# Post-Graduate Degree Programme (CBCS)

# in

# ZOOLOGY

# SEMESTER-IV

# ELECTIVE THEORY PAPER

# CELL AND DEVELOPMENTAL BIOLOGY

# ZDSE(MJ)T-403

# SELF LEARNING MATERIAL

**DIRECTORATE OF OPEN AND DISTANCE LEARNING**
**UNIVERSITY OF KALYANI**
**KALYANI, NADIA,**
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**May 2024**

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

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

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

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

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

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



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

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# ELECTIVE THEORY PAPER (ZDSE(MJ)T -403)

## CELL AND DEVELOPMENTAL BIOLOGY

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# UNIT- I

## Genetic regulation during development: Gradients in early embryogenesis in *Drosophila*

**Objective:** This module unit focuses on the study of genetic regulation in the development of *Drosophila*, specifically the gradients involved in embryogenesis. This will enable the reader to gain a deeper understanding of pattern formation in *Drosophila*. By the end of this unit, the reader will be able to comprehend the hierarchical interactions between the maternal and zygotic gene activities that contribute to the creation of the anterior-posterior axis during *Drosophila*'s development.

### *Drosophila* as a model organism

The fly *Drosophila* has been the foremost model organism for the study of the genetics of animal development. Like other insects, it begins its development with a series of nuclear divisions generating a syncytium, and a large amount of early patterning occurs in this single giant multinucleate cell. The pattern originates with asymmetry in the egg, organized both by localized deposits of mRNA inside the egg and by signals from the follicle cells around it. Positional information in the multinucleate embryo is supplied by four intracellular gradients that are set up by the products of four groups of maternal-effect genes called egg-polarity genes. These control four distinctions fundamental to the body plan of animals: dorsal versus ventral, endoderm versus mesoderm and ectoderm, germ cells versus somatic cells, and head versus rear.

Most of the genes controlling the pattern of the body in *Drosophila* turn out to have close counterparts in higher animals, including ourselves. In fact, many of the basic devices for defining the body plan and patterning individual organs and tissues are astonishingly similar. Thus, quite surprisingly, the fly has provided the key to understanding the molecular genetics of our development.

### Series of segmental units:

The timetable of *Drosophila* development, from egg to adult, is summarized in **Fig.1** The period of embryonic development begins at fertilization and takes about a day, at the end of which the embryo hatches out of the eggshell to become a *larva.* The larva then passes through three stages, or *instars,* separated by moults in which it sheds its old coat of cuticle and lays down a larger one. At the end of the third instar, it pupates. Inside the *pupa,* a radical remodelling of the body takes place—a process called ***metamorphosis*.** Eventually, about nine days after fertilization, an adult fly, or imago, emerges.

The fly consists of a head, with mouth, eyes, and antennae, followed by three thoracic segments (numbered T1 to T3), and eight or nine abdominal segments

(numbered A1 to A9). Each segment, although different from the others, is built according to a similar plan. Segment T1, for example, carries a pair of legs, T2 carries a pair of legs plus a pair of wings, and T3 carries a pair of legs plus a pair of halteres—small knob-shaped balancers important in flight, evolved from the second pair of wings that more primitive insects possess.

***Drosophila* development starts as a syncytium:**

The egg of *Drosophila* is about 0.5 mm long and 0.15 mm in diameter, with a clearly defined polarity. Like the eggs of other insects, but unlike vertebrates, it begins its development in an unusual way: a series of nuclear divisions without cell division creates a syncytium. The early nuclear divisions are synchronous and extremely rapid, occurring about every 8 minutes. The first nine divisions generate a cloud of nuclei, most of which migrate from the middle of the egg toward the surface, where they form a monolayer called the *syncytial blastoderm*. After another four rounds of nuclear division, plasma membranes grow inward from the egg surface to enclose each nucleus, thereby converting the syncytial blastoderm into a *cellular blastoderm* consisting of about 6000 separate cells (**Fig. 2**). About 15 of the nuclei populating the extreme posterior end of the egg are segregated into cells a few cycles earlier; these *pole cells* are the germ-line precursors (primordial germ cells) that will give rise to eggs or sperm.

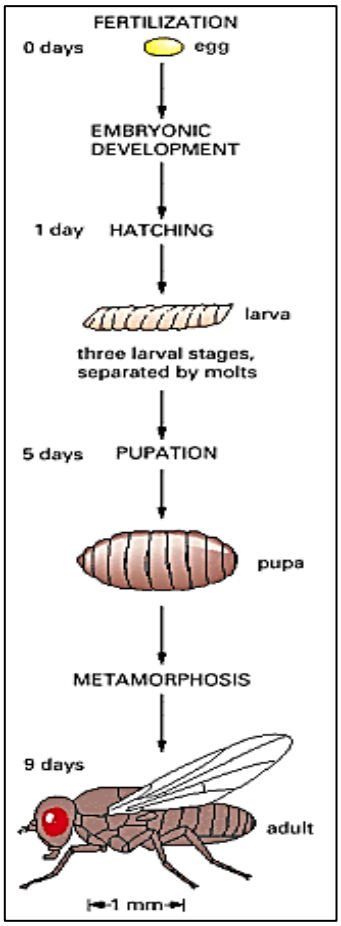



**Fig. 1:** *Drosophila* development from egg to adult fly.

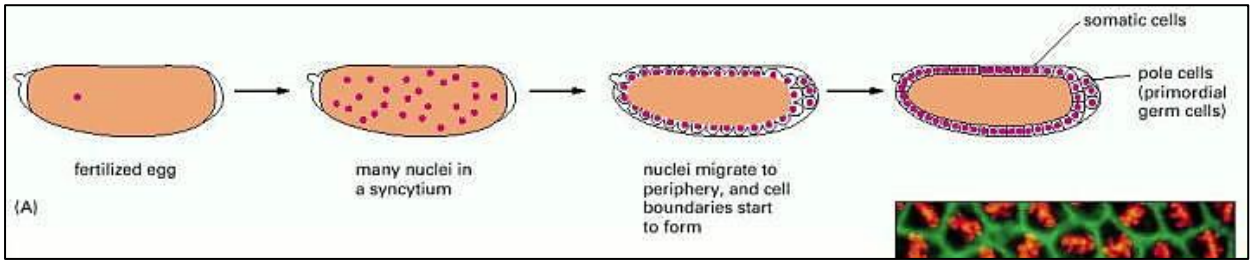



**Fig. 2** *Egg from fertilization to the cellular blastoderm stage*

**The role of egg polarity genes:**

The early development of *Drosophila* represents a rather extreme variant. The main axes of the future insect body are defined before fertilization by a complex exchange of signals between the unfertilized egg, or oocyte, and the follicle cells that surround it in the ovary (**Fig.3**). Then, in the syncytial phase following fertilization, an exceptional amount of patterning occurs in the array of rapidly dividing nuclei, before the first partitioning of the egg into separate cells. Here, there is no need for the usual forms of cell-cell communication involving transmembrane signaling; neighbouring regions of the early *Drosophila* embryo can communicate using gene regulatory proteins and mRNA molecules that diffuse or are actively transported through the cytoplasm of the giant multinuclear cell.

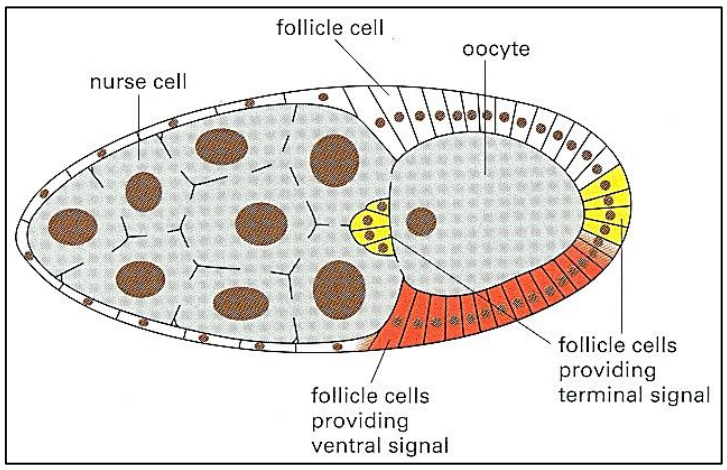



**Fig. 3** *oocyte in its follicle*

In the stages before fertilization, the anteroposterior axis of the future embryo becomes defined by three systems of molecules that create landmarks in the oocyte (**Fig.4**). Following fertilization, each landmark serves as a beacon, providing a signal, in the form of a morphogen gradient, that organizes the developmental process in its neighbourhood.

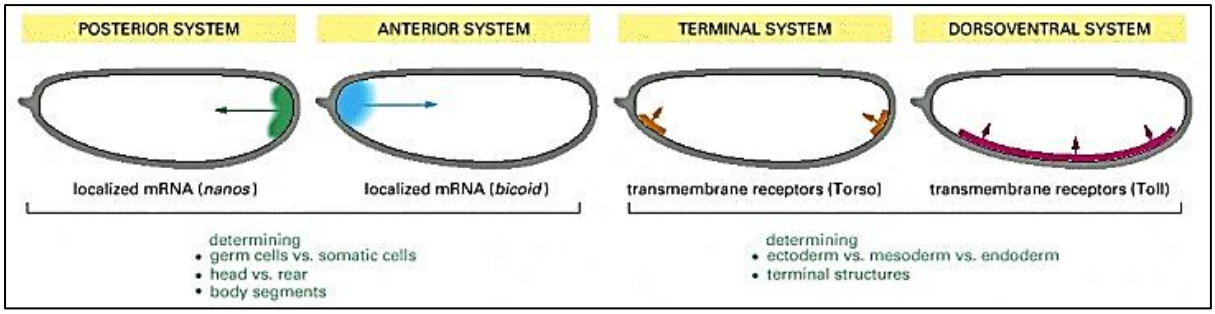



**Fig.4** *The organization of the four egg-polarity gradient systems*

Two of these signals are generated from localized deposits of specific mRNA molecules. The future anterior end of the embryo contains a high concentration of mRNA for a gene regulatory protein called Bicoid; this mRNA is translated to produce Bicoid protein, which diffuses away from its source to form a concentration gradient with its maximum at the anterior end of the egg. The future posterior end of the embryo contains a high concentration of mRNA for a regulator of translation called Nanos, which sets up a posterior gradient in the same way. The third signal is generated symmetrically at both ends of the egg, by local activation of a transmembrane tyrosine kinase receptor called Torso. The activated receptor exerts its effects over a shorter range, marking the sites of specialized terminal structures that will form at the head and tail ends of the future larva and also defining the rudiments of the future gut. The three sets of genes responsible for these localized determinants are referred to as the anterior, posterior, and **terminal** sets of **egg-polarity** genes.

A fourth landmark defines the dorsoventral axis (see Fig.4): a protein that is produced by follicle cells underneath the future ventral region of the embryo leads to localized activation of another transmembrane receptor, called Toll, in the oocyte membrane. The genes required for this function are called dorsoventral egg-polarity genes.

All the egg-polarity genes in these four classes are maternal-effect genes: it is the mother's genome, not the zygotic genome, that is critical. Thus, a fly whose chromosomes are mutant in both copies of the *bicoid* gene but who is born from a mother carrying one normal copy of *bicoid* develops perfectly normally, without any defects in the head pattern. However, if that daughter fly is a female no functional *bicoid* mRNA can be deposited into the anterior part of her own eggs, and all of these will develop into headless embryos regardless of the father's genotype.

Each of the four egg-polarity signals—provided by Bicoid, Nanos, Torso, and Toll—exerts its effect by regulating (directly or indirectly) the expression of genes in the nuclei of the blastoderm. The use of these particular molecules to organize the egg is not a general feature of early animal development—indeed, only *Drosophila* and closely related insects possess a *bicoid* gene. And Toll has been coopted here for dorsoventral patterning; its more ancient and universal function is in the innate immune response.

**Dorsoventral signaling genes as a gradient**

Once inside the nucleus, the Dorsal protein turns on or off the expression of different sets of genes depending on its concentration. The expression of each responding gene depends on its regulatory DNA—specifically, on the number and affinity of the binding sites that this DNA contains for Dorsal and other regulatory proteins. In this way, the regulatory DNA can be said to *interpret* the positional signal provided by the Dorsal protein gradient, to define a dorsoventral series of territories—distinctive bands of cells that run the length of the embryo (**Fig.5** A). Most ventrally—where the concentration of Dorsal protein is highest—it switches on, for example, the expression of a gene called *twist* that is specific for mesoderm. Most dorsally, where the concentration of Dorsal protein is lowest, the cells switch on *decapentaplegic (dpp)*. And in an intermediate region, where the concentration of Dorsal protein is high enough to repress *dpp* but too low to activate *twist,* the cells switch on another set of genes, including one called *short gastrulation (sog).*

Products of the genes directly regulated by the Dorsal protein generate in turn more local signals that define finer subdivisions of the dorsoventral axis. These signals act after cellularization and take the form of conventional extracellular signaling molecules. In particular, *dpp* codes for the secreted Dpp protein, which forms a local morphogen gradient in the dorsal part of the embryo. The gene *sog*, meanwhile, codes for another secreted protein that is produced in the neurogenic ectoderm and acts as an antagonist of Dpp. The opposing diffusion gradients of these two proteins create a steep gradient of Dpp activity. The highest Dpp activity levels, in combination with certain other factors, cause the development of the most dorsal tissue of all—extraembryonic membrane; intermediate levels cause the development of dorsal ectoderm; and very low levels allow the development of neurogenic ectoderm (Fig.5 B).

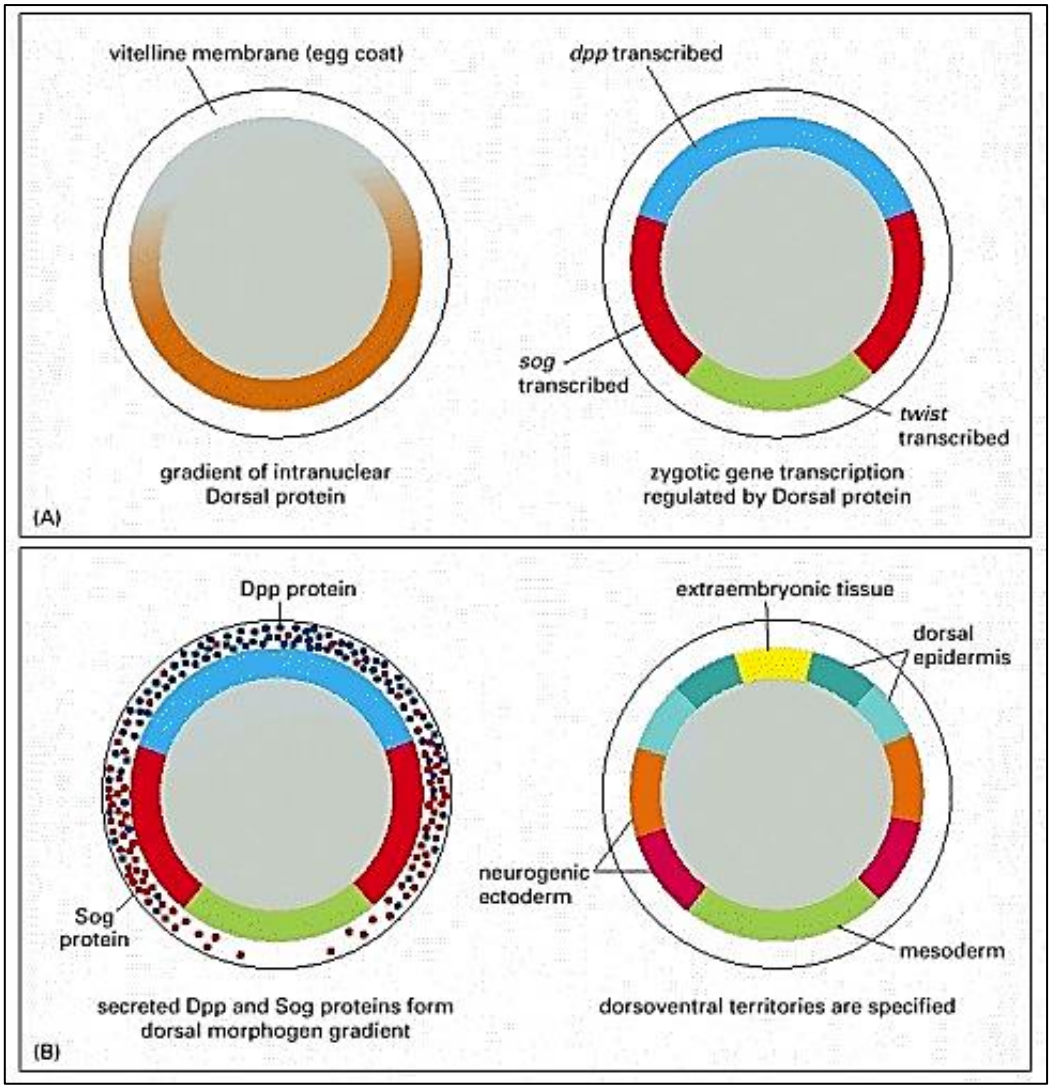



**Fig.5** *Morphogen gradients patterning the dorsoventral axis*

(A) The gradient of Dorsal protein defines three broad territories of gene expression, marked here by the expression of three representative genes—*dpp*, *sog*, and *twist*.

(B) Slightly later, the cells expressing *dpp* and *sog* secrete, respectively, the signal proteins Dpp (a TGFβ family member) and Sog (an antagonist of Dpp). These two proteins diffuse and interact with one another (and with certain other factors) to set up a gradient of Dpp activity that guides a more detailed patterning process.

**Probable questions:**

1. Differentiate maternal genes and zygotic genes.
2. Give the structure of an oocyte of *Drosophila*.
3. Discuss the egg-polarity gradient systems in the development of *Drosophila*.
4. What is the role of dorsoventral signaling genes in *Drosophila* development?

**Suggested Literature:**


1. Developmental Biology: Michael J.F. Barresi Scott F. Gilbert, (12 th Ed)
2. Principles of Development: Lewis Wolpert and Cheryll Tickle (4th Ed.)
3. Principles of Genetics. 3rd Ed. D. Peter Snustad and Michael J. Simmons. Wiley Publication.
4. iGenetics: A molecular approach. 3rd Ed. Peter J. Russell. Pearson International Edition.

## UNIT- II

## Cell fate and differentiation, signaling pathways

**Objective:** This area of developmental biology is dedicated to the study of cell fate, differentiation, and signaling in development. Understanding these concepts can help readers gain insight into how cells are differentiated and how signaling plays a role in developmental processes. After studying this topic, readers should be able to understand how differentiation determines the function and specific type of a cell, the different types and functions of signaling molecules, and how these molecules control cell differentiation.

## Cell fate and differentiation

Within the field of developmental biology, one goal is to understand how a particular cell develops into a final cell type, known as **fate determination**. Within an embryo, several processes play out at the cellular and tissue level to create an organism. These processes include cell proliferation, differentiation, cellular movement and programmed cell death. Each cell in an embryo receives molecular signals from neighboring cells in the form of proteins, RNAs and even surface interactions. Almost all animals undergo a similar sequence of events during very early development, a conserved process known as embryogenesis. As embryos mature, more complex fate determination occurs as structures appear, and cells differentiate, beginning to perform specific functions. Under normal conditions, once cells have a specified fate and have undergone cellular differentiation, they generally cannot return to less specified states. The determination of a cell to a particular fate can be broken down into two states where the cell can be **specified (committed)** or **determined**. In the state of being committed or specified, the cell type is not yet determined and any bias the cell has toward a certain fate can be reversed or transformed to another fate. If a cell is determined, the cell's fate cannot be reversed or transformed. In general, this means that a cell **determined** to differentiate into a brain cell cannot be transformed into a skin cell. Determination is followed by differentiation, the actual changes in biochemistry, structure, and function that result in specific cell types. Differentiation often involves a change in appearance as well as function.

Differentiation is the process by which a cell becomes a functional, mature cell of a specific type. As you may remember, signalling molecules play a key role in the differentiation of different cell types in the developing embryo. But how do they work? A cell doesn't just automatically differentiate when it encounters a signaling molecule. It's quite a bit more complicated than that, and for good reason, too. With hundreds of potentials signalling molecules that a cell could encounter, it has to be able to respond to a real signal and ignore a chance encounter with a stray molecule.

In addition, the cell has to incorporate information from more than one type of signaling molecule to activate the correct differential program.

## Signaling in development

Starting from only a single cell, the fertilized egg, all the diverse cell types of the body are produced and organized into tissues and organs. Both cell differentiation and the development of body structures must be regulated by intricate pathways of cell-cell signaling that coordinate the activities of individual cells and ultimately give rise to organisms as complex as human beings.

## The receptor Tyrosine Kinase/Ras/Raf/MAP Kinase pathway in *Drosophila*

Signaling by receptor tyrosine kinases that activate the Ras/Raf/MAP kinase pathway regulates the development and differentiation of many types of cells. A well-studied example in vertebrates is provided by the differentiation of neurons, which is mediated by activation of the nerve growth factor receptor (a receptor tyrosine kinase) and subsequent stimulation of the Ras/Raf/ERK pathway. However, the key role of this pathway in development has been demonstrated most clearly by genetic analysis of the model organisms *Drosophila* and *C. elegans*.

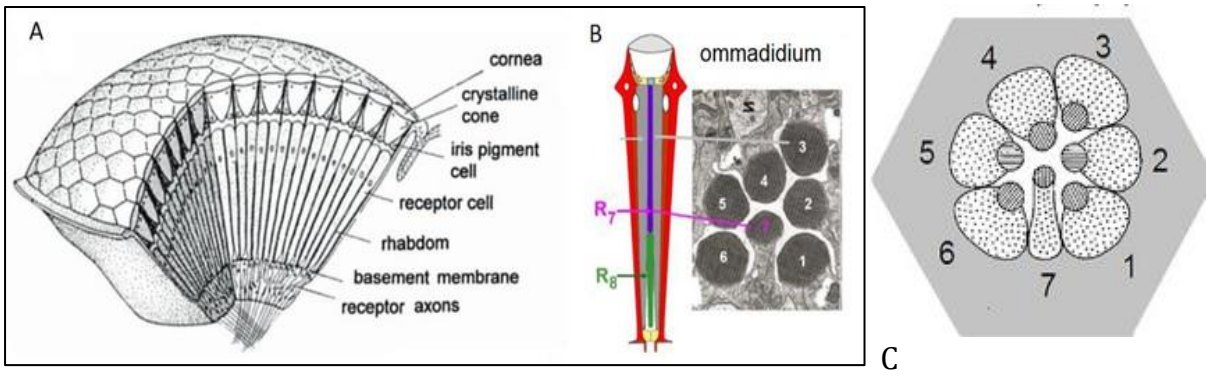



Fig.1 (**A**) *Drosophila eyes are composed of about 800 modular units, ommmadia. (**B**) Each ommatidium contains a lens system and underneath it eight photoreceptor cells: the outer receptors, R1-R6, and the inner receptors, R7/R8. In the electron micrograph, which numbers each cell's rhabdomere (light sensitive part), R8 is not shown because it lies directly below R7. (**C**) Schematic of the intracellular pupil*

Signaling by the Ras/Raf/MAP kinase pathway plays a key role in the development of the compound eye of *Drosophila*, which also illustrates the role of direct cell-cell signaling in differentiation. The *Drosophila* compound eye consists of about 800 individual units, **(Fig.1)** each of which contains eight photoreceptor neurons (R1 through R8) and 12 lens cells. The photoreceptor neurons develop in a fixed order, beginning with the differentiation of R8. R8 then induces two neighbouring cells to become the R2 and

R5 photoreceptors. Next, R2 induces neighbouring cells to become R1 and R3, and R5 induces neighbouring cells to become R4 and R6. The final step is the differentiation of R7, which is induced by interaction with R8. Lens cells then develop from those cells that do not differentiate into photoreceptors.

## Notch signaling

The **Notch** pathway is another highly conserved signaling pathway that controls cell fate during animal development. Like the signaling pathway leading to differentiation of the R7 photoreceptor neuron in *Drosophila*, Notch signaling is an example of direct cell-cell interactions during development. It functions at all stages of development to regulate cell proliferation, survival, and differentiation in organisms ranging from *Drosophila* and *C. elegans* to humans.

