13 Regeneration

- Limb regeneration
- Regeneration in *Hydra*

"Even when some of us were removed by force, others took our place."

Many of the cells in the adult body, such as muscle and nerve cells, are the same cells as were originally generated during embryonic development. But some tissues, such as blood and epithelia, are continually being replaced by stem cells (see, for example, Section 9.6). We have also seen many examples of the capacity of the embryo to self-regulate when parts of it are removed or rearranged (see, for example, Sections 3.5 and 6.1). Here we look at the related phenomenon of **regeneration** in adult organisms. Regeneration is the ability of the fully developed organism to replace tissues, organs and appendages by growth or remodeling of somatic tissue. Plants have remarkable powers of regeneration: a single somatic plant cell can give rise to a complete new plant. Some animals also show great ability to regenerate: small fragments of animals such as starfish, planarians (flatworms), and *Hydra* can give rise to a whole animal (Fig. 13.1). The ability of animals like *Hydra* and planarians to regenerate may be related to their ability to reproduce asexually. A remarkable case of regeneration is claimed for ascidians, whose blood cells alone have been reported to give rise to a

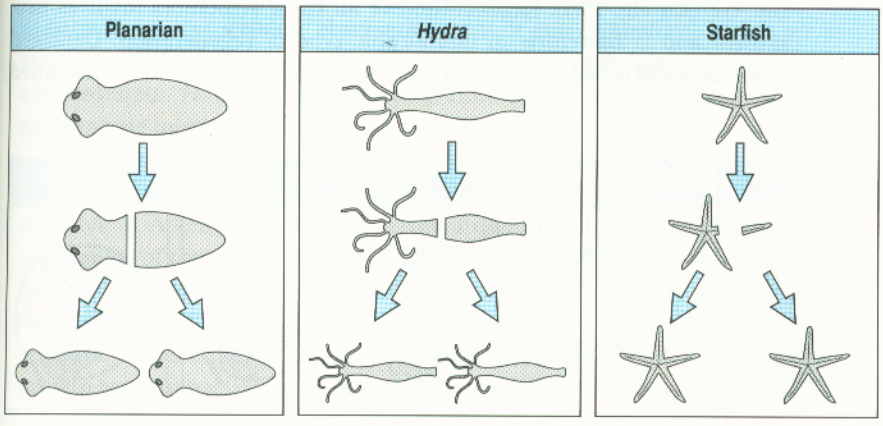


Fig. 13.1 Regeneration in some invertebrate animals. A planarian, *Hydra*, and a starfish all show remarkable powers of regeneration. When parts are removed or a small fragment isolated, a whole animal can be regenerated.

Fig. 13.2 The capacity for regeneration in urodele amphibians. The emperor newt can regenerate its dorsal crest (1), limbs (2), retina and lens (3 and 4), jaw (5), and tail (not shown).

fully functional organism. But just why some animals can regenerate while others are unable to do so is not clear.

Among vertebrates, newts and other urodele amphibians show a remarkable capacity for regeneration (Fig. 13.2). The newt lens, for example, regenerates from the pigmented epithelium of the iris (Fig. 13.3). Some insects and other arthropods can also regenerate lost appendages, such as legs. The regenerative powers of mammals are much more restricted. The mammalian liver can regenerate if a part of it is removed, the antlers of male deer regenerate each year, and fractured bones can mend by a regenerative process. But mammals cannot regenerate lost limbs, although they do have a limited capacity to replace the ends of digits, and nematodes and rotifers cannot regenerate at all.

The issue of regeneration raises several major questions. Why are some animals able to regenerate and others not? What is the origin of the cells that give rise to the regenerated structures? What mechanisms pattern the regenerated tissue and how are these related to the patterning processes that occur in embryonic development? We will focus on two systems in which regeneration has been intensively studied: regeneration of the whole animal in *Hydra*, and limb regeneration in insects and amphibians. Plant regeneration was discussed briefly in Chapter 7 and that of the nervous system in Chapter 11.

A distinction can be drawn at the outset between two types of regeneration. In one—**morphallaxis**—there is little new growth, and regeneration occurs mainly by the repatterning of existing tissues and the re-establishment of boundaries. Regeneration in *Hydra* is a good example of morphallaxis. By contrast, regeneration in the newt limb, for example, depends on the growth of new, correctly patterned structures, and this is known as **epimorphosis**. Both types of regeneration can be illustrated with reference to the French flag pattern (Fig. 13.4). In morphallaxis, new boundary regions are first established and new positional values are specified in relation to them; in epimorphosis, new positional values are linked to growth from the cut surface. In both cases, the origin of the progenitor cells for the regenerated tissue is a key issue.

Limb regeneration

Urodele amphibians such as newts and axolotls show a remarkable capacity for regenerating body structures such as tails, limbs, jaws, and the lens of the eye (see Fig. 13.2). Regeneration of all these structures involves new growth and is therefore an epimorphic type of regeneration. Damaged insect legs and other appendages can also regenerate. This has been studied

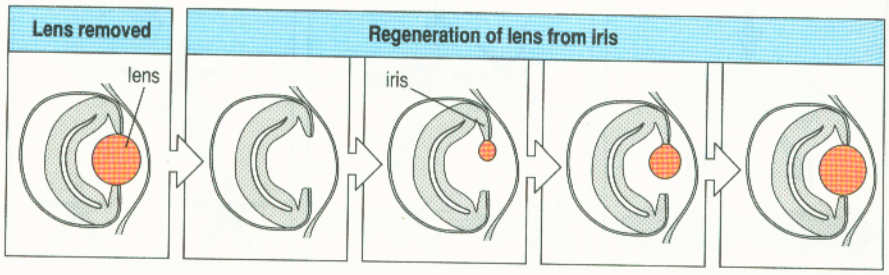


Fig. 13.3 Lens regeneration. Removal of the lens from the eye of a newt results in regeneration of a new lens from the dorsal pigmented epithelium of the iris.

mainly in the larval cockroach, which has relatively large legs that are easy to manipulate. Regeneration of a structure such as an adult vertebrate limb, which contains a variety of fully differentiated cell types in a highly organized arrangement, raises a central question relating to the origin of the cells that give rise to the regenerated structure: are there special reserve cells or do cells dedifferentiate and change their character? As we shall see, the fully differentiated cells of the mature vertebrate limb return to the cell cycle, dedifferentiate, and then redifferentiate into different cell types. Regeneration of insect appendages will also be considered.

13.1 Vertebrate limb regeneration involves cell dedifferentiation and growth

Following amputation of a newt limb, there is a rapid migration of epidermal cells over the wound surface, which is essential for subsequent outgrowth, and a **blastema** begins to form, which will give rise to a regenerated limb (Fig. 13.5). The blastema is formed from cells beneath the wound epidermis which lose their differentiated character and start to divide. As the limb regenerates over a period of weeks, these cells differentiate into cartilage, muscle, and connective tissue. The blastemal cells are derived locally from the mesenchymal tissues of the stump, close to the site of amputation. They particularly come from the dermis but also from the cartilage and muscle. This raises the question as to whether cells differentiating into cartilage and muscle in the blastema are remaining true to type or whether, for example, multinucleate skeletal muscle cells in the stump undergo dedifferentiation and then give rise to other cell types, such as cartilage, during regeneration. In other words, is transdifferentiation (see Section 9.18) occurring during limb regeneration, as it occurs in the transdifferentiation of pigmented iris epithelial cells into lens cells in the regenerating eye lens of a newt (see Fig. 13.3)? The answer, at least in the newt, is yes. If cultured newt limb muscle myotubes, which are multinucleate and have stopped dividing, are labeled in culture with a retrovirus expressing alkaline phosphatase, and are introduced into regenerating limbs, strongly labeled mononucleate cells can be observed in blastemas after 1 week. The majority of the myotubes give rise to mononucleate cells. These mononucleate cells proliferate, and there is some evidence that they later give rise to cartilage as well as new muscle.

These results reinforce the view that the early blastema is an environment that causes cells to dedifferentiate. The cells in this special environment then act as progenitor cells for the regenerating limb. This is further illustrated by the striking observation that if iris epithelium cells are grafted to different sites on the newt's body, they maintain their differentiated state. However, if they are grafted into a limb blastema they differentiate into lens cells.

The ability of muscle cells to re-enter the cell cycle is a special feature of newt limb regeneration, since mature muscle cells normally never divide (Chapter 9). A general feature of vertebrate muscle differentiation is the withdrawal of the muscle precursor cells from the cell cycle after myoblast fusion, which involves the dephosphorylation of the protein product of the *Rb* gene (see Section 9.5). Cultured mouse muscle cells lacking the Rb protein can re-enter the cell cycle. The regenerating newt cells contain Rb protein but it is inactivated by phosphorylation, so the cells can re-enter the

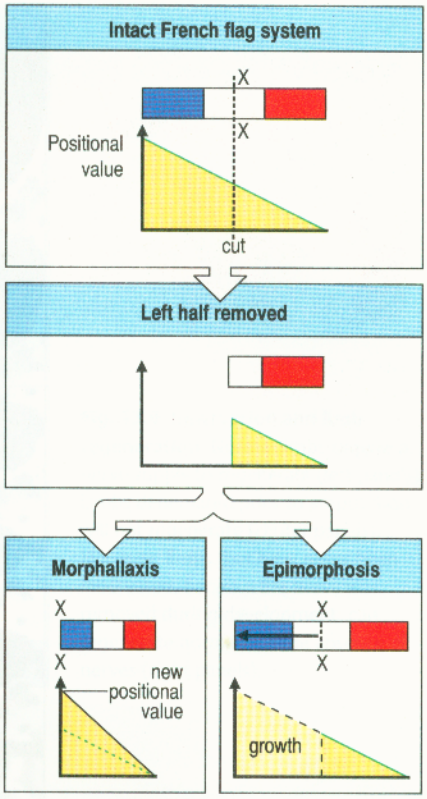


Fig. 13.4 Morphallaxis and epimorphosis. A pattern such as the French flag may be specified by a gradient in positional value (see Fig. 1.22). If the system is cut in half it can regenerate in one of two ways. In regeneration by morphallaxis, a new boundary is established at the cut and the positional values are changed throughout. In regeneration by epimorphosis, new positional values are linked to growth from the cut surface.

