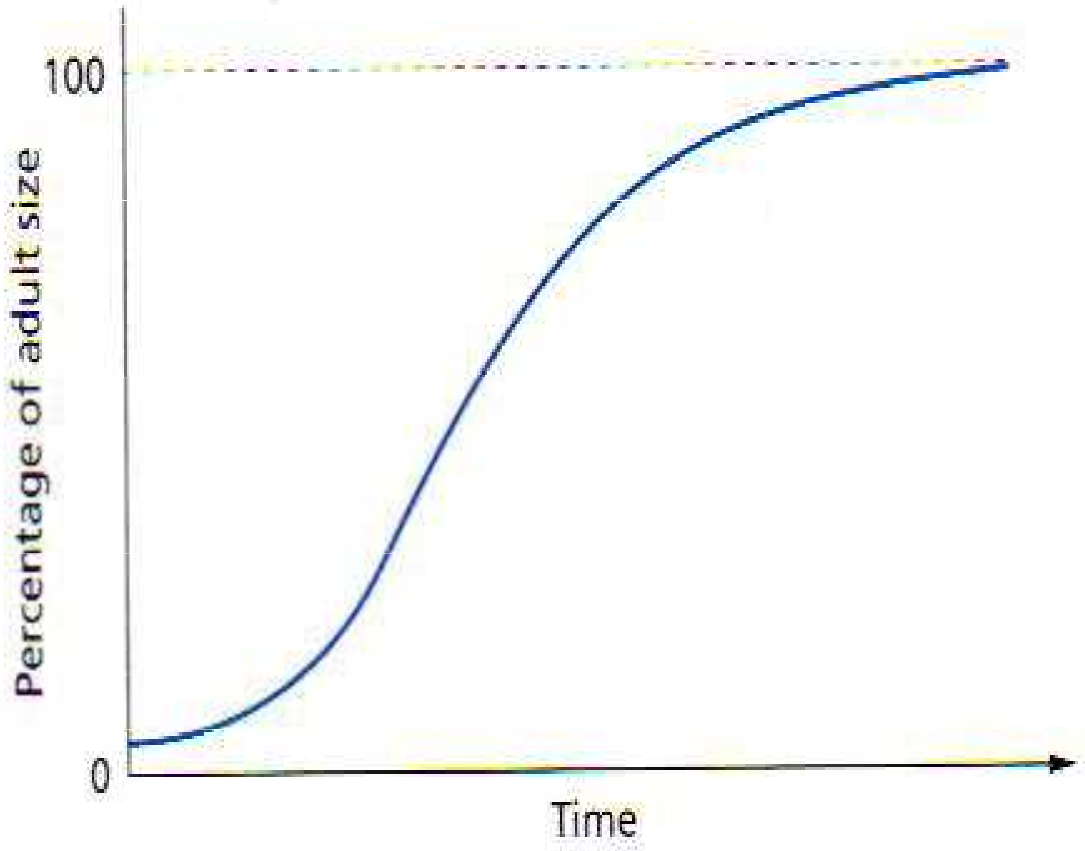
7. GROWTH, REGENERATION AND EVOLUTION

SIZE AND PROPORTION

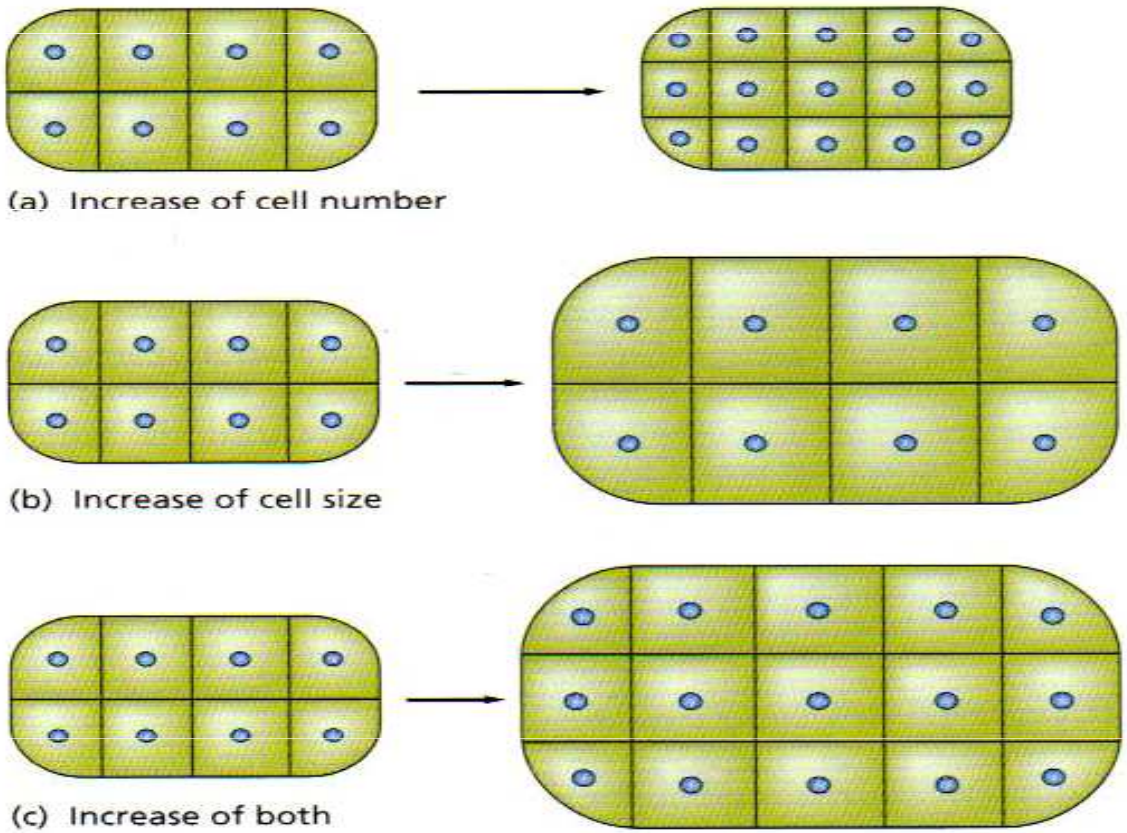
Growth is one of the least well understood aspects of development. By the time the general body plan is formed the whole embryo is about 1 mm long with each organ as a small rudiment. In a human, there is approximatelly 10^9-fold increase of volume when compared to 1 mm long embryo. This increase is driven by **cell division**, secretion of **extracellular matrix** and increase of **cell size**. The intracellular mechanisms that drive cell division are well understood as are the actions of growth factors, but overall growth control remains largely mysterious.

Such mysteries relate to the final size and why most animals stop growing (with exception of fish that have indeterminate growth) when reaching it. The cells in tissue culture grow exponentially in the presence of excess nutrients but this is rarely found in mammals. One general explanation of the final size is the limited nutrient supply that is restricted by the amount of terminal capillaries which can not grow as fast as the volume of the 3D object.

The other main issue is the coordination of growth between parts of the body. Small differences in growth rate between body parts over a 10^9-fold expansion would lead to very large changes in relative proportions. This suggests that there must be exist a mechanism that senses the overall size of the organism and regulates the growth of individual parts accordingly. The relationship between the growth of two parts of an organism can be represented as equation $y=bx^{\alpha}$ where y is the size of one part, x the size of the other and b and α are constants. There are many exceptions from this equation.

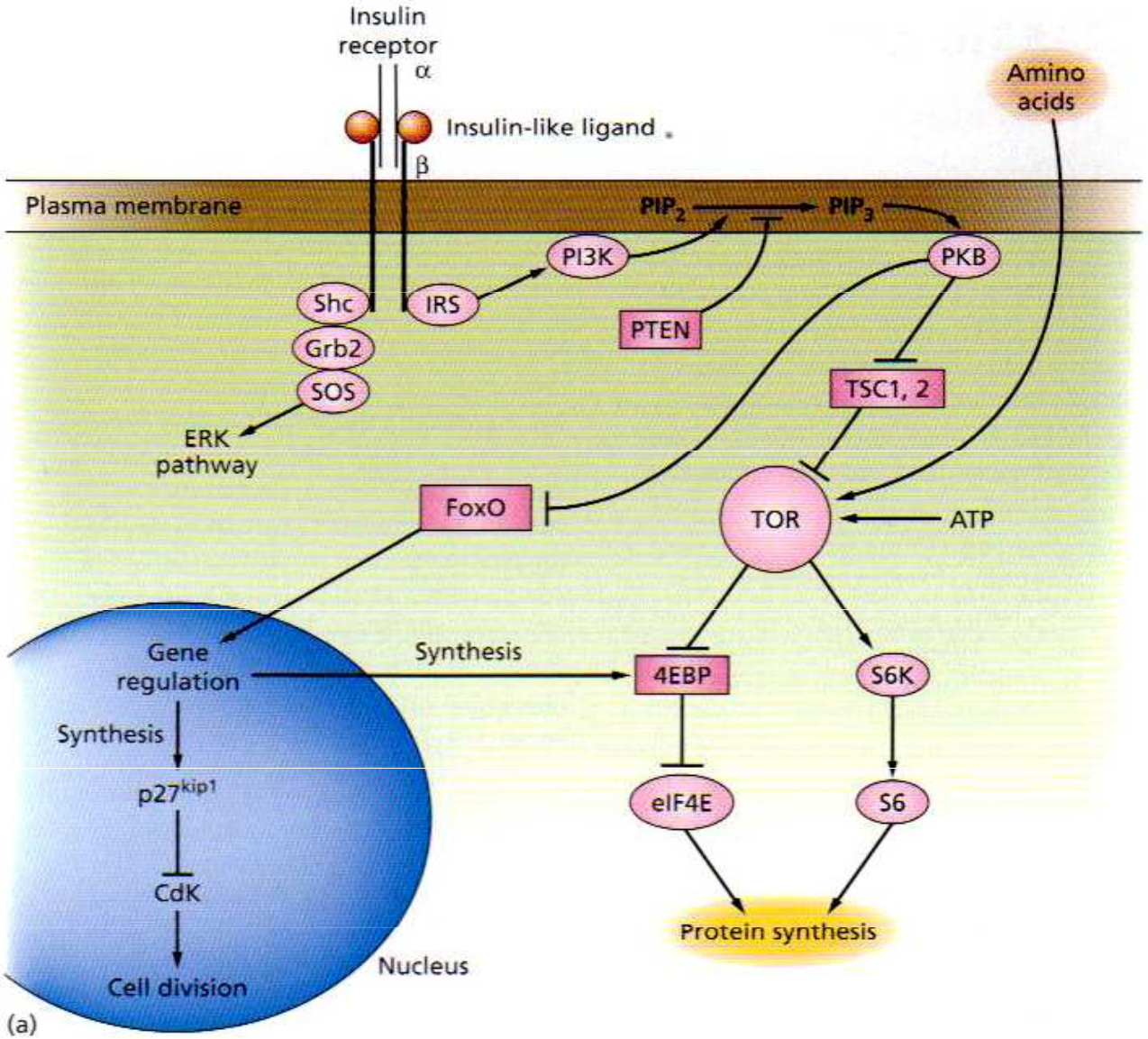


BIOCHEMISTRY OF GROWTH: Although the cell division is fundamental it is not the only factor driving growth. The cell division can be accelerated by overexpression of **E2F transcription factor** which is important for expression of S-phase genes. Conversely, the cell division rate can be slowed by expression of **Retinoblastoma** (Rb) protein, that inhibits endogenous E2F. But neither of these manipulations have much effect on overall size and proportion of the organ since the rate of cell size increase does not change. In normal situation, the rate of cell division must be coupled to cell size so that change in growth rate leads to altered volume of tissue.



INSULIN/IGF SIGNALING:
Insulin/IGF pathway together with the **TOR** (**target of rapamycin**) systém represent key mediators of cell size. The activation of both pathways leads to increased uptake of glucose, fatty acids and increased sythesis of glycogen. The pathway activity also promotes cell growth by suppressing its inhibitor **FoxO**.