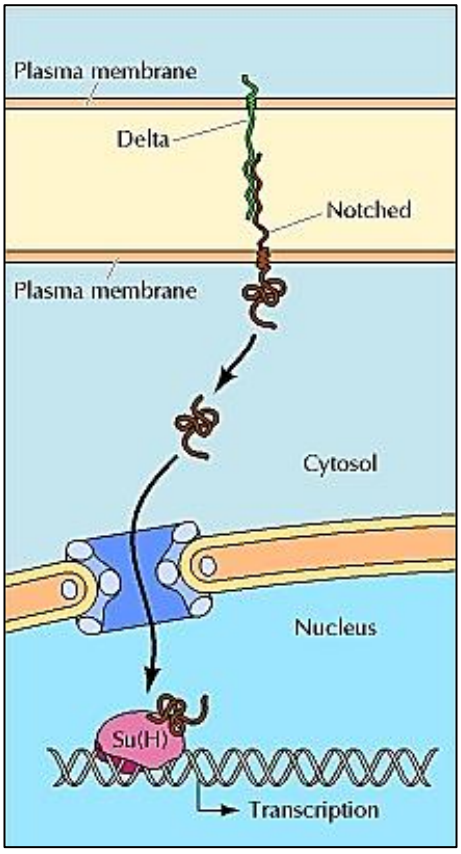



**Fig. 2** *Notch serves as a receptor for direct cell-cell signaling by transmembrane proteins (e.g., Delta) on neighboring cells. The binding of Delta leads to proteolytic cleavage of Notch, releasing the Notch intracellular domain, which translocates to the nucleus and interacts with a transcription factor [Su(H) or CBF-1] to induce gene expression.*

Notch is a large protein with a single transmembrane domain that serves as a receptor for signaling by transmembrane proteins (e.g., Delta) on the surface of adjacent cells **(Fig.2)** Stimulation of Notch initiates a novel and direct pathway of transcriptional activation. In particular, ligand binding leads to proteolytic cleavage of Notch, and the intracellular domain of Notch is then translocated into the nucleus. The Notch

intracellular domain then interacts with another transcription factor (called Su(H) in *Drosophila* or CBF-1 in mammals) and induces expression of its target genes. As in the Wnt signaling pathway, the Notch target genes include genes encoding other transcriptional regulatory proteins, which act to determine cell fate.

**Probable questions:**

1. Define the fate of a cell
2. What is the cell fate state of a cell?
3. Discuss signaling mechanism that regulates *Drosophila* development

**Suggested Literature:**


1. Developmental Biology: Michael J.F. Barresi Scott F. Gilbert, (12 th Ed)
2. Principles of Development: Lewis Wolpert and Cheryll Tickle (4th Ed.)
3. Principles of Genetics. 3rd Ed. D. Peter Snustad and Michael J. Simmons. Wiley Publication.
4. iGenetics: A molecular approach. 3rd Ed. Peter J. Russell. Pearson International Edition.

# Unit III
# Role of microtubules in development, Axis specification

**Objective**: This module aims to explain the significance of microtubules in the development and axis specification in embryos. Upon completion, you will have a better grasp of microtubules and how they contribute to the formation of the embryo's axis. You will understand what microtubules are and their function in the development process. By the end of this module, you will have a comprehensive understanding of the topic.

## Role of microtubule and axis specification in *Drosophila*

Microtubules, with intermediate filaments and microfilaments, are the components of the cell skeleton that determine the shape of a cell. Microtubules are involved in different functions including the assembly of mitotic spindle, in dividing cells, or axon extension, in neurons. In the first case, microtubules are highly dynamic, while in the second case, microtubules are quite stable, suggesting that microtubules with different physical properties (stability) are involved in different functions.

Drosophila oocytes develop within cysts containing 16 cells that are interconnected by cytoplasmic bridges. Although the cysts are syncytial, the 16 cells differentiate to form a single oocyte and 15 nurse cells, and several mRNAs that are synthesized in the nurse cells accumulate specifically in the oocyte. Shortly after the formation of the 16-cell cysts, a prominent microtubule organizing center (MTOC) is established within the syncytial cytoplasm, and at the time the oocyte is determined, a single microtubule cytoskeleton connects the oocyte with the remaining 15 cells of each cyst. The formation of the polarized microtubule cytoskeleton is required for oocyte differentiation, and this structure mediates the asymmetric accumulation of mRNAs within the syncytial cysts.

## Localization of maternal mRNAs

The body axes of the fruit fly are established in mid-oogenesis by the localization of three mRNA determinants, *bicoid*, *oskar*, and *gurken*, within the oocyte. General mechanisms of RNA localization and cell polarization, applicable to many cell types, have emerged from the investigation of these determinants in *Drosophila* oogenesis. Localization of these RNAs is dependent on the germline microtubules, which reorganize to form a polarized array at mid-oogenesis in response to a signaling relay between the oocyte and the surrounding somatic follicle cells.

In *Drosophila*, asymmetric RNA localization is essential for patterning the embryo and partitioning the embryonic cytoplasm into future somatic and germline lineages. This process begins in the oocyte, where MT-based transport restricts key RNAs to specific cortical regions. MT-dependent localization of three major mRNA determinants at mid-oogenesis establishes the embryonic body axes. Localization of *bicoid* (*bcd*)

and *Oskar* (*osk*) mRNAs to the anterior and posterior, respectively, establishes the anterior-posterior (A/P) axis), and dorsal-ventral (D/V) axis determination depends on asymmetric localization of *gurken* (*grk*) mRNA at the future dorsal anterior corner.

## Axis specification

Embryonic axis formation in *Drosophila* is the direct consequence of symmetry-breaking events that take place throughout oogenesis. Oogenesis starts with the first asymmetric division of a germline stem cell to generate a new stem cell and a cystoblast. Four divisions of the cystoblast will generate a cyst of 16 cells, one of which will become the oocyte. The events leading to oocyte specification and patterning of the oocyte and surrounding follicular epithelium provide an attractive model for the study of basic cellular processes such as cytoskeletal dynamics, cell to cell signalling, RNA localisation and translational control.

Patterning the anteroposterior axis involves the sequential and hierarchical activation of several different groups of genes. Maternal gene products are responsible for the first stage of this process, which is axis specification.

Setting up the anteroposterior axis involves three different classes of maternal genes each controlling the development of a different part of the embryo by establishing a distinct signalling center. One class of genes establishes an anterior signaling center that controls head and thorax development. Another establishes a posterior signaling center that controls the development of the abdomen. In both cases, the principal genes are transcribed in the nurse cells and products are deposited in the egg, as maternal mRNAs, where they become asymmetrically localized.

## Anterior and posterior signaling centers

There are about 50 maternal genes involved in anteroposterior axis specification but four genes are particularly important because their products form gradients along the axis and play a predominant role in patterning the nuclei of the syncytial blastoderm. These genes are *bicoid, hunchback, nanos and caudal*. The maternal bicoid mRNA is synthesized in the nurse cells and is localized at the anterior pole of the egg. After fertilization, the message is translated, producing a gradient of Bicoid protein along the anteroposterior axis, with the highest concentration at the anterior pole. Bicoid is a transcription factor that activates several downstream zygotic genes including *hunchback.* The anteroposterior gradient of Bicoid therefore sets up a similar gradient of Hunchback protein, also a transcription factor, with the highest concentration at the anterior pole of the egg. Both Bicoid and Hunchback regulate downstream genes that control anterior-specific developmental processes. Maternal *nanos* mRNA is localized to the posterior pole of the egg and like *bicoid* mRNA, is translated after fertilization to form a protein gradient. The gradient of the Hunchback protein is therefore established by a dual mechanism: stimulation of zygotic *hunchback* transcription by Bicoid in the anterior

of the embryo and inhibition of maternal *hunchback* translation by Nanos in the posterior. The final gene in this quartet, *caudal*, encodes a transcription factor that controls the development of posterior structures. Like *hunchback*, maternal *caudal* mRNA is also distributed uniformly in the egg so its translation must be blocked in the anterior of the embryo. This function is carried out by Bicoid, therefore establishing a gradient of Caudal protein running from posterior to anterior. Bicoid is bifunctional: it acts as both a transcription factor to activate *hunchback* and a translational inhibitor to repress *caudal*. Conversely, while Nanos can act as a translational inhibitor, it does not act as a transcription factor. As a result of the interactions between these four genes, the syncytial embryo has gradients of Bicoid and Hunchback running from anterior to posterior and a gradient of Caudal running from posterior to anterior.

**Probable questions:**

1. What are microtubules and their function?
2. State the role of microtubules in *Drosophila* development.
3. Discuss the axis specification in the Drosophila embryo.

**Suggested Literature:**


1. Developmental Biology: Michael J.F. Barresi Scott F. Gilbert, (12 th Ed)
2. Principles of Development: Lewis Wolpert and Cheryll Tickle (4th Ed.)
3. Principles of Genetics. 3rd Ed. D. Peter Snustad and Michael J. Simmons. Wiley Publication.
4. iGenetics: A molecular approach. 3rd Ed. Peter J. Russell. Pearson International Edition.

# Unit IV
# Role of Gap genes, pair-rule genes, segment polarity genes, axis formation

**Objective**: This unit covers the topic of *Drosophila* development and segment formation in embryos, with a focus on the role of gap genes, pair-rule genes, and segment polarity genes. The reader will gain knowledge about the impact of zygotic genes and segmentation. By the end of this topic, the reader will be able to comprehend the specific functions performed by the zygotic genes in *Drosophila* development, especially those that contribute to the early patterning in the embryo.

## Three classes of segmentation genes

After the initial gradients of *Bicoid* and *Nanos* are created to define the anteroposterior axis, the **segmentation genes** refine the pattern. Mutations in any one of the segmentation genes alter the number of segments or their basic internal organization without affecting the global polarity of the embryo. Segmentation genes are expressed by subsets of cells in the embryo, so their products are the first components that the embryo's genome, rather than the maternal genome, contributes to embryonic development. They are therefore called *zygotic-effect genes* to distinguish them from the earlier maternal-effect genes described in the module of Unit III.

The segmentation genes fall into three groups according to their mutant phenotypes and the stages at which they act (**Fig.1**). First come a set of at least six **gap genes**, whose products mark out coarse subdivisions of the embryo. Mutations in a gap gene eliminate one or more groups of adjacent segments, and mutations in different gap genes cause different but partially overlapping defects. In the mutant *Krüppel,* for example, the larva lacks eight segments, from T1 to A 5 inclusive.

The next segmentation genes to act are a set of eight **pair-rule genes**. Mutations in this cause a series of deletions affecting alternate segments, leaving the embryo with only half as many segments as usual. While all the pair-rule mutants display this two-segment periodicity, they differ in the precise positioning of the deletions relative to the segmental or parasegmental borders. The pair-rule mutant *even-skipped (eve),* for example, lacks the whole of each odd-numbered parasegment, while the pair-rule mutant *fushi tarazu (ftz)* lacks the whole of each even-numbered parasegment, and the pair-rule mutant *hairy* lacks a series of regions that are of similar width but out of register with the parasegmental units.

Finally, there are at least 10 **segment-polarity genes**. Mutations in these genes produce larvae with a normal number of segments but with a part of each segment deleted and replaced by a mirror-image duplicate of all or part of the rest of the segment. In *gooseberry* mutants, for example, the posterior half of each segment (that is, the anterior half of each parasegment) is replaced by an approximate mirror image of the adjacent anterior half-segment.

**Expression of segmentation genes**

The products of the egg-polarity genes provide the global positional signals in the early embryo. These cause particular gap genes to be expressed in particular regions. The products of the gap genes then provide a second tier of positional signals that act more locally to regulate finer details of patterning through the expression of yet other genes, including the pair-rule genes (**Fig.1**). The pair-rule genes, in turn, collaborate with one another and with the gap genes to set up a regular periodic pattern of expression of segment-polarity genes, and the segment-polarity genes collaborate with one another to define the internal pattern of each segment. The strategy, therefore, is one of sequential induction. By the end of the process, the global gradients produced by the egg-polarity genes have triggered the creation of a fine-grained pattern through a hierarchy of sequential, progressively more local, positional controls.

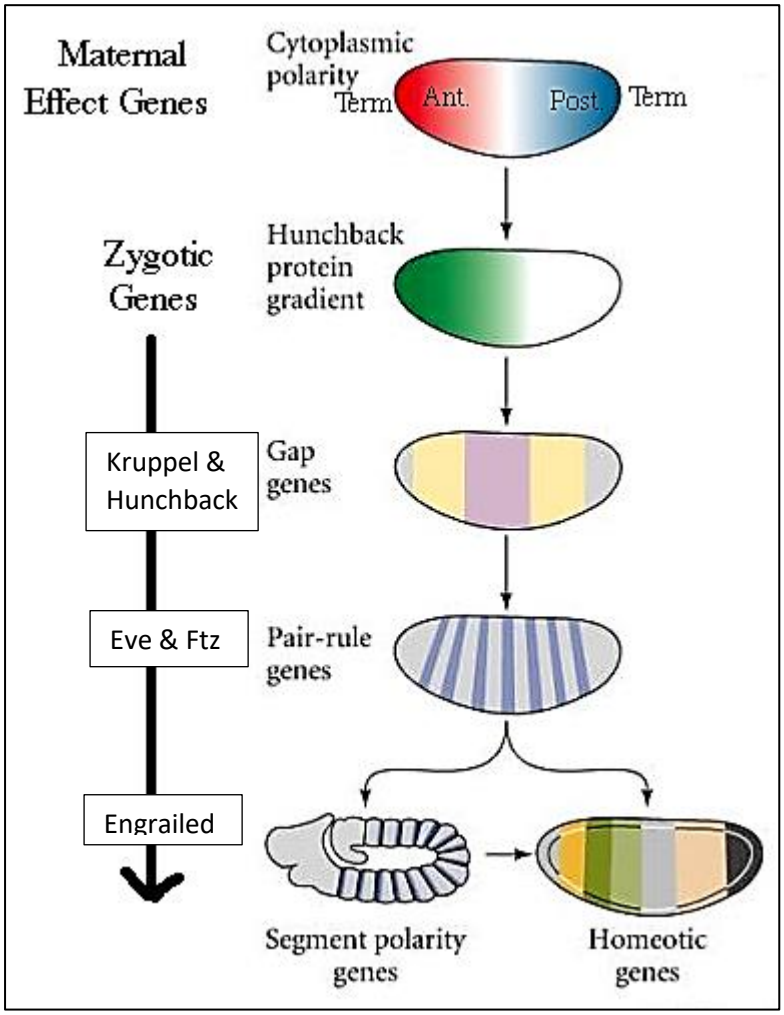



Fig.1 *The regulatory hierarchy of egg-polarity, gap, segmentation, and homeotic selector genes*

**About Gap genes**

The gap genes are the first zygotic genes to be expressed along the anteroposterior axis in the Drosophila embryo. They are called gap genes because they are expressed in broad domains corresponding to several contiguous segments of the larva. The mutant phenotypes reflect the loss of these segments as well as other, more specific defects. The gap genes are expressed transiently in the syncytial blastoderm and all of them encode transcription factors. The gap genes have at least three regulatory roles: a) they regulate the expression of each other in complex feedback loops that establish sharp boundaries between expression domains; b) they regulate the expression of downstream pair-rule/segment polarity genes to establish the basic segmental body plan; c) they regulate downstream homeotic selector genes to give each segment a positional identity along the anteroposterior axis.

**About pair-rule genes**

The pair-rule genes are the first genes to be expressed in spatially periodic patterns in the *Drosophila* embryo and are collectively responsible for patterning the expression of the "segment-polarity" genes, which organize and maintain segmentally reiterated compartment boundaries termed "parasegment boundaries".

There are 7 canonical pair-rule genes: *hairy*, *even-skipped (eve) runt* , *fushi tarazu* (*ftz*), *odd-skipped* (*odd*), *paired* (*prd*), and *sloppy-paired* (*slp*). 5 of these genes (*hairy*, *eve*, *runt*, *ftz*, and *odd*) are known as the "primary" pair-rule genes because they are expressed earlier than the 2 "secondary" pair-rule genes, *prd* and *slp*.

The position of the future parasegments is established in the syncytial *Drosophila* embryo by the expression of pair-rule genes, all of which encode transcription factors. Most of the pair-rule genes are expressed initially as a series of seven transverse stripes along the anteroposterior axis. There are 14 parasegments, and the pair-rule genes are expressed in domains representing alternating parasegments.

The regular pattern of pair-rule gene expression is controlled in two stages. First, the primary pair-rule gene *even-skipped*, *hairy,* and *runt* is activated by the gap gene transcription factor. Second, the products of the primary pair-rule gene regulate the secondary pair-rule gene such as *fushi tarazu* and *odd skipped*. This establishes pair-rule expression domains in complementary patterns.

**About Segment polarity genes**

The segment polarity genes are expressed in the cellular blastoderm as 14 transverse stripes, under the control of complementary pair-rule gene products. The function of the segment polarity genes is to establish the boundaries between Para segments and generate the pattern within each Para segment. Therefore, a mutation in these genes generates a disturbance to the underlying patterning mechanism as shown by abnormal denticle patterns. Examples of segment polarity genes reflecting the

appearance of segments that lack denticle belts include *naked, smooth, fused,* etc or have denticle belts throughout are a *hedgehog*, *gooseberry, porcupine,* etc.

The segment polarity genes function in the cellular blastoderm and encode secreted proteins, receptors, and intracellular signalling components as well as transcription factors.

The segment polarity genes essentially carry out three functions

- They establish an anterior and posterior compartment within each parasegment. Where the anterior and posterior compartments meet, a boundary is generated.
- They maintain parasegment boundaries.
- They generate an anteroposterior pattern within each parasegment.

**Probable questions:**

1. What are gap genes?
2. State the role of pair-rule genes in *Drosophila* axis patterning.
3. How do the segment polarity genes help in patterning the body of the *Drosophila* embryo?

**Suggested Literature:**


1. Developmental Biology: Michael J.F. Barresi Scott F. Gilbert, (12 th Ed)
2. Principles of Development: Lewis Wolpert and Cheryll Tickle (4th Ed.)
3. Principles of Genetics. 3rd Ed. D. Peter Snustad and Michael J. Simmons. Wiley Publication.
4. iGenetics: A molecular approach. 3rd Ed. Peter J. Russell. Pearson International Edition.

# Unit V
# Role of homeotic genes; homeodomains; Hox genes and HOM-c genes

**Objective**: This unit covers the topic of homeotic genes, homeodomains, Hox genes, and HOM-c genes. It provides information on the genes involved in Drosophila development. By studying this topic, readers will learn how these genes help in establishing the positional identity of segments during *Drosophila* development.

## *Drosophila* Segment

Each segment of the *Drosophila* larva is unique, both in terms of its ventral denticle pattern and its internal organization. Furthermore, different segments give rise to different appendages in the adult fly. The unique properties of a given segment depend upon its position along the anteroposterior axis. The establishment of regional differences between segments requires a set of homeotic selector genes, which confer positional values on the segments.

## Homeotic genes

Homeotic genes occupy a central position in the hierarchy of genes controlling the early steps of embryonic development in Drosophila. They are master regulator genes and are involved in the specification of the individual identity of each segment of the insect's body. When homeotic genes are over-activated or inactivated by mutations, body structures may develop in the wrong place—sometimes dramatically so! Most animal homeotic genes encode transcription factor proteins that contain a region called the **homeodomain** and are called ***Hox* genes**.

Most homeotic genes of Drosophila are located in two large gene clusters, the Antennapedia complex (ANT-C) and the Bithorax complex (BX-C). These genes are expressed in two partially overlapping domains whose positions along the anterior-posterior axis of the organism are colinear with their position within each complex. Five genes in the ANT-C are involved in the specification of the identity of some segments of the head and of that of the first and second thoracic segments. The three genes of the BX-C determine the identities of the posterior compartment of the second and third thoracic segments and the eight abdominal segments.

As an example, let's look at a homeotic gene called *Antennapedia*. Normally, *Antennapedia* is expressed in what will become the second segment of a fly's thorax, starting when the fly is a tiny embryo and persisting into the adult fly (**Fig.1**). There, the gene acts as a master regulator, turning on the genetic program that makes the fly's second pair of legs and other segment-specific structures.

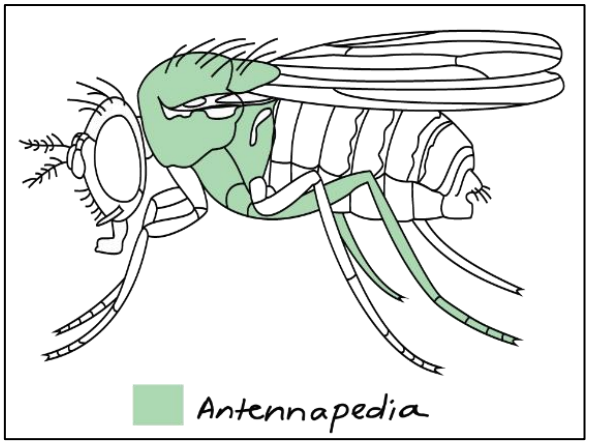
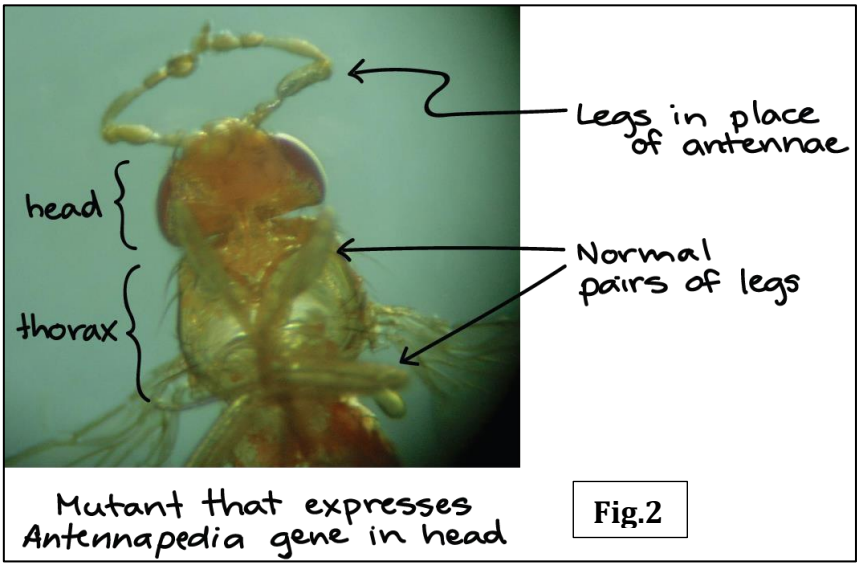



**Fig.1** *Normal fly showing Antennapedia expression.*

If *Antennapedia* stays where it's supposed to and does its job, we get a nice, normal-looking fly with all its appendages in the right place. But what happens if a genetic mutation causes expression of the *Antennapedia* gene to expand into the fly's head? This type of mutation causes legs to grow from the fly's head in place of antennae! (**Fig.2**) In other words, the gene activates its normal, second-segment leg development program, but in the wrong part of the fly.




Another fly homeotic gene with dramatic effects is the *Ultrabithorax* gene. This gene is expressed strongly in the third segment of the thorax, which bears the fly's rearmost pair of legs. *Ultrabithorax* expression in this region of the fly starts early in development and continues throughout the fly's life.

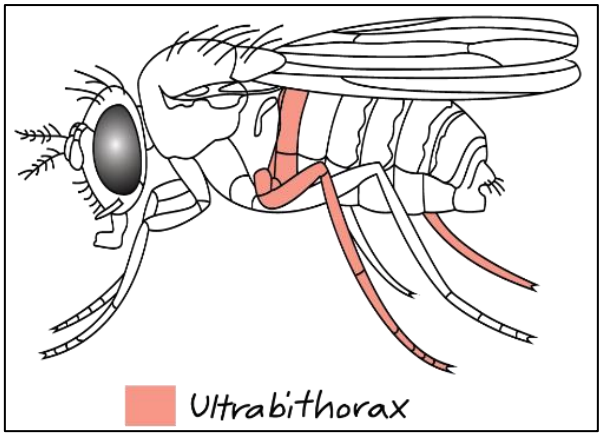



**Fig.3** *Normal fly showing Ultrabithorax expression.*

Wings usually form only in the second segment of the thorax, not in the third, which instead makes small structures called halteres that help the fly balance (**Fig.3).** The job of *Ultrabithorax* is to repress second-segment identity and formation of wings in the third segment. When *Ultrabithorax* is inactivated in the developing third segment due to mutations, the halters will be converted to a second set of wings, (**Fig.4**) neatly positioned behind the normal set.