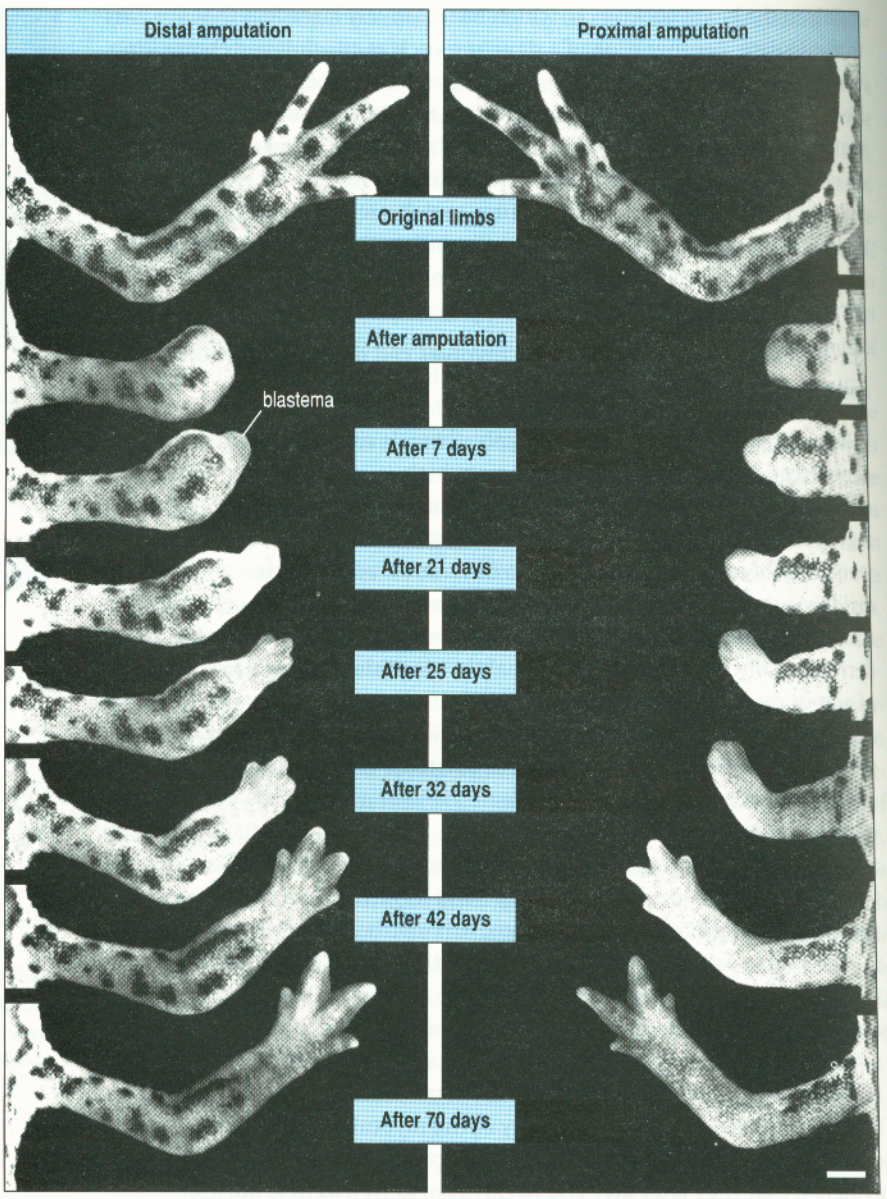


Fig. 13.5 Regeneration of the forelimb in the red-spotted newt *Notophthalmus viridescens*. The left panel shows the regeneration of a forelimb after amputation at a distal (mid-radius/ulna) site. The right panel shows regeneration after amputation at a proximal (mid-humerus) site. At the top, the limbs are shown before amputation. Successive photographs were taken at the times shown after amputation. Note that the blastema gives rise to structures distal to the cut. Scale bar = 1 mm.

cell cycle and divide. The ability of newt muscle cells to enter the cell cycle is signaled by local activation of thrombin, a proteolytic protein that is more familiar as part of the blood clotting cascade. The regeneration of the newt lens from the dorsal margin of the iris correlates with thrombin activity in that region.

Growth of the blastema is dependent on its nerve supply (Fig. 13.6) and the overlying wound epidermis, which may play a role similar to the apical ectodermal ridge in limb development (Section 10.3). In limbs in which the nerves have been cut before amputation, a blastema forms but fails to grow. The nerves have no influence on the character or pattern of the regenerated structure; it is the amount of neural innervation, not the type of nerve that matters. Nerve cells, therefore, seem to be providing some essential growth factor. Members of the neuregulin family of growth factors are likely candidates and their local application can result in regeneration of denervated limbs.

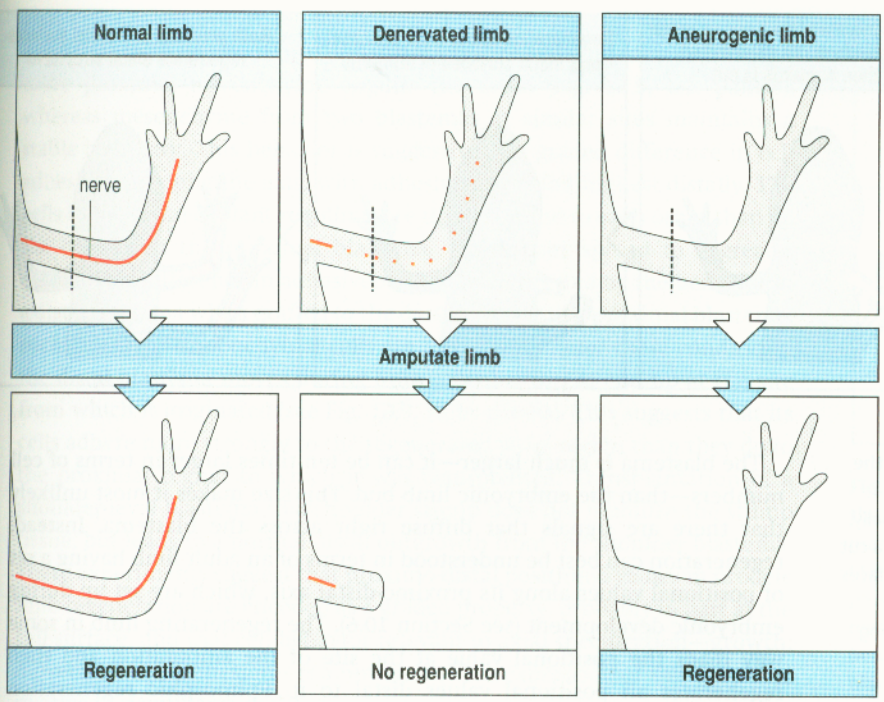


Fig. 13.6 Innervation and limb regeneration. Normal limbs require a nerve supply to regenerate (left panels). Limbs denervated prior to amputation will not regenerate (center panels). However, limbs that have never been innervated, because the nerve was removed during development, can regenerate normally in the absence of nerves (right panels).

An interesting phenomenon, as yet unexplained, is that if embryonic limbs are denervated very early in their development and so have never been exposed to the influence of nerves, they can regenerate in the complete absence of any nerve supply (see Fig. 13.6, right panel). If such an aneurogenic limb is innervated, it rapidly becomes dependent on nerves for regeneration. This evidence suggests that the dependence on the nerve is imposed on the limb after the ingrowth of the nerve.

13.2 The limb blastema gives rise to structures with positional values distal to the site of amputation

Regeneration always proceeds in a direction distal to the cut surface, allowing replacement of the lost part of the limb. If the hand is amputated at the wrist, only the carpals and digits are regenerated, whereas if amputation is through the middle of the humerus, everything distal to the cut (including the distal humerus) is regenerated. Positional value along the axis is therefore of great importance. The blastema has considerable morphogenetic autonomy. If it is transplanted to a neutral location that permits growth, such as the dorsal crest of a newt larva or even the anterior chamber of the eye, it gives rise to a regenerate appropriate to the position from which it was taken.

The growth of the blastema and the nature of the structures it gives rise to are dependent on the site of the amputation and not on the nature of the more proximal tissues. The limb is not, however, simply 'trying' to replace missing parts. This was shown in a classic experiment in which the distal end of a newt limb that had been amputated at the wrist was inserted into the belly of the same animal so as to establish a blood supply to it. The limb was then cut mid-humerus. Both surfaces regenerated distally, even though the part attached to the belly already had a radius and ulna (Fig. 13.7).

