Pattern formation in biology: a comparison of models and experiments

Rep. Prog. Phys. 55 (1992) 797-849. Printed in the UK

Pattern formation in biology: a comparison of models and experiments

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Abstract

How are the complex structures of a higher organism generated in such a reproducible way? Models of biological pattern formation are given in the form of nonlinear partial differential equations that describe production and decay rates as well as the diffusion of substances involved in pattern formation. As shown by comparison between expected and observed regulatory behaviour, these models describe many experimental observations in detail. According to these models, the following processes play a key role.

(i) Primary pattern formation results from short ranging autocatalysis and long ranging inhibition. Monotonically graded distributions of substances can be generated that can be used by cells to develop appropriate to their position within the organism. Periodic or stripe-like distributions can be generated in the same way by different diffusion rates and life times of the substances involved.

(ii) Cells obtain a stable state of differentiation by direct or indirect autoregulation of genes accompanied by a mutual competition among alternative genes. In this way, only one of several alternative genes can remain active within a particular cell. Which of the genes becomes activated can be under the control of a gradient generated by the mechanism mentioned above.

(iii) By mutual long range stabilization of cell states, a controlled neighbourhood of structures can be achieved. Segmentation such as seen in insects is proposed to result by a cyclic mutual activation of such locally self-stabilizing cell states.

(iv) Boundaries between regions generated by these mechanisms can obtain organizing properties for the finer subdivision of an organism. Substructures such as eyes, legs or wings are proposed to be initiated around the intersection of two borders. This mechanism accounts for the pair-wise initiation of these structures at the correct positions and with the correct handedness.

Many such elementary steps are required for development of a higher organisms. To allow the generation of complex patterns in a reproducible way, is assumed that these elementary steps are coupled to each other in a hierarchical way. The patterns of one level exert a strong influence on the subsequent pattern. Therefore, each subsequent pattern has a precise spatial relationship to pattern of the hierarchically higher level. An application of this scheme to the early *Drosophila* development is given.

This review was received in its present form in September 1991.

0034-4885/92/060797+53$18.00

Contents

1. The problem of pattern formation

A higher organism consist of many different cell types that have a precise spatial relation to each other. Starting with a single cell, the fertilized egg, this complex structure is reformed in each life cycle. Nature accomplishes the generation of order and complexity that seems to be forbidden by the laws of physics. The similarity of identical twins indicate how precisely this process is controlled by the genes. Reference to the genes, however, does not provide *per se* an explanation for the generation of spatial structures since, as a rule, with each cell division both daughter cells obtain the same genetic information. This leads to the question of how different parts of the developing organism can become different from each other. How is it achieved that some parts form the head and others the tail? How are arms and legs initiated at precise positions with a predictable handedness, which leads to the ever finer branching structure of a vein system or of a growing nerve?

A widespread tool in physics for the investigation of complex systems is to introduce perturbations and to observe the subsequent regulations. The same general approach has been applied to developing organisms to study the principles on which development is based. Tissue fragments have been removed or transplanted either to ectopic positions or to organisms that are in a different developmental stage. The genetic material has been changed by mutagenesis etc. The new tools of molecular biology have opened a second inroad. It may even be tempting to assume that a complete understanding can be achieved just by measuring the distribution of the relevant molecules at all stages of development. As the rule, however, this shifts only the problem. If a local concentration of a particular substance elicits a particular structure we need insights why precisely at this position this concentration maximum has been formed. Models and theories provide the necessary step from the observation to the paradigm regardless whether the regulatory behaviour or the distributions of molecules have been observed.

In physics a general consensus exists that theories are required. It is clear that one cannot deduce the quantum mechanics directly from the observation of the spectral lines of a hydrogen atom or Newton's law from the observation of planets. It appears to be an almost hopeless enterprise to develop a theory for a process as complex as the development of a higher organism. However, experiments have shown that the process of development can be separated into a sequence of discrete steps that can be regarded in a first approximation as independent from each other. For instance, it has been shown for insects that the formation of the anteroposterior (head to tail) axis can be regarded as independent of the dorsoventral (back to belly) axis. Therefore, meaningful models and theories can be developed that describe individual basic steps. The link of these steps is subject of a second approximation.

Since models for biological pattern formation are expected to contain nonlinear reactions with strong positive feedback terms, our intuition about the properties of the models is necessarily insufficient. Only a mathematical formulation combined with computer simulations allow a comparison between theory and experiment. In this way, models can be checked and if necessary, modified. We have developed several model for basic steps as well as for their linkage. The present article provides a review of these models. They represent certainly a personal view of how development is controlled. Models will be discussed for primary pattern formation, for the position-dependent

activation of genes, for the generation of filament-like branching structures, for the segmentation of animals as well as for the initiation of legs and wings at precise positions within the developing embryo. These models will be compared with most typical experimental observations. The common theme is how originally identical cell become different from each other in a reproducible way. Other important aspects of development such as the shaping of tissue or the differential control of cell proliferation will not be considered.

1.1. The egg cannot contain the final pattern

Although many eggs have some overall structure, the final pattern cannot already be present within the egg in a hidden form. If it were so, the egg must also contain the pattern of the egg of the next generation and so on, a process that soon comes necessarily to an end due to the atomic nature of matter.

At an early stage, many embryos can be fragmented into two parts and each part forms a complete organism. The sea urchin embryo at the 16 cell stage is an example. This indicates that in these cases the embryo is not a mosaic-like arrangement of differently determined cells that have a fixed further pathway. Instead, a communication must exist between different parts, such that the removal of some parts become detected and the missing parts replaced.

1.2. Organizing regions

Some small specialized regions obviously play a decisive role for the overall organization of the developing organism. Such organizing regions direct pattern formation in the surrounding tissue. One of such regions is the dorsal lip of an amphibian embryo (Spemann and Mangold 1924). The embryo first forms a blastula, a hollow sphere. In the process of gastrulation, an invagination of cells starts at a particular position that leads to a tube-like structure within the cavity. The first invaginating cells at the dorsal side have such special properties. A transplantation to the opposite (ventral) side causes the formation of a second embryo. By using tissue for transplantation that differ in their pigmentation from that of the host it has been shown that the secondary embryo is formed mostly not from the transplant but from host tissue. Obviously, the transplanted cells have an instructive influence on the surrounding tissue. The resulting pattern has no discontinuity at the border between the transplanted and the host cells.

A similar organizing region is located at the posterior pole of many insect eggs. Pinching off about 10% of an egg of a dragonfly suppresses development everywhere in the egg (Seidel 1929). A shift of material located at the posterior pole of a leaf hopper causes a dramatic change in the resulting pattern in a region much larger than that directly involved in the manipulation (Sander 1976).

Small regions with an organizing influence on the surrounding tissue are also found in the generation of substructures. For instance, at the posterior margin of a chick wing bud a small nest of cells exists that organizes the anteroposterior pattern of the limb. Transplantation of these cells into a more anterior position causes limb duplications (figure 1). Only very few cell have to be transplanted.

1.3. The concept of positional information

To account for the long range effect of small specialized regions and for the spatial

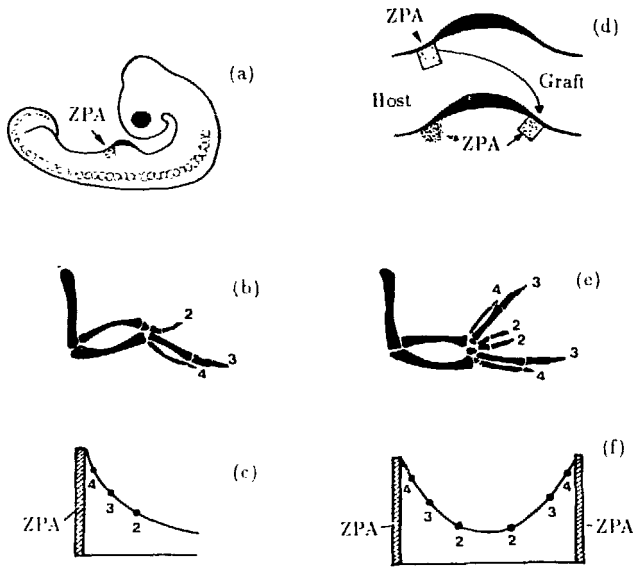

Figure 1. The positional information concept and mirror-image duplications (*a*) Chicken embryo with a wing bud. (*b*) normal structure of a wing, 2–4 denotes the digits from anterior to posterior. (*c*) According to the positional information concept (Wolpert 1969, Tickle *et al* 1975), the normal structure results from a graded distribution of a morphogen that spreads out from specialized cells at the posterior margin of the wing bud (ZPA, zone of polarized activity). The cells respond to this signal in a concentration-dependent manner. (*d*), (*e*) Grafting ZPA-cells to a more anterior position leads to a duplication of digits with an arrangement 432234. In terms of the positional information scheme, the operation leads to an U-shaped distribution (*f*) and thus to a symmetrical structure.

continuity observed after many experimental interferences, the concept of 'positional information' has been developed (Wolpert 1969).

Organizing regions are assumed to be the source of morphogenetic substances. By diffusion and decay graded distributions of such substances are generated. The local concentrations are a measure for the distance from the organizing region. The cells are assumed to interpret this positional information by a concentration-dependent response. A local source of a morphogenetic substance behaves as an organizing region since after transplantation the surrounding tissue becomes exposed to a graded concentration profile and follows the same pathway as the tissue that normally surrounds the organizing region. Figure 1 provides an explanation of the limb duplication experiment mentioned above in the view of the positional information concept. However, the assumption of a localized source shifts the problem. The question arises of how a localized source can emerge in an initially homogeneous tissue or at a particular position within the developing organism. Further, a local source has no handedness but, for instance, the limb has a handedness. Models will be discussed that suggest possible mechanisms.

2. Pattern formation by autocatalysis and lateral inhibition

Pattern formation starting from almost homogeneous initial conditions is very common in inorganic nature; sand dunes, rivers, clouds, lightning are examples. We have proposed that primary pattern formation proceeds by local self-enhancement coupled

to an antagonistic effect of long range (Gierer and Meinhardt 1972, Gierer 1981, Meinhardt 1982). A similar proposal has been made by Segel and Jackson (1972) for ecological systems. Patterns are formed since small deviations from a homogeneous distribution have a strong positive feedback such that the deviations grow further. A long range antagonistic effect localizes this increase and suppresses it at larger distances. The self-enhancing element is easy to see in the examples of inorganic pattern formation mentioned above. Erosion proceeds faster at an initial injury since more water collects there. A sand dune provides a windshield that accelerates the deposition of more sand. Since the total amount of water or sand is limited, a local accumulation must be accompanied by an overall decrease elsewhere.

A possible realization of this general principle based on biochemical feasible interactions is shown in figure 2. A short-ranging substance—the activator—promotes its own production (autocatalysis) as well as that of its rapidly diffusing antagonist, the inhibitor. The concentrations of both substances can be in a steady state. A general increase of the activator is compensated via an increase in the inhibitor concentration. However, such an equilibrium is locally unstable. Any local increase of the activator will increase further due to the autocatalysis since the surplus of the inhibitor diffuses rapidly into the surroundings of this local increase. It inhibits the activator production there while the local activator elevation grows further (figure 2). The following partial differential equation describes a possible interaction. It relates the change per time unit of the activator a and the inhibitor h as function of the concentrations present.

$$\frac{\partial a}{\partial t} = \frac{\rho a^2}{h} - \mu a + D_a \frac{\partial^2 a}{\partial x^2} + \rho_0 \tag{1a}$$

$$\frac{\partial h}{\partial t} = \rho a^2 - \nu h + D_h \frac{\partial^2 h}{\partial x^2} + \rho_1 \tag{1b}$$

where t is time, x is the spatial coordinate, D_a and D_h are the diffusion coefficients, μ and ν the decay rates of a and h. The source density ρ describes the ability of the cells to perform the autocatalysis. A small activator-independent activator production ρ_0 can initiate the system at low activator concentrations. The total time course can be calculated by an integration of these equations, starting from certain initial conditions.

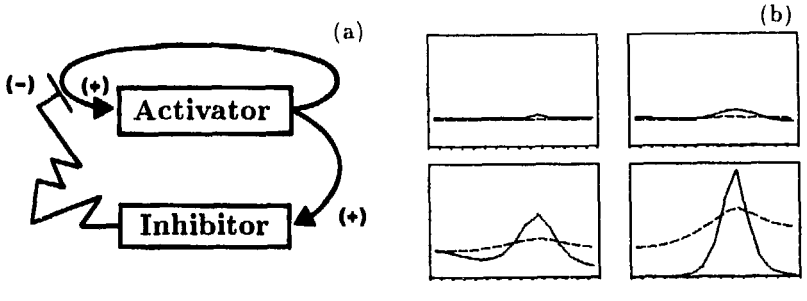

Figure 2. Pattern formation by autocatalysis and long-range inhibition (*a*) Reaction scheme. An activator catalyses its own production and that of its highly diffusing antagonist, the inhibitor. (*b*) Stages in pattern formation after a local perturbation. Computer simulation in a linear array of cells. A homogeneous distribution of both substances is unstable. A minute local increase of the activator (——) grows further until an equilibrium is reached with the surrounding cloud of inhibitor (- - - -).

For computer simulation, these equations have been approximated by difference equations for discrete cells. For instance, (1*a*) is approximated by

$$da_{i,t} = \frac{\rho a_{i,t}^2}{h_{i,t}} - \mu a_{i,t} + D_a(a_{i-1,t} + a_{i+1,t} - 2a_{i,t}) + \rho_0 \tag{1c}$$

where $i = 1, 2, \ldots$ is the number of the cell in a linear array of cells. This allows the calculation of the activator and inhibitor distribution at a somewhat later time. The activator concentration at the time $t+1$ is given by

$$a_{i,t+1} = a_{i,t} + da_{i,t}.$$

The total time course is calculated from a repetition of many such iterations. In all simulations shown the boundaries are assumed to be impermeable.

A condition for the formation of stable patterns is that the activator diffuses much slower than the inhibitor and has a longer time constant, i.e. $D_a \ll D_h$ and $\mu < \nu$. As discussed below in detail, the patterns that can be generated by this type of interaction correspond with many patterns observed in living systems. In small or growing fields, monotonic gradients are formed that are appropriate to supply positional information. In fields large compared with the range of both substances, spatial periodic patterns will result. The mechanism has regulatory properties that allow, for instance, reformation of a graded concentration profile after an interference.

In contrast, if $D_a > D_h$ and $\mu > \nu$ oscillations and travelling waves can result. The pigmentation patterns on shells of molluscs will be discussed later as example of this mode of pattern formation (see figures 12–14 below).

2.1. Local instability and global stability

Some very simple calculations should provide some intuition why an interaction as given by (1) can lead to stable patterns. For simplicity, we assume all constants equal to 1, disregard diffusion and assume that even the inhibitor concentration is constant. Equation 1(*a*) would be simplified to

$$\frac{da}{dt} = a^2 - a. \tag{2a}$$

In this simplified version, the activator has a steady state ($da/dt = 0$) at $a = 1$. However, this steady state is unstable since for any concentration of a larger than 1, $a^2 - a$ will be positive and the concentration of a will further increase and vice versa. The reason for this instability lies in the over-exponential autocatalytic production in conjunction with a normal exponential decay.

Now let us include the action of the inhibitor. Disregarding again any constants and diffusion, 1(*b*) would be simplified to

$$\frac{dh}{dt} = a^2 - h \tag{2b}$$

which has a steady state at $h = a^2$. If we assume that the inhibitor equilibrates relatively rapid to a changed activator concentration, we can express the change of activator concentration as function of the activator concentration alone:

$$\frac{da}{dt} = \frac{a^2}{h} - a \approx \frac{a^2}{a^2} - a = 1 - a. \tag{2c}$$

Therefore, if we include the action of the inhibitor, we obtain a steady state at $a = 1$

that is stable since if a is larger than 1, $1-a$ is negative and the concentration will return to the steady state at $a=1$.

To see why an interaction according to (1a, b) can generate a pattern we have to take into consideration that the inhibitor is assumed to diffuse much faster compared with the activator. Let us assume an array of cells, all at the steady state concentrations of a and h, except one cell which should have a slightly increased activator concentration. It will produce also more of the inhibitor but since the inhibitor diffuses rapidly into the surroundings, the inhibitor can be regarded in the first approximation as constant. It is the average activator concentration that is decisive for the inhibitor production. As mentioned, if the inhibitor remains constant, any deviation from the activator steady state will grow further, the steady state being unstable. However, after a substantial increase of the activator maximum, the inhibitor concentration can no longer be regarded as constant. As shown above, the action of the inhibitor leads to the stabilization of the autocatalysis. A new stable patterned steady state will be reached. Thus, the formation of a stable pattern depends on a local instability and a global stability.

These considerations can be expressed in more general terms (Gierer and Meinhardt 1974, Gierer 1981). If a and h changes according to the equations

$$\frac{\partial a}{\partial t} = f(a, h) \tag{3a}$$

$$\frac{\partial h}{\partial t} = g(a, h) \tag{3b}$$

and h equilibrates rapidly over a large area by diffusion or convection, h can be approximated as a function of the average a concentration $\bar{a}$ in a region from which the inhibitor is derived. The change of the activator concentration can be written as

$$\frac{\partial a}{\partial t} = f(a, h(\bar{a})). \tag{3c}$$

This may have a uniform steady state solution at $a_0 = \bar{a}$ at which $\partial a/\partial t = 0$. Patterns are formed if a slight local increase over this steady state concentration grows further, i.e. if

$$\left(\frac{\partial f}{\partial a}\right)_{a_0} > 0. \tag{3d}$$

The inhibitor will lead to a globally stable pattern if

$$\left(\frac{\partial f}{\partial a}\right)_{a_0} + \left(\frac{\partial f}{\partial h}\frac{\partial h}{\partial a}\right)_{a_0} < 0. \tag{3e}$$

It is easy to see that (1a, b) satisfies these conditions. Other possible molecular realizations will be discussed further below.

