CHAPTER: 11

PATTERNING AND MORPHOGENESIS IN LIMB DEVELOPMENT

Limb organizing centers

The limb has three organizing centers - the apical ectodermal ridge (AER), the zone of polarizing activity (ZPA) and the dorsal ectoderm- that help to pattern the proximodistal, anteroposterior and dorsoventral limb axes, respectively. As well as patterning the axes, these organizers also interact with each other to maintain limb outgrowth.

Specifying the proximodistal axes

The proximo-distal limb axis emerges progressively as mesenchyme cells drop out of the progress zone and differentiate. The type of structure formed by these differentiating cells may depend on the amount of time they have spent in the progress zone, and hence the number of division cycles they have undergone in response to signaling from the AER.

Specifying the anteroposterior axis

The anteroposterior limb axis is specified by the zone of polarizing activity in the posterior mesenchyme, as grafting this region to the anterior side of the limb bud can induce duplication andrnirror image reversal of the axis. The secreted protein Sonic hedgehog can substitute for the activity of the ZPA and appears to act in a dose-dependent manner to specify the fates of anteroposterior structures, such as the different digits of the hand.

Positional values along the proximodistal and anteroposterior axes

Along both the anteroposterior and proximodistallimb axes, the 5' *HoxA* and *HoxD* genes are expressed in overlapping concentric patterns. These *Hox* genes play an important role in the regional specification of different skeletal elements along the two axes, as gene knockouts cause the deletion or respecification of particular limb structures.

Specifying the dorsoventral axis

The dorsoventral limb axis is specified by the secreted protein Wnt7a, which is synthesized in the dorsal ectoderm. This activates a transcription factor called Lmxl in the dorsal mesenchyme. The inactivation of either gene generates biventral limbs, while overexpression throughout the limb generates bidorsallimbs.

Interaction and dependence in axis pattering

There is a significant interaction and interdependence between the three signaling pathways. The AER is maintained by Sonic hedgehog secreted by the ZPA. The maintenance of the ZPA is dependent on both FGFs secreted by the AER and Wnt7a secreted by the dorsal ectoderm. Furthermore, FGFs secreted by the AER are required in addition to Sonic hedgehog to establish the nested pattern of *HoxD* gene expression along the anteroposterior axis.

Other patterning Influences

There is evidence that the limb is pre-patterned before the induction of the three signaling centers, as the absence of the AER and ZPA in certain mutants does not prevent the regionalized expression of asymmetrically expressed genes, such as

those of the *HoxD* cluster. Furthermore, there is evidence that the limb has significant self-organizing ability, as shown by the development of recognizable digits in disaggregated and recombined limb buds.

Morphogenesis in the limb

The final structure of the limb depends largely on the regulation of *cell* death, controlled by the opposing activities of BMPs (which promote apoptosis) and BMP antagonists (which inhibit it). Complementary patterns of BMPs and their antagonists are seen in the limb, marking the interdigital and internal necrotic zones that separate the digits and the two bones of the zeugopodium, and the positions where joints will form.

The vertebrate Hox genes

Vertebrate genomes contain four copies of the Drosophila homeotic complex, designated Hox-A, Hox-B, Hox-C and Hox-D. Although there are significant differences between the vertebrate and fly complexes, the similarities are remarkable. The same types of homeobox gene are present, allowing classification into 13 cognate groups (paralogous subgroups) based on homeobox structure Further more, the genes are arranged in more or less the same order along the chromosome and are expressed , in a similar manner, with the most 3' genes expressed in the most anterior domains and the most 5' genes in the most posterior domains. Since the divergence of Drosophila and vertebrates, the fly HOM-C has been split into two subcomplexes, whilst the vertebrate cluster has undergone a 5' end expansion and has been duplicated in its entirety to generate four complexes. Each of the four complexes has suffered individual losses, which may be different between species. For instance, the hatched boxes in the above figure represent Hox-C genes present in humans but missing in mice.

► The similarity between the Drosophila and vertebrate homeobox-containing genes, in terms of both structure and expression patterns is strong evidence for a conserved function. This has been confirmed by the use of cloned human HOX genes to rescue Drosophila homeotic mutants, and targeted disruptions of mouse Hox genes do indeed generate partial homeotic transformations . Deletion of the Hoxc-8 gene, for instance, results in transformation of lumbar vertebrae into vertebrae with a more anterior characteristic (in this case, a thoracic vertebra, complete with a rib). The Paralogous Hox genes appear to have overlapping but nonidentical functions and may cooperate with each other in certain cases. Individual gene knock-outs of genes in paralogous subgroup 3, for instance, cause different types of disturbances to the structures of the neck, but when combined in the same mouse, severe defects are observed including missing vertebrae.