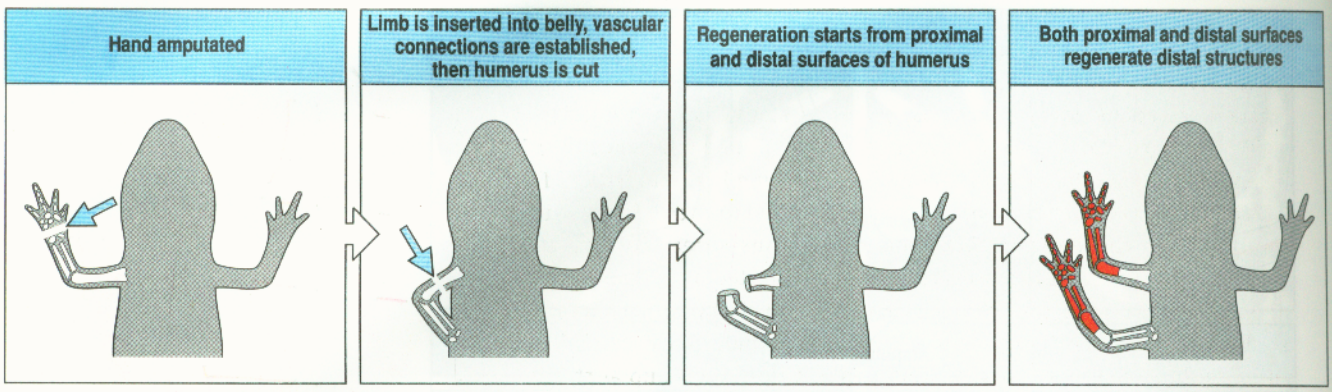
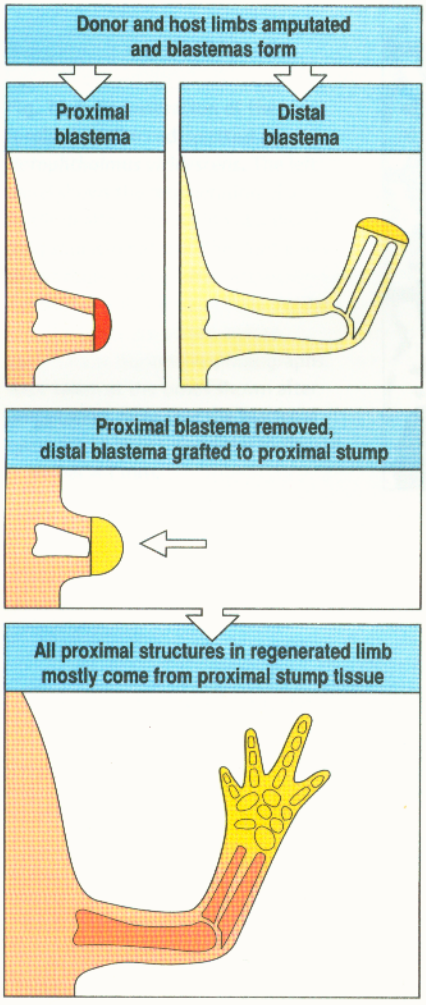


Fig. 13.7 Limb regeneration is always in the distal direction. The distal end of a limb is amputated and the limb inserted into the belly. Once vascular connections are established, a cut is made through the humerus. Both cut surfaces regenerate the same distal structures even though, in the case of one of the regenerating limbs, distal structures are already present.



The blastema is much larger—it can be ten times larger in terms of cell numbers—than the embryonic limb bud. This size makes it most unlikely that there are signals that diffuse right across the blastema. Instead, regeneration can best be understood in terms of an adult limb having a set of positional values along its proximo-distal axis, which are set up during embryonic development (see Section 10.6). The regenerating limb in some way reads the positional value at the site of the amputation and then regenerates all positional values distal to it. Epimorphic regeneration involves the retention of embryonic processes, like the ability to specify new positional values.

The ability of cells to recognize a discontinuity in positional values is illustrated by grafting a distal blastema to a proximal stump. In this experiment, the forelimb's stump and blastema have different positional values, corresponding to shoulder and wrist, respectively. The result is a normal limb in which structures between the shoulder and wrist have been generated by **intercalary growth**, predominantly from the proximal stump, while the cells from the wrist blastema mostly give rise to the hand (Fig. 13.8).

During limb development in the embryo, the Hoxa genes are expressed with temporal and spatial co-linearity along the proximo-distal axis, as we saw in Chapter 10. In the regenerating axolotl limb, however, two Hoxa genes from the 3′ and 5′ ends of the complex are expressed in the stump cells at the same time—24 to 48 hours after amputation. This suggests that the most distal region of the blastema is specified first and that regeneration involves intercalation of positional values between this distal region and the stump. The timing of expression of the Hoxd complex is similar to that in embryonic development, *Hoxd-8* being switched on earlier than *Hoxd-11*, although the early expression of *Hoxd-11* would fit better with the intercalation model as it is in the most distal region of the limb. *Sonic hedgehog* is expressed in a small region at the posterior margin of the blastema but its function is not known. However, limb duplication induced by treatment with retinoic acid is also preceded by anterior expression of *Sonic hedgehog*.

While the nature of the positional values is not known, there is evidence

Fig. 13.8 Proximo-distal intercalation in limb regeneration. A distal blastema grafted to a proximal stump results in intercalation of all the structures proximal to the distal blastema. Almost all of the intercalated region comes from the proximal stump.

that cell-surface properties are involved. When mesenchyme from two blastemas from different proximo-distal sites are confronted in culture, the more proximal mesenchyme engulfs the distal (Fig. 13.9, left panels), whereas mesenchyme from two blastemas at similar sites maintains a stable boundary. This behavior is suggestive of a graded difference in cell adhesiveness along the axis, with adhesiveness being highest distally. The cells in the distal explant remain more tightly bound to each other than do the cells from the proximal blastema, which thus spread to a greater extent. This difference in adhesiveness is also suggested by the behavior of a distal blastema when grafted to the dorsal surface of a proximal blastema, such that their mesenchymal cells are in contact. Under these conditions, the distal blastema moves during limb regeneration to end up at the site from which it originated (see Fig. 13.9, right panels). This suggests that its cells adhere more strongly to the regenerated wrist region than they do to the proximal region to which it was transplanted. Transplantation of a shoulder-level blastema to a shoulder stump does not mobilize the stump tissue, but leads to a normal distal outgrowth from the shoulder blastema. These experiments suggest that proximo-distal positional values in urodele limb regeneration are encoded as a graded property, probably in part at the cell surface, and that cell behavior relevant to axial specification—growth, movement, and adhesion—is a function of the expression of this property, relative to neighboring cells.

Maintaining the continuity of positional values by intercalation is a fundamental property of regenerating epimorphic systems and we consider it further in relation to the cockroach leg, below. Even normal regeneration by outgrowth from a blastema could be considered the result of intercalation between the cells at the level of amputation and those with the most distal positional values, as specified by the wound epidermis. It is not clear to what extent blastemal cells inherit a particular positional value, for example, from their differentiated precursors, and to what extent they are subject to signals that induce the appropriate expression of positional value. The precise relationship between Hox gene expression and positional identity is not understood, either for limb embryonic development or regeneration.

Although mammals cannot regenerate whole limbs, many, including young children, can regenerate the ends of their digits. In mice and children, the level from which digits are able to regenerate is limited to the base of the claw or nail, respectively. This probably reflects the presence of stem cells in the nail bud rather than cell dedifferentiation. In mammalian limb development, the homeobox gene *Msx-1* is expressed in the progress zone of the embryonic limb bud, and in mice it continues to be expressed in the tips of the digits even after birth. Since the region in which digit regeneration in mice can occur corresponds with that of *Msx-1* expression, this gene may be required for the generation of new positional values.

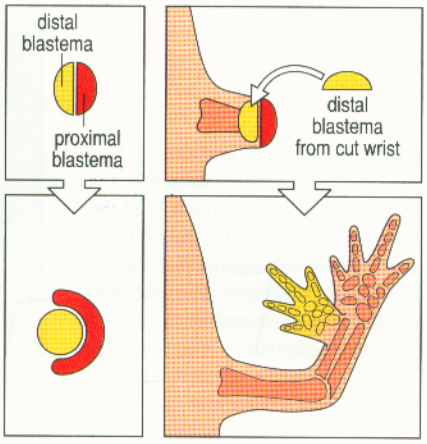


Fig. 13.9 Cell-surface properties vary along the proximo-distal axis. Left panels: when mesenchyme from distal and proximal blastemas is placed in contact in culture, the proximal mesenchyme engulfs the distal mesenchyme, which has greater adhesion between its cells. Right panels: if a distal blastema (in this case from a cut wrist) is grafted to the dorsal surface of a more proximal blastema, the regenerating wrist blastema will move distally to a position on the host limb that corresponds to its original level and regenerates a hand.

13.3 Retinoic acid can change proximo-distal positional values in regenerating limbs

We have already seen that retinoic acid is present in developing vertebrate limbs and how experimental treatment with retinoic acid can alter positional values in the developing chick limb (see Section 10.5). It also has striking effects on regenerating amphibian limbs.