IGF regulates the growth at the level of whole organism. IGF is made by many tissues upon the **growth hormone** action and stimulates the bone growth via increasing the proliferation of **growth plate cartilage** (growth zones of the long bones).

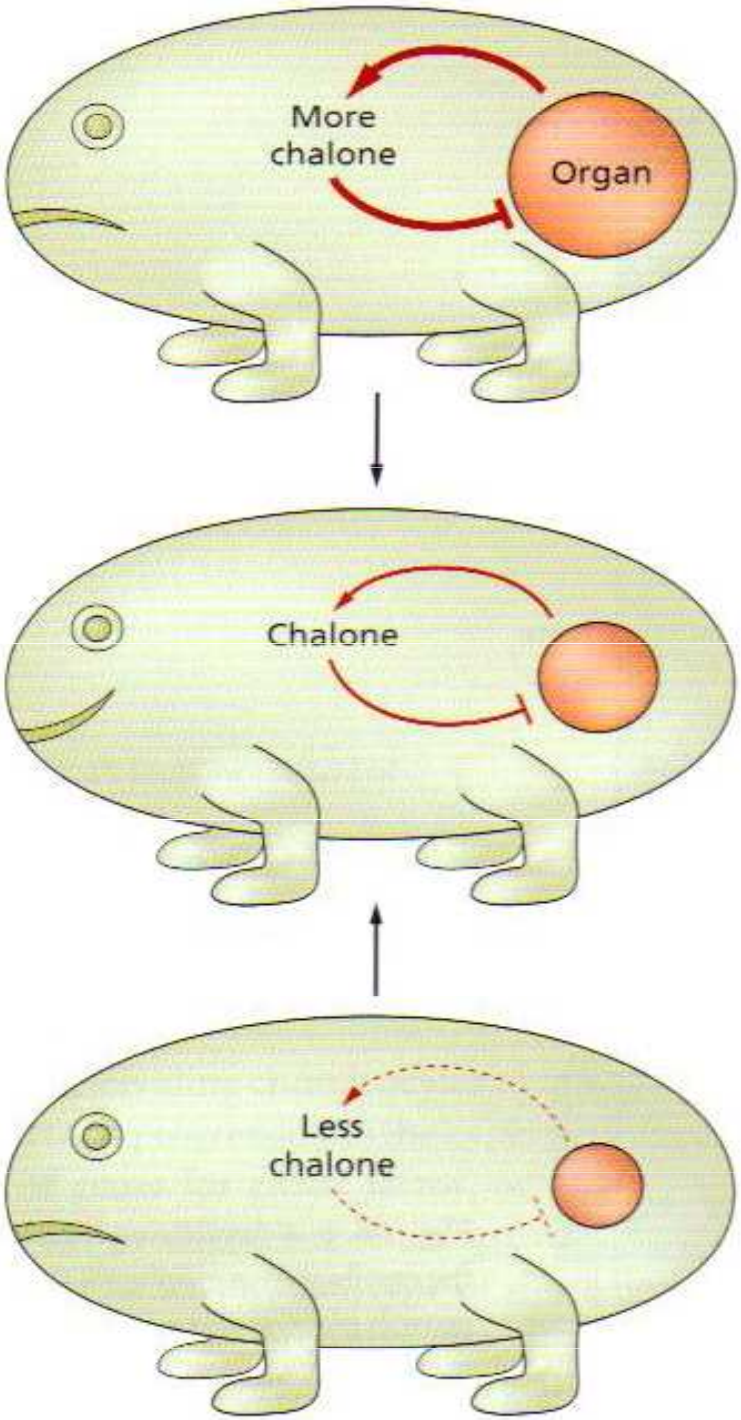


	Receptor	PI3K	PKB	TSC	TOR	FoxO	Protein synthesis
+ Ligand	Active	Active	Active	Inactive	Active	Inactive	Up
– Ligand	Inactive	Inactive	Inactive	Active	Inactive	Active	Down

(b)

CONTROL OF RELATIVE PROPORTION:

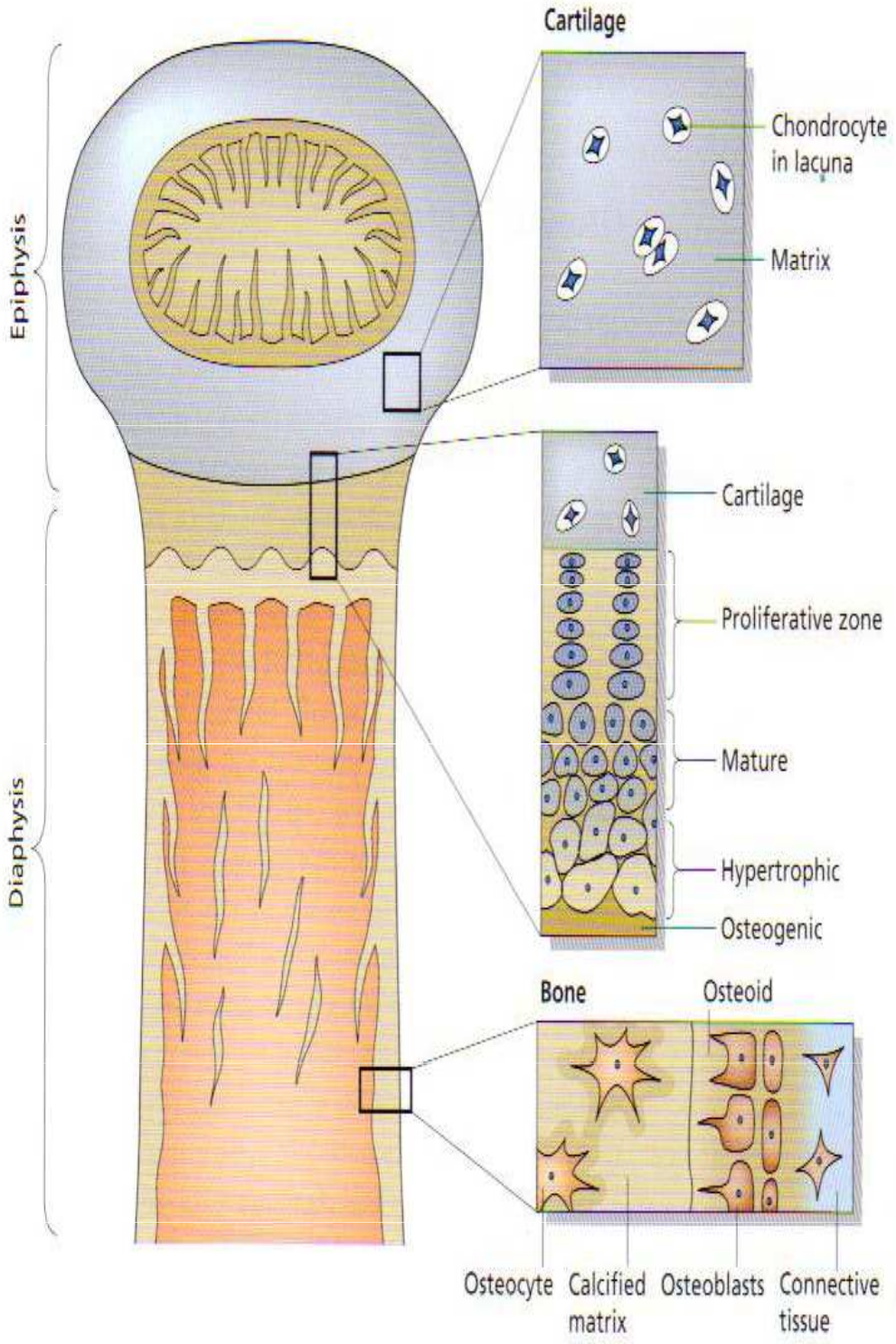
This is an important problem in growth control, even if the overall size of the organism is correct. How can it be guaranteed that different body parts expand in proportion to one another? The coordinated growth of body parts is explained by **feedback inhibition** of each body part on itself (chalone model). Arguing against the chalone, the grafts sometimes appear to grow autonomously. There are however examples of chalone action, such as **myostatin** (growth and differentiation factor 8, GDF8), that is produced by developing muscle cells and reduces both myoblast divisions and enlargement of myofibers.

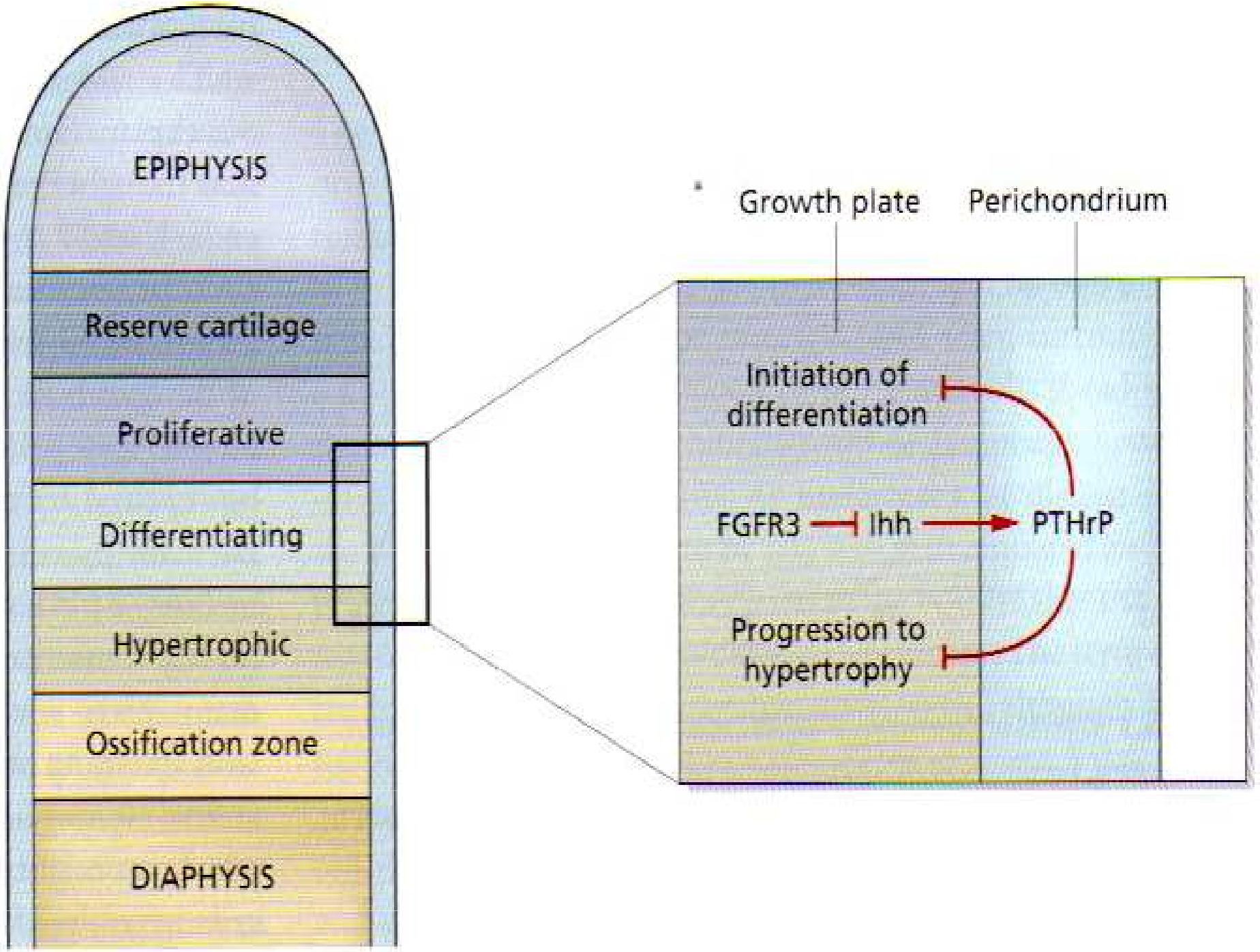


GROWTH IN STATURE: The height of human being is regulated by a long-bone growth. Long bones grow from their cartilage element that contains the zone of cartilage proliferation. Proliferating chondrocyte matures into the hyperthrophic chondrocyte that undergoes apoptosis and resulting space is invaded by **osteoblasts**. During the hyperthrophy, chondrocytes enlarge their volume. Chondrocyte proliferation and enlargement each contribute about 50% to the bone growth.