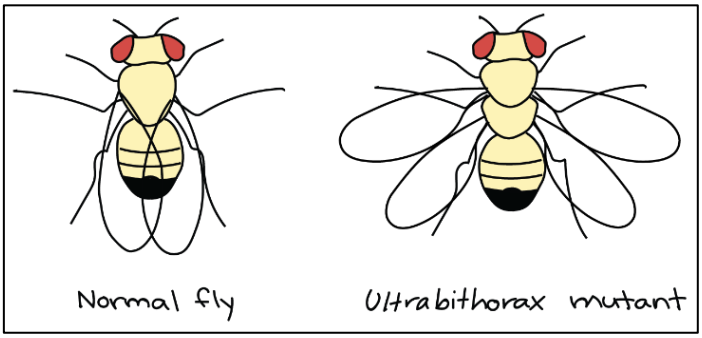



**Fig. 4** A *normal and an Ultrabithorax mutant fly*

## Overview of HOX genes

HOX genes are referred to as the **HOM-C** (for Homeotic Complex). Historically **HOM-C genes** have referred to **Drosophila** homologs, while Hox **genes** refer to vertebrates.

Hox proteins encode and specify the characteristics of 'position', ensuring that the correct structure form in the correct places of the body. For example, Hox genes in insects specify which appendages form on a segment (for example, legs, antennae, and wings in fruit flies), and Hox genes in vertebrates specify the types and shapes of vertebrae that will form. In segmented animals, Hox proteins thus confer segmental or positional identity but do not form the actual segments themselves.

*Antennapedia* and *Ultrabithorax* are not the only homeotic genes in a fruit fly. In fact, a whole set of different homeotic genes act in different regions of the fly's body, ensuring that each segment takes on its correct identity. These genes are typically expressed in the regions they regulate, starting early in embryonic development, and they continue to be expressed in the adult fly.

The diagram below (**Fig.5**) shows eight major homeotic genes in flies. The upper part of the diagram shows where each gene is most strongly expressed in the mature fly, while the lower part of the diagram shows where the genes are located on the chromosome. The order of the genes on the chromosome more or less mirrors their order of expression along the head-tail axis of the fly.

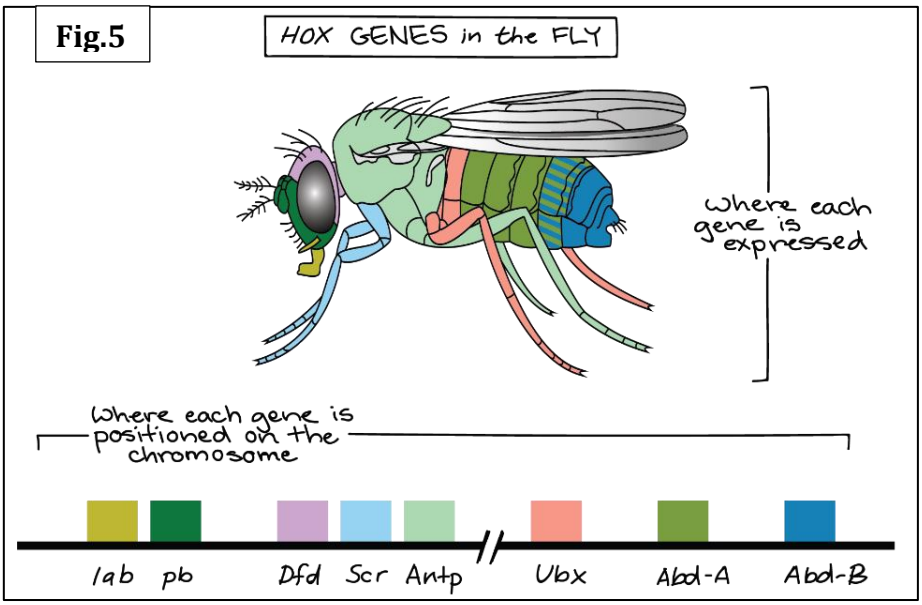



**Fig: 5** *The break mark (//) in the chromosome indicates that these two clusters of genes are separated by a long intervening region that's not shown.*

What exactly are these homeotic genes? Each gene encodes a transcription factor that is expressed in a specific region of the fly starting early in its development as an embryo. The transcription factors change the expression of target genes to enact the genetic “program” that's right for each segment.

The homeotic transcription factors shown in the diagram above all contain a -binding protein region called the **homeodomain**, which is encoded by a segment of DNA called the **homeobox**. Because they contain a homeobox, homeotic genes of this class are sometimes called *Hox genes* for short. Molecular studies have revealed that the proteins coded by the homeotic genes share a 60 amino acid motif, within the homeotic transcription factor protein, the homeobox, whose helix-turn-helix structure enables them to bind as transcription factors to specific DNA sequences in the cis-acting regulatory regions of their target genes. If a mutation occurs in the homeobox of any of

the homeotic genes, an organism will not develop correctly. For example, in fruit flies (*Drosophila*), mutation of a particular homeotic gene results in altered transcription, leading to the growth of legs on the head instead of an antenna; this is known as the antennapedia mutation.

To be clear, not all homeobox-containing genes are necessarily homeotic genes. Other, non-homeotic genes contain the same protein motif.

Also, not all homeotic genes have to contain a homeobox. Although many animals' homeotic genes do contain a homeobox, plant homeotic genes often have a different, characteristic segment called the MADS box.

**Probable questions:**

1. What is the function of homeotic genes?
2. What is the Hox gene in *Drosophila*?
3. How are homeotic genes in *Drosophila* regulated?
4. Discuss about HOM-c in *Drosophila*.

**Suggested Literature:**


1. Developmental Biology: Michael J.F. Barresi Scott F. Gilbert, (12 th Ed)
2. Principles of Development: Lewis Wolpert and Cheryll Tickle (4th Ed.)
3. Principles of Genetics. 3rd Ed. D. Peter Snustad and Michael J. Simmons. Wiley Publication.
4. iGenetics: A molecular approach. 3rd Ed. Peter J. Russell. Pearson International Edition.

# Unit VI

## Dosage compensation in *Drosophila* and in mammals

**Objective:** In this unit we will discuss about what is dosage compensation and how it plays roles in *Drosophila* and in mammals.

### Dosage compensation in mammals

***Dosage Compensation and Sex-Chromatin Bodies:***

In man it has been found that Y-chromosomes are genetically inert in comparison to the X-chromosomes and other chromosomes and only a few genes are present in the human Y-chromosome.

The chromosome numbers of male and female human, it appears that females contain a higher dose of functional gene containing chromosome than males (Female chromosome numbers = 44 + XX and Male chromosome number = 44 + XY).

This mechanism of compensating the differential doses of functional sex chromosomes in male and female human is affected by the inactivation of one X-chromosome in the normal female. The genetically inactive X- chromosome or condensed X-chromosome is called heteropychnotic X-chromosome or heterochromatin or sex-chromatin body or Barr body (according to the name of the geneticist M. L. Barr who first observed it) or Drum-stick (according to the shape of the inactive X-chromosome).

Among the two X-chromosomes in females, which X-chromosome becomes inactive is a matter of chance, but it should be remembered that once an X- chromosome has become inactivated, all cells arising from that cell will keep the same inactive X-chromosome.

In humans, inactive form of X-chromosome as a Barr-body have been observed by the sixteenth day of gestation. X-chromosome inactivation occurs in human when two or more X-chromosomes are present.

**Details about Dosage Compensation or Lyon's Hypothesis:**

The inactive X hypothesis or the Lyon's hypothesis or the Dosage Compensation is widely known from 1961 which states that only one of the two X chromosomes in the homogametic sex is functional while the other condenses and is inactivated. The X inactivated in some cells would be that from the father, in other cells it would be that from the mother.

Hence any tissue in the body of a woman would be a mosaic of cells which would show dominance of all genes having diffusible products but would remain a fine-grained mosaic for other intracellular differences.

Such a mosaic of cells might be difficult to demonstrate, particularly among rigid tissues, although cells which can be separated and cloned might show antigenic differences. This hypothesis has stimulated many new investigations, some of which are currently being completed.

***Objectives behind the Proposition of Lyon's Hypothesis:***

**Lyon was impressed by three observations relating to X chromosome:**

1. In normal mammalian females, one of the two X's is genetically inactive in the somatic cells (single active X-hypothesis).

2. Inactivation is random i.e., irrespective of paternal and maternal origin (random inactivation).

3. (a) The inactivation occurs during early ontogeny (early ontogenic differentiation) and (b) The particular X which has thus become inactivated, remains inactive in all the succeeding cell generation (fixed differentiation).

## Dosage compensation in *Drosophila*

Dosage compensation is the crucial process that equalizes gene expression from the X chromosome between males (XY) and females (XX). Dosage compensation in Drosophila increases the transcription of genes on the single X chromosome in males to equal that of both X chromosomes in females. This is done by the site-specific histone acetylation and activation of genes along the length of male X chromosome by male-specific lethal complex (MSL). Sex determination and dosage compensation in Drosophila are parallel process and regulated during the embryonic development. If the X/A ratio is equal to 1, a regulatory cascade leads to female sexual development. In female, the presence of the Sxl gene product prevents the translation of the msl2 mRNA and assembly of the MSL complex. If the X/A ratio is only 0.5, absence of the cascade leads by default to male sexual development and to the formation of the MSL complex or Dosage Compensation Complex (DCC). (Fig 1)

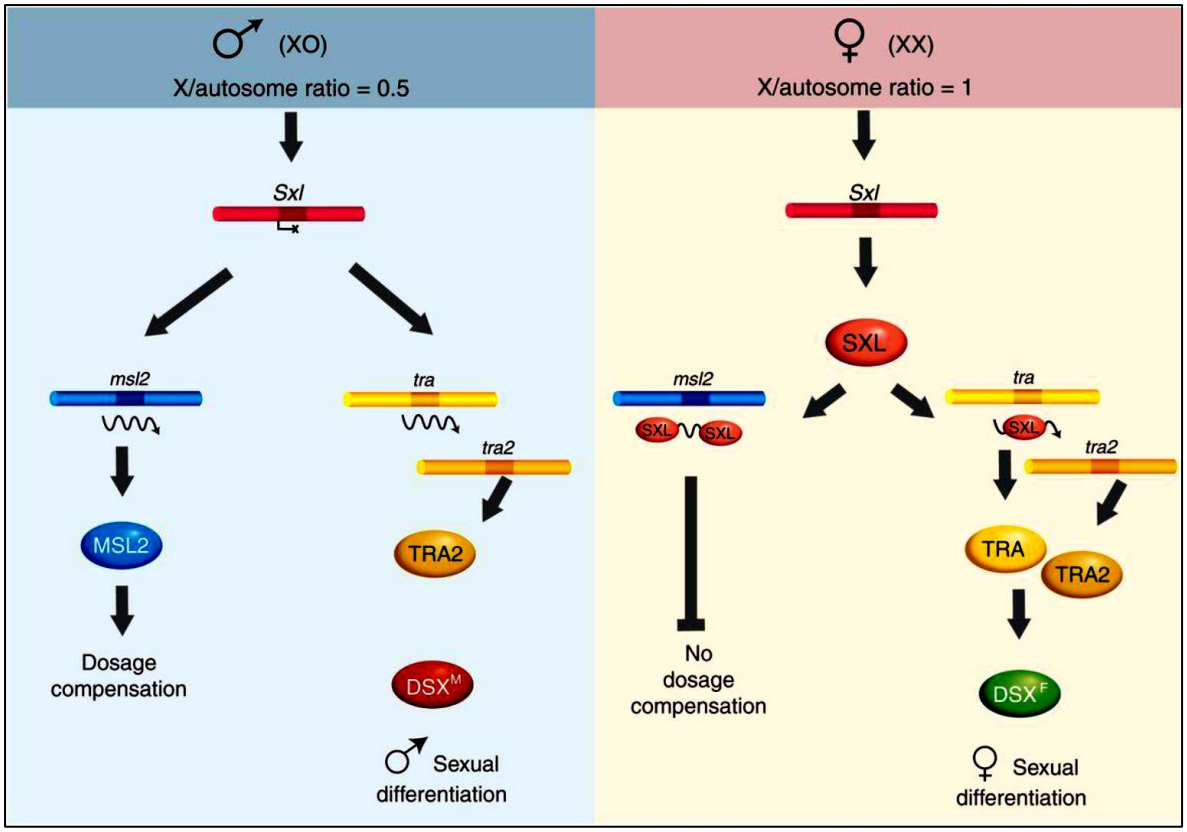



Fig 1: Diagram of the control of sex determination and dosage compensation. [source: Cold Spring Harb Perspect Biol. 2015]

**Probable questions:**

1. What is dosage compensation?
2. State Lyon's hypothesis?
3. What is barr body?
4. How dosage compensation and sex determination is related in Drosophila?

**Suggested Literature:**

1. Developmental Biology: Michael J.F. Barresi Scott F. Gilbert, (12 th Ed)
2. Principles of Development: Lewis Wolpert and Cheryll Tickle (4th Ed.)
3. Principles of Genetics. 3rd Ed. D. Peter Snustad and Michael J. Simmons. Wiley Publication.
4. iGenetics: A molecular approach. 3rd Ed. Peter J. Russell. Pearson International Edition.

# Unit VII

## Sex determination, developmental mutations in *Drosophila*

**Objective:** In this unit we will discuss about what is sex determination. We also discuss about sex determination and developmental mutations in Drosophila in this unit.

## Introduction:

Genotypic Sex determination: In genotypic sex determination system, the sex chromosomes play the decisive role in the inheritance and determination of sex, and it may occur in one of the two ways:

1)In the Y-chromosome mechanism of sex determination (e.g., human), the Y chromosome of the heterogametic sex is active in determining the sex of an individual. Individuals carrying the Y-chromosome are genetically male, while individuals lacking the Y-chromosomes are genetically female.

2)In the X-chromosome autosome balance system (e.g., Drosophila) the main factor in sex determination is the ratio between the number of X-chromosomes and the number of sets of autosomes. In this system the Y-chromosomes has no effect on sex determination, but is required for male fertility.

Chromosomal Sex-Determination Systems:

- XX-XO system: • XX – female • XO – male Example: Grasshoppers
- XX-XY system: • XX – female • XY – male Example: Mammals
- ZZ-ZW system: • ZZ – male • ZW – female Example: Birds, snakes, butterflies, some amphibians, and fishes.
- Haplodiploidy system: • Haploid set – male • Diploid set – female Example: Bees, wasps, and ants

## Sex Determination in *Drosophila melanogaster*

Sex in Drosophila is determined by the ratio of number of X chromosomes (X) to that of the number of sets of autosomes (A) - Genic Balance System, proposed by Calvin Bridges, 1926.

• X:A ratio (X, number of X chromosomes; A, number of haploid sets of autosomes)

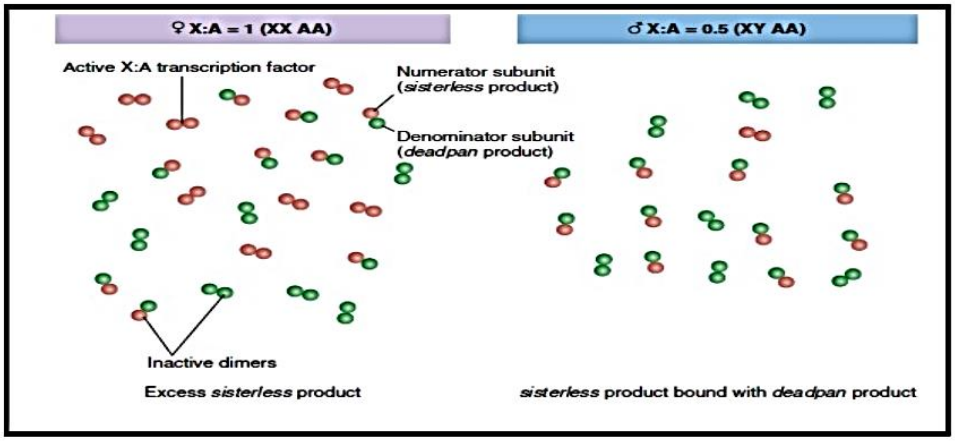
**Table 4.1 Chromosome complements and sexual phenotypes in *Drosophila***

| Sex-Chromosome Complement | Haploid Sets of Autosomes | X : A Ratio | Sexual Phenotype |
|---|---|---|---|
| XX | AA | 1.0 | Female |
| XY | AA | 0.5 | Male |
| XO | AA | 0.5 | Male |
| XXY | AA | 1.0 | Female |
| XXX | AA | 1.5 | Metafemale |
| XXXY | AA | 1.5 | Metafemale |
| XX | AAA | 0.67 | Intersex |
| XO | AAA | 0.33 | Metamale |
| XXXX | AAA | 1.3 | Metafemale |

Table 4-1
*Genetics: A Conceptual Approach, Third Edition*


## Mechanism:X:A ratio determination

The X chromosome are the sisterless numerator genes sis-a, sis-b, and sis-c,runt and on an autosome is the deadpan (dpn) denominator gene. The numerator genes are expressed to produce protein subunits that can form either homodimers or heterodimers with the subunit encoded by the denominator gene. In females, the excess of numerator proteins produces numerator numerator dimers that function as transcription factors to activate Sxl. In males, Sxl expression from PE does not occur because sufficient numerator-numerator transcription factors are absent: No SXL protein is produced in males.



In *Drosophila,* and in insects in general, one can observe **gynandromorphs**—animals in which certain regions of the body are male and other regions are female (Figure 1). This can happen when an X chromosome is lost from one embryonic nucleus. The cells descended from that cell, instead of being XX (female), are XO (male). Because there are no sex hormones in insects to modulate such events, each cell makes its own sexual "decision." The XO cells display male characteristics, whereas the XX cells display female traits. This situation provides a beautiful example of the association between insect X chromosomes and sex.

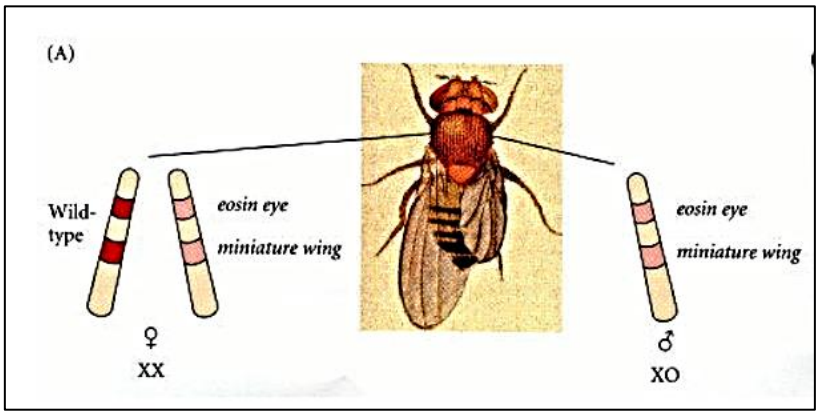



Fig 1: Gynandromorphs. (A) Gynandromorph of *D. melanogaster* in which the left side is female (XX) and the right side is male (XO). The male side has lost an X chromosome bearing the wild-type alleles of eye color and wing shape, thereby allowing the expression of the recessive alleles' *eosin eye* and *miniature wing* on the remaining X chromosome.

Several genes with roles in sex determination have been found. Loss-of-function mutations in most of these genes—*Sex-lethal* (*Sxl*), *transformer* (*tra*), and *transformer-2* (*tra2*)—transform XX individuals into males. Such mutations have no effect on sex determination in XY males. Homozygosity of the *intersex* (*ix*) gene causes XX flies to develop an intersex phenotype having portions of male and female tissue in the same organ. The *doublesex* (*dsx*) gene is important for the sexual differentiation of both sexes. If *dsx* is absent, both XX and XY flies turn into intersexes.The positioning of these genes in a developmental pathway is based on

(1) the interpretation of genetic crosses resulting in flies bearing two or more of these mutations and

(2) the determination of what happens when there is a complete absence of the products of one of these genes. Such studies have generated the model of the regulatory cascade seen in fig 2.

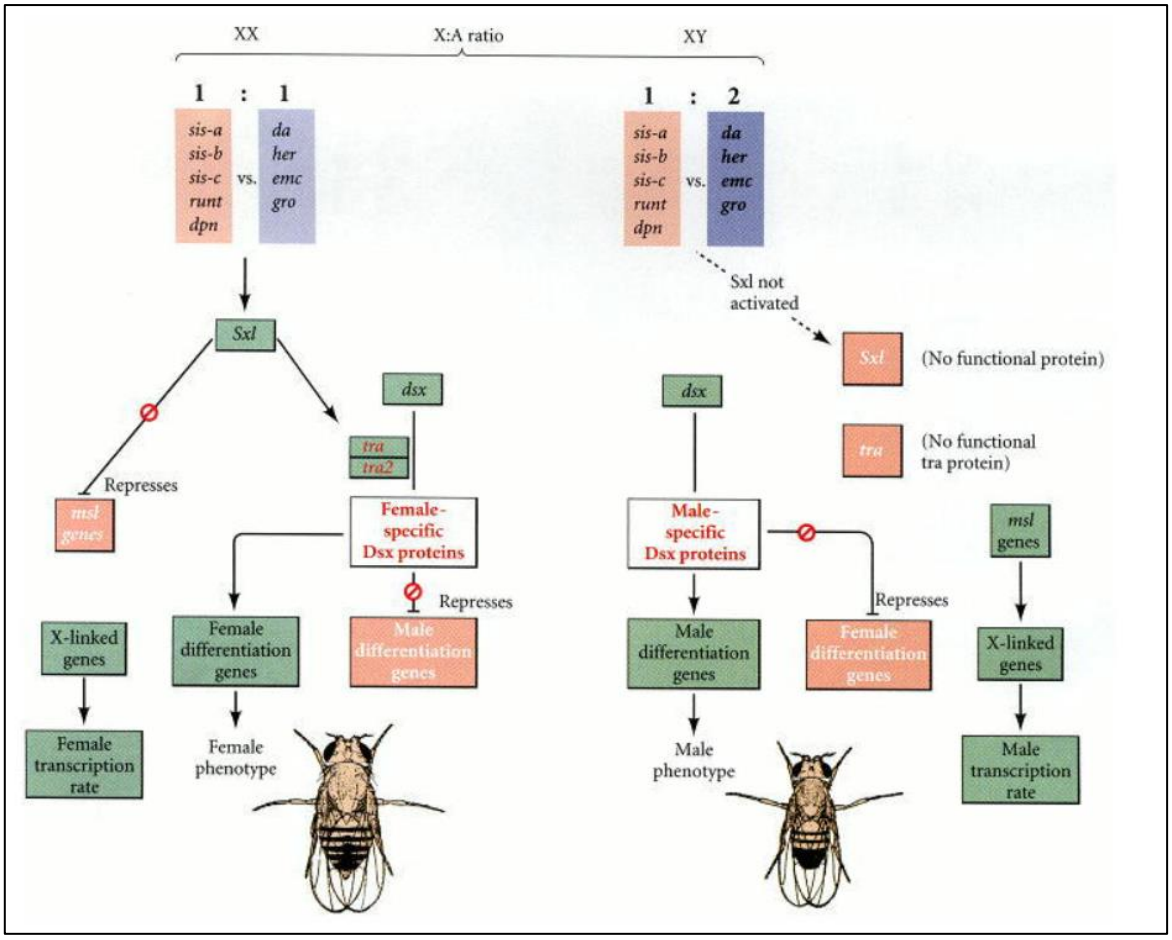



Fig 2: Proposed regulation cascade for *Drosophila* somatic sex determination. Arrows represent activation, while a block at the end of a line indicates suppression. The *msl* loci, under the control of the *Sxl* gene, regulate the dosage compensatory transcription of the male X chromosome.

## The sex-lethal gene as the pivot for sex determination

### Interpreting the x:a ratio

The first phase of *Drosophila* sex determination involves reading the X:A ratio. It appears that high values of the X:A ratio are responsible for activating the feminizing switch gene ***Sex-lethal*** (***Sxl***). In XY cells, *Sxl* remains inactive during the early stages of development In XX *Drosophila, Sxl* is activated during the first 2 hours after fertilization, and this gene transcribes a particular embryonic type of *Sxl* mRNA that is found for only about 2 hours more. Once activated, the *Sxl* gene remains active because its protein product is able to bind to and activate its own promoter.