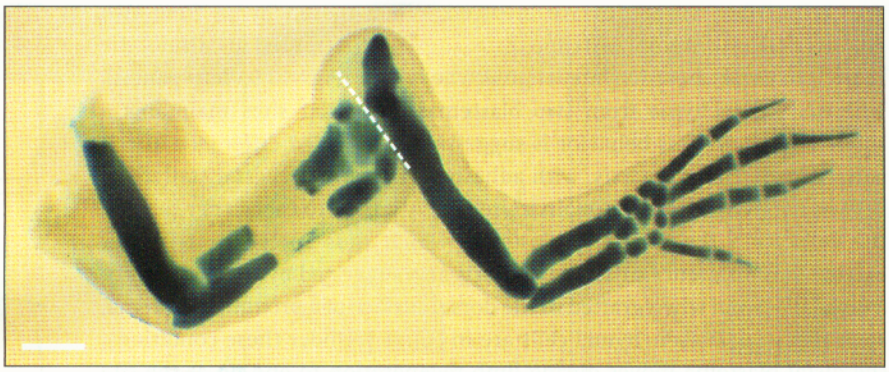


Fig. 13.10 Retinoic acid can proximalize positional values. A forelimb amputated at the level of the hand, as indicated by the dotted line, and then treated with retinoic acid, regenerates structures corresponding to a cut at the proximal end of the humerus. Scale bar = 1 mm.

Exposing a regenerating limb to retinoic acid results in the blastema becoming proximalized; that is, the limb regenerates as if it had originally been amputated at a more proximal site. For example, if a limb is amputated through the radius and ulna, treatment with retinoic acid will result not only in the regeneration of the elements distal to the cut, but also in the production of an extra complete radius and ulna. The effect of retinoic acid is dose dependent, and with a high dose it is possible to regenerate a whole extra limb, including part of the shoulder girdle, on a limb from which only the hand has been amputated (Fig. 13.10). Retinoic acid can therefore alter the proximo-distal positional value of the blastema, making it more proximal. Retinoic acid can also, under some experimental conditions, shift positional values along the anterior–posterior axis in a posterior direction.

In untreated regenerating limbs, endogenous retinoic acid is present in a distinct pattern, although there is no direct evidence that it is involved in regeneration. There is an antero-posterior gradient of retinoic acid in the blastema, and there is also a higher concentration in distal blastemas than in proximal blastemas, suggesting a proximo-distal gradient as well. The wound epidermis is a strong source of retinoic acid.

Retinoic acid is known to act through a variety of receptors. There are a number of different types of receptors in the limb but only one of them (δ_2) is involved in changes in positional values. By constructing a chimeric

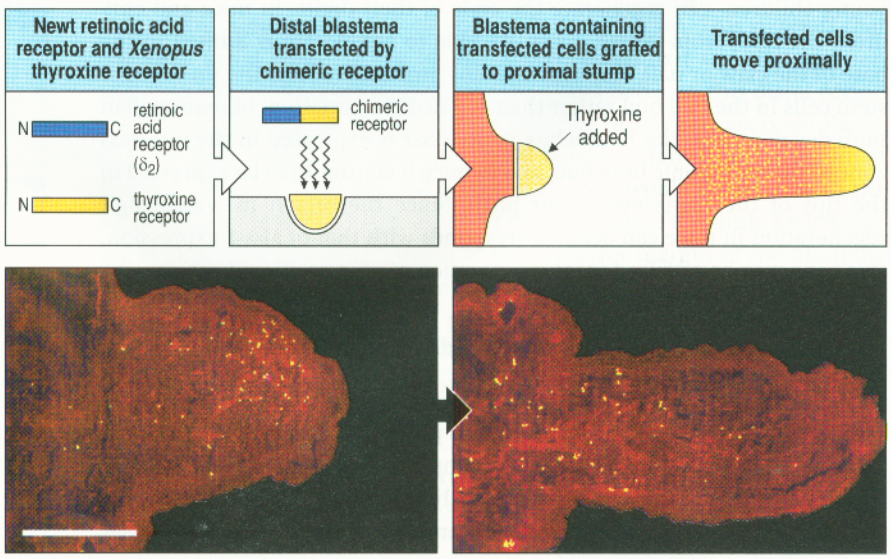


Fig. 13.11 Retinoic acid proximalizes the positional value of individual cells. Some of the cells of a newt distal blastema are transfected by a chimeric receptor, through which retinoic acid receptor function can be activated by thyroxine. This blastema is grafted to a proximal stump and treated with thyroxine. During intercalary growth, the transfected cells, which have been labeled, move proximally because their positional values have been proximalized by the activation of the retinoic acid receptor. The photographs illustrate proximalization of the transfected cells. Scale bar = 0.5 mm. *Photographs from Pecorino, L.T., et al.: 1996.*

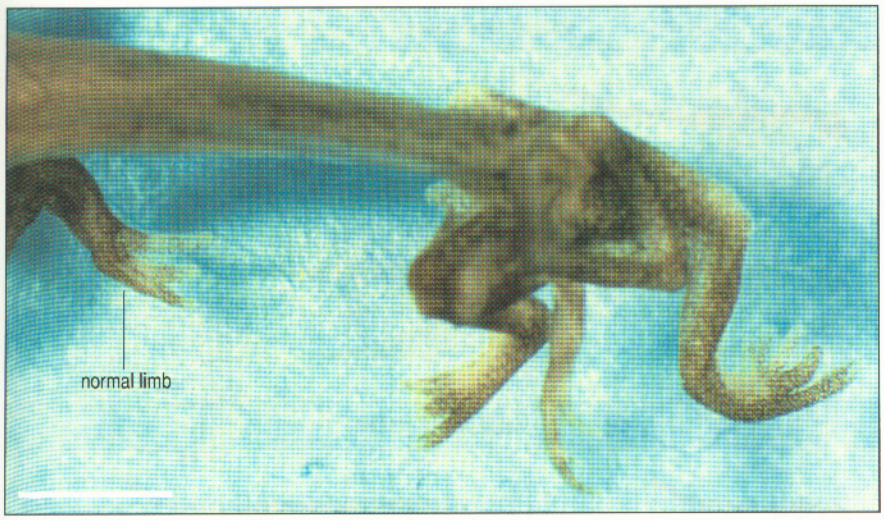


Fig. 13.12 Retinoic acid can induce additional limbs in regenerating tail of a frog, *Rana temporaria*. Treatment of the regenerating tail with retinoic acid at the time when hindlimbs are developing, results in the appearance of additional hindlimbs in place of a regenerated tail. Scale bar = 5 mm. *Photograph courtesy of M. Maden.*

receptor from a δ_2 retinoic acid receptor and a thyroxine receptor, the retinoic acid receptor can be activated selectively by thyroxine, and the effects of its activation studied experimentally (Fig. 13.11). In these experiments, cells in the distal blastema are transfected with the chimeric receptor, which is then grafted to a proximal stump and treated with thyroxine. The transfected cells behave as if they have been treated with retinoic acid. The result is a movement of the transfected blastemal cells to more proximal regions in the intercalating regenerate. This shows that activation of the retinoic acid pathway can proximalize the positional values of the cells, which then respond by translocation to more proximal sites.

Another remarkable effect of retinoic acid is its ability to bring about a homeotic transformation of tails into limbs in tadpoles of the frog *Rana temporaria*. If the tail of a tadpole is removed it will regenerate. Treatment of regenerating tails with retinoic acid at the same time as hindlimbs are developing results in the appearance of additional hindlimbs in place of a regenerated tail (Fig. 13.12). We have, as yet, no satisfactory explanation for this result, but it has been speculated that the retinoic acid alters the antero-posterior positional value of the regenerating tail blastema to that of the site along the antero-posterior axis where hindlimbs would normally develop.

13.4 Insect limbs intercalate positional values by both proximo-distal and circumferential growth

The legs of some insects such as the cockroach can regenerate. The structure of insect legs is different from those of vertebrates as the key structural feature is the ectodermally derived external cuticle. Nevertheless, intercalation of missing positional values, as already described for the proximo-distal axis of the amphibian limb (see Section 13.2), seems to be a general property of regenerating epimorphic systems. When cells with disparate positional values are placed next to one another, intercalary growth occurs in order to regenerate the missing positional values. Intercalation is particularly clearly illustrated by limb regeneration in the cockroach. Intercalation also occurs in *Drosophila* leg and wing imaginal discs.

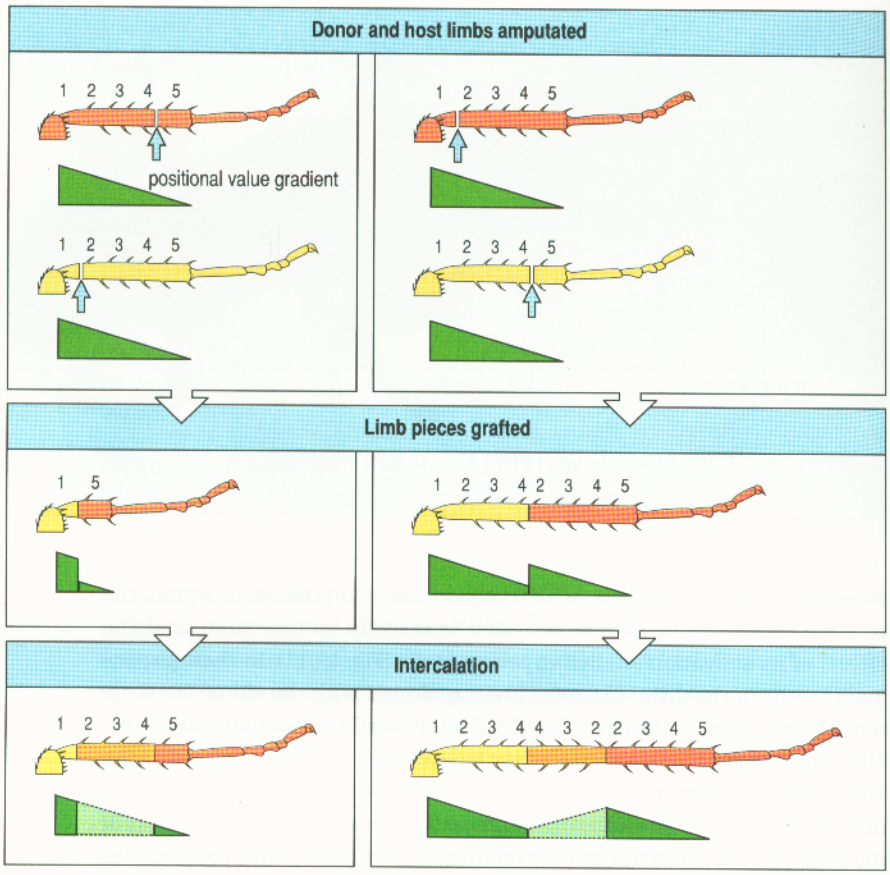


Fig. 13.13 Intercalation of positional values by growth in the regenerating cockroach leg. Left panels: when a distally amputated tibia (5) is grafted to a proximally amputated host (1), intercalation of the positional values 2–4 occurs, irrespective of the proximo-distal orientation of the grafts, and a normal tibia is regenerated. Right panels: when a proximally amputated tibia (1) is grafted to a distally amputated host (4), however, the regenerated tibia is longer than normal and the regenerated portion is in the reverse orientation to normal, as judged by the orientation of surface bristles. The reversed orientation of regeneration is due to the reversal in positional value gradient. The proposed gradient in positional value is shown under each figure. After French, V., *et al.*: 1976.