In the cartilage development, **Sox9** represents the key transcription factor, whereas the transcription factor driving osteogenesis is **Cbfa1** (Runx2).

Within the growing cartilage, the proliferation of chondrocytes is maintained by **Ihh positive feedback loop**, and negative regulated by fibroblast growth factor **(FGF)** system.




EPIPHYSIS
Reserve cartilage
Proliferative
Differentiating
Hypertrophic
Ossification zone
DIAPHYSIS
Growth plate
Perichondrium
Initiation of differentiation
FGFR3
Ihh
PTHrP
Progression to hypertrophy

AGING: Is aging a developmental process or not? The argument in favor is that both events are **pre-programme**d and under developmental control. The argument against is that such events are due to the **random mutational damage**. Seems that both positions are correct to some extent. Remarkably, the aging can be manipulated through alteration of the **insulin signaling**, that is also critical for growth regulation. This was originally found in *D. elegans* during the studies of so called **dauer larva** (non-feeding stage that develops when nutrients are limited). The animals with loss-of-function of the insulin pathway genes develop dauer whereas hypomorphic alleles of the same genes live much longer than normal animals. The general anti-aging effect of insulin signaling lies in repression of **FoxO**, that is a transcription factor necessary for production of proteins reducing the oxidative stress such as catalase and superoxide dismutase.

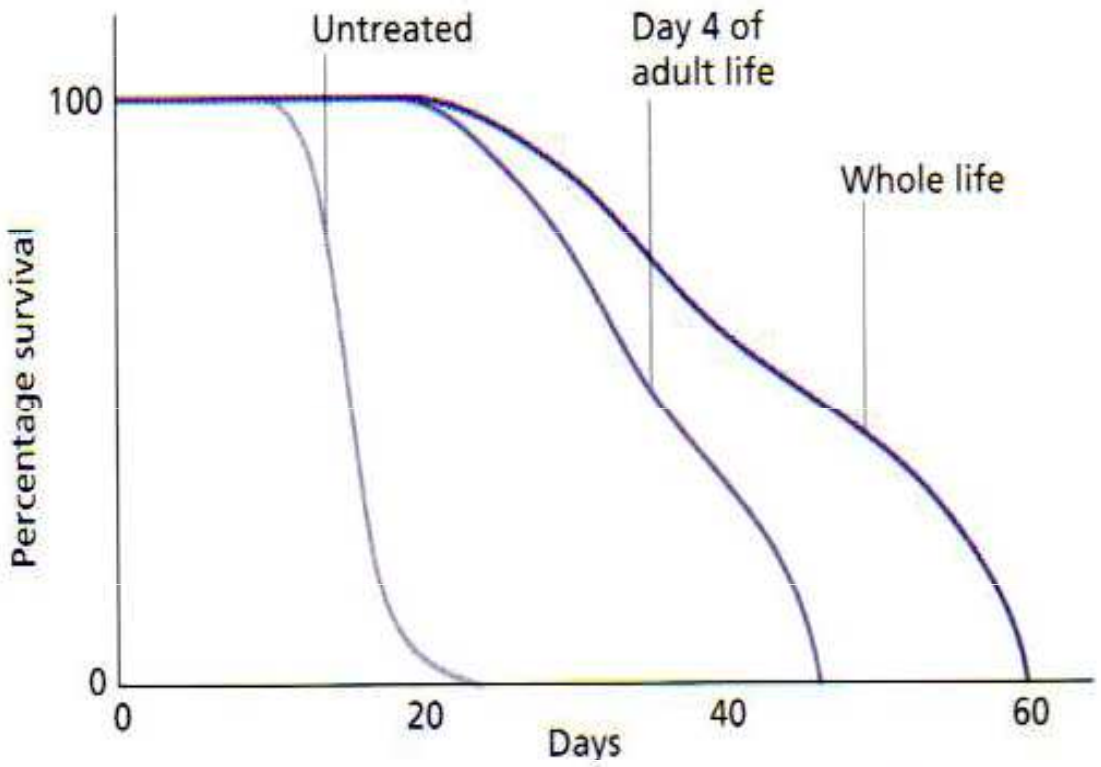
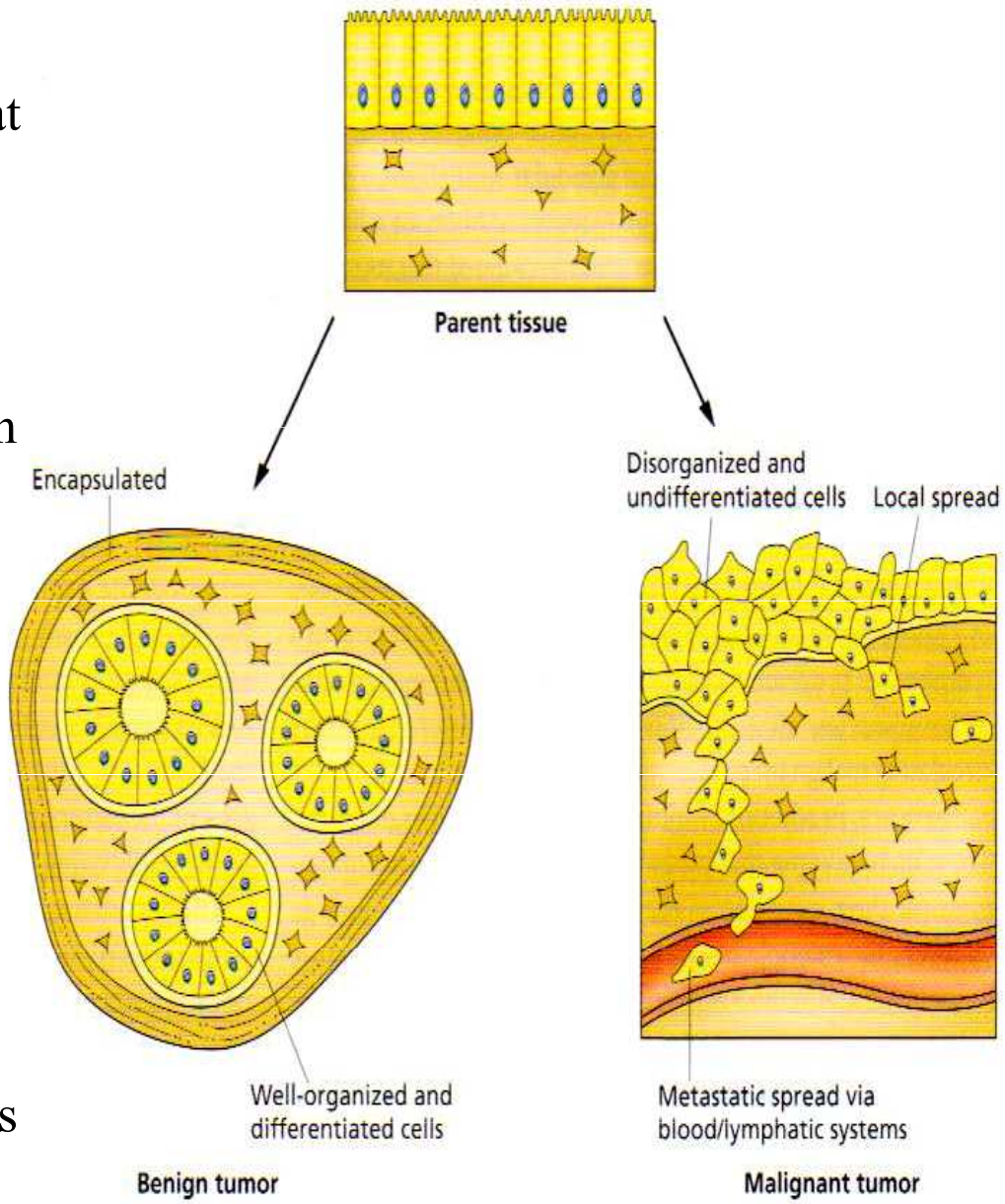


Fig. 18.7 Effects of downregulating the insulin pathway on the lifespan of *C. elegans*. Feeding with RNAi to insulin receptor will extend life substantially if started before the last molt, but the effect falls off the later in adult life the treatment is started.

POSTNATAL DISORDERS OF GROWTH AND DIFFERENTIATION

Hyperplasia: excessive production of cells that are normally differentiated. Physiological – mammary epithelia in pregnancy. Pathological – goiter – the hyperplasia of the thyroid due to the lack of iodine. **Metaplasia:** the conversion of one tissue to another, usually associated with tissue regeneration provoked by trauma or infection. The examples are squamous metaplasia of the bronchus that is common for smokers, intestinal metaplasia in stomach that is associated with ulcers, and cartilage metaplasia in surgical scars. **Neoplasm:** fundamentally a growth of the body's own tissue to an inappropriate extent or in an inappropriate place. Benign tumor is localized to the site of origin, often surrounded by fibrous capsule. Malignant tumor sends its cells to the surroundings or, through blood of lymphatic system, to distant tissues. The term cancer is usually used for malignant tumors.



REGENERATION OF MISSING PARTS

DISTRIBUTION OF REGENERATIVE CAPACITY

The studies of the ability to re-grow missing parts is fascinating frontier of the developmental biology with many unsolved problems.

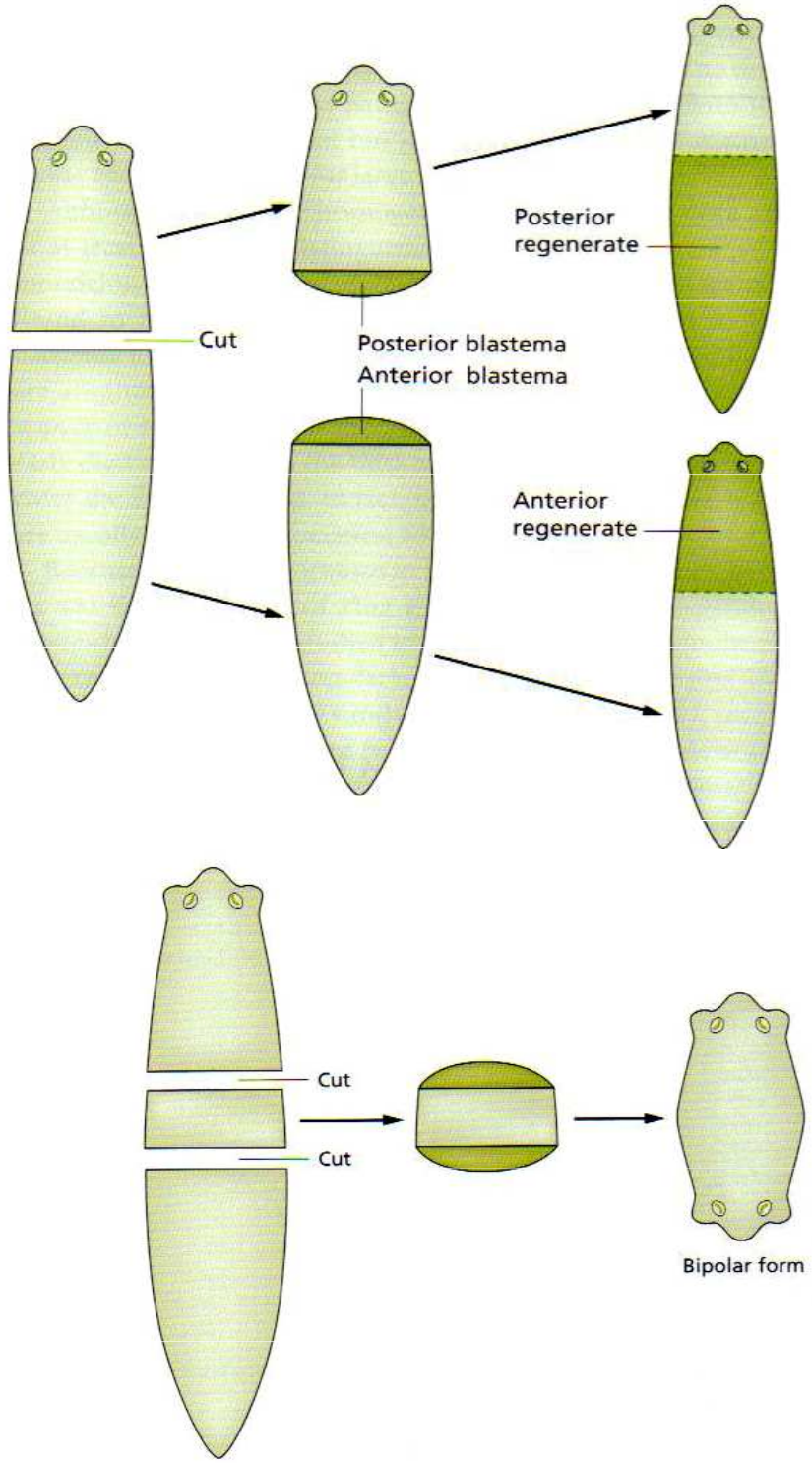
The most dramatic type of regeneration is the whole body regeneration – an ability to re-grow the main body axis following a severing of the main body such as demonstrated in nemertean worms. Planarians and similarly *Hydra* can re-grow new body from an anterior facing cut. The whole body regeneration is bi-directional and is usually associated with asexual reproduction, where body fragments into two parts that can reconstitute the whole individual.