CHAPTER: 12

REGENERATION

Regeneration is of three types:

1. Physiological Regeneration

There is a constant loss of many kinds of calls due to wear and tear caused by day-to-day activities. The replacement of these cells is known as physiological regeneration

Example:

⇨Replacement of R.B.C's

The worn out R.B.C's are deposited in the spleen and new R.B.C's regularly produced from the bone marrow cells, since the life span of R.B.C's is only 120days.

⇨Replacement of Epidermal Cells of the Skin

The cells from the outer layers of epidermis are regularly peeled off by wear and tear. These are constantly being replaced by new cells added by the malpighian layer of the skin.

2. Reparative Regeneration

This is the replacement of lost parts or repair of damaged body organs. In this type of regeneration, wound is repaired or closed by the expansion of the adjoining epidermis over the wound.

Example:

⇨Regeneration of limbs in salamanders

⇨Regeneration of lost tail in lizard

⇨Healing of wound

⇨Replacement of damaged cells.

3. Autotomy

In some animals like starfish, some part of the body is broken off on being threatened by a predator. This phenomenon of self-mutilation of the body is called autotomy

Example:

⇨Crabs break off their leg on approaching of the enemy

⇨Holothurians throw off their internal viscera

⇨Starfish breaks off an arm

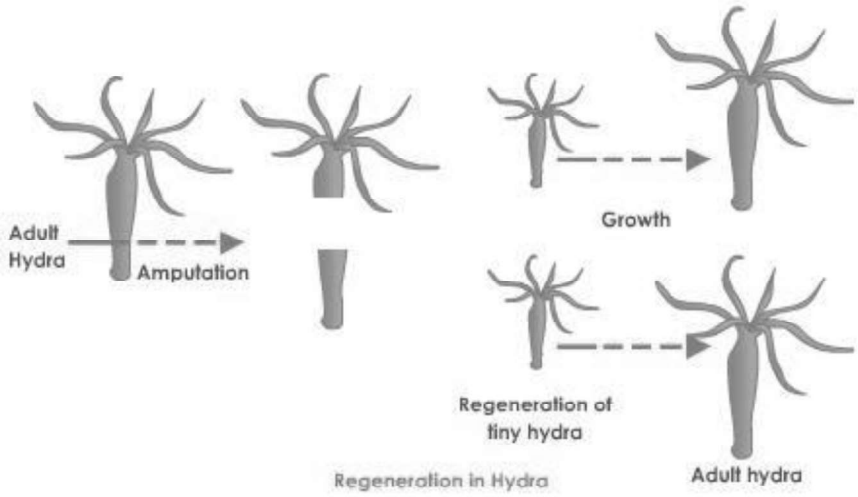
Regenerative capacity in Animal Group

The capacity of regeneration varies in its extent in various animal groups. Regenerative capacity is very high among the protozoan, sponges and coelenterates.

Invertebrates

- **In sponges**, the entire body can be reconstructed from isolated body cells. The cells rearrange and reorganize to form bilayered sponge body wall.
- **Regeneration was first discovered in hydra by Tremble (1740). Even 1/1000th part of the body regenerate into new organisms.**
- **In hydra and planaria**, small fragments of the body can give rise to a whole animal. When a hydra or a planaria is cut into many pieces, each individual part regenerates into a whole individual.

- **Some annelids like earthworms** are able to regenerate some segments removed from the anterior and posterior ends of the body.
- **Some molluscs can regenerate** only the eyes and heads while squids can also regenerate their arms.



- Many arthropods (e.g., spiders, crustaceans, insect larvae, etc) can regenerate limbs only. Regeneration is faster in the young than in the adults. Regenerated part may not always be similar to the part lost. This type of regeneration is called heteromorphosis.
- Echinoderms (like starfish, brittle star, sea lilly)exhibit autotomy. They can regenerate arms and parts of te body.
- **Vertebrates**
- **Fishes:** Lamprey can regenerate its lost tail. Some fishes have the ability to regenerate parts of its fins.
- **Amphibians:** The regeneration power is well marked in urodel amphibians like salamanders, newts and their axolotl larvae. They can regenerate limbs, tail, external gills, jaws, parts of eye like lens and retina. Tail and limb regeneration is found in the larval stages of frogs and toads.
- **Reptiles:** Lizards exhibit autotomy. When threatened, the lizard detaches its tail near the base to confuse its predator and later regenerates a new tail. The new tail differs from the old one in its shape, absence of vertebrae and the kind of scales covering it.
- **Birds:** Regeneration is restricted to parts of the beak.
- **Mammals:** Regeneration is restricted to tissues only. External parts are not regenerated. Skin and skeletal tissues possess great power of regeneration. The liver has the maximum capacity of regeneration. If one kidney is damaged or removed, the other enlarges to compensate the lost kidney. This is called as compensatory hypertrophy.
- **Regeneration is an usual form of asexual reproduction in several lower groups of animals.**

Three Types of Regeneration based on Cellular Mechanism

1. The first mechanism involves the dedifferentiation of adult structures of form an undifferentiated mass of cells that then becomes respecified. This type of regeneration is called **epimorphosis** and is characteristic of regenerating limbs. 2. **The second mechanism is called morphallaxis**. Here, regeneration occurs through the re-patterning of existing tissues, and there is little new growth. Such regeneration is seen in hydras.