This female-specific activation of *Sxl* is thought to be stimulated by **"numerator proteins"** encoded by the X chromosome. These constitute the X part of the X:A ratio. These numerator proteins include Sisterless-a and Sisterless-b. These proteins bind to the "early" promoter of the *Sxl* gene to promote its transcription shortly after fertilization.

The **"denominator proteins"** are autosomally encoded proteins such as Deadpan and Extramacrochaetae. These proteins block the binding or activity of the numerator proteins). The denominator proteins may actually be able to form inactive heterodimers with the numerator proteins (Figure 3). It appears, then, that the X:A ratio is measured by competition between X-encoded activators and autosomally encoded repressors of the promoter of the *Sxl* gene.

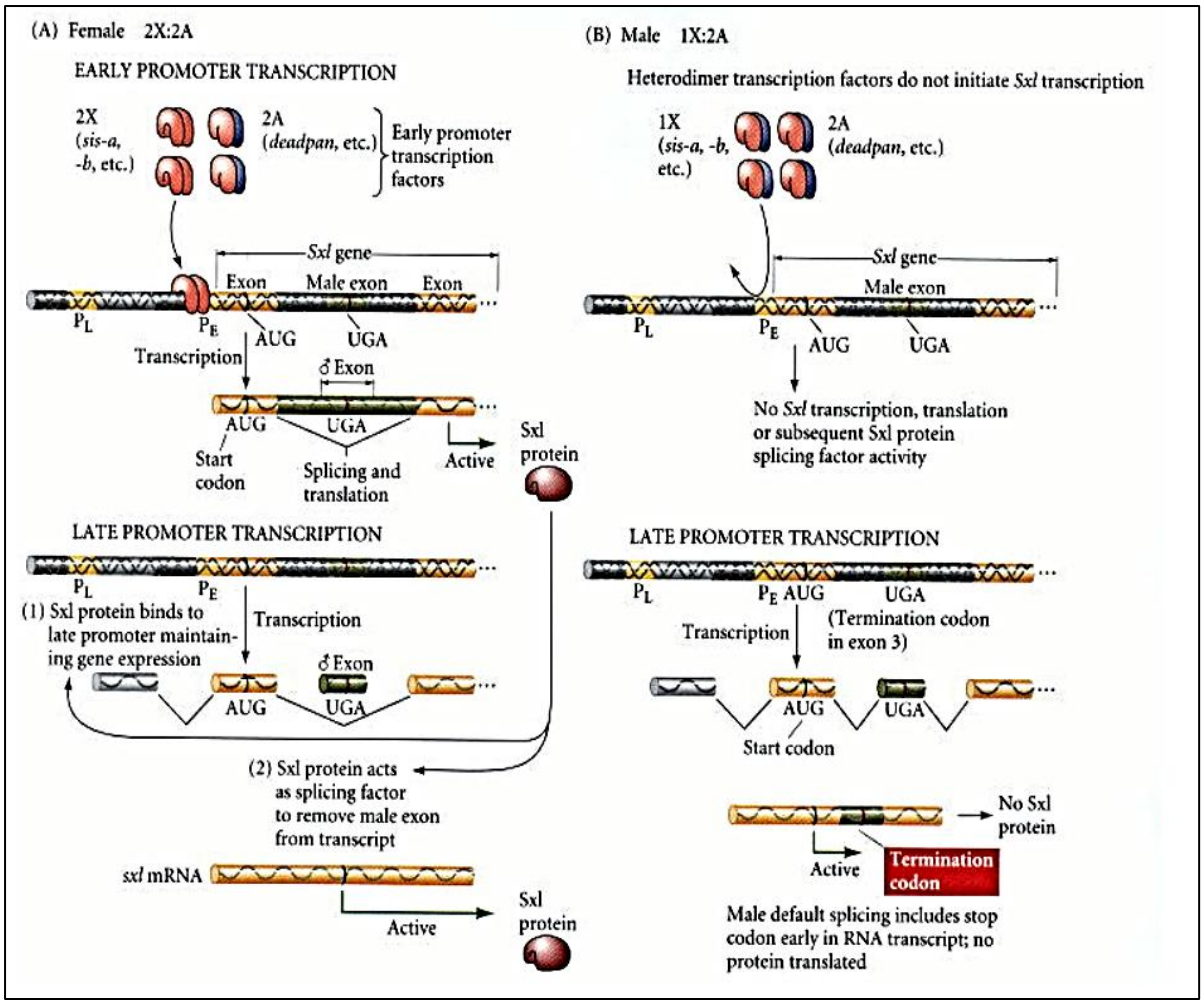



Fig 3: The differential activation of the *sxl* gene in females and males. (A) In wild-type *Drosophila* with two X chromosomes and two sets of autosomes (2X:2A), the numerator proteins encoded on the X chromosomes (sis-a, sis-b, etc.) are not all bound by inhibitory denominator proteins derived from genes (such as *deadpan*) on the autosomes. The numerator proteins activate the early promoter of the *Sxl* gene. Eventually, in both males and females, constitutive transcription of *sxl* starts from the late promoter. If Sxl is already available (i.e., from early transcription), the *Sxl* pre-mRNA is spliced to form the functional female-specific message. (B) In wild-type *Drosophila* with one X chromosome and two sets of autosomes (1X:2A), the numerator proteins are bound by the denominator proteins and cannot activate the early promoter. When the *Sxl* gene is transcribed from the late promoter, RNA splicing does not exclude the male-specific exon in the mRNA. The resulting message encodes a truncated and nonfunctional peptide,

since the male-specific exon contains a translation termination codon. (After Keyes et al. 1992.)

## Maintenance of *sxl* function

Shortly after *Sxl* transcription has taken place, a second, "late" promoter on the *Sex-lethal* gene is activated, and the gene is now transcribed in both males and females. However, analysis of the cDNA from *Sxl* mRNA shows that the *Sxl* mRNA of males differs from *sxl* mRNA of females. This difference is the result of differential RNA processing. Moreover, the Sxl protein appears to bind to its own mRNA precursor to splice it in the female manner. Since males do not have any available Sxl protein when the late promoter is activated, their new *Sxl* transcripts are processed in the male manner. The male *Sxl* mRNA is nonfunctional. While the female-specific *Sxl* message encodes a protein of 354 amino acids, the male-specific *Sxl* transcript contains a translation termination codon (UGA) after amino acid 48. The differential RNA processing that puts this termination codon into the male-specific mRNA is shown in Figures 3B and 4. In males, the nuclear transcript is spliced in a manner that yields eight exons, and the termination codon is within exon 3. In females, RNA processing yields only seven exons, and the male-specific exon 3 is now spliced out as a large intron. Thus, the female-specific mRNA lacks the termination codon.

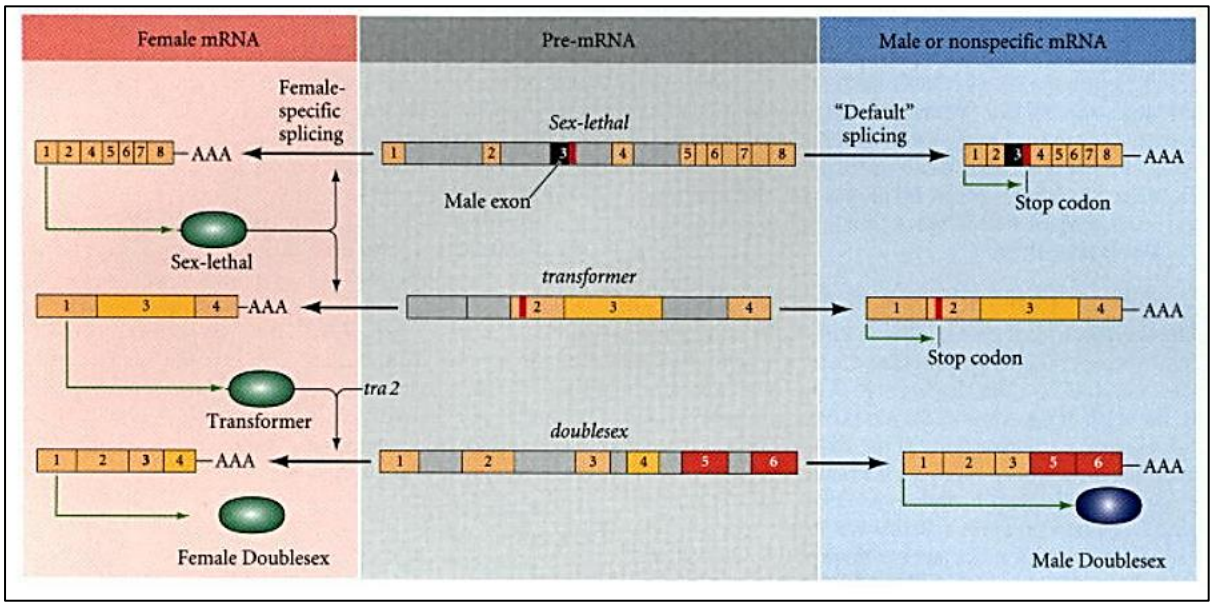



Fig 4: The pattern of sex-specific RNA splicing in three major *Drosophila* sex-determining genes. The pre-mRNAs are located in the center of the diagram and are identical in both male and female nuclei. In each case, the female-specific transcript is shown at the left, while the default transcript (whether male or nonspecific) is shown to the right. Exons are numbered, and the positions of the termination codons and poly(A) sites are marked. (After Baker 1989.)

## The transformer genes

The *Sxl* gene regulates somatic sex determination by controlling the processing of the ***transformer*** (***tra***) gene transcript. The *tra* message is alternatively spliced to create a female-specific mRNA as well as a nonspecific mRNA that is found in both females and males. Like the male *sxl* message, the nonspecific *tra* mRNA contains a termination codon early in the message, making the protein nonfunctional. In *tra,* the second exon of the nonspecific mRNA has the termination codon. This exon is not utilized in the female-specific message (see Figure 4).

## Doublesex: The switch gene of sex determination

The ***doublesex*** (***dsx***) gene is active in both males and females, but its primary transcript is processed in a sex-specific manner. This alternative RNA processing appears to be the result of the action of the *transformer* gene products on the *dsx* gene. If the Tra2 and female-specific Tra proteins are both present, the *dsx* transcript is processed in a female-specific manner. The female splicing pattern produces a female-specific protein that activates female-specific genes (such as those of the yolk proteins) and inhibits male development. As discussed in Chapter 5, if functional Tra is not produced, a male-specific transcript of *dsx* is made. This transcript encodes an active protein that inhibits female traits and promotes male traits.

The functions of the Doublesex proteins can be seen in the formation of the *Drosophila* genitalia. Male and female genitalia in *Drosophila* are derived from separate cell populations. In male (XY) flies, the female primordium is repressed, and the male primordium differentiates into the adult genital structures. In female (XX) flies, the male primordium is repressed, and the female primordium differentiates. If the *dsx* gene is absent (and thus neither transcript is made), both the male and the female primordia develop, and intersexual genitalia are produced. Similarly, in the fat body of *Drosophila,* activation of the genes for egg yolk production is stimulated by the female Dsx protein and is inhibited by the male Dsx protein.

The result of the sex determination cascade comes down to what type of mRNA is going to be processed from the *dsx* transcript. If the X:A ratio is 1, then *Sxl* makes a female-specific splicing factor that causes the *tra* gene transcript to be spliced in a female-specific manner. This female-specific protein interacts with the Tra2 splicing factor to cause the *doublesex* pre-mRNA to be spliced in a female-specific manner. If the *doublesex* transcript is not acted on in this way, it will be processed in a "default" manner to make the male-specific message.

**Description of the Regulatory Cascade:**

• Early in embryogenesis in the female, the numerator dimer transcription factor activates transcription of the Sxl gene from PE (promoter early), one of two promoters for this gene, the other being a more upstream promoter, PL.

• The premRNA transcribed from PE has eight exons; exons 2 and 3 are skipped to produce the mature mRNA consisting of exons E1, 4, 5, 6, 7, and 8. Translation of this mRNA produces the SXL early protein.

• In males, Sxl expression from PE does not occur because sufficient numerator transcription factors are absent: No SXL protein is produced in males.

• Later in embryogenesis (after gastrulation), Sxl is transcribed constitutively from the late promoter, PL, in all cells, regardless of the X:A ratio. This transcription does not depend on the numerator transcription factors.

• The pre-mRNA produced is longer than the transcript from PE and is subject to alternative splicing depending on the presence or absence of SXL early protein.

• In females, the SXL early protein binds to the Sxl pre-mRNA and causes regulated splicing: exons E1 and 3 are skipped, resulting in a mature mRNA with exons L1, 2, 4, 5, 6, 7, and 8.

• Translation of this mRNA produces the SXL late protein.

• In males, the absence of SXL early protein results in default splicing of the pre-mRNA and a mature mRNA is produced that includes exon 3.

• Exon 3 has a stop codon in frame with the start codon at the beginning of exon 2, so no functional SXL late protein is produced in males.

• These events set the switch to either female or male differentiation.

• A cascade of alternative splicing events follows. • In the female embryo, SXL late protein regulates splicing of transformer (tra) pre-mRNA.

• In this case, a stop codon-containing exon segment upstream of and contiguous with exon 2 is removed, resulting in an mRNA with exons 1, 2, and 3. Translation of this mRNA produces the active TRA protein.

• In males, default splicing occurs as a result of the absence of SXL late protein. This means that the stop codon-containing segment is not removed. Translation of the resulting mRNA halts at the stop codon in that segment; no functional TRA protein is produced.

• TRA protein is also an RNA splicing regulator. The target is the pre-mRNA of the doublesex (dsx) gene

• In females, TRA-regulated splicing gives rise to female dsx mRNA. This mRNA encodes the DSX-F (F for female) protein, a transcription factor that represses male-specific gene expression in all cells. As a result, female-specific somatic cell differentiation occurs.

• In males, the absence of functional TRA protein results in default splicing of the dsx premRNA to produce male dsx mRNA. This mRNA encodes the DSX-M (M for male) protein, a transcription factor that represses female specific gene expression in all cells. As a result, male specific somatic cell differentiation occurs. Knockout mutants of dsx have a mixture of male and female characteristics, which occurs because of the lack of repression of male- and female-specific genes.

## Developmental mutations in *Drosophila*

Mutation is a sudden, hereditary change in the genetic make-up of an organism. Mutation is of two types gene mutations or point mutations and chromosomal mutations.

Gene consists of few segments of DNA; gene mutations include changes in the number or arrangement of nucleotides. Thus, gene mutations alter or modify the expressions of a particular gene. Sickle cell anemia, chlorophyll deficiencies in plants and albinism (loss of pigment) are caused by gene mutations. Naturally occurring mutations are known as spontaneous mutations. In 1910, Morgan found few white eyed Drosophila in a population of no⁻ mal red eyed Drosophila. In Drosophila many mutant forms such as white eye, black body, vestigial wings arose through spontaneous mutations.

Mutations caused by mutagenic agents like X-ray, Ultra-violet rays, mustard gas, formaldehyde, caffeine, phenol etc. are known as induced mutations. In contrast to spontaneous mutations, the frequency of induced mutations is high.

### What is a Mutant Phenotype?

Each and every observable character or trait of an individual (i.e. its phenotype) is controlled by specific gene(s). Whenever a gene undergoes a change, the message of the gene is altered, and this will have an effect on the product and, therefore, the function of that gene. As a consequence of this, the phenotype, which was under the control of this gene is going to be changed. This altered phenotype is called a 'mutant phenotype', and the event of the gene undergoing such a change is termed 'mutation'. A mutation, thus, is a heritable change in the genetic material. Every gene can undergo mutation, but different mutations have different types of effects on the phenotype and, moreover, they have different mutation rates. In any species, including humans, every individual is a carrier of mutations for one or the other gene. For instance, a few mutant phenotypes in man are curly hair, baldness, eye colours, albinism, sickle-cell anaemia, xeroderma pigmentosum, polydactyly, hemophilia, night blindness etc. Similarly, mutations and mutant phenotypes are seen in all species including Drosophila, maize, worms, frogs, mouse etc. Mutations occur spontaneously in nature, but at a very low rate. Chemical, physical and biological agents can also induce mutations in the laboratory. The father of genetics, Gregor Mendel, exploited the normal and mutant forms of the pea plant and gave us important generalizations concerned with the norms of inheritance.

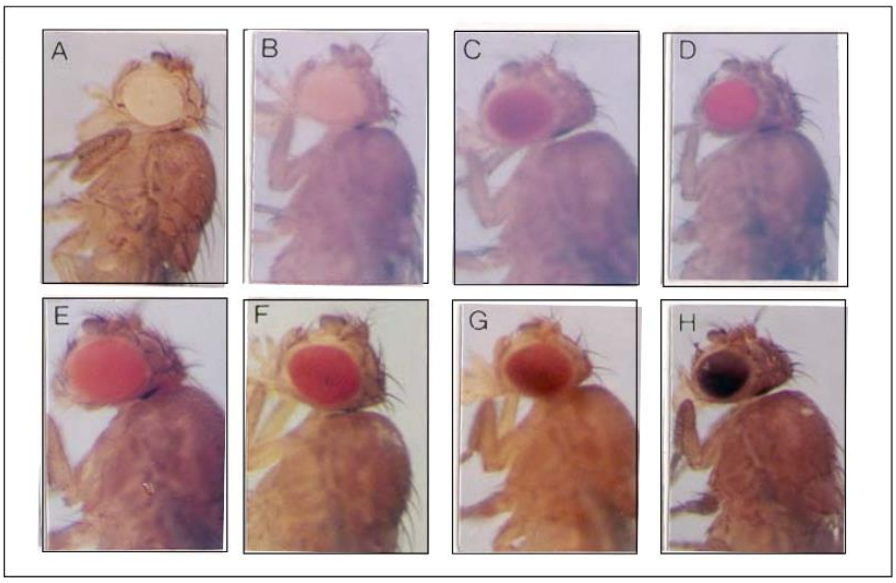
**What is so Special about Mutant Phenotypes of *D. melanogaster*?**

The Drosophila Stock Centre at Mysore has about 2000 genetic stocks of D. melanogaster; each stock breeds true for a particular mutant phenotype(s). For instance, 'white eye' stock means a homozygous strain which breeds true for this phenotype. Such genetically defined mutant strains are a prerequisite for a systematic analysis of inheritance of characters and for the genetic dissection of phenotypes. The genetic repertoire of D. melanogaster includes a wide variety of mutants of importance to the understanding of genetics, development, behaviour and cell biology.

## Description of a Few Mutants of *D. Melanogaster*

1) Eye Mutations (Figure 2A-H): The normal eye colour of D. melanogaster, is red (Figure 1A-B) due to colored pigments. In a mutant these pigments are absent and, hence, the eye appears white. In other mutants, depending on the presence or absence of one or the other pigment, the eye shows different shades of colour. The mutant phenotype and the respective gene symbol in brackets for a few eye mutants are as follows: white (w) (Figure 2A), white apricot (wa) (Figure 2B), brown (bw) (Figure 2C), scarlet (st) (Figure 2D), cinnabar (cn) (Figure 2E), vermilion (v) (Figure 2F), rosy (ry) (Figure 2G), sepia (se) (Figure 2H)

***Figure 2A-H.***
***Eye colour mutants:***
***(A) white***
***(B) white apricot***
***(C) brown***
***(D) scarlet***
***(E) cinnabar***
***(F) vermilion***
***(G) rosy***
***(H) sepia.***




2) Wing Mutations (Figure 3A-I): D. melanogaster, a dipteran, has a pair of wings, which have a definite shape and structure, as well as orientation on the body (Figures 1A-B; 3D). Because of mutations in the genes, which determine these features, many wing mutants are available. Wings may be cut to points and edges scalloped – cut (ct) (Figure 3A); wings can be extremely reduced – vestigial (vg) (Figure 3B); wings small and spoon

like – microptera (mp) (Figure 3C); wing margins scalloped with thickened veins – scalloped (sd) (Figure 3E); wing veins do not reach margins – veinlet (ve) (Figure 3F); wings without crossveins – crossveinless (cv) (Figure 3G); wings obliquely truncated – dumpy (dp) (Figure 3H); and wings that have a network of extra veins – plexus (px) (Figure 3I).

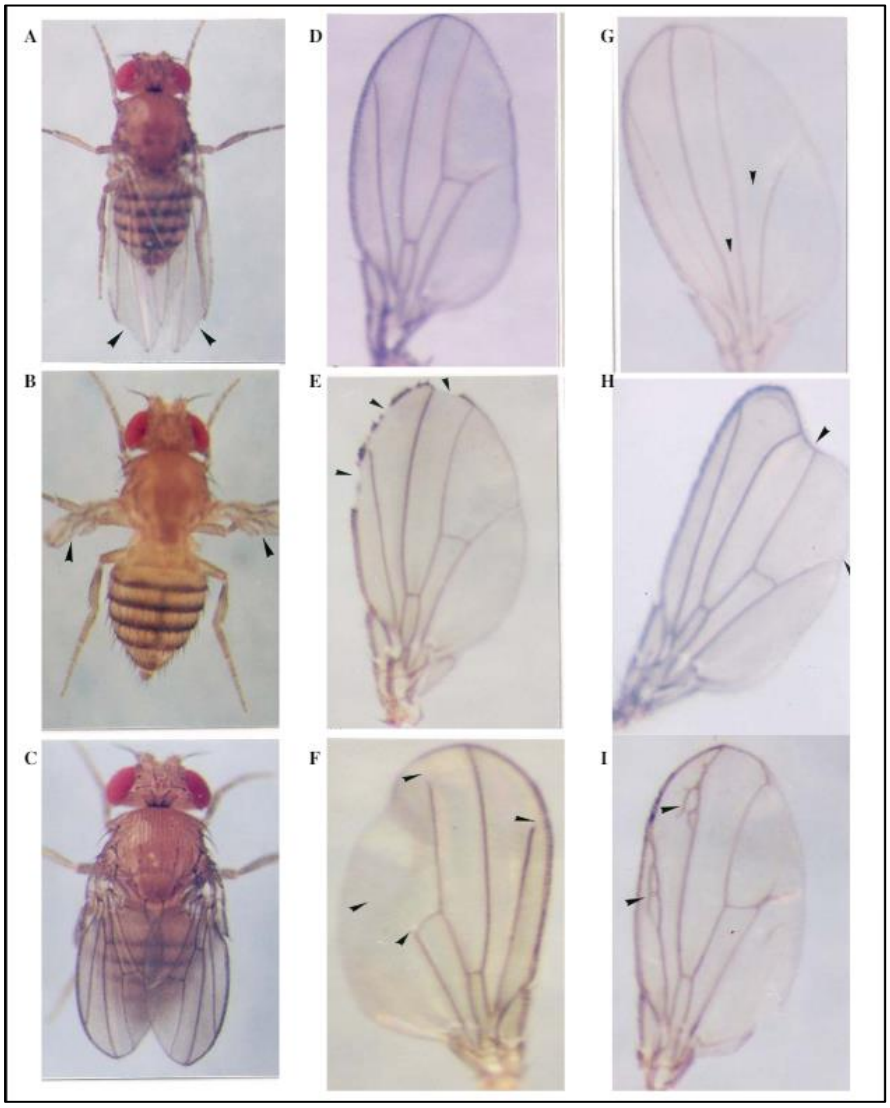



Figure 3A-I. Wing mutants: (arrowhead points the mutant region of the wing). (A) cut wing (B) vestigial (C) microptera (D) normal wing (E) scalloped (F) veinlet (G) crossveinless (H) dumpy (I) plexus.

3) Body colour mutations (Figure 4A-C): The normal body colour of *D. melanogaster* is grey (Figure 1A-B). Here again, due to mutations in the concerned genes, the colour of the body can change giving rise to mutants like yellow body colour – yellow (y) (Figure 4A); black pigment on the body – black (b) (Figure 4B); and body colour shining black – ebony (e) (Figure 4C).

