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Models of Biological Pattern Formation: From Elementary Steps to the Organization of Embryonic Axes

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An inroad into an understanding of the complex molecular interactions on which development is based can be achieved by uncovering the minimum requirements that describe elementary steps and their linkage. Organizing regions and other signaling centers can be generated by reactions that involve local self-enhancement coupled to antagonistic reactions of longer range. More complex patterns result from a chaining of such reactions in which one pattern generates the prerequisites for the next. Patterning along the single axis of radial symmetric animals including the small freshwater polyp hydra can be explained in this way. The body pattern of such ancestral organisms evolved into the brain of higher organisms, while trunk and midline formation are later evolutionary additions. The equivalent of the hydra organizer is the blastopore, for instance, the marginal zone in amphibians. It organizes the anteroposterior axis. The Spemann organizer, located on this primary organizer, initiates and elongates the midline, which is responsible for the dorsoventral pattern. In contrast, midline formation in insects is achieved by an inhibitory signal from a dorsal organizer that restricts the midline to the ventral side. Thus, different modes of midline formation are proposed to be the points of no return in the separation of phyla. The conversion of the transient patterns of morphogenetic signaling into patterns of stable gene activation can be achieved by genes whose gene products have a positive feedback on the activity of their own gene. If several such autoregulatory genes mutually exclude each other, a cell has to make an unequivocal decision to take a particular pathway. Under the influence of a gradient, sharply confined regions with particular determinations can emerge. Borders between regions of different gene activities, and the areas of intersection of two such borders, become the new signaling centers that initiate secondary embryonic fields. As required for leg and wing formation, these new fields emerge in pairs at defined positions, with defined orientation and left–right handedness. Recent molecular-genetic results provide strong support for theoretically predicted interactions. By computer simulations it is shown that the regulatory properties of these models correspond closely to experimental observations (animated simulations are available at www.eb.tuebingen.mpg.de/meinhardt).

I. Introduction

The formation of a higher organism within each life cycle is a most fascinating process. With modern molecular-genetic techniques it is possible to monitor simultaneously the mutual interference of hundreds of genes. However, it is notoriously

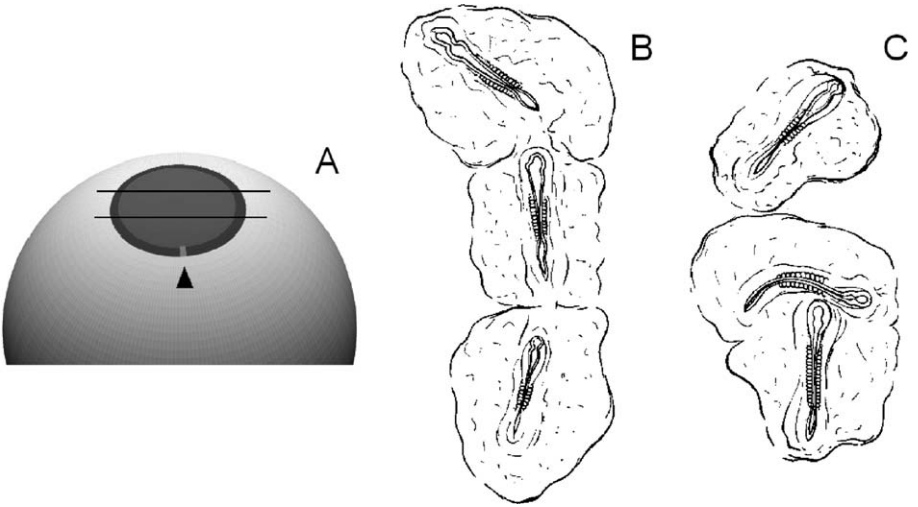

Figure 1 An example of pattern regulation. (A) The early chick embryo has the shape of a disk that is located on the huge yolk. The triangle marks the normal position of the organizing region, Koller's sickle. (B–C) Fragmentation of such a disk at an early stage can lead to complete embryo formation in each fragment (Lutz, 1949; Spratt and Haas, 1960). The fragment that contains the organizer forms an embryo in the normal orientation. In the other fragments the orientation is more or less arbitrary. Nevertheless, in all embryos the correct mutual orientation of the anteroposterior and the mediolateral axes (as indicated by the paired somites) reestablished (B, C after Lutz, 1949).