A cockroach leg is made up of a number of distinct segments, arranged along the proximo-distal axis in the order coxa, femur, tibia, tarsus. Each segment seems to contain a similar set of proximo-distal and circumferential positional values, and will intercalate the missing positional values. When a distally amputated tibia is grafted onto a host tibia that has been cut at a more proximal site, localized growth occurs at the junction between graft and host, and the missing central regions of the tibia are intercalated (Fig. 13.13, left panels). In contrast to amphibian regeneration, there is a predominant contribution from the distal piece. As in the amphibian, however, regeneration is a local phenomenon and the cells are indifferent to the overall pattern of the tibia. Thus, when a proximally cut tibia is grafted onto a more distal site, making an abnormally long tibia, regenerative intercalation again restores the missing positional values, making the tibia even longer (Fig. 13.13, right panels). The regenerated portion is in the reverse orientation to the rest of the limb, as indicated by the direction in which the bristles point, suggesting that the gradient in positional values also specifies cell polarity, as in insect body segments (see Chapter 5). These results also show that when cells with non-adjacent positional values are placed next to each other, the missing values are intercalated by growth to provide a set of continuous positional values.

A similar set of positional values is present in each segment of the limb. Thus, a mid-tibia amputation, when grafted to the mid-femur of a host, will heal without intercalation. But grafting a distally amputated femur onto a proximally amputated host tibia results in intercalation, largely femur in

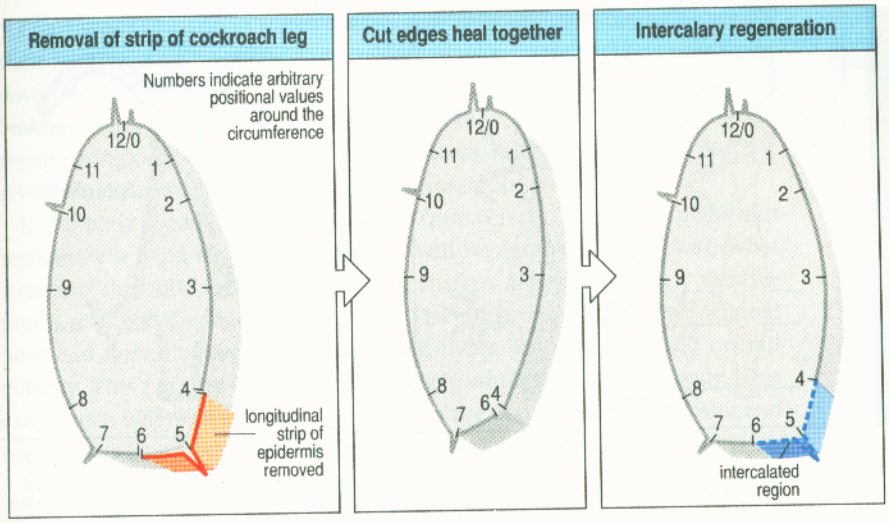


Fig. 13.14 Circumferential intercalation in the cockroach leg. The leg is seen in transverse section. When a piece of cockroach ventral epidermis is removed (left panel), the cut edges heal together (center panel). When the insect molts and the cuticle regrows, circumferential positional values are intercalated (right panel). The positional values are arranged around the circumference of the leg, rather like the hours on a clock face. After French, V., *et al.*: 1976.

type. There must be other factors making each segment different, rather like the segments of the insect larva.

Intercalary regeneration also occurs in a circumferential direction. When a longitudinal strip of epidermis is removed from the leg of a cockroach, normally nonadjacent cells come into contact with one another, and intercalation in a circumferential direction occurs after molting (Fig. 13.14). Cell division occurs preferentially at sites of mismatch around the circumference. One can treat positional values in the circumferential direction as a clock face, with values going continuously 12, 1, 2, 3 . . . 6 . . . 9 . . . 11. As in the proximo-distal axis, there is intercalation of the missing positional values.

Summary

Urodele amphibians can regenerate amputated limbs and tails. Stump tissue at the site of amputation first dedifferentiates to form a blastema, which then grows and gives rise to a regenerated structure. The dedifferentiated cells of the blastema are the progenitors of the regenerate. Regeneration is usually dependent on the presence of nerves, but limbs that have never been innervated are capable of regeneration. Regeneration

Summary: regeneration of an amphibian limb

amputation of a newt limb

⇩

local dedifferentiation of stump tissue to form a blastema

⇩

the blastema grows to form distal structures
provided nerves are present

⇩

graft of distal blastema to proximal stump

⇩

intercalation of missing proximo-distal positional values from stump tissue growth

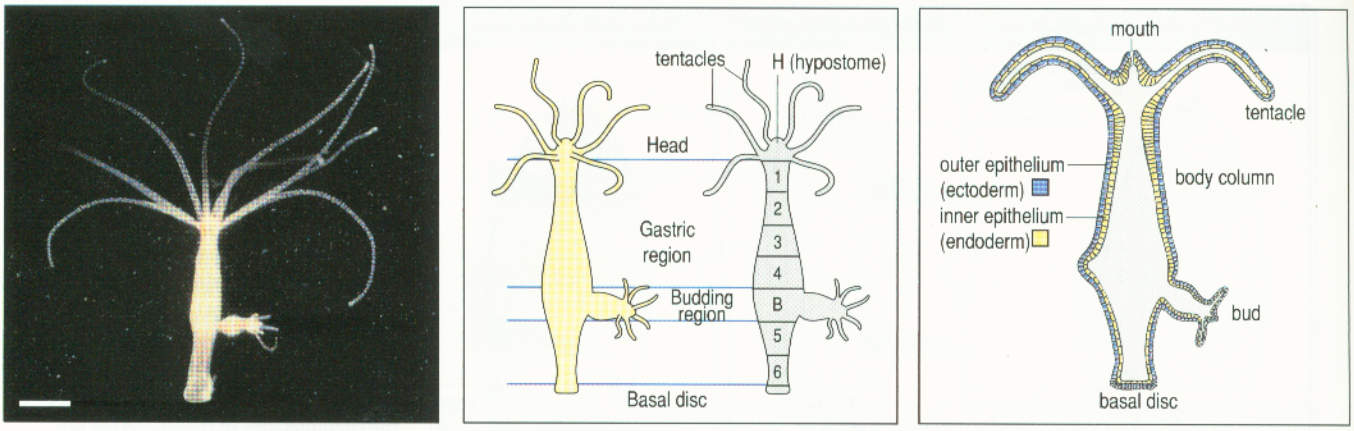


Fig. 13.15 ***Hydra.*** This freshwater coelenterate (photograph, left panel) has a head with tentacles and mouth at one end and a sticky foot at the other. It can reproduce by budding. For the purposes of grafting experiments, the body column is divided into a series of regions, as indicated in the center panel. The body wall is made up of two epithelial layers, corresponding to the ectoderm and endoderm found in other organisms featured in this book (right panel). Scale bar = 1 mm.

Photograph courtesy of W. Müller, from Müller, W.A.: 1989.

always gives rise to structures with positional values more distal than those at the site of amputation. When a blastema is grafted to a stump with different positional values, proximo-distal intercalation of the missing positional values occurs. Retinoic acid proximalizes the positional values of the cells of the blastema. Insect limbs can also regenerate, intercalation of positional values occurring in both proximo-distal and circumferential directions.

Regeneration in *Hydra*

Hydra is a freshwater coelenterate consisting of a hollow tubular body about 0.5 cm long, with a head region at one end (the distal end) and a basal region at the other (proximal) end, with which it can stick to surfaces (Fig. 13.15). The head consists of a small conical hypostome where the mouth opens, surrounded by a set of tentacles, which are used for catching the small animals on which *Hydra* feeds. Unlike most of the animals discussed so far in this book, which have three germ layers, *Hydra* has only two. The body wall is composed of an outer epithelium, which corresponds to the ectoderm, and an inner epithelium, which corresponds to the endoderm. These two layers are separated by a basement membrane. There are about 20 different cell types in *Hydra*, which include nerve cells, secretory cells, and nematocysts that are used to capture prey.