Neither insects nor vertebrates show any bidirectional regeneration, some however, can mono-directionally regenerate the appendages. In insects, the regeneration of external appendages is confined to Hemimetabola that progress to adulthood through a larval forms. No regeneration of any adult structures is possible in Holometabola, which mature through abrupt metamorphosis in a pupal phase. Analogically, the amphibians regenerate limbs, tails, and jaws whereas the higher vertebrates can not regenerate with exception of deer antlers.

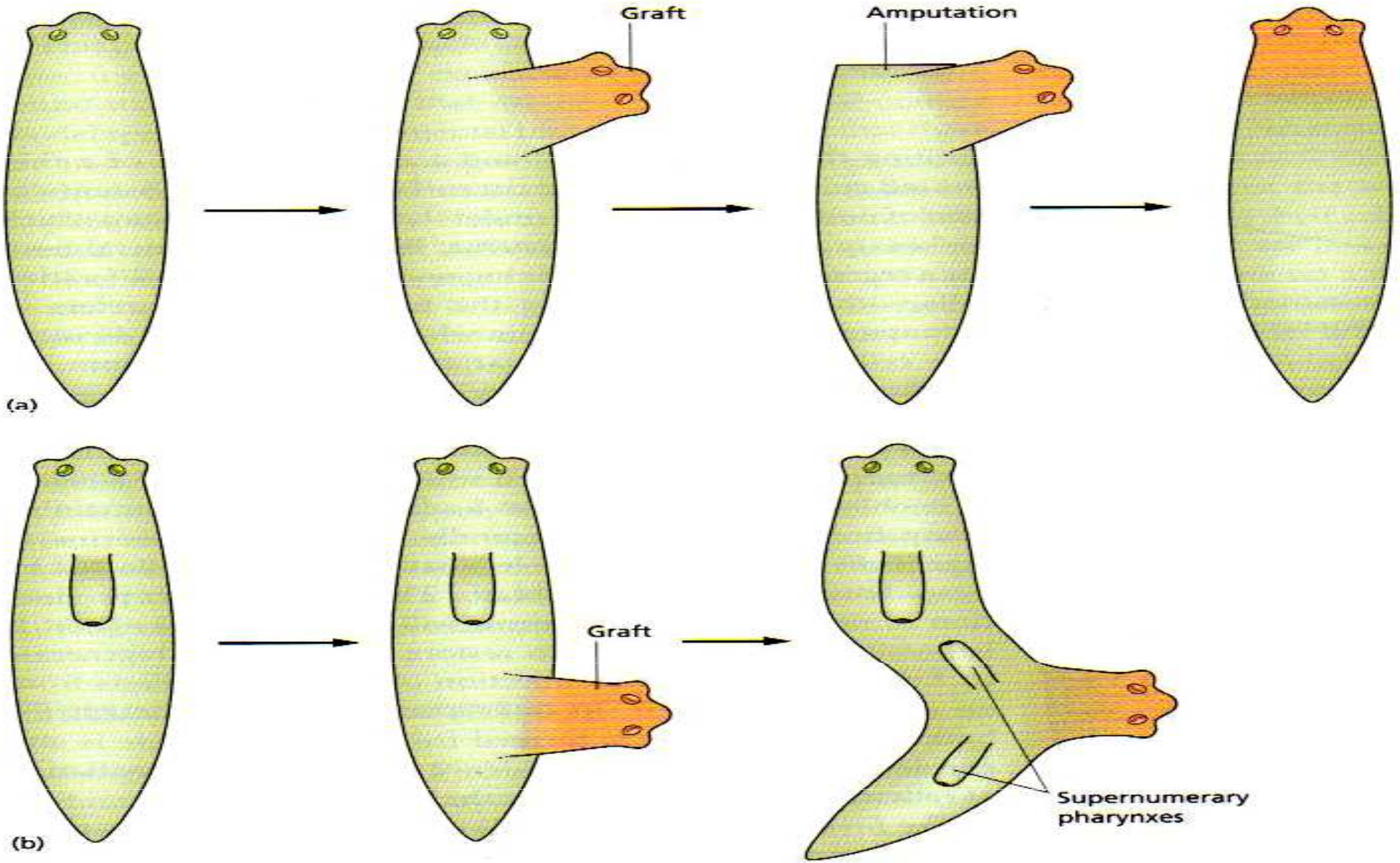
Among mammals, the so called regeneration of some damaged organs, such as liver or kidney, is rather a hyperplasia since the organs restores its volume but not its shape.

REGENERATION IN PLANARIANS: Being the simplest animals with bilateral symmetry, planarians are in a constant cell turnover. Their bodies contain up to 20% of so called neoblasts, characterized by the expression of ATP-dependent RNA helicase similar to Drosophila vasa protein. Neoblasts divide and contain the population of totipotent cells that can form all 15 cell types of the planarian tissues.

Following transection, there is a muscular contraction limiting the area of the cut followed by the formation of the wound epithelia that makes up regeneration blastema. The blastema enlarges and redifferentiates to form missing structures. The mechanism of a polarity decision, whether to be a head or tail, is poorly understood and does not likely involve the Hox genes.



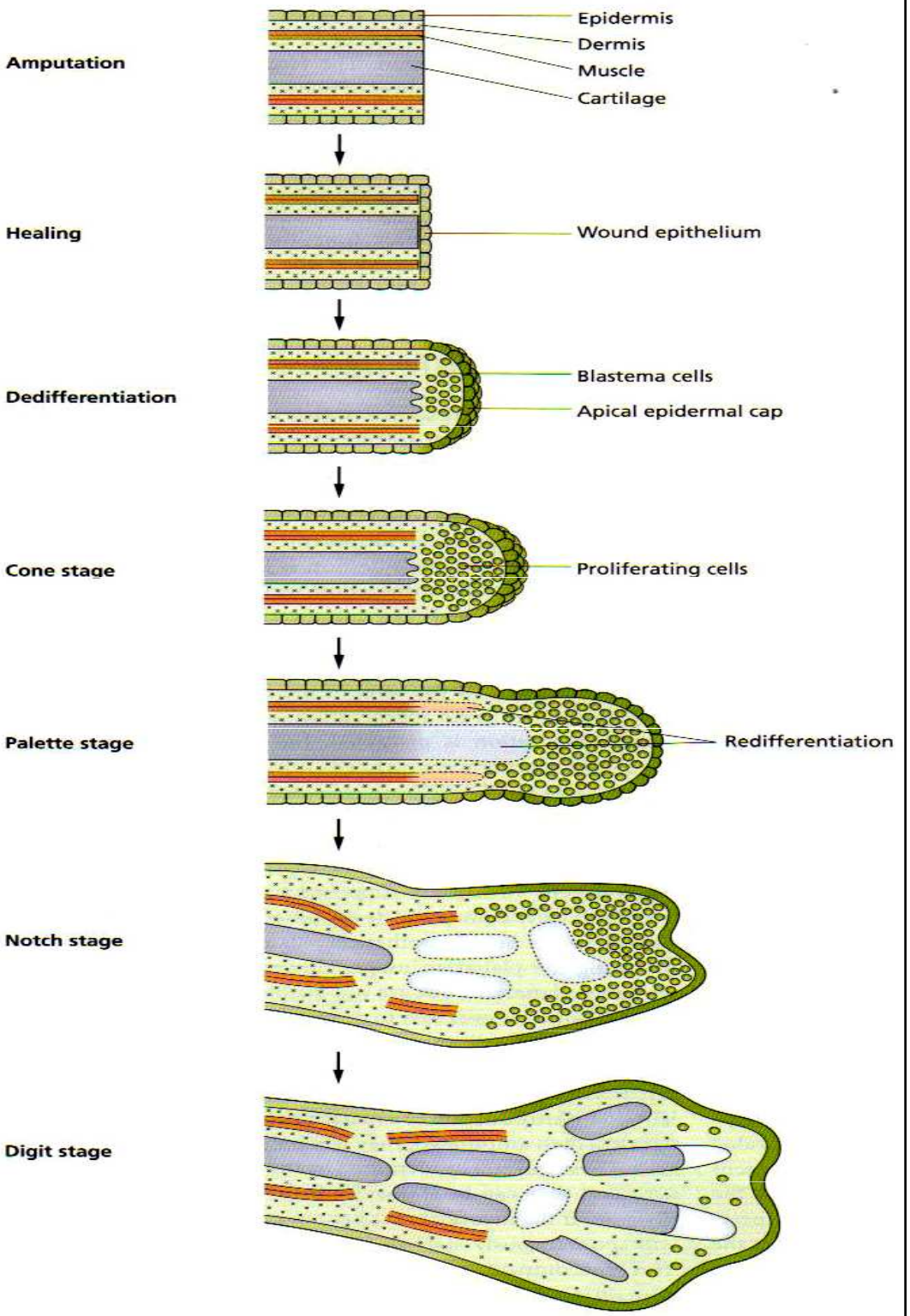
There is evidence for long term interactions in planarian regeneration, such as suppression of head regeneration by grafted head and induction of extra pharynges by grafted head. The nature of signals involved in these interactions is presently unknown.



VERTEBRATE LIMB REGENERATION:

Among the vertebrates only certain amphibians can regenerate limbs after surgical removal. These include anuran tadpoles that can regenerate limbs before they reach the metamorphosis as well as many urodele species that regenerate limbs during both larval and adult life.

After limb amputation, a wound epithelia forms via migration of epidermal cells over the cut surface followed by dedifferentiation of an underlying tissue. The blastema consists of loose-packed mesenchymal cells surrounded by thick epidermal jacket. The blastema proliferates and then the limb structures redifferentiate in the proximal-distal sequence.