2.2. Turing's mechanism

The possibility of generating a pattern by the reaction of two substances with different diffusion rates was discovered by Rashewsky (1940) and Turing (1952). The involvement of diffusion seems to contradict our intuition since with diffusion one associates a smoothing out of any local accumulation of molecules, not the creation of differences. However, as we have seen, the different diffusivities of the substances can lead to a

restriction of an increase at one location and to its decrease in the surroundings. Turing exemplified the mechanism he proposed by the following set of equations (Turing 1952, p 42).

$$\frac{dx}{dt} = 5x - 6y + 1 \tag{4a}$$

$$\frac{dy}{dt} = 6x - 7y + 1 \text{ (+diffusion)}. \tag{4b}$$

Both equations, (4*a*) and (4*b*), look very similar. It is not immediately obvious why such interaction leads to a pattern. However, a similar consideration as undertaken above for (1) shows that the basis of the Turing pattern is also autocatalysis and lateral inhibition. From (4) we can calculate that a steady state is given at $y = 1$ and $x = 1$. If we regard y as constant when there is a small local deviation from the steady state—remember that y is the rapidly diffusing substance—we obtain

$$\frac{dx}{dt} = 5x - 5 \tag{4c}$$

which is again positive for any $x > 1$. Therefore, due to the self-enhancement, the deviation will grow further. To show how the interaction of y stabilizes the system, we have to calculate the steady state of y as function of x. By setting $dy/dt = 0$, we obtain from (4*b*)

$$y = \frac{6x + 1}{7}. \tag{4d}$$

Inserting this into (4*a*) we obtain:

$$\frac{dx}{dt} = \frac{1 - x}{7} \tag{4e}$$

indicating a steady state at $x = 1$. This state is stable since we get a negative change of x for any $x > 1$. This elementary calculation shows that also in Turing's mechanism the local instability results from a short-range autocatalysis. The long-range substance assures that the total system remains stable. An increase in one area depends on a decrease in the surrounding. Therefore, Turing's mechanism can generate basically the same types of pattern as the lateral inhibition mechanism, i.e. graded concentration profiles and isolated maxima (Bard and Lauder 1974, Lacalli and Harrison 1978).

The mechanism proposed by Turing has an essential drawback: its molecular basis is not reasonable. According to (4*a*), the number of x molecules disappearing per time unit is assumed to be proportional to the number of y molecules but independent of the number of x molecules. In other words, x molecules can disappear although no x molecules are present. This can lead to negative concentrations. Turing had seen this problem and proposed to ignore negative concentrations. However, such a cut-off can cause an unlimited concentration increase. In any case, such a cut-off is not necessary if the number of decaying molecules is proportional to the number of molecules present. This requires, as given in (1), nonlinear production terms.

2.3. Formation of graded concentration profiles

A very important step in early embryogenesis is the generation of the primary body axes. One side of the developing organism must become different from the other. It

is a property of the mechanism outlined above that in growing fields polar concentration profiles will be formed whenever a critical size is exceeded (see figure 8 below). In a growing field a high concentration on one side and a low concentration at maximum distance is the first pattern that emerges since a non-marginal maximum requires space for two slopes, a space that is not available if the critical size is just reached. If the size is smaller, any incipient activator pattern will be smoothed out due to the rapid redistribution of the activator within the small field. Thus, the critical size depends on the range of the activator molecules. The resulting polar pattern can be stabilized also during further growth (see figure below 9).

Since a homogeneous activator-inhibitor distribution represents an unstable situation, any inhomogeneity can initate pattern formation. The stimulus can be very unspecific. Any asymmetry in oxygen supply, in the pH or in temperature could be sufficient. Local disadvantages would shift the maximum towards an opposite region. Pattern initiation can result from fluctuations of the activator concentration or from an inhomogeneity in the source density, i.e. the abilility of the cells to perform the pattern forming reaction (ρ in equation (1)). According to the model, such stimulus only orients the pattern. The pattern itself is fairly independent of the initial stimulus (figure 3). Therefore, no precision is required for the initiating signal. A stronger initiating asymmetry has the advantage that the pattern reaches much faster the final steady state since no time-consuming competition is required between opposite sides of the field. In addition, the danger is lower that in a somewhat larger field a symmetric instead of a polar pattern is formed.

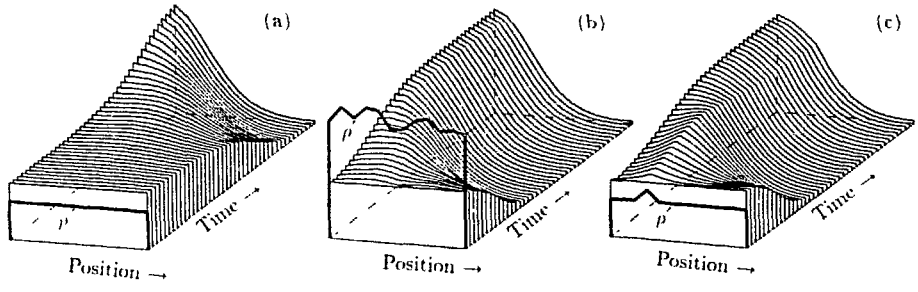

Figure 3. Generation of a polar pattern and its orientation by asymmetries in the source density. Computer simulation in a linear array of cells. Concentrations are plotted as function of time. (*a*) Small random fluctuation of the source density (ρ in equation (1)) are sufficient to initiate pattern formation. In a field with a size comparable to the range of the activator only a marginal maximum can be formed since space for two slopes is not available. The result is a polar pattern. (*b*) Any asymmetry can accelerate the patterning process, but the resulting pattern is independent of the source density. The asymmetry only orients the pattern. (*c*) Even a non-marginal perturbation leads to a polar pattern. If the diffusion range would be smaller, the tendency to form marginal maxima is lost (figure 2).

Eggs with strong as well as no initial internal asymmetries are known. An example for the latter is the brown algae *Fucus* (Jaffe 1968). Any external asymmetry, for instance, gradients in pH, in temperature, in illumination, in the streaming of the surrounding water or the proximity of other *Fucus* eggs are able to orient the outgrowth, underlining the instability of the system. In contrast, in amphibians, the position of the sperm entry polarizes the egg. The point of invagination during gastrulation, the classical organizer of Spemann and Mangold (1924) is formed opposite the sperm

entry (see Gerhart *et al* 1980). However, after removal of this intrinsic asymmetry by dissociation and re-aggregation of cells, the pattern can be formed as well (Nieuwkoop 1973). Thus, the initiating asymmetry is used, but it is not necessary for pattern formation.

2.4. Pattern regeneration

Many developing systems show pattern regulation. As mentioned, the separation of an early sea urchin embryo in two parts can lead to two complete embryos. The regeneration of a new head after head removal in *Hydra* will be discussed further below. An activator-inhibitor system shows substantial pattern regulation. With the removal of the site of high activator concentration, the site of inhibitor production is removed too. The remnant inhibitor decays until a new activator maximum is triggered in the remaining cells from a low level activator production (ρ_0 in equation (1)). The pattern is restored in a self-regulatory way (figure 4).

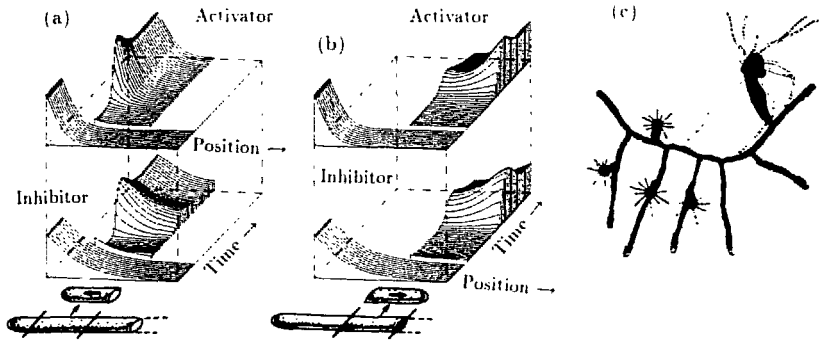

Figure 4. Regeneration. (*a*) After removal of the activated region, a new activator maximum can be formed after the decay of the remnant inhibitor. (*b*) if the fragment does not contain the activated region, regeneration can be accompanied by a polarity reversal since the new maximum can appear in the region bearing the lowest inhibitor concentration. (*c*) Biological example: Colony of a marine hydroid *Eirena*. Polyps are connected by branching tubes. Fragments of these tubes form new tips, the polarity depends on the distance from the original tip (drawing below the simulations).

During pattern regeneration, the polarity may be reverted. This has been observed after fragmentation of the sea urchin mentioned above or in regenerating tips of solons in colonial hydrozoa (Müller and Plickert 1982) (figure 4). According to the model, whether polarity is maintained depends on the activator and inhibitor distributions in the remaining tissue. If the fragment has been obtained from a distant position in relation to the former activator maximum, the activator distribution is flat. In contrast, the inhibitor distribution has its lowest concentration at the largest distance from the former maximum. This will be the site where the new peak will be formed and the polarity of the pattern will be reversed. As will be discussed below, gradients in the source density lead to a maintenance of the polarity upon regeneration, as is observed in *Hydra* (see figure 9 below).

2.5. Unspecific induction of a second embryonic axis

After the discovery that the dorsal lip of an amphibian blastopore acts as an organizer and that a second embryo can be induced after its transplantation to the ventral side

(see section 1.2), a great optimism arose that substances controlling development could be isolated. However, further investigations showed disappointingly that very unspecific stimuli can induce a secondary embryo.

The occurrence of unspecific induction is a straightforward consequence of the proposed activator-inhibitor scheme. By an unspecific manipulation one can only lower a substance concentration, for instance by destruction of molecules due to irradiation or by a leakage through an injury. At larger distances from an existing activator maximum, the inhibitor concentration is low in any case. Any further decrease may result in the onset of autocatalysis. A second activator maximum will be formed that is independent of the triggering stimulus since the activator concentration increases until it is in equilibrium with the surrounding cloud of inhibition. The resulting maximum is indistinguishable from a maximum triggered by application of the genuine autocatalytic substance.

2.6. Generation of periodic patterns

In many developmental situations, periodic structures are formed. The leaves on a growing plant, the bristles on insects or the spacing of feathers are examples. In terms of the model, periodic structures will be formed if the range of the inhibitor is smaller than the total field. If the pattern is formed when the field has already a substantial size, the resulting pattern will be somewhat irregular, only a maximum and minimum distance will be maintained (figure 5). The cilia on the surface of a *Xenopus* embryo (an African frog) or the stomata of leaves (figure 5) are biological examples of this type of pattern. In contrast, if the field grows new maxima will be formed whenever

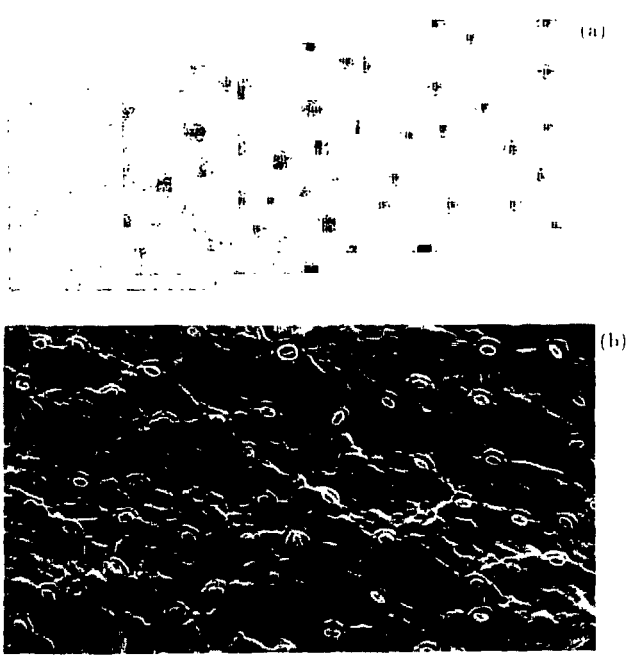

Figure 5. Periodic patterns. If the size of the field is much larger than the range of the antagonist a periodic pattern results. (*a*) Due to the initiation by random fluctuations the spacing is somewhat irregular, but a maximum and a minimum distance is maintained. (*b*) Biological example: the pattern of stomatas (leaf openings).

the distance to existing maxima become too large. The resulting pattern will be very regular (figure 6).

To generate a regular periodic structure even though the field has already a large size, biology uses in some situations an interesting trick, that is to say a simulated growth. A determination wave moves over the tissue from one side to the other. Pattern formation starts only after this wave has been passed. Thus, the zone in which pattern formation can take place enlarges in a systematic way as if growth were present. Such a moving zone has been found in *Drosophila* during development of the very regular compound eye (Tomlinson 1988). Similarly, the initiation of feather primordia on birds begins at an anterior-dorsal position and moves backwards and more ventrally in a wave-like manner (Sengel 1976).

If a tissue that bears a periodic structure grows further by cell divisions at random positions, the distances between existing maxima increase. At large distances to existing maxima the inhibitor concentration can become so low that autocatalysis is no longer repressed and new maxima are inserted. Wigglesworth (1940) has investigated the bristle pattern of the bug *Rhodnius*. After each mould, new bristles are inserted that keep a distance from existing bristles. Similarly, during growth of leaves, new stomatas (see figure 5) are inserted. The insertion of new maxima in a growing array of cells can be seen in figure 8(*b*) below.

2.7. Formation of stripe-like distributions require saturation of self-enhancement and activator diffusion

Stripe-like patterns, i.e. structures with a long extension in one dimension and a short extension in the other, are formed at many instances during embryogenesis. Proverbial are the stripes of zebras. The activation patterns of genes involved in the segmentation of the early *Drosophila* embryo (see figures 21 and 22 below) or the ocular dominance columns, the patterns of the preferential connection of the left and the right eye in

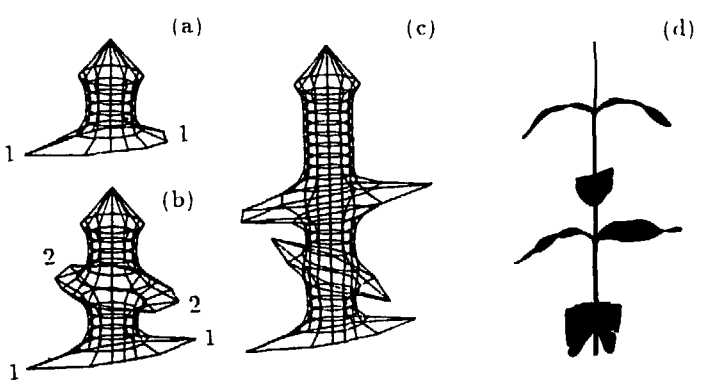

Figure 6. Formation of a regularly spaced periodic pattern during growth (*a*)-(*c*). Simulation of a decussate leaf arrangement. Assumed is a cylindrical arrangement of cells, growing by doubling the uppermost row of cells. The distance from the central cylinder is a measure for the activator concentration. An activator maximum and thus an inhibitory zone at the tip of the shoot is assumed. The first pair of maxima (1) appears at a certain distance from the tip. Their positions are determined by random fluctuations. After sufficient distance is achieved from existing maxima, a ring-shaped activated region is formed that disintegrate into a further pair of peaks (2) (after Meinhardt 1974, 1982). Each subsequent pair appears at right angle with respect to the previous one. (*d*) Example of a natural decussate leaf arrangement.

the visual cortex (Hubel *et al* 1977) may serve as an example. To provide positional information along the shorter dorsoventral axes of *Drosophila* a region of high concentration must be generated which has a long extension along anteroposterior, but a short extension along the dorsoventral axis. How can it be achieved that regions of high concentrations are generated that have different extensions along different axes?

Stripe-like distributions can be generated by the mechanism described above if activator production has an upper bound (Meinhardt 1988, 1989). If activator autocatalysis saturates at a relatively low concentration the inhibitor production is limited too and the mutual competition between neighbouring cells is reduced. More cells remain activated, although at a lower level. Thus, an activated cell has to tolerate an activated cell in its neighbourhood, independent of the range of inhibition. An example for the required saturation term is given by the following modification of (1*a*)

$$\frac{\partial a}{\partial t}=\frac{\rho a^2}{h(1+\kappa a^2)}-\mu a+D_a\Delta a+\rho_0. \tag{5}$$

Stripe formation requires, in addition to the saturation, a modest diffusion of the activator. Due to this diffusion, activated regions tend to occur in large coherent patches since, if a cell is activated, the probability is high that the neighbouring cell also becomes activated. On the other hand, it is necessary that activated cells are close to non-activated cells into which the inhibitor can diffuse, otherwise no activation above average would be possible. These two seemingly contradictory requirements, coherent patches and proximity of non-activated cells, are satisfied if a stripe-like pattern is formed (figure 7). Each activated cell is bordered by other activated cells but non-activated cells are not too far away.

If initiated by random fluctuations, the stripes have random orientations too. As will be discussed further below, preceding pattern forming events can be used to orient the stripes such that a regular and predictable pattern is generated.

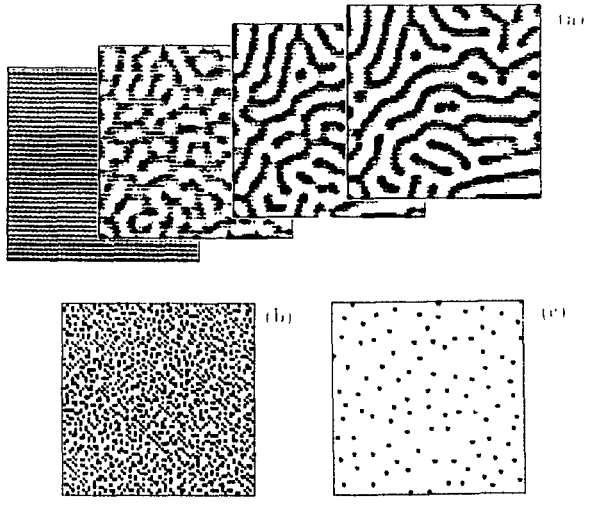

Figure 7. Stages in the formation of stripes. (*a*) If the autocatalytic reaction saturates at low activator concentrations, more cells remain activated although at a lower level. Stripes are the preferred pattern since activated cells have activated cells as neighbours and non-activated cells are nearby into which the inhibitor can diffuse. (*b*) Without activator diffusion, activated cells appear with a salt-and-pepper distribution. (*c*) Without saturation, fewer activated cells (with higher concentrations) emerge in separate patches.

2.8. Possible molecular realization of autocatalysis and lateral inhibition

The activator-inhibitor mechanism as given in (1*a*, *b*) is of course only one example of the many possible molecular realizations that satisfy the general criterion (3*d*, *e*). A minor modification would be that the inhibitor acts in a non-linear way. Then, its production can be linear. This offers the possibility that the inhibitor is the more rapidly diffusing decay product of the activator. The rate at which the activator disappears corresponds to the production rate of the inhibitor.

$$\frac{\partial a}{\partial t} = \frac{\rho a^2}{h^2} - \mu a + D_a \frac{\partial^2 a}{\partial x^2} + \rho_0 \tag{6a}$$

$$\frac{\partial h}{\partial t} = \mu a - \nu h + D_h \frac{\partial^2 h}{\partial x^2}. \tag{6b}$$

The inhibitor can act also by an acceleration of the activator destruction. However, then the lifetime and therefore the range of the activator would change during pattern formation. The shortening of the lifetime can lead to oscillations.