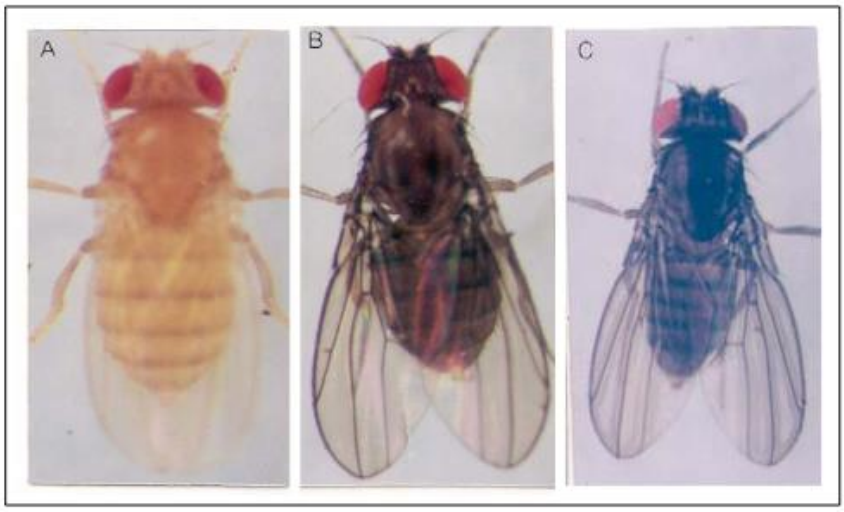



Figure 4A-C. Body colour mutants: A) yellow (B) black (C) ebony

4) Dominant Mutations (Figure 6A-B and 7A-D): Mutations thus far discussed are recessive to normal phenotypes; hence the lower-case letters are adopted to represent the symbol of the gene. However, there are mutations which are dominant over normal features. Some of them are Curly wing (Cy) (Figure 6A), short stumpy Bristles (BL) (Figure 6A), wings are held at 45° to the body – Dichaete (D) (Figure 6B), lateral margins of wings reduced giving narrowed shape – Lyra (Ly) (Figure 6B).

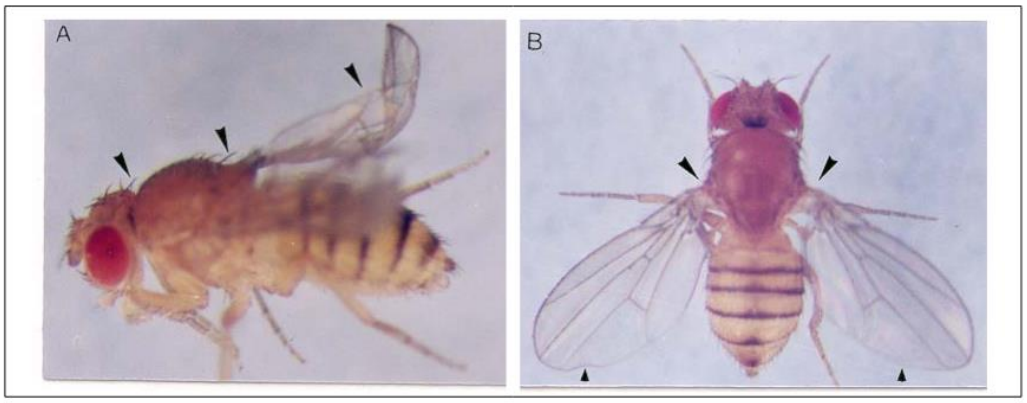



Figure 6A-B. Dominant mutants: (arrowhead indicates the mutant region). (A) Curly wing, short stumpy, Bristles (B) Wings held at 45°–Dichaete; narrow wings – Lyra.

5) An additional feature of these is that each one of them is lethal in homozygous state, meaning that flies of genotypes like Cy/Cy, Bl/Bl, D/D, and Ly/Ly, do not survive. Hence, these mutants have to be maintained in heterozygous condition, such as Cy/Cy+, Bl/Bl+, D/D+, Ly/Ly+. This does not, however, mean that all dominant mutants are lethal in homozygous condition.

Most of the mutants enjoy total **penetrance** and **expressivity**. Differences in environmental conditions or in genetic backgrounds may cause individuals, which are genetically identical at a particular locus, to exhibit different phenotypes. The ability of a given gene or gene combination to be expressed phenotypically is called penetrance. A

trait, though penetrant, may be quite variable in its expression. The degree to which a penetrant genotype is actually expressed is called expressivity.

**Probable questions:**

1. What is dosage compensation?
2. State the role of *sxl* gene in sex determination in Drosophila.
3. State the role of ***doublesex*** (***dsx***) gene in sex determination in Drosophila***.***
4. Why *dsx* gene is considered as the switch gene of sex determination.
5. What is a Mutant Phenotype?
6. What is so Special about Mutant Phenotypes of *D. melanogaster*?
7. State penetration and expressivity in mutation of *Drosophila.*
8. Describe Body colour mutations in *Drosophila melanogaster*.

**Suggested Literature:**


1. Developmental Biology: Michael J.F. Barresi Scott F. Gilbert, (12 th Ed)
2. Principles of Development: Lewis Wolpert and Cheryll Tickle (4th Ed.)
3. Principles of Genetics. 3rd Ed. D. Peter Snustad and Michael J. Simmons. Wiley Publication.
4. iGenetics: A molecular approach. 3rd Ed. Peter J. Russell. Pearson International Edition.
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8. D L Lindsley and G G Zimm, The Genome of Drosophila melanogaster, Academic Press, San Diego, USA, 1992.

# Unit VIII

## Development of *C. elegans*; Asymmetric cell divisions and cell-cell interaction. Signaling pathways in vulval induction

**Objective**: This unit on Developmental Biology focuses on the development of *Caenorhabditis elegans*, including its cell division and cellular interactions. It also covers the signaling pathways involved in vulval induction. By studying this topic, you will gain knowledge about the development of *C. elegans* and the related phenomena. You will learn about how *C. elegans* develops, how cells divide asymmetrically, the interactions that take place within the cell, and specifically how the vulva develops through the induction of the relevant pathway.

## *Caenorhabditis elegans* – a model:

As an adult, *C. elegans* consists of only about 1000 somatic cells and 1000–2000 germ cells (exactly 959 somatic cell nuclei plus about 2000 germ cells are counted in one sex; (exactly 1031 somatic cell nuclei plus about 1000 germ cells in the other) (**Fig.1**). The body plan of the worm is simple: it has a roughly bilaterally symmetrical, elongate body composed of the same basic tissues as in other animals (nerve, muscle, gut, skin), organized with mouth and brain at the anterior end and anus at the posterior. The outer body wall is composed of two layers: the protective epidermis, or "skin," and the underlying muscular layer. A tube of endodermal cells forms the intestine. A second tube, located between the intestine and the body wall, constitutes the gonad; its wall is composed of somatic cells, with germ cells inside it.

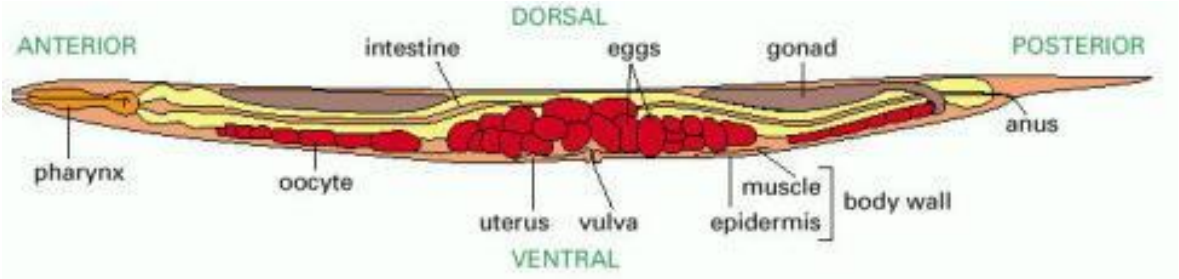



**Fig.1** A *side view of an adult hermaphrodite*

*C. elegans* has two sexes—a hermaphrodite and a male. The hermaphrodite can be viewed most simply as a female that produces a limited number of sperm: she can reproduce either by self-fertilization, using her sperm, or by cross-fertilization after the transfer of male sperm by mating. Self-fertilization allows a single heterozygous worm to produce

homozygous progeny. This is an important feature that helps to make *C. elegans* an exceptionally convenient organism for genetic studies.

## Life Cycle:

*C. elegans* begins life as a single cell, the fertilized egg, which gives rise, through repeated cell divisions, to 558 cells that form a small worm inside the eggshell. After hatching, further divisions result in the growth and sexual maturation of the worm as it passes through four successive larval stages separated by molts **(Fig.2)**. After the final molt to the adult stage, the hermaphrodite worm begins to produce its own eggs. The entire developmental sequence, from egg to egg, takes only about three days.

The embryo of the nematode *Caenorhabditis elegans* progresses through several distinctive phases in developing towards the first larval stage when the embryonic worm first emerges from the eggshell.

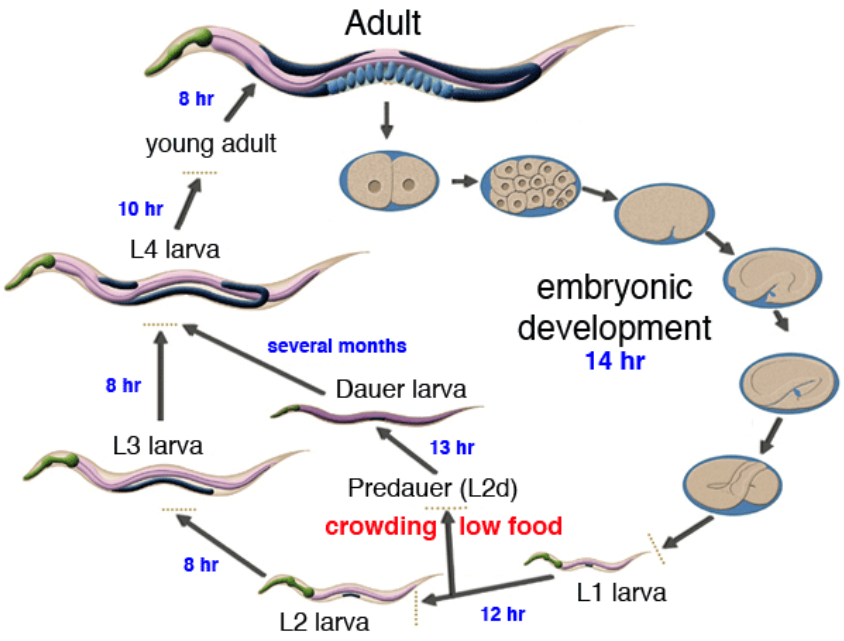



**Fig.2 Lifecycle** *and timing of developmental events of C. elegans*

## Post embryonic development

- Under environmental conditions favorable for reproduction, hatched larvae develop through four larval stages – L1, L2, L3, and L4 – in just 3 days at 20 °C before molting to an adult.
- When conditions are stressed, as in food insufficiency, excessive population density, or high temperature, *C. elegans* can enter an alternative third larval stage, L2d, called the Dauer stage (Dauer is German for permanent).

- Dauer larvae are stress-resistant; they are thin and their mouths are sealed with a characteristic dauer cuticle and cannot take in food. They can remain in this stage for a few months and exit the dauer stage and maturing to adulthood when conditions are more favourable.
- The stage ends when conditions improve to favor further growth of the larva, now moulting into the L4 stage, even though the gonad development is arrested at the L2 stage.
- Each stage transition is punctuated by a molt of the worm's transparent cuticle.

## **Asymmetric cell divisions**:

Asymmetric cell divisions play a key role in generating cell and tissue complexity during development. The mechanisms by which a single cell can divide to yield two cells with different developmental potentials have been studied in a wide range of organisms. An important feature of many asymmetric cell divisions is the spatial orientation of the daughter cells relative to each other and to the body axis.

Cleavage divisions in *C. elegans* are asymmetrical. They establish the principal embryonic axes and produce six **founder cells**. (**Fig.3A**) The first cleavage generates an anterior **AB cell** and a posterior **$P_1$** cell. Before cleavage occurs, uniformly distributed **P granules** migrate to the posterior and are incorporated into the $P_1$ cell. The P-cell lineage is like that of a stem cell. The $P_1$ cell undergoes three further asymmetrical divisions, in each case producing a further P cell and the somatic progenitor cells EMS, C, and D. At each division, the P granules segregate into the P cell. The P4 cell contains all the P granules and gives rise to all germ cells in the embryo. The EMS cell also divides asymmetrically to produce **E** and **MS** cells. The AB, C, D, E, MS, and $P_4$ cells are the **founder** cells. Each founder cell then cleaves equally and synchronously at a unique rate, continuing to do so until zygotic transcription begins (when there are about 100 cells in the embryo).

The descendants of each founder cell divide at specific times in ways that are nearly identical from individual to individual. In this way, exactly 558 cells of the newly hatched larva are generated. The descendants of the founder cells can be observed through the transparent cuticle and are named according to their positions relative to their sister cells. For instance, ABal is the "left-hand" daughter cell of the Aba cell, and ABa is the "anterior" daughter cell of the AB cell.

Gastrulation begins when there are just 28 cells in the embryo, starting with the ingression of the two daughters of the E founder cell that go on to form the gut (**Fig.3 B**) The P4 cell is next, followed by the MS-derived cells that go on to form body muscle and neurons among other cell types. Some cells from the remaining lineages (AB, C and D) also ingress during gastrulation which is completed after about 4 hours when there are 300-400 cells in the embryo.

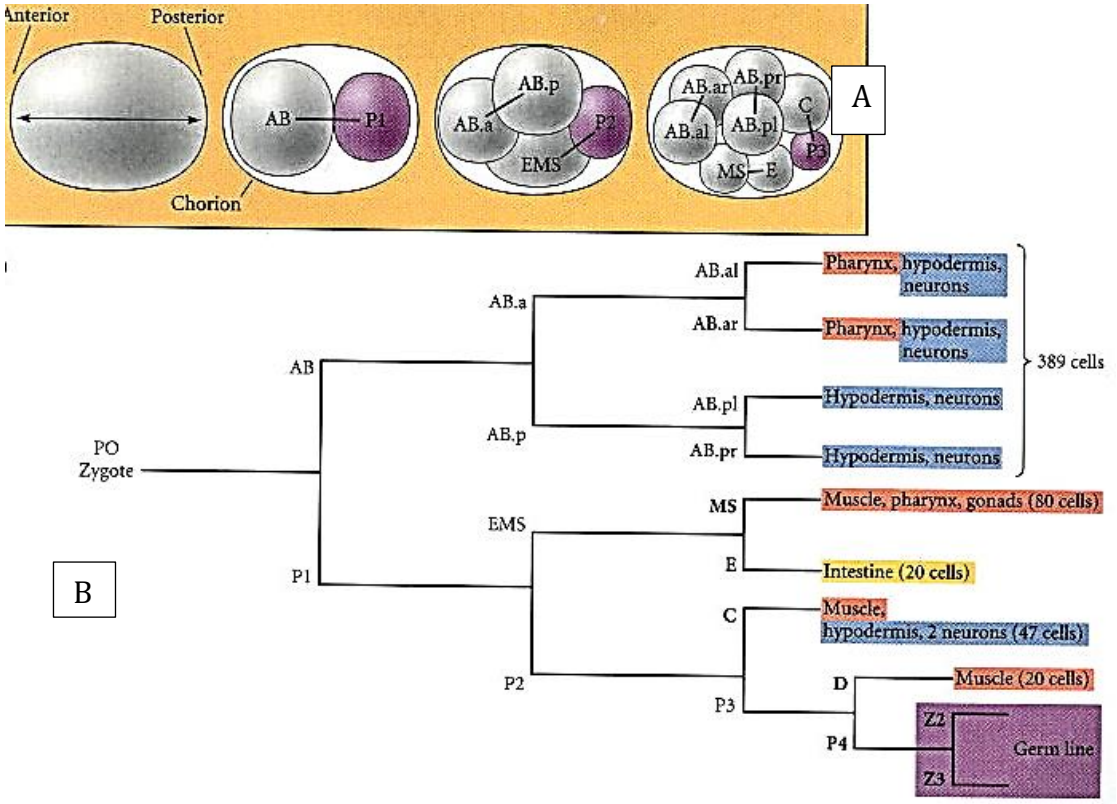



**Fig.3** *(A) Early development, as the egg is fertilized and moves toward the vulva. The p-lineage are stem cells that will eventually form the germ cells. (B) Abbreviated cell lineage chart. The germ line segregates into the posterior portion of the most posterior (P) cell. The first three cell divisions produce the AB, C, MS, and E lineages. The number of derived cells (in parentheses) refers to the 558 cells present in the newly hatched larva. Some of these continue to divide to produce the 959 somatic cells of the adult.*

## Cell-cell interactions

In the *C. elegans* four-cell embryo, the EMS blastomere receives a polarizing signal from its neighbor, the germline precursor P2. This signal, encoded by the mom-2/Wnt gene, acts in a position-dependent manner to induce and orient the asymmetric division of the EMS cell. As a consequence, the anterior daughter of EMS adopts a mesodermal fate and the posterior daughter adopts an endodermal fate. The MOM2/Wnt signal polarizes the EMS cell division by regulating known Wnt pathway components including MOM-5 (Frizzled), APR-1 (adenomatous polyposis coli-related) and WRM-1 (β-catenin/Armadillo).

The *C. elegans* embryo uses both autonomous and conditional modes of specification. Conditional specification can be seen in the development of the endoderm cell lineage. At the 4-cell stage, the EMS cell requires a signal from its neighbor (and sister), the P2

blastomere. Usually, the EMS cell divides into an MS cell (which produces mesodermal muscles) and an E cell (which produces the intestinal endoderm). If the P2 cell is removed at the early 4-cell stage, the EMS cell will divide into two MS cells, and endoderm will not be produced. If the EMS cell is recombined with the P2 blastomere, however, it will form an endoderm; it will not do so, however, when combined with ABa, ABp, or both AB derivatives.

The P2 cell produces a signal that interacts with the EMS cell and instructs the EMS daughter that is next to it to become the E cell. This message is transmitted through the Wnt signaling cascade (**Fig.4**) The P2 cell produces the *C. elegans* homologue of a Wnt protein, the MOM-2 peptide. The MOM-2 peptide is received in the EMS cell by the MOM-5 protein, the *C. elegans* version of the Wnt receptor protein, Frizzled. The result of this signaling cascade is to down-regulate the expression of the *pop-1* gene in the EMS daughter destined to become the E cell. In *pop-1*-deficient embryos, both EMS daughter cells become E cells.

The P2 cell is also critical in giving the signal that distinguishes ABp from its sister, ABa (**Fig.4**). ABa gives rise to neurons, hypodermis, and the anterior pharynx cells, while ABp makes only neurons and hypodermal cells. However, if one experimentally reverses their positions, their fates are similarly reversed, and a normal embryo is formed. In other words, ABa and ABp are equivalent cells whose fate is determined by their positions within the embryo. Transplantation and genetic studies have shown that ABp becomes different from ABa through its interaction with the P2 cell. In an unperturbed embryo, both ABa and ABp contact the EMS blastomere, but only ABp contacts the P2 cell. If the P2 cell is killed at the early 4-cell stage, the ABp cell does not generate its normal complement of cells. Contact between ABp and P2 is essential for the specification of ABp cell fates, and the ABa cell can be made into an ABp-type cell if it is forced into contact with P2.

Moreover, these studies show that this interaction is mediated by the GLP-1 protein on the ABp cell and the APX-1 (anterior pharynx excess) protein on the P2 blastomere. In embryos whose mothers have mutant *glp-1,* ABp is transformed into an ABa cell. The GLP-1 protein is a member of a widely conserved family called the Notch proteins, which serve as cell membrane receptors in many cell-cell interactions, and it is seen in both the ABa and ABp cells - one of the most important ligands for Notch proteins such as GLP-1 is another cell surface protein called Delta. In *C. elegans,* the Delta-like protein is APX-1, and it is found on the P2 cell. This APX-1 signal breaks the symmetry between ABa and ABp, since it stimulates the GLP-1 protein solely on the AB descendant that it touches, namely, the ABp blastomere. In doing this, the P2 cell initiates the dorsal-ventral axis of *C. elegans,* and it confers on the ABp blastomere a fate different from that of its sister cell.

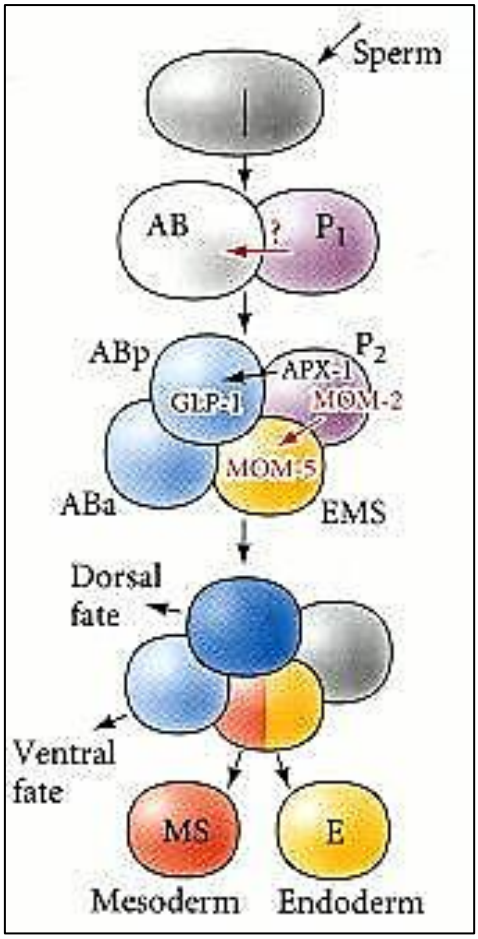



**Fig.4** *Cell-cell signaling in the 4-cell embryo of C. elegans. The P2 cell produces two signals: (1) the juxtacrine protein APX-1 (Delta), which is bound by GLP-1 (Notch) on the ABp cell, and (2) the paracrine protein MOM-2 (Wnt), which is bound by the MOM-5 (Frizzled) protein on the EMS cell.*

## Vulval induction Signaling pathways

Vulval development in the nematode *Caenorhabditis elegans* is a beautifully simple system for asking how cells choose between different possible fates. Vulval fate patterning involves just seven cells: a signaling cell (the anchor cell), which initiates vulval fate induction, and six vulval precursor cells which can respond to that inductive signal by generating vulval descendants (**Fig.5**). Each vulval precursor cell is capable of adopting any of three different fates: 1°, central vulva; 2°, lateral vulva; or 3°, non-vulval. In wild-type animals, an invariant pattern of fates is seen: 3° 3° 2° 1° 2° 3°. The vulval precursor cell closest to the anchor cell, termed P6.p from its cell lineage position, adopts the 1° fate, the flanking vulval precursor cells, P5.p and P7.p, adopt the 2° fate, and vulval precursor cells farther away from the anchor cell adopt the 3° fate (**Fig.5**).

The precise pattern of vulval precursor cell fates is controlled by a combination of Ras-mediated 'inductive' signaling and Notch-mediated 'lateral' signaling, so vulval

development is also a good system for studying the molecular interactions between these two signaling pathways. The inductive signal from the anchor cell is an EGF-like growth factor, LIN-3, which stimulates a canonical receptor tyrosine kinase–Ras–MAP kinase cascade in nearby vulval precursor cells and promotes both the 1° and 2° vulval fates (**Fig.5**).

A lateral signal between neighboring vulval precursor cells stimulates the LIN12/Notch pathway which promotes the 2° fate in the signal-receiving cell. The recent molecular identification of the lateral signal and of various gene targets of lateral signaling have clarified the role of lateral signaling and its relationship to the inductive Ras pathway. There has been a long-running debate about the relationship between inductive and lateral signaling, part of the larger debate about the roles of long-range and short-range signals in cell fate patterning. The original 'graded signal' model proposed that the inductive signal is graded and acts like a morphogen: high levels induce the 1° fate in P6.p, and lower levels induce the 2° fate in P5.p and P7.p.

But this model cannot be true in its simplest form, because mosaic analysis showed that LET-23, the receptor for the inductive signal, is required only in P6.p for normal vulval patterning. These data led to the 'sequential signal' model, where the inductive signal induces the 1° fate in P6.p, which subsequently produces a lateral signal to induce the 2° fate in P5.p and P7.p. It is also possible that the combination of the lateral signal and a graded inductive signal cooperate to ensure 'a perfect vulva every time'.