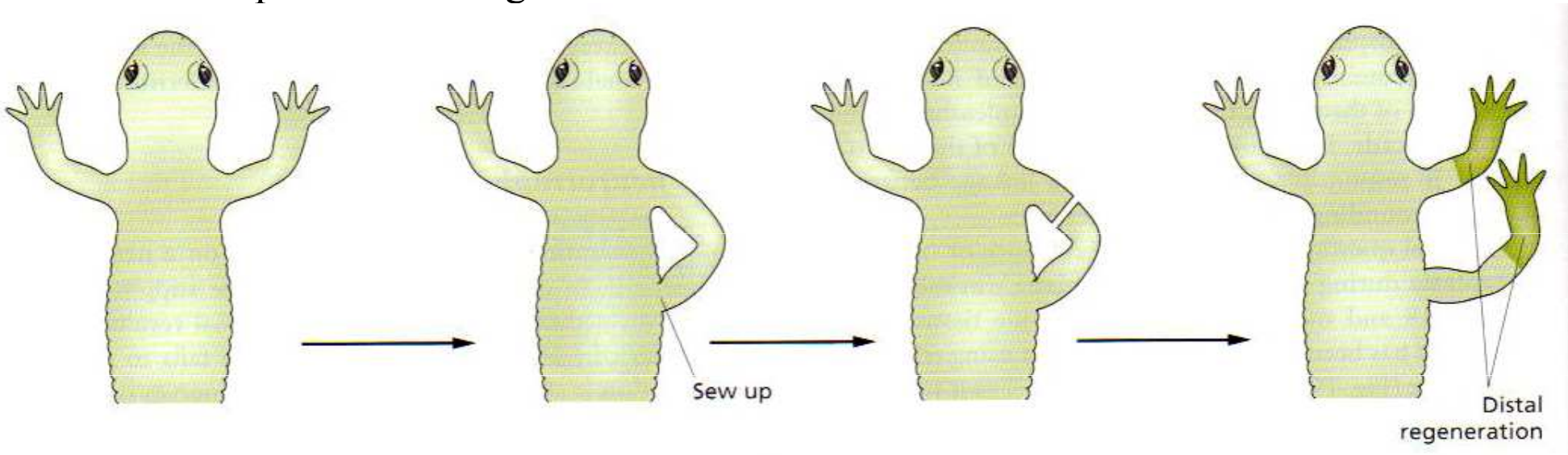


THE SOURCE OF REGENERATING CELLS: The blastema cells originate from the cells no further than few milimeters from the cut surface – **local origin**. The cells for regeneration may stem from the reserve cells **- neoblasts** – that are hypothetical pluripotent cells scattered throughout the tissue. Or they may originate from differentiated cells through a process of dedifferentiation. This is evidenced, for instance, by newt myofibers that, upon limb amputation, break into the mononucleate cells and start proliferation.

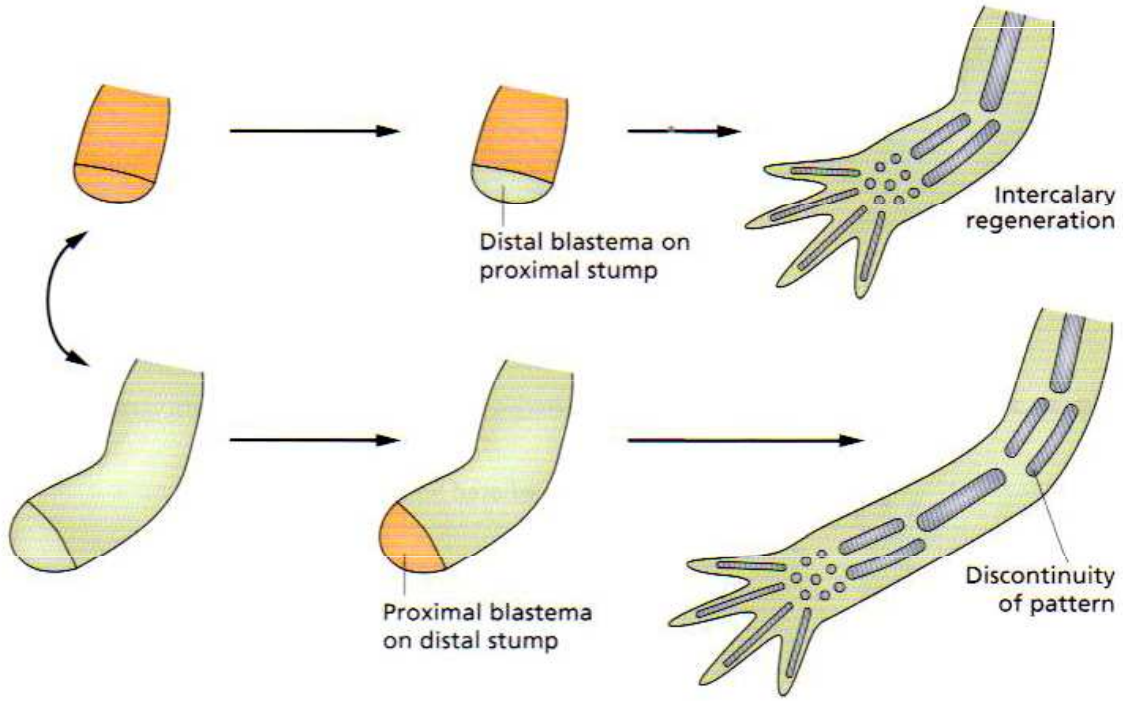
Are the regenerating cells uni-, pluri- or totipotent? The experiments show that there are three tissue lineages, epidermal, muscle and connective tissue. Only connective tissue is capable of extensive hypoplasia. For instance, cartilage can give rise to dermis, tendons, ligaments and fibrous tissues.

THE NEUROTROPIC FACTOR: The limb regeneration is absolutely dependent on nerve supply without which the regeneration stops at the blastema stage. The function of the nerve is to release the mitogenic factors that normally function to keep neurons alive (neurotropic). These factors include FGFs and neuregulins.

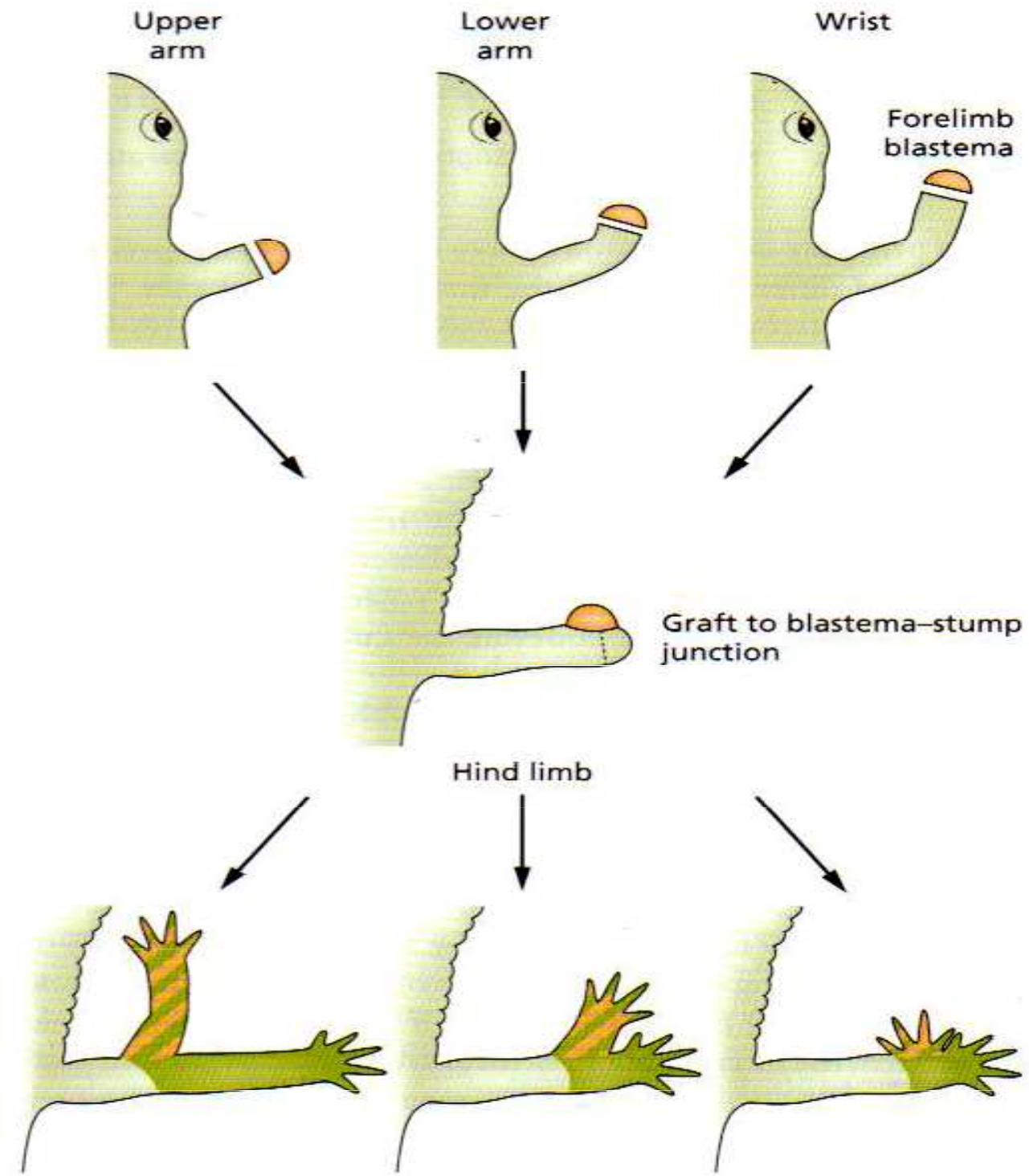
REGENERATION OF REGIONAL PATTERN: The pattern of regeneration ensures the all parts that are distal to the cut are replaced. Proximal regeneration does not occur, even when the cut is proximal facing.



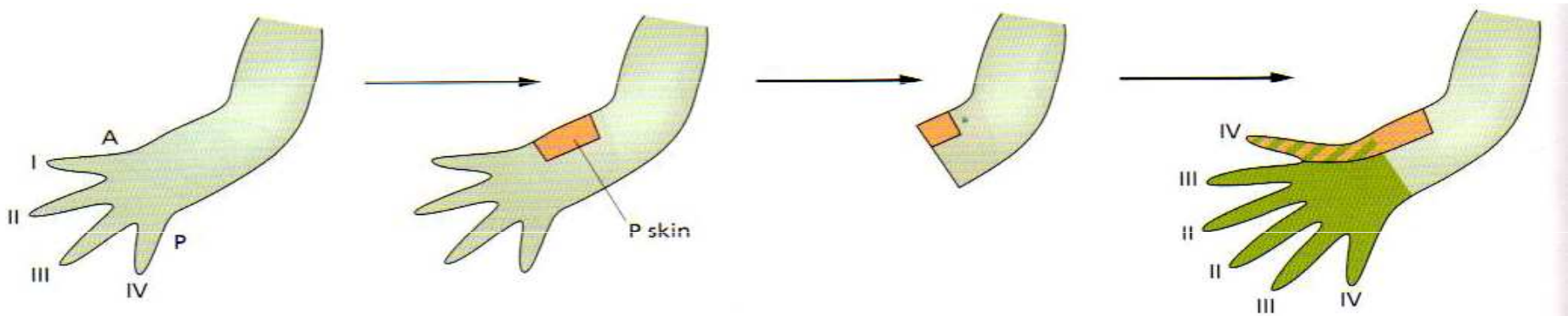
In the amphibian limb the intercalary regeneration is also possible, that means regeneration at the tissue junction between parts that are not normally neighbors.