difficult to deduce from such a plethora of data the functioning of underlying complex networks. Long before the molecular-genetic methods became available, we followed a different approach by asking what type of molecular machinery would be required at least to account for the observed patterns, including pattern regulation after experimental interference. It turned out that interactions employing relatively few components are able to describe elementary steps in surprising detail. In order to find the appropriate hypothetical interactions a mathematical formulation of the reactions was mandatory since our intuition is often unreliable to predict the behavior of systems that are based on strong positive and negative feedback loops.

The final complexity of an organism does not already exist in a mosaic-like fashion in the egg. For instance, each cell of an eight-cell mouse embryo can give rise to a complete embryo. Likewise, after an early partition of the disk-shaped chick embryo, complete embryos can emerge in each fragment (Fig. 1). Obviously, communication between the cells is essential to achieve this spatial organization, and an interruption of this communication can lead to a dramatic rearrangement of the main body axes. It follows that axes formation has a strong self-organizing aspect. Most surprisingly, even after such a severe perturbation, the two main body axes, anteroposterior (AP) and dorsoventral (DV), still have the correct orientation relative to each other, indicating a strong coupling between the system that patterns these two axes.

Simple radial-symmetric animals including the freshwater polyp hydra or the small sea anemone *Nematostella* are evolutionary ancestral organisms, close to the branch point where bilaterality was invented. Since mechanisms in development are so well preserved during evolution (de Jong *et al.*, 2006), it is reasonable to assume that these animals provide a key to understanding the patterning along a single axis and provide information about the steps that occurred towards more evolved bilateral-symmetric body plans.

Hydra tissue is famous for its almost unlimited capability for regeneration (Trembley, 1744; von Rosenhof, 1755; see also Gierer, 1977; Bode, 2003). Even more dramatic, hydra tissue can be dissociated into individual cells and, after reaggregation, these clumps of cells again form viable organisms (Gierer *et al.*, 1972; Fig. 2). Obviously, pattern formation does not require any initiating asymmetry. The small cone-shaped region around the gastric opening, the so-called hypostome, has organizing capabilities (Browne, 1909). A small tissue fragment from this region transplanted into the body column of another animal can induce the formation of a secondary body axes. Although Ethel Browne did not use explicitly the term 'organizer,' she discovered a phenomenon that became of central interest 15 years later with the discovery of the amphibian organizer (Spemann and Mangold, 1924; see Lenhoff, 1991). Thus, hydra can be used as a guide to find the corresponding interactions that allow *de novo* organizer formation and its regeneration.

Hydra is also a convenient model organism to study more complex patterning steps. In many developmental systems particular structures are formed with a precise spatial relation. In hydra the primary organizer, the hypostome, is surrounded by a necklace of tentacles. Since the tentacles resemble a periodic pattern, hypostome and tentacle formation is governed by two separate but coupled pattern-forming systems, providing an inroad into the question of how to induce two structures next to each other. Why do tentacles appear close to each other around a narrow ring, but do not form with a similar spacing along the body column?

Hydra is under the control of two antipodal organizing regions, the head and the foot. Both appear at maximum distance from each other. Again, this is a frequent occurrence; shoot and root in plants or head and tail in planarians are other examples. Which interaction enforces a maximum distance from each other but allows, nevertheless, both terminal structures to be formed close to each other at early stages or during regeneration of small fragments?

In the first part of this paper such elementary steps in pattern formation will be discussed and compared with more recent molecular-genetic observations.