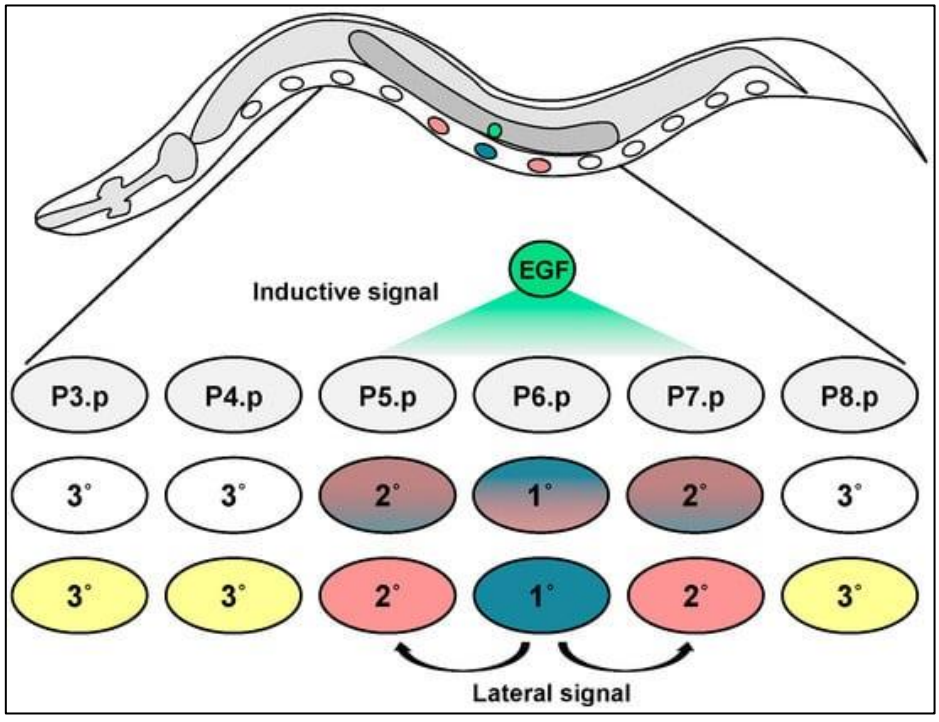



**Fig.5** *Overview of the C. elegans VPC fate patterning. The six naïve VPCs are numbered P3.p through P8.p. P6.p, closest to the Anchor Cell (AC), receives the highest level of EGF inductive*

*signal and assumes 1° fate. P5.p and P7.p receive lower levels of inductive signal and lateral Notch signal from the P6.p to assume a 2° fate. P3.p, P4.p, and P8.p receive insufficient inductive and lateral signals and adopt nonvulval fates.*

## The morphogen gradient model becomes the graded signal plus the lateral signal model

Combining cell lineage analysis with ablation of selected cells with a laser microbeam revealed the presence of cell-cell signaling events between the AC and VPCs and among VPCs . From an elegant combination of these approaches arose the Morphogen Gradient Model (**Fig.6 (**A) (C)). The AC induces equipotent VPCs to assume their fate. P6.p, the VPC closest to the AC, typically becomes 1°. Isolated VPCs (generated by ablation of other VPCs with a laser microbeam) assume 1° or 2° fate based on distance from the source of signal; VPCs close to the AC become 1°, while those distal from the AC become 2°. This observation led to the model that it is the dose of a "morphogen" signal that dictates VPC fate.

**Schematic summary of vulva induction in C. elegans** (**Fig.7**)

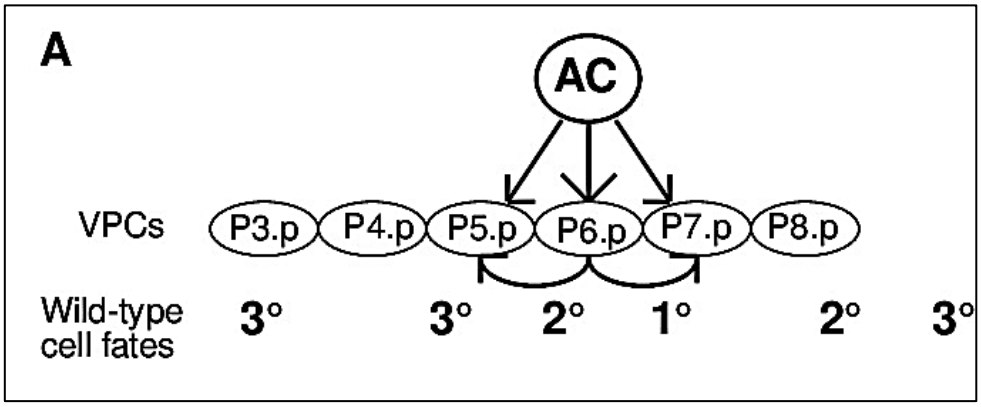
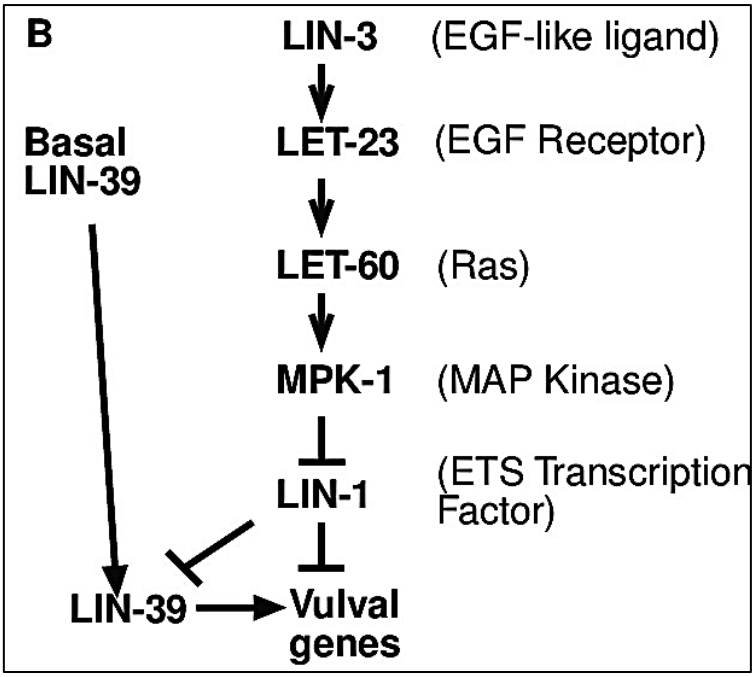



**Fig.7** (A) *Cell signaling events controlling VPCs fate decisions. The graded AC signal (arrows between AC and VPCs), along with lateral signaling among the VPCs (arrows pointing from P6.p to P(5,7).p) ensure the invariant pattern 3°-3°-2°-1°-2°-3°. Cells with a 1° and 2° fate generate 8 and 7 cells respectively, all of which participate in the formation of the vulva. 3° cells remain epidermal.*




(B) *A model for vulva induction*. Vulval induction is mediated by a conserved EGF/Ras/MAPK signaling pathway. lin-3 encodes the EGF-like ligand which is expressed in the AC and signals the underlying VPCs ; let-23 encodes an EGF-receptor tyrosine kinase ; let-60 encodes a Ras molecule acting as a molecular switch, mpk-1/sur-1 encodes a MAP kinase and lin-1 encodes an ETS-domain transcription factor which functions to inhibit vulva formation . In P(5-7).p, AC signaling inactivates lin-1 which then leads to vulva formation. lin-1 represses a number of 'vulval genes' including lin39 . A basal level of lin-39 expression is required for the increase of lin-39 expression in response to Ras signaling .

**Probable questions;**

1. What is a Dauer larva?
2. Discuss asymmetric cell division that occurs in *C. elegans.*
3. What is p granules?
4. How the vulva develops in *C. elegans.*
5. Discuss different signaling involves in vulva induction in C*. elegans.*

**Suggested Literature:**

1. Developmental Biology: Michael J.F. Barresi Scott F. Gilbert, (12 th Ed)
2. Principles of Development: Lewis Wolpert and Cheryll Tickle (4th Ed.)
3. Principles of Genetics. 3rd Ed. D. Peter Snustad and Michael J. Simmons. Wiley Publication.
4. iGenetics: A molecular approach. 3rd Ed. Peter J. Russell. Pearson International Edition.

**Unit IX**

**Epigenetic regulation of the genetic material in *C. elegans***

**Objective**: This unit of Developmental Biology focuses on the epigenetic regulation of genetic material in *C. elegans*. By the end of this topic, you will gain knowledge about the epigenetic regulation in this animal and a better understanding of its genetic material and its regulatory mechanisms.

## Epigenetics:

Epigenetics is defined as the study of heritable changes in gene expression that are not accompanied by changes in the DNA sequence. Epigenetic mechanisms include histone post-translational modifications, histone variant incorporation, non-coding RNAs, and nucleosome remodeling and exchange. In addition, the functional compartmentalization of the nucleus also contributes to epigenetic regulation of gene expression.

The basic subunit of chromatin is the nucleosome, composed of the highly conserved core histones H2A, H2B, H3, and H4. Epigenetic mechanisms can modify this structure in several ways. Depending on the chromosomal context, core histones can be replaced by histone variants, including H3.3, H2A.Z, or H2A.X, each of which is associated with specialized functions. The flexible N- and C-terminal tails of core histones are also subjected to a variety of post-translational modifications, including acetylation, methylation, phosphorylation, ubiquitination, sumoylation, ADPribosylation, and biotinylation. These modifications, alone or in combination, correlate with transcriptional repression or activation. In addition, chromatin-associated factors such as Trithorax (Trx-G) and Polycomb group (PcG) proteins function to maintain cellular memory of transcriptional states of developmentally important genes through many rounds of cell division. Transcriptional regulation by PcG and Trx-G group proteins is achieved through both histone methyltransferase activities of individual components, as well as specific binding to and interpretation of these histone marks. PcG and Trx-G group proteins are also able to recruit additional proteins that greatly expand their repertoire of covalent histone modifications.

Histone post-translational modifications are carried out by a variety of complexes containing histone-modifying enzymes. Histone methyltransferases (HMTs), histone demethylases (HDMs), histone acetyltransferases (HATs), histone deacetylases (HDACs), and histone kinases not only modify histone tails, but also act as recruiters of protein complexes and nucleosome remodelers. Histone tail modifications are in turn associated with specific chromatin-binding proteins or protein complexes (readers) that modify chromatin structure. Small non-coding RNAs, nucleosome exchange and remodeling by ATP-dependent complexes, and histone variant incorporation, are additional

mechanisms that can alter the degree of chromatin compaction. In addition, nuclear organization has recently emerged as another important player in epigenetic regulation.

## *C. elegans* enzymes modifying core histones and their biological function

During *C. elegans* development, important roles for chromatin modifiers have been identified in vulval cell fate specification, lifespan determination, genome stability, and embryonic, germline and foregut development. The *C. elegans* genome contains 38 genes predicted to encode histone lysine methyltransferase (HMT) activity, based on the presence of a conserved SET domain. The first of these genes to be identified through a genetic screen were mes-2 and mes-4. MES-2 is the ortholog of the Drosophila Polycomb group protein Enhancer of zeste E(z), while MES-4 is a nuclear SET domain protein related to mouse NSD1 (nuclear receptor binding SET-Domain 1. MES-2 physically interacts with both MES-6, homologue of the Pc group protein Extra sex combs (Esc), and MES-3 (a novel protein), in a repressive complex with H3K27 HMT activity similar to mammalian and fly E(z)/ESC complexes. MES deficiency results in maternal-effect sterility, derepression of high copy transgenes in the germline, and specific loss of the H3K27me3 heterochromatin mark from the X chromosome. In addition, the absence of MES activity suppresses the synthetic multivalve (synMuv) phenotype associated with the inactivation of a large group of histones modifying enzymes which act redundantly in vulval cell fate specification.

The *C. elegans* vulva represents a simple system in which to study how chromatin factors influence specific developmental pathways. synMuv genes fall into three classes encoding chromatin-associated factors that act redundantly for vulval cell fate specification. Simultaneous inactivation of synMuv genes from any two different classes leads to ectopic EGF/RTK/Ras signaling and the formation of ectopic vulvae (the Multivulva phenotype). synMuv genes include the homologue of mammalian class I histone deacetylases (HDA-1), histone methyltransferases (MET-2; MET-1), and homologues of the evolutionarily conserved NuRD/Mi-2 nucleosome remodelling and histone deacetylase complex (LET-418/Mi-2, LIN-53/RbAp48, MEP-1, and HDA1/HDAC-1. HDA-1 is sumoylated in *C. elegans*, and both SUMO and the E2SUMO ligase UBC9 are also members of the synMuv group, suggesting that SUMOylation may regulate the activity of the NuRD/Mi-2 complex in vulval cell fate specification.

### Maintenance of germline continuity

MES-4 has been shown to transmit memory of gene expression from one generation of germ cells to the next by maintaining H3K36 methylation of germline expressed loci in embryos. Genome-wide analysis showed that in early embryos, MES-4 binds to the all five autosomes, where it is abundantly present on the most highly transcribed genes, but is under-represented on the X chromosome.

Chromatin regulators are also involved in the maintenance of somatic cell fate. synMuv genes antagonize germline fate in somatic cells, and in their absence germline-specific genes are ectopically expressed in the soma. Interestingly, MES-4 is required for somatic cells to acquire germ cell fate in the absence of synMuv activity (**Fig. 1**). Somewhat surprisingly, recent results show that transformation of germ cells into somatic cells only requires CHE-1, a transcription factor that specifies the fate of somatic cells, and loss of the chromatin regulatory factor LIN-53.

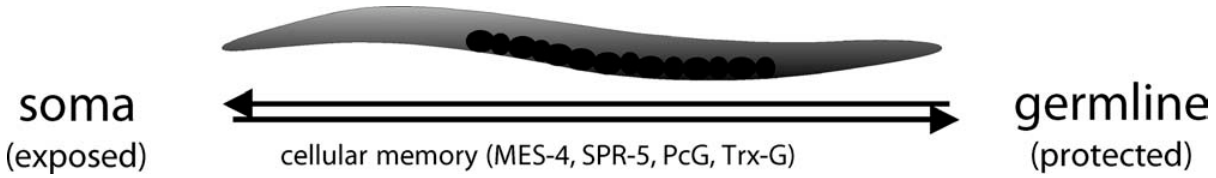



**Fig.1** *Cellular identity in C. elegans is the sum of the interplay between Polycomb (PcG) and Trithorax (Trx-G) group proteins, as well as histone modifying enzymes (MES-4, SPR-5). The soma (grey) influences germline cell fate decisions and conversely, the germline (black) can control soma-specific gene expression.*

Information obtained from genome-wide histone modification profiling, combined with the power of genetic approaches in *C. elegans*, will undoubtedly provide novel insight into the underlying molecular mechanisms and the functional importance of epigenetic regulation. Given the high degree of conservation between chromatin modifiers across species, the information derived will likely contribute to a better understanding of epigenetic regulation in normal development and longevity.

**Probable questions**

1. What is epigenetics?
2. Discuss different genes found in *C. elegans* and state their regulation.

**Suggested Literature:**

1. Developmental Biology: Michael J.F. Barresi Scott F. Gilbert, (12 th Ed)
2. Principles of Development: Lewis Wolpert and Cheryll Tickle (4th Ed.)
3. Principles of Genetics. 3rd Ed. D. Peter Snustad and Michael J. Simmons. Wiley Publication.
4. iGenetics: A molecular approach. 3rd Ed. Peter J. Russell. Pearson International Edition.

# Unit X

# Growth: Definition, pattern, factors influencing growth and development

**Objective**: This unit covers the topic of growth, including its pattern and the various factors that influence it. By reading through this material, you will gain a better understanding of how cells and tissues grow and develop, as well as the different stages involved in the process. You will also become familiar with the typical patterns of growth curves and learn about the factors that can impact growth and development. By the end of this topic, you should feel equipped to grasp the concepts of growth and development and appreciate the intricacies of this important biological process.

## Overall Growth

Growth generally means an increase in size. This phenomenon is particularly observed during embryonic development. The process may be studied from two points of view -

i) to study the increase that takes place in the whole embryo or parts of it

ii) to study the defined process of growth to set up a general pattern and rule from which the different facts as they appear during the development of particular animals.

This is of course very complex considering the whole situation that occurs during the growth of an organism.

## Definition:

Grey (1931) speaks of growth as "essentially concerned with the formation of new living material". Madewar (1941) states that what results from biological growth is itself typically capable of growing.

According to Watson and Lowery, "Growth means an increase in the physical size of the whole or any of its parts." It can be measured in terms of centimeters and kilograms or metabolic balance i. e. retention of hydrogen and calcium in the body. Juan Comas defines it "as the objective manifestation of hypertrophy and hyperplasia of the organism constituent tissues and is determined by post-natal body size." This increase in body size is limited by predetermined constitutional and hereditary factors. It is however influenced by exogenous factors like diet, climate, race, environment, etc.

Weiss (1949) defined growth as "the increase in that part of the molecular population of an organic system which is synthesized within that system" and it means the multiplication of that part of the molecular population which is capable of further continued reproduction.

Thus to formulate a precise concept of growth it should be confined to the increase in the amount of the system that is capable of growing. The main problem that requires a study is

a) At what rate does this increase take place and

b) How does this rate change as time passes?

When the rates of multiplication/unit mass remain constant it may be formulated as $1/w \times dw/dt = k$---------- (1)

or $dw/dt = kw$ ---------- (1a)

where 'w' is the weight or mass of the system. This grows faster and faster at an exponential rate. i.e. always increasing and the final equation may be represented as

$w = w_0.e^{kt}$ (Where w = final weight, $w_0$ = initial weight, t = time, k = constant)

or, $\log w = \log w_0 e^{kt}$

or, $\log w = \log w_0 + \log e^{kt}$

or, $\log w = \log w_0 + kt$ ---------- (2) [log e = 1]

or $dw/dt = d(w_0 e^{kt})/dt$

or, $dw/dt = w_0 k e^{kt}$ ---------- (3)

Now from (1)

$1/w \times dw/dt = 1/ w_0 e^{kt} . dw/dt$ ----- (4) [$\because w = w_0 e^k$]

Putting the differentiating coefficient of dw/dt from (3) we get

$1/w.dw/dt = 1/ w_0 e^{kt} . w_0 k e^{kt} = K$ which is the same as (1)

This behavior is very common in biological systems.

## Patterns of growth

A growth pattern refers to the population curve of a species. Depending on the limiting factors of the environment, the curve follows a specific shape such as a J or an S curve.

Ecologists who study populations use several mathematical methods to understand the growth patterns of any given population of organisms. The increase in the population of any group of organisms depends on several factors that decide the population growth curve. Based on the parameters of the equations, accurate predictions can be made about how quickly the numbers can increase and where it tapers off.

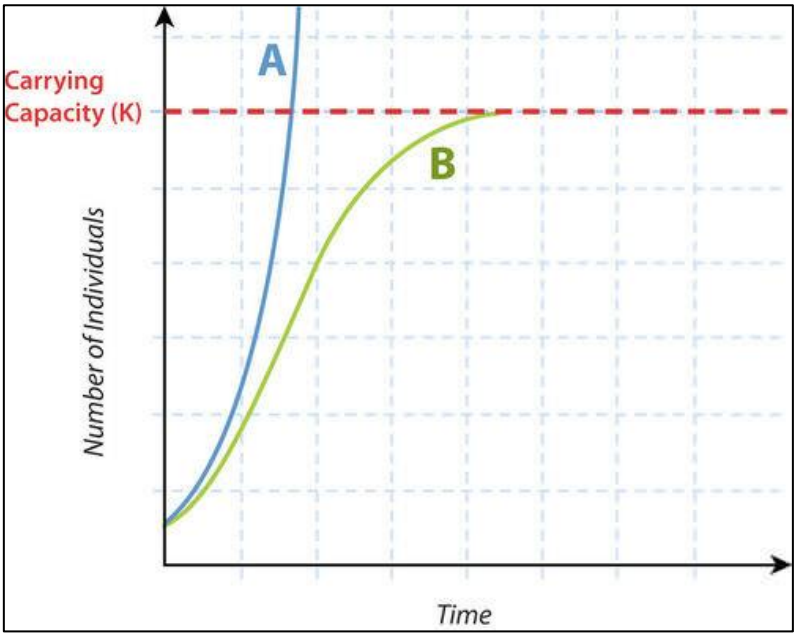
## What are the different population growth curves?

There are two types of growth curves: the j-shaped growth curve and the s-shaped growth curve. Both types of growth curves fit population growth models that have different environmental pressures.

## Exponential growth

One of the easily observable examples of exponential growth occurs in bacteria that divide rapidly within an hour. If there are 1000 bacteria on a plate, in the next hour, there will be 2000. In the 3rd hour, there will be 4000 bacteria, and by the 4th hour, there will be 8000. The characteristics of exponential growth are:

- They occur in ideal environments where the resources are relatively unlimited.
- There is no competition or limit to the exponential growth
- The population starts small and grows rapidly as time progresses, giving a J-type exponential growth curve.
- Exponential growth usually occurs in regions that have newly been colonized.




### Logistic growth

Logistic growth is seen in most populations living in realistic conditions with limited space and resources. Since neither space nor resources are infinite, the growth rate starts

to taper as the population density reaches a stage where it runs out of food or is poisoned by its waste.

The characteristics include the logistic growth curve:

- Start rapidly as a J curve and flatten as it curves hits the environment's carrying capacity.
- Carrying capacity refers to the maximum population of a species the environment can support.
- As the population reaches the carrying capacity (denoted by k), the curve begins to take an "S" shape.
- Logistical growth is seen in all stable populations living in a finite geographic area.

## The process of selecting growth factors that affect population growth.

In living in stable environments, the population growth is controlled by the carrying capacity factor (k). As shown in the figure for logistic growth, these organisms have populations that are k-selected. Their numbers increase until the point where the population tapers off as the limits of space and resources are reached.

In populations that live in unstable environments where the whole population can be disturbed or destroyed in a short time, the growth pattern is said to be r-selected. The variable r is the growth rate defined earlier as the difference between the birth and death rates. The (r) usually corresponds to the first part of the exponential growth curve before the growth is interrupted/reset by unstable environmental factors.

## Factors affecting growth and development

The integrated nature of growth and maturation is largely maintained by a constant interaction of genes, hormones, nutrients and other factors. These factors also influence physical performance. Some are hereditary in origin. Others, such as season, dietary restriction, severe psychological stress, originate in the environment and simply affect the rate of growth at the time they are acting. Others again, such as socio-economic class, reflect a complicated mixture of hereditary and environmental influences and probably act throughout the whole period of growth.

## Genetic control

The height, weight, or body build of a child or an adult always represents the result of both the genetical and environmental forces, together with their interaction. It is a long way from the possession of certain genes to the acquisition of a height of 2m. The gene depends for its expression firstly on the internal environment created by all the other genes, and secondly on the external environment. The control of body size is certainly a complicated affair involving many genes, yet a disturbance in a single gene or group of

genes may produce a widespread and drastic effect, as in the condition of achondroplasia, which is inherited as a simple dominant. On the other hand, the effects may be quite restricted and specific.

## Environmental:

Environmental factors play a crucial role in growth and development. Exposure to toxins, pollution, and infectious diseases can negatively affect growth and development. For example, exposure to lead, mercury, and other toxins can impair cognitive development and lead to learning disabilities. Similarly, infectious diseases such as measles, mumps, and rubella can lead to developmental delays and other health complications. Access to clean water, sanitation, and healthcare are also essential environmental factors that influence growth and development. Poor access to these resources can increase the risk of illness and malnutrition, which can impair growth and development. Inadequate nutrition can lead to stunted growth, weakened immune systems, and cognitive impairments.

## Endocrine regulation

It refers to the process by which hormones are released into the bloodstream by various glands in the body, to control and coordinate the activity of different organs and tissues**.** Hormones are regarded as growth-promoting substances. Probably all the endocrine glands influence growth. Most of the hormones are secreted by the endocrine glands and play a significant role in regulating the pattern of growth and development as per instructions of the genes. The most important hormone controlling growth from birth up to adolescence is growth hormone or somatotrophin. This is a polypeptide secreted by the pituitary. It helps the growth of bones and thereby increases the height of persons. Growth hormone controls the rate at which growth takes place up to the time of steroid-induced adolescent spurt. Its administration causes the amino acids to be incorporated into tissues to form new proteins. It also causes an overall growth rate of most tissues including the brain.

Thyroid hormone plays a vital role throughout the whole of growth. The activity of the thyroid, judged by the basal metabolic rate, decreases gradually from birth to adolescence. In hypothyroidism growth is delayed, skeletal maturity, dental maturity and growth of the brain are all affected.