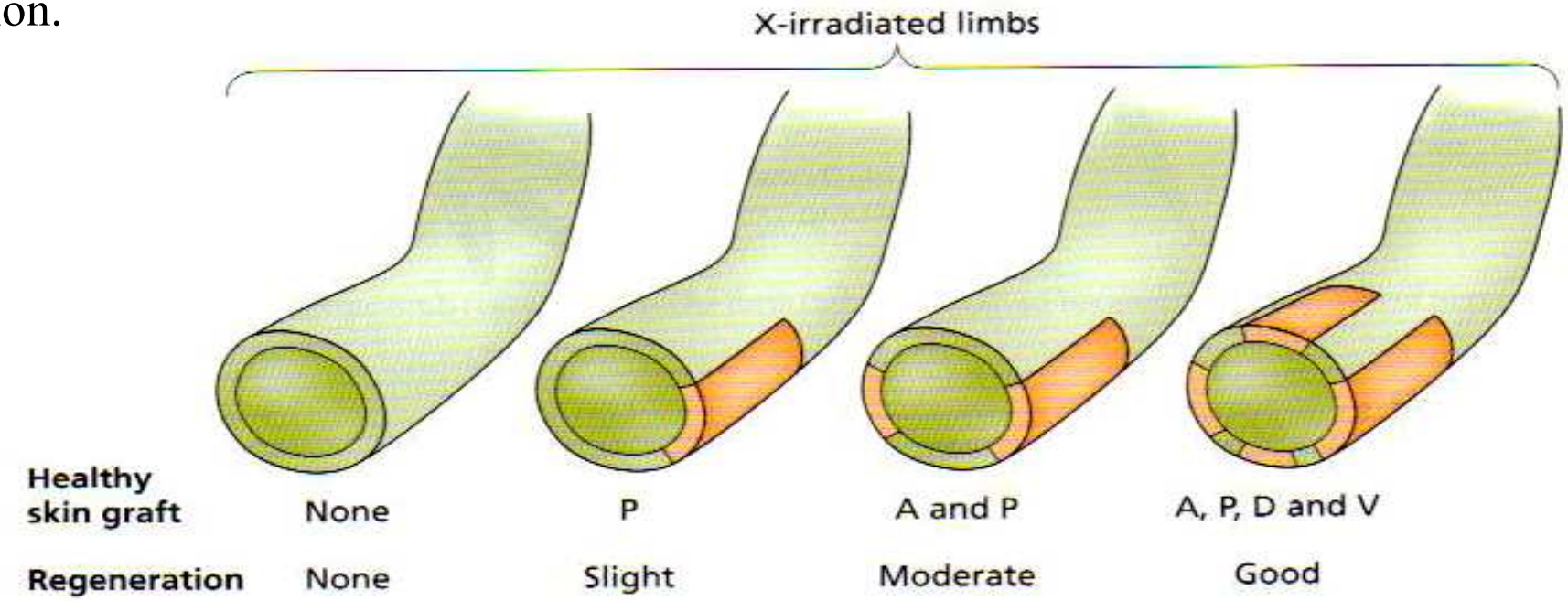
Experiments show that limb tissue carry specific codes for regional identity, called **positional values**, that specify which structure will be formed on differentiation. Molecular candidates for positional value signals are CD59 (membrane-anchored inhibitor of complement) and Meis transcription factors.



The limb is however asymmetrical in three dimensions and thus needs three sets of codes to specify the pattern of differentiation in the three anatomical axes: proximodistal, anteroposterior and dorsoventral. There are two well-established principles that summarize the operation of these interactions in the anteroposterior and dorsoventral axes. The principle of **intercalation** states that when blastema is from two regions with discontinuity in their codes, the regenerate will fill in the gap with structures that would normally form in between the two regions.



The other principle is that some degree of **discontinuity** of codes is necessary to initiate distal regeneration.



EVOLUTION AND DEVELOPMENT

The interface between the evolution and development has long history since the **Ernst Haeckel's** theory of Ontogeny Recapitulating the Phylogeny. This theory fits well with the Lamarkian view of evolution in which heritable changes could arise from the life experience. Later on, at the beginning of 20th century, the Neodarwinism became generally accepted. This is a synthesis of **Darwin'**s evolutionary theory of natural selection, **Mendel**'s genetics and the quantitative mathematical theory of genetics developed by **Fisher**, **Wright** and **Haldane**.

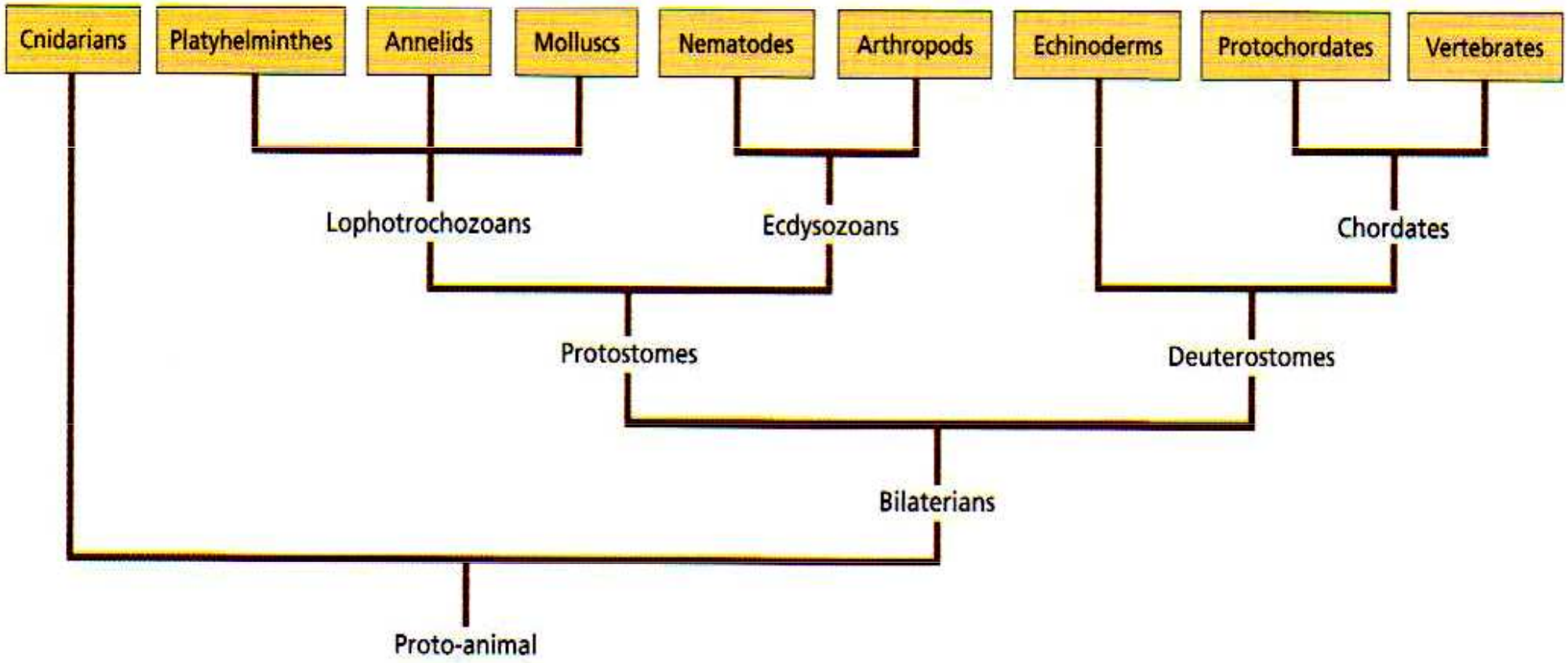
According to Neodarwinism, morphological changes appear gradually as a result of action of number of mutations each having a small effect. Changes arising from natural selection are called adaptive evolution or adaptation. Lately, in the second part of the 20th century, it became obvious that a great deal of changes in DNA is not adaptive but neutral evolution, consisting of accumulation of mutations of no selective consequence that spread through the population by the effect of random sampling of alleles from one generation to next (genetic drift).

The molecular understanding of development had an important impact on evolutionary biology because it answered questions that could not be answered before. The most impressive example is the ability to reconstruct a credible model of remote ancestors way beyond the reach of comparative morphology. Developmental biology also contributed to understanding of phylogeny of animals.

MACROEVOLUTION: (=evolution above the species level) Phyla – the highest animal taxa (currently 35). The highest animal taxa are the phyla (singular phyllum). Presently there are some 35 phyla. The vertebrates and insects, the most important groups for developmental biology are not phyla but classes. Vertebrates belong to phylum Chordata whereas insects form the largest class of phylum Arthropoda.

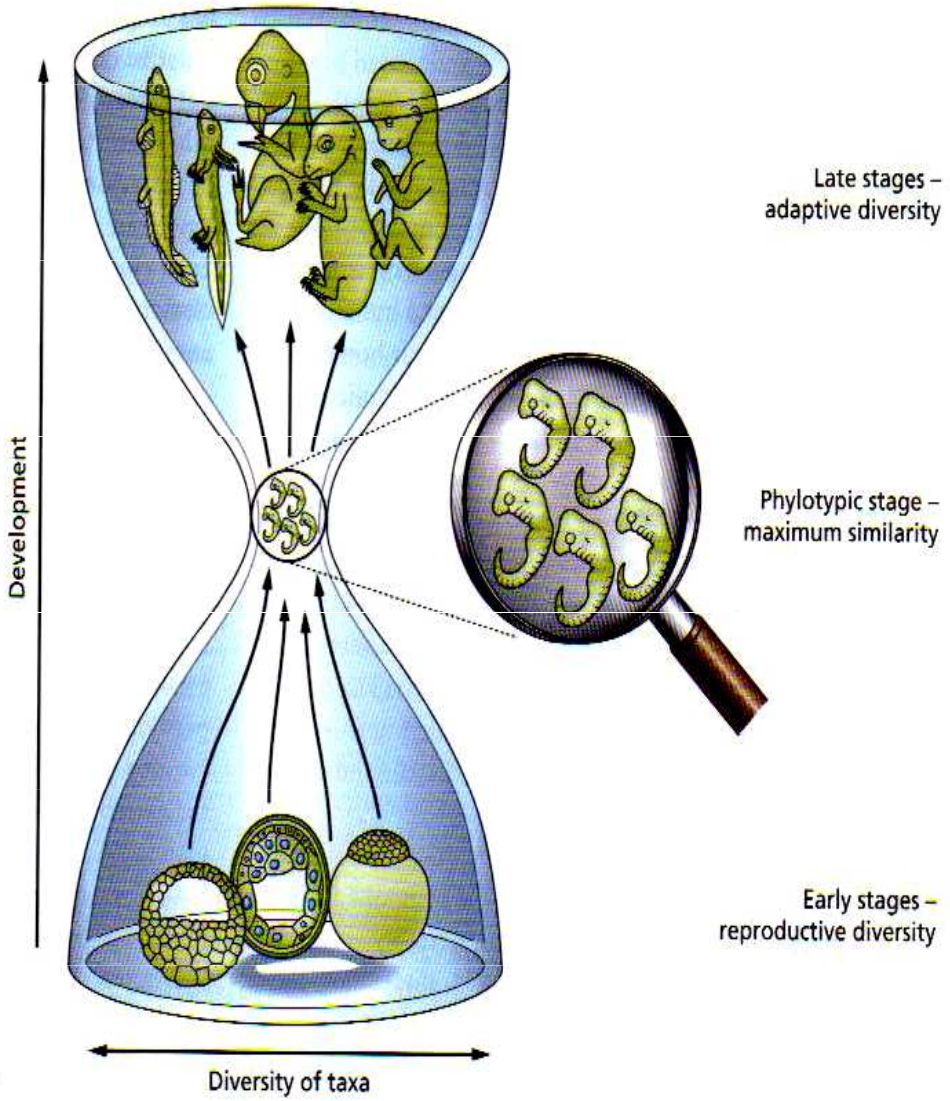
A classification systém – **taxonomy** – can be completely arbitrary but still useful when it allows for unambiguous identification of specimens. There is hovewer an attempt to make taxonomy congruent with the actual evolutionary history of the organisms. According to this a taxon should be **clade** consisting of complete set of descendants from a common ancestor. Certain well-known groups are not clades but are tolerated for the reason of familiarity. For instance reptiles are not a clade because they do not contain birds that are descendants from within the reptiles and not a separate group. Regardless the classification (and opinionated old professors) there is only one "true tree" that follows exactly the bifurcating branches as they occurred in evolution. This is called **phylogenetic tree** whether it deals with phyla themselves or with lower-level taxa. Each node of the phylogenetic tree should correspond to a population of **real ancestors** that split to give rise to the two branches. Importantly, this is only literally true at the level of **species**, because what actually evolves are species (in a sense of interbreeding population of organisms).