A. The Body Pattern of Hydra-Like Ancestral Organisms Evolved into the Brain of Higher Organisms

A step of primary importance in the development of higher organisms is the generation of the main body axes, anteroposterior (AP), dorsoventral (DV) and, in ver-

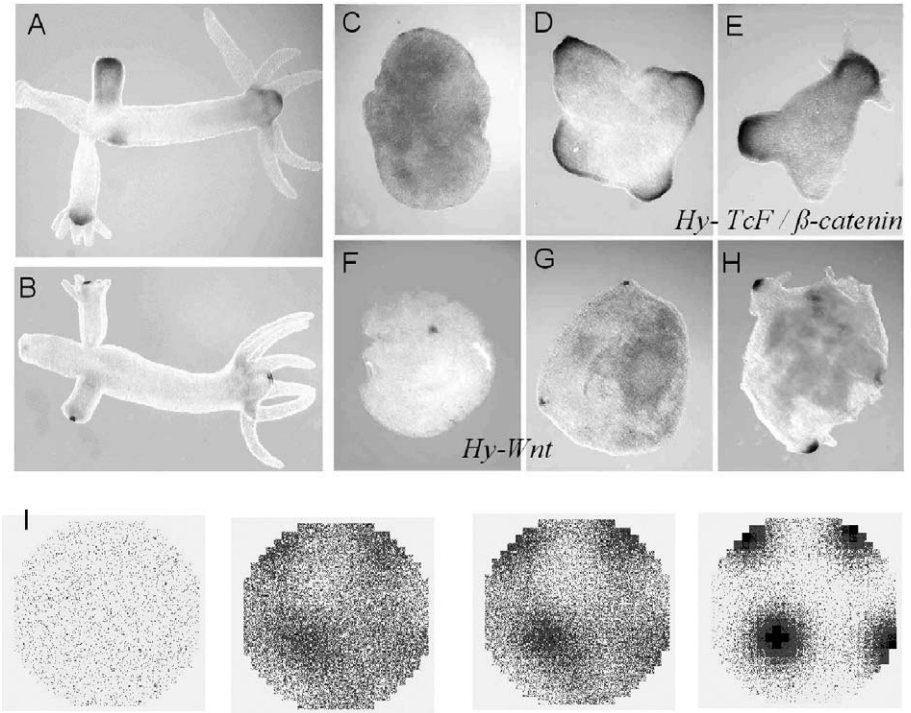

Figure 2 The canonical *Wnt* pathway is involved in organizer formation in the freshwater polyp hydra. From classical experiments it was known that the region around the opening of the gastric column, the cone-shaped hypostome, has organizer activity. (A) *TCF* (and β-catenin) expression occurs in a graded fashion at the tip and also precedes the formation of a new axis during bud formation (Hobmayer *et al.*, 2000). (B) *Hy-Wnt* expression is more sharply confined to the tip. (C–E) In reaggregating cells, *Hy-TCF* (and β-catenin) appears first uniformly distributed and become subsequently more restricted to regions that eventually form the new heads. (F–H) In contrast, *Hy-Wnt* appears directly in sharp spots that form the future oral opening. (I) This nested pattern formation can be accounted for by the assumption that both *Hy-*β*-*catenin*/Hy-Tcf* (gray) and *Hy-Wnt* (black) are pattern forming systems and that a high *Hy-*β*-*catenin concentration is the precondition to trigger *Hy-Wnt*. Thus, *Wnt* peaks require a high *Hy-*β*-*catenin*/Hy-Tcf* level and appear as sharp spots at the highest level of the more graded *Hy-*β*-*catenin*/Hy-Tcf* distributions. Such a superimposed patterning allows the specification of a large region for head formation and provides nevertheless a sharp signal, e.g., for the opening of the gastric column (Photographs by courtesy of B. Hobmayer, T. Holstein and colleagues; see Hobmayer *et al.*, 2000.)

tebrates, left–right (LR). Radially-symmetric organisms themselves provide a key to understanding the essential inventions required for the transition from radial- to bilateral-symmetric body plans. For long it was unclear whether the single axis of hydra corresponds to any of the main body axes in higher organisms, and, if so, to which axis and in which orientation. Almost all components involved in higher organisms to pattern the AP as well as the DV axes are already present in hydra. However, systems

that control the orthogonal axes in higher organisms, e.g., *WNT* for the AP axis and *Chordin/BMP* for the DV axis, are expressed in hydra along the only existing axis. Thus, bilaterality is proposed to be achieved by a realignment of at least two already existing, originally parallel axial systems and not by the invention of a new signaling system (Meinhardt, 2004a). Some coelenterates already show pronounced deviations from radial symmetry (Martindale, 2005).