## Nutritional

Growth is closely related to nutrition. A sufficiency of food is essential for normal growth. An adequate supply of calories is naturally essential for the normal growth of humans and the need varies with the phase of development.

Nine different amino acids have been claimed to be essential for growth and the absence of any one will result in disordered or stunted growth. Other factors are also essential for growth. For example, zinc plays a part in protein synthesis and is a constituent of certain enzymes; a deficiency of zinc causes stunting, interference with sexual development and falling out of hair.

Iodine is needed for the manufacture of the thyroid hormones. Bone will not grow properly without an adequate supply of calcium, phosphorus and other inorganic constituents such as magnesium and manganese. Iron is required for the production of haemoglobin.

Vitamins play an important part in growth. Vitamin A is thought to control the activities of osteoblasts. In vitamin C deficiency the intercellular substance of bone is inadequately formed. Vitamin D deficiency is the cause of rickets.

Malnutrition during childhood delays growth, and malnutrition in the years proceeding adolescence delays the appearance of the adolescent spurt. Growth studies have demonstrated that malnutrition may cause serious impairment of growth. The term malnutrition generally refers to the effects of an inadequate intake of calories or other major dietary components such as proteins. Malnutrition may also result from diseases that decrease the appetite or interfere with digestion and assimilation. A majority of malnourished children fail to achieve their full genetic potential of body growth (both linear and ponderal) and are thus stunted or wasted or both.

## Social and Emotional Factors:

Social and emotional factors also play an essential role in growth and development. Parental care and support, exposure to stress, and access to education and opportunities can influence cognitive and emotional development. Positive social interactions and nurturing environments can promote healthy social and emotional development. In contrast, exposure to neglect, abuse, and other adverse experiences can lead to mental health issues, behavior problems, and developmental delays.

## Cultural Factors:

Cultural beliefs, values, and practices can also influence growth and development. For example, cultural norms regarding diet, physical activity, and gender roles can affect health outcomes. In some cultures, certain foods are believed to promote growth and development, while in others, physical activity is emphasized as a means of promoting healthy growth. Additionally, cultural beliefs about gender roles and expectations can impact cognitive and emotional development.

The physical growth of human beings is affected by cultural factors. Culture differs from ethnic group to ethnic group. The body growth differences correlate with varied cultural

groups. The physical growth of the body follows some adaptations in different geographical areas of distribution of the groups

**In conclusion o**verall, growth and development are influenced by a complex interplay of factors, and a comprehensive understanding of these factors is essential for promoting healthy development in individuals of all ages. Understanding the various factors that influence growth and development can help identify potential risk factors and promote interventions that support healthy development. By addressing environmental, social, and cultural factors that influence growth and development, we can promote healthy development and improve overall health outcomes.

**Probable questions**

1. How growth can be defined biologically?
2. Differentiate logistic with exponential growth.
3. What are the factors that affect growth?
4. How does malnutrition affect growth and development?
5. Discuss in brief the endocrine regulation of growth.

**Suggested Literature:**


1. Developmental Biology: Michael J.F. Barresi Scott F. Gilbert, (12 th Ed)
2. Principles of Development: Lewis Wolpert and Cheryll Tickle (4th Ed.)
3. Principles of Genetics. 3rd Ed. D. Peter Snustad and Michael J. Simmons. Wiley Publication.
4. iGenetics: A molecular approach. 3rd Ed. Peter J. Russell. Pearson International Edition.

# Unit XI

# Post embryonic development- larval forms in *Xenopus*, environmental regulation of normal development

**Objective**: This unit focuses on post-embryonic development in *Xenopus*. It covers the role of the environment in regulating normal development and explains the various larval forms of *Xenopus*. Readers will gain a clear understanding of how environmental factors influence the animal's development by the end of the topic.

## Larva:

**Larva**, is a stage in the development of many animals, occurring after birth or hatching and before the adult form is reached. These immature, active forms are structurally different from the adults and are adapted to a different environment.

In some species the larva is free-living and the adult is an attached or nonmobile form; in others the larva is aquatic and the adult lives on land. In forms with nonmobile adults, the mobile larva increases the geographic distribution of the species. Such larvae have well-developed locomotor structures. A larva sometimes functions as a food gatherer—in many species, the larval stage occurs at a time when food is abundant—and has a well-developed alimentary system. It stores food so that the transformation to the adult stage can occur. Some larvae function in both dispersion and nutrition.

The amount of time in the life cycle spent in the larval stage varies among species. Some have long larval periods, either hatching early, metamorphosing into adults late, or both. Some organisms have a short-lived larval phase or no larvae at all.

## Larval forms:

Nieuwkoop and Faber developed a new method for staging the development of *Xenopus* embryos. Instead of relying on the number of hours or length of the larvae, they based their system on external and internal features at a stable temperature. This system is known as the NF staging system and can be applied to various *Xenopus* species.

Although the length of tadpoles at every NF stage may vary among different species of *Xenopus,* there are internal and external developmental milestones that can be used to stage embryos in most species. These milestones include early cell divisions (NF stage 2-6), the start of gastrulation (NF stage 10), the beating of the heart (NF stage 33 and 34), gut coiling (NF stage 41-46), and limb development (NF stage 48-58).

## *Xenopus* Larva (NF Stage 22-26)

During the developmental stages of Xenopus, its larval forms are identified as NF stages 22-26. Early tailbud embryos at NF stages 22-26 (**Fig.1.A-E**) can be identified through certain anatomical landmarks such as the degree of dorsal curvature in the embryo, the number of pharyngeal bulges, the three-dimensionality and pigmentation of the developing eye, and the number and extent of anteriorly segregated, chevron-like somites.

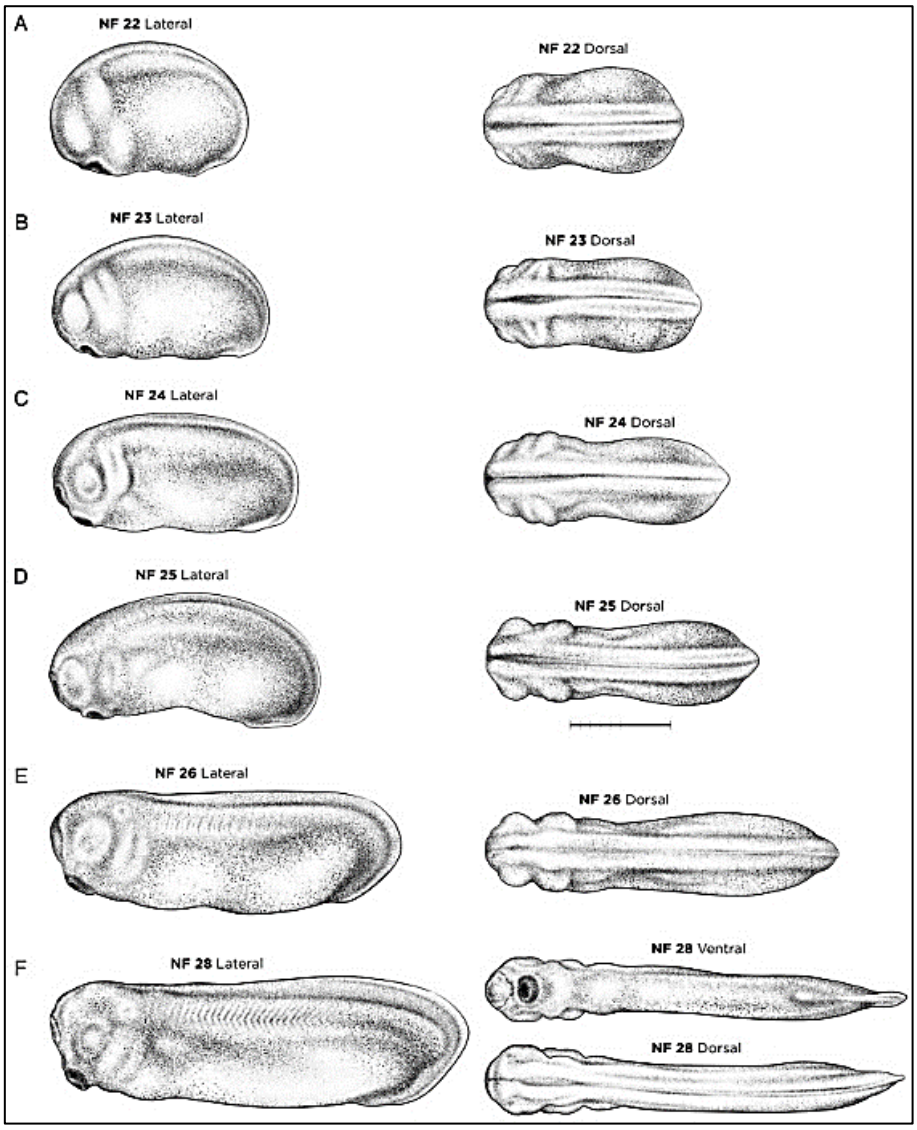



**Fig.1** *Early tailbud-stage X. laevis embryos. (A) NF stage 22. (B) NF stage 23. (C) NF stage 24. (D) NF stage 25. (E) NF stage 26. (F) NF stage 28. Orientation for dorsal and ventral views is anterior left; lateral views have dorsal up and anterior left.*

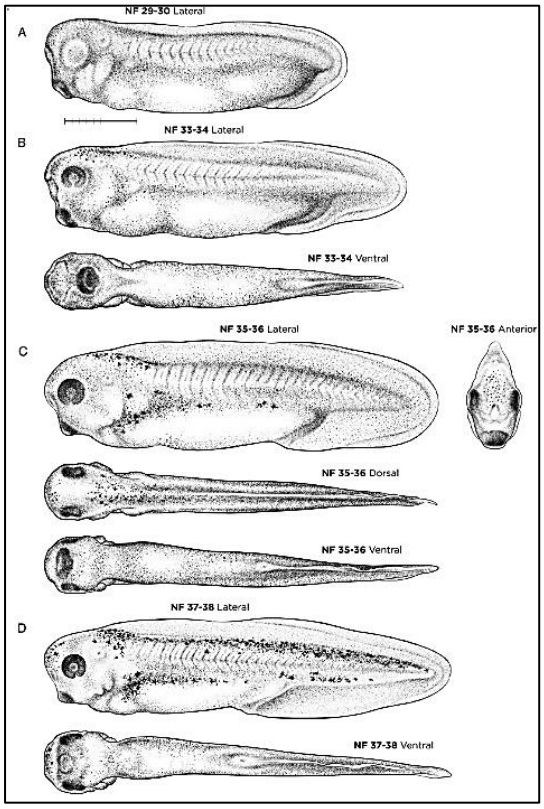
This period is marked by somites, heart, and pronephric kidney formation, and the embryo starts to lengthen and become slenderer. Although somite segmentation may not be visible under a light microscope until approximately NF stages 27-28, molecular

markers such as myod1 can be used to determine the boundary between formed somites and presomitic mesoderm from NF stages 22 onwards.

During the embryonic development of *Xenopus*, stage 23 (**Fig. 1B**) is referred to as a 'coffee bean' embryo. At this stage, it develops an inverted Y-shape and a hatching gland on the anterior 'forehead'. This gland expresses cxcl14, astl3a.1, and pax3. Key neurobehavioral milestones for tailbud stages include motor reactions to external stimuli at stage 24 and spontaneous movements at stage 26 (**Fig.1E**). At stage 28, (**Fig. 1F**) embryos that have been liberated from their vitelline membrane as a result of the ciliary beating across the epidermis.

At NF stage 28 (as shown in **Fig. 1F**), there are both external and internal landmarks that can be used to identify the developmental stage of the embryo. External landmarks include a fully pigmented cement gland, a fin with an outer transparent and an inner translucent band that extends to the cloaca, and a unique 'nose shape' to the extending tailbud. Internal landmarks include four distinct streams of dlx2-positive migrating cranial neural crest cells, segregation of the epibranchial placodes (which express neurog2, foxi2 and pax2), and the first appearance of pax2- and lhx1-expressing nephrostomes.

## NF Stage 29-38: Late tail bud and free–swimming tadpoles



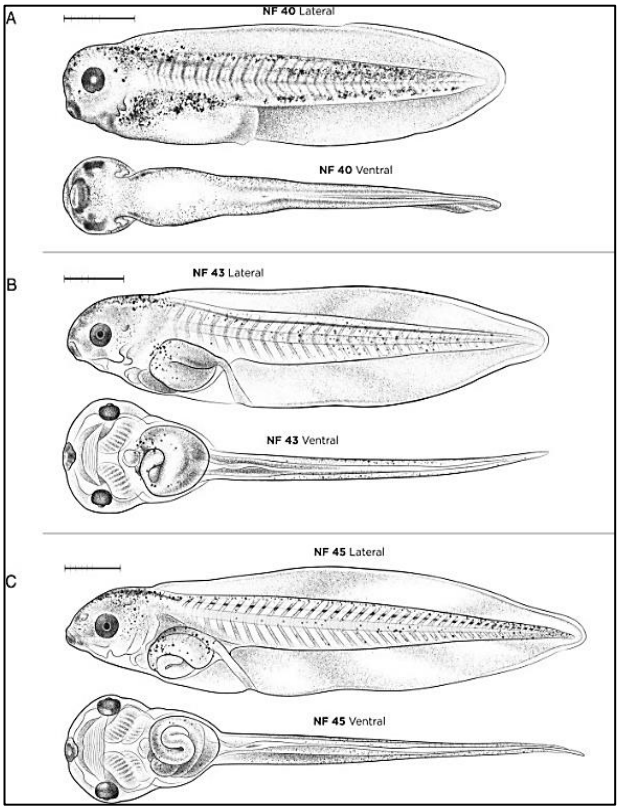



**Fig.2** (Left) *Late tailbud-stage and free-swimming tadpole X. laevis embryos. (A) NF stage 29-30. (B) NF stage 33-34. (C) NF stage 35-36. (D) NF stage 37-38. Orientation for dorsal and ventral views is anterior left; lateral views have dorsal up and anterior left.*

**Fig.3** (Right) *Free-swimming and gut-coiling stages of X. laevis tadpoles. (A) NF stage 40. (B) NF stage 43. (C) NF stage 45. Orientation for ventral views is anterior left; lateral views have dorsal up and anterior left.*

Staging of late tailbud stages is best accomplished after the removal of the vitelline membrane, focusing on eye morphology, tail and gut development, and the spread of melanophores across the body. At NF stage 29-30, (Fig. 2A) the developing eye cup forms a gray disc, and its darkening color and degree of choroid fissure closure can be easily assessed at NF stage 33-34 (darker above, still gray ventrally, open C-shape) (Fig.2B) , NF stage 35-36 (completely black, edges almost touching) (Fig. 2C), and NF stage 37-38 (fissure surfaces touching, but still slightly open) (Fig. 2D). As embryos lengthen as a result of tail growth, their abdominal area simultaneously 'shortens' as undifferentiated gut endoderm begins to form distinct fore-, mid- and hindgut domains. The 'tail length to gut length' ratio is used as an external landmark.

### *NF stages 40-46: free swimming tadpole*

Morphogenesis of the digestive and respiratory systems are the major milestones occurring during the late free-swimming tadpole and pre-metamorphosis stages (**Figs 3**A-C). Many of these events can be readily observed as the body wall of tadpoles becomes more transparent. The foregut gives rise to the esophagus, trachea, lungs, stomach, duodenum, liver, pancreas and gall bladder, whereas the midgut and hindgut give rise to the intestine and cloaca, respectively. At NF stage 40 (**Fig. 3**A), external landmarks include the completely closed choroidal fissure and clearly visible blood circulating through the gills. NF stage 40 tadpoles will take gulps of air from the surface at this stage, although their lungs are not yet functional. Tadpoles start to feed at NF stage 45 (**Fig.3**C) and food can be seen in the gut. As gut development and elongation proceed through NF stages 43-47 (**Figs 3**B, C), stereotypical gut coiling and asymmetry of the viscera/organs are best viewed ventrally.

## Environmental signals and normal development

### Larval settlement

During the early stage of marine larvae development, environmental signals play a crucial role. These signals may not be consistent but are necessary for further development. To begin metamorphosis, a free-swimming marine larva usually needs to settle near a food source or a firm substrate. If either of these gives off soluble molecules,

the larvae can use them as cues to settle. Molluscs have specific cues for settlement, which can be provided by prey or substrate molecules.

The red abalone, also known as *Haliotis rufescens*, is a species that has been extensively studied about larval settlement. These larvae only settle when they come into physical contact with coralline red algae. Interestingly, even a brief contact is sufficient for the competent larvae to stop swimming and begin their metamorphosis. Although the chemical agent responsible for this change has not been identified yet, a receptor that recognizes an algal peptide may be involved in inducing metamorphosis in competent larvae. It is important to note that larvae that are not competent to begin metamorphosis do not seem to possess this receptor. Scientists believe that the receptor is linked to a G protein that is similar to those found in vertebrates. The activation of this G protein may be necessary to induce larval settlement and metamorphosis.

## Blood meal

Female mosquitoes require a blood meal to produce eggs. Before feeding, they do not produce vitellogenin yolk protein. In *Aedes aegypti* mosquitoes, the blood meal stimulates the brain to secrete the egg development neurosecretory hormone (EDNH), also known as ovarian ecdysteroidogenic hormone (OEH). This hormone triggers the ovary to produce ecdysteroids, which signal the fat body cells to create vitellogenin for the oocytes. Vitellogenin is essential for egg production. Therefore, without a blood meal, there is no vitellogenin, and no eggs are produced.

Female *Rhodinus prolixus*, a type of blood-sucking bug, produces a new batch of eggs with each blood meal they consume. The blood serves two purposes - it provides the amino acids required for vitellogenin synthesis, and the physical stretching of the abdomen by the blood triggers the endocrine stimuli that activate juvenile hormone secretion by the corpora allata. Juvenile hormone stimulates vitellogenin synthesis in the ovary and fat body. In addition, a single large blood meal triggers the molt. If this bug feeds on many small meals, it will survive, but it will not molt or grow. In such cases, mammals provide the environmental cues for part of the insect's development.

## Probable environmental differences as cues for development

Organisms can incorporate predictable components present in their environment, such as gravity, into their development. Likewise, species often use predictable changes, like seasonal variations in temperature and daylight length, to adjust their development according to the environment. Additionally, some organisms' development is affected by the stresses of gravitational pressure.

Several species of aphids have a fascinating life cycle wherein an egg hatched in the spring gives rise to several generations of parthenogenetically (asexually) reproducing females. During the autumn, however, a particular type of female is

produced whose eggs can give rise to both males and sexual females. These sexual forms mate and their eggs can survive the winter. When the overwintering eggs hatch, each one gives rise to an asexual female.

Many species of insects have developed a mechanism called diapause. Diapause involves the temporary suspension of development, which can occur at any stage of the insect's life cycle, including embryonic, larval, pupal, or adult stage, depending on the insect species. The hickory aphid's overwintering eggs are a good example of the diapause mechanism. In some species, diapause is optional and only occurs when triggered by specific environmental conditions.

In some species, diapause is a necessary stage of the life cycle. This is often observed in insects living in temperate regions, where diapause is triggered by changes in the photoperiod, i.e. the duration of daylight and darkness. The critical day length is the point at which 50% of the population enters diapause, and this usually occurs suddenly. The critical day length is genetically determined.

**Probable questions:**

1. What is NF stage?
2. Discuss different larval stage of *Xenopus.*
3. How environmental factors regulate normal development?

**Suggested Literature:**


1. Developmental Biology: Michael J.F. Barresi Scott F. Gilbert, (12 th Ed)
2. Principles of Development: Lewis Wolpert and Cheryll Tickle (4th Ed.)
3. Principles of Genetics. 3rd Ed. D. Peter Snustad and Michael J. Simmons. Wiley Publication.
4. iGenetics: A molecular approach. 3rd Ed. Peter J. Russell. Pearson International Edition.

# Unit XII

# Promising field of tissue repair and restoration, factors; Field action in regeneration

**Objective**: This section of the Developmental Biology course focuses on a promising area of tissue repair and restoration, as well as the factors involved in such processes. The content will also cover the field action in regeneration. After completing this topic, the reader will be able to understand the mechanisms of tissue repair and restoration, as well as gain an understanding of the field action in regeneration.

## Tissue repair (TR) and restoration

The term "repair," when used in the context of the healing of damaged tissue, is defined as the restoration of tissue architecture and function after an injury. It encompasses two separate processes: regeneration and replacement. Regeneration refers to a type of healing in which new growth completely restores portions of damaged tissue to its normal state. Replacement refers to a type of healing in which severely damaged or non-regenerable tissues are repaired by the laying down of connective tissue, a process commonly referred to as scarring. While a few types of tissue injury (such as minor paper cuts) can sometimes be healed in such a way that no permanent damage remains, most of our tissue repair consists of both regeneration and replacement. Tissue repair may restore some of the original structures of the damaged tissue (such as epithelial layers), but may also result in structural abnormalities that impair organ function (such as the scar formed in the healing of a myocardial infarction).

Whether the healing of a wound proceeds down the regeneration or the replacement pathway (or both) depends, in part, on the type of tissue in which it occurs. Certain tissues of the body are more capable of cellular proliferation (and hence regeneration) than others. In this regard, there are three types of tissues: continuously dividing tissues, quiescent tissues and nondividing tissues. Continuously dividing tissues (also known as labile tissues) are comprised of cells that are constantly proliferating to replace dead or sloughed-off cells. Examples of such tissues include epithelia (such as skin, gastrointestinal epithelium and salivary gland tissue) and hematopoietic tissues. These tissues contain pools of stem cells, which have enormous proliferative and self-renewing ability, and which give rise to more than one type of cell. Replicating asymmetrically, each stem cell gives rise to one daughter cell that differentiates and matures and another daughter cell that remains undifferentiated and capable of beginning another self-renewing cycle.

Some tissues, known as quiescent tissues (or stable tissues) are composed of cells that normally exist in a non-dividing state but may enter the cell cycle in response to certain stimuli, such as cell injury. Tissues falling into this category include parenchymal

cells of the liver, kidney and pancreas, mesenchymal cells such as fibroblasts and smooth muscle cells, endothelial cells and lymphocytes. It should be noted that the liver, unlike other quiescent tissues, has a relatively robust proliferative capacity.

When a lobe of the liver is resected for donation, for example, the remaining liver cells proliferate at such a rate that the liver reaches a size similar to that before resection. While this process is commonly described as regeneration, it is more accurately viewed as compensatory growth, since the original lobe itself does not regrow. A few types of tissue are composed of cells that have left the cell cycle permanently and are therefore unable to proliferate. These nondividing tissues (or permanent tissues) include cardiac and skeletal muscle. Tissue repair in these tissues always leaves permanent evidence of injury, such as a scar.

## Actual player involved

Stem cells are unique for two reasons: (1) they have the ability to self-renew and (2) they have the capacity to generate more than one cell type. Self-renewal occurs either by symmetric replication (in which a stem cell gives rise to two daughter stem cells, equally capable of self-renewal) or asymmetric replication (in which one daughter cell remains a self-renewing stem cell and the other daughter cell differentiates and matures).

The capacity of a stem cell to give rise to multiple lineages of cells is most striking in embryonic stem cells. These cells, which are denoted as pluripotent, are capable of generating cells from any of the tissues of the body. Adult (or somatic) stem cells are designated as multipotent, and give rise to a more restricted array of cell types. As expected, somatic stem cells have been found in continuously dividing tissues, such as skin, gastrointestinal epithelial lining, cornea and hematopoietic tissue. However, they have also been discovered in certain quiescent tissues, such as liver, pancreas and adipose tissue, in which they do not normally produce differentiated cells. Most surprising is the recent discovery of stem cells residing in certain parts of the central nervous system, an organ system whose tissues have long been thought to be incapable of proliferating.

Beyond the stem cell, three other types of cells are critical to the process of tissue repair: fibroblasts, endothelial cells and macrophages. In most wounds, complete replacement of wounded tissue to its original, unharmed state is impossible.

## Process:

One of the main actions in the tissue repair script is cell proliferation. In order to heal after injury—whether by regeneration or scarring—cells must enter and progress through the cell cycle, a tightly regulated process that consists of two main activities: DNA replication and mitosis. Continuously proliferating cells are always moving through the cell cycle, whereas quiescent cells must be called into the cell cycle by growth factors or

cytokines (via receptor-mediated signal transduction) or by ECM components (via integrins).

