia, and congenital heart disease. Infants with this disorder rarely live beyond the first year of life. Trisomy 18 is associated with intrauterine growth restriction, clenched hands, rocker bottom feet, and congenital heart disease. Similar to trisomy 13, infants with the syndrome also rarely live beyond the first year of life. Other chromosomal abnormalities including interstitial deletions, interstitial duplications, and unbalanced translocations are often associated with congenital anomalies. The most common deletions have named clinical syndromes with which they are associated.

In addition to gross chromosomal abnormalities, there are multiple single gene defects that can result in congenital malformations. Many of these genes include developmentally important transcription factors and genes important in intermediary metabolism. Teratogenic agents cause approximately 7% of congenital malformations. A teratogenic agent is a chemical, infectious agent, physical condition, or deficiency that, on fetal exposure, can alter fetal morphology or subsequent function. Teratogenicity depends upon the ability of the agent to cross the placenta. Certain medications such as heparin cannot cross the placenta due to its high molecular weight and are therefore not teratogenic. The embryo is most susceptible to teratogenic agents during periods of rapid differentiation. The stage of development of the embryo determines susceptibility to teratogens. The most critical period in the development of an embryo or in the growth of a particular organ is during the time of most rapid cell division. The critical period for each organ is pictured below. For instance, the critical period for brain growth and development is from three to 16 weeks. However the brain's differentiation continues to extend into infancy. Teratogens can produce mental retardation during both embryonic and fetal periods.

Specific types of major malformations and the times of development usually associated with exposure to the teratogenic agent are outlined in the table below. Each organ of an embryo has a critical period during which its development may be disrupted. The type of congenital malformation produced by an exposure depends upon which organ is most susceptible at the time of the teratogenic exposure. For instance, high levels of radiation produce abnormalities of the central nervous system and eyes specifically at eight to 16 weeks after fertilization. Embryological timetables such as the one above are helpful in studying the etiology of human malformations. However, it is wrong to assume that malformations always result from a single event occurring during a single critical sensitive period or that one can determine the exact day on which a malformation was produced.

A teratogen is any agent that can induce or increase the incidence of a congenital malformation. Recognition of human teratogens offers the opportunity to prevent exposure at critical periods of development and prevent certain types of congenital malformations. In general, drugs, food additives, and pesticides are tested to determine their teratogenicity to minimize exposure of pregnant women to teratogenic agents. To prove that a specific agent is teratogenic means to prove that the frequency of congenital malformations in women exposed to the agent is prospectively greater than the background frequency in the general population. These data are oftentimes not available for humans and thus cannot be determined in an unbiased fashion. Therefore, testing is often done in animal models and often times administered at higher than the usual therapeutic doses. There are clearly species differences between teratogenic effects, limiting this testing in animals. Based upon either anecdotal information on exposures in humans or on the basis of testing in animals, drugs are classified as to their teratogenic potential. It should be emphasized that less than 2% of congenital malformations are caused by drugs or chemicals. There are small numbers of drugs that have been positively implicated as teratogenic agents that should

Nicotine does not produce congenital malformations but nicotine does have a effect on fetal growth. Maternal smoking is a well-established cause of intrauterine growth restriction. Heavy cigarette smokers were also more likely to have a premature delivery. Nicotine constricts uterine blood vessels and causes decreased uterine blood flow thereby decreasing the supply of oxygen and nutrients available to the embryo. This compromises cell growth and may have an adverse effect on mental development.

Alcohol is a common drug abused by women of childbearing age. Infants born to alcoholic mothers demonstrate prenatal and postnatal growth deficiency, mental retardation, and other malformations. There are subtle but classical facial features associated with fetal alcohol syndrome including short palpebral fissures, maxillary hypoplasia, a smooth philtrum, and congenital heart disease. Binge drinking also likely has a harmful effect on embryonic brain developments at all times of gestation.

Tetracycline, the type of antibiotic, can cross the placental membrane and is deposited in the embryo in bones and teeth. Tetracycline exposure can result in yellow staining of

the primary or deciduous teeth and diminished growth of the long bones. Tetracycline exposure after birth has similar effects. Anticonvulsant agents such as phenytoin produce the fetal hydantoin syndrome consisting of intrauterine growth retardation, microcephaly, mental retardation, distal phalangeal hypoplasia, and specific facial features.

Anti-neoplastic or chemotherapeutic agents are highly teratogenic as these agents inhibit rapidly dividing cells. These medications should be avoided whenever possible but are occasionally used in the third trimester when they are urgently needed to treat the mother.

Retinoic acid or vitamin A derivatives are extremely teratogenic in humans. Even at very low doses, oral medications such as isotretinoin, used in the treatment of acne, are potent teratogens. The critical period of exposure appears to be from the second to the fifth week of gestation. The most common malformations include craniofacial dysmorphisms, cleft palate, thymic aplasia, and neural tube defects.

The tranquilizer **thalidomide** is one of the most famous and notorious teratogens. This hypnotic agent was used widely in Europe in 1959, after which an estimated 7000 infants were born with the thalidomide syndrome or meromelia. The characteristic features of this syndrome include limb abnormalities that span from absence of the limbs to rudimentary limbs to abnormally shortened limbs. Additionally, thalidomide also causes malformations of other organs including absence of the internal and external ears, hemangiomas, congenital heart disease, and congenital urinary tract malformations. The critical period of exposure appears to be 24 to 36 days after fertilization.

Infectious agents can also cause a variety of birth defects and mental retardation when they cross the placenta and enter the fetal blood stream. Congenital rubella or German measles consists of the triad of cataracts, cardiac malformation, and deafness. The earlier in the pregnancy that the embyro is exposed to maternal rubella, the greater the likelihood that it will be affected. Most infants exposed during the first four to five weeks after fertilization will have stigmata of this exposure. Exposure to rubella during the second and third trimester results in a much lower frequency of malformation, but continues to pose a risk of mental retardation and hearing loss.

Congenital cytomegalovirus infection is the most common viral infection of the fetus. Infection of the early embryo during the first trimester most commonly results in spontaneous termination. Exposure later in the pregnancy results in intrauterine growth retardation, micromelia, chorioretinitis, blindness, microcephaly, cerebral calcifications, mental retardation, and hepatosplenomegaly.

Ionizing radiation can injure the developing embryo due to cell death or chromosome injury. The severity of damage to the embryo depends on the dose absorbed and the stage of development at which the exposure occurs. Study of survivors of the Japanese atomic bombing demonstrated that exposure at 10 to 18 weeks of pregnancy is a period of greatest sensitivity for the developing brain. There is no proof that human congenital

malformations have been caused by diagnostic levels of radiation. However, attempts are made to minimize scattered radiation from diagnostic procedures such as x-rays that are not near the uterus. The standard dose of radiation associated with a diagnostic x-ray produces a minuscule risk to the fetus. However, all women of childbearing age are asked if they are pregnant before any exposure to radiation.

Maternal medical conditions can also produce teratogenic risks. Infants of diabetic mothers have an increased incidence of congenital heart disease, renal, gastrointestinal, and central nervous system malformations such as neural tube defects. Tight glycemic control during the third to sixth week post-conception is critical. Infants of mothers with phenylketonuria who are not well controlled and have high levels of phenylalanine have a significant risk of mental retardation, low birth weight, and congenital heart disease.

Mechanical forces can also act as teratogens. Malformations of the uterus may restrict fetal movements and be associated with congenital dislocation of the hip and clubfoot. Oligohydramnios can have similar results and mechanically induce abnormalities of the fetal limbs. These abnormalities would be classified as deformations or abnormal forms, shapes, or positions of body parts caused by physical constraints. Amniotic bands are fibrous rings and cause intrauterine amputations or malformations of the limbs as well. These abnormalities would be classified as disruptions or defects from interference with a normally developing organ system usually occurring later in gestation.

Most common congenital malformations have familial distributions consistent with multifactorial inheritance. Multifactorial inheritance may be presented by a model in which liability to a disorder is a continuous variable that is dependent on a combination of environmental and genetic factors. Development of the malformation is dependent upon passing a threshold that is the sum of a combination of many of these factors. Traits that demonstrate this mode of inheritance include cleft lip, cleft palate, neural tube defects, pyloric stenosis, and congenital dislocation of the hip.

Environmental Teratology: It is estimated that approximately 10–15% of congenital structural anomalies are the result of the adverse effect of environmental factors on prenatal development. This means that approximately 1 in 250 newborn infants have structural defects caused by an environmental exposure and, presumably, a larger number of children have growth retardation or functional abnormalities resulting from nongenetic causes, in other words, from the effects of teratogens. A teratogen is defined as any environmental factor that can produce a permanent abnormality in structure or function, restriction of growth, or death of the embryo or fetus. A dose-response relationship should be demonstrated in animals or humans so that the greater the exposure during pregnancy, the more severe the phenotypic effects on the fetus. Factors comprise medications, drugs, chemicals, and maternal conditions or diseases, including infections.

Teratogenic exposures during prenatal development cause disruptions regardless of the developmental stage or site of action. Most structural defects caused by teratogenic exposures occur during the embryonic period, which is when critical developmental events are taking place and the foundations of organ systems are being established. Different organ systems have different periods of susceptibility to exogenous agents.

i. **Radiation:** Ionizing radiation can injure the developing embryo due to cell death or chromosome injury. There is no proof that human congenital malformations have been caused by diagnostic levels of radiation. The most critical exposure period is 8–15 week after fertilization. Before implantation, the mammalian embryo is insensitive to the teratogenic and growth-retarding effects of radiation and sensitive to the lethal effects. The risks of 1-rad (0.10Gy) or 5-rad (0.05Gy) acute exposure are far below the spontaneous risks of the developing embryo because 15% of human embryos abort, 2.7 – 3.0% of human embryos have major malformations, 4% have intrauterine growth retardation, and 8–10% have early- or late-stage onset genetic disease. Permanent growth retardation is more severe after midgestation radiation.

Because of its extended periods of organogenesis and histogenesis, the central nervous system (CNS) retains the greatest sensitivity of all organ systems to the detrimental effects of radiation through the later fetal stages. In utero radiation produces microcephaly and mental retardation. Later in life there is increased incidence of hematopoietic malignancies and leukemia.

ii. **Infectious Agents:** The lethal or developmental effects of infectious agents are the result of mitotic inhibition, direct cytotoxic effects, or a vascular disruptive event on the embryo or fetus. However, a repair process may result in scarring or calcification, which causes further damage by interfering with histogenesis. Infections that do not result in congenital malformations but do cause fetal or neonatal death include enteroviruses (coxsackie virus, poliovirus and echovirus) and hepatitis, variola, vaccina, and mumps viruses. Non-radioactive in-situ hybridization of formalin-fixed, paraffin-embedded placental and fetal tissue, using virus-specific DNA or RNA probes, is helpful for diagnosing fetal virus infections such as cytomegalovirus, parvovirus B-19, and varicellazoster virus that cause fetal hydrops, placentitis, and abortion.

iii. **Varicella:** Varicella (or chickenpox) is a highly infectious disease, usually occurring in childhood. By adulthood, more than 95 percent of Americans have had chickenpox. Eighty-five to ninety-five percent of pregnant women are immune to chickenpox, which means that there is no need to be concerned about this during pregnancy, even if the woman is exposed to someone with chickenpox. Nearly seven women out of 10,000 will develop chickenpox during pregnancy, however, because they are not immune.

The disease is caused by the varicellazoster virus (VZV), which is a form of the herpes virus. Transmission occurs from person-to-person by direct contact or through the air.

Chicken pox is contagious from 1 to 2 days before the appearance of the rash until the blisters have dried and become scabs. Once a person is exposed to the virus, chickenpox may take up to 14 to 18 days to develop. When a woman has a varicella infection during the first 20 wk of pregnancy, there is a 2% chance that the baby will have a group of defects called the congenital varicella syndrome, which includes scars, defects of muscle and bone, malformed and paralyzed limbs, small head size, blindness, seizures, and mental retardation. This syndrome is rarely seen if the infection occurs after 20 weeks of pregnancy.

Another time that there is a concern about a varicella infection is in the newborn period, if the mother develops the rash during the period from 5 days before to 2 days after delivery. Between 25% and 50% of newborns will be infected in this case, and they develop a rash between 5 and 10 days after birth. Up to 30% of infected babies will die if not treated. If the mother develops a rash between 6 and 21 days before delivery, the baby faces some risk of mild infection.

iv. **Mumps virus**: Mumps virus during pregnancy does not cause malformations, but endocardial fibroelastosis has been noted in infants with a positive mumps antigen skin test; this relationship has not been consistent.

v. **Influenza virus**: There is no compelling evidence to incriminate influenza virus infection during pregnancy as a cause of malformations.

vi. **Parvovirus:** Human parvovirus B-19 is able to cross the placenta and results in fetal infection, which may occur whether the mother is symptomatic or asymptomatic. It is associated with a higher than average fetal loss and may lead to spontaneous abortion in the first trimester, hydrops fetalis in the second trimester, and stillbirth at term. Generalized myocarditis, myositis of skeletal muscles, and abnormalities of the eyes are reported. Human parvovirus B-19 has an affinity for the erythropoietic tissue of the host and is therefor associated with fetal anemia leading to cardiac failure.

Mechanism of teratogenesis:

The Mechanism of Teratogenesis fall into broad categories based on the etiology of congenital malformations:

(a) Errors in genetic programming based on deviations in the genotypes of the embroy or the low probability for error of the normal genotype ; and

(b) Environmental agants or factors that interact with an embryo during the period of development (drugs, chemicals, rediation, hyperthermia, infections, abnormal maternal metabolic states, or mechanical factors).

The etiology of human malformations includes both genetic and environmental factors.

Most developmental defects have complex etiology following from interactions of gene environment lifestyle factors.

• Recognizing a teratogen is a very different problem than understanding its mechanism of action.

• Mechanistic information is essential to understanding how drugs and chemicals perturb development.

• Identifies important molecular initiating events for which rapid and cost efficient screens can be developed.

• Understanding mechanisms is needed for appropriate intervention and preventive public health strategies.

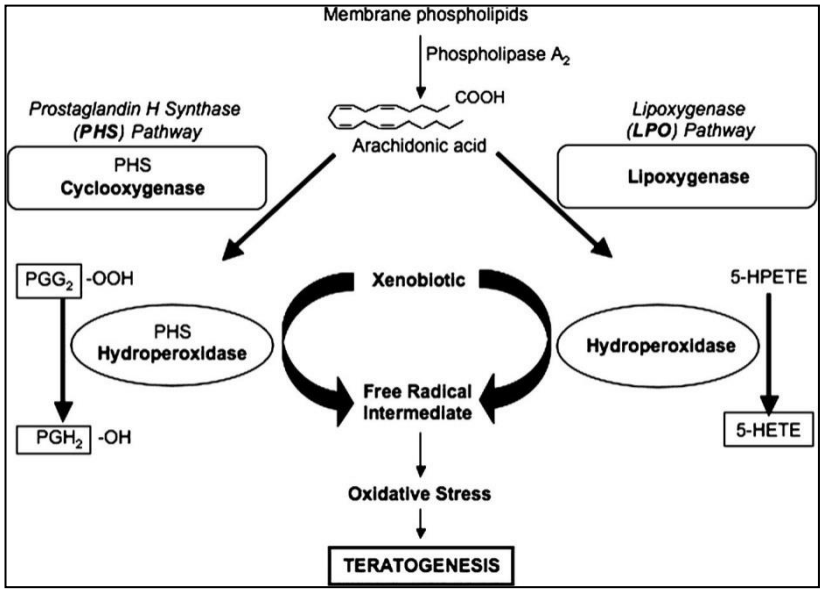


Figure: Mechanism of teratogen.

What defines a teratogenic mechanism?

*The means by which a lesion is produced and propagated through a series of measurable events in development.

* Starts with exposure (eg, maternal) and ends with an adverse developmental outcome (eg, malformation).

* Implies detailed molecular knowledge of the initial point of chemical-biological interaction (initiating event).

* Considers downstream pathogenesis that can be linked to dynamic changes in cell fate and behavior.

Large number of teratogens and adverse developmental outcomes makes it difficult to pinpoint unifying mechanisms. Principal teratogenic mechanisms based on associations of major birth defects with medications used by women of reproductive age:

1. Folate antagonism
2. Neural crest cell disruption
3. Endocrine disruption
4. Oxidative stress
5. Vascular disruption
6. Specific receptor- or enzyme-mediated teratogenesis.

Various mechanisms are involved in teratogenic effects:

i. Interference with Nucleic Acids:

Various teratogenic agents interfere with nucleic acid replication, transcription, or RNA translation. These include alkylating agents, antimetabolites, intercalating agents and amino acid antagonists.

ii. Inhibition of Enzymes:

Inhibitors of enzymes, e.g. 5-flourouracil, may induce malformation through interference with differentiation or growth by inhibiting thymidylate synthatase. Other examples include 6-aminonicotinamide, which inhibits glucose-6-phosphate dehydrogenase, and folate antagonists which inhibit dihydrofolate reductase.

iii. Deficiency of Energy Supply and Osmolarity:

Certain teratogens can affect the energy supply for the metabolism by restricting the availability of substrates either directly (e.g., dietary deficiencies) or through the presence of analogs for antagonists of vitamins, essential amino acids, and others.

In addition, hypoxia and agents i.e., CO and CO_2, can be teratogenic by depriving the metabolic process of the required O_2 and probably also by the production of osmolar imbalances. These can induce edema, which, in turn, cause mechanical distortion and tissue ischemia. Physical agents that can cause malformations include radiation, hypothermia, hyperthermia and mechanical trauma.

It shall not be out of place to mention that the mode of action of many teratogens is yet uncertain. Furthermore, a potential teratogen may or may not exert teratogenic effects depending on such factors as bio-activating mechanism, stability and detoxifying capability of the embryonic tissues. Appropriate experimental testing for the teratogenicity of toxicants is, therefore, essential.

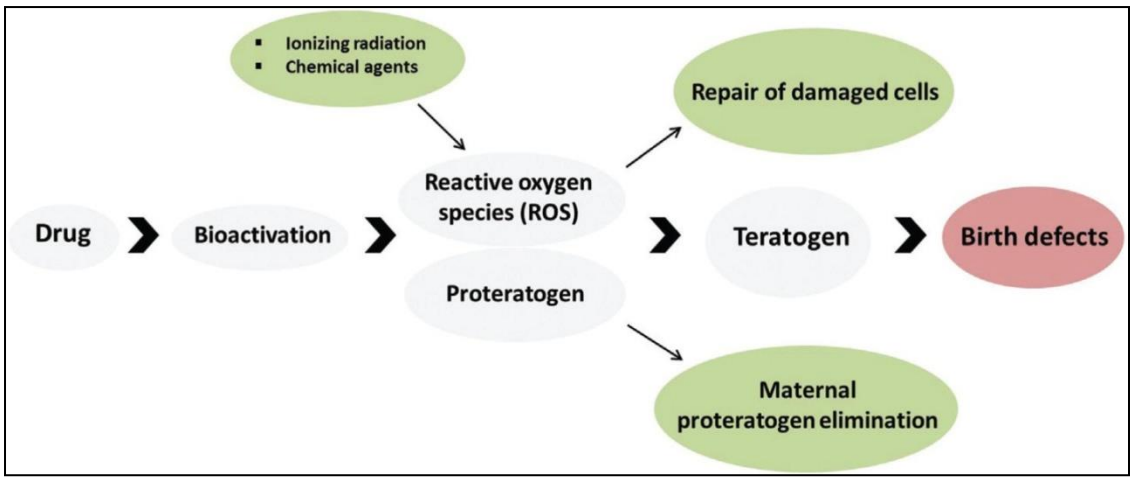


Figure: Mechanism of action of teratogen.

Probable questions:

1. What is teratology?
2. What is teratogen? Give example.
3. Which agents are responsible for genetic teratogenesis?
4. Which agents are responsible for environmental teratogenesis?
5. Write down the mechanism of teratogenesis.

Suggested readings:

1. Carlson BM. 2014. Human Embryology and Developmental Biology. 5th Edn. Elsevier.
2. Das N. 2012. Fundamental Concept of Developmental Biology. New Central Book Agency
3. Gardner DK. 2006. In Vitro Fertilization: a Practical Approach. CRC Press.
4. Gilbert S.F. 2010. Developmental Biology, IX Edition, Sinauer Associates, Inc., Publishers
5. Schoenwolf GC, Bleyl SB, Brauer PR, Francis-West PH. 2009. Ladesn's Human Embryology. Elsevier

UNIT VI

Contribution of teratology to Developmental Biology

Objective: In this unit we will discuss about contribution of teratology to Developmental Biology.

Introduction

Teratology, branch of the biological sciences dealing with the causes, development, description, and classification of congenital malformations in plants and animals and with the experimental production, in some instances, of these malformations.