As the expression patterns of more and more genes became available, the situation became more and more difficult to interpret. Around the gastric opening genes are expressed that are characteristic for both head *and* tail formation, *Goosecoid* and *Brachyury* (Broun *et al.*, 1999; Technau and Bode, 1999). This apparent discrepancy can be resolved by the assumption that the body pattern of a hydra-like ancestor evolved into the most anterior (and most important) part of higher organisms, the brain and the heart (Meinhardt, 2002). The *Otx* gene, in vertebrates, characteristic for the fore- and midbrain, is expressed in the hydra all over the polyp with the exception of the most terminal regions (Fig. 3). This suggests that in an ancestral radial-symmetric organism the posterior end was at a position that corresponds in vertebrates roughly to the midbrain/hindbrain border. Thus, although in hydra the region around the gastric opening with the tentacles is commonly called 'the head,' it represents the most posterior part as indicated by *Wnt* and *Brachyury* expression. The recently observed highly conserved patterning in the brain of such distantly related organisms as insects and vertebrates (Hirth *et al.*, 2003; Lowe *et al.*, 2003; Sprecher and Reichert, 2003) is proposed to have its origin in the preserved body pattern of a common radially-symmetric ancestor.

In later parts of this paper it will be shown that this relation is a key to understanding the different modes in the generation of bilateral-symmetric body plans. In the final part mechanisms will be discussed that lead to insertion of new structures within the frame of the body axes, such as legs and wings or of branching structures such as blood vessels and tracheae.

II. Primary Pattern Formation by Local Self-Enhancement and Long-Ranging Inhibition

The observation that patterns can emerge in an initially more or less uniform assembly of cells (Fig. 2) raises the question of what type of molecular interaction would be able to generate local concentration maxima. In a pioneering paper, Alan Turing (Turing, 1952) showed that pattern formation is possible by an interaction of two components with different diffusion rates, now collectively called reaction–diffusion systems. However, most reactions in which two substances interact have no pattern-forming capability whatsoever, even if they spread with different rates. We have shown that pattern formation is possible if, and only if, a locally restricted self-enhancing reaction is coupled with an antagonistic reaction that acts on a longer range (Gierer and Meinhardt, 1972; Gierer, 1977; Meinhardt, 1982;

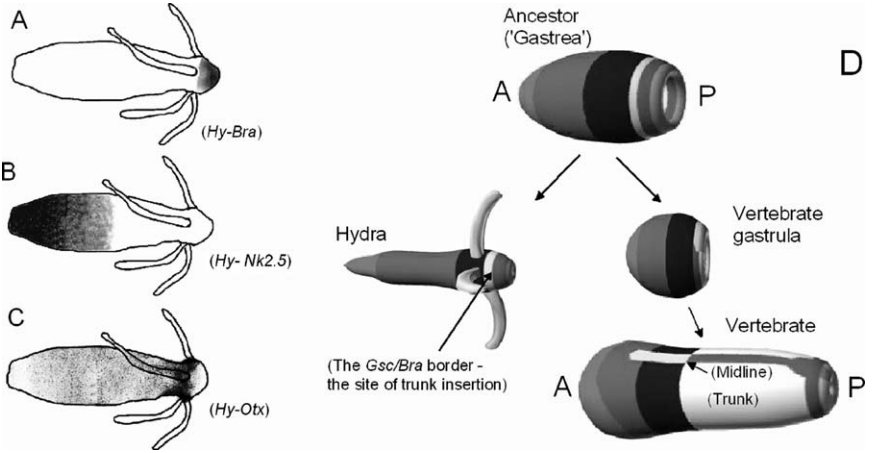

Figure 3 Gene expression of well-known genes in hydra suggests that the body pattern of radial-symmetric ancestors evolved into the head pattern of higher organisms. (A) *Brachyury* is expressed in hydra around the gastric opening (Technau and Bode, 1999). In higher organisms, *Brachyury* marks the blastoporal opening, which is always most posterior. (B) In the antipodal foot region *Nkx2.5* is expressed (Grens *et al.*, 1996). In higher organisms *Nkx