The antagonistic effect can also result from the depletion of a substrate $s(x)$ which is consumed during the autocatalytic activator production.

$$\frac{\partial a}{\partial t} = \rho s a^2 - \mu a + D_a \frac{\partial^2 a}{\partial x^2} + \rho_0 \tag{7a}$$

$$\frac{\partial s}{\partial t} = \sigma - \rho s a^2 (-\nu s) + D_s \frac{\partial^2 s}{\partial x^2}. \tag{7b}$$

This reaction has similarities with the so-called Brusselator reaction (Prigogine and Lefever 1968, Lefever 1968) but is somewhat simpler. It has some properties distinctly different from the activator-inhibitor mechanism. In growing systems new maxima are formed preferentially by a split and movement of existing maxima (figure 8), in contrast to the generation of new maxima at a distance to existing maxima in the activator-inhibitor system. The reason is as follows. With growth, the substrate concentration increases in the enlarging space between the activated regions since the substrate is not used up there. This can lead to a higher activator production at the side of an

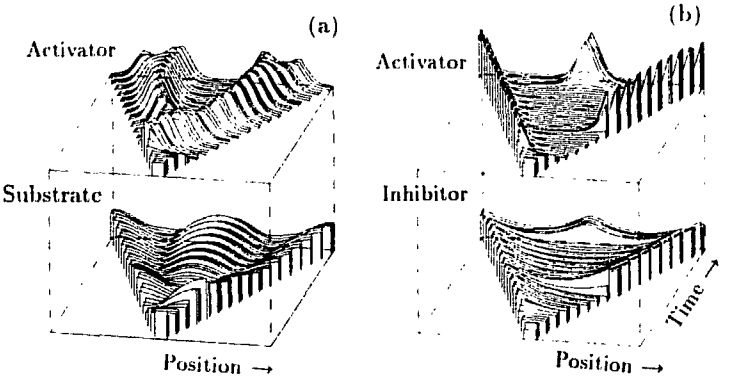

Figure 8. Antagonistic reactions by depletion or inhibition. (*a*) The antagonistic reaction results from the depletion of a substrate *s* that is required for autocatalysis (7). Upon growth, new maxima are formed by a split and shift of existing maxima. (*b*) In contrast, if the antagonstic reaction results from an inhibitor (1), in a growing field new maxima appear at a distance to the existing one.

existing maximum, i.e. the maximum begins to wander towards higher substrate concentrations until a new optimum position is obtained. Lacalli (1981) has simulated pattern formation during growth of the unicellular algae by such a mechanism.

A further difference between both types of reactions is that unspecific induction as discussed above for the activator-inhibitor system is unlikely to occur in an activator-substrate mechanism. By an unspecific manipulation such as irradiation or leakage through an injury, one can only destroy molecules, but not increase their concentration. Neither lowering of the activator nor of the substrate but lowering of the inhibitor concentration can trigger a new maximum.

Pattern formation does not require a molecule with direct autocatalytic regulation. The autocatalysis can be a property of the system as a whole. For instance, if two substances, a and b exist and a inhibits b and vice versa, a small increase of a above an equilibrium leads to a stronger repression of the b production by a. This, in turn, leads to a further increase of a, in the same way as if a were autocatalytic. The same holds for b. a and b together form a switching system in which either a or b is high. The switch of the λ phage between the lytic and the lysogenic phase is based on such an inhibition of an inhibition (Ptashne *et al* 1980). To allow pattern formation, a long ranging signal is required that interferes with the mutual competition of the two substances, For instance, if a has won the a-b competition in a particular region, b must win in the surroundings. A possible realization would be that the a molecules control the production of a substance c which, in turn, either inhibits the a or promotes b production. These modes are equivalent since in competing systems a self-limitation is equivalent with a support of the competitor. In ($8a$-c) an interaction is described in which the diffusible antagonistic substance c is produced under control of the a molecules and which undermines the repression of b production by the a molecules. No direct autocatalytic interaction is assumed:

$$\frac{\partial a}{\partial t} = \frac{\rho}{\kappa + b^2} - \mu_a a + D_a \frac{\partial^2 a}{\partial x^2} + \rho_0 \tag{8a}$$

$$\frac{\partial b}{\partial t} = \frac{\rho}{\kappa + a^2/c^2} - \mu_b b + D_b \frac{\partial^2 b}{\partial x^2} + \rho_0 \tag{8b}$$

$$\frac{\partial c}{\partial t} = \gamma a - \mu_c c + D_c \frac{\partial^2 c}{\partial x^2}. \tag{8c}$$

A more general discussion of possible mechanisms and their equivalence is provided by Gierer (1981).

Much further theoretical work has been done on such reaction-diffusion mechanism and their application for biological pattern formation. Murray (1981, 1989) has discussed similar mechanism for the animal coat markings. The possible involvement of strain and mechanical forces in self-enhancement (Oster and Murray, 1989, Goodwin and Trainor, 1985) or the role of calcium as one of the components for pattern formation within single cells (Harrison and Hillier 1985) has been discussed.

Although the theory describes correctly the dynamic behaviour of primary pattern formation and pattern regulation, its direct verification has to wait for future results. So far, the molecular machinery is known for none of the systems that can exhibit *de novo* pattern formation. However, in some of these systems promising approaches towards a molecular understanding are under way. In *Xenopus* the growth factor TGFβ can induce tissue to behave as tissue from an organizing region (Cooke 1989). At least in tissue culture, TGFβ shows autocatalytic enhancement of its own production (Van

Obberghen-Schilling *et al* 1988), displaying in this way the theoretically predicted behaviour. In the slime mould *Dictyostelium*, cAMP is autocatalytically amplified. The self-amplification is antagonized by a cAMP-dependent receptor phosphorylation (Vaugham and Devreotes 1988). In the latter case travelling waves result instead of a stable pattern since the inhibition is local while the self-amplification can spread (see figure 12 below). In contrast, non-biological pattern formation of the Turing type has been demonstrated in the laboratory (Ouyang and Swinny 1991).

3. Coupling of several reaction-diffusion systems in order to generate specific patterns

Although, as shown above, many essential patterns can be generated by a mechanism based on autocatalysis and long range inhibition, there are other patterns that require a coupling between several reactions or at least some modifications of the mechanism discussed above. Examples of such patterns are the maintenance of a monotonic gradient during growth, the formation of a gradient across the short extension of a field, and the regulation of the size of a particular structure in relation to the total size of an organism.

3.1. The wavelength problem: maintenance of a graded concentration profile during growth

Usually the size of morphogenetic fields increases during the growth of the embryo. In a reaction as given by (1), a graded concentration profile can be maintained only over a range of about a factor two. With increasing field size, a tendency exists to change from a monotonic into a symmetric and a ultimately in a periodic distribution (figure 8). This is inappropriate if the graded concentration should be used as positional information for the determination of the primary body axes in the growing embryo.

Several possibilities exist to circumvent this problem. The conversion of the dynamically regulated pattern into a stable pattern by a concentration-dependent gene activation will be discussed further below (figure 16). Alternatively, the appearance of secondary maxima can be suppressed. If the inhibitor has a small activator-independent production term (ρ_1 in equation (1(b)) the nominator in (1a) remains finite even at very low activator concentrations. Then, in addition to semi-stable steady state, a second stable state exists a low activator concentrations. This can inhibit the onset of autocatalysis at low activator concentrations. The field can grow without secondary maxima appearing. However, this mechanism is inappropriate for systems that show regulation over a large range of sizes since an activator maximum cannot regenerate after its complete removal.

3.2. Stabilization of a monotonic gradient by a feedback on the source density—the freshwater polyp Hydra as example

Pattern regulation and growth are not mutually exclusive. For instance, the fresh water polyp *Hydra* maintains its polar structure over substantial growth but, nevertheless, a fragment 1/10 of the normal body size is still able to regenerate. *Hydra* has presumably the longest history in the study of regeneration (Trembley 1744). Head regeneration of a fragment occurs always at the side pointing towards the original head. Morgan (1904) interpreted similar observations with *Tubularia* by assuming that a systematic change in the ability for head regeneration exists and that a competition takes place.

The tissue orginally closer to the head always has an advantage and wins the competition. The relative position is decisive.

The range of dominance of the activated over the non-activated region, the apical dominance, can be increased by an order of magnitude if a feedback of the activator on the source density (ρ in equation (1)) exists (Meinhardt and Gierer 1974). Usually the sources of the activator and inhibitor synthesis are assumed to be homogeneously distributed, except of small random fluctuations (figure 3). If, however, an increased activator concentration leads to an increase of the source density, the source density obtains a graded distribution too. A possible addition to (1) or (7) for the change of the source density ρ is given by (9):

$$\frac{\partial \rho}{\partial t} = \gamma(a - \rho) + D_\rho \frac{\partial^2 \rho}{\partial x^2}. \tag{9}$$

With increasing distance from the activated region, the source density becomes lower. Conseqently it is less likely that the inhibition emanating from the existing maximum can be overcome, such that a secondary maximum can appear. Thus, the dominance of an existing maximum is enlarged and a polar pattern can be maintained although substantial growth occurs (figure 9).

Since the wavelength of a pattern forming system is no longer determined by the activator diffusion, the activator diffusion can be very small. Therefore, small fragments can also regenerate a pattern. The graded distribution of the source density provides information about the polarity of the tissue. Small fragments regenerate an activator maximum in the region of the relatively highest source density (figure 3), i.e. according to the original polarity. This requires that the source density has a longer time constant in comparison with the activator. The source density does not change significantly during regeneration. The simulation in figure 9 shows the rapid regeneration of a fragment according to the orginal polarity. This should be compared with a regeneration in a system with a homogeneous source density (figure 4). Since after regeneration the ρ-gradient will be restored in the course of time, this gradient will not be diluted out in repeating rounds of regenerations.

It may appear surprising that a feedback of the activator on the source density as given in (9) does not lead to an explosion in the activator concentration in that more activator causes a higher source density which, in turn, would lead to an increased activate concentration. However, in (1) or (7) the activator (but not the inhibitor or the substrate concentration) is independent of ρ over a wide range. Thus, an increase of ρ leads not to an increase in a but a source density gradient leads to a more rapid development of the activator pattern since symmetry breaking is no longer required (see figure 3).

In *Hydra* the source density is presumably the density of a certain cell type to which the activator and inhibitor synthesis is restricted. An increased activator concentration could lead to an increased proliferation or differentiation, such that more cells of this type are formed. The 'head activator' isolated from *Hydra* and other tissues (see Schaller *et al* 1989) may be a factor involved in the generation of a graded source density. Addition of this small peptide leads to the differentiation of more nerve cells from stem cells while, in turn, under normal condition, the head activator is mostly produced by the nerve cells.

The source gradient would be a morphogenetic gradient in the terms of Morgan (1904), a gradient in the tendency to form a particular structure. It becomes decisive in a competitive situation, for instance during regeneration. In this case, the *relative*

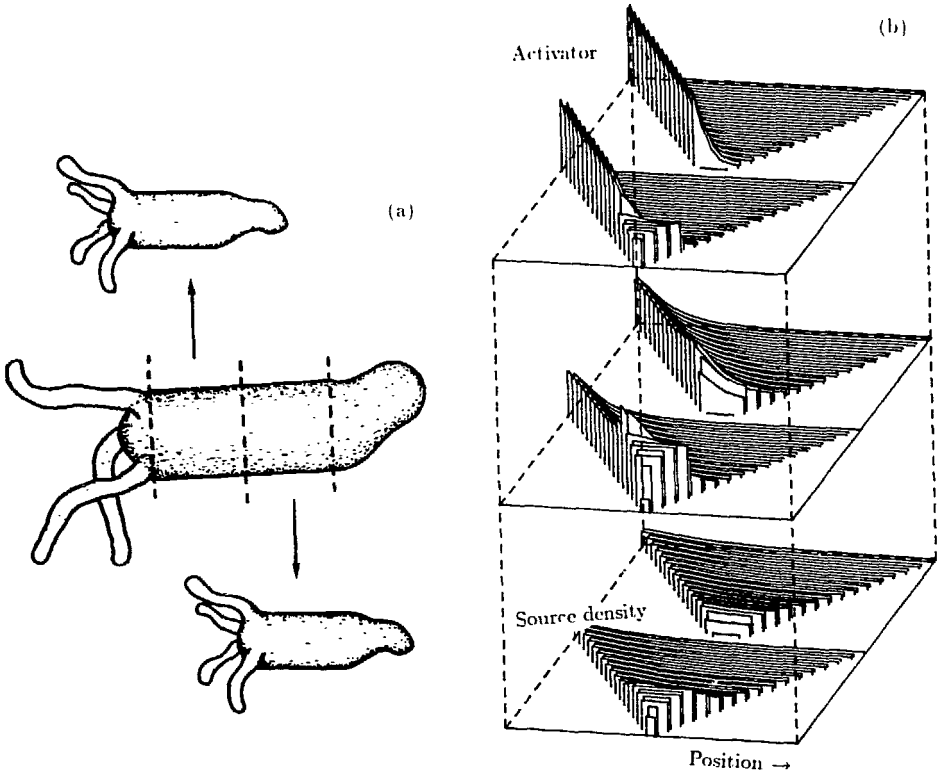

Figure 9. Stabilization of polar distributions by a feedback of the activator on the source density—a model for the maintenance of apical dominance in *Hydra*. (*a*) Small fragments of a *Hydra* regenerate according to the original polarity. Depending on the cuts, the same tissue forms either a head or a foot, but the polarity is always maintained. (*b*) The tendency to form periodic structures upon growth can be reduced if the activator has a feedback on the source density (compare with figure 8(*b*)). The source density becomes graded which, in turn, stabilizes the polar distribution. At a region of low source density, the initiation of secondary maxima is unlikely. The graded source density provides the long-lasting information about the polarity of the system. A small fragment regenerates a pattern according to the original polarity (see figure 3).

concentration is decisive. Such a concept of morphogenetic gradient should not be mixed up with a morphogen gradient used as positional information (see figure 1) where the local *absolute* concentration of a substance determines which structure will be formed.

3.3. Bipolar morphogenetic fields

Pattern formation in *Hydra* is not only controlled from the head, but also from the opposite end, the foot. A second pattern formation system exists which controls the foot formation. Both pattern forming systems show features of lateral inhibition. The induction of a second foot close to an existing foot is inhibited by the existing foot (Cohen and MacWilliams 1975). *Hydra* is by no means a singular case being controlled from both ends. For instance, the early *Drosophila* embryo is controlled by sources of morphogenetic substances localized at the anterior and at the posterior pole (Nüsslein-Volhard *et al* 1987).

A single gradient generated by a local source at one side of a field has an inherent drawback if used as positional information. Supposing impermeable boundaries, the gradient at the side not containing the source is necessarily shallow and the absolute concentration depends on the total size of the field. A possible way out of this problem is the use of only a certain concentration range while regions exposed to higher or lower concentrations do not participate. This concentration range is present in fields (e.g. eggs) of different sizes. A precise regulation of the absolute level of the gradient is not required since the critical portion of the concentration range would be present anyway although at a lightly shifted position. A biological example for the employment of this strategy are presumably the short germ insects (Sander 1976). Underneath of the shell of an insect egg a monolayer of cells, the so-called blastoderm, is formed which gives rise to the embryo proper by space-dependent gene activation (see below). In short germ insects, only a small portion of the blastoderm contributes to the formation of the embryo. The remaining cells degenerate. The region utilized is close to the posterior pole which is, as known from other experiments, the source region of a gradient.

Another circumvention of the problem of size-regulation of gradients consists in the formation of two gradients of opposite slopes, having their origins at opposite sides of the field. Each gradient directs cell determination in the corresponding part of the developing organism.

Several possibilities exist to generate two different maxima at opposite ends of a field. For instance, if two activator-inhibitor systems exist and the two inhibitors show some cross-reaction, the corresponding two maxima will appear at the largest possible distance from each other (figure 10).

Bipolar systems can be used to generate morphogen distributions that are in a wide range independent of the total size. One of the system acts as a source, the other as the sink of the morphogen in addition to an overall decay. Around the source, the concentration depends on the production, decay and diffusion rate. At the sink, the concentration will be kept at a low level.

In *Hydra*, the situation is even more complicated. The head and the foot system not only inhibit each other mutually such that they appear at opposite ends of the animal. On very long range the head system seems to activate the foot system. The formation of a foot requires the presence of a head (Ando *et al* 1989, Müller 1990). By such a dual system of medium range inhibition and very long range activation a coexistence of the head and the foot system can be achieved. If only a mutual inhibition were at work, the danger would be so high that in smalls animal the head system suppresses the foot system or vice versa.

3.4. How to keep the main body axes orthogonal to each other

For a developing organism it is absolutely essential that the main body axes—anterior to posterior and dorsal to ventral (back to belly)—be aligned perpendicular to each other. This requires a coupling between both pattern forming systems. Otherwise the anteroposterior and the dorsoventral pattern could arise along the same axis of the embryo and any reasonable development would be abolished. This requires that the high point of the second system appears in an equatorial zone in relation to the first. Several possibilities exist.