Congenital malformations arise from interruption in the early development of the organism. Malformations in human infants, for example, may occur because the infant's genotype contains mutant genes or includes an abnormal number of chromosomes; they also may occur if early in pregnancy the mother has had German measles (rubella), has taken some injurious drug, or has been exposed to an injurious dosage of radiation. Experimental studies suggest similar types of factors can cause malformations in animals and plants.

Contribution of teratology in developmental biology

Teratology is the branch of medical science which studies the causes and underlying mechanisms of congenital birth defects. A teratogen can consist of any chemical, infectious or environmental agent capable of disrupting normal embryo-fetal development following exposure during pregnancy. Understanding the biological processes by which a teratogen causes congenital malformations requires the integration of fundamental scientific knowledge from a diverse range of disciplines including toxicology, developmental and molecular biology. Research within the field of teratology, can be utilised to inform the pharmaceutical industry and government agencies of preventative measures and treatment strategies for pregnant females and their offspring. This scientific knowledge can be further translated into a clinical setting within the specialties of prenatal medicine, obstetrics, neonatology and paediatrics. Alcohol, one of the oldest intoxicants in the world, has long been documented as a human teratogen and is a primary focus within this research stream.

While embryologists could look at embryos to describe the evolution of life and how different animals form their organs, physicians became interested in embryos for more practical reasons. About 2% of human infants are born with a readily observable anatomical abnormality. These abnormalities may include missing limbs, missing or extra digits, cleft palate, eyes that lack certain parts, hearts that lack valves, and so forth. Physicians need know the causes of these birth defects in order to counsel parents as to

the risk of having another malformed infant. In addition, the different birth defects can tell us how the human body is normally formed. In the absence of experimental data on human embryos, we often must rely on nature's "experiments" to learn how the human body becomes organized. Some birth defects are produced by mutant genes or chromosomes, and some are produced by environmental factors that impede development.

Abnormalities caused by genetic events (gene mutations, chromosomal aneuploidies and translocations) are called malformations. Malformations often appear as syndromes (from the Greek, "running together"), where several abnormalities are seen concurrently. For instance, a human malformation called piebaldism is due to a dominant mutation in a gene (KIT) on the long arm of chromosome 4. The syndrome includes anemia, sterility, unpigmented regions of the skin and hair, deafness, and the absence of the nerves that cause peristalsis in the gut. The common feature underlying these conditions is that the KIT gene encodes a protein that is expressed in the neural crest cells and in the precursors of blood cells and germ cells. The Kit protein enables these cells to proliferate. Without this protein, the neural crest cells—which generate the pigment cells, certain ear cells, and the gut neurons—do not multiply as much as they should (resulting in underpigmentation, deafness, and gut malformations), nor do the precursors of the blood cells (resulting in anemia) or the germ cells (resulting in sterility).

Probable questions:

1. Describe the contribution of teratology to Developmental Biology.

Suggested readings:

1. Gilbert S.F. 2010. Developmental Biology, IX Edition, Sinauer Associates, Inc., Publishers
2. Schoenwolf GC, Bleyl SB, Brauer PR, Francis-West PH. 2009. Ladesn's Human Embryology. Elsevier

UNIT VII

Ageing: Cellular basis of aging, Causes of aging

Objective: In this unit we will discuss about Ageing: Cellular basis of aging, Causes of aging.

Introduction

Aging is a progressive physiological change in an organism that lead to senescence, or a decline of biological functions and of the organism's ability to adapt to metabolic stress.

Aging takes place in a cell, an organ, or the total organism with the passage of time. It is a process that goes on over the entire adult life span of any living thing. Gerontology, the study of the aging process, is devoted to the understanding and control of all factors contributing to the finitude of individual life. It is not concerned exclusively with debility, which looms so large in human experience, but deals with a much wider range of phenomena. Every species has a life history in which the individual life span has an appropriate relationship to the reproductive life span and to the mechanism of reproduction and the course of development. How these relationships evolved is as germane to gerontology as it is to evolutionary biology. It is also important to distinguish between the purely physicochemical processes of aging and the accidental organismic processes of disease and injury that lead to death.

Gerontology, therefore, can be defined as the science of the finitude of life as expressed in the three aspects of longevity, aging, and death, examined in both evolutionary and individual (ontogenetic) perspective. Longevity is the span of life of an organism. Aging is the sequential or progressive change in an organism that leads to an increased risk of debility, disease, and death. Senescence consists of these manifestations of the aging process.

The viability (survival ability) of a population is characterized in two actuarial functions: the survivorship curve and the age-specific death rate, or Gompertz function. The relation of such factors as aging characteristics, constitutional vigour, physical factors, diet, and exposure to disease-causing organisms to the actuarial functions is complex. There is, nevertheless, no substitute for them as measures of the aging process and of the effect of environmental or genetic modifiers.

- ## Maximum Life Span and Life Expectancy

The **maximum life span** is a characteristic of the species. It is the maximum number of years a member of that species has been known to survive. The maximum human life span is estimated to be 121 years (Arking 1998). The life spans of tortoises and lake trout are both unknown, but are estimated to be more than 150 years. The maximum life span of a domestic dog is about 20 years, and that of a laboratory mouse is 4.5 years.

If a *Drosophila* fruit fly survives to eclose (in the wild, over 90% die as larvae), it has a maximum life span of 3 months.

However, a person cannot expect to live 121 years, and most mice in the wild do not live to celebrate their first birthday. The **life expectancy**, the amount of time a member of a species can expect to live, is not characteristic of species, but of populations. It is usually defined as the age at which half the population still survives. A baby born in England in the 1780s could expect to live to be 35 years old. In Massachusetts during that same time, the life expectancy was 28 years. This was the normal range of human life expectancy for most of the human race in most times. Even today, the life expectancy in some areas of the world (Cambodia, Togo, Afghanistan, and several other countries) is less than 40 years. In the United States, a child born in 1986 can expect to live 71 years if male and 78 years if female.

For humans in industrialized countries, life expectancy has increased significantly. Indeed, at the beginning of the 20th century, life expectancy in those countries was between 30 and 45 years. At the century's close, life expectancy averaged about 67 years, thanks in large part to improvements in health care, nutrition, and standards of living. In the early 21st century, demographic projections suggested that life expectancy for men and women who maintained the healthiest lifestyle patterns would continue to increase. In the first decade of the 21st century in the United States, centenarians—those who live to age 100 or older—were the fastest-growing segment of the population.

- ## Cellular basis of aging

Cellular aging is the result of a progressive decline in the proliferative capacity and life span of cells and the effects of continuous exposure to exogenous factors that cause accumulation of cellular and molecular damage.

- ## Causes of aging

The general senescent phenotype is characteristic of each species. But what causes it? This question can be asked at many levels. We will be looking primarily at the cellular level of organization. Even here, there is evidence for many different theories, and there is not yet a consensus on what causes aging.

1. Wear-and-tear theory

The "wear-and-tear" theory assumes that animals and cells, like machines, simply wear out. Animals, however, unlike machines, have some ability to repair themselves, so that this theory does not fit the facts of a biological system. A corollary to the wear-and-tear theory is the presumption that waste products accumulate within cells and interfere

with function. The accumulation of highly insoluble particles, known as “age pigments,” has been observed in muscle cells in the heart and nerve cells of humans and other animals.

2. Cross-linking theory

With increasing age, tendons, skin, and even blood vessels lose elasticity. This is due to the formation of cross-links between or within the molecules of collagen (a fibrous protein) that give elasticity to these tissues. The “cross-linking” theory of aging assumes that similar cross-links form in other biologically important molecules, such as enzymes. These cross-links could alter the structure and shape of the enzyme molecules so that they are unable to carry out their functions in the cell.

3. Autoimmune theory

Another theory of aging assumes that immune reactions, normally directed against disease-producing organisms as well as foreign proteins or tissue, begin to attack cells of the individual’s own body. In other words, the system that produces antibodies loses its ability to distinguish between “self” and foreign proteins. This “autoimmune” theory of aging is based on clinical rather than on experimental evidence.

4. Oxidative damage theory

Reactions that take place within cells can result in the oxidation of proteins and other cellular molecules. Oxidation entails the loss of electrons from these molecules, causing them to become unstable and highly reactive and leading to their eventual reaction with and damage of cell components such as membranes. Such reactive molecules are known as free radicals—any atom or molecule that has a single unpaired electron in an outer shell.

Oxidative damage (oxidative stress) accumulates with age, and this has given rise to the free radical theory of aging, which is concerned in particular with molecules known as reactive oxygen species (ROS). This theory was first proposed in the 1950s by American gerontologist Denham Harman and was supported in part by evidence that antioxidant proteins, which neutralize free radicals, are more abundant in aging cells, indicating a response to oxidative stress.

The initial free radical theory of aging was later extended to include ROS derived from cellular organelles known as mitochondria, which are the primary sites of energy production in most eukaryotic organisms (eukaryotic cells are cells with clearly defined nuclei). The mitochondrial theory of aging was based on the idea that there exists within mitochondria a vicious oxidation cycle, in which the mutation of mitochondrial DNA impairs the function of proteins in the organelle’s respiration machinery, thereby enhancing the production of DNA-damaging oxygen radicals. This in turn results in the accumulation of mutations in mitochondrial DNA and a bioenergetic impairment, characterized by the failure of mitochondria to produce sufficient energy for cells to carry out their daily activities, which leads to tissue dysfunction and degeneration.

A similar mitochondrial theory of aging proposes a mechanism in which electrons leaking from the electron transport chain (ETC), the central component of the organelle's respiration machinery, produce ROS and then damage ETC proteins and mitochondrial DNA, leading to further increases in intracellular ROS levels and a decline in mitochondrial function.

Another consideration is the molecular inflammatory theory of aging, whereby the activation of redox- (oxidation-reduction-) sensitive transcription factors (molecules that control gene activity) by age-related oxidative stress causes increased expression of proinflammatory genes, leading to inflammation in various tissues. This inflammatory cascade is exaggerated during aging and has been linked to many age-associated pathologies, including cancer, cardiovascular disease, arthritis, and several neurodegenerative diseases. Chronic inflammation, whether due to diet, infection, stress, or other factors, can potentially accelerate the aging process.

Mammals under calorie restriction produce fewer ROS and age slower. Such effects of calorie restriction have been attributed to its ability to lower the steady state of oxidative stress, slow the accumulation of age-associated oxidative damage, and increase metabolic efficiency.

A common phenomenon in all of the aforementioned theories is that ROS serve as a contributing factor to many age-associated diseases.

5. Mitochondrial genome damage

The mutation rate in mitochondria is 10–20 times faster than the nuclear DNA mutation rate (Johnson et al. 1999). It is thought that mutations in mitochondria could (1) lead to defects in energy production, (2) lead to the production of ROS by faulty electron transport, and/or (3) induce apoptosis. Age-dependent declines in mitochondrial function are seen in many animals, including humans (Boffoli et al. 1994). A recent report (Michikawa et al. 1999) shows that there are "hot spots" for age-related mutations in the mitochondrial genome, and that mitochondria with these mutations have a higher replication frequency than wild-type mitochondria. Thus, the mutants are able to outcompete the wild-type mitochondria and eventually dominate the cell and its progeny. Moreover, the mutations may not only allow more ROS to be made, but may make the mitochondrial DNA more susceptible to ROS-mediated damage.

6. Telomere shortening

Telomeres are repeated DNA sequences at the ends of chromosomes. They are not replicated by DNA polymerase, and they will shorten at each cell division unless maintained by **telomerase**. Telomerase adds the telomere onto the chromosome at each cell division. Most mammalian somatic tissues lack telomerase, so it has been proposed (Salk 1982; Harley et al. 1990) that telomere shortening could be a "clock"

that eventually prohibits the cells from dividing any more. When human fibroblasts are cultured, they can divide only a certain number of times, and their telomeres shorten. If these cells are made to express telomerase, they can continue dividing (Bodnar et al. 1998; Vaziri and Benchimol 1998).

However, there is no correlation between telomere length and the life span of an animal (humans have much shorter telomeres than mice), nor is there a correlation between human telomere length and a person's age (Cristofalo et al. 1998). Telomerase-deficient mice do not show profound aging defects, which we would expect if telomerase were the major factor in determining the rate of aging (Rudolph et al. 1999). It has been suggested that telomere-dependent inhibition of cell division might serve primarily as a defense against cancer rather than as a kind of "aging clock."

7. Genetic aging programs

Several genes have been shown to affect aging. In humans, Hutchinson-Gilford progeria syndrome causes children to age rapidly and to die (usually of heart failure) as early as 12 years. It is caused by a dominant mutant gene, and its symptoms include thin skin with age spots, resorbed bone mass, hair loss, and arteriosclerosis. A similar syndrome is caused by mutations of the *klotho* gene in mice (Kuro-o et al. 1997). The functions of the products of these genes are not known, but they are thought to be involved in suppressing the aging phenotypes. These proteins may be extremely important in determining the timing of senescence.

In *C. elegans,* there appear to be at least two genetic pathways that affect aging. The first pathway involves the decision to remain a larva or to continue growth. After hatching, the *C. elegans* larva proceeds through four instar stages, after which it can become an adult or (if the nematodes are overcrowded or if there is insufficient food) can enter a nonfeeding, metabolically dormant **dauer stage**. It can remain a dauer larva for up to 6 months, rather than becoming an adult that lives only a few weeks. When it comes out of the dauer stage, it will live as long as if it had never been a dauer larva. In the dauer stage, adult development is suppressed, and extra defenses against ROS are synthesized. If some of the genes involved in this pathway are mutated, adult development is allowed, but the ROS defenses are still made. The resulting adults live twice to four times as long as wild-type adults (Figure 1: Friedman and Johnson 1988). The second pathway involves the gonads. Germ cells appear to inhibit longevity, while the somatic cells of the gonads act to prolong the life of the nematode (Hsin and Kenyon 1999).

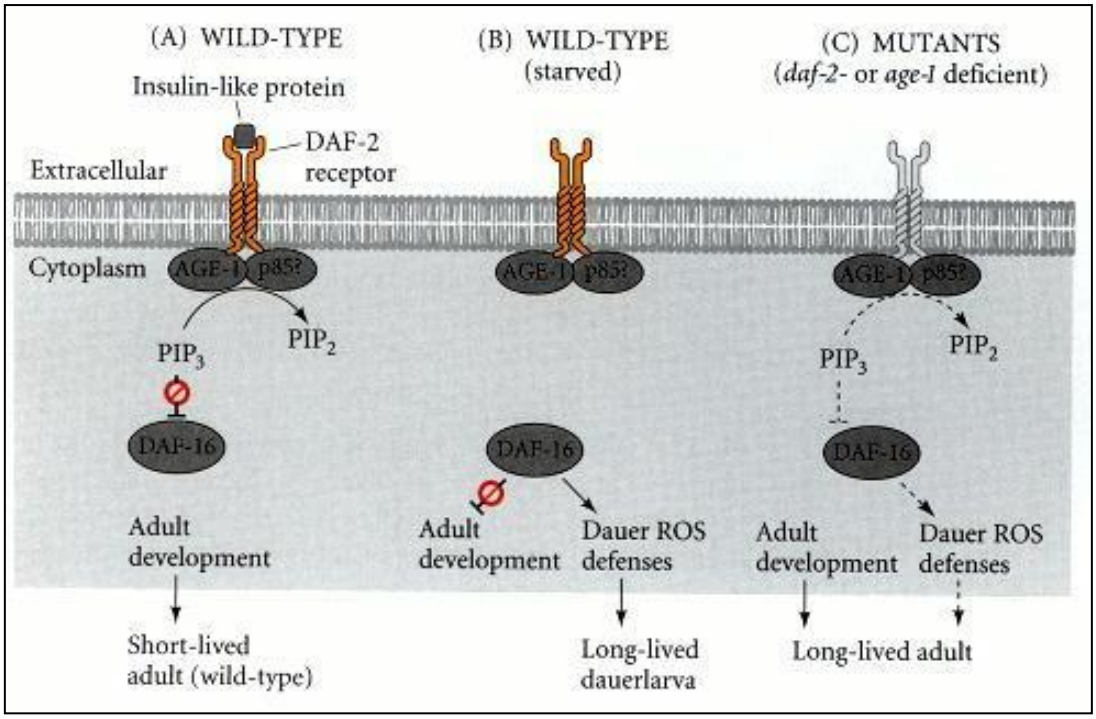


Figure 1: Proposed mechanism for extending the life span of *C. elegans* through the dauer larva pathway. (A) Wild-type animal in a favorable environment makes a ligand that activates a pathway that inhibits the DAF-16 protein. This allows metamorphosis to the adult stage. (B) If a wild-type larva is starved, the ligand is not made, and DAF-16 creates a long-lived dauer larva by repressing the pathway to adult development and by increasing the ROS-degrading enzymes. (C) In mutants deficient in *age-1* (or *daf-2*), DAF-16 is only partially inhibited. Adult development is permitted, and the ROS-degrading enzymes are also made. This creates a long-lived adult.

8. Changes in structural tissues

The structural integrity of the vertebrate organism depends on two kinds of fibrous protein molecules, collagen and elastin. Collagen, which constitutes almost one-third of the body protein, is found in skin, bone, and tendons. When first synthesized by cells called fibroblasts, collagen is in a fragile and soluble form (tropocollagen). In time this soluble collagen changes to a more stable, insoluble form that can persist in tissues for most of an animal's life. The rate of collagen synthesis is high in youth and declines throughout life, so that the ratio of insoluble to soluble collagen increases with age. Insoluble collagen then builds up with age as a result of synthesis exceeding removal, much like another fibrous tissue, the crystalline lens of the eye. With increasing age, the number of cross-linkages within and between collagen molecules increases, leading to crystallinity and rigidity, which are reflected in a general body stiffness. There is also a decrease in the relative amount of a mucopolysaccharide (i.e., the combination of a protein and a carbohydrate) ground substance; a measure of this, the hexosamine–collagen ratio, has been investigated as an index of individual differences in the rate of

aging. An important consequence of these changes is decreased permeability of the tissues to dissolved nutrients, hormones, and antibody molecules.

The rate of aging of collagen is related to the overall metabolic activity of the animal; rats kept on low-calorie diets have more youthful collagen than fully nourished rats of the same age.

Elastin is the molecule responsible for the elasticity of blood vessel walls. With age, progressive loss of elasticity of vessels occurs, presumably because of fragmentation of the elastin molecule.

The cross-linkage of collagen is chemically similar to the cross-linkages that occur in skins when they are tanned to leather. This similarity has stimulated proposals that chemicals that inhibit cross-linkage in tanning will retard aging.

9. Tissue cell loss and replacement

The tissues of the body fall into two groups, according to whether or not there is continuous renewal of tissue cells. At one extreme are nonrenewal tissues such as nerves and voluntary muscles, in which few new cells are formed (at least in mammals) after a certain stage of growth. In renewal tissues such as the intestinal epithelium and the blood, on the other hand, some cell types live only one or a few days and must be replaced hundreds of times in the life span of even a short-lived animal such as the rat. Between these limits lie many organs, such as liver, skin, and endocrine organs, that have cells that are replaced over periods ranging from a few weeks to several years in humans.

A peripheral nerve is a convenient object to study because the total number of fibres in the nerve trunk can be counted. This has been done for the cervical and thoracic spinal nerve roots of the rat, the cat, and humans. In the ventral and dorsal spinal roots of humans, the number of nerve fibres decreases about 20 percent from age 30 to age 90. In the cat, the rat, and the mouse, however, the data do not consistently indicate a decrease of number of spinal root fibres with age. In humans the number of olfactory nerve fibres, which serve the sense of smell, decreases by age 90 to about 25 percent of the number present at birth, and the number of optic nerve fibres, serving vision, decreases at a nearly comparable rate.

There is a striking decrease in the number of living cells in the cerebral cortex of the brain of humans with age. The cerebellar cortex of the rat and human is about as susceptible to age deterioration as is the cerebral cortex. Other parts of the brain are not so obviously marked by aging.

There is, in short, a tendency for the higher and more recently evolved levels of the nervous system to undergo more severe aging loss than do other regions, such as the brainstem and spinal cord. It is not yet known how much of the loss of brain cells results from conditions within the brain itself and how much result from extrinsic

causes, such as deterioration of the blood circulation. The nutrition and maintenance of nerve cells, or neurons, in the central nervous system depends to a considerable extent on neuroglia, small cells that surround the neurons. The absolute number of these cells apparently does not decrease with age, but some of the microscopic changes seen in the neurons of old persons are similar to the changes produced by starvation or physical exhaustion.

It has been shown that after an attack of measles, the virus remains in the host's body for the remainder of life and infrequently gives rise to a rapidly progressing degeneration of the cerebral cortex. This virus or other inapparent viruses may also be responsible for the individual differences in onset of senility in humans.