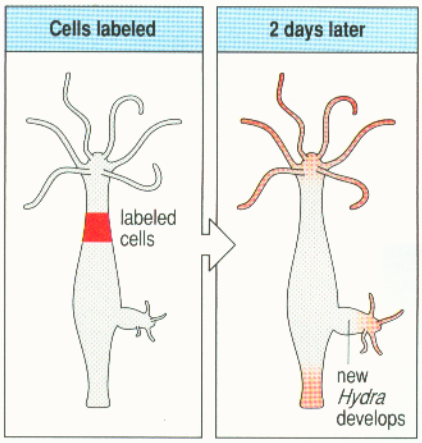


Fig. 13.16 **Growth in *Hydra*.** The cells in the body column of a *Hydra* are continually dividing and being displaced. If a group of cells in region 1 are labeled (left panel), 5 days later the labeled cells have been displaced to the tentacles and basal disc, where they are lost, and to the budding zone where they form a new *Hydra* (right panel).

13.5 *Hydra* grows continuously but regeneration does not require growth

Well-fed *Hydra* are in a dynamic state of continuous growth and pattern formation. The cells of both epithelial layers proliferate steadily and, as the tissues grow, cells are displaced along the body column toward the head or foot (Fig. 13.16). In order for an adult *Hydra* to maintain a constant size, excess cells must be continually lost. Cell loss occurs at the tips of the tentacles and at the basal disc of the foot bud. Most of the excess cell production is taken up by the asexual budding of new *Hydra* from the body column. Budding occurs about two-thirds of the way down the body column; the body wall evaginates by a morphogenetic change in cell shape, generated locally by the cells in the bud region, to form a new column that develops a head at the end and then detaches as a small new *Hydra*.

Continuous growth in *Hydra* means that cells are continually changing their relative positions and are forming new structures as they move up or down the body column. Moreover, new *Hydra* are generated asexually by budding from the body wall. There must, therefore, be mechanisms for repatterning cells during this dynamic process. It is these mechanisms that give *Hydra* its remarkable capacity for regeneration.

If the body column of a *Hydra* is cut transversely, the lower piece will regenerate a head but the upper piece will regenerate a foot. Thus, what structure the cells regenerate at a cut surface depends on their relative position within the regenerating piece. The cut surface nearest the original head end forms a head—this shows that *Hydra* has a well-defined overall polarity. This polarity is maintained even in small pieces of the body. This can be seen when a short piece is cut out of the body column; the distal end regenerates a head while the proximal end becomes the basal disc.

Regeneration in *Hydra* does not require growth and is thus said to be morphallactic. When a short fragment of the column regenerates, there is no initial increase in size and the regenerated animal will be a small *Hydra*. Only after feeding will the animal return to a normal size. The lack of a growth requirement for regeneration is shown in heavily irradiated *Hydra*; no cell divisions occur in these animals but they can still regenerate more or less normally.

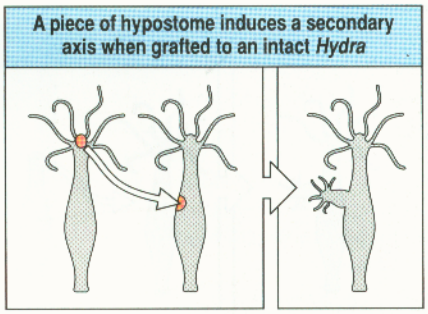


Fig. 13.17 The hypostome can induce a new head and body in *Hydra*. When an excised fragment of hypostome is grafted into the gastric region of another, intact *Hydra*, it can induce the formation of a complete new secondary axis with a head and tentacles.

13.6 The head region of *Hydra* acts both as an organizing region and as an inhibitor of inappropriate head formation

At the beginning of this century, it was shown that grafting a small fragment of the hypostome region of a *Hydra* into the gastric region of another *Hydra* induced a new head, complete with tentacles, and a body axis (Fig. 13.17). Similarly, transplantation of a fragment of the basal region induced a new body column with a basal disc at its end. *Hydra* therefore have two organizing regions, one at each end. The hypostome and the basal disc act as organizing regions (like the Spemann organizer in amphibians and the polarizing regions in vertebrate limb buds). These organizing regions at the ends give *Hydra* its overall polarity.

Grafting experiments also show that, as part of its organizing function, the hypostome produces an inhibitor of head formation whose effectiveness drops with distance from the head (Fig. 13.18). This inhibition normally prevents inappropriate head formation in the intact animal. When a body piece from just below the head (region 1 in Fig. 13.15) is grafted into the gastric region, it rarely induces a new head and is usually simply absorbed into the body. But if the head of the host is removed at the time of grafting, the graft can then induce a new axis and head. This suggests that the removal of the head results in the loss of some factor that is inhibiting head formation. This inhibitory effect falls off with distance from the head:

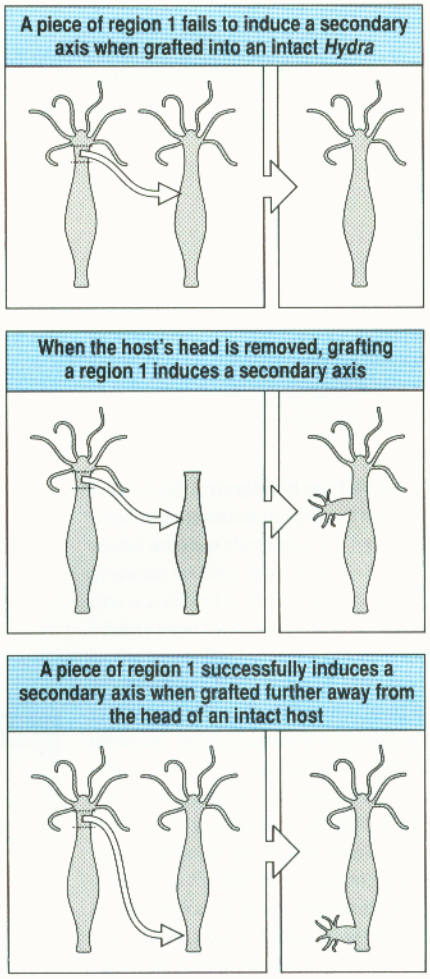


Fig. 13.18 The head region of *Hydra* produces an inhibitory signal that falls off with distance. Region 1 does not induce a head when grafted into the gastric region of an intact *Hydra* (top panel), indicating the presence of an inhibitory signal in the host. If the host's head is removed and a piece of region 1 is then grafted into the host, a secondary axis is induced (middle panel), indicating that the head region is the source of the inhibitory signal. Region 1 can induce a new axis in the foot region of an intact *Hydra* because the inhibitory signal grows weaker further away from the head (bottom panel).

when a region 1 is grafted to near the foot it can induce a head, even when the original head is still in place (see Fig. 13.18, bottom panel). These experiments suggest that the formation of extra heads is normally prevented in *Hydra* by a lateral inhibition mechanism (see Sections 1.14 and 11.2) acting through a gradient of inhibitory signal with its highest concentration at the head end. An opposing gradient inhibiting foot regeneration appears to be produced by the basal disc. These gradients are dynamic and when, for example, the head is removed, the concentration of the inhibitor falls.

13.7 Head regeneration in *Hydra* can be accounted for in terms of two gradients

One can account for the results of most of the regeneration experiments in *Hydra* in terms of an interaction between a gradient of head inhibitor and a gradient of positional value that determines the character of the different regions along the body column. The gradient in positional value appears to determine both head-inducing ability and resistance to inhibition. A gradient in resistance to inhibition can be recognized by differences in the level of inhibitor required to suppress head formation by different regions of the body. This gradient in resistance decreases with distance from the head and is thought to represent a gradient of positional value with a high point at the head end. Thus, there is insufficient inhibitor near the foot to prevent a region 1 transplant forming a head when transplanted to this site, but sufficient inhibitor to prevent a region 5 from doing so.

The gradient in head-inducing ability also runs from a high point at the head end to a low point at the basal end. Evidence that this is determined by a gradient in positional value is provided by the difference in time required for different regions to acquire head-inducing properties after amputation. Region 1 from an intact *Hydra* will not induce an axis when transplanted into the gastric region of another *Hydra*. If, however, the head of the donor *Hydra* is amputated, the region 1 can induce a new axis if taken about 6 hours after head removal (Fig. 13.19). The further down the axis the amputation is made, the longer it takes for the remaining cells to acquire head-like inducing properties. A region 5 can take up to 30 hours.

A simple model for these gradients assumes that the head inhibitor is a secreted factor, made by the head, that diffuses down the body column and is degraded at the basal end. The gradient in positional value is assumed to be an intrinsic property of cells. In this model, both gradients are linear—their values decrease at a constant rate with distance from the head. We further assume that, provided the level of inhibitor is greater than the threshold set by the positional value, head regeneration is inhibited. Removal of the head results in the concentration of inhibitor falling, as the inhibitor is degraded and cannot be replaced. The decrease in inhibitor concentration is greatest at the cut end, and when the inhibitor falls below the threshold concentration set by the local positional value, the positional value increases to that of the head end (Fig. 13.20). Thus, the first key step in this morphallactic regeneration, when the head region is removed, is the specification of a new head region at the cut surface.