Orthogonal positional information systems can emerge reliably in a self-regulatory manner if simple cross-inhibitions exist. The mechanism is especially simple if one of

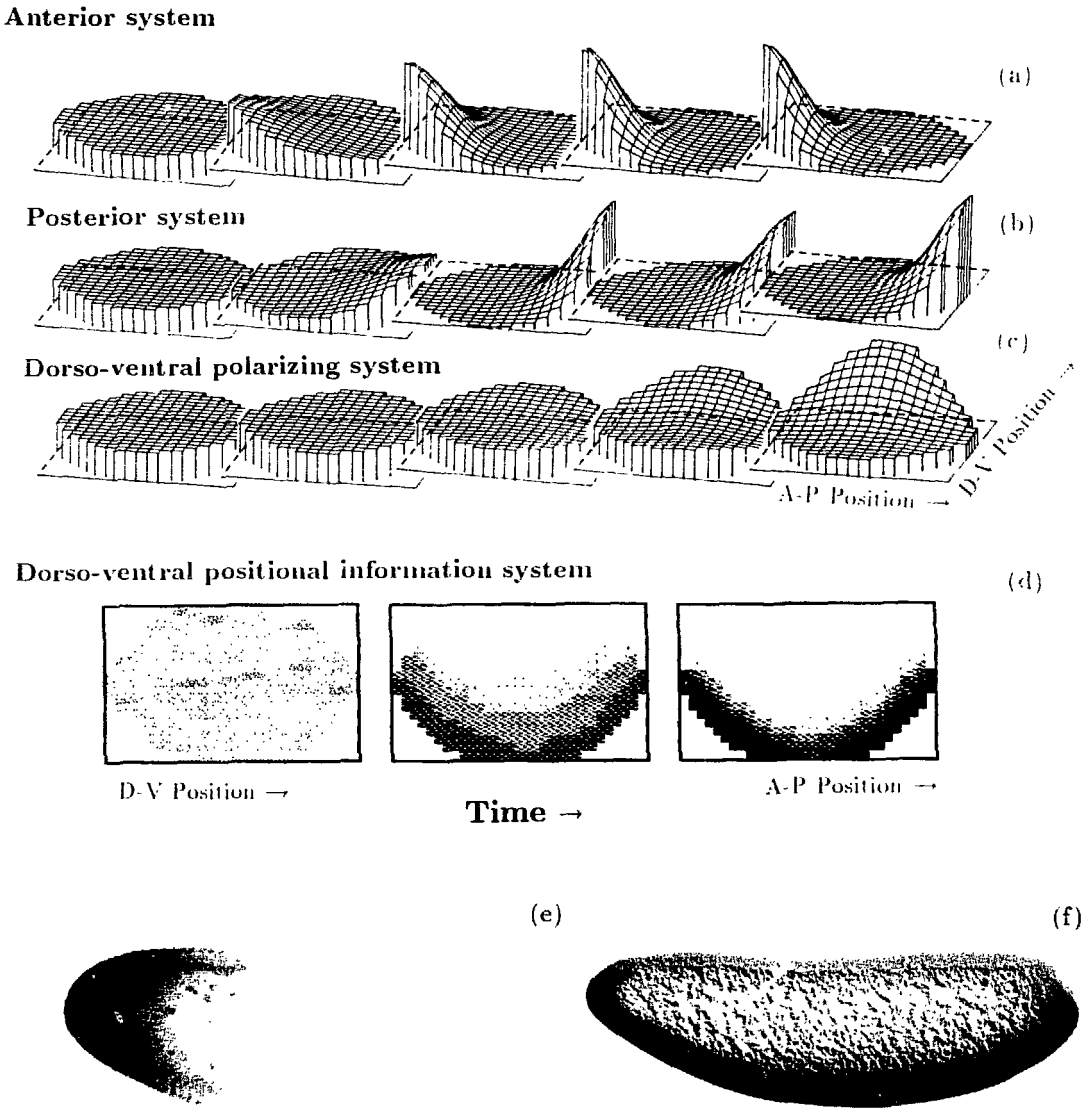

Figure 10. Orthogonal coordinate systems. Positional information for the two main body axes (anteroposterior, AP, and dorsoventral, DV) can emerge with an orthogonal orientation by three activator-inhibitor systems that are coupled by small cross-inhibitions. (*a*), (*b*) First a bipolar field is formed with a high concentration at the anterior and the posterior end. (*c*) The DV-pattern develops, due to longer time constants, somewhat slower. The maxima of the A and P pattern repel the DV-peak. Thus the resulting DV peak appears at an equatorial position. (*d*) This peak can be used to orient a stripe forming system to the dorsal side. The stripe of high concentration stretches from one pole to the other and forms a gradient perpendicular to the AP axis. (*e*), (*f*) Biological examples: (*e*) the graded distribution of the *bicoid* protein. It organizes the anterior part of the AP axis of a *Drosophila* embryo (Driever and Nüsslein-Volhard 1988, 1989). (*f*) The *dorsal* gradient determines the dorsoventral pattern (Roth *et al* 1989). The actual formation of these gradients is even more complicated and requires more intervening steps than expected by the model. (Photographs kindly supplied by Christiane Nüsslein-Volhard and Siegfried Roth).

the axes is organized by bipolar gradients. As discussed above, a cross-inhibition between two systems can lead to two . iaxima at opposite positions (figure 10). If no other constraints are imposed there maxima appear at the largest possible distance from each other. In other words, such a system orients itself along the longest extension of the available field. I assume that such a double gradient organizes the anteroposterior (AP) axis.

The dorsoventral (DV) axis requires an additional pattern forming system. It can also be of the activator-inhibitor type. If a cross-inhibition exists between the AP- and the DV-system, the high point of the DV-system will appear at the largest possible distance from the two maxima generating the AP-axis, i.e. in relation to the AP-poles in an equatorial zone. In the simulation shown in figure 10 pattern formation is initiated by random fluctuation only. Due to a shorter time constant, the AP-pattern develops somewhat faster and directs the DV-pattern into the orthogonal orientation.

Another possibility would be that an initially spherical embryo becomes first subdivided into two hemispheres. The border region between both hemispheres has the shape of a disk or a ring. Some sort of equator is formed. If a second pattern forming system is confined to this region, it will have an orientation perpendicular to the first. Such a mechanism seems to be realized in sea urchins and amphibians.

3.5. Formation of a gradient along the short extension of a field

In a lateral inhibition mechanism, a tendency exists to form the highest and lowest concentration at the maximum distance from each other, i.e. along the largest extension of a field. If the extensions of the field are very different, one of the gradients must be oriented along the shorter extension. A positional information system for the short axis does not require a high point of a gradient but a region of a high concentration that has a stripe-like extension along the long axis.

Such a system exists in *Drosophila* (figure 10(*f*)). As shown above, stripes are a stable pattern if autocatalysis saturates (figure 7). However, a stripe-forming reaction requires different parameters in comparison with a reaction that organizes an axis. For the formation of a coherent activated region at a marginal position an activator range comparable to the field size but no saturation is necessary (figure 8). In contrast, stripe formation requires very low activator diffusion but saturation. The stripes will have an arbitrary orientation within a larger field (figure 7). Therefore, from the model one would expect that two reactions are involved in the organization of the short dimension of the field, one that generates the overall polarity and a second one that generates the stripe and thus the actual positional information. The first reaction determines the position at which the stripe should be formed. If, for instance, the DV-system shown in figure 10(*c*) has an inhibitory influence on a stripe-forming system, a single stripe is formed that stretches from the anterior to the posterior pole of the egg (figure 10(*d*)). In *Drosophila*, clear evidence for such superposition of two systems exist (Schüpbach 1987, for simulation see Meinhardt 1989) although the details of the molecular machinery are not yet fully understood.

3.6. Size-regulation may require superposition of two patterns

In developing organisms, the size of a particular structure can be regulated in relation to the total size of the organism. The regulation of the size of a *Hydra* head (Bode and Bode 1980) and of the ratio of prestalk/prespore cells in slime molds (MacWilliams

and Bonner 1979) may serve as examples. The formation of size-regulated gradients as a possible mechanism has been discussed already. Another possibility would be the generation of a more step-like distribution in which the region bearing the high concentration is a certain fraction of the total field.

In an activator-inhibitor or an activator-substrate model the number of activated cells will be a certain fraction of the total number of cells if the autocatalysis saturates and the antagonist diffuses throughout the field (Gierer and Meinhardt 1972). In the presence of saturation ($\kappa > 0$ in equation (5)) the increase of the activator at one position can only proceed on the expense of the activator concentration elsewhere. The lower the saturation level the more cells remain activated, although at a lower level. In the steady state the number of activated cells is a certain fraction of the number of non-activated cells since the latter provide space into which the inhibitor can escape or from which substrate can be obtained. In other words, the size of the activated regions is regulated in relation to the total field.

In order that this size regulation works properly, the diffusion range of the activator must be zero or very small while that of the antagonistic substance, the substrate or the inhibitor, must be high. The low activator diffusion is necessary otherwise a graded zone of transition between activated and non-activated cells would be formed which cannot adapt to changing sizes as long as the diffusion is not size-dependent. Also an unwanted stripe formation could occur. In contrast, the range of the antagonistic substance must cover the whole field, otherwise a removal of tissue at one end cannot lead to a regulatory response at the other.

However, the low diffusion of the activator causes another problem. Since under this condition the activator is more or less cell-restricted, a cell neighbouring an activated cell has not a higher chance to become activated than any other cell. In other words, the tendency to produce coherent activated regions is lost in favour of a mosaic-like pattern of activated and non-activated cells (figure 7(*b*)). A way out of this problem requires another pattern which controls at which position the activated cells are formed. Thus, according to this view, one pattern controls *where* a particular structure is formed and a second one controls *how large* the structure will be. For instance, a graded source density can exist (see above) which leads to a preferential (and thus coherent) activation in the region of high source density. A model on this basis has been proposed for the size-regulated regeneration of a *Hydra* head (MacWilliams 1982).

Another possibility would be that cells become activated at more or less random positions but in the correct ratio to the non-activated cells. Under the control of a second pattern the activated cells move to a particular position and form thus a coherent mass of activated cells. Such a mechanism seems to be realized in a classical model system for size regulation, in prestalk/prespore cell patterning of the slime mold *Dictyostelium discoideum* (Gerisch 1968). Slugs of different sizes produce these cell types in a ratio of about 1:5, the prestalk cells form the tip of a moving slug. After separation of a slug into two fragments, the correct ratio becomes restored (figure 11(*a*)). This behaviour is well reproduced in the simulations (figure 11(*b–d*)).

The separation into a mechanism that determines the size and into another one that determines the position of a structure may appear as a non-necessary complication. However, according to the model, to achieve both tasks with a single mechanism would require an unsatisfactory compromise. If the diffusion of the autocatalytic substance is low, the size-regulation would be good but the danger of a decay into isolated regions would be high and vice versa. The separation of both task allows an independent

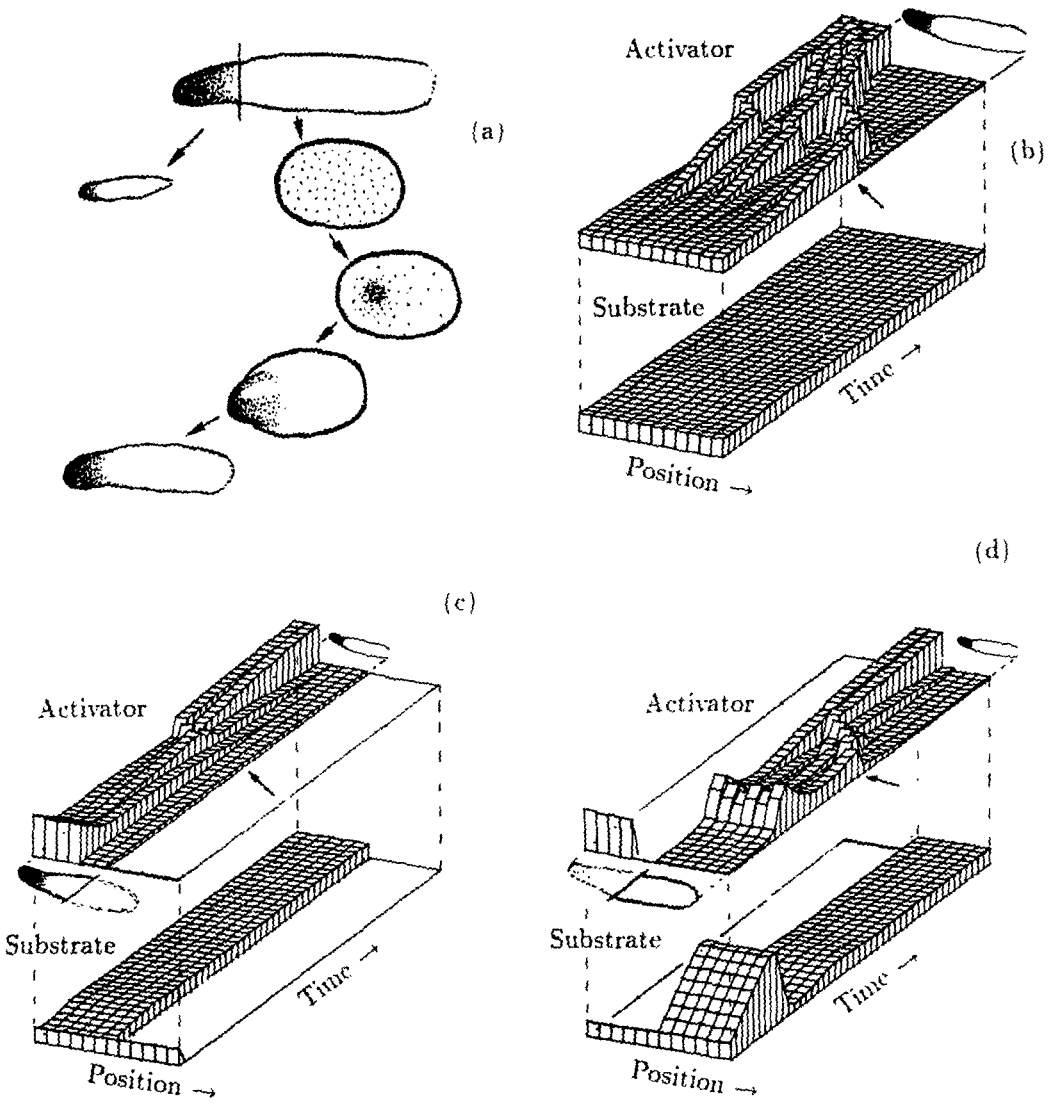

Figure 11. Size-regulation by saturation of autocatalysis. (*a*) Biological example: The slug of the slime mold *Dictyostelium discoideum* consists of two cell types, the prestalk cells (black) and the prespore cells. Both cell types are present in a size-independent proportion. After fragmentation, the correct ratio of both cell types is restored in each fragment. In an isolated prespore region, prestalk cells become detectable at scattered positions and sort out later on under control of a separate mechanism. (*b*) Model. If autocatalysis saturates, the number of activated cells becomes a certain proportion of the total number of cells. To be close to experimental observations, calculations have been done with an activator-substrate model (7). At the steady state, the number of activated cells—the substrate consumer—depends on the total number of cells, the substrate producers. Since the activator is assumed to be non-diffusible, small random fluctuations in the source density are decisive as to which cells will win the competition and become activated. A separate system (not shown) is required for the sorting out of activated cells. The latter needs not to be size-regulated since only the orientation of the cell movement must be determined. The initiation of the sorting towards the left side is indicated by an arrow. (*b*), (*c*) Pattern regulation with correct proportioning after removal of activated (*c*) or non-activated (*b*) cells. (After Meinhardt 1983c).

optimalization. The biological example shows that a better system is preferred even if this requires a more elaborate mechanism.

4. Patterns on the shells of molluscs: a natural picture book to study nonlinear pattern forming mechanisms

The shells of many molluscs are decorated with pigmentation patterns of great beauty. The formation of these patterns proceeds in most species in a strictly linear manner since new pattern elements are added only along a narrow zone at the growing edge of the shell. The second dimension is a time record of what has happened at the growing edge. In other words, the shells are space-time plots. They preserve the complete history of their formation. This allows the reconstruction of an obviously very dynamic processes. Based on reaction-diffusion mechanisms as described above, we have proposed a model for shell pigmentation (Meinhardt 1984, Meinhardt and Klingler 1986, 1987). Detailed simulations for specific species illustrate the similarity of the natural and simulated patterns. A model based on similar principles but underlining the possible role of the nervous system has been proposed by Ermentrout *et al* (1986).

The pigmentation patterns on shells of molluscs are obviously not under a great evolutionary pressure. They could be modified without endangering the species. Freed from functional constraints, the pattern forming reactions show a wide range of behaviour. Since the patterns are records in time, oscillatory patterns are used as well. Three examples will be given here to illustrate that very complex patterns result if one pattern modifies the parameter of a second pattern forming system. In the first example it will be shown that a regular shift between an oscillating and a steady state mode of activation can lead to travelling waves of unusual properties. The second example shows that a second pattern can lead to stable regional differences in the time constants of the primary reaction. The last example illustrates that an antagonistic reaction can be in itself a complete pattern forming system, causing a permanent change between pigmentation and non-pigmentation in space and time.

Basic elements of shell patterns are lines perpendicular, parallel and oblique to the growing edge. Keeping in mind the space-time character of the shell pattern, lines perpendicular to the growing edge indicate the formation of a spatial periodic pattern of pigment production along the edge which is stable in time. They can be simulated by the autocatalysis-lateral inhibition mechanism in the normal mode (see figure 8). Lines parallel to the growing edge indicate an oscillating pigment production. All cell of the mantel gland at the growing edge enter more or less simultaneously into the pigment producing phase which is followed by an inactive (refractory) period until the next pigment production takes place. As mentioned, oscillations occur if the time constant of the antagonistic reaction is longer than that of the activator. High diffusion rates lead to a synchronization of the oscillating cells.

4.1. Travelling waves generate oblique lines

Oblique lines originate from travelling waves of pigment production. Such waves arise if an activated region 'infects' its neighbouring region. After a certain time delay required to obtain full activation, such cell infects again its neighbouring region, and

so on. In the time record of the shell wandering regions of high pigment production are oblique lines.

In terms of the models worked out above, travelling waves arises if the activator has a small diffusion range and the antagonistic substance is nearly non-diffusible. In addition, the antagonist must have a longer time constant such that burst-like activations occurs.

Characteristic for many such shell patterns is a W-like arrangement of the oblique lines. The ∧-like element of this pattern result from a spontaneous trigger of a cell followed by an 'infection' of both neighbouring regions. In other words, a spontaneous trigger of a cell gives rise to two diverging oblique lines. If two waves collide, both waves become extinct since such wave would have to enter into a region that is in the refractory state due the other wave. This leads to the ∨-like elements of the pattern (figure 12).