The renewal tissues are typically made up of a population of proliferative cells, which retain the capability for division, and a population of mature cells, produced by the proliferative cells and with limited life spans. The production of cells must balance the steady loss and also compensate quickly for unusual losses caused by injury or disease, so each renewal tissue has one or more channels of feedback control to adjust production to demand. Aging of renewal tissues is expressed in several ways, including decrease in the number of proliferative cells, decrease in the rate of cell division, and decrease in responsiveness to feedback signals. Changes of these factors in the blood-forming tissues of the mouse are small, yet the blood-forming tissues do suffer an aging deficit, for the ability to respond to extreme or repeated demand is significantly reduced in older mice.

The intact skin has a cell turnover time of several weeks, with the capability, shared by all renewal tissues, of temporarily increasing the rate of cell production by a large factor in response to injury. The rate of wound healing decreases with age, rapidly at first and more slowly as age increases.

One of the most regular and striking aging processes is the decrease in the ability to focus on both close and distant objects. This loss in visual accommodation is the result in part of a weakening of the ciliary muscle of the eye and of a decrease in the flexibility of the lens. A further contributing factor, however, is that the lens continues to grow throughout life at a rate that diminishes with age. This growth is the result of continuous division of epithelial cells near an imaginary midline of the lens, giving rise to fresh cells that differentiate into the precisely aligned lens fibres. Once formed, the fibres remain permanently in place.

An important feature of the renewal mechanism is the stem cell. These cells, which may normally continue to divide at a low rate throughout life, under conditions of increased demand enter a compensatory proliferative phase during which they divide rapidly. Blood-forming tissue has a stem cell population that responds to injury readily in youth, but its capacity diminishes with age. The increased incidence of anemia in old age and the reduced capacity to respond to blood loss have been attributed to depletion of the blood-forming stem cells. Stem cell populations have not been identified with certainty

in other proliferative tissues. The intestinal mucosa, in particular, has a high cell-division rate without any clear indication of a reserve population of stem cells.

Probable questions:

1. What is aging?
2. What do you mean by cellular basis of aging?
3. Describe the Cross-linking theory of aging.
4. Describe the Oxidative damage theory of aging.
5. Discuss how Telomere shortening is responsible for aging.
6. Discuss how Changes in structural tissues is responsible for aging.

Suggested readings:

1. Carlson BM. 2014. Human Embryology and Developmental Biology. 5th Edn. Elsevier.
2. Das N. 2012. Fundamental Concept of Developmental Biology. New Central Book Agency
3. Gardner DK. 2006. In Vitro Fertilization: a Practical Approach. CRC Press.
4. Gilbert S.F. 2010. Developmental Biology, IX Edition, Sinauer Associates, Inc., Publishers,
5. Schoenwolf GC, Bleyl SB, Brauer PR, Francis-West PH. 2009. Ladesn's Human Embryology. Elsevier

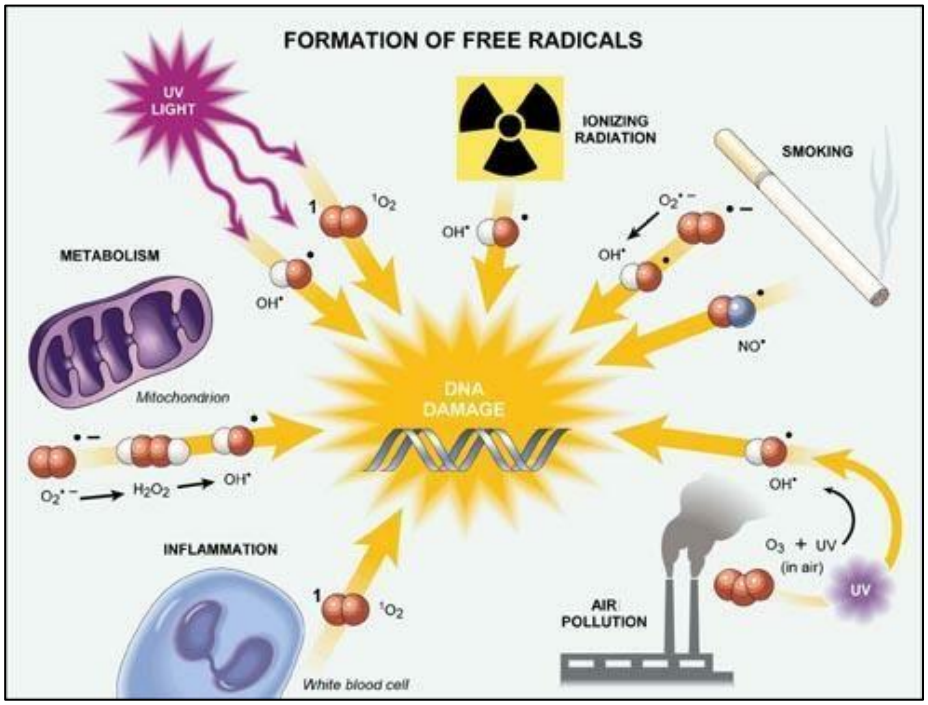
UNIT VIII

Free Radical Theory of Aging

Objective: In this unit we will discuss about Free Radical Theory of Aging.

Free Radical Theory of Aging (FRTA)

The **free radical theory of aging** (**FRTA**)was conceived by Denham Harman in the 1950s, when prevailing scientific opinion held that free radicals were too unstable to exist in biological systems.The free radical theory of aging states that organisms age because cells accumulate free radical damage over time. A free radical is any atom or molecule that has a single unpaired electron in an outer shell. While a few



free radicals such as melanin are not chemically reactive, most biologically relevant free radicals are highly reactive.For most biological structures, free radical damage is closely associated with oxidative **damage**.The free radical theory proposes that ageing is the cumulative result of oxidative damage to the cells and tissues of the body that arises primarily as a result of aerobic metabolism.

Several lines of evidence have been used to support this hypothesis including the claims that:

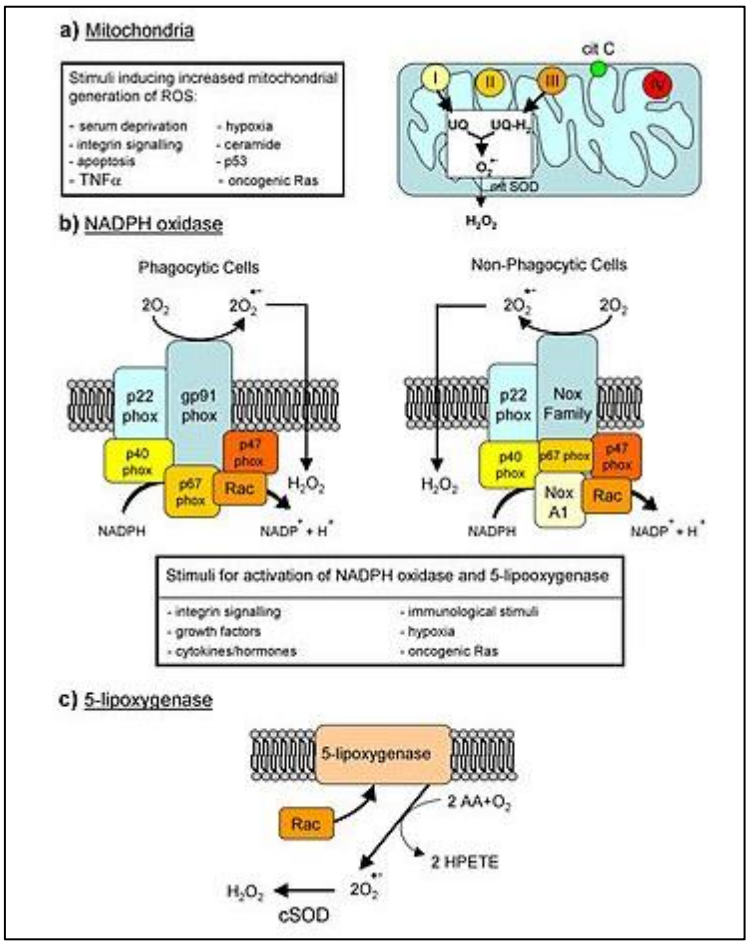
(1) Variation in species life span is correlated with metabolic rate and protective antioxidant activity;

(2) Enhanced expression of antioxidative enzymes in experimental animals can produce a significant increase in longevity;

(3) Cellular levels of free radical damage increases with age; and

(4) Reduced calorie intake leads to a decline in the production of reactive oxygen species and an increase in life span.

The free radical theory may also be used to explain many of the structural features that develop with ageing including the lipid peroxidation of membranes, formation of age pigments, cross-linkage of proteins, DNA damage and decline of mitochondrial function. Free radicals only occur in trace quantities in biological tissues, their cellular levels and actions cannot be measured in vivo, and definitive proof that oxidized molecules are the primary cause of ageing is lacking. Moreover, ageing is also likely to be a multifactorial process and not reducible to any one single cause.



The free radical theory is only concerned with free radicals such as superoxide (O_2^-), but it has since been expanded to encompass oxidative damage from other Reactive Oxygen Species **(ROS)** such as hydrogen peroxide **(H_2O_2)**, or peroxynitrite **($OONO^-$)**.

The free radical theory was expanded to include not only aging, but also age-related diseases. Free radical damage within cells has been linked to a range of disorders

including cancer, arthritis, atherosclerosis, Alzheimer's disease, and diabetes. There has been some evidence to suggest that free radicals and some reactive nitrogen species trigger and increase cell death mechanisms within the body such as **apoptosis**and in extreme cases **necrosis**.

In 1972, **Harman** modified his original theory to what became known as the **mitochondrial theory of aging**. In its current form, this theory proposes that reactive oxygen species that are produced in the mitochondria, causes damage to certain macromolecules including lipids, proteins and most importantly mitochondrial DNA. This damage then causes mutations which lead to an increase of ROS production and greatly enhance the accumulation of free radicals within cells.

Mitochondrial theory of aging was first proposed in 1978, and shortly thereafter the mitochondrial free radical theory of aging was introduced in 1980. The theory implicates the mitochondria as the chief target of radical damage, since there is a known chemical mechanism by which mitochondria can produce Reactive oxygen species(ROS), mitochondrial components such as mtDNA are not as well protected as nuclear DNA, and by studies comparing damage to nuclear and mtDNA that demonstrate higher levels of radical damage on the mitochondrial molecules. Electrons may escape from metabolic processes in the mitochondria like the Electron transport chain, and these electrons may in turn react with water to form ROS such as the superoxide radical, or via an indirect route the hydroxyl radical. These radicals then damage the mitochondria's DNA and proteins, and these damage components in turn are more liable to produce ROS byproducts. Thus a positive feedback loop of oxidative stress is established that, over time, can lead to the deterioration of cells and later organs and the entire body.

Epigenetic oxidative redox shift (EORS) theory of aging:

Brewer proposed a theory which integrates the free radical theory of aging with the insulin signalling effects in aging. Brewer's theory suggests "sedentary behaviour associated with age triggers an oxidized redox shift and impaired mitochondrial function". This mitochondrial impairment leads to more sedentary behaviour and accelerated aging. (Refer note on **Cellular Basis Of Aging**).

Probable questions:

1. Describe the free radical theory of aging.

Suggested readings:

1. Carlson BM. 2014. Human Embryology and Developmental Biology. 5th Edn. Elsevier.
2. Das N. 2012. Fundamental Concept of Developmental Biology. New Central Book Agency
3. Gardner DK. 2006. In Vitro Fertilization: a Practical Approach. CRC Press.
4. Gilbert S.F. 2010. Developmental Biology, IX Edition, Sinauer Associates, Inc., Publishers,
5. Schoenwolf GC, Bleyl SB, Brauer PR, Francis-West PH. 2009. Ladesn's Human Embryology. Elsevier

UNIT IX

Role of anti-oxidant enzymes in the process of aging, aging related disorders

Objective: In this unit we will discuss about Role of anti-oxidant enzymes in the process of aging, aging related disorders.

Introduction

The term antioxidant originally was used to refer specifically to a chemical that prevented the consumption of oxygen. In the late 19^{th} and early 20^{th} century, extensive study was devoted to the uses of antioxidants in important industrial processes, such as the prevention of metal corrosion, the vulcanization of rubber, and the polymerization of fuels in the fouling of internal combustion engines.

Early research on the role of antioxidants in biology focused on their use in preventing the oxidation of unsaturated fats, which is the cause of rancidity.

Antioxidant activity could be measured simply by placing the fat in a closed container with oxygen and measuring the rate of oxygen consumption. However, it was the identification of vitamins A, C, and E as antioxidants that revolutionized the field and led to the realization of the importance of antioxidants in the biochemistry of living organisms.

Research into how vitamin E prevents the process of lipid peroxidation led to the identification of antioxidants as reducing agents that prevent oxidative reactions, often by scavenging reactive oxygen species before they can damage cells.

- **Classification of Antioxidant:**

The body has several mechanisms to counteract oxidative stress by producing antioxidants, either naturally generated in situ (endogenous antioxidants), or externally supplied through foods (exogenous antioxidants).

The roles of antioxidants are to neutralize the excess of free radicals, to protect the cells against their toxic effects and to contribute to disease prevention. Endogenous compounds in cells can be classified as enzymatic and non-enzymatic antioxidants.

A. <u>Enzymatic Antioxidants:</u>

The major enzymatic antioxidants directly involved in the neutralization of ROS and RNS are superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione reductase (GRx).

I. Superoxide Dismutase (SOD):

Superoxide dismutase (SOD) scavenges both extracellular and intracellular superoxide anion and prevents lipid peroxidation of the plasma membrane. It is first line enzyme of defense against free radicals, catalyzes the dis-mutation of superoxide anion radical (O_2) into hydrogen peroxide (H_2O_2) by reduction.

The oxidant formed (H_2O_2) is transformed into water and oxygen (O_2) by catalase or glutathione peroxidase. SOD also prevents premature hyper activation and capacitation induced by superoxide radicals before ejaculation.

II. Catalase (CAT):

Catalase detoxifies both intracellular and extracellular H_2O_2 to water and oxygen (Baker et al., 1996). In addition, catalase activates NO-induced sperm capacitation, which is a complex mechanism involving H_2O_2.

III. Glutathione Peroxidase (GPx) /Reductase System (GRx):

This system forms an excellent protection against lipid peroxidation of plasma membrane of spermatozoa. It scavenges lipid peroxides thereby arresting the progressive chain reaction of lipid peroxidation. It also scavenges hydrogen peroxide (H_2O_2), which is responsible for the initiation of lipid peroxidation. Glutathione reductase (GRx) stimulates the reduction of glutathione disulfide to reduced glutathione.

Glutathione reductase, a flavoprotein enzyme, regenerates reduced glutathione (GSH) from oxidized glutathione (GSSG), with NADPH as a source of reducing power. Besides hydrogen peroxide, GPx also reduces lipid or non-lipid hydro peroxides while oxidizing glutathione (GSH). This ensures a steady supply of the reductive substrate (NADPH) to glutathione peroxidase.

B. Non-Enzymatic Antioxidants:

The non-enzymatic antioxidants are also divided into metabolic and nutrient antioxidants. Metabolic antioxidants belonging to endogenous antioxidants, are produced by metabolism in the body, such as lipoid acid, glutathione, L-arginine, coen-zyme Q10, melatonin, uric acid, bilirubin, metal- chelating proteins, transferrin, etc.

While nutrient antioxidants belonging to exogenous antioxidants, are compounds which cannot be produced in the body and must be provided through foods or supplements, such as vitamin E, vitamin C, carotenoids, trace metals (selenium, manganese, zinc), flavonoids, omega-3 and omega-6 fatty acids, etc. copper and iron.

- **Role of anti-oxidant enzymes in the process of aging**

Aging is a complex process where most antioxidant enzymes, including peroxidases, undergo a marked change. The main endogenous antioxidants are enzymes that reduce the danger of free radicals, i.e., SOD, glutathione peroxidase (GPx) and CAT

OXIDATIVE STRESS IN AGING The "Free Radical Theory of Aging" as first presented by Harman (1) postulated that oxygen radicals generated in metabolic pathways damaged cells and increased their vulnerability to death. It also postulated that it is the incessant accumulation of structural damage that disrupts functions at a macromolecular level and is the underlying cause of aging. Since it was first proposed, there have been many modifications to this theory. From a number of studies, it has also become apparent that neither gross structural damage to cellular components, nor decreased repair capacity can completely account for cellular dysfunction and death. However, even if free radical reactions do not account for all aspects of aging, they appear to underlie many aspects of aging and to play a major role in the onset and progression of many human diseases. Free radical reactions probably account for certain aspects of adult respiratory distress syndrome, age-associated diseases such as diabetes, ischemic injury associated with organ transplant, stroke and heart disease and various late-onset neurodegenerative diseases.

Aging is usually associated with increasing levels of oxidation. Conversely, the antioxidant defenses only rarely increase during aging; they are known to decline in some tissues during aging. In most cases, however, the antioxidant defenses do not change with age. It has been demonstrated repeatedly that the relative rate of oxidant generation increases with age, which correlates with age-associated changes in cellular redox state that are also commonly seen during aging. For example, the rates of superoxide (O_2-) and H_20_2 generation increase in the cells of aging organisms while glutathione concentration declines progressively with advancing age. Furthermore, it has been demonstrated that species longevity correlates inversely with the rate of free radical generation and that over expression of Cu/Zn SOD (SOD-l) and catalase can extend the lifespan and metabolic potential in *Drosophila*. In spite of this, the full extent of oxidative involvement in the regulation of longevity is only beginning to be understood. The underlying causes of aging-associated increases in oxidative stress are unknown. In vivo, age-associated decreases in the activities of cytochrome c oxidase, NADH dehydrogenase and to a lesser extent succinate dehydrogenase activities have been reported in a wide variety of mammalian species including humans. These changes are believed to play an important role in aging-dependent increases in oxidation *in vivo* although they do not necessarily occur in all or even most of the cells of a given tissue. Those aging-associated decreases in cytochrome c oxidase that occur in vivo appear to result from age dependent changes in lipid-protein interactions. Furthermore, restoring young levels of mitochondrial membrane cardiolipin in rats by treatment with acetyI-L-carnitine restores cytochrome c oxidase activity to the level seen in young animals. While the majority of studies show that oxidant generation increases with age, there are

some instances in which oxidant generation fails to increase and may even decline during aging. The reasons for these discrepancies are unknown, but assay conditions appear to be a major factor. Considering the effects of membrane changes on the activities of key mitochondrial enzymes, it also seems probable that tissue differences in membrane composition as well as the diets of experimental animals could to some extent determine whether age-associated changes in oxidant generation are observed.

i) Oxidative Stress, Aging and Neurodegenerative Disease Of central biological interest to studies of aging are the cellular mechanisms that measure physiological time in order to signal initiation or termination of critical events at various stages of life. An understanding of these mechanisms is crucial to elucidating the mechanisms of late-onset degenerative diseases associated with aging. Although aging is progressive, some age-associated changes and disease states appear to occur suddenly rather than gradually. For example, aging is the major risk factor for late-onset neurodegenerative diseases such as Parkinson's disease, Alzheimer's disease, Huntington's disease and amyotrophic lateral sclerosis (ALS; Lou Gehrig's Disease). Furthermore, even when expressed as a dominant trait, penetrance is rarely seen during the first several decades of life. This suggests that some disease genes may cause disease only when the level of cellular damage has reached a critical level or when the genetic background in the cells has undergone age-associated changes that are permissive to the disease state. Interestingly, the late-onset neurodegenerative diseases are frequently associated with impaired function of the mitochondrial respiratory complexes or defects in cellular machinery that removes metabolically-generated oxidants. For example, cytochrome c oxidase activity is diminished in some cases of ALS and Alzheimer's, while NADH dehydrogenase (complex I) is increased by as much as 55% in patents with ALS. Changes in the abundance and activity of other respiratory complexes are also associated with ALS, Alzheimer's, Huntington's and Parkinson's disease and are discussed in detail by Bowling and Beal. Possibly the most compelling evidence of oxidative stress involvement in neurodegenerative diseases stems from the fact that defects in superoxide dismutase, an enzyme associated with oxidant removal, appear to be the cause of one form of ALS.

ii.) SOD-1 in Familial Amyotrophic Lateral Sclerosis (ALS) Amyotrophic Lateral Sclerosis is an adult-onset, progressive, paralytic disorder that leads to paralysis and death largely as a result of degeneration of motor neurons in cortex, brain stem and spinal cord. While the majority of ALS cases occur sporadically, about 10-15% is inherited as an autosomal dominant trait. About 15-20% of the familial cases (2% of all cases) appear to arise because of mutations in the copper/zinc superoxide dismutase gene. A summary of known SOD-1 defects associated with ALS is presented in Table 1. A complete discussion of all of these mutations is beyond the scope of this discussion; however, more detailed discussions do exist and the interested reader is referred to these. Interestingly, no mutations that cause ALS have ever been found in exon 3, although one silent mutation has been observed in exon 3 of a human SOD-1 in transgenic mice. The effects of these mutations on SOD protein are known. Normally,

the copper/zinc form of SOD protein (SOD-l) exists as a dimer; it contains a large 13-sheet that consists of 8 strands in an antiparallel arrangement. About 50% of the SOD-1 residues are contained in this 13-sheet, which, seen in three dimensions, appears as a cylinder or barrel (13-barrel). There are seven loops in SOD; loops I and V are short 13-hairpin connections between adjacent 13-strands.