3. A third type of regeneration is an intermediate type, and can be through of as **compensatory regeneration**. Here, the cells divide, but maintain their differentiated functions. They produce cells similar to themselves and do not form a mass of undifferentiated tissue. **This type of regeneration is characteristic of the mammalian liver.**

1. Epimorphosis:

In contrast to morphallaxis, **epimorphosis requires active cellular proliferation prior to the replacement of the lost body part.**

Epimorphosis can be further subdivided into dedifferentiation-*dependent* and dedifferentiation-*independent* subclasses. Planarian, which are flatworms, regenerate using a dedifferentiation-*independent* mechanism in which preexisting stem cells, known as neoblasts, begin to proliferate and migrate to the injured site in response to injury.

These cells then form a mass of proliferating cells, known as the regeneration blastema, that will later differentiate into the specialized cells that comprise the regenerated structure.

Most tissue regeneration in mammals also belongs to the dedifferentiation-*independent* subclass. For example, mammals can regenerate their muscle, bone, epithelia of the skin and gut, blood, and some neurons by activating preexisting stem cells or progenitor cells.

Vertebrate limb regeneration involves cell dedifferentiation and growth.

Regeneration of a Limb of a Newt

When an adult salamander limb is amputated, the remaining cells are able to reconstruct a complete limb, with all its differentiated cells arranged in the proper order. In other words, the new cells construct only the missing structures and no more. For example, when a wrist is amputated, the salamander forms a new wrist and not a new elbow.

⇨Upon limb amputation, a plasma clot forms, and within 6 to 12 hours, epidermal cells from the remaining stump migrate to cover the wound surface, forming the **wound epidermis**. This single-layered structure is required for the regeneration of the limb, and it proliferates to form the **apical ectodermal cap**. Thus, in contrast to wound healing in mammals, no scar forms, and the dermis does not move with the epidermis to cover the site of amputation. The nerves innervating the limb degenerate for a short distance proximal to the plane of amputation

⇨During the next 4 days, the cells beneath the developing cap undergo a dramatic dedifferentiation: bone cells, cartilage cells, fibroblasts, myocytes, and neural cells lose their differentiated characteristics and become detached from one another The formerly well-structured limb region at the cut edge of the stump thus forms a proliferating mass of indistinguishable, dedifferentiated cells just beneath the apical ectodermal cap. This dedifferentiated cell mass is called the **regeneration blastema**. These cells will continue to proliferate, and will eventually redifferentiate to form the new structures of the limb

⇨The creation of the blastema depends upon the formation of single, mononucleated cells.It is probable that the macrophages that are released into the wound site secrete metallo-proteinases that digest the extracellular matrices holding epithelial cells together The proliferation of the salamander limb regeneration blastema is dependent on the presence of nerves. a minimum number of nerve fibers must be present for regeneration to take place. It is thought that the neurons release mitosis-stimulating factors that increase the proliferation of the blastema cells

⇨**Glial growth factor** (**GGF**) is known to be produced by newt neural cells, is present in the blastema, and is lost upon denervation. When this peptide is added to a denervated blastema, the mitotically arrested cells are able to divide again

⇨**A fibroblast growth factor** may also be involved. FGFs infused into denervated blastemas are able to restore mitosis.

⇨Another important neural agent necessary for cell cycling is **transferrin**, an iron-transport protein that is necessary for mitosis in all dividing cells (since ribonucleotide reductase, the rate-limiting enzyme of DNA synthesis, requires a ferric ion in its active site). When a hindlimb is severed, the sciatic nerve transports transferrin along the axon and releases large quantities of this protein into the blastema. Neural extracts and transferrin are both able to stimulate cell division in denervated limbs, and chelation of ferric ions from a neural extract abolishes its mitotic activity regeneration blastema resembles in many ways the progress zone of the developing limb.

⇨The dorsal-ventral and anterior-posterior axes between the stump and the regenerating tissue are conserved, and cellular and molecular studies have confirmed that the patterning mechanisms of developing and regenerating limbs are