4.2. Formation of branches: the sudden formation of backwards waves

Many species form oblique lines that branch. Figure 12 shows as an example a portion of the shell of the snail *Olivia porphyria.* Keeping in mind the space-time character of the shell pattern, branches indicate the sudden formation of a backwards wave. The initiation of such a backward wave requires a modification of the normal serial trigger mechanism. From a particular moment onwards and for a short time interval, the activated cells must remain in the activated state until the refractory period of the

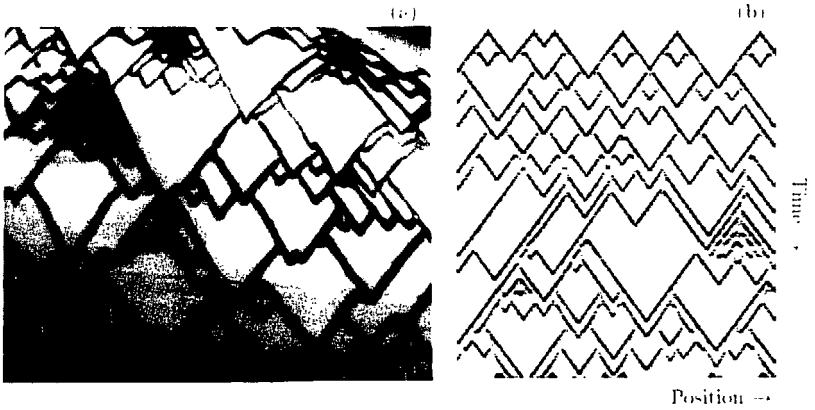

Figure 12. Formation of branching lines on shells of a tropical snail. (*a*) Shell of *Olivia Porphyria.* The oblique lines are time records of travelling waves of pigment production along the growing edge of the shell. Branches indicate the sudden formation of backwards waves. (*b*) Model: travelling waves result from an activator-inhibitor system. The activator has a longer diffusion range but a shorter time constant than the inhibitor. Branching occurs by a temporary transition from an oscillatory to a steady state activator production whenever the number of travelling waves drops below a critical level. The controlling agent is a hormone-like substance (not shown) which is rapidly distributed within the organism and which is produced at a rate proportional to the local activator concentration. The hormone thus provides a measure of how many travelling waves are present. The hormone stabilizes the inhibitor. Below a certain hormone concentration the inhibitor life time becomes so short that the just activated cells switch from the oscillatory into a steady state mode of activator production. Groups of cells remain activated. Backwards waves are initiated by re-infection after the refractory period of the neighbouring still oscillating cells is over (after Meinhardt and Klingler 1987).

neighbouring region is over. Then, these neighbouring cells can be re-infected and the backwards wave is initiated.

The temporary shift from the oscillating into a steady state activation can result, for instance, from a change in the decay rate of the inhibitor, from $\nu < \mu$ to $\nu > \mu$ and back to $\nu < \mu$. Of course, this requires a signal as to where and when this transition should occur. As mentioned, as a rule, travelling waves annihilate each other during a collision. Branching is one of the possible mechanisms to maintain the number of travelling waves. In the simulation of figure 12 this has been achieved by the assumption of a hormone-like substance that is produced by all activated cells. The hormone is assumed to be rapidly distributed in the organism by the blood stream. Its concentration is thus a measure of the total number of activated cells. The signal is available in each cell along the growing edge. The hormone has an stabilizing influence on the inhibitor. With a decreasing number of travelling waves, the lifetime of the inhibitor becomes smaller and smaller until the cells shift from the oscillatory into the steady state mode of activation. Cells that are activated at this very moment remain activated. After the refractory period of neighbouring cells (that still proceeds through their oscillatory cycles) is over, these neighbouring cell are re-infected and backward waves are initated. This causes an increase of the number of travelling waves and thus an increase in the hormone concentration and inhibitor lifetime. All cells return to the oscillating mode and the formation of a branch is completed. A further condition for such pattern formation is that the refractory period is much shorter then the time interval after which a spontaneous activation can occur, a property that can be adjusted in (1) by an appropriate choice of ρ_0 and ρ_1.

The simulation reproduces many details of the natural pattern. Branchings are simultaneously initiated at many positions. In the model, this results from the global control of the homogeneously distributed hormone. In the final pattern, this leads to the v-like elements with the same length between branch initiation and the tip. While the original wave proceeds in an unperturbed way, the branches appear frequently only loosely connected to the original line. In the simulation, this effect results from the passage through the temporary steady state activation which is lower than that achieved during the pulse-like activation in the oscillatory regime. Sometimes, a little hook appears close to the branch initiation. This results from an incipient intiation of a wave parallel to the primary wave. Usually this wave does not survive since the medium is not yet excitable enough. If this wave survives, however, a line close to the original line is formed together with the branch. If by chance large non-pigmented regions are formed, the density of pigmented lines increases in other parts of the shell.

4.3. Superposition of a spatially stable and a periodic pattern

Many shell pattern can be explained by assuming a superposition of two patterns, one that is stable in time and one that oscillates. The first pattern controls the parameter of the second. While the first pattern is invisible, the second produces the proper pigment. In the simulation of *Natica enzona* (figure 13) an activator-substrate model (7) has been assumed. The spatially stable pattern is assumed to modulate the substrate production σ, the activator lifetime μ as well as the source density ρ. This has the consequence that regions of high and low oscillation frequencies alternate. The resulting pattern consist of broad bands perpendicular to the growing edge that contain pigmentation lines of different spacing. Bands with large and small spacing alternate. The regions of the highest oscillation frequencies form pace maker regions from which waves

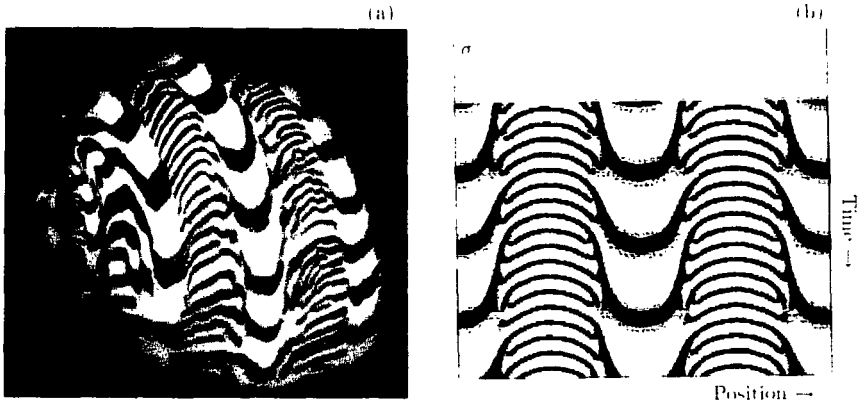

Figure 13. Shell patterning resulting from superposition of stable and periodic patterns (*a*) Shell of *Natica enzona*. (*b*) Model: the pigment production indicate higher oscillation frequencies in some broad bands in comparison to the intervening regions, indicating the existence of a spatially stable pattern that influences the oscillation frequency. Similated with (7). The substrate production σ as well as the decay rate of the activator μ and the source density ρ is assumed to be a function of position. These parameters have the same space dependency as that shown for σ. Regions at which these parameters are high oscillate more rapidly. This leads to bands in which the pigment lines have a smaller spacing. At the border between these bands, the pigment lines partially merge (after Meinhardt and Klingler 1987).

spread out. In regions of low oscillation frequencies the cells fail to follow such rapid triggering. Some pulses are skipped. This has as the consequence that some of the pigmentation lines merge while others terminate at the borders between bands of high and low frequency oscillations.

4.4. Superposition of two time-dependend patterns

The pattern on the shell of *Conus marmoreus* (figure 14) is characterized by a pigmented background with drop-like unpigmented regions. The long straight black-to-white border almost perpendicular to the direction of growth at the beginning of these 'drops'

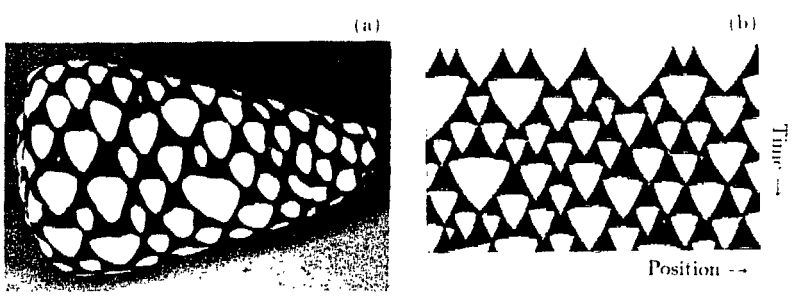

Figure 14. Shell patterning by superposition of two time-dependend patterns (*a*) Shell of *Conus marmoreus*. (*b*) Model: A first reaction is tuned to a steady state activation and leads to the spread of pigment production. A second reactions (not visible in the natural pattern) extinguishes the first. It is triggered whenever cells have beeen activated for a certain period (shaded regions at the top of the white drops). Due to a high diffusion of the extinguishing reaction pigment production stops almost simultaneously over larger regions. The white area becomes black again in the course of time by the spread of activation from regions in which the first system survived.

indicates a nearly simultaneous cessation of the activation in large regions. The simulation of figure 14 has been made under the following assumptions. The pigmentation reaction is tuned to obtain a stable steady state. Due to the activator diffusion, a small activated region will enlarge. A second reaction extinguishes the first. The second reaction produces a short pulse-like activation. The substrate required for the extinguishing reaction is produced only if pigment is produced, i.e. if the first reaction is in its activated state. After pigment has been produced for a certain time interval, the burst-like extinguising reaction is triggered. Since the components of the extinguishing reaction spread by diffusion, this burst-like activation occurs almost simultaneously in a large region. In the real pattern this reaction is only indirectly visible by the sudden black–white transition at the beginning of a white drop. The pigmentation will survive at regions that has been activated too shortly to have sufficient substrate accumulated for the extinguishing reaction. Due to the spread of the pigmentation reaction from the regions in which activation has survived the region becomes pigmented again in the course of time while in other regions new pigment-free regions emerge, and so on.

5. Biological switches, cell states and the interpretation of positional information

In higher organisms, the pattern generated by a reaction–diffusion mechanism is necessarily transient since, due to growth, the polar pattern cannot be maintained over the whole expanse of the growing organism. This requires that at an appropriate stage the cells make use of position-specific signals, i.e. that they become determined for a particular pathway by activating particular genes. Afterwards the cells maintain this determination whether or not the evoking signal is still present.

The simplest system with a long term memory consist of a substance that feeds back on its own production rate in a nonlinear way. To avoid an unlimited growth the production must saturate at high concentrations. The interaction described by (10) provides an example for a reaction with threshold behaviour.

$$\frac{\partial g}{\partial t}=\frac{cg^2}{(1+\kappa g^2)}-\mu g+m. \tag{10}$$

At low g concentration, the linear decay rate is dominating and the g concentration decreases further to zero. Above a threshold, the nonlinear production term dominates. The concentration of g increases further until a steady state at high concentration is reached due to the saturation. If the signal m is above a certain threshold, only the high state is stable (figure 15(a)). The system remains at this state even if m becomes small later on (Meinhardt 1976). Figure 15(b) shows the switching from low to high g concentration in a portion of a field under the influence of a graded m concentration.

For several developmental systems evidence exist that a single morphogen gradient controls the activation of several genes in a concentration-dependent manner (see figure 1), leading to a region-specific gene activation. To see which type of mechanisms can be involved, it is helpful to realize the formal similarities of the activation of a particular gene and the formation of a pattern. In pattern formation, a particular substance should be produced at a particular location while this production is suppressed at other locations. Correspondingly, cell determination requires the activation of a particular gene out of a set of alternative genes while the remaining genes of the set should be suppressed. Gene activation may thus be regarded as a pattern formation in the gene space.

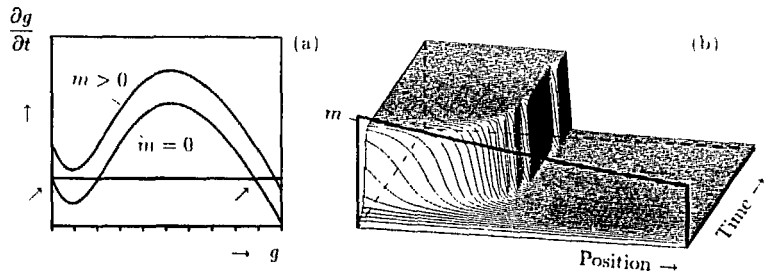

Figure 15. (*a*) Switching behaviour of a substance with non-linear, saturating feedback on its own production rate. In a reaction according to (10) in the absence of a morphogen m, two stable states exist (arrows). At higher m concentration the low state becomes unstable. (*b*) If a chain of cells is exposed to a graded m distribution, cells exposed to a certain m concentration switch irreversible into the high state. Regions of high and low g concentration appear. No zone of transition exists although the controlling signal has a smoothly graded distribution.

In analogy to the activator-inhibitor system for pattern formation, one can formulate the following set of equations for activation of particular genes via their gene products g_i (Meinhardt 1978, 1982).

$$\frac{\partial g_i}{\partial t} = \frac{c_i g_i^2}{r} - \mu g_i + \frac{\delta m g_{i-1}}{r} \tag{11a}$$

$$\frac{\partial r}{\partial t} = \sum_i c_i g_i^2 - \nu r. \tag{11b}$$

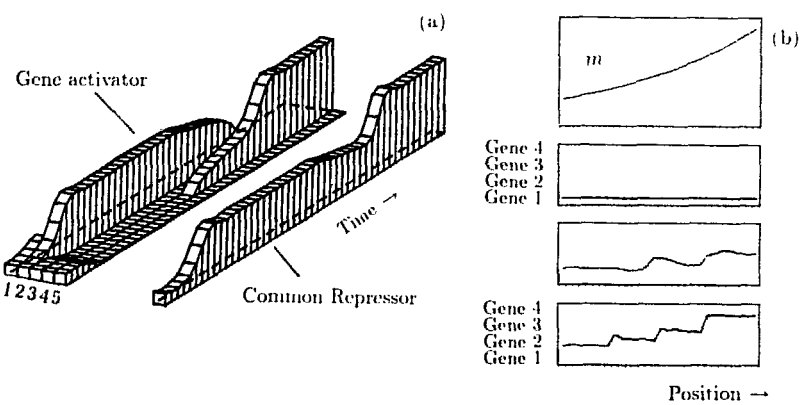

Figure 16. Formation of cell states and space-dependent gene activation. (*a*) A set of genes (1, 2 ... 5) is assumed whose products feed back on the activation of the corresponding genes. In addition, all genes compete with each other by a repressor (11*a*, *b*). Only one of the genes can be active within one cell. In this simulation, initially gene 2 wins this competition due to random fluctuations. By an external signal (not shown), gene 4 becomes activated. Full activation results from auto-regulation and is accompanied by the repression of the previously active gene. (*b*) Stages in the activation of several genes under a morphogen control. Initially, gene 1 is turned on in every cell. The morphogen (m) is assumed to provoke the transition from one gene to the next, the represssor (r, step-like curve) slows down this transition (11*a*). The repressor concentration increases with the activation of a subsequent gene (since in equation (11) it has been assumed that $c_{i+1} > c_i$). Thus, the activation of higher and higher genes comes to rest at a particular gene. Which one it is depends on the local morphogen concentration. The result is an ordered sequence of gene activities in space. Although the positional information is graded, the activation of the genes is an all-or-nothing event.

Each gene product g_i is autocatalytic, but also produces and reacts upon the represssor r, $i = 1 \ldots n$, where n is the number of alternative pathways. Due to the competition via a common repressor (or via a direct negative influence of the alternative genes), only one gene of the set can be active in a particular cell (figure 16(a)). Several possibilities exist for a coupling between the gene switching system and the morphogen gradient m. In the example given in (11a), the cells switch from one activated gene (g_{i-1}) to the next (g_i). The number of steps depends on the morphogen concentration. Since each gene feeds back on its own activation, a gene remains active, independent of whether the signal is present or not. The result of this 'interpretation' of positional information is that in groups of neighbouring cells a particular gene is active. An abrupt transition from one activated gene to the subsequent one takes place between neighbouring cells despite the smooth distribution of the morphogen. The result is an ordered sequence of gene activities in space (figure 16(b)). Meanwhile, many genes with autocatalytic properties (autoregulation) have been found (for a review see Serfling 1989). The predicted mechanism, the maintenance of the determined state by a feedback of a gene on its own activity, appears to be a general process.

6. Formation of filament-like branching structures

A feedback of a position-dependent gene activation on the pattern that has caused its activation can lead to very complex patterns. As an example the formation of filament-like branching structures will be discussed. This pattern is very common in almost all higher organisms. The venation of leaves, the tracheae of insects, the blood or lymph vessels as well as neurons are examples. How can such complex patterns emerge?

In contrast to the branching structures on shells, these patterns are generated in a two- or three-dimensional space. A travelling wave that started at a particular point would not lead to a line but to an enlarging ring or a sphere. Therefore, a different mechanism is required.

We have seen how a local high activator concentration can be generated and how it can be used as the signal to cause stable gene activation if a threshold is exceeded. The exposed cells differentiate and become, for instance, a part of a vascular system. I will assume further that differentiated cells repel the signal. The signal will be shifted into a neighbouring cell which will differentiate and become a part of the vascular system, too. A repetition of this process—differentiation, shift of the signal, differentiation—leads to a strand of differentiated cells behind a wandering activator maximum (figure 17).

For the simulation, the following set of equations have been used (Meinhardt 1976)

$$\frac{\partial a}{\partial t} = \frac{\rho a^2 s}{h} - \mu a + D_a \Delta a + \rho_0 y \tag{12a}$$

$$\frac{\partial h}{\partial t} = \rho a^2 s - \nu h + D_h \Delta h + \rho_1 y \tag{12b}$$

$$\frac{\partial s}{\partial t} = \sigma - \gamma s - \varepsilon s y + D_s \Delta s \tag{12c}$$

$$\frac{\partial y}{\partial t} = \frac{y^2}{1 + f y_2} - \mu_y y + \rho_y y. \tag{12d}$$

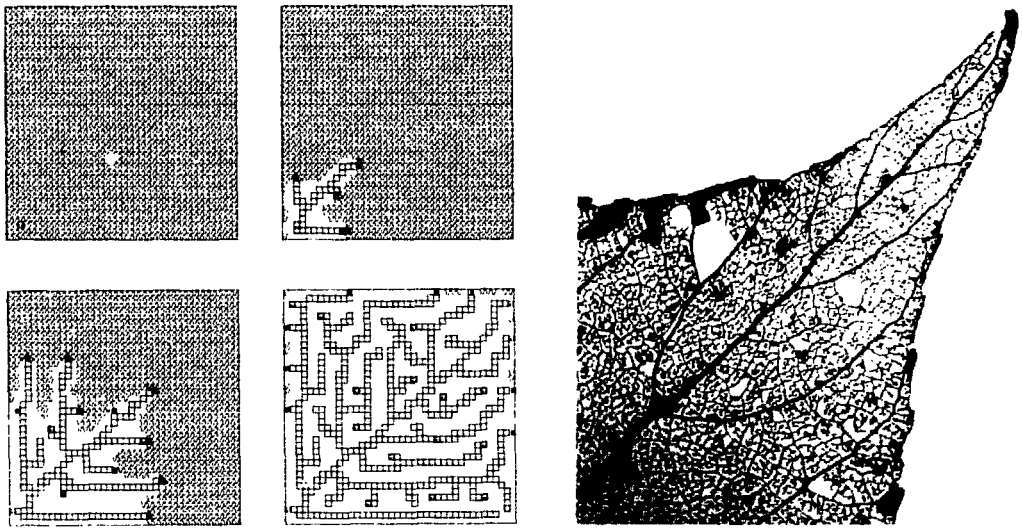

Figure 17. Formation of a net-like structure. The interaction of four substances is sufficient to generate a structure with branching filaments. A signal for the local elongation of the filament is generated by an activator (black dots)/inhibitor system. In this simulation the signal is used to differentiate cells (squares). Differentiated cells remove a substrate (wavy lines). Since the activator/inhibitor system depends on the substrate, the activator maximum is shifted to that neighbouring cell which has the largest distance from other differentiated cells, which is usually the tip of the filament. The patterning process comes to rest if a certain density of the filaments is reached.