## Regeneration vs repair

Regeneration involves restitution of tissue components; repair involves "patching" rather than restoring. The amount of regeneration vs. repair that occurs depends on the proliferative capacity of the cells, the integrity of the stromal framework and the duration of the injury and inflammatory response.

Repair by connective tissue involves the influx of debris-removing inflammatory cells, formation of granulation tissue (a substance consisting of fibroblasts and delicate capillaries in a loose extracellular matrix), and conversion of said granulation tissue into fibrous tissue that is remodeled over time to form a scar. There are five major components in this process: inflammation, new vessel formation, fibroblast proliferation, collagen synthesis and scar remodeling.

## Stimulus for tissue repair

TR is characterized by cell division to increase the number of cells, differentiation, and specification of the newly divided cells, angiogenesis, that is, regeneration of blood vessels to restore blood supply, and regeneration of extracellular matrix (ECM), which holds the tissue together. TR is a complex and comprehensive process that encompasses various aspects of tissue rebuilding and is governed by intricate molecular signaling (**Fig. 1**).

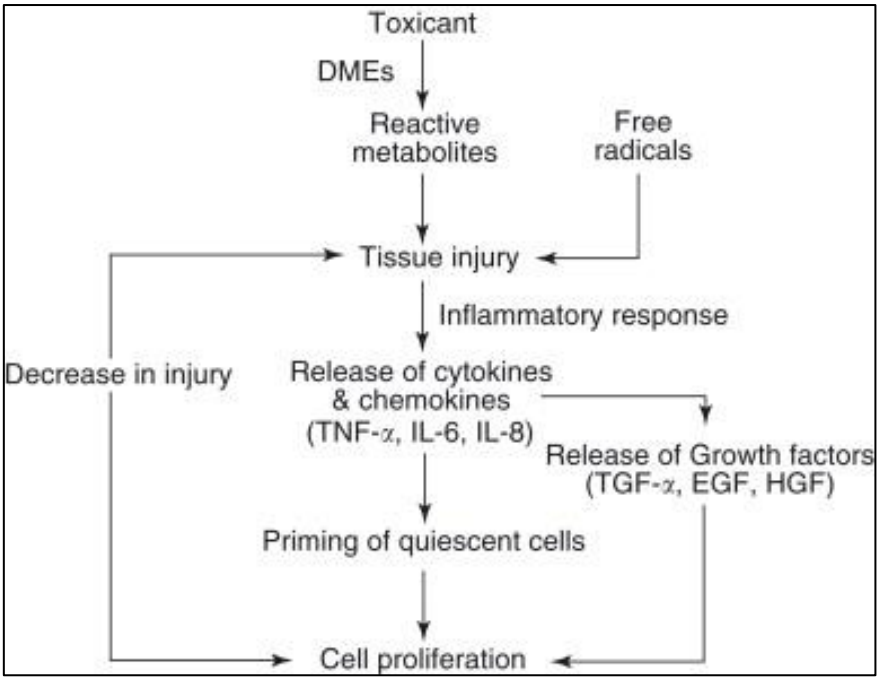


**Fig.1** *Schematic representation of stimulation of tissue repair. Chemicals and drugs are metabolized in the tissue to their reactive metabolites, which initiate injury. An inflammatory response follows the injury-stimulating release of cytokines and chemokines, which prime the quiescent cells to enter cell cycle. Additional stimulation to complete cell division comes from growth factors. As cell proliferation increases, the dead tissue is replaced by viable cells and injury regresses*.

## Field action in regeneration

Regeneration involves growth and differentiation and is related to i) the stump or remaining part of the animal and ii) the final complete form. During this process, the concept of field action is often applied to explain the various processes. In hydra and other allied coelenterate, a portion of the body can give rise to an entire individual. If a hydra is cut into 2 pieces, a) with hypostome and b) with the basal disc, a) will regenerate a basal disc and b) a hypostome. When an incision is made more towards the hypostome the 2 pieces resulting are unequal.

a) the piece with hypostome is small and

b) the piece with the basal disc is large

The experiment shows the clusters of cells that in the first experiment form a basal disc and forms a hypostome i.e. these cells are competent to form a basal disc but are suppressed by the dominance of the existing hypostome which in a normal hydra gradually falls off towards the basal disc. This suggests that the hypostome sets up a high level of some activity or concentration of some substances which gradually diminishes towards the basal disc. At the same time, it has been observed that the rate of regeneration gradually decreases when the cut is made more towards the basal disc.

These experiments indicate the presence of two gradients leading to two fields.

1. An intrinsic regeneration capacity within the cell and i.e. a field of competence.
2. Presence of dominance within the section of a body i.e. a field of hypostome regeneration.

Birth and Spiegelman suggest that the competition between different regions for physiologically necessary substances (such as nutrients and oxygen) drives the mechanism of the individuation field.

When producing a new hydranth, it is necessary to transport a raw material known as 'K' into the hydranth material 'R'. If two hydranths are created at the same time from a common supply of 'K', the development of one hydranth will likely hinder the growth of the other. If one of the hydranths (A) manages to gain an advantage over the other or is more effective at drawing supplies of 'K' from the pool, it will dominate over the other (B). Although the dominant hydranth may be slightly inhibited, it will have a strong suppressive effect on the other hydranth.

The Spiegelman hypothesis explains the phenomenon by relating the production of 'R' to the amount of raw material used and its efficiency. (A graphical representation and mathematical equation are required (not shown here) to relate $dR/dt$ with $dR_1/dt$ and $dR_2/dt$.)

**Probable questions**

1. Differentiate tissue repair and regeneration.
2. What is compensatory regeneration? Explain with an example.
3. Discuss field action in regeneration through mathematical expression.

**Suggested Literature:**


1. Developmental Biology: Michael J.F. Barresi Scott F. Gilbert, (12 th Ed)
2. Principles of Development: Lewis Wolpert and Cheryll Tickle (4th Ed.)
3. Principles of Genetics. 3rd Ed. D. Peter Snustad and Michael J. Simmons. Wiley Publication.
4. iGenetics: A molecular approach. 3rd Ed. Peter J. Russell. Pearson International Edition.

# Unit XIII

# Sonic Hedgehog and limb-enhance specification of anteroposterior pattern

**Objective**: This unit of Developmental Biology focuses on the Sonic Hedgehog gene and its role in determining the anteroposterior pattern of limbs. By studying this gene, you will gain a deeper understanding of how it enhances limb development and specifies the anteroposterior pattern. By the end of this topic, you will be equipped with all the necessary information to comprehend the function and importance of the Sonic Hedgehog gene in limb specification.

## The Hedgehog

The hedgehog (hh) gene plays a crucial role in regulating segmental and imaginal disc patterning in the fruit fly, Drosophila melanogaster. Unlike invertebrates, which have only one hh gene, vertebrates have a family of genes that are similar to the hh gene. All hedgehog genes encode signaling molecules that help in both short and long-range patterning processes during embryonic development.

The expression pattern of Sonic Hedgehog has been studied in several species. Studies in rodents have shown that Shh is essential for anteroposterior patterning of the limb buds besides other patterning or development.

## Specification of the Anterior-Posterior Limb Axis

The gene responsible for encoding Sonic Hedgehog (Shh), a protein that is secreted, is expressed in a small group of mesenchyme cells located at the posterior margin of the vertebrate limb bud, known as the polarizing region or zone of polarizing activity. Detailed research has shown that Shh has the properties of a polarizing region morphogen that specifies positional values across the anteroposterior axis of the limb, such as the thumb to little finger axis. Shh has also been found to control the width of the limb bud by promoting the proliferation of mesenchyme cells and regulating the anteroposterior length of the apical ectodermal ridge, which is responsible for limb bud outgrowth and the development of structures along the proximo-distal axis of the limb, such as the shoulder to digits' axis. It has been observed that Shh signaling can specify antero-posterior positional values in limb buds both in a concentration-dependent (paracrine) and time-dependent (autocrine) fashion.

Several experiments (Saunders and Gasseling 1968; Tickle et al. 1975) suggest that the anterior-posterior axis is specified by a small block of mesodermal tissue near the posterior junction of the young limb bud and the body wall. This region of the mesoderm has been called the **zone of polarizing activity** (**ZPA**). When this tissue is

taken from a young limb bud and transplanted into a position on the anterior side of another limb bud (**Fig.1**), the number of digits of the resulting wing is doubled. Moreover, the structures of the extra set of digits are mirror images of the normally produced structures. The polarity has been maintained, but the information is now coming from both an anterior and a posterior direction.

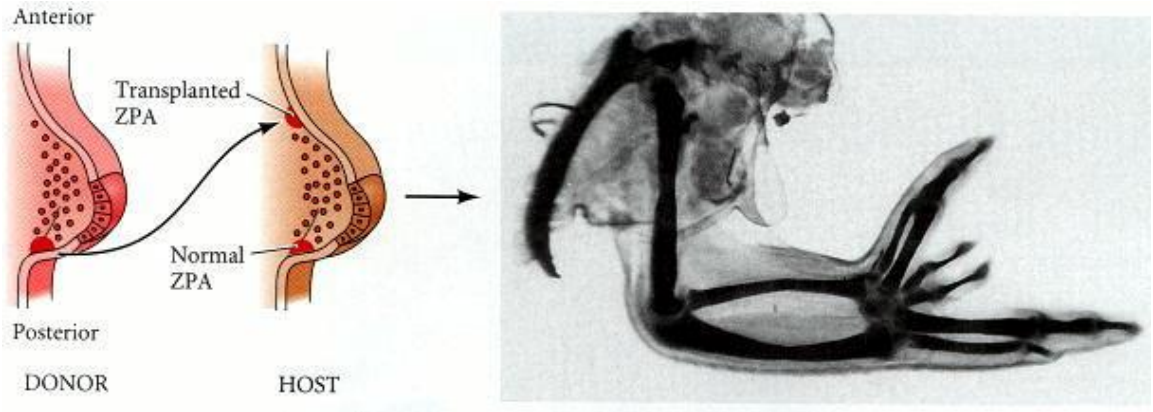



**Fig.1** *When a ZPA is grafted to the anterior limb bud mesoderm, duplicated digits emerge as a mirror image of the normal digits*.

## Sonic Hedgehog and ZPA

As evidence that this association between the ZPA and *sonic hedgehog* was more than just a correlation, Riddle and co-workers (1993) demonstrated that the secretion of Sonic hedgehog protein is sufficient for ZPA activity. They transfected embryonic chick fibroblasts (which normally would never synthesize this protein) with a viral vector containing the *shh* gene (**Fig. 2**). The gene became expressed and translated in these fibroblasts, which were then inserted under the anterior ectoderm of an early chick limb bud. Mirror-image digit duplications like those induced by ZPA transplants were the result. More recently, beads containing Sonic Hedgehog protein were shown to cause the same duplications. Thus, Sonic Hedgehog appears to be the active agent of the ZPA.

## Specification of the posterior limb bud

It is currently unknown why the activation of sonic hedgehog genes occurs only in cells located in the posterior limb bud and not in those located more anteriorly. The activation of the sonic hedgehog gene appears to be initiated by an FGF protein secreted by the newly formed apical ectodermal ridge. Specifically, FGF8 is capable of activating Sonic Hedgehog. However, it is unclear why FGF8 does not activate all the mesenchyme cells beneath the AER. This may be due to the differential competence of certain mesenchyme cells to respond to the FGF signal.

## The action of Sonic Hedgehog

When Sonic Hedgehog was first discovered as a regulator of the ZPA (zone of polarizing activity) in limb development, scientists believed that it acted as a morphogen. This meant that it diffused from the ZPA, where it was produced, and created a concentration gradient from the posterior to the anterior of the limb bud. However, recent research has shown that the Sonic Hedgehog protein, or its active amino-terminal region, does not diffuse outside the ZPA. Instead, it works by triggering a series of other proteins, such as BMP2 and BMP7, which in turn create a gradient of BMPs that emanate from the ZPA and specify the formation of digits.

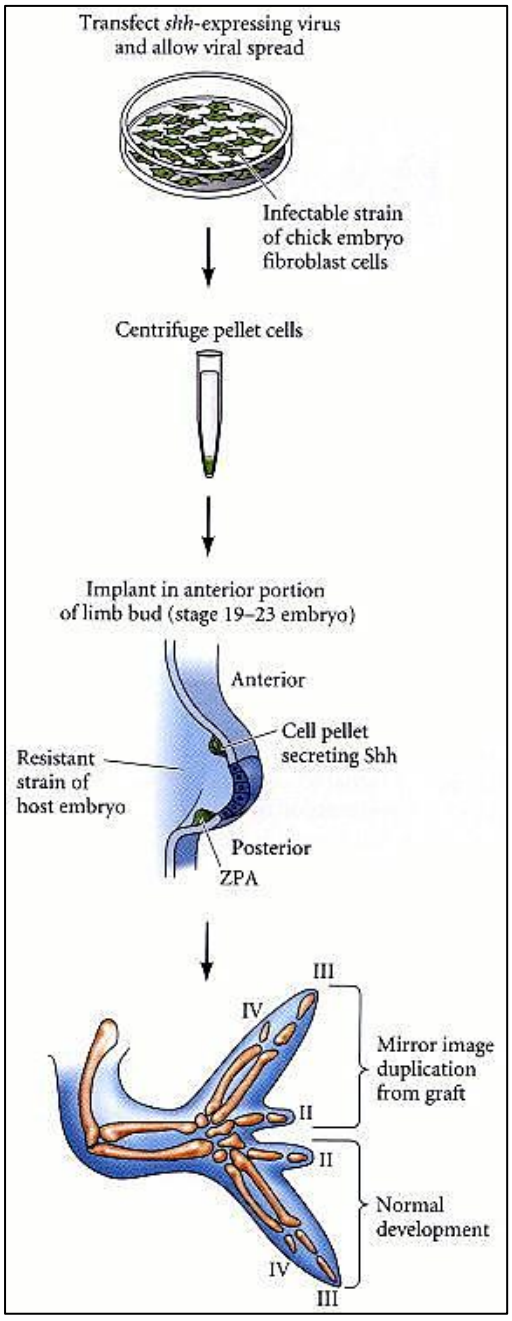



Fig.2 *Assay for polarizing activity of Sonic hedgehog. The sonic hedgehog gene was inserted adjacent to an active promoter of a chicken virus, and the recombinant virus was placed into cultured chick embryo fibroblast cells (CEF). The virally infected cells were pelleted and implanted into the anterior margin of a limb bud of a chick embryo. The resulting limbs produced mirror-image digits, showing that the secreted protein had polarizing activity.*

Regardless of how it happens, Sonic Hedgehog, either alone or with the help of the BMP cascade, is responsible for regulating the expression of the 5´ Hox D genes. The shift from phase I to phase II Hox expression patterns coincides with the expression of Sonic hedgehog in the ZPA. Additionally, if the ZPA or other cells that secrete Sonic hedgehog are transplanted to the anterior margin of the limb bud at this stage, it leads to the formation of mirror-image patterns of Hox D gene expression and results in mirror-image digit patterns.

**Probable questions**

1. State the relation between Sonic Hedgehog and ZPA.
2. Describe specification of the posterior limb bud in Hedgehog

**Suggested Literature:**


1. Developmental Biology: Michael J.F. Barresi Scott F. Gilbert, (12 th Ed)
2. Principles of Development: Lewis Wolpert and Cheryll Tickle (4th Ed.)
3. Principles of Genetics. 3rd Ed. D. Peter Snustad and Michael J. Simmons. Wiley Publication.
4. iGenetics: A molecular approach. 3rd Ed. Peter J. Russell. Pearson International Edition

# Unit XIV

## Interaction between positional information and self-organization mechanism; mechanism of Shh signaling

**Objective**: This unit explains how positional information mechanisms interact during development and the mechanism of Shh signaling as well. By the end of this topic, you will have a comprehensive understanding of the Shh signaling mechanism and how it takes place during development, as well as the interaction between positional information and the self-organization mechanism.

A widely used mechanism for pattern formation is based on positional information: cells acquire positional identities as in a coordinate system and then interpret this information according to their genetic constitution and developmental history. In Drosophila maternal factors establish the axes and set up a maternal system of positional information on which further patterning is built. There is a cascade of gene activity that leads both to the development of periodic structures, of the segments and to their acquiring a unique identity. This involves the binding of transcription factors to regulatory regions of genes to produce sharp thresholds. Many of the genes involved in these processes, particularly the Hox complex, are also involved in specifying the body axis and limbs of vertebrates. There are striking similarities in the mechanisms for specifying and recording positional identity in Drosophila and vertebrates.

Vertebrate limbs appear as small buds produced by the epithelial-to-mesenchymal transition of trunk epithelial cells at specific locations along the body. These buds produce a small number of digits, e.g. three in the chick forelimb and five in mouse limbs, that are morphologically distinct along the anterior-posterior (a-p) axis. Classical grafting experiments indicated that positional information, in the form of a Shh gradient, instructs the morphological identity of digits. While in principle positional information could be interpreted to generate a periodic pattern, mutations in components of the Shh signaling pathway result in polydactyly due to an increase in cell proliferation and a larger limb rather than in altered periodicity of the pattern. This indicates that Shh regulates the size of the digit field but is not required for digit patterning per se. Moreover, loss of positional identity, through dissociation, reaggregation, and grafting of limb cells, does not block digit formation. Thus, generating digits and specifying morphological identities are largely independent processes.

The idea that digit patterning is self-organized, and might be described by a reaction-diffusion model, is supported by several observations.

**First**, time-course analysis of the emergence of digit condensations in the developing limb indicates that digits appear as regularly spaced condensations in a defined temporal order as the digit field grows.

**Second**, an allelic series of mutations 10 in the Hoxa13, Hoxd11-13, and Gli3 genes produces mouse mutant embryos with a gradual increase in digit number, ranging from five up to thirteen. While this phenotype may reflect the changing wavelength of a Turing-like pattern of stripes, the observation that digits may be initiated as spots and extend with growth, rather than being specified de novo as stripes, suggested a different interpretation of these phenotypes: spots develop with the same spacing along the a-p axis but at a more distal position within the limb, where the digit field is broader. In this view, digits appear at regular intervals and their number is defined by the size of the digit field, as a Turing-like system would predict.

**Third**, using Sox9 as an early digit marker, dissociated limb cells could form periodic patterns ex vivo. Together, these data support the view that a Turing-like system may be at play.

## Molecular dynamics underlying this patterning system

A transcription factor, Sox9, and two signals, BMP and Wnt, were recently proposed to be part of a three-node Turing system. Indeed, Sox9 expression, marking the future digits, is in phase with BMP activity (phosphorylated Smad) but out of phase with Wnt signaling activity (β-catenin). Loss and/or inhibition of Sox9, BMP, and Wnt activities disrupt patterning with BMP activating and Wnt repressing Sox9 expression. Absent a full characterization of the interactions between Sox9, BMP, and Wnt, an exploration of possible network structures identified a minimal topology that could support digit patterning.

## About Shh

Shh is one of the three vertebrate hedgehog genes (Indian, Desert, and Sonic) homologous to the *Drosophila* hedgehog. Shh acts as a morphogen and induces different cell fates at different concentration thresholds, low concentrations induce ventral neurons, high induce motor neurons, and very high floor plate cells. Inhibition of Shh signaling stops differentiation.

### Signaling Mechanism

The activation of the Shh signal requires binding of Shh to the Ptc-mediated Smoothened (Smo) (Ptc-smo) receptor complex and induction of downstream signaling cascade (**Fig.1**). This is a heterodimeric receptor complex. Ptc-Smo heterodimeric receptor complex consists of two trans-membrane subunits, namely Ptc and Smo. Ptc gene codes for a 1286 amino acid protein having at least seven putative transmembrane α helices, which plays a major role in the downstream Shh signaling. In *Drosophila*, Ptc is integral for the correct patterning of segments, devoid of which all cells attain segment border cell characteristics.

Binding studies using labeled Shh have shown that the Hh receptor is encoded by Ptc. Ptc contains a sterol-sensing domain (SSD), which interacts with the cholesterol-modified Shh. The binding of Shh signaling protein to Ptc regulates the activity of Smo. Shh-free Ptc has been found to act sub-stoichiometrically to suppress Smo activity, and thus it is critical in specifying the level of signaling activity.

The Ptc suppressor normally functions as a transmembrane molecular transporter. Ptc is also believed to indirectly inhibit Smo activity, possibly through changes in the distribution or concentration of a small inhibitor molecule. Smo is a member of the Frizzled (Fz) family of seven-pass trans-membrane receptors. As a response to the binding of Shh with Ptc, Smo is activated and stabilized. The activated Smo initiates the Shh downstream signaling cascade by encoding membrane proteins, which are similar to G protein-coupled receptors. Smo is also believed to encode a receptor of the Shh signal. It generates intracellular signals that regulate several protein kinases, which activate a class of transcription factors known as cubitus interruptus (Ci) proteins and glioblastoma (Gli) proteins.

## Shh transduction

Genetic studies have identified that transduction of the Hh-encoded signal is mediated by the activity of four segment polarity genes, Ptc, fused (Fu), costal-2 (Cos-2) and (Ci). Transcriptional activation of Hh target genes requires Ci, a 155 kDa cytoplasmic zinc finger protein (Ci155). Three Gli proteins Gli1, Gli2 and Gli3 are expressed in vertebrates, in overlapping domains and are partially redundant. Gli2 and Gli1 show activator functions that are dependent on Hh. Gli2 and Gli3 are proteolyzed to produce a repressor form, which can inhibit Hh expression. Hh regulates Gli3 repressor activity, while Gli2 is independent. The three Gli proteins in vertebrates allow complex responses within target fields. The Hh signal cellular response depends on both the level of ligand exposure and the individual Gli genes expressed. Cos2 is a kinesin-like protein, which is associated with microtubule. Cos2 is mainly the motor domain that binds to ATP and microtubules and hydrolyses ATP.

Fu is a segment polarity gene, which is phosphorylated in response to Hh signaling and involves an activation loop for Fu transcription. Fu binds to Cos2 by its carboxy-terminal and phosphorylate it.

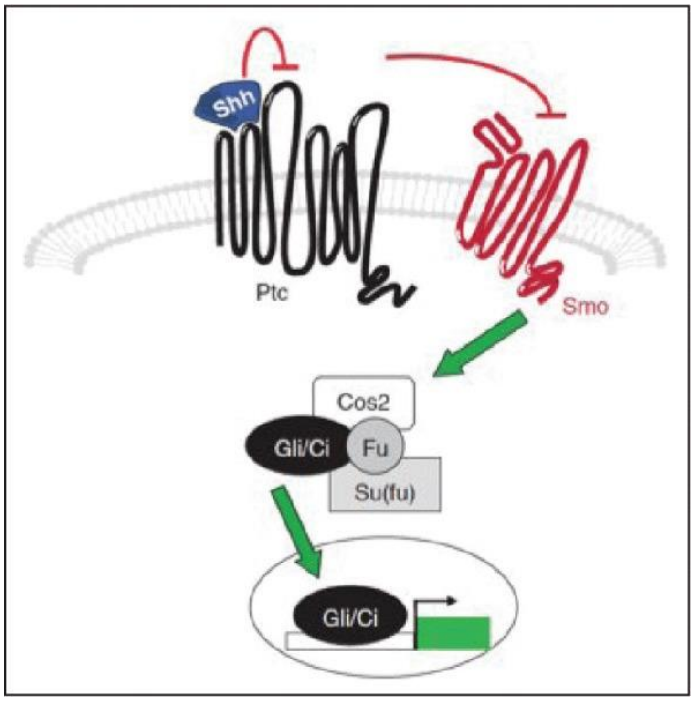



**Fig.1** *The Sonic Hedgehog (Shh) signaling pathway: Activation of signaling pathway is connected to various transmembrane proteins (Ptc, Smo), transcription factors (Ci/Gli) and protein kinases (Su(fu), fu).*

**Probable questions:**

1. What is positional information?
2. How does positional information help in pattern formation?
3. Discuss the mechanism of Shh signaling.

**Suggested Literature:**

1. Developmental Biology: Michael J.F. Barresi Scott F. Gilbert, (12 th Ed)
2. Principles of Development: Lewis Wolpert and Cheryll Tickle (4th Ed.)
3. Principles of Genetics. 3rd Ed. D. Peter Snustad and Michael J. Simmons. Wiley Publication.
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