Aging related disorders

- **Ageing explained**

At the biological level, ageing results from the impact of the accumulation of a wide variety of molecular and cellular damage over time. This leads to a gradual decrease in physical and mental capacity, a growing risk of disease and ultimately death. These changes are neither linear nor consistent, and they are only loosely associated with a person's age in years. The diversity seen in older age is not random. Beyond biological changes, ageing is often associated with other life transitions such as retirement, relocation to more appropriate housing and the death of friends and partners.

- **Common health conditions associated with ageing**

Common conditions in older age include hearing loss, cataracts and refractive errors, back and neck pain and osteoarthritis, chronic obstructive pulmonary disease, diabetes, depression and dementia. As people age, they are more likely to experience several conditions at the same time.

Older age is also characterized by the emergence of several complex health states commonly called geriatric syndromes. They are often the consequence of multiple underlying factors and include frailty, urinary incontinence, falls, delirium and pressure ulcers.

I. Cardiovascular system

What's happening?

The most common change in the cardiovascular system is stiffening of the blood vessels and arteries, causing your heart to work harder to pump blood through them. The heart muscles change to adjust to the increased workload. Your heart rate at rest will stay about the same, but it won't increase during activities as much as it used to. These changes increase the risk of high blood pressure (hypertension) and other cardiovascular problems.

II. Bones, joints

What's happening?

Bones tend to become less dense. Moderate loss of bone density is called **osteopenia** and severe loss of bone density (including occurrence of a fracture due to loss of bone density) is called **osteoporosis.** With osteoporosis, bones become weaker and more likely to break. In women, loss of bone density speeds up after menopause because less estrogen is produced. Estrogen helps prevent too much bone from being broken down during the body's normal process of forming, breaking down, and re-forming bone.

Bones become less dense partly because they contain less calcium (which gives bones strength). The amount of calcium decreases because the body absorbs less calcium from foods. Also, levels of vitamin D, which helps the body use calcium, decrease slightly. Certain bones are weakened more than others. Those most affected include the end of the thighbone (femur) at the hip, the ends of the arm bones (radius and ulna) at the wrist, and the bones of the spine (vertebrae).

Changes in vertebrae at the top of the spine cause the head to tip forward, compressing the throat. As a result, swallowing is more difficult, and choking is more likely. The vertebrae become less dense and the cushions of tissue (disks) between them lose fluid and become thinner, making the spine shorter. Thus, older people become shorter.

The cartilage that lines the joints tends to thin, partly because of the wear and tear of years of movement. The surfaces of a joint may not slide over each other as well as they used to, and the joint may be slightly more susceptible to injury. Damage to the cartilage due to lifelong use of joints or repeated injury often leads to **osteoarthritis**, which is one of the most common disorders of later life.

Ligaments, which bind joints together, and tendons, which bind muscle to bone, tend to become less elastic, making joints feel tight or stiff. These tissues also weaken. Thus, most people become less flexible. Ligaments and tendons tend to tear more easily, and when they tear, they heal more slowly. These changes occur because the cells that maintain ligaments and tendons become less active.

III. Muscles

What's happening?

The amount of muscle tissue (muscle mass) and muscle strength tend to decrease beginning around age 30 and continuing throughout life. Some of the decrease is caused by physical inactivity and decreasing levels of growth hormone and testosterone, which stimulate muscle development. Also, muscles cannot contract as quickly because more fast-contracting (fast-twitch) muscle fibers are lost than slow-contracting (slow-twitch) muscle fibers. However, aging's effects reduce muscle

mass and strength by no more than about 10 to 15% during an adult's lifetime. In the absence of disease, most of the loss beyond that 10 to 15% is preventable with regular exercise. More severe muscle loss (called **sarcopenia**, which literally means loss of flesh) results from disease or extreme inactivity, not from aging alone.

Older people retain enough muscle mass and strength for all necessary tasks. Many older people remain strong athletes. They compete in sports and enjoy vigorous physical activity. However, even the fittest notice some decline as they age.

IV. Digestive system

What's happening?

Age-related structural changes in the large intestine can result in more constipation in older adults. Other contributing factors include a lack of exercise, not drinking enough fluids and a low-fiber diet. Medications, such as diuretics and iron supplements, and certain medical conditions, such as diabetes, also might contribute to constipation.

V. Bladder and urinary tract

What's happening?

Your bladder may become less elastic as you age, resulting in the need to urinate more often. Weakening of bladder muscles and pelvic floor muscles may make it difficult for you to empty your bladder completely or cause you to lose bladder control (urinary incontinence). In men, an enlarged or inflamed prostate also can cause difficult emptying the bladder and incontinence.

Other factors that contribute to incontinence include being overweight, nerve damage from diabetes, certain medications, and caffeine or alcohol consumption.

VI. Memory and thinking skills

What's happening?

Your brain undergoes changes as you age that may have minor effects on your memory or thinking skills. For example, healthy older adults might forget familiar names or words, or they may find it more difficult to multitask.

VII. Eyes and ears

What's happening?

As people age, the following occur:

- The lens stiffens, making focusing on close objects harder.

- The lens becomes denser, making seeing in dim light harder.
- The pupil reacts more slowly to changes in light.
- The lens yellows, changing the way colors are perceived.
- The number of nerve cells decrease, impairing depth perception.
- The eyes produce less fluid, making them feel dry.

A change in vision is often the first undeniable sign of aging.

Changes in the lenses of the eye can cause or contribute to the following:

- **Loss of near vision:** During their 40s, most people notice that seeing objects closer than 2 feet becomes difficult. This change in vision, called presbyopia, occurs because the lens in the eye stiffens. Normally, the lens changes its shape to help the eye focus. A stiffer lens makes focusing on close objects harder. Ultimately, almost everyone gets presbyopia and needs magnifying reading glasses. People who need glasses to see distant objects may need to wear bifocals or glasses with variable-focus lenses.

- **Need for brighter light:** As people continue to age, seeing in dim light becomes more difficult because the lens tends to become less transparent. A denser lens means that less light passes through to the retina at the back of the eye. Also, the retina, which contains the cells that sense light, becomes less sensitive. So for reading, brighter light is needed. On average, 60-year-olds need 3 times more light to read than 20-year-olds.

- **Changes in color perception:** Colors are perceived differently, partly because the lens tends to yellow with aging. Colors may look less bright and contrasts between different colors may be more difficult to see. Blues may look more gray, and blue print or background may look washed out. These changes are insignificant for most people. However, older people may have trouble reading black letters printed on a blue background or reading blue letters.

The pupil of the eye reacts more slowly to changes in light. The pupil widens and narrows to let more or less light in, depending on the brightness of the surroundings. A slow-reacting pupil means that older people may be unable to see when they first enter a dark room. Or they may be temporarily blinded when they enter a brightly lit area. Older people may also become more sensitive to glare. However, increased sensitivity to glare is often due to darkened areas in the lens or to cataracts.

Most changes in hearing are probably due as much to a lifetime of noise exposure as to aging. Exposure to loud noise over time damages the ear's ability to hear. Nonetheless, some changes in hearing occur as people age, regardless of their exposure to loud noise.

It is important to see a doctor to determine whether hearing loss is due to cerumen (ear wax) impaction because this is easily treated.

As people age, hearing high-pitched sounds becomes more difficult. This change is considered age-associated hearing loss (**presbycusis**). For example, violin music may sound less bright.

The most frustrating consequence of presbycusis is that words become harder to understand. As a result, older people may think that other people are mumbling. Even when other people speak more loudly, older people still have difficulty understanding the words. The reason is that most consonants (such as k, t, s, p, and ch) are high-pitched, and consonants are the sounds that help people identify words. Because vowels are lower-pitched sounds, they are easier to hear. So older people may hear "Ell me exaly wha you wan oo ee," rather than "Tell me exactly what you want to keep." To help, other people need to articulate consonants more clearly, rather than simply speak louder. Understanding what women and children say may be more difficult than understanding what men say because most women and children have higher-pitched voices. Gradually, hearing lower pitches also becomes more difficult.

VIII. Teeth

What's happening?

Your gums might pull back from your teeth. Certain medications, such as those that treat allergies, asthma, high blood pressure and high cholesterol, also can cause dry mouth. As a result, your teeth and gums might become slightly more vulnerable to decay and infection.

IX. Skin

What's happening?

With age, your skin thins and becomes less elastic and more fragile, and fatty tissue just below the skin decreases. You might notice that you bruise more easily. Decreased production of natural oils might make your skin drier. Wrinkles, age spots and small growths called skin tags are more common.

X. Weight

What's happening?

How your body burns calories (metabolism) slows down as you age. If you decrease activities as you age, but continue to eat the same as usual, you'll gain weight. To maintain a healthy weight, stay active and eat healthy.

XI. Brain and nervous system

The number of nerve cells in the brain typically decreases. However, the brain can partly compensate for this loss in several ways:

- As cells are lost, new connections are made between the remaining nerve cells.
- New nerve cells may form in some areas of the brain, even during old age.

- The brain has more cells than it needs to do most activities—a characteristic called redundancy.

Levels of the chemical substances involved in sending messages in the brain tend to decrease, but some increase. Nerve cells may lose some of their receptors for these chemical messages. Blood flow to the brain decreases. Because of these age-related changes, the brain may function slightly less well. Older people may react and do tasks somewhat more slowly, but given time, they do these things accurately. Some mental functions—such as vocabulary, short-term memory, the ability to learn new material, and the ability to recall words—may be subtly reduced after age 70.

After about age 60, the number of cells in the spinal cord begins to decrease. Usually, this change does not affect strength or sensation.

As people age, nerves may conduct signals more slowly. Usually, this change is so minimal that people do not notice it. Also, nerves may repair themselves more slowly and incompletely. Therefore, in older people with damaged nerves, sensation and strength may be decreased.

XII. Blood production

The amount of active bone marrow, where blood cells are produced, decreases. Therefore, fewer blood cells are produced. Nonetheless, the bone marrow can usually produce enough blood cells throughout life. Problems may occur when the need for blood cells is greatly increased—for example, when **anemia** or an infection develops or bleeding occurs. In such cases, bone marrow is less able to increase its production of blood cells in response to the body's needs.

XIII. Immune system

The immune system has the enormous task of recognizing self from non-self.

- Not all components of the immune system may be equally affected by aging, but dysfunction known to accompany ageing increases susceptibility to a number of disabling diseases having different aetiologies.
- Increasing incidence of tumourigenesis occurs with age.
- Levels of circulating antibodies begin to decline; therefore infectious diseases occur more frequently and with greater consequences in older people, e.g. pneumonia, influenza, urinary tract infections.

Probable questions:

1. What is aging?
2. Write short notes on Enzymatic Antioxidants.
3. Write short notes on Superoxide Dismutase.
4. Discuss the role of anti-oxidant enzymes in the process of aging.
5. Discuss about Common health conditions associated with ageing.
6. State the changes in Bones and joints with aging.
7. What is osteoarthritis?
8. What is sarcopenia?
9. What is cataract?
10. What are the symptoms of presbycusis?

Suggested readings:

1. Carlson BM. 2014. Human Embryology and Developmental Biology. 5th Edn. Elsevier.
2. Das N. 2012. Fundamental Concept of Developmental Biology. New Central Book Agency
3. https://www.mdpi.com/2076-3921/11/7/1224#:~:text=Aging%20is%20a%20complex%20process,peroxidase%20(GPx)%20and%20CAT.

UNIT X

Differentiation: Cell aggregation and differentiation in *Dictyostelium*

Objective: In this unit we will discuss about Differentiation: Cell aggregation and differentiation in *Dictyostelium*.

Introduction

One of the central aims of the study of development is to understand how distinct cellular behaviours (e.g. division, differentiation, apoptosis and movement) are coordinated in space and in time to result in reproducible pattern formation and morphogenesis. Coordination of these cellular behaviours requires extensive communication between cells of different types and cells and their environment. The social amoebae *Dictyostelium discoideum*, a simple genetically tractable organism situated at the threshold of single and multicellular organisms in the evolutionary tree of life, is well suited for the study of these interactions because its genome has been sequenced and it is amenable to experimental manipulation through targeted gene disruption and replacement. *Dictyostelium* cells normally live as single cells in the soil leaf litter where they feed on bacteria and divide by binary fission. Under starvation conditions up to several hundreds of thousands cells aggregate chemotactically to form a multicellular structure (the slug) that, directed by light and temperature gradients, migrates to the soil surface to form a fruiting body. The fruiting body is composed of a stalk supporting a mass of spores. The spores disperse and under suitable conditions germinate to release amoeba, closing the life cycle. I review our understanding of the signalling mechanisms coordinating cellular behaviours responsible for pattern formation and morphogenesis.

- ## The control of cell movement during development

As *Dictyostelium* development occurs under starvation conditions, only limited cell divisions occur during multicellular development. Morphogenesis primarily results from the arrangement of differentiating cells in a regulative spatial pattern. Key questions are: which signals guide the movement behaviour of thousands of cells during development, and how do movement and differentiation interacts?

Aggregation

Starvation induces changes in the gene-expression programme that result in the cells acquiring the ability to produce, secrete and degrade cAMP. Through the expression of the cAMP receptor, the cells also acquire the ability to respond chemotactically to cAMP

gradients. It has emerged that chemotaxis results from the polarisation of the cytoskeletal dynamics persistently along the cAMP gradient. Unstimulated amoeboid cells are changing shape continuously by extension and retraction of pseudodpods in all directions resulting in a random walk. In the presence of an external gradient of a chemoattractant such as cAMP, the cells persistently extend successive pseudopods in the direction of rising cAMP concentration while suppressing the extension of lateral pseudopods, which results in an efficient movement up the gradient.

Much current research is directed towards understanding how cells detect cAMP gradients, polarise and move in response to it. Pseudopod extension is driven by actin polymerisation, which provides the driving force for extension and simultaneous disassembly of the myosin thick filaments in the cortex at the site of extension as well as localised delivery of membrane and or proteins to allow extension to occur. Cells also need to pull up their back end and suppress the extension of lateral pseudopods which involves members of the myosin I family and is dependent on internal cAMP levels. To move forward the cells must gain traction from the substrate on which they are moving, which involves the formation of multiple transient (10–20s) actin contact sites that have been shown to transduce traction forces to the substrate. It appears that cells may undergo alternating phases of actin-driven extension at the front and myosin-driven contraction at the back. Much work is directed towards the investigation of the molecular mechanism resulting in the molecular mechanism underlying signal detection and its translation in directed movement, and has been reviewed recently elsewhere.

Aggregation is caused by periodic cAMP synthesis and secretion by cells in an aggregation centre. Detection and amplification of this signal by surrounding cells coupled with desensitisation of the cAMP-producing cells results in the propagation of waves of cAMP from the aggregation centre outward (Figure 1). These waves of cAMP guide the chemotactically moving cells towards the aggregation centre, where they accumulate into a three dimensional aggregate: the mound. Initially, the cells move towards the aggregation centre as individuals, but after 10–20 waves have passed they form bifurcating aggregation streams, in which the cells make head-to-tail contacts via calcium-independent adhesion molecules, contact site A and side-to-side contacts via a calcium dependent contact molecule (Dd cadherin).

Stream formation is dependent on the localisation of aggregation stage adenylyl cyclase (ACA) in the rear of the aggregating cells, resulting in polarised cAMP secretion from the back of the cells. cAMP wave propagation can be observed indirectly at the population level via the observation of propagating optical density waves that are associated with the periodic surges in cell movement of groups of cells in the direction of the cAMP signal during the rising phase of the cAMP wave, or at the individual cell level by following the localized translocation of PIP3 at the leading edge of the cell. During aggregation the cAMP signals propagate as target patterns or spiral waves from the aggregation centre outward. Cells stay polarised as long as the cAMP signal is rising in time. However, upon passage of the wave, the chemotactic response adapts, which prevents the cells from turning around and chasing after the cAMP waves once they

have passed. Adaptation of the chemotactic pathways, involving depolarisation of the cells may involve activation of a newly discovered RGS-domain containing kinase as well as the activation of PkA. The number of cells in aggregation streams appears to be controlled by the local concentration of a secreted extracellular high molecular weight complex protein complex, counting factor, which through modulation of movement and adhesion may control the numbers of cells that stably migrate in an aggregation stream.

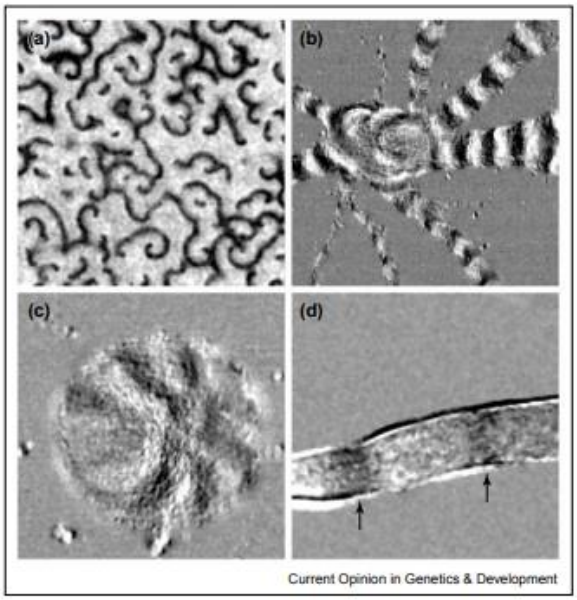


Fig: Dictyostelium development is controlled by propagating cAMP waves. These waves possess distinct geometries typical for the different stages of development. The cAMP waves are detected indirectly as optical density waves associated with the chemotactic movement of the cells in response to the cAMP waves. Groups of cells moving directly during the rising phase of the waves scatter more light than non-moving, less polarised cells during the falling phase of the waves. (a) Spiral waves typical for the early aggregation phase, when the cells are still in a monolayer on agar. (b) Waves in a streaming aggregate. In the body of the aggregate, multi-armed spiral waves rotates counter clockwise, throwing off individual wave fronts that propagate from the centre outward to the periphery of the aggregate directing inward movement of the cells. (c) Multi-armed waves in a mound. In this image, the waves rotate counter clockwise, directing the clockwise movement of the cells in the mound. (d) A slug migrating from right to left, showing two optical density waves, indicated by arrows, which travel from left to right. These waves direct the migration of the prespore cells in the direction of the tip from right to left.

Differentiation

A major goal is to understand the relationship between cell movement and the signals that control differentiation. These signals must be able to maintain the correct proportion of the prespore and prestalk cell types in an environment of extensive cell movement and changes in shape of the slug. In the slug, the different cell types are arranged in a simple axial pattern: pstA (prestalk A) cells in the tip, a band of pstO cells, prespore cells with intermingled anterior-like cells and rearguard cells that are

precursors of the basal disk in the back of the slug. It seems evident that this requires adaptive signalling dynamics but the signals and the details of their regulation are not understood in detail. cAMP pulses control the expression of aggregation-stage genes necessary for cAMP relay and cell–cell contact and can control prespore cell specific gene expression in later development. Prespore cells, in turn, produce DIF, which controls the differentiation of pstO cells. DIF spreads by simple diffusion from the prespore zone in adjacent regions where it controls the differentiation of prestalk O cells and possibly rear guard cells, in agreement with the fact that Dif-dependent pstO translocation of StatC is seen in both zones. Cells in the pstA zone express ACA and studies investigating the cAR1-dependent nuclear translocation of the transcription factor statA have shown that cAM P levels are high in the tip, whereas cAMP levels are lower elsewhere in the slug, compatible with the idea that all the prestalk cells in the tip and only the anterior-like cells in the posterior part of the slug relay the cAMP signal. It is not known what maintains the expression of ACA in the cells in the tip and in anterior-like cells, but it could involve signalling through newly discovered orphan serpentine receptors, as deletion of these receptors results in defective tip formation. The mechanisms of cell-type proportioning and detailed signal-transduction pathways to cell-type specific gene expression still need to be resolved. The switch from migrating slugs to fruiting body formation (culmination) appears to be controlled by a fall in ammonia concentration. The identification of several ammonia transporters, some of which are expressed in the very tip and when deleted lead to a slugger (slugs that fail to culminate) phenotype, supports the importance of ammonia as a morphogen. Ammonia signals most likely through the histidine kinase DhkC to the response regulator domain of the internal cAMP phosphodiesterase RegA, which is a major determinant in the control of intracellular cAMP levels. High ammonia is expected to result in activation of regA and low internal cAMP levels. A drop in ammonia is expected to result in a rise of intracellular cAMP and stalk-cell differentiation.