The concentration of y within a cell is an indicator of whether a cell is differentiated or not. As mentioned above, only two stable states are possible. The concentration of y is either high or low. At a certain concentration of the activator sufficient y is produced to turn on the autocatalysis of y and the cell switches from low to high y production and remains at the high y level even after a decrease of the activator concentration (see figure 15). To achieve a shift of the activator maximum away from the differentiated cells, it is assumed that all differentiated cells remove a substance s that is produced by all cells and on which the activator autocatalysis depends. Therefore, after differentiation of the cell, the maximum is shifted to those neighbouring cells that contain the highest substrate concentration. Usually, these are the cells in front of the tip of the incipient filament. The filament becomes elongated in this way. Deflection of a growing filament can result from other nearby filaments or from the margins of the fied.

Branches are formed whenever activator maxima become sufficiently remote from each other during elongation of filaments. Then, the inhibitor concentration can become locally so low that a new activator maxima is triggered along an existing vein due to the term $\rho_0 y$. After removal of some filaments, the system is able to regenerate the missing veins (or whatever it is) since in these regions, s is no longer removed and the rising s concentrations attract activator maxima from the non-injured region.

Leaf venation is frequently regarded as an example for a fractal process containing similar structures at very different scales. According to the model, there is only one process that works at a single scale only. However, since the leaves grow also the spaces between the veins enlarge and from a certain size onwards, new filaments grow into these regions, in a similar way as in the regeneration mentioned above.

In conclusion, by superposition of several reactions pattern forming systems can emerge that have a far richer repertoire then the components. This allows a tailoring of systems such that requirements for specific developmental situations can be met.

7. Formation of more complex patterns: the superposition of sequential and periodic patterns in segmentation

Segmentation is a widespread pattern in the animal kingdom. Looking at an earthworm makes the periodic character of segmentation evident. However, as a rule, the segments are different from each other. For instance, in insects only the three thoracic segments bear legs. Only the second and in some species the third thoracic segment (meso- and metathrax) forms wings. Certain abdominal segments form the gonads. In other words, segments can have an individuality. The individual segments form a spatially ordered sequence of structures. Therefore, segmentation results from the superposition of a periodic and a sequential pattern and models will be discussed for both processes and their linkage.

7.1. Formation of the periodic structures is the primary event

A key issue is the question of what is the signal to form a segment border. One could envisage that the juxtaposition of cells with different segmental identities causes a border in between. Experimental evidence, however, indicate that this is not the case. In *Drosophila* mutations in the *Ultrabithorax* (Lewis 1978, Akam 1987) or in the *polycomb* gene complex (Denell and Frederick 1983) can lead to identical segments, but the segmentation proceeds as normal. This rules out that a segment border is formed whenever cells with different segmental identities, e.g. mesothoracic and metathoracic cells are juxtaposed. The formation of the periodic pattern must be the primary event. This makes sense also from an evolutionary point of view since in lower segmented animals like centipedes the segments are presumably more or less identical, suggesting that the repetition of identical elements was the primary achievement and to make segments different from each other was an evolutionary later addition.

7.2. Pattern formation within a segment: dynamic regulation of a neighbourhood of structures

The pattern within a segment has characteristic landmarks. Information about how the pattern *within* a segment is regulated has been obtained from surgical interference in hemimetabolous insects. There are insects that have from the beginning an appearance similar to the adults but no larval stages. The juvenile forms proceed through several moulds. Creating a discontinuity by grafting a piece of the sclerotic cuticula together with the underlying ectoderm to a different position within the segment provokes pattern regulation (Locke 1959). After one or two moulds, a new stable pattern becomes established. The result of such regulation is a restoration of the normal neighbourhood of structures. The discontinuity disappears. However, the resulting pattern can be dramatically different from the natural pattern. Figure 18 shows an experiment of Bohn (1970), taking cockroach legs as an example. If we denote the normal sequence of structures within a segment as 123 . . . 9, a combination of a stump 12345678 and a grafted piece 456789 leads to the structure 123456787**65**456789.

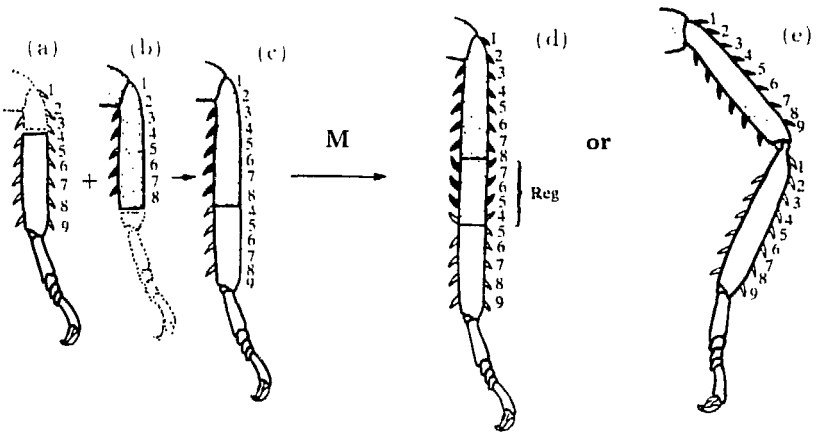

Figure 18. Intercalary regeneration. Experimental observation (Bohn 1970; French 1976). Grafting a large distal part (*a*) onto a large proximal stump (*b*) leads to a discontinuity in the neighbourhood of structures. The elements of the tibia of a cockroach leg are arbitrary denoted with 1, 2 . . . 9. The correct neighbourhood becomes restored either by a duplication of the surplus elements (Reg) with polarity reversal or by insertion of an supernumerary joint. After one or two moulds (M) each element is juxtaposed with a natural neighbour although the normal pattern is not restored.

Although the leg was already too long after the operation, even more structures became inserted by intercalary regeneration (written in bold face). After this intercalation each structure has a neighbour that would be also a neighbour in the unperturbed situation. Obviously, it is not the natural sequence of elements but the normal neighbourhood that is regulated. This result rules out mechanisms postulating that a segment is organized by a gradient generated by a source at one segment border and a sink at the other since, after a manipulation such as described above, a restoration of the normal monotonic sequence would be expected. As indicated by the reversed orientation of the spines (figure 18), the surplus structures are intercalated with a reversed polarity. Thus, the polarity of the pattern within a segment results from the sequence of elements and not from the alignment of polar cells since polarity reversal occurs without rotation of parts.

7.3. Mutual activation of cell states that locally exclude each other

The possibility to generate a sequence of structures by gene activation under the influence of a gradient has been outlined above (equation (11), figure 16). In such a mechanism the cells do not communicate directly with each other to obtain the correct determination. They measure the local concentration of a substance and behave accordingly. Due to this lack of communication, as a rule, a discontinuity can not be repaired, especially if at later stages the signalling gradient is no longer available. The cells just remain in the once obtained state. A gradient mechanism is therefore inconvenient to account for a dynamic regulation of a correct neighbourhood.

As shown above, self-activation of genes together with a mutual repression of alternative genes leads to stable states of determination. If two (or more) such states not only exclude each other locally but activate each other mutually over long ranges, these cell states stabilize each other in a symbiotic manner. Neighbouring cell states need each other close by while the local exclusiveness assures that these states do not merge or overlap (Meinhardt and Gierer 1980). The most stable state is reached if a

region in which a particular gene is active is bordered by regions in which the genes for the correct neighbouring structures are active; a possible interaction is

$$\frac{\partial g_i}{\partial t} = \frac{c_i g_i'^2}{r} - \mu g_i + D_g \frac{\partial^2 g_i}{\partial x^2} \qquad \text{with } g_i' = g_i + \delta^- s_{i-1} - \delta^+ s_{i+1} \tag{13a}$$

$$\frac{\partial s_i}{\partial t} = \gamma(g_i - s_i) D_s \frac{\partial^2 s_i}{\partial x^2} \tag{13b}$$

$$\frac{\partial r}{\partial t} = \sum_i c_i'^2 - \nu r \tag{13c}$$

where s_i describes the diffusible substances required for the long range support. The simulation in figure 19 shows that the model is able to account for the intercalation of missing elements, if necessary with a polarity reversal. Due to the possibility that missing elements can be inserted, more complex structures can be formed from simpler one. For instance, the sequence 123456789 can be initiated by a sequence 159 since

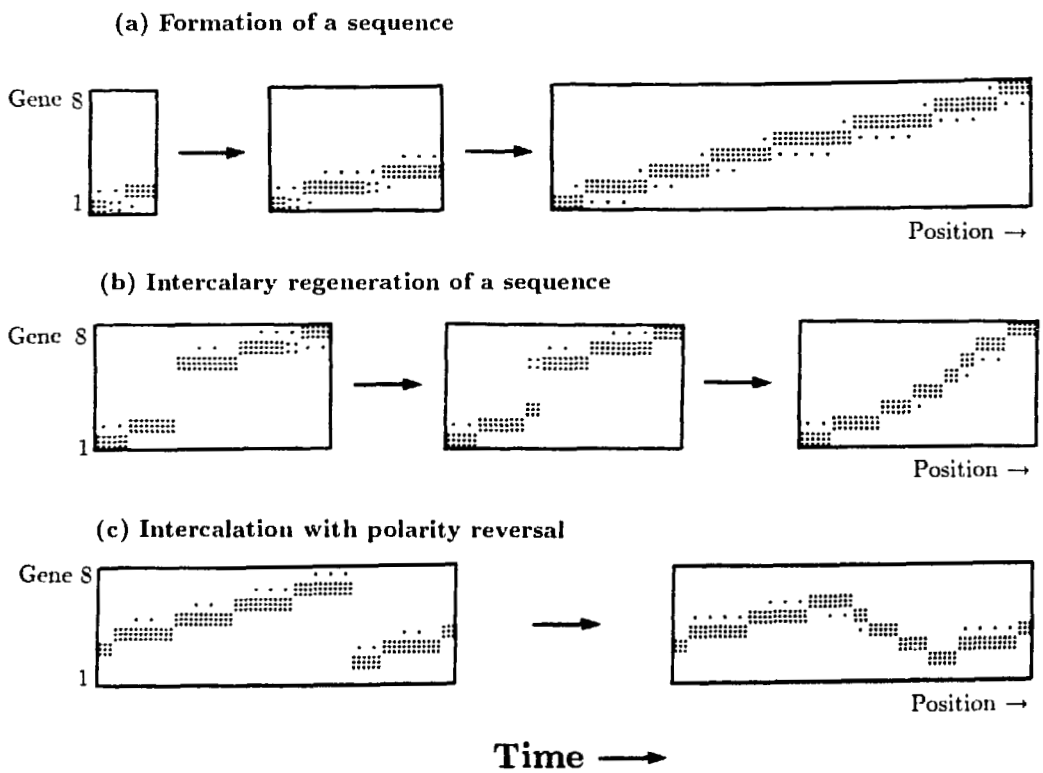

Figure 19. Mutual activation of cell states—formation of a controlled neighbourhood of structures. Assumed are feedback loops (genes 1–8) that locally exclude each other but activate each other on long range (13). The density of dots indicates the activation of a particular feedback loop as function of position. (*a*) In a growing array of cells whenever sufficient cells in a particular cell state have been formed, the subsequent cell state becomes activated due to the lateral activation. (*b*) If by a manipulation groups of cells become juxtaposed which are usually not neighbours, the pattern discontinuity is repaired. (*c*) This intercalation can lead to supernumerary structures and polarity reversal (compare with the experiment figure 18, after Meinhardt and Gierer 1980).

the structures 234 and 678 would be inserted by intercalation at the correct positions. At least three elements have to act as founders since two elements would not have any polarity.

It is a feature of these interactions that they can generate stripes. A stripe-like gene activation causes long borders between the regions. A particular cell type is close to the cell types that are required for stabilization. This facilitates the mutual support by the diffusible substances. A stripe-like arrangement is therefore especially stable. Many genes involved in the segmentation of *Drosophila* are expressed in narrow stripes (see figures 21 and 22 below).

7.4. Long range activation of neighbouring structures can be cyclic

Segments are essentially periodic structures. According to the model the mutual activation can be cyclic in that state 1 activates state 2, state 2 activates state 3 and so on until state n and n activates state 1. Such scheme leads to a periodic structure without a discontinuity at borders. The repetitive nature of pattern within segments has been demonstrated experimentally (Bohn 1970). The experiment shown in figure 18 has a similar result if parts of different leg segments are grafted together. Thus, the pattern of a leg is formed in a combinatorial way. Information must exist as to what segment a cell belongs and, further, at which position within a segment it is. The latter information is repeated in each segment. While the pattern within a segment is best described by a lateral activation scheme, the overall structure and the sequence of segments can be best explained by a positional information scheme (see figure 26 below). Thus, even within the same organ different mechanisms of pattern formation can be at work.

7.5. Segmentation: the repetitive pattern of (at least) three cell states

An important source of information concerning segmentation was the genetic analysis of the development of the fruit fly *Drosophila*. Almost simultaneously with the delimitation of the segments, the segments become subdivided into an anterior and a posterior compartment. The progeny of single cells genetically marked at an early stage (blastoderm) contribute either to the anterior or to the posterior half of a segment but not to both (Garcia-Bellido *et al* 1973). The borders between these two compartments are well defined and reproducible even if by genetic tricks the cells in one compartment proliferate more rapidly than in the other.

The simplest periodic structure would consist of an alternation between two cell states, A and P. The two compartments found in *Drosophila* appear to support such a view. However, such a binary sequence has no polarity, in contrast to the biological observation. No signal would be available that indicates at which AP-border a segment border is to be formed; the grouping could be AP/AP/AP or . . A/PA/PA/P. . Both sequences would have opposite polarity. This problem has two possible solutions: (i) an organization with a repeat length of two segments exists, consisting for instance of a pattern . . OEOE . . ; O and E would each cover the domain of a future segment and would eventually coincide with either an even (E) or an odd (O) numbered segment. Each O and E state would be subdivided into an A and a P region. The signal to form a border would be an O/E but not an A/P confrontation. (ii) Segmentation results from the periodic alternation of (at least) three cell states, for instance S, A and P. A segment border would be formed whenever P and S cells are juxtaposed

(... P/SAP/SAP/S ...). A cyclic sequence of (at least) three cell states has necessarily a polarity since each cell state has anteriorly a different neighbour than posteriorly.

A decision between these alternatives can be made on the basis of existing experiments. According to the first model grafting tissue into a neighbouring segment should lead to a segment border around the grafted tissue due to the resulting O/E confrontation. According to the second model, transplantation of fragments from an anterior position of a segment into a posterior position or vice versa should lead to a segment border, independent whether the graft is implanted into the same or a neighbouring segment. In insects, the latter prediction is in agreement with experiment (Wright and Lawrence 1981) while experiments with the segmentation of the vertebrate hindbrain indicate that there the first mechanism is realized (Guthrie and Lumbsden 1991).

The cyclic repetition of three cell states (.. P/SAP/SAP/...) is only the simplest possibility to get a polar structure. The repetition of four cell states would work as well. In a repetitive sequence it is more or less arbitrary what is regarded as the primary unit. Work on lower crustaceans (see Dohle and Scholz 1988) have suggested that the primary building block of segments do not span from one segment border to the next (SAP), but from the P-region of one segment to the A region of the subsequent segment (P/SA). These units have been called parasegments (Martinez-Arias and Lawrence 1985). Molecular-genetic evidence exists that the genes responsible for the cell states forming the parasegment borders are activated very early and that a third element *patch* is used in between somewhat like a space holder which becomes filled in by other cell states during further development (Nakano *et al* 1989, Hooper and Scott 1989). As will be shown further below, not only is the P/S border used (as a signal for the segment border), the more elementary A/P border is a prerequisite to form legs or wings.

The repetition of three cell states as the basis of segmentation is presumably not restricted to insects. Leeches show many small rings at the surface of the body, the so-called annuli. In many leeches every third annulus contains a cluster of nerve cells, each supplying three annuli with branching nerves. I would expect that each annuli corresponds to one of the three basic cell states mentioned above (see Shankland 1991).

8. Hierarchical pattern formation to obtain a precise number segments

In *Drosophila* precisely 14 segments are formed. This number is largely independent of variations in the egg size. How can be such precision achieved? The phenotypes that are formed if a gene involved in segmentation is mutated (figure 20) indicate that the formation of segments proceeds in a hierarchical way. Four pattern forming events follow each other. Each subsequent pattern depends on the preceding one (Nüsslein-Volhard and Wieschaus 1980, Ingham 1988).

The first level is responsible for the formation of a coordinate system within the egg. Relatively shallow gradients are formed by localized sources at both egg poles. The anterior one, *bicoid*, has been shown in figure 10 as an example. In addition, a steep symmetrical pattern exist with two high regions marking both poles (see Nüsslein-Volhard *et al* 1987).

These positional information systems, formed under the control of the maternal genome, cause the space-dependent activation of the so-called gap genes. This name has been given since large gaps result in the segmentation if one of these genes is lost due to a mutation (figure 20(*c*)).