When the positional value has increased to that of a normal head region, the cells start to make inhibitor and so prevent head formation in other body regions. The level of inhibitor will always first fall below the threshold

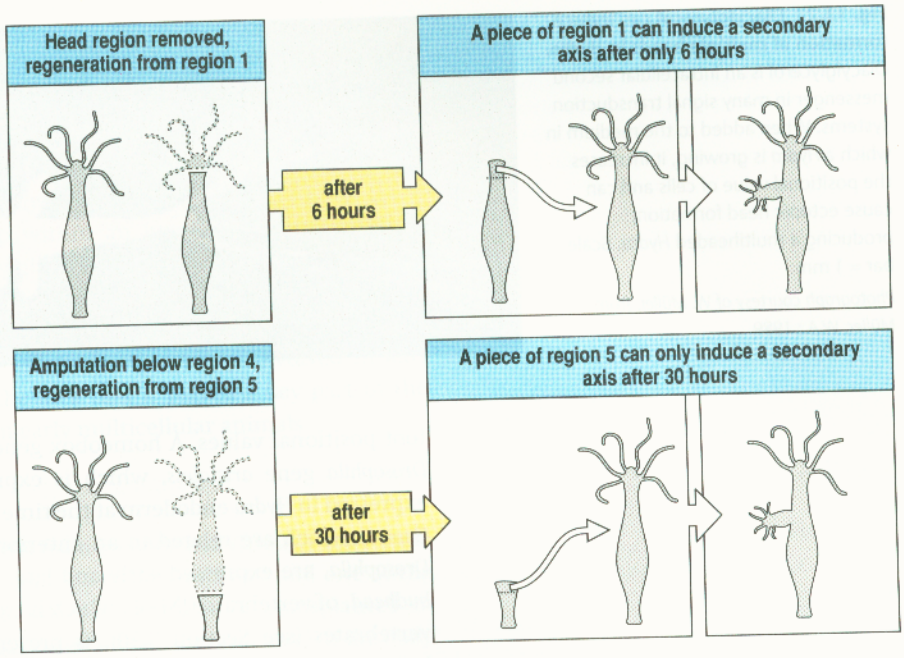


Fig. 13.19 The time needed to acquire head-like inducing properties following amputation increases with distance from the head. Top panels: a region 1 can induce a secondary axis if grafted into an intact *Hydra* 6 hours after head removal from the host. Bottom panels: if amputation is made lower down, it can take up to 30 hours for the region's cells to acquire head-like inducing properties.

for head inhibition where the positional value is highest, thus maintaining polarity. Once a new head has been specified and the inhibitory gradient re-established, the gradient in positional value also returns to normal, but this can take more than 24 hours. Morphallactic regeneration results in a smaller *Hydra* which, after feeding, will eventually grow back to a normal size

Addition of diacylglycerol—an intracellular second messenger in many signal transduction systems—to the medium in which *Hydra* is growing, increases positional value throughout the body column and can cause ectopic head formation, producing a multiheaded *Hydra* (Fig. 13.21). Diacylglycerol is produced in the phosphatidylinositol signaling pathway; this pathway is affected by lithium, which when added to the medium can cause ectopic foot ends to form, apparently by lowering positional values throughout the body column. The molecular nature of the inhibitor remains to be discovered, but there is evidence for peptide signals.

13.8 Genes controlling regeneration in *Hydra* are similar to those expressed in animal embryos

Hox genes, which control body pattern in many animals, are also involved in patterning *Hydra*. Some Hox genes are expressed along the body axis of coelenterates in a regional pattern and may be involved in specifying head-

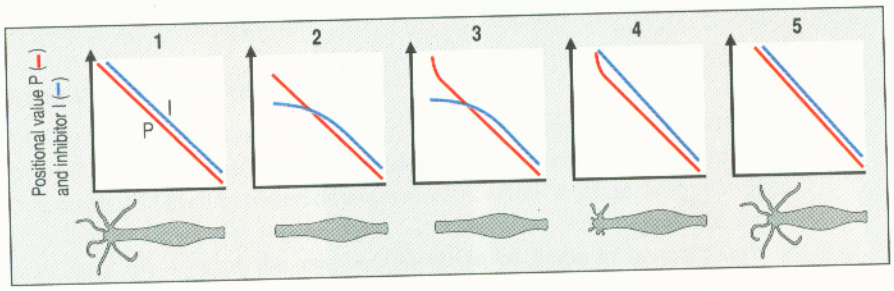


Fig. 13.20 A simplified model for head regeneration in *Hydra*. This model assumes that there are two gradients running from head to foot. One is a diffusible molecule (I) that inhibits head formation and is produced by the head. The other is a gradient of positional value (P) that is an intrinsic property of the cells. When the head is removed, the concentration of inhibitor falls at the cut surface (graph 2) until a threshold is reached at which the positional value increases to that of the head region (graph 3). This then re-establishes the inhibitor gradient (graph 4). The overall gradient in positional value takes a much longer time to return to normal (graph 5).

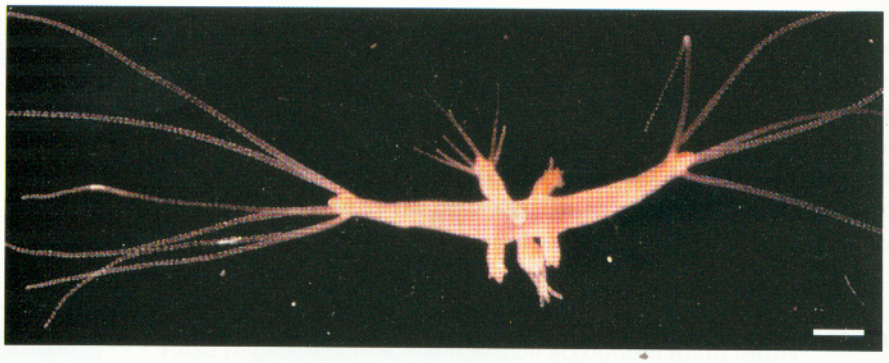


Fig. 13.21 Diacylglycerol can induce formation of multiple heads in *Hydra*. Diacylglycerol is an intracellular second messenger in many signal transduction systems. When added to the medium in which a *Hydra* is growing, it increases the positional value of cells and can cause ectopic head formation, producing a multiheaded *Hydra*. Scale bar = 1 mm.

Photograph courtesy of W. Müller, from Müller, W.A.: 1989.

foot positional values. A homeobox gene of the paired type related to the *Drosophila* gene *aristaless*, which is expressed in the head of the fly, is expressed in *Hydra* endoderm at the time of head determination. *Cnox-1* and *Cnox-2*, which are related to an anterior member of the Hox complex of *Drosophila*, are expressed early and late, respectively. The *Hydra* homolog, *budhead*, of vertebrate *HNF-3b*, which is expressed in the organizer region of vertebrates (see Section 3.20), is present in developing heads. A *Hydra* homolog of *goosecoid* is expressed just above where tentacles will appear. When injected into an early *Xenopus* embryo it can induce a partial secondary axis. This suggests that the roles of those genes in tissue that can act as an organizer have been retained over many millions of years of evolution. In addition, the *Hydra* homolog of the T-box gene *Brachyury* is expressed in the hypostome and in buds is expressed at an early stage in the region of the future hypostomes. Its function is not known, but its presence has evolutionary implications as *Hydra* has no mesoderm, the usual tissue in which *Brachyury* is expressed and suggests that the head corresponds to the proximal end, the blastopore, of other animals.

Wnt and β-catenin are both present in *Hydra*, and the Wnt signaling pathway is of particular importance. The *Hydra* homolog of β-catenin, Hyβ-Cat, shows a high degree of conservation in its role in the Wnt pathway, as injection of *Hy*β-Cat mRNA into ventral *Xenopus* blastomeres at the eight-cell stage was able to induce complete secondary body axes. These

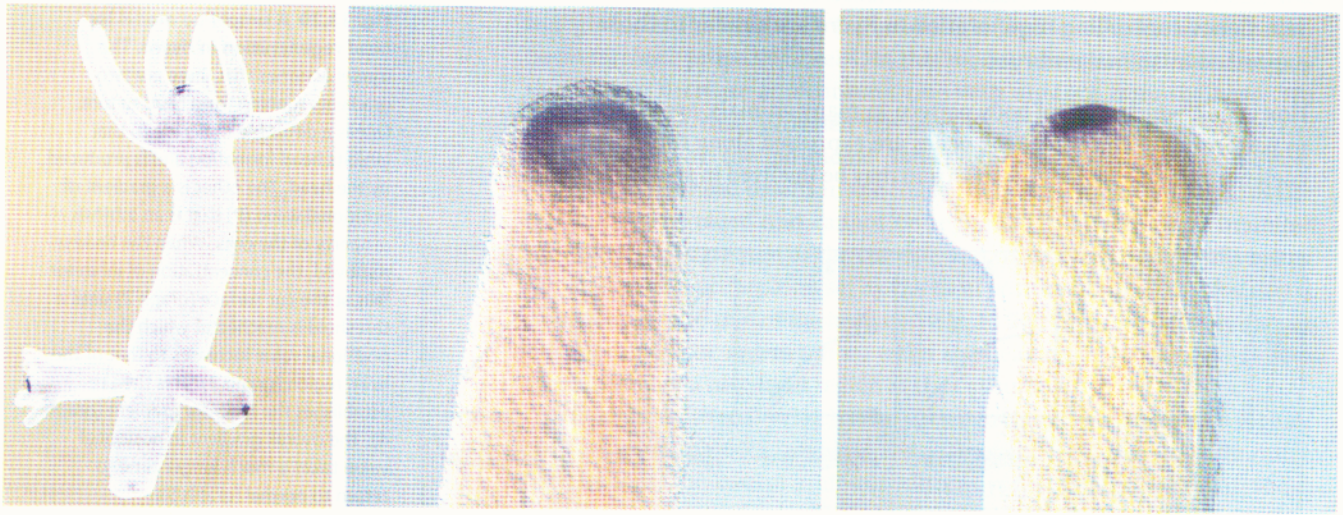


Fig. 13.22 Wnt expression in *Hydra* and in a regenerating tip at 1 hour and 48 hours.