There are two types of similarity between different organisms – **homology** and **analogy**. Homologous structures not only look similar but also are descendent from a common ancestor possessing the ancestral version of the structure in question. An example is the **tetrapod limb** – many types of vertebrates from crocodiles to humans have limbs that are similar in the arrangement of the bones and muscles and general position in the body. By contrast, when two structures are analogous, there is no common ancestry and the parts look similar because the pressure of natural selection had forced a convergence of structure to meet the need for a similar function. An example is the insect wing versus the wing of bird. Note, however, that although the wings themselves can not be homologous, the genes or genetic pathways involved in formation of insect and bird wing may be homologous.



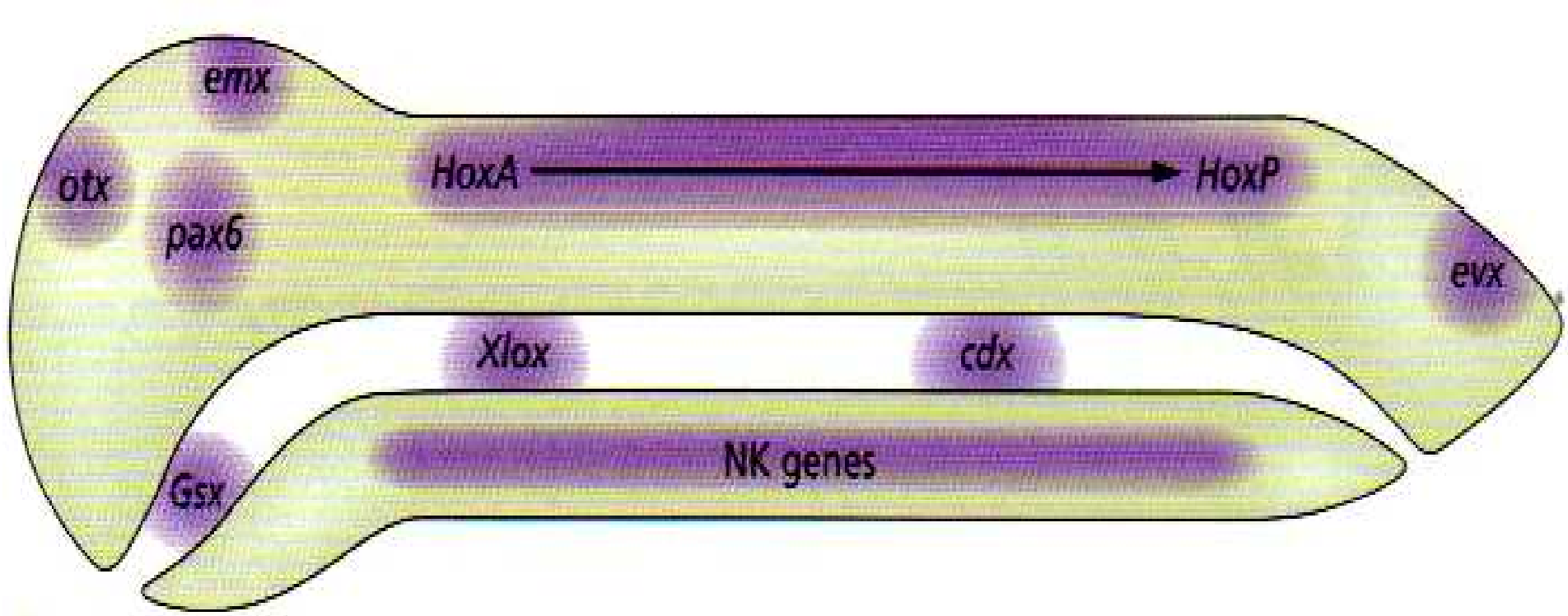
THE PRIMORDIAL ANIMAL

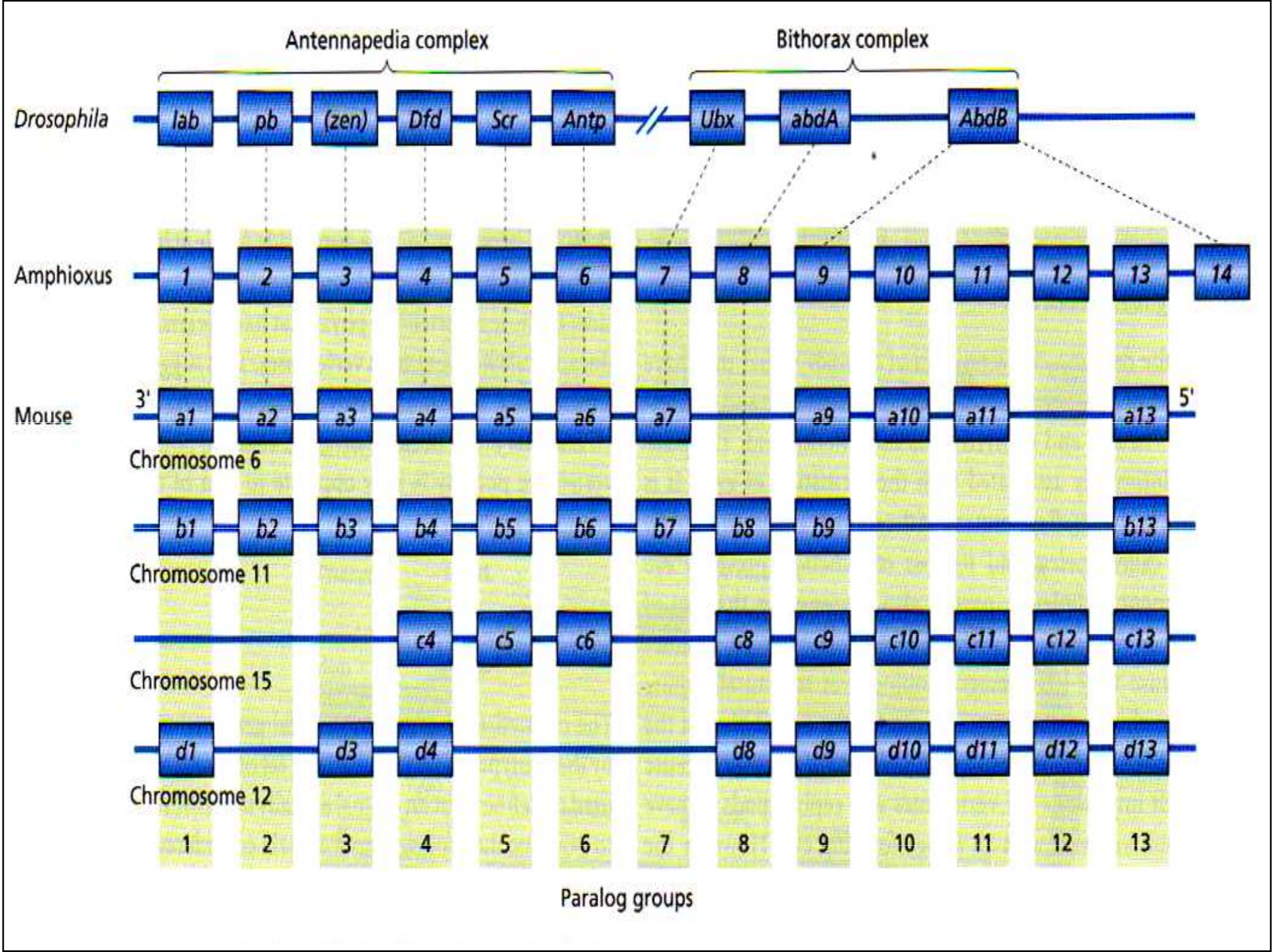
The most important result of evolutionary developmental biology is the ability to uncover previously invisible homologies through the study of expression and function of the genes that make the **body plan**. The body plan refers to the idea that is possible to abstract the essential features of anatomical organization from a wide range of organisms. For instance, the expression of the **brachyury** gene in vertebrates occurs in differently shaped reagions in *Xenopus*, chick and mouse but they all correspond to what was previously a mesoderm. Thus the mesoderm is a real cell state definable by the expression of brachyury.



THE PHYLOTYPIC STAGE: A stage in development at which all members of taxon show maximal morphological similarity. Among vertebrates, the phylotypic stage is the **tailbud** stage when all vertebrates have a dorsal nerve cord, segmented somites, ventral heart, and a set of pharyngeal arches.

THE ZOOTYPE: Animal phyla have been defined in such way that each of them corresponds to a different body plan which means that it is difficult to find any morhological homologies among them. However the developmental biology now makes it possible to compare the expression patterns of key developmental genes between phyla. Some of theese seem to be conserved across the whole animal kingdom. They are active around the phylotypic stage for all the main animal groups examined. The totality of common expression domains is called the **zootype**. This cryptic anatomy of developmental gene expression patterns defines of what an animal actually is.




Antennapedia complex
Bithorax complex
Drosophila
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pb
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Dfd
Scr
Antp
Ubx
abdA
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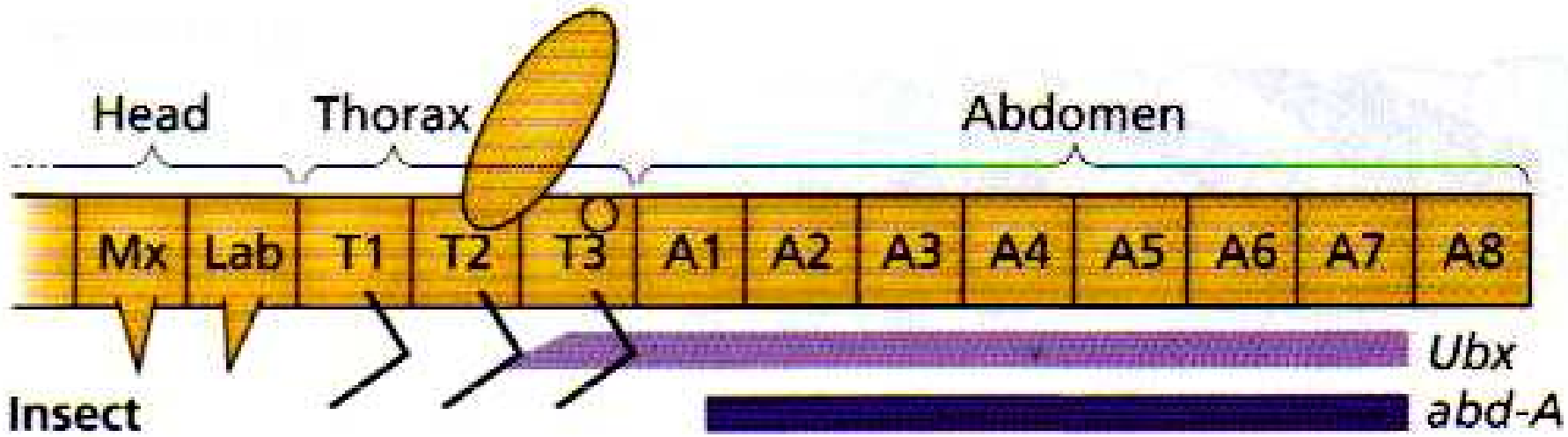
Paralog groups

SENSE ORGAN AND CELL TYPES: One of the defining characteristics of animals is that they have their sense organs concentrated at anterior end, representing a „head“. The ***pax6*** gene is essential for formation of eyes, its loss in Drosophila leads to eyeless fly, its partiall loss in mouse leads to small eyes phenotype. The *pax6* is also expressed in eye of cephalopods. Cephalopods are significant in having the eyes quite similar to vertebrates and this has always been an example of **analogy**, because it was difficult to comprehend a common ancestor with image-forming eyes. But the *pax6* expression suggests that they really are **homologous** structures al least to extent that the ancestor had some sort of photoreceptor made with the help of *pax6*. An example of developmental control gene conservation between vertebrates and insects can be the **myogenic gene family**, which prototype ***MyoD*** controls muscle development in all animals.