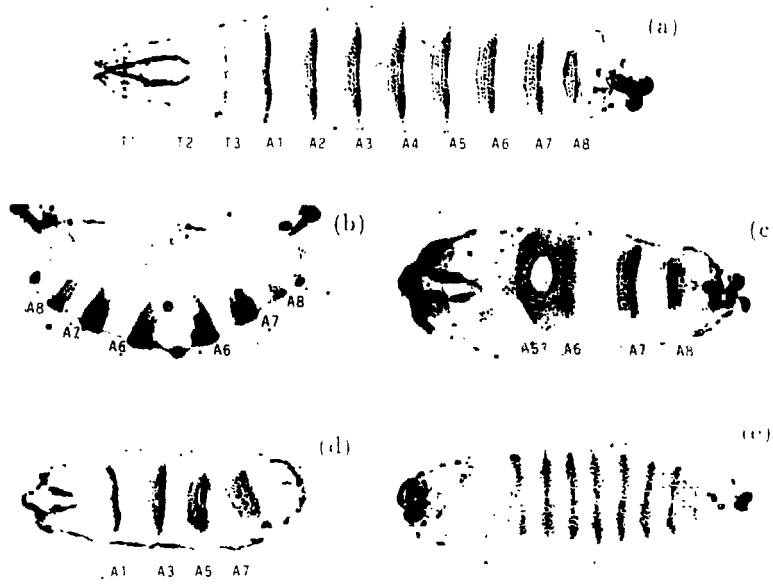

Figure 20. Examples of the four classes of mutations that affects segmentation in Drosophila (Nüsslein-Volhard 1977, Nüsslein-Volhard and Wieschaus 1980). (*a*) Wild type larvae. T1-T3 thoracic, A1-A8 abdominal segments. (*b*) In a *bicaudal* embryo the whole coordinate system is changed. A mirror-symmetrical embryo with two abdomen results. (*c*) *Krüppel*, a gap mutant: a single region of about 7 segments is missing (T1-A4). (*d*) *hairy*, a pair rule mutant: homologous pattern elements around every second segment border are deleted; the remaining denticle belts indicate the maintenance of the polarity. (*e*) *gooseberry*, a segment polarity mutant. Certain pattern elements are deleted in every segment, the remaining pattern of each segment is duplicated. The polarity of the segments is lost. (Negatives of dark field photographs, kindly provided by Christiane Nüsslein-Volhard.)

The gap genes, in turn, control the activation of the pair rule genes. If one of the pair rule genes is missing, a periodic defect appears in each second segment. Mutations exist that leads to a similar deletions either in the even- or the odd numbered segments (or around the corresponding segment borders). The remaining pattern maintains its polarity. The occurrence of these mutations has been a great surprise since before their discovery no other biological observation has indicated an underlaying pattern with a repeat length of two segments.

The lowest level in the hierarchy of genes involved in segmentation are the segment polarity genes. A mutation leads to a deletion of structures within each segment. The polarity of the segments is lost. The pattern consists of a repetition of mirror-symmetric structures (figure 20(*e*)).

8.1. How to couple pattern forming reactions in a hierarchical way

A cascade of pattern forming reaction provides a way to obtain a fine grained pattern in a very reproducible way. Initially, a relatively crude pattern is formed that controls the formation of the next finer pattern, and so on. A corresponding theory can be summarized here only very briefly (see Meinhardt 1986).

Imagine that we obtain under the influence of the maternal positional information a primary basic subdivision into regions characterized by regional activation of the gap genes (see figure 16). To avoid confusion by the strange and arbitrary names that

have been given to the corresponding genes, I call these I, II, III and IV. These regions would be in itself more or less homogeneous. It may appear straightforward to assume that each of these regions become further subdivided into two or three subregions. However, the polarity must be transmitted throughout the cascade in order to make sure that the final segments have the correct polarity. Each of the region regions I, II, III... has *per se* no polarity but the borders between the regions have. For this reason, I have proposed that not the regions itself but the borders between these regions, resulting for instance from an overlap of the regions I and II, II and III and so on, act as a scaffold and organize the subsequent pattern. This has led to the prediction (Meinhardt 1985) that the region in which a gap gene is transcribed is only half as large as the gap in the sequence of segments that is caused by a mutation of that gene. Imagine that the gene responsible for region III is broken. This has the consequence that neither the II/III border nor the III/IV border is present. Thus, in addition to the III region, half of the II region and half of the IV region would be lost too. The resulting gap would be twice as large as the III region itself. This prediction has been shown to be correct. The *Krüppel* gene, for instance, is expressed in a region of about 3.5 future segments (Knipple *et al* 1985, figure 21) while in a *Krüppel* mutant at least 7 segments are missing (figure 20(*c*)).

As mentioned above, a periodic structure with polarity requires the repetition of at least three elements. In the pair rule mutants, the remaining pattern still maintains

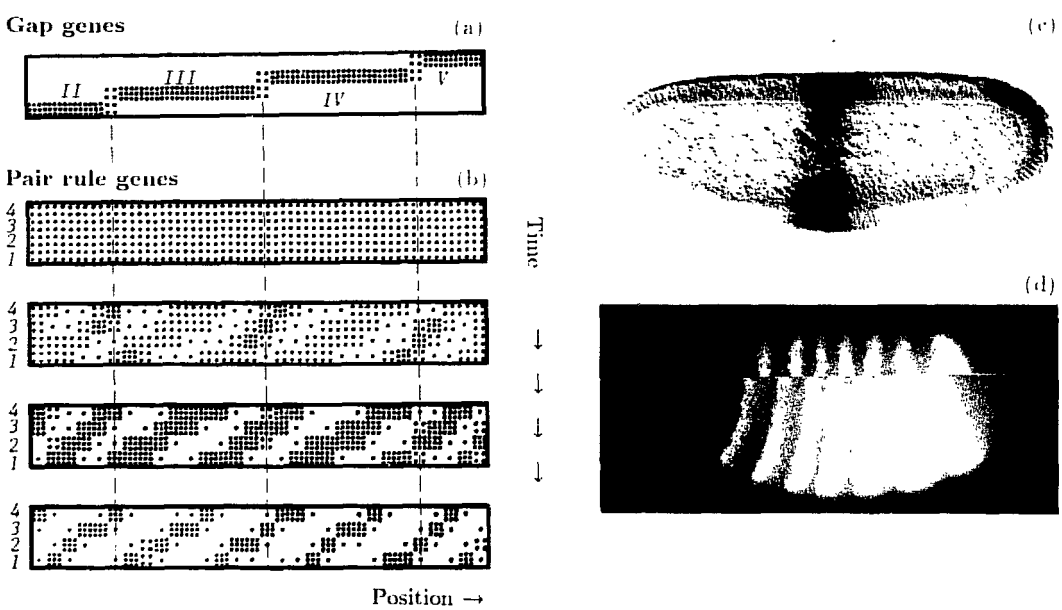

Figure 21. Conversion of the sequential pattern of gap gene activity into the periodic pattern of pair rule gene activation. (*a*) assumed pattern of gap gene activity (II-V, see figure 16). (*b*) The borders between the gap genes activate the pair-rule genes (1-4). For instance, the II/III border activates a 341-sequence. These are the first regions of increased gene activities (see dotted lines). The polarity residing in the sequence of gap genes is transmitted. Depending whether all pair rule genes compete with each other, each of the gene can be active in one half or in one quarter of the repeat length. (*c*) Biological examples: transcription of the *Krüppel* gene (corresponding to region III in (*a*) is restricted to a small band (Knipple *et al* 1985). (*d*) The *fushi tarazu* (top) and the *even-skipped* genes are transcribed in complementary periodic patterns (corresponding to genes 2 and 4 in simulation (*b*) (Hafen *et al* 1984, Frasch *et al* 1987; photographs kindly supplied by Herbert Jäckle and Manfred Frasch).

the original polarity. For this reason I have proposed that the pair rule pattern results from the repetition of at least four basic building blocks. If one of the four elements is missing, three elements remain and the polarity is maintained. Although about 8 pair rule genes are known, it has turned out that only a mutation in four of them leads to a change in the gene expression of the remaining ones (Carroll and Scott 1985), indicating that these genes generate the primary pair rule pattern. These four genes are expressed in two binary sequences, to be called ..131313.. and ..242424.. which are phase-shifted against each other. While the individual sequence would has no polarity, the two superimposed sequences ..12341234.. have polarity (figure 21).

The theory has predicted that the pair rule genes are selectively activated at particular positions in relation to the gap genes and further that the activation at each particular position results from the synergistic action of two gap genes. This has been experimentally verfied (Pankratz *et al* 1990, Howard and Struhl 1990, Hülskamp and Tautz 1991). A further prediction was that a particular region of gap gene expression is insufficient to give rise to a periodic pattern (since a single region does not contain borders). This has been also verified. If all maternal positional information is removed by mutations, the gap gene *Krüppel* is activated everywhere in the egg, but no periodic pattern is formed (Gaul and Jäckle 1987).

On the other hand, from the model I had expected that only a few points of the periodic pattern are fixed by the hierarchically higher gap gene pattern and that the remaining pattern becomes filled in due to the self-organizing capacity of the pair rule pattern. Experiments indicate however a very detailed initiation of the pair rule pattern by the gap gene pattern (Howard *et al* 1988, Goto *et al* 1989).

As predicted by the model, at least two of the pair rule genes have a strong feedback on their own activation. However, no long-ranging substance is yet known that could be the signal for the spacing of the stripe-like gene expression. Therefore, it is not yet clear whether the autocatalysis is used for a switching behaviour (figures 15, 16) or for a lateral activation mechanism (eqation (13), figure 19).

For the activation of the segment polarity genes by the pair rule genes, a doubling in the spatial periodicity is required. The same cell state must be activated at two different positions in the double segment pattern. Figure 22 shows a simulation of such doubling in the spatial frequency.

In terms of the threefold subdivision of segments, the SAP model, a mutation in a gene involved in the generation of this pattern is expected to cause a loss of one of the three cell states. The remaining two alternating cell states would still form a periodic pattern but the polarity would be lost, in agreement with the observation (see figure 20(*e*)).

8.2. Observation on the molecular level support the theory of segmentation

Recently, the model of mutual activation of locally exclusive cell states for segmentation has found much experimental support. The *engrailed* gene is a key gene for the P state. It is expressed in narrow stripes (figure 22, Kornberg *et al* 1985). Its activation is presumably autocatalytic (Condie and Brower 1989). Additionally required for its activation is a substance of longer range, the product of the *wingless* gene (Baker 1988) which becomes activated in the cells that border the *engrailed* expressing cells at the anterior side. The *wingless* protein is secreted and can be detected in neigbouring cells (van den Heuvel *et al* 1989). Similarly, the activity of the *wingless* gene depends on the activity of the *engrailed* gene in the neighbourhood (Martinez-Arias *et al*; 1988,

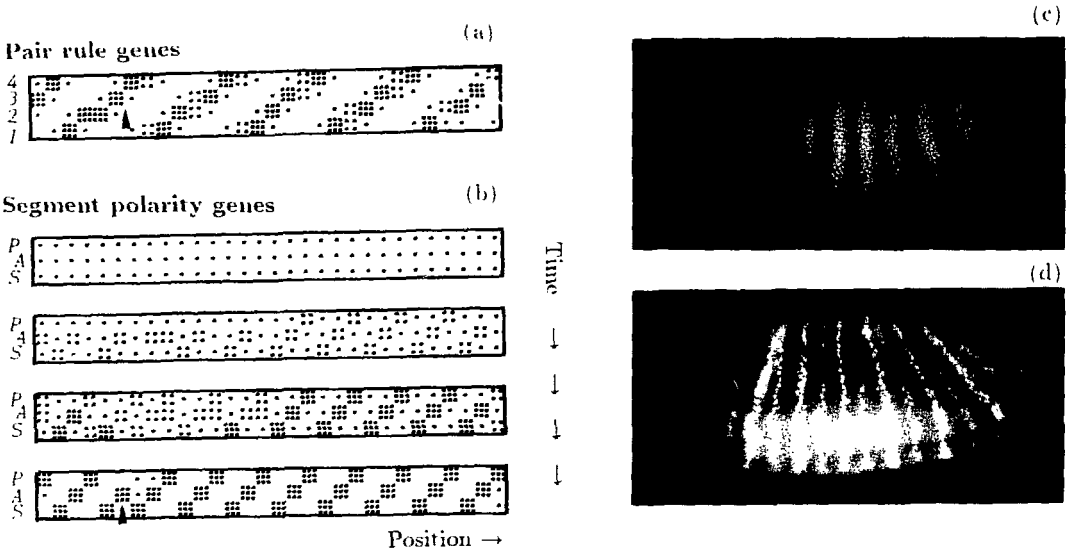

Figure 22. Frequency doubling: Induction of the single segment pattern (cell states S, A and P) by the double segment pattern (1234-pattern). (*a*) The pattern of pair-rule gene activities (see figure 21). (*b*) Stages in the activation of the SAP pattern. The S, the A and the P cell states are activated at two different positions of the 1234 pattern, A at 1 and 3, S at 2 and 4 and P at the $\frac{1}{2}$ and the $\frac{3}{4}$ border. The result is a doubling of the spatial frequency such as required for the double segment-single segment transition. If not enough cells carry a particular activation in the double segment pattern the resulting single segment pattern may contain a locally symmetric pattern (arrow heads), a situation frequently encountered in mutants. (*c*), (*d*) Biological example for frequency doubling: the seven stripes of the *even-skipped* (*c*) and the 14 stripes of the *engrailed* (*d*) transcription (corresponding to gene 2 and gene P in the simulation; photographs kindly suppled by Manfred Frasch).

see Ingham and Nakano 1990 for review). Thus, a mutual long range activation is required for the stabilization of the two neighbouring cell states.

If the *engrailed* gene is completely lost in a mutant, the periodic character of segmentation is lost altogether. According to the lateral activation mechanism discussed above, cell states need each other for mutual stabilization. Therefore, a loss of one element can lead to the loss of other elements.

8.3. Formation of a precise number of different segments during terminal outgrowth

The simultaneous formation of the segments by a cascade of pattern forming events is a later evolutionary invention. In lower arthropodes, annelides, leeches and in short germ insects such as grasshoppers (figure 23), segmentation results from a sequential addition of segments at the posterior end of the embryo until the correct number is reached. An example is the formation of the 32 segments of the leech.

The lateral activation scheme accounts for the addition of segments during localized cell proliferation. In terms of the SAP-model introduced above, by proliferation at the posterior end cells of the same specificity are added. Whenever, for instance, too much A cells have been formed, at a distance from the S/A border the support of the A cells by the S cells will become low, but the support of the P state by the many A cells will be high and some cells will switch from the A state to the P state and so on. The result will be a periodic . . .P/SAP/SA. . pattern (figure 23).

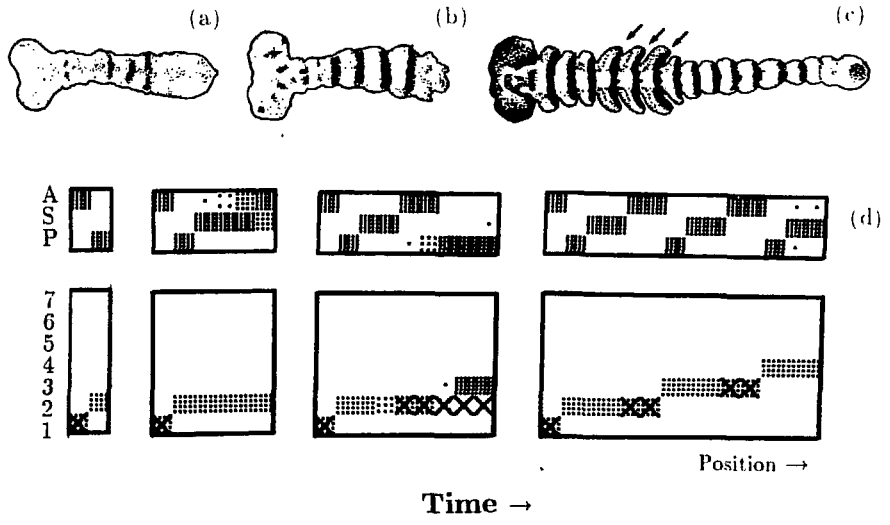

Figure 23. Formation of a periodic and sequential pattern by marginal growth. (*a*)-(*c*) Stages in the development of a grasshopper embryo. At the posterior end, cell proliferates at a high rate. In the course of development, more and more segments are added at the posterior end. In the posterior third of each segment the gene *engrailed* (black stripes) is transcribed (Patel *et al* 1989). The segments are different from each other. Legs are formed only in the three thoracic segments at the anterior *engrailed* border (leg buds are marked by arrows). Model: by addition of cells at the right side, the A, S or P region become enlarged until the subsequent gene becomes activated. A periodic pattern with polarity results. Further, if cells are in the A-stage, the subsequent element of the sequential pattern (1, 2. .7) becomes activated but blocked. Only after transition into the P state, this block is released and the activation of the subsequent gene takes place. The resulting 1, 2, 3... pattern is in precise register with the . .SAP/SAP. . pattern. The A/P border is a prerequisite for leg formation (see figure 26) (*a*)-(*c*) drawn after photographs kindly supplied by Nipam H Patel).

In the leech initially more then the final 32 segments are formed. The few surplus segments are later removed by a programmed cell death (Fernandez and Stent 1982, Shankland 1991). The number $32 = 2^5$ may suggest a digital counting mechanism. This is misleading since, for instance, the polychaet *Clymenelly torquata* has 22 segments. The latter animal regenerates removed segments such that a pattern of 22 segments will be restored independent of the number of segments removed (Moment 1951, Goss 1969).

These observations indicate that some sort of counting mechanism must be available. Its molecular basis not yet clear. It is suggestive that the formation of the periodic pattern causes also a sequential pattern 1, 2, 3 . . . that provides an individuality for each segment. If counting is possible, the outgrowth can be terminated if the last gene has been activated.

It is possible to generate a sequential pattern of gene activation that is precisely in register with the periodic pattern, for instance the SAP-pattern mentioned above. The periodic alternation between the cell states at the growing posterior end can be used in a mechanism analogous to the escape-mechanism of a grandfather's clock. There, the periodic movement of the pendulum controls the switch mechanism that leads to a controlled sequential advancement of the pointer step by step. In terms of gene switching, in one state the transition from one gene to the next is prepared but the actual transition is blocked. In the other, the actual transition takes place but no further

transition is prepared. Thus, with each full SAP cycle, there will be one and only one transition to the next gene that controls identity.

In *Drosophila* genes such as *Antennapedia* or *Ultrabithorax* are known to determine segmental identity (see Akam 1987). The switch from one gene to the next does not take place at the segment border but in the frames of the parasegments, i.e. not in the frame . .P/SAP/S. . but in the frame . .A/PSA/P. . . This is very remarkable since, for instance, legs and wings are also formed at these A/P borders of parasegments. Thus, the anterior and the posterior part of a wing are under control of different genes that controls segmental identity.