Conclusions

Multicellular *Dictyostelium* morphogenesis results from the chemotactic movement of thousands of differentiating cells coordinated by propagating waves of the chemoattractant cAMP produced by these cells. The dynamical interactions between the cAMP waves and the resulting chemotactic movement of the cells is formally sufficient to explain aggregation, stream and mound formation. Slug formation and culmination require the emergence of prespore and several prestalk cell types that show distinct cAMP signalling and movement properties. Only prestalk and anterior-like cells relay the cAMP signals while both prestalk and prespore cells move chemotactically in response to these signals. However, prestalk cells move more effectively than prespore cells, resulting in cell sorting. Many of the molecular details underlying the polarisation response to cAMP gradients and the mechanisms of actual movement, cell–cell contact and differentiation are beginning to be uncovered. It would appear that direct cell–cell

contact mediated signalling will play an important role in the modulation of these processes, as is the case in higher organisms. *Dictyostelium* will thus, besides being a system of choice to investigate the molecular mechanisms underlying cell polarity and chemotaxis, be an excellent model system to investigate the principles underlying multicellular tissue organisation and cell-type proportioning

Probable questions:

1. Discuss about differentiation in *Dictyostelium sp.*
2. Discuss about Cell aggregation in *Dictyostelium sp.*

Suggested readings:

1. Carlson BM. 2014. Human Embryology and Developmental Biology. 5th Edn. Elsevier.
2. Das N. 2012. Fundamental Concept of Developmental Biology. New Central Book Agency
3. Cornelis J Weijer: Dictyostelium morphogenesis Current Opinion in Genetics & Development 2004, 14:392–398
4. Kimmel AR: dictyBase: a new Dictyostelium discoideum genome database. Nucleic Acids Res 2004, 32(Suppl): D332-D333.
5. Iranfar N, Fuller D, Loomis WF: Genome-wide expression analyses of gene regulation during early development of Dictyostelium discoideum. Eukaryot Cell 2003, 2:664-670.
6. Saran S, Meima ME, Alvarez-Curto E, Weening KE, Rozen DE, Schaap P: cAMP signaling in Dictyostelium. Complexity of cAMP synthesis, degradation and detection. J Muscle Res Cell Motil 2002, 23:793-802.
7. Soll DR, Wessels D, Heid PJ, Voss E: Computer-assisted reconstruction and motion analysis of the three-dimensional cell. Scientificworldjournal 2003, 3:827-841.
8. Thompson CR, Bretscher MS: Cell polarity and locomotion, as well as endocytosis, depend on NSF. Development 2002, 129:4185-4192.
9. Singleton CK, Zinda MJ, Mykytka B, Yang P: The histidine kinase dhkC regulates the choice between migrating slugs and terminal differentiation in Dictyostelium discoideum. Dev Biol 1998, 203:345-357

UNIT XI

FTIR based identification of early lineage commitment in differentiating cells

Objective: In this unit we will discuss about FTIR based identification of early lineage commitment in differentiating cells.

What is FTIR?

Fourier Transform Infrared Spectroscopy, also known as FTIR Analysis or FTIR Spectroscopy, is an analytical technique used to identify organic, polymeric, and, in some cases, inorganic materials. The FTIR analysis method uses infrared light to scan test samples and observe chemical properties.

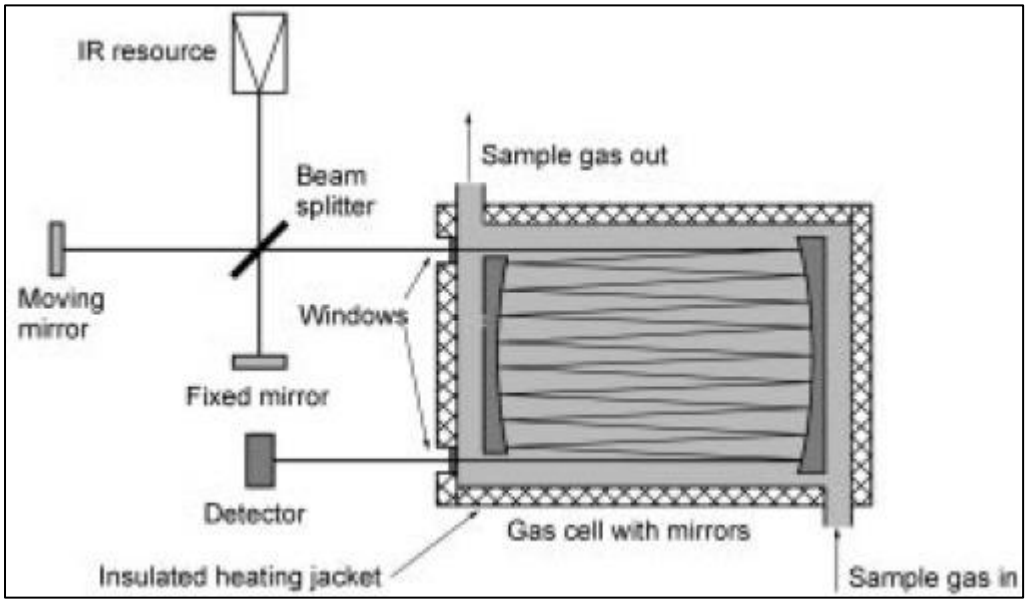
What are the applications of FTIR spectroscopy?

It is suitable for analysis of solid, liquid and biotechnological pharmaceutical forms. Fourier-transform infrared (FTIR) spectroscopy has become a valuable analytical technique for structural or functional studies related to foods as a rapid, cost-efficient, and sensitive physicochemical fingerprinting method.

Principle

Fourier transform infrared (FTIR) spectroscopy, FTIR spectrometers rely on the same basic principle as NDIR analyzers, i.e., the fact that many gases absorb IR radiation at species-specific frequencies. However, FTIR spectroscopy is a disperse method, which means that measurements are performed over a broad spectrum instead of a narrow band of frequencies. Figure 2.26 shows a schematic of a FTIR spectrometer. A blackbody source emits IR radiation over a range of wavenumbers, typically between 4000 and 400 cm^{-1} for (fire) gas analysis. The IR beams passes through an interferometer, which consists of a beam splitter, a fixed mirror and a moving mirror. The interferometer is a cleverly designed optical device that separates the spectral components of the beam in time. The beam then passes through the sample gas cell before it hits the detector. To increase the sensitivity, a system of mirrors in the cell significantly increases the path length. An interferogram is recorded consisting of the detector signal as a function of time. The corresponding absorbance spectrum is obtained from a Fourier transform (a mathematical transformation from the time to the frequency domain) of the interferogram. The concentration of IR-active gases in the sample can be determined from the absorbance spectrum. In order to measure the concentration of a

specific gas it is necessary first to obtain reference spectra for certified mixtures with different concentrations of the gas in N_2.



2.26: Fourier transform infrared spectrometer

FTIR spectroscopy has the advantage that a large number of gases can be measured with one analyzer. The technique is commonly used to measure simultaneously the concentrations of CO, HBr, HCN, HCl, HF, NO and SO_2 because these gases form the basis for smoke toxicity regulations of the International Maritime Organization (Orvis and Janssens, 1999). However, many other gases and vapors such as CH_4, C_2H_2, C_2H_4, C_2H_6, C_3H_4O (acrolein), CO_2, $COCl_2$ (phosgene), COF_2 (carbonyl fluoride), H_2O and NO_2 have been routinely measured as well (Speitel, 2002). Another advantage of FTIR spectroscopy is that it is semi-continuous since an interferogram can typically be obtained in less than five seconds.

The analysis of FTIR spectra is very difficult because the interference between overlapping species, the effect of unknown components in the gas mixture and errors due to noise all have to be accounted for. At least a dozen mathematical techniques of varying complexity have been proposed to accomplish the task (Pottel, 1996). Classical multivariate chemometrical techniques such as classical least squares (CLS), partial least squares (PLS) and implicit non-linear regression (INLR) appear to be most widely used but alternative techniques such as quantitative target factor analysis (QTFA) have also been found suitable (Hakkarainen *et al.,* 2000). The accuracy of the measurements is strongly affected by system characteristics such as cell volume, path length and the type of detector and by operating parameters such as sample flow rate; sample line temperature; cell temperature and pressure; spectrum resolution, etc. A first attempt at specifying optimum operating conditions ('Nordtest Method NT FIRE 047 – Combustible products smoke gas concentrations, continuous FTIR analysis') lacked detail in several areas (Bulien, 1996, 1997). Subsequent research in Europe (Hakkarainen, 1999) recently resulted in the development of an international standard

guide for analyzing fire gases with FTIR spectroscopy ('ISO DIS 19702 – Toxicity testing of fire effluents – Guide for analysis of gases and vapours in fire effluents using FTIR gas analysis').

Probable questions:

1. What is the full form of FTIR?
2. Describe the principle of FTIR technology.

Suggested readings:

1. Carlson BM. 2014. Human Embryology and Developmental Biology. 5th Edn. Elsevier.
2. Das N. 2012. Fundamental Concept of Developmental Biology. New Central Book Agency
3. Cornelis J Weijer: Dictyostelium morphogenesis Current Opinion in Genetics & Development 2004, 14:392–398
4. Kimmel AR: dictyBase: a new Dictyostelium discoideum genome database. Nucleic Acids Res 2004, 32(Suppl):D332-D333.
5. Iranfar N, Fuller D, Loomis WF: Genome-wide expression analyses of gene regulation during early development of Dictyostelium discoideum. Eukaryot Cell 2003, 2:664-670.

UNIT XII

Reversibility of differentiated state, criteria for dedifferentiation, metaplasia and transdifferentiation, modulation

Objective: In this unit, you will learn about Reversibility of differentiated state, criteria for dedifferentiation, metaplasia and transdifferentiation, modulation.

Introduction

- ***Differentiation***

The cells derived from root apical meristem (RAM) and shoot apical meristem (SAM) and cambium differentiate, mature to perform specific functions. This act leading to maturation is termed differentiation. They, undergo a few or major structural changes both in their cell walls and protoplasm.

For example, to form, a trachery element the cells lose its protoplasm but develops a very strong, elastic, lignocellulosic secondary cell wall is best suited to carry water to long distances even under extreme tension.

Similarly, cells designated to be mesophyll come to possess many chloroplasts so as to, perform photosynthesis. On one hand, a parenchyma in hydrophytes develop large schizogenous interspaces for mechanical support, buoyancy and aeration, but on the other hand, in a potato tuber (or perennating organs) develops more amyloplasts.

- ***Dedifferentiation:***

Dedifferentiation is a cellular process by which cells grow in reverse, from a partially or terminally differentiated stage to a less differentiated stage within their own lineage. In general, the phenomenon is manifested by a change in the shape, gene expression pattern, protein expression pattern and function.

In plants, the living differentiated cells can regain the capacity to divide mitotically under certain conditions. The sum of events, that bestow this capacity to divide once again, are termed dedifferentiation. A dedifferentiated tissue can act as meristem (e.g., interfascicular vascular cambium, wound meristem, cork cambium).

Criteria for Differentiation:

1. The cells that have lost their capability to divide and attain a specific function are known as differentiation.

2. Differentiation is the process in which cells and tissues gain several characteristics to perform a specific function.
3. Once a cell is differentiated, it becomes committed to a particular fate and loses its capacity to divide.
4. The fate-committed cells revert to a state where they re-enter the cell cycle under cellular divisions.
5. It is widely noticed in plants.
6. Plants undergo dedifferentiation of parenchymal cells to form meristem tissue.
7. As a result, the differentiated parenchymal cells form cork cambium and interfascicular cambium.

- **Redifferentiation:**

1. The cells divide and produce cells that once again lose their dividing capacity but mature to carry out particular tasks.
2. Redifferentiation is the event of losing the ability to divide by dedifferentiated cells.
3. Example: the secondary xylem and phloem formation in vascular cambium.

Sl. No.	Dedifferentiation	Redifferentiation
1.	The process by which a cell loses its ability to divide and attains a specific function is known as differentiation.	The phenomenon by which cells divide and produce cells that once again loses their dividing capacity but mature to perform specific functions.
2.	It is manifested by a gene expression pattern, a change in the shape, protein expression pattern, and function.	It is both the process and the result of developing additional new characters.
3.	Example: Interfascicular cambium and meristems-cork cambium formation from fully differentiated parenchymal cells	Example: Formation of secondary xylem and secondary phloem, secondary cortex cork from the interfascicular cambium, and cork cambium.

What Is Transdifferentiation?

Transdifferentiation is another form of irreversible change in the cellular structure. It is the conversion of one cell type into another. In transdifferentiation, a

differentiated cell committed to developing a particular organ system is converted to another differentiated cell from a varied lineage. Transdifferentiation of cells occurs due to genetic changes brought about by external or internal factors mediating the changes in the genetic expression. This can also include dedifferentiation, cell division, or conversion of cells. This phenomenon was first observed in the silk moth during metamorphosis by Selman and Kafatos, who coined the term 'transdifferentiation.' Over the years, when advanced studies were made at an anatomical and histological level where conversion and presence of foreign tissue were observed, such changes were termed 'metaplasia.'

What Is Metaplasia?

Metaplasia refers to the reversible conversion of a mature differentiated cell to another differentiated cell. Transdifferentiation and metaplasia are more broad terms referring to similar changes; however, transdifferentiation occurs more at a cellular level, whereas metaplasia happens at the tissue level. Metaplasia is a form of cellular response toward any injury or adverse events. In metaplasia, there is complete reprogramming of the precursor (stem) cells rather than just a structural change, as seen in transdifferentiation. Therefore, it is an essential step in tissue repair and regeneration.

One of the most commonly cited examples of metaplasia is that which happens in smokers' trachea (airway) and bronchi. A healthy individual's trachea is lined by cells with certain hair-like projections. These cells are specialized to protect the airway by helping to clear out foreign particles and also help secrete mucus. But in smokers, these cells are replaced by layers of flattened cells. This metaplastic change happens so that the airway is protected from the toxic chemicals of the smoke. However, the crucial protective functioning of the primary cells is lost in this change. Hence metaplasia acts as a double-edged sword. Moreover, this metaplastic change in smokers is considered to be a predisposing factor for lung cancer. Though metaplasia does not always indicate cancer, there is an increasing propensity toward cancerous changes.

Metaplasia in human pathology

In human histopathology, it is not unusual to find foci of a particular tissue in the wrong places. Examples are the occurrence of bone in soft connective tissue or of squamous patches in an epithelium that is normally glandular in histology. Metaplasias nearly always arise in tissues that have been subjected to chronic trauma, infection or abnormal hormonal stimulation, hence under-going continuous regeneration. This association with regeneration is similar to the examples in arthropods. In some cases, it is not clear from the static preserved pathological specimens whether the ectopic tissue developed *in situ* or migrated from elsewhere. Obviously, the migrating-tissue situation is not relevant to our present concerns. But the glandular metaplasias, in which patches of one tissue are found embedded in the epithelium of another, are highly unlikely to

arise by migration. These glandular metaplasias can be found particularly in the gut and in the female reproductive system, perhaps because each of these two systems consists of a series of organs arranged as a tube, with each organ being lined with a histologically different epithelium. When a patch of metaplasia occurs, it is often composed of the tissue type normally derived from a neighbouring region in the embryo. For example, intestinal metaplasia of the stomach entails the occurrence of patches of intestinal tissue in the gastric mucosa; the intestine and stomach develop from adjacent territories of the endoderm in the early embryo. Less obvious is the condition known as cystitis glandular is, in which colonic type tissue arises in the urinary bladder. This is a separate organ to the intestine in the adult, but is derived from neighbouring endoderm in the embryo, as the urinary bladder forms from the proximal part of the allantoic evagination of the hindgut. Some metaplasias have a clinical significance because they predispose individuals to the development of cancer. For example, the bronchi are lined with columnar epi-\thelium, but smokers often have patches of squamous metaplasia and it is from within these patches that lung cancer usually arises. Adenocarcinoma of the oesophagus usually arises in areas of Barrett's metaplasia, a condition in which the normally squamous epithelium of the lower oesophagus becomes converted to columnar type, with gastric and intestinal differentiation patterns. In such cases the metaplasia can be regarded as the first step in a multistep progression to cancer.

Modulation

What is Modulation?

Modulation is the process of converting data into radio waves by adding information to an electronic or optical carrier signal. A carrier signal is one with a steady waveform -- constant height, or amplitude, and frequency.

Two signals are involved in the modulation process. Message signals are also known as baseband signals. Baseband signals are the band of frequencies representing the original signal. This is the signal to be transmitted to the receiver. The frequency of such a signal is usually low. The other signal involved in this is a high-frequency sinusoidal wave. This signal is called the carrier signal. The frequency of the carrier signals is almost always higher than that of the baseband signal. The amplitude of the baseband signal is transferred to the high-frequency carrier. Therefore, modulation can be defined as, the process of superimposing a low-frequency signal on a high-frequency carrier signal, or, the process of varying the RF carrier wave in accordance with the information in a low-frequency signal

Such a higher frequency carrier wave can travel farther than the baseband signal. But that's not the only advantage of modulation. In the next section, we will discuss the various advantages of modulation.

Biological modulation

In many of our studies, we chose biocompatible semiconductors, such as silicon or silicon carbide nanostructures, which can target a single cell or subcellular component. Our methods have the potential to overcome the limitations of current metal electrode-based devices such as bulk and cell membrane disruption while avoiding the need for genetic modifications. We have identified and quantified the physicochemical outputs from the photo-thermal, -faradic, and -capacitive effects of nanostructured semiconductors at biointerfaces.

A) Neuromodulation

Neural stimulation methods remain a cornerstone technique in neuroscience. Besides the traditional electrode-based methods and optogenetics, semiconductor-based biomaterial interfaces have enabled wireless, non-genetic, multiscale, high-resolution, random-access photomodulation of neural activities. We have an active research program that aims to study the mechanisms and to validate the efficacy of recently developed nanostructured semiconductor-based neuromodulation tools.

- Photothermal neuromodulation

My lab set out to demonstrate that silicon's photothermally induced electric effect could be applied to living cells. We synthesized a deformable and porous type of silicon with molecular-level feature sizes. We introduced the silicon particles over dorsal root ganglia (DRG) neuron cultures and illuminated the cell-membrane-supported particles, eliciting action potentials (signals) in individual neurons (**Fig. 1A-B**). We also successfully delivered a train of light pulses and repeatedly excited neurons with a one-pulse-one-spike fidelity. This confirmed that the photothermally induced electric effect could indeed be applied to living cells. Silicon's photothermal effect at the neuron-silicon interface does not require direct physical contact as the heating can be effective for a distance up to one hundred micrometres. This makes it ideal for use in situations such as peripheral nerve stimulations where extracellular matrix or other cellular barriers would usually impede tight biointerfaces.

- Photoelectrochemical neuromodulation

For greater efficiency in neuromodulation, a tight interface between the silicon device and the neuron is required. When direct access to the cells is available, the preferred neuromodulation approach would be to use electrons and holes (*i.e.,* the charge carriers) that are generated by light, the way a photoelectrochemical device works.

To investigate the biological applicability of silicon's photoelectrochemical effect, my lab used coaxial *p*-type/intrinsic/*n*-type silicon (PINS) nanowires to wirelessly and photoelectrochemically modulate primary rat DRG neuron excitability (**Fig. 1C-D**). Our results showed that atomic gold on the nanowires enhances the photoelectrochemical process through which the action potentials in rat DRG neurons were elicited.

Essentially, atomic gold reduces the kinetic barrier necessary for the photoelectrochemical current generation, thereby playing the role that a catalyst would play in traditional photoelectrochemical devices.

- Formulation of a rational design principle for semiconductor-based modulation tools

  Efficient biological modulation requires accurate designs for tight cell-device interfaces. We identified a biology-guided two-step design principle for establishing tight intra-, inter-, and extra-cellular silicon-based interfaces in which silicon and the biological targets have matched mechanical properties and efficient signal transduction. To gain a biophysical understanding of the different biological modulations that silicon could induce, my lab developed a set of matrices to quantify and differentiate the capacitive, faradaic, and thermal outputs from different silicon materials in saline. We confirmed that we could use light to (non-genetically) modulate intracellular calcium dynamics, cytoskeleton-based transport and structures, and cellular excitability, highlighting the diverse utility of these new interfaces. In particular, we showed that flexible and freestanding silicon mesh can modulate brain activities and simple animal behaviors such as induced limb motion from anaesthetized mice (**Fig. 1E-F**).