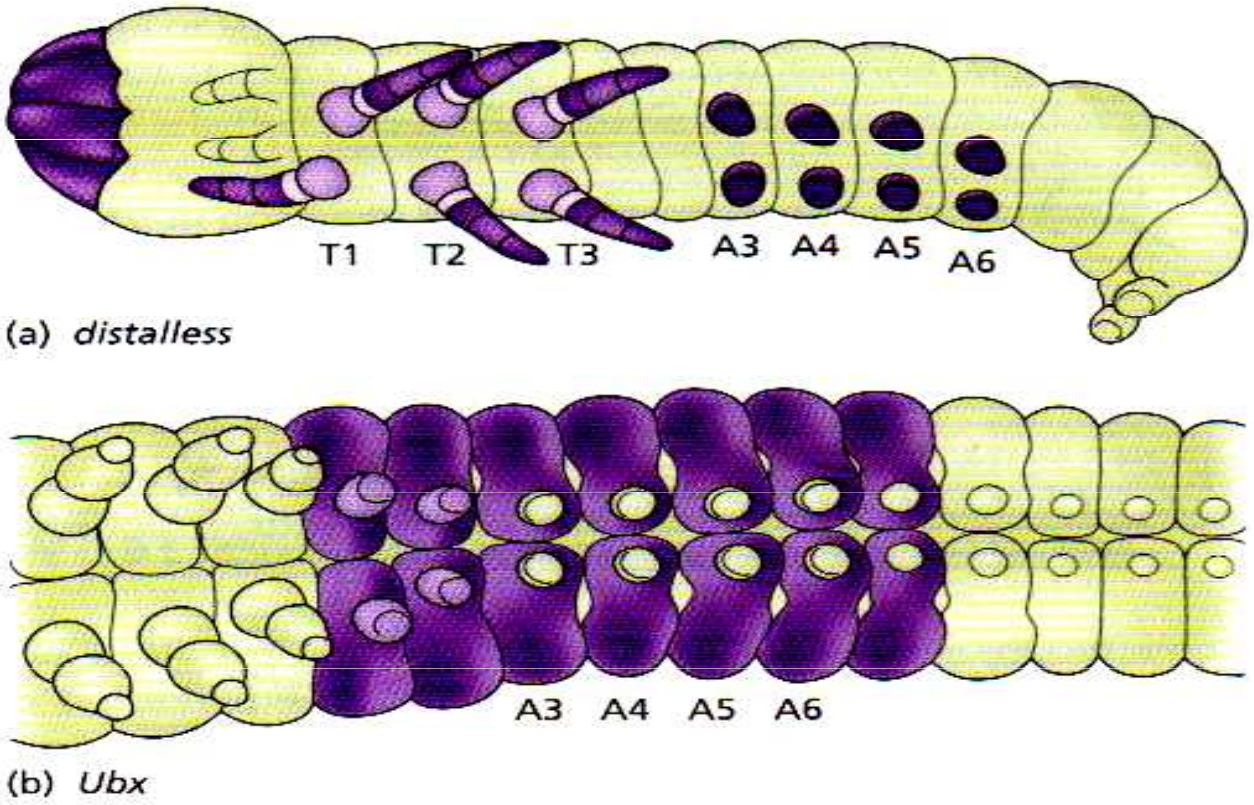
WHAT IS AN ANIMAL? The activity of developmental genes represented by zootype is a set of characters that can be used to define what animal actually is. It is however necessary to examine the most basal animals to find whether this notion is congruent with the traditional zoology. Cnidarians have traditionally been considered as basal animals since they only posses two germ layers (ecto- and endoderm) and that they are radially instead of bilaterally symmetrical. Cnidarians do have Hox genes with at least some of them foming a linked cluster. Moreover, they sometimes posses elements of bilateral symmetry and develop a cell layer called the entocodon, that expresses homologs of mesoderm-specific genes (*twist*, *snail* and *mef2*). In any case, the molecular analysis of cnidarians have shown that they correspond to the zootype and thus are definitely animals. The latter can not be concluded about the most basal animals in modern phylogenies – the Porifera. Since they do not have any orthologs of Hox genes, they do not share the animal zootype and should not be considered as animals.

WHAT REALLY HAPPENED IN EVOLUTION: Another area where developmental biology can assist in solution of evolutionary problems is to find out what really happened in evolution. An evidence about this can be obtained by comparing the expression pattern of key developmental genes that give rise to two different morphologies, one ancestral and one derived.

Segmented body plans: The arthropods represent very large phylum containing four classes: insects, crustaceans, myriapods (centipedes) and chelicerates (spiders and scorpions). Each class has fundamentally different body plan but, by both morphological and molecular phylogeny, they are a clade that evolved from common ancestor. The changes in body pattern between the classes originate in differences of Hox gene expression, that controls the identity of each body segment. In particular, the boundary between thorax and abdomen is controled by genes *Ubx* and *abdA* in *Drosophila* that inhibit formation of legs in the abdomen through repression of *distalless* homeobox gene that is required for development of all appendages.

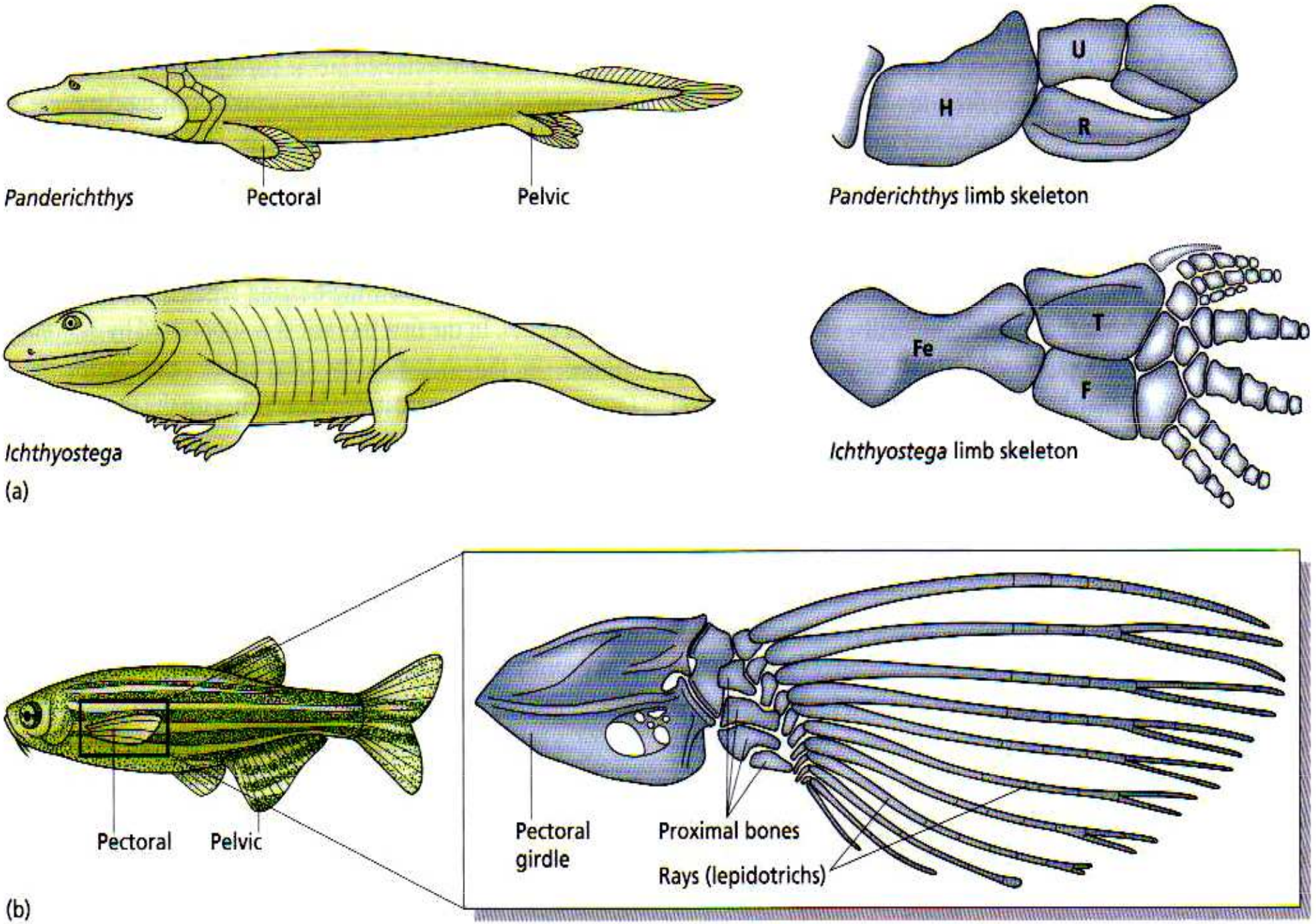


Atavism – a mutation that yields a morphology characteristic of an ancestor. Since much of the diversification of arthropods depended on suppression of appendages by Hox activity, it is expected that loss-of-function mutations of Hox genes will have atavistic phenotypes. Example is the four-winged *Drosophila* resulting from complete loss of *Ubx* from the haltere disc. Atavisms may sometimes become established as wild-types thus leading to **reversal** of the evolution. For instance the caterpillars of butterflies have legs on four of their abdominal segments (A3-A6). It is thought that primordial arthropod had legs on most segments, like modern myriapods. When the expression of *Ubx*, *abdA* and *distalless* are examined in betterflies it appears that the Hox genes are turned off in the leg buds that allows for distalless expression and formation of atavistic legs.



VERTEBRATE LIMBS: The vertebrate limb has a distinquished history in evolutionary biology. It a homologous structure across the tetrapoda and provides a striking example of **allometry** – a differential growth.

It is believed that the first tetrapods resembled the creature called *Panderichthys*, whose paired fins possesed skeletal elements similar to proximal elements of modern limbs but no **autopodium** (hand or foot). The paired fins of modern fish still contain the basic developmental machinery required to make a limb. Zebrafish fin buds have an early phase of outgrowth that is similar to tetrapod limb bud, regulated by the same genes, *fgfs*, *shh*, *wnt2b* and *tbx5*. These patterns suggest that the basic machinery for specifying the limb bud was present in primitive fish fins and therefore predated the tetrapods by a considerable period of time.


Panderichthys
Pectoral
Pelvic
U
H
R
Panderichthys limb skeleton
Ichthyostega
Fe
T
F
Ichthyostega limb skeleton
(a)
Pectoral
Pelvic
Pectoral girdle
Proximal bones
Rays (lepidotrichs)
(b)

LIMB PRESENCE AND POSITIONING: Some tetrapods such as snakes, whales and flightless birds have lost their limbs. Often a rudimentary portion of the limb skeleton survives indicating that the ancestor had more substantial limbs. It is believed that the position of the limbs on the lateral plate is specified by the anteroposterior patterning systém of the whole body. This includes Hox genes but also the ParaHox and NK clusters. In the chick, *Hoxc6* and *Hoxc8* are expressed in the lateral plate between the two limb buds and repress limb formation in this region. It python that has no trace of forelimbs but a rudiment of hindlimbs, the *Hoxc8* and *8* expression extends all the way to the head but stops just short of the rudimentary hindlimbs.