9. Structures with a handedness: initiation of legs and wings

Substructures such as legs, wings, eyes, etc, appear pairwise at precise positions, they have a defined rotational asymmetry (handedness) and a reproducible orientation in relation to the main body axes of the embryo. As for other developmental systems, many informations about how these structures are formed have been obtained from experiments in which normal development has been perturbed by transplantations, excisions, etc. Developing legs and wings of vertebrates and imaginal disk of insects (the larval primordia of insect appendages) are frequently employed model systems. These experiments have revealed that from very early stages onwards the primordial structures have their own coordinate system and a high degree of autonomous regulation. An early amphibian limb bud can be transplanted into the head region and a complete limb develops there. Harrison (1921) concluded from his experiments that first a more or less homogeneous limb field is formed that becomes subsequently specified first along the anteroposterior (AP) and later along the dorsoventral (DV) axis.

However, some of Harrisons result was a surprise and difficult to integrate into a scheme of axis determination under the influence of the main body axes. Excision of tissue from the flank of an axolotl embryo and re-implantation at the same side at a more posterior position leads to the formation of a supernumerary limb with a reversed anteroposterior but a normal dorsoventral polarity (figure 24). In other words, if this operation is performed on the right side, an additional left limb will be formed although no tissue has been rotated or transposed from one side to the other.

At that time many interpretations of biological phenomena have been influenced by physical and mechanistic considerations. A magnet can be divided into pieces and each piece maintains its polarity. After isolation, a magnetic south pole 'regenerates' a north pole. In analogy, polarity of a tissue was assumed to result from the alignment of many polar cells. Clearly, the polarity reversal observed in Harrisons experiment contradicts such a view since a shift should not induce a polarity reversal.

9.1. Generation of positional information at borders of differently determined cells

In contrast to Harrison's idea I have proposed that secondary embryonic fields are formed around borders that result from a preceding pattern forming event (Meinhardt 1983a, b). As discussed above for segmentation, borders between differently determined cells are very convenient to organize the subsequent hierarchical level. Let us first regard only one dimension. Imagine that an embryo has been subdivided into several regions, among them are the regions A and P. If, for instance, a diffusible substance m is produced in the P region, the A cells will become exposed to a m-gradient that

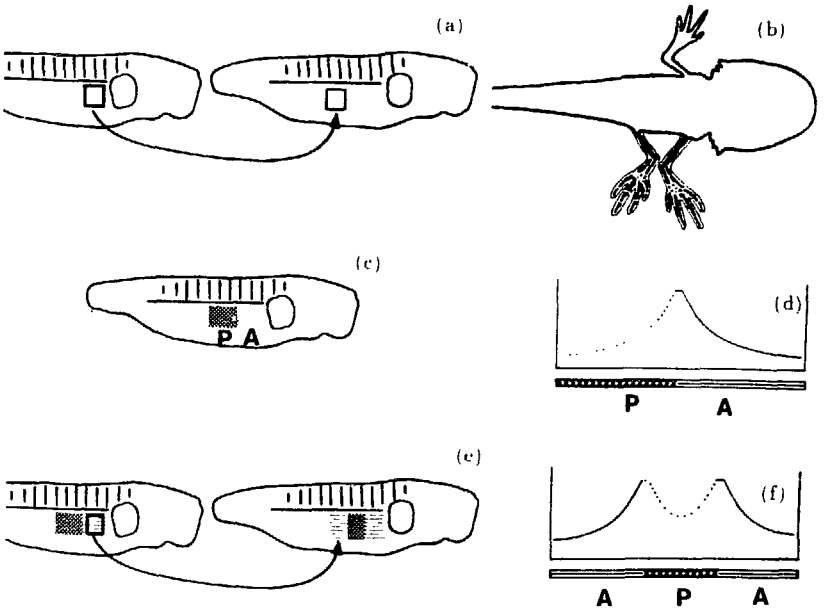

Figure 24. Formation of a supernumerary limb after transplantation of flank tissue in axolotl (Harrison 1921, Slack 1976, 1977). (*a*) The operation, (*b*) the result: a supernumerary limb is formed with reversed anteroposterior orientation and reversed handedness. (*c*), (*d*) Model: it is assumed that the A-tissue is able to produce a limb if bordered by P tissue. By cooperation of A and P tissue a signalling substance is produced at the common border to which only the A cells can respond (see also figure 1). (*f*) After transplantation of A tissue to a more posterior position, two separated A-regions exist that are exposed to opposite gradients.

has its highest point at the A/P border. If only the A-cells responded in a concentration-dependent manner, the *m*-gradient would be appropriate to provide the A-cells with unique positional information. Alternatively, by a cooperation of A and P cells specialized cells can appear at the common border that, in turn, produce the morphogen. In the latter case, a symmetrical morphogen distribution will be formed that is centred over the border. Nevertheless, the resulting pattern can be asymmetric or only partially symmetric since the A and P cells can respond in a different manner to the same morphogen, or response may be restricted to one of the two cell types.

This model provides a straightforward explanation for the puzzeling appearing polarity reversal mentioned above (figure 24). If we assume that the A region forms the limb and that it is organized by an A/P border, transplantation of A-cells to a more posterior position leads to an A/P/A configuration. Both A regions can form a limb since they are both bordered by a P region. However, in the transplanted A region, the P region is unusually located at the anterior side of the A region, i.e. the morphogen gradient has the opposite polarity. Thus, Harrison's observation is a very strong indication that neither the global axes of the embryo nor the orientation of polar cells but the local neighbourhood of determined cells is decisive for the polarity of the developing limbs.

9.2. An intersection of two borders is required to specify a particular position and handedness

A primary subdivision of an embryo along the AP axis would lead to areas of different

gene activities and thus to borders that surround the embryo in a belt-like fashion. To specify the position of any particular structure along such a border, an intersection with a second border is required that runs from anterior to posterior at a particular dorsoventral level (figure 25). If the embryo has the shape of a tube or an ellipsoid, then for any reasonable subdivision along the AP and DV axis, the intersections appear in pairs, one on the left, the other on the right side of the embryo (figure 25(*d*)). Due to the natural inside-outside asymmetry of the embryo, each intersection has a handedness. For instance, posterior dorsal, anterior dorsal and anterior ventral is arranged clockwise on the right and counterclockwise on the left side. The positional information systems generated by interactions across such borders necessarily have the correct orientations with respect to the preceding subdivisions along the main body axes since they strictly depend on them.

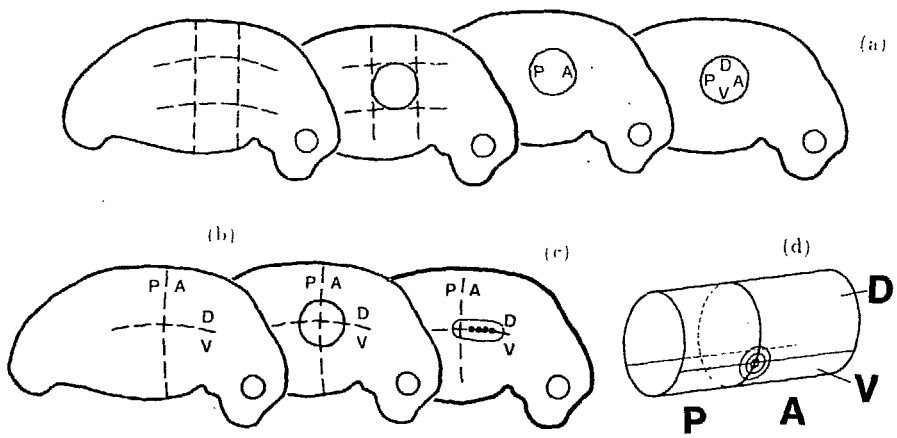

Figure 25. Formation of a limb field. (*a*) Traditional view: Formation of the limb field precedes its internal organization. Primary embryonic subdivision along the AP and DV axes lead to the specification of the initially uniform limb field. Subsequently, this field obtains step-wise an internal organization along the AP and DV axis. (*b*) Boundary model: The limb field is formed around the intersection of two borders generated by a preceding step. A limb field therefore has an internal organization from the beginning. (*c*) In the vertebrate limb, the structures are mainly derived from the anterior side of the border. The DV border determines the plane at which the digits (black dots) are formed. (*d*) Viewed an embryo as a cylinder, an intersection of the A/P and the D/V border is formed at two positions. They have an opposite handedness. The model thus accounts for the bilateral disposition of limbs.

9.3. Generation of cartesian and polar coordinate systems

This model accounts for the generation of radial-symmetric (polar) as well as for cartesian coordinate systems. Cooperation of two pairs of tissues can lead to a more or less point-like source region. The resulting morphogen distribution would have the shape of a cone that provides information about the distance from the centre (see figure 26). In addition, since the field is generated over the touching point of four quadrants or three sectors, a coarse information about the angular position exists. As shown below, such a coordinate system is required for insect legs. On the other hand, by cooperation of two pairs of tissues, two roof-like distributions can be generated along the two borders. This lead to a cartesian coordinate system since the distances from two orthogonal lines are measured.

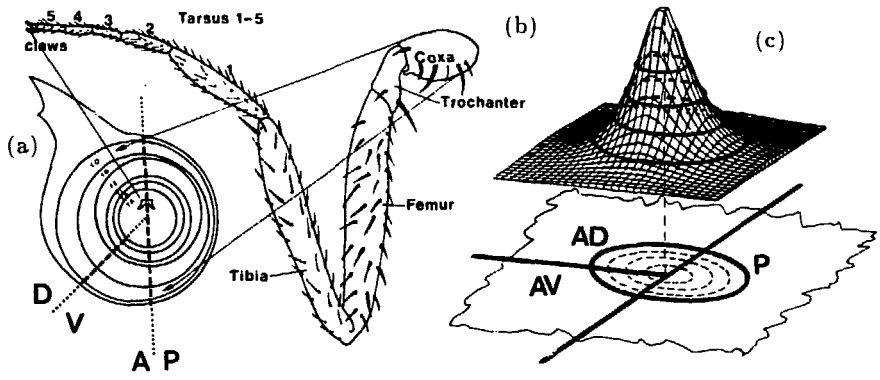

Figure 26. Generation of the coordinate system for an insect leg. In the imaginal disks (*a*), the structures of an adult insect leg (*b*) are already determined in concentric rings. Distal structures are laid down in the centre, more proximal structures follow in concentric rings (Schubiger 1968). (*c*) Model: Production of a substance requires cooperation of two pairs of differentiated cells (A/P and D/V). Production is thus limited to a point-like region at the intersection of the two borders. The local concentration is a measure for the distance from that border. Since this point is surrounded by four different quadrants, also an information about the angular position is available.

9.4. A coordinate system for the vertebrate limb

The vertebrate limb system was used already in the introduction to illustrate the positional information concept (figure 1). Several problems exist in such a model which has been neglected so far but which are resolved by the boundary model. The cells in the ZPA region are regarded as localized source of a morphogen. The resulting gradient has been drawn only to one side (figure 1(*c*)). But a localized source would generate a graded profile in all directions. The resulting distribution would have the shape of a cone. Expected would be a set of structures arranged in concentric rings. This is correct for the arrangement of primordial segments of insects (figure 26) but the digits are arranged in a plane. According to an application of the boundary model to vertebrate limbs (Meinhardt 1983a), the organizing ZPA region is formed at an intersection of an A/P and a D/V border. The digits are formed along the D/V border in the A region (figure 25(*c*)). Which of the digits is formed depends on the distance from the P border. Thus, in the view of the boundary model, the simple, normally unilateral gradient is a reasonable approximation. The boundary model accounts for many non-trivial regulatory phenomena such as different regeneration capacity of experimentally produced double posterior or double anterior limb buds or the formation of supernumerary limbs with a DV–VD polarity change within the hand after cut, rotation and reimplantation (Meinhardt 1983a). Genes have been cloned which are expressed very early in complementary regions (Oliver *et al* 1988, 1989). The common border is located in the region that forms later the forelimb. This could be the A/P border postulated by the model. Genes that mark the D/V border are not yet available.

9.5. Formation of insect legs

In *Drosophila* and other holometabolous insects, epithelial structures such as legs, wings, halteres, eyes and antennae are derived from nests of cells, the imaginal disks (see Gehring and Nöthiger 1973). During pupation, these disks form the adult structures while larval tissue degenerates. In *Drosophila*, the cells of the imaginal disks are almost

completely determined before pupation begins. Small disk fragments implanted into a metamorphosing larvae differentiate according to their original position. This allows one to reconstruct fate maps of the disks. Schubiger (1968) has shown that the primordia of the leg segments are arranged in the disk as a series of concentric rings with the claws, the most distal structure, in the centre and the more proximal structures (thorax and coxa) at the periphery (figure 26). The thin tube-like leg results from an evagination of the disks (a process that is similar to the pulling out of a telescope antenna).

The compartment borders discussed above cross the leg and wing disks (Garcia-Bellido *et al* 1973, figure 26). If, as I have proposed, the compartment borders organize secondary fields, it is expected that the concentric structures of the leg disks are centred around the intersection of two borders and the distal-most structure, the two claws, coincide with the intersection itself. Indeed, one of the claws belongs to the anterior, the other to the posterior compartment (Steiner 1976). In the leg disk, only the anterior compartment is subdivided into a dorsal and ventral compartment. The D-V border points towards the distalmost structures of the disk, although its precise position there is not known.

9.6. Formation of borders precedes formation of imaginal disks

A central prediction of the boundary model is that the formation of the borders precedes the formation of the disks. This prediction is supported by many observations. Steiner (1976) found that the progeny of a single cell, genetically marked at the blastoderm stage, can contribute to leg *and* wing formation. However, these cells will be confined either to the anterior or to the posterior compartment. This rules out the idea that disks are initially homogeneous and subsequently become separated into anterior and posterior compartments. Instead, the primary event is the formation of the border and later on cells from both sides of the border are allocated for disk formation.

In the recent years probes have become available that allow detection of localized gene expression involved in disk determination. The *engrailed* (*en*) gene, the key gene for the formation of the posterior compartment (Lawrence and Morata 1976), is already expressed in narrow stripes about one cell wide during the blastoderm stage, i.e. long before any imaginal disk can be present (Kornberg *et al* 1985). It is a member of the segment polarity group mentioned above (figures 20, 22). The same is true for the *wingless* gene (Baker 1988) which is expressed in similar stripes just anterior to the *en* stripes. Both genes are primarily involved to generate the repetitive pattern of cell states that leads to segmentation, but as the name '*wingless*' emphasizes, they are also required to form appendages, in full agreement with the stipulation that a primary subdivision into discrete regions is the precondition to form secondary fields.

9.7. Regeneration and formation of supernumerary cockroach legs

In hemimetabolous insects no metamorphosis takes place. Juvenile and adult forms have a similar appearance. Leafhoppers belong to this class. Perturbations in the epidermal structures at juvenile stages can regulate during one or several molds. The cockroach leg as model system has been mentioned already (figure 18).

Removal of a cockroach leg can lead to its regeneration. If we assume a compartmental subdivision in the leg similar to that in *Drosophila*, the three compartments have the shape of narrow stripes that touch each other only at the tip. According to the model, removal of the leg and would closure leads to a new point where all

compartments are close to each other and thus to a new positional information system that allows a re-specification of the lost structures. Not only the complete leg but also some of the surrounding tissue can be removed and leg regeneration still occurs (Bohn 1974). This supports the view that secondary fields are smaller than the regions that generate the fields at their borders. In his study of leg regeneration, Bohn has also removed tissue that under normal circumstances never forms a leg. The surprising observation was that excision of epidermal tissue close to the anterior segment border can cause the formation of a supernumerary leg. According to the model for segmentation outlined above segmentation results from the repetition of (at least) three cell states, called S, A and P. With the removal of tissue at the anterior part of the segment, the S region and thus the region that separates two A/P pairs is removed. After would healing this leads to a new A/P confrontation, although with reversed polarity (P/A) (figure 27; Meinhardt 1984). Moreover, since the AP but not the DV orientation is reversed, the supernumerary limb will have the opposite handedness. These expectations are in full agreement with the experimental observation.

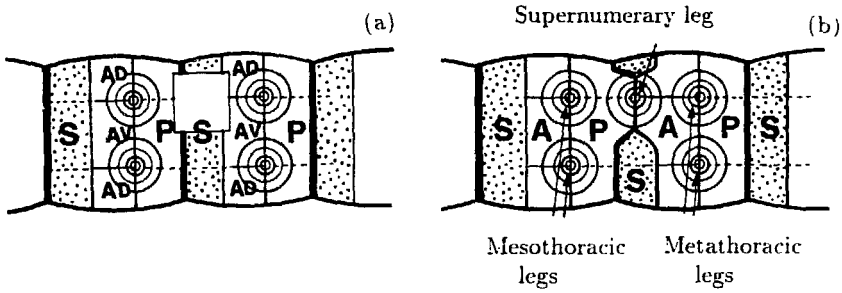

Figure 27. Induction of a supernumerary leg in the cockroach by removal of epidermis from the anterior side of a thoracic segment (Bohn 1974). (*a*), (*b*) Model: Segmentation is assumed to result from the repetition of at least three structures, S, A and P. A ventral view of the thoracic segments is shown. Legs (concentric circles) are formed pairwise at the intersections of the A/P and the D/V borders. Removal of the tissue from anterior part (the S region; white rectangle) and would healing leads to a new P/A confrontation. A new intersection with reversed AP polarity and thus a supernumerary limb is formed. Since the DV-level is not changed, the handedness is reversed.

In summary, the organization of secondary fields by borders formed in a preceding pattern forming event enables a reliable fine structuring of a developing organism; newly determined structures necessarily have the correct spatial relationship (position and handedness) to the structures laid down in a preceding step. The process can be reiterated leading to an organization with progressively finer scale. No autocatalysis and lateral inhibiton is required to generate the signal system, but autoregulation is required for the maintenance of region-specific gene activation.

10. Conclusion

It was my intention to show that the emergence of pattern during development can be explained by relatively simple coupled biochemical interactions. All the ingredients used, mutual activation and inhibition of biochemical reactions and of course diffusion are known to exist in other biochemical systems. An explanation of many phenomena

can be given without the addition of unreasonable assumptions. The computer simulations show the consistency of the models. Many elements postulated by the models meanwhile found direct support by the new molecular-genetic techniques. Although the models for the more complex structures such as segmentation have already a considerably complexity, the investigations on the molecular level have revealed that the actual realizations are even more complex, partly to gain a greater robustness, to synchronize a particular step with other processes, or perhaps even due to an evolutionary load. The models presented are minimal models only.

I hope I have convinced the reader that development of an organism is a tractable process open to theoretical analysis as is common in physics. I also hope that an understanding of these processes does not diminish the admiration for the steps that lead ultimately to the beginning of a life.

Acknowledgments

I wish to express my sincere thanks to Professor Alfred Gierer. Much of the basic work described in this review emerged from a fruitful collaboration over many years.

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