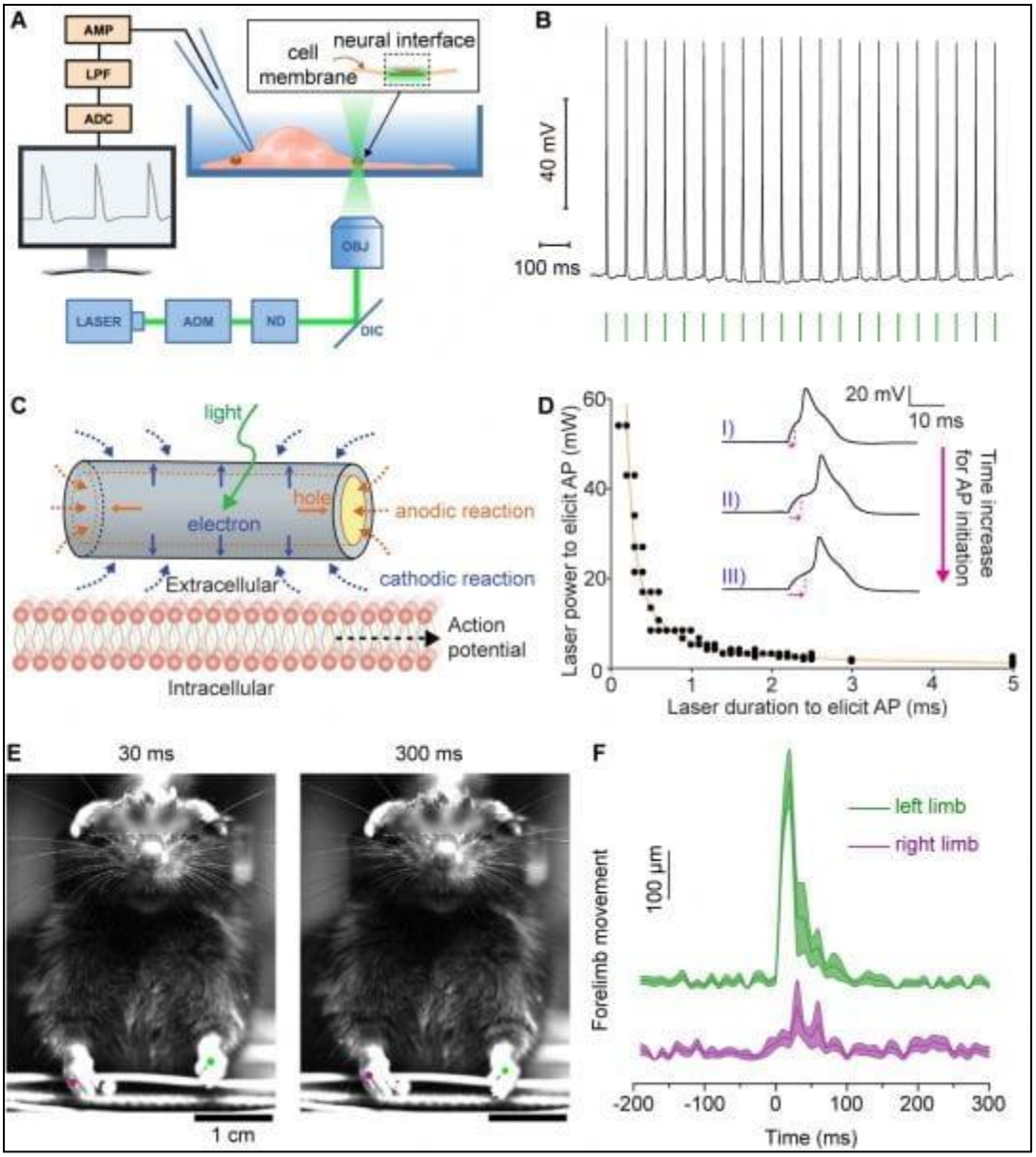


Figure 1. Neuromodulation. (A-B) Nanoporous silicon-based neuromodulation through an optocapacitance mechanism. (C-D) Coaxial silicon nanowire for photoelectrochemical neuromodulation. (E-F) Multilayered silicon membranes for neuromodulation at the animal level.

B) Cardiac modulation

The electrical conduction system of the heart allows for the coordinated contraction of cardiomyocytes to produce heartbeats. Abnormalities in this system can lead to delayed mechanical activation of specific regions of the heart or pathologically slow heart rates (bradyarrhythmias). Thus, therapies that can either resynchronize the heart or increase the overall beating frequency of the heart are necessary for the treatment of these disorders.

Probable questions:

1. What is differentiation?
2. What is dedifferentiation?
3. Write down the criteria of dedifferentiation?
4. What is Metaplasia?
5. What is transdifferentiation?
6. Write short notes on modulation.

Suggested readings:

1. Carlson BM. 2014. Human Embryology and Developmental Biology. 5th Edn. Elsevier.
2. Das N. 2012. Fundamental Concept of Developmental Biology. New Central Book Agency
3. Gardner DK. 2006. In Vitro Fertilization: a Practical Approach. CRC Press.
4. Gilbert S.F. 2010. Developmental Biology, IX Edition, Sinauer Associates, Inc., Publishers,
5. Schoenwolf GC, Bleyl SB, Brauer PR, Francis-West PH. 2009. Ladesn's Human Embryology. Elsevier

UNIT XIV

Developmental regulatory networks (vertebrates): Signalling and development, Molecular mechanism of dorsoventral axis formation and three signal model of mesoderm induction in *Xenopus*

Objective: In this unit we will discuss about Developmental regulatory networks (vertebrates): Signalling and development, Molecular mechanism of dorsoventral axis formation and three signal model of mesoderm induction in *Xenopus.*

The Progressive Determination of the Amphibian Axes

Vertebrate axes do not form from localized determinants in the various blastomeres, as in *Drosophila.* Rather, they arise progressively through a sequence of interactions between neighboring cells. Amphibian axis formation is an example of regulative development. In case of regulative development (1) an isolated blastomere has a potency greater than its normal embryonic fate, and (2) a cell's fate is determined by interactions between neighboring cells. Such interactions are called inductions. That such inductive interaction was responsible for amphibian axis determination.

It was demonstrated by the laboratory of Hans Spemann at the University of Freiburg. The experiments of Spemann and his students framed the questions that experimental embryologists asked for most of the twentieth century, and they resulted in a Nobel Prize for Spemann in 1935. More recently, the discoveries of the molecules associated with these inductive processes have provided some of the most exciting moments in contemporary science.

The experiment that began this research program was performed in 1903, when Spemann demonstrated that early newt blastomeres have identical nuclei, each capable of producing an entire larva. His procedure was ingenious: Shortly after fertilizing a newt egg, Spemann used a baby's hair taken from his daughter to lasso the zygote in the plane of the first cleavage. He then partially constricted the egg, causing all the nuclear divisions to remain on one side of the constriction. Eventually, often as late as the 16-cell stage, a nucleus would escape across the constriction into the non-nucleated side. Cleavage then began on this side, too, whereupon Spemann tightened the lasso until the two halves were completely separated. Twin larvae developed, one slightly older than the other (Figure 10.17). Spemann concluded from this experiment that early amphibian nuclei were genetically identical and that each cell was capable of giving rise to an entire organism.

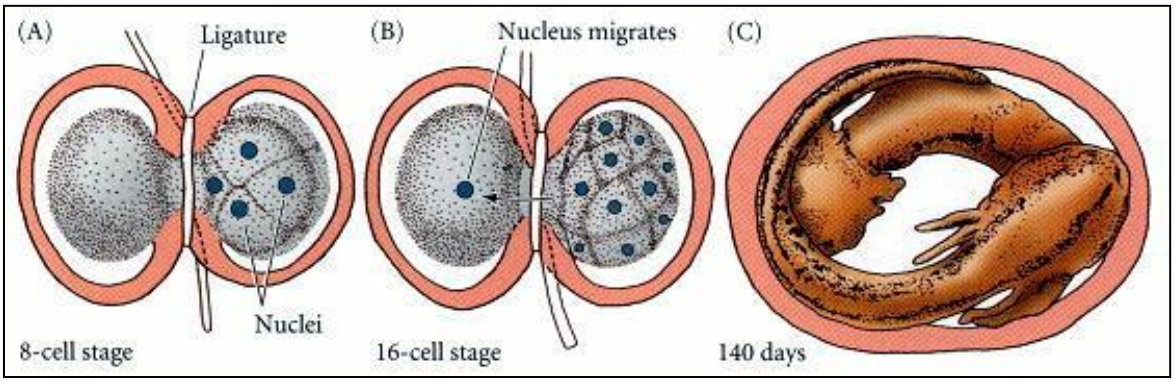


Figure 10.17: Spemann's demonstration of nuclear equivalence in newt cleavage. (A) When the fertilized egg of the newt *Triturus taeniatus* was constricted by a ligature, the nucleus was restricted to one-half of the embryo. The cleavage on that side of the embryo reached the 8-cell stage, while the other side remained undivided. (B) At the 16-cell stage, a single nucleus entered the as yet undivided half, and the ligature was constricted to complete the separation of the two halves. (C) After 140 days, each side had developed into a normal embryo. (After Spemann 1938.)

However, when Spemann performed a similar experiment with the constriction still longitudinal, but perpendicular to the plane of the first cleavage (separating the future dorsal and ventral regions rather than the right and left sides), he obtained a different result altogether. The nuclei continued to divide on both sides of the constriction, but only one side—the future dorsal side of the embryo—gave rise to a normal larva. The other side produced an unorganized tissue mass of ventral cells, which Spemann called the *Bauchstück*—the belly piece. This tissue mass was a ball of epidermal cells (ectoderm) containing blood and mesenchyme (mesoderm) and gut cells (endoderm), but no dorsal structures such as nervous system, notochord, or somites (Figure 10.18).

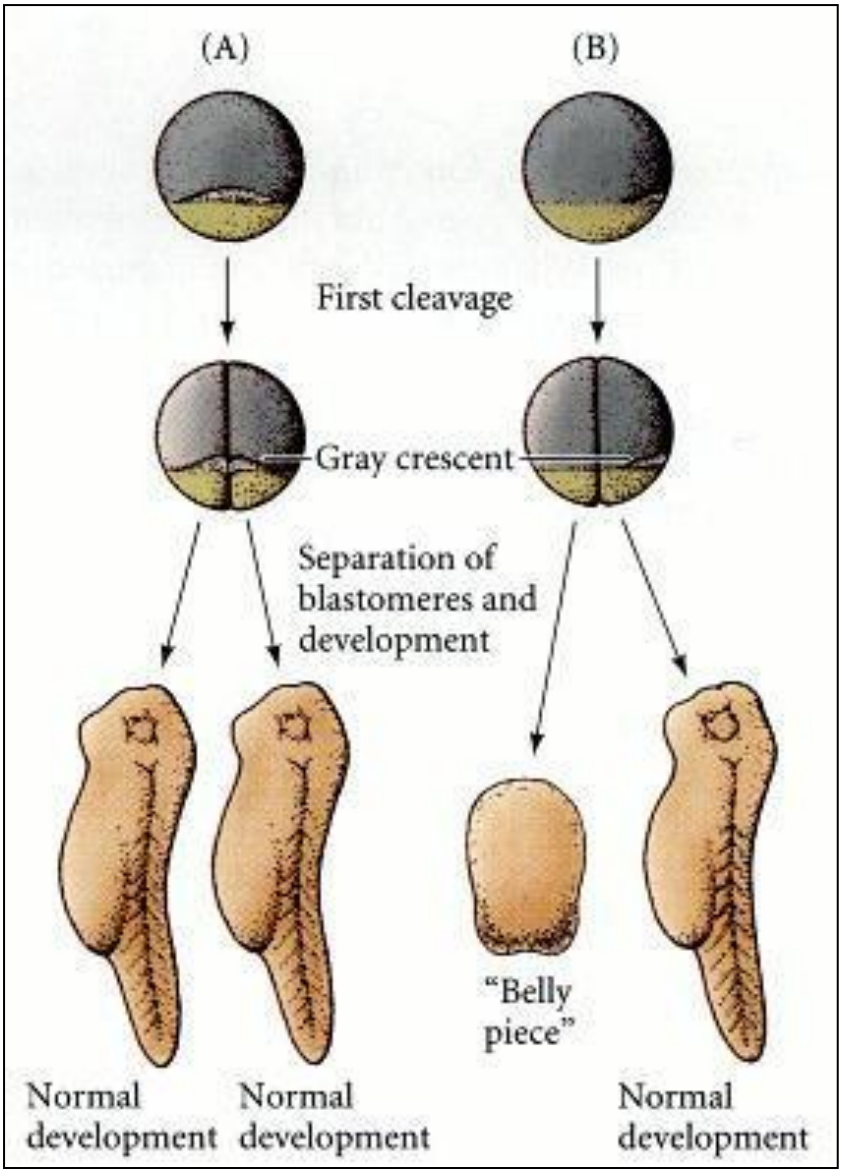


Figure 10.18: Asymmetry in the amphibian egg. (A) When the egg is divided along the plane of first cleavage into two blastomeres, each of which gets one-half of the gray crescent, each experimentally separated cell develops into a normal embryo. (B) When only one of the two blastomeres receives the entire gray crescent, it alone forms a normal embryo. The other half produces a mass of unorganized tissue lacking dorsal structures. (After Spemann 1938.)

Why should these two experiments give different results? One possibility was that when the egg was divided perpendicular to the first cleavage plane, some *cytoplasmic* substance was not equally distributed into the two halves. Fortunately, the salamander egg was a good place to test that hypothesis.

There are dramatic movements in the cytoplasm following the fertilization of amphibian eggs, and in some amphibians these movements expose a gray, crescent-shaped area of

cytoplasm in the region directly opposite the point of sperm entry. This area has been called the **gray crescent.** Moreover, the first cleavage plane normally splits the gray crescent equally into the two blastomeres. If these cells are then separated, two complete larvae develop. However, should this cleavage plane be aberrant (either in the rare natural event or in an experiment), the gray crescent material passes into only one of the two blastomeres. Spemann found that when these two blastomeres are separated, only the blastomere containing the gray crescent develops normally.

It appeared, then, that something in the gray crescent region was essential for proper embryonic development. But how did it function? What role did it play in normal development? The most important clue came from the fate map of this area of the egg, for it showed that the gray crescent region gives rise to the cells that initiate gastrulation. These cells form the dorsal lip of the blastopore. The cells of the dorsal lip are committed to invaginate into the blastula, thus initiating gastrulation and the formation of the notochord. Because all future amphibian development depends on the interaction of cells rearranged during gastrulation, Spemann speculated that the importance of the gray crescent material lies in its ability to initiate gastrulation, and that crucial developmental changes occur during gastrulation.

In 1918, Spemann demonstrated that enormous changes in cell potency do indeed take place during gastrulation. He found that the cells of the *early* gastrula were uncommitted, but that the fates of *late* gastrula cells were determined. Spemann demonstrated this by exchanging tissues between the gastrulae of two species of newts whose embryos were differently pigmented (Figure 10.19).

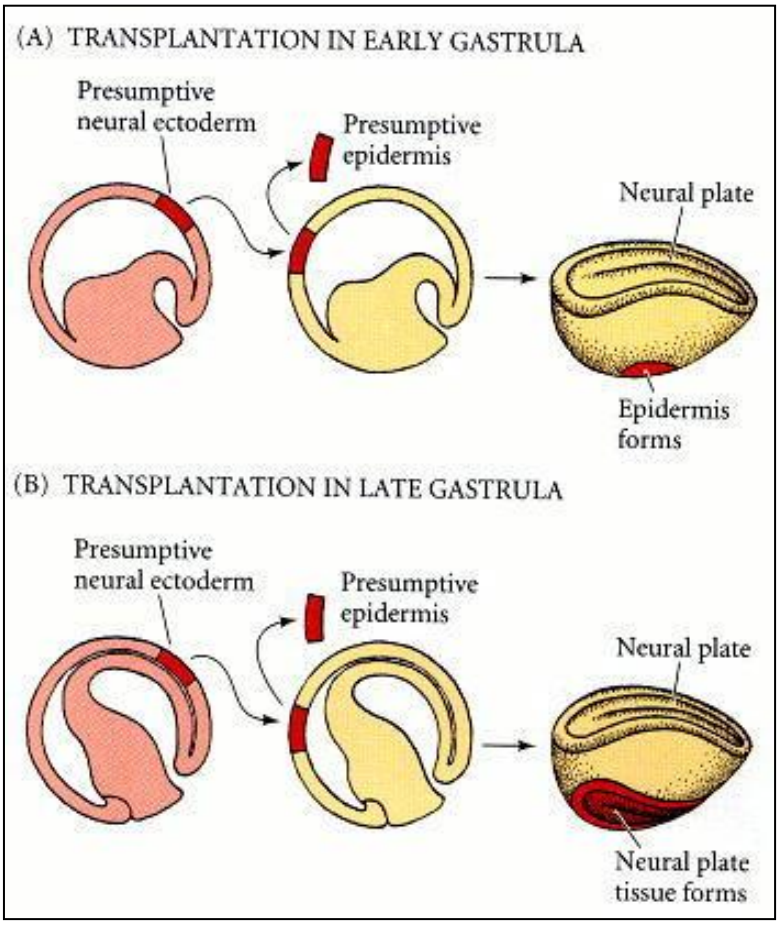


Figure 10.19: Determination of ectoderm during newt gastrulation. Presumptive neural ectoderm from one newt embryo is transplanted into a region in another embryo that normally becomes epidermis. (A) When the tissues are transferred between early gastrulas, the presumptive neural tissue develops into epidermis, and only one neural plate is seen. (B) When the same experiment is performed using late-gastrula tissues, the presumptive neural cells form neural tissue, thereby causing two neural plates to form on the host.

When a region of prospective epidermal cells from an *early* gastrula was transplanted into an area in another early gastrula where the neural tissue normally formed, the transplanted cells gave rise to neural tissue. When prospective neural tissue from early gastrulae was transplanted to the region fated to become belly skin, the neural tissue became epidermal. Thus, these early newt gastrula cells were not yet committed to a specific fate. Such cells are said to exhibit **conditional** (i.e., regulative or dependent) **development** because their ultimate fates depend on their location in the embryo. However, when the same interspecies transplantation experiments were performed on *late* gastrulae, Spemann obtained completely different results. Rather than differentiating in accordance with their new location, the transplanted cells exhibited **autonomous** (or independent, or mosaic) **development**. Their prospective fate was determined, and the cells developed independently of their new embryonic location. Specifically, prospective neural cells now developed into brain tissue even when placed in the region of prospective epidermis, and prospective epidermis formed skin even in the region of the prospective neural tube. Within the time separating early and late gastrulation, the potencies of these groups of cells had become restricted to their eventual paths of differentiation. Something was causing them to become determined to epidermal and neural fates. What was happening?

The Mechanisms of Axis Formation in Amphibians

The experiments of Spemann and Mangold showed that the dorsal lip of the blastopore, and the notochord that forms from it, constituted an “organizer” that could instruct the formation of new embryonic axes. But the mechanisms by which the organizer was constructed and through which it operated were totally unknown. Indeed, it is said that Spemann and Mangold's paper posed more questions than it answered. Among these questions were:

- How did the organizer get its properties? What caused the dorsal blastopore lip to differ from any other region of the embryo?
- What factors were being secreted from the organizer to cause the formation of the neural tube and to create the anterior-posterior, dorsal-ventral, and left-right axes?

- How did the different parts of the neural tube become established, with the most anterior becoming the sensory organs and forebrain, and the most posterior becoming spinal cord?

The origin of the Nieuwkoop center

The major clue in determining how the dorsal blastopore lip obtained its properties came from the experiments of Pieter Nieuwkoop (1969, 1973, 1977). He and his colleagues in the Netherlands demonstrated that the properties of this newly formed mesoderm were induced by the vegetal (presumptive endoderm) cells underlying them. He removed the equatorial cells (i.e., presumptive mesoderm) from a blastula and showed that neither the animal cap (presumptive ectoderm) nor the vegetal cap (presumptive endoderm) produced any mesodermal tissue. However, when the two caps were recombined, the animal cap cells were induced to form mesodermal structures such as notochord, muscles, kidney cells, and blood cells (Figure 10.21).

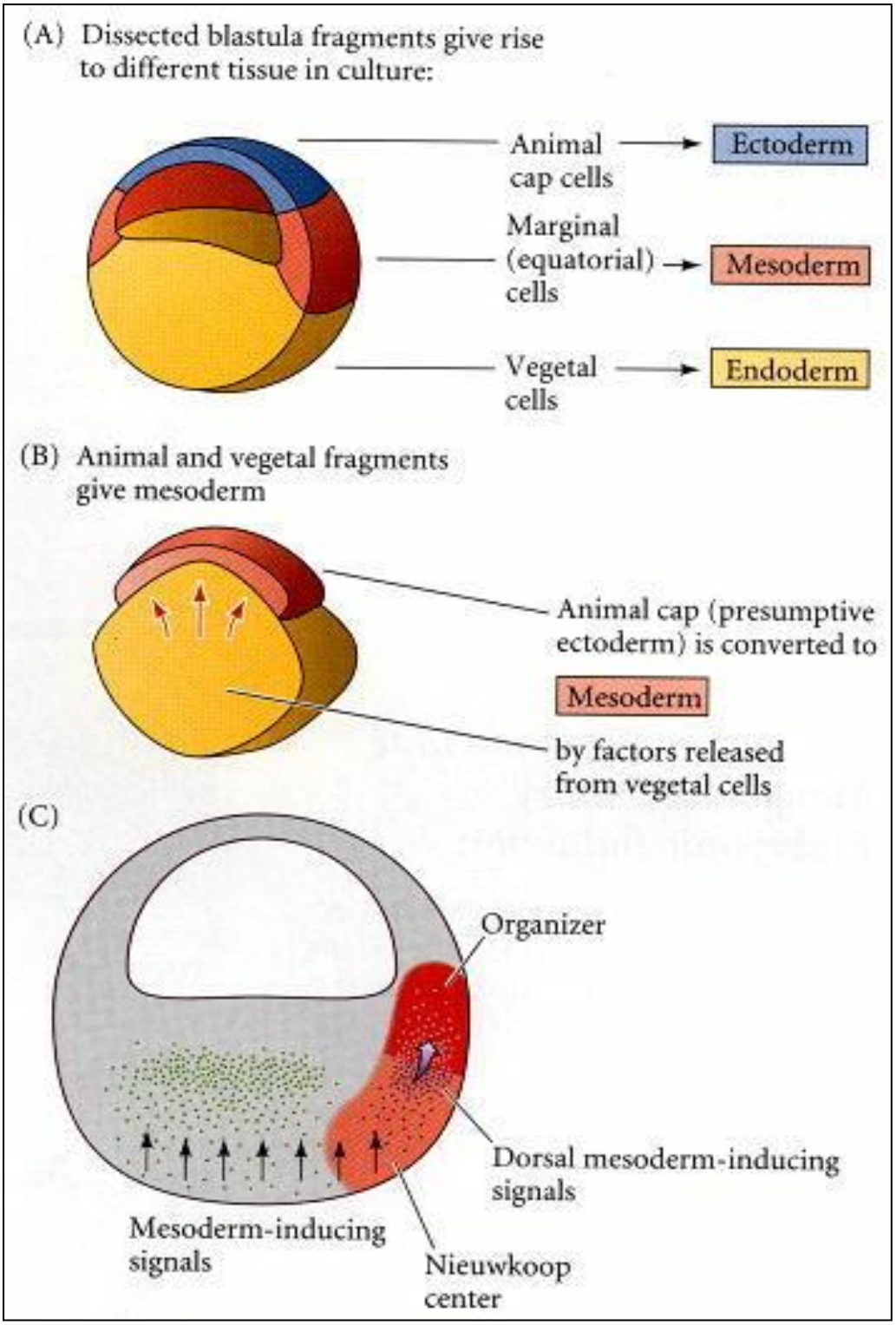


Fig: 10.21: Summary of experiments by Nieuwkoop and by Nakamura and Takasaki (1970), showing mesodermal induction by vegetal endoderm. (A) Isolated animal cap cells become a mass of ciliated epidermis, isolated vegetal cells generate gutlike tissue, and isolated equatorial (marginal zone) cells become mesoderm. (B) If animal cap cells are combined with vegetal cap cells, many of the animal cells generate mesodermal tissue. (C) Model for mesoderm induction in *Xenopus.* A ventral signal (probably FGF2 or BMP4) is released throughout the vegetal region of the embryo. This induces the marginal cells to become mesoderm. On the dorsal side (away from the point of sperm entry), a signal is released by the vegetal cells of the Nieuwkoop center.

This dorsal signal induces the formation of the Spemann organizer in the overlying marginal zone cells. The possible identity of this signal will be discussed later in this chapter. (C after De Robertis et al. 1992.)

The polarity of this induction (whether the animal cells formed dorsal mesoderm or ventral mesoderm) depended on the dorsal-ventral polarity of the endodermal (vegetal) fragment. While the ventral and lateral vegetal cells (those closer to the side of sperm entry) induced ventral (mesenchyme, blood) and intermediate (muscle, kidney) mesoderm, the dorsalmost vegetal cells specified dorsal mesoderm components (somites, notochord), including those having the properties of the organizer. The dorsalmost vegetal cells of the blastula, which are capable of inducing the organizer, have been called the **Nieuwkoop center** (Gerhart et al. 1989).

The Nieuwkoop center was demonstrated in the 32-cell *Xenopus* embryo by transplantation and recombination experiments. First, Gimlich and Gerhart (Gimlich and Gerhart 1984; Gimlich 1985, 1986) performed an experiment analogous to the Spemann and Mangold studies, except that they used blastulae rather than gastrulae. When they transplanted the dorsalmost vegetal blastomere from one blastula into the ventral vegetal side of another blastula, two embryonic axes were formed (see Figure 10.11B). Second, Dale and Slack (1987) recombined single vegetal blastomeres from a 32-cell *Xenopus* embryo with the uppermost animal tier of a fluorescently labeled embryo of the same stage. The dorsalmost vegetal cell, as expected, induced the animal pole cells to become dorsal mesoderm. The remaining vegetal cells usually induced the animal cells to produce either intermediate or ventral mesodermal tissues (Figure 10.22). Thus, dorsal vegetal cells can induce animal cells to become dorsal mesodermal tissue.

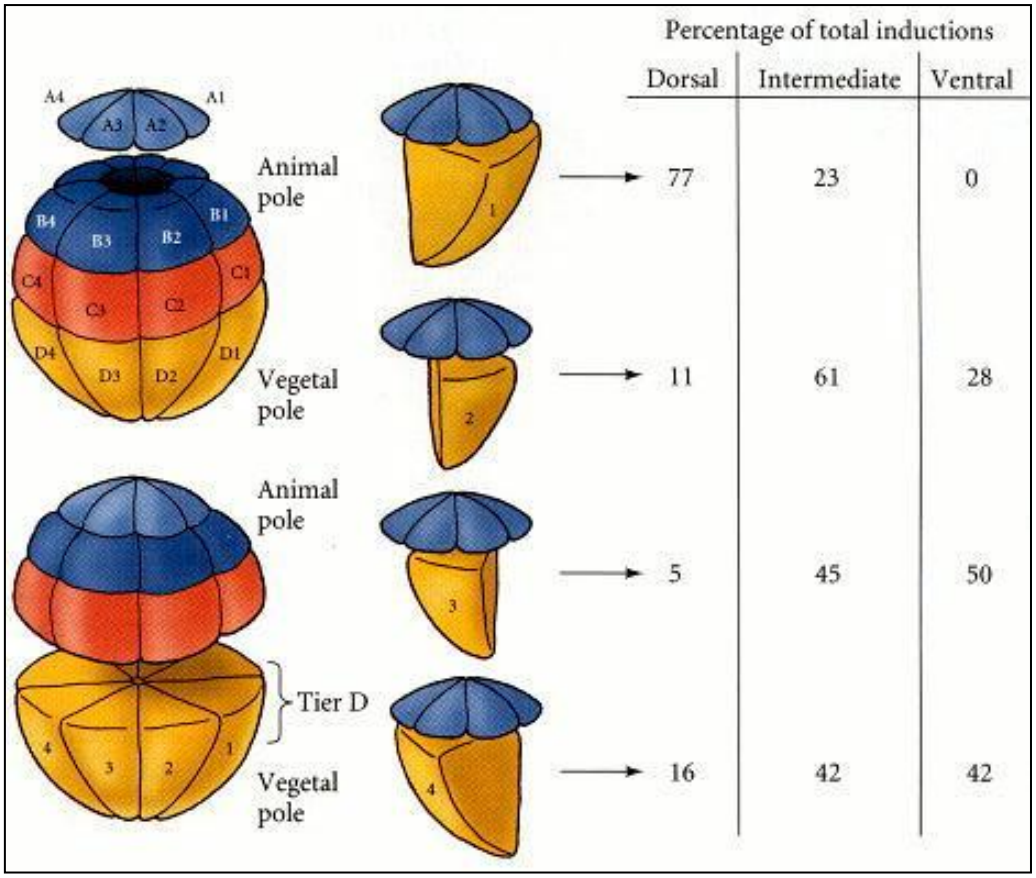


Figure 10.22: The regional specificity of mesoderm induction can be demonstrated by recombining cells of 32-cell *Xenopus* embryos. Animal pole cells were labeled with fluorescent polymers so that their descendants could be identified, then combined with individual vegetal blastomeres. The inductions resulting from these recombinations are summarized at the right. D1, the dorsalmost vegetal blastomere, was the most likely to induce the animal pole cells to form dorsal mesoderm. (After Dale and Slack 1987.)

The Nieuwkoop center is created by the cytoplasmic rotation that occurs during fertilization. When this rotation is inhibited by UV light, the resulting embryo will not form dorsal-anterior structures such as the head or neural tube (Vincent and Gerhart 1987). However, these UV-treated embryos can be rescued by transplantation of the dorsalmost vegetal blastomeres from a normal embryo at the 32-cell stage (Dale and Slack 1987; see Figure 10.11A). If eggs are rotated toward the end of the first cell cycle so that the future ventral side is upward, two Nieuwkoop centers are formed, leading to two dorsal blastopore lips and two embryonic axes (see Figure 10.10). Therefore, the specification of the dorsal-ventral axis begins at the moment of sperm entry.

The molecular biology of the Nieuwkoop center

In *Xenopus,* the endoderm is able to induce the formation of mesoderm by causing the presumptive mesodermal cells to express the *Xenopus Brachyury* (*Xbra*) gene. The

mechanism of this induction is not well understood (see Harland and Gerhart 1997), but the Xbra protein is a transcription factor that activates the genes that produce mesoderm-specific proteins. While all the vegetal cells appear to be able to induce the overlying marginal cells to become mesoderm, only the dorsalmost vegetal cells can instruct the overlying dorsal marginal cells to become the organizer. The major candidate for the factor that forms the Nieuwkoop center in these dorsalmost vegetal cells is **β-catenin.**

β-catenin is a multifunctional protein that can act as an anchor for cell membrane cadherins or as a nuclear transcription factor (see Chapter 6). In *Xenopus* embryos, β-catenin begins to accumulate in the dorsal region of the egg during the cytoplasmic movements of fertilization. β-catenin continues to accumulate preferentially at the dorsal side throughout early cleavage, and this accumulation is seen in the nuclei of the dorsal cells (Figure 10.23A-D; Schneider et al. 1996; Larabell et al. 1997). This region of β-catenin accumulation originally appears to cover both the Nieuwkoop center and organizer regions. During later cleavage, the cells containing β-catenin may reside specifically in the Nieuwkoop center (Heasman et al. 1994a; Guger and Gumbiner 1995).

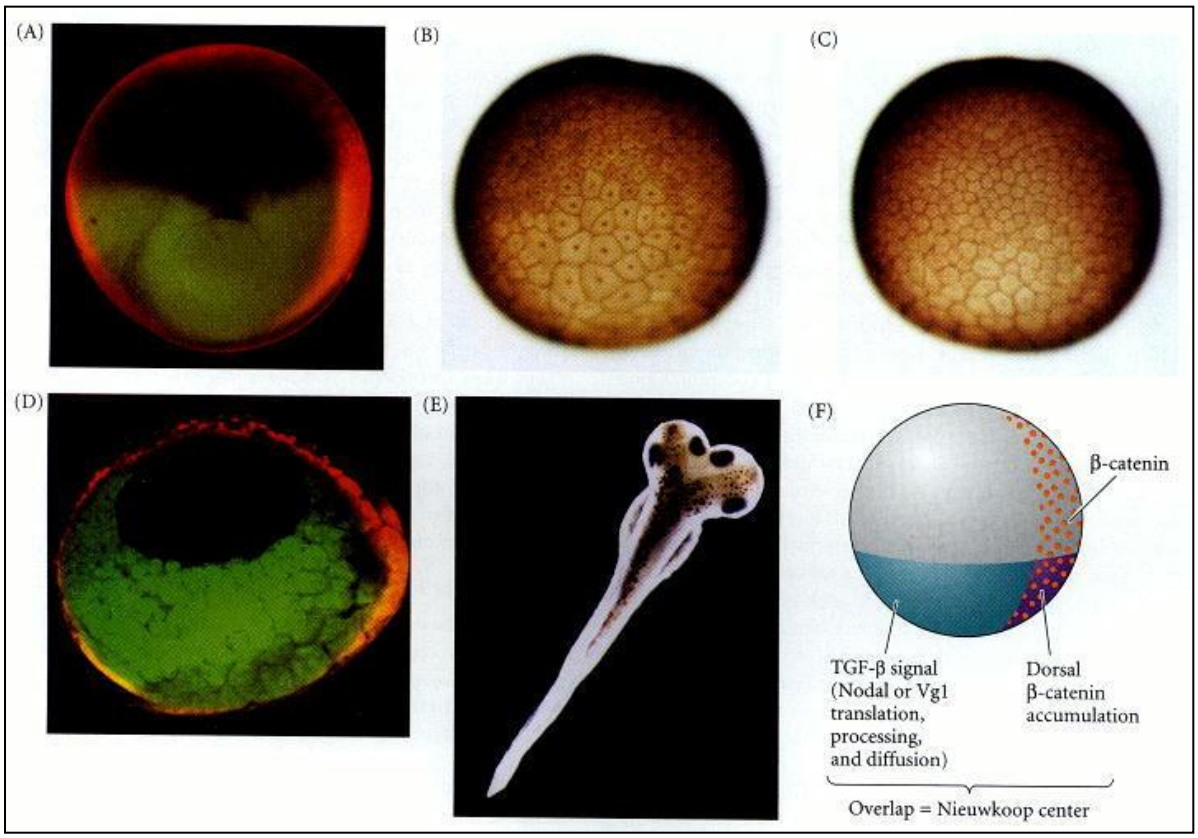


Figure 10.23

The role of Wnt pathway proteins in dorsal-ventral axis specification. (A-D) Differential translocation of β-catenin into *Xenopus* blastomere nuclei. (A) Early 2-cell stage of *Xenopus,* showing β-catenin (orange) predominantly at the dorsal surface. (B) Presumptive dorsal side of a *Xenopus* blastula stained for β-catenin shows nuclear localization. (C) Such nuclear localization is not seen on the ventral side of the same embryo. (D) β-catenin dorsal

localization persists through the gastrula stage. (E) Dorsal axis formation caused by the injection of both blastomeres of a 2-cell *Xenopus* embryo with dominant inactive GSK-3. Dorsal fate is actively suppressed by wild-type GSK-3. (F) Irenic model whereby the Nieuwkoop center (characterized by *siamois* gene expression and the ability to induce dorsal mesoderm) is created by the synergy of the activation of β-catenin dorsally and the activation of the TGF-β signal vegetally. (A, D from R. T. Moon; B and C from Schneider et al. 1996, photographs courtesy of P. Hausen; E from Pierce and Kimelman 1995, photograph courtesy of D. Kimelman.)

β-catenin is necessary for forming the dorsal axis, since experimental depletion of β-catenin transcripts with antisense oligonucleotides results in the lack of dorsal structures (Heasman et al. 1994a). Moreover, the injection of exogenous β-catenin into the ventral side of the embryo produces a secondary axis (Funayama et al. 1995; Guger and Gumbiner 1995). β-catenin is part of the Wnt signal transduction pathway and is negatively regulated by the glycogen synthase kinase 3 (GSK-3; see Chapter 6). GSK-3 also plays a critical role in axis formation by suppressing dorsal fates. Activated GSK-3 blocks axis formation when added to the egg (Pierce and Kimelman 1995; He et al. 1995; Yost et al. 1996). If endogenous GSK-3 is knocked out by a dominant negative protein in the ventral cells of the early embryo, a second axis forms (Figure 10.23E).

So how can β-catenin become localized to the future dorsal cells of the blastula? Labeling experiments (Yost et al. 1996; Larabell et al. 1997) suggest that β-catenin is initially synthesized (from maternal messages) throughout the embryo, but is degraded by GSK-3-mediated phosphorylation specifically in the ventral cells. The critical event for axis determination may be the movement of an inhibitor of GSK-3 to the cytoplasm opposite the point of sperm entry (i.e., to the future dorsal cells). One candidate for this agent is the Disheveled protein. This protein is the normal suppressor of GSK-3 in the Wnt pathway and it is originally found in the vegetal cortex of the unfertilized *Xenopus* egg. However, upon fertilization, Disheveled is translocated along the microtubular array to the dorsal side of the embryo (Figure 10.24; Miller et al. 1999). Thus, on the dorsal side of the embryo, β-catenin should be stable, since GSK-3 is not able to degrade it; while in the ventral portion of the embryo, GSK-3 should initiate the degradation of β-catenin.

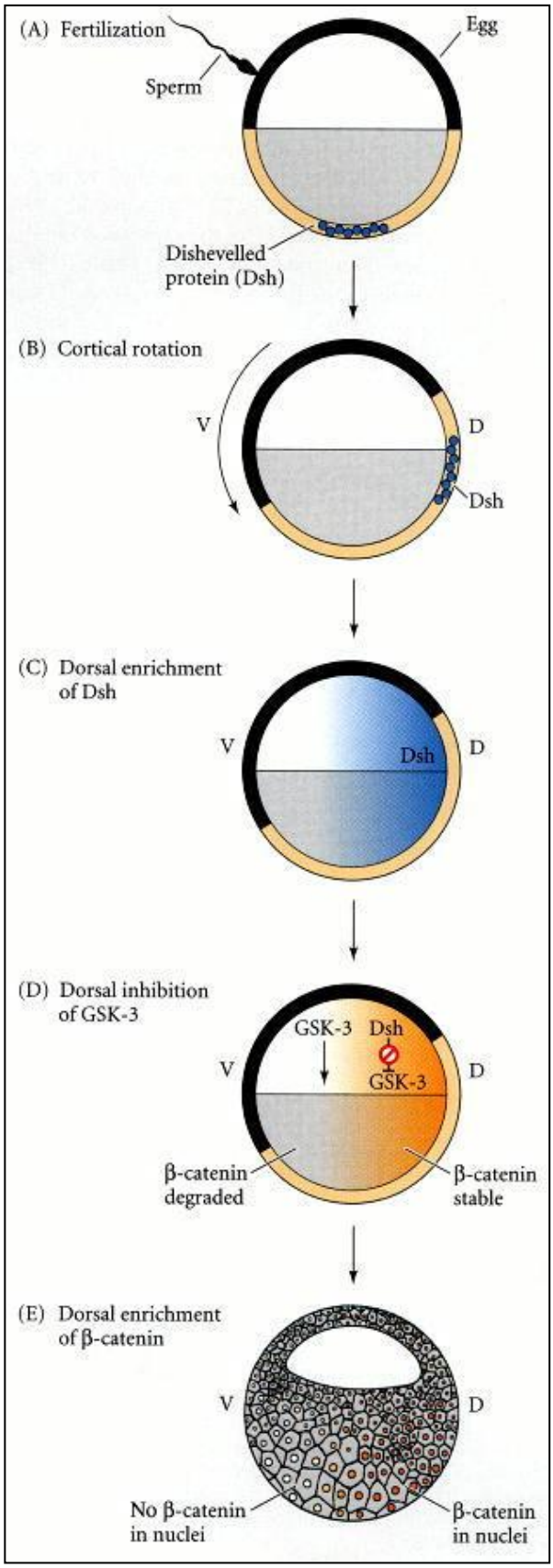


Figure 10.24

Model of the mechanism by which the Disheveled protein stabilizes β-catenin in the dorsal portion of the amphibian egg. (A) Disheveled (Dsh) associates with a particular set of proteins at the vegetal pole of the unfertilized egg. (B) Upon fertilization, these protein vesicles are translocated dorsally along subcortical microtubule tracks. (C) Disheveled is then released from its vesicles and is distributed in the future dorsal third of the 1-cell embryo. (D) Disheveled binds to and blocks the action of GSK-3, thereby preventing the degradation of β-catenin on the dorsal side of the embryo. (E) The nuclei of the blastomeres in the dorsal region of the embryo receive β-catenin, while the nuclei of those in the ventral region do not.

β-catenin is a transcription factor that can associate with other transcription factors to give them new properties. It is known that *Xenopus* β-catenin can combine with a ubiquitous transcription factor known as **Tcf3**, and that a mutant form of Tcf3 lacking a β-catenin binding domain results in embryos without dorsal axes (Molenaar et al. 1996). The β-catenin/Tcf3 complex appears to bind to the promoters of several genes whose activity is critical for axis formation. One of these genes is *siamois,* which is expressed in the Nieuwkoop center immediately following the midblastula transition. If this gene is ectopically expressed in the ventral vegetal cells, a secondary axis emerges on the former ventral side of the embryo, and if cortical rotation is prevented, *siamois* expression is eliminated (Lemaire et al. 1995; Brannon and Kimelman 1996). The Tcf3 protein is thought to inhibit *siamois* transcription when it

binds to that gene's promoters in the absence of β-catenin. However, when the Tcf3/β-catenin complex binds to its promoter, *siamois* is activated (Figure 10.25; Brannon et al. 1997).

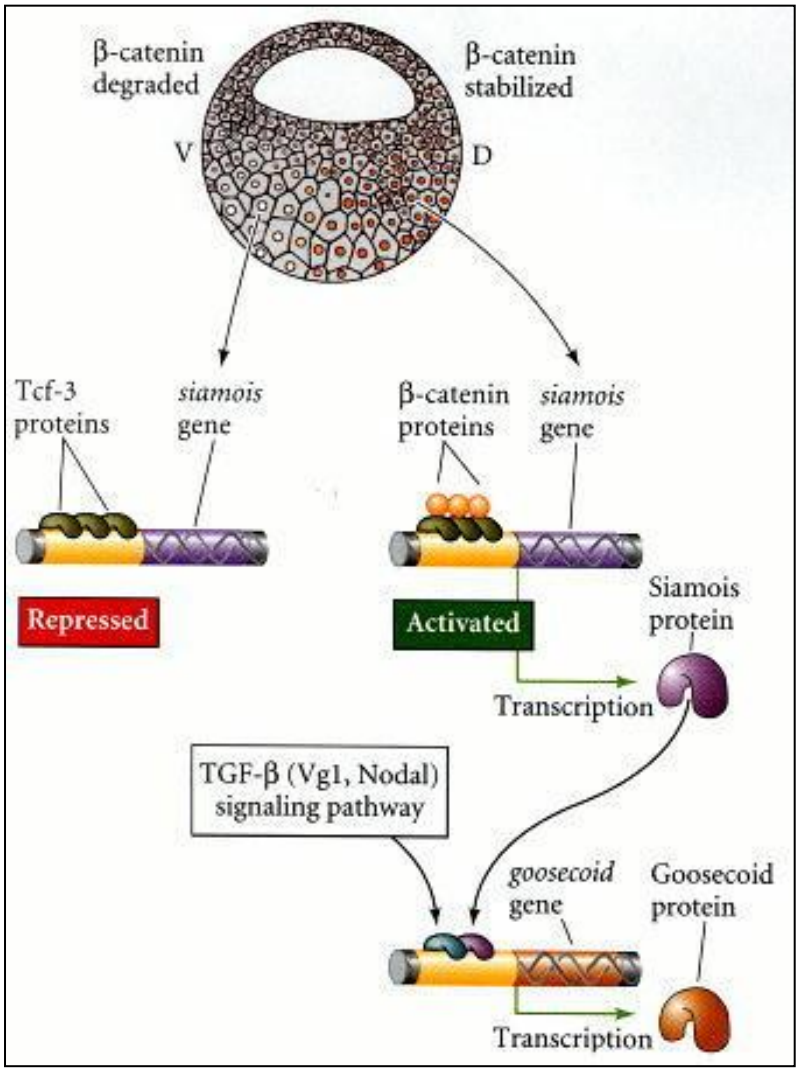


Figure 10.25

Summary of events hypothesized to bring about the induction of the organizer in the dorsal mesoderm. Cortical rotation causes the translocation of Disheveled protein to the dorsal side of the embryo. Dsh binds GSK-3, thereby allowing β-catenin to accumulate in the future dorsal portion of the embryo. During cleavage, β-catenin enters the nuclei and binds with Tcf3 to form a transcription factor that activates genes encoding proteins such as Siamois. Siamois and Lim-1, a transcription factor activated by the TGF-β pathway, function together to activate the *goosecoid* gene in the organizer. Goosecoid is a transcription factor that can activate genes whose proteins are responsible for the organizer's activities. (After Moon and Kimelman 1998).

The Functions of the Organizer

While the Nieuwkoop center cells remain endodermal, the cells of the organizer become the dorsal mesoderm and migrate underneath the dorsal ectoderm. There, the dorsal mesoderm induces the central nervous system to form. The properties of the organizer tissue can be divided into five major functions:

1. The ability to become dorsal mesoderm (prechordal plate, chordamesoderm, etc.)
2. The ability to dorsalize the surrounding mesoderm into lateral mesoderm (when it would otherwise form ventral mesoderm)
3. The ability to dorsalize the ectoderm into neural ectoderm

4. The ability to initiate the movements of gastrulation
5. The ability to cause the neural plate (the induced neural ectoderm) to become the neural tube

In *Xenopus* (and other vertebrates), the formation of the anterior-posterior axis follows the formation of the dorsal-ventral axis. Once the dorsal portion of the embryo is established, the movement of the involuting mesoderm establishes the anterior-posterior axis. The mesoderm that migrates first through the dorsal blastopore lip gives rise to the anterior structures; the mesoderm migrating through the lateral and ventral lips forms the posterior structures.

It is now thought that the cells of the organizer ultimately contribute to four cell types—pharyngeal endoderm, head mesoderm (prechordal plate), dorsal mesoderm (primarily the notochord), and the dorsal blastopore lip (Keller 1976; Gont et al. 1993). The pharyngeal endoderm and prechordal plate lead the migration of the organizer tissue and appear to induce the forebrain and midbrain. The dorsal mesoderm induces the hindbrain and trunk. The dorsal blastopore lip forms the dorsal mesoderm and eventually becomes the chordaneural hinge that induces the tip of the tail.

When the organizer was first described, it started one of the first truly international scientific research programs: the search for the organizer molecules. Researchers from Britain, Germany, France, the United States, Belgium, Finland, Japan, and the Soviet Union all tried to find these remarkable substances (see Gilbert and Saxén 1993). R. G. Harrison (quoted by Twitty 1966, p. 39) referred to the amphibian gastrula as the "new Yukon to which eager miners were now rushing to dig for gold around the blastopore." Unfortunately, their picks and shovels proved too blunt to uncover the molecules involved. The analysis of organizer molecules had to wait until recombinant DNA technologies enabled investigators to make cDNA clones from blastopore lip mRNA and to see which of these clones encoded factors that could dorsalize the embryo.

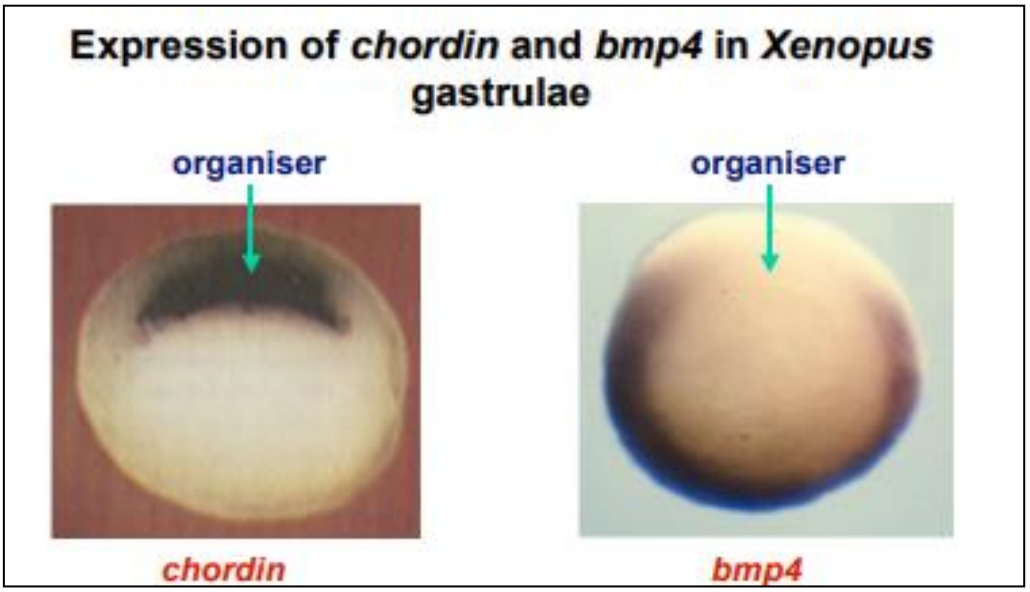
The formation of the dorsal (organizer) mesoderm involves the activation of several genes. The secreted proteins of the Nieuwkoop center are thought to activate a set of transcription factors in the mesodermal cells above them. These transcription factors then activate the genes encoding the secreted products of the organizer.

Organizer signals

Following the discovery of the Spemann organizer, amphibian embryologists spent many years trying to identify the molecules involved. However, the technologies available at the time (1930-1960) were inadequate for the task and no progress was made. The first organizer signal was not identified until 1992, using molecular techniques that were only developed in the previous 10 years. Smith and Harland cloned organizer specific mRNAs and injected them into UV-irradiated Xenopus embryos. As described above (section 1.3), UV-irradiation of the vegetal pole soon after fertilization blocks cortical rotation and the formation of the Nieuwkoop centre, the

resulting embryos lack all dorsal mesoderm. An mRNA that rescued dorsal development was identified and found to be localized to the Spemann organizer. It encodes a novel protein that Smith and Harland called Noggin. Injection of *noggin* mRNA into ventral blastomeres induced a partial secondary axis that lack head structures, indicating that it mimicked the trunk organizer. Noggin protein dorsalized isolated ventral mesoderm and induced neural tissue in animal caps. Unfortunately, the amino acid sequence of Noggin provided few clues as to how it might work.

Two additional proteins, Chordin and Follistatin, with similar properties to Noggin were subsequently identified and their mRNAs were also found to be localized to the Spemann organizer.



Chordin induces a secondary dorsal axis (lacking head structures), while both Chordin and Follistatin neuralize animal caps. Inhibition studies (injecting antisense morpholino oligonucleotides that block translation of target mRNAs) have shown that all three proteins are necessary for the normal function of the organizer. Chordin and then Noggin are probably the most important, since they give the strongest phenotypes when depleted. However, depletion of all three proteins is necessary for full ventralization of the embryo. Follistatin was of special interest because its mode of action was known from mammalian physiology, where it binds to extracellular Activin. Follistatin bound Activin cannot activate its receptors. Subsequent studies showed that it also bound and inhibited BMP7, suggesting that a member of the TGF-ß family promoted ventral development in amphibian embryos and that dorsal development required inhibition of this signal by the organizer. It also suggested that Chordin and Noggin might act in a similar manner and biochemical studies demonstrated that they do indeed inhibit members of the TGFß family, in both cases BMP2 and BMP4 (but not Activin). Transcripts for bmp4 are uniformly expressed in Xenopus blastulae but are lost from the Spemann organizer and neural plate during gastrulation. Injection of bmp4 mRNA into the organizer causes a catastrophic loss dorsal structure, the resulting embryos only differentiating ventral mesoderm (blood) and ectoderm (epidermis). Moreover, a

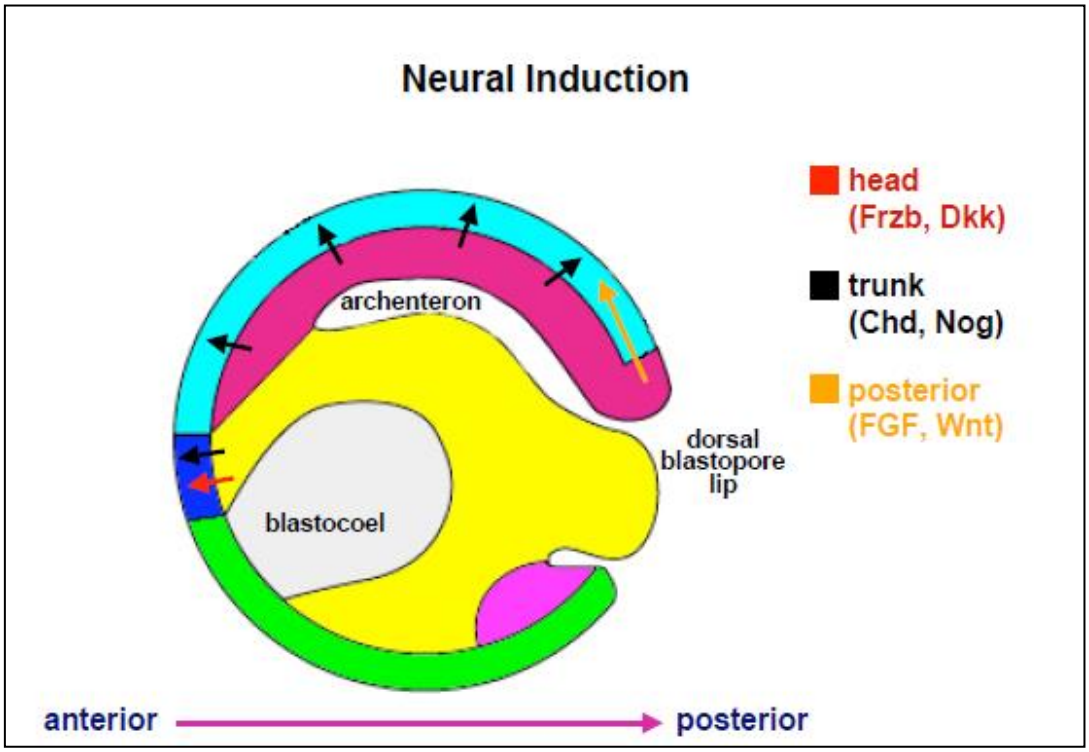
dominant negative BMP receptor (dnBMPR1), which specifically inhibits BMPs, dorsalizes *Xenopus* embryos. It induces a secondary dorsal axis, lacking head structures, when expressed in ventral blastomeres and neural tissue when expressed in animal caps. BMP4, but not Activin, can also restore epidermal differentiation to dissociated animal caps, which would otherwise differentiate as neural tissue (In contrast to whole animal caps, which form epidermis, dissociated animal caps form neural tissue, presumably because an epidermalizing factor (BMP4) is diluted by dissociation). Taken together, the data suggested that BMP4 specifies ventral fates in both the ectoderm and mesoderm (and probably the endoderm as well) and that dorsal fates are activated in the absence of this signal. (In the nervous system this is known as the neural default model). Genetic studies in zebrafish support this conclusion. Mutations that disturb dorsal-ventral patterning during gastrulation always affect the BMP signalling pathway and either dorsalize (e.g. mutations in *bmp2* and *bmp7*) or ventralize (e.g. mutations in chordin) embryos.

Head-induction

Although BMP antagonists induce a secondary dorsal axis when expressed in ventral blastomeres, the axis always lacks the anterior regions of the head (although they frequently contain a hindbrain and otic vesicles). BMP antagonists therefore mimic the trunk organizer. How then does an embryo get its full head? Clearly the organizer must release additional signals. The hunt for genes specifically expressed in the Organizer identified ***frzb***, ***cereberus***, and ***dickkopf1* (*dkk1*)**, all of which appear to be required for head development. Transcripts for *cerberus* are localized to the anterior endoderm, while transcripts for *frzb* and *dkk1* are localized to the prechordal plate (mesoderm anterior to the notochord). Thus all three genes are specifically expressed in tissues that contribute to the head. They have only weak dorsalizing activity but they neuralize animal caps, cooperating with BMP inhibitors to promote strong neuronal differentiation. Coinjecting ***chordin*** and ***frzb*** mRNA gives secondary axes that are much more complete that than injecting *chordin* alone, with eyes usually being formed. However, the eyes are always cyclopic (fused across the midline), indicating that the most anterior regions (the forebrain) are still missing. Almost identical results are obtained

After coinjecting *chordin* and *dkk1* mRNAs, although the heads are often fully formed. Moreover, inhibition of Dkk1 function results in embryos that lack anterior head structures. Cerberus, on the other hand, will induce a fully formed head when injected alone. As described in section 2.3, Cerberus is a protein that binds, and inhibits, members of the Wnt, BMP, and Nodal families of extracellular signaling molecules, indicating that inhibition of Wnt and/or Nodal signaling, as well as BMP signaling, may be necessary for head induction. FrzB has a very similar amino acid sequence to the N-terminal domain of Frizzled receptors, which is known to be responsible for binding Wnt signaling molecules. This suggested that FrzB might be an inhibitor of Wnt signaling, binding and preventing Wnts from activating their receptors. Biochemical experiments proved that this was indeed the case and subsequent studies showed that

Dkk1 also inhibits Wnt signalling. Thus inhibition of Wnt signaling appears to be the key event in head induction. Inhibition of Nodal signalling may also be required for full head development, as suggested by experiments with the Nodal-binding domain of Cerberus (Cer-S). Embryos injected with Chordin, FrzB, and Cer-S have fully formed heads. The ability to inhibit BMP, Wnt, and Nodal signaling may explain the formation of a fully formed second head when *Cerberus* mRNA is injected into ventral blastomeres. These experiments suggest that inhibition of BMP, Nodal and Wnt signaling pathways are required for head induction in amphibians.



Although inhibiting BMPs induces only trunk tissues in axis duplication assays, it induces anterior (head) neural tissue in isolated animal caps. This paradox can be explained if the embryo produces posteriorizing signals that inhibit the development of anterior structures, transforming anterior into posterior fates. One candidate for a posteriorizing signal is Wnt3a, which blocks head development when over-expressed in *Xenopus* embryos. Wnt3a will also transform anterior neural tissue, induced in animal caps by Chordin, into posterior neural tissue. The role of Wnt inhibitors in head induction may be to prevent this transformation, allowing anterior neural tissue to differentiate. Other candidate molecules for posteriorizing signals include FGF4 and Retinoic Acid (RA). Both molecules transform anterior neural tissue into posterior neural tissue in animal caps lacking BMP signalling. *Fgf4* expression is localized to posterior and tail bud mesoderm, where it activates expression of the T-box transcription factor *xbra*. This gene has an essential role in the development of posterior structures, as evident from the "no tail" phenotype of mouse and zebrafish embryos

mutant for the *brachyury* gene. A similar phenotype is seen in *Xenopus* embryos expressing a dominant-negative mutation for *xbra*. An almost identical phenotype is obtained with the dominant-negative FGF receptor, which blocks FGF4 signalling and induction of *xbra* expression. (*xbra* is a direct target of FGF signalling in *Xenopus* blastulae and early gastrulae) FGF also activates expression of *Xenopus* caudal genes that in turn activate expression of posterior *hox* genes, such as *hoxb-9*. As a consequence, overexpression of FGF4 leads to more posterior specification of the nervous system and a reduction of anterior fates. A similar phenotype is also observed following addition of RA to embryo culture media during gastrulation. A posterior (high) to anterior (low) gradient of RA has been detected in the dorsal mesoderm of *Xenopus* neurulae, and high concentrations of RA have been shown to activate the expression of posterior *hox* genes.

Probable questions:

1. What is Nieuwkoop center?
2. What is organizer?
3. What is grey crescent?
4. Describe the role of organizer in axis formation in *Xenopus*.
5. Describe the role of Nieuwkoop center in axis formation in *Xenopus*.
6. Write down the role of beta catenin in axis formation in *Xenopus.*
7. Write down the functions of the Organizer.

Suggested readings:

1. Carlson BM. 2014. Human Embryology and Developmental Biology. 5th Edn. Elsevier.
2. Das N. 2012. Fundamental Concept of Developmental Biology. New Central Book Agency
3. Spemann, H. 1938. *Embryonic Development and Induction.* Yale University Press, New Haven
4. Smith J C , Slack J M W . Dorsalization and neural induction: Properties of the organizer in *Xenopus laevis*. J. Embryol. Exp. Morphol. 1983; 78:299–317.
5. Recanzone G , Harris W A . Demonstration of neural induction using nuclear markers in *Xenopus*. Wilhelm Roux Arch. Dev. Biol. 1985; 194:344–354.

6. Gerhart J C , Danilchik M , Doniach T , Roberts S , Rowning B , Stewart R . Cortical rotation of the *Xenopus* egg: Consequences for the anteroposterior pattern of embryonic dorsal development. Development [Suppl.] 1989; 107:37–51.
7. Dale L, Slack J M W. Regional specificity within the mesoderm of early embryos of *Xenopus laevis*. Development. 1987; 100:279–295.
8. Harland R M, Gerhart J. Formation and function of Spemann's organizer. Annu. Rev. Cell Dev. Biol. 1997; 13:611–667.
9. Schneider S , Steinbeisser H , Warga R M , Hausen P . β-catenin translocation into nuclei demarcates the dorsalizing centers in frog and fish embryos. Mech. Dev. 1996;57:191–198.
10. Guger K A , Gumbiner B M . β-catenin has wnt-like activity and mimics the Nieuwkoop signaling center in *Xenopus* dorsal-ventral patterning. Dev. Biol. 1995;172:115–125.
11. Miller J R , Rowning B A , Larabell C A , Yang-Snyder J A , Bates R L , Moon R T . Establishment of the dorsal-ventral axis in *Xenopus* embryos coincides with the dorsal enrichment of Dishevelled that is dependent on cortical rotation. J. Cell Biol. 1999;146:427–437.

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