Photographs courtesy of T.W. Holstein

contained the anterior-most structures, such as eyes and cement gland, which were indistinguishable from those induced by *Xenopus* β-catenin. *In situ* hybridization experiments revealed that expression of HyWnt, the *Hydra* Wnt homolog, is restricted to the apical tip of the body axis in adult polyps; this apical tissue is the *Hydra* head organizer (Fig. 13.22, opposite). During head regeneration, both *Hydra* catenin and Wnt genes were expressed at the regenerating tip within an hour after head removal. During budding, a significant upregulation of Hyβ-Cat expression occurred in a ring-like domain in the prospective budding zone before tissue evagination started. This high expression level was maintained until bud detachment. These results demonstrate that Wnt signaling is involved in axis formation in *Hydra* and support the idea that it played a key part in the evolution of axial differentiation in early multicellular animals.

Summary

Hydra grows continually, losing cells from its ends and through the formation of buds. Two organizing regions, one at the head end and one at the basal end, pattern the body and maintain polarity. If transplanted to another site, a head region can induce a new body axis and head. If the body of an intact *Hydra* is severed in two, it regenerates by morphallaxis, which does not require new growth. Regeneration initially involves the respecification of cells at the cut end as 'head', leading to the establishment of an organizing region. The head region produces an inhibitor that prevents other regions forming a head. The concentration of inhibitor decreases with distance from the head. There is also a gradient in positional value that determines the threshold at which head inhibition occurs. When the head is removed, the inhibitor level in the rest of the body falls, and a new head region develops where the positional value is highest, thus maintaining polarity.

Summary: regeneration in *Hydra*

Hydra grows continuously and loses cells from its ends and by budding

⇩

the head produces a gradient of inhibitor that prevents other heads forming; the head also specifies a gradient in positional values

⇩

local reduction in concentration of inhibitor

⇩

local increase in positional value

⇩

formation of new head without new growth

SUMMARY TO CHAPTER 13

Regeneration is the ability of an adult organism to replace a lost part of its body. The capacity to regenerate varies greatly among different groups of organisms and even between different species within a group. Mammals have very limited powers of regeneration, whereas newts can regenerate limbs, jaws, and the lens of the eye. Regeneration of limbs in animals such as

amphibians may in part reflect the retention or reactivation of embryonic mechanisms. The coelenterate *Hydra* regenerates by morphallaxis: when its head is removed, the level of inhibitor normally produced by the head falls, resulting in a new head being specified at the cut end, without any growth having occurred. Regeneration of limbs in amphibians and insects is by epimorphosis: a blastema forms by dedifferentiation of stump cells and the blastemal cells divide and differentiate to regenerate distal structures. Intercalation of positional values can occur when normally non-adjacent values are placed next to each other. In these examples of regeneration the cellular mechanisms are related to those operating in normal development.

REFERENCES

Introduction

Goss, R.J.: *Principles of Regeneration*. London and New York: Academic Press, 1969.

Rinkevich, B., Shlemburg, Z., Fishelson, L.: **Whole-body protochordate regeneration from totipotent blood cells.** *Proc. Natl Acad. Sci. USA* 1995, **92**: 7695–7699.

13.1 Vertebrate limb regeneration involves cell dedifferentiation and growth

Brockes, J.P.: **Amphibian limb regeneration: rebuilding a complex structure.** *Science* 1997, **276**: 81–87.

Gardiner, D.M., Carlson, M.R., Roy, S.: **Towards a functional analysis of limb regeneration.** *Semin. Cell Dev. Biol.* 1999, **10**: 385–393.

Kumar, A., Velloso, C.P., Imokawa, Y., Brockes, J.P.: **Plasticity of retrovirus-labelled myotubes in the newt limb regeneration blastema.** *Dev. Biol.* 2000, **218**: 125–136.

Tanaka, E.M., Gann, A.A.F., Gates, P.B., Brockes, J.P.: **Newt myotubules re-enter the cell cycle by phosphorylation of the retinoblastoma protein.** *J. Cell Biol.* 1997, **136**: 155–165.

Tanaka, E.M., Drechel, D.N., Brockes, J.P.: **Thrombin regulates S-phase re-entry by cultured newt myotubes.** *Curr. Biol.* 1999, **9**: 792–799.

Wang, L., Marchioni, M.A., Tassava, R.A.: **Cloning and neuronal expression of a type III newt neuregulin and rescue of denervated nerve-dependent newt limb blastemas by rhGGF2.** *J. Neurobiol.* 2000, **43**: 150–158.

13.2 The limb blastema gives rise to structures with positional values distal to the site of amputation

Gardiner, D.M., Bryant, S.V.: **Molecular mechanisms in the control of limb regeneration: the role of homeobox genes.** *Int. J. Dev. Biol.* 1996, **40**: 797–805.

Gardiner, D.M., Carlosn, M.R.J., Roy, S.: **Towards a functional analysis of limb regeneration.** *Semin. Cell Dev. Biol.* 1999, **10**: 385–393.

Reginelli, A.D., Wang, Y.Q., Sassoon, D., Muneoka, K.: **Digit tip regeneration correlates with *Msx-1* (*Hox7*) expression in fetal and newborn mice.** *Development* 1995, **121**: 1065–1076.

Stocum, D.L.: **A conceptual framework for analyzing axial patterning in regenerating urodele limbs.** *Int. J. Dev. Biol.* 1996, **40**: 773–783.

Torok, M.A., Gardiner, D.M., Shubin, N.H., Bryant, S.V.: **Expression of HoxD genes in developing and regenerating axolotl limbs.** *Dev. Biol.* 1998, **200**: 225–233.

13.3 Retinoic acid can change proximo-distal positional values in regenerating limbs

Bryant, S.V., Gardiner, D.M.: **Retinoic acid, local cell–cell interactions and pattern formation in vertebrate limbs.** *Dev. Biol.* 1992, **152**: 125.

Maden, M.: **The homeotic transformation of tails into limbs in *Rana temporaria* by retinoids.** *Dev. Biol.* 1993, **159**: 379–391.

Pecorino, L.T., Entwistle, A., Brockes, J.P.: **Activation of a single retinoic acid receptor isoform mediates proximo-distal respecification.** *Curr. Biol.* 1996, **6**: 563–569.

Scadding, S.R., Maden, M.: **Retinoic acid gradients during limb regeneration.** *Dev. Biol.* 1994, **162**: 608–617.

13.4 Insect limbs intercalate positional values by both proximo-distal and circumferential growth

French, V.: **Pattern regulation and regeneration.** *Phil. Trans. Roy. Soc. Lond., Biol. Sci.* 1981, **295**: 601–617.

13.5 *Hydra* grows continuously but regeneration does not require growth

Hicklin, J., Wolpert, L.: **Positional information and pattern regulation in Hydra: the effect of gamma-radiation.** *J. Embryol. Exp. Morph.* 1973, **30**: 741–752.

Otto, J.J., Campbell, R.D.: **Tissue economics of *Hydra*: regulation of cell cycle, animal size and development by controlled feeding rates.** *J. Cell Sci.* 1977, **28**: 117–132.

13.6 The head region of *Hydra* acts both as an organizing region and as an inhibitor of inappropriate head formation

Wolpert, L., Hornbruch, A., Clarke, M.R.B.: **Positional information and positional signaling in *Hydra*.** *Am. Zool.* 1974, **14**: 647–663.

13.7 Head regeneration in *Hydra* can be accounted for in terms of two gradients

Bosch, T.C.G., Fujisawa, T.: **Polyps, peptides and patterning.** *BioEssays* 2001, **23**: 420–427.

Hassel, M., Albert, K., Hofheinz, S.: **Pattern formation in *Hydra vulgaris* is controlled by lithium-sensitive process.** *Dev. Biol.* 1993, **156**: 362–371.

MacWilliams, W.: **Hydra transplantation phenomena and the mechanism of *Hydra* head regeneration. II. Properties of the head activation.** *Dev. Biol.* 1983, **96**: 239–257.

Müller, W.A.: **Pattern formation in the immortal *Hydra***. *Trends Genet.* 1996, **12**: 91–96.

13.8 Genes controlling regeneration in *Hydra* are similar to those expressed in animal embryos

Broun, M., Sokol, S., Bode, H.R.: **Cngsc, a homologue of goosecoid, participates in the patterning of the head, and is expressed in the organizer region of Hydra**. *Development* 1999, **126**: 5245–5254.

Galliot, B.: **Conserved and divergent genes in apex and axis development in cnidarians**. *Curr. Opin. Genet. Dev.* 2000, **10**: 629–637.

Hobmayer, B., Rentzsch, F., Kuhn, K., Happel, C.M., Cramer von Laue, C., Snyder, P., Rothbächer, U., Holstein, T.W.: **WNT signalling molecules act in axis formation in the diploblastic metazoan *Hydra***. *Nature* 2000, **407**: 186–189.

Shenk, M.A., Gee, L., Steele, R.E., Bode, H.R.: **Expression of *Cnox-2*, a Hom/Hox gene, is suppressed during head formation in *Hydra***. *Dev. Biol.* 1993, **160**: 108–118.

Technau, U., Bode, H.R.: ***HyBra1*, a *Brachyury* homologue, acts during head formation in *Hydra***. *Development* 1999, **126**: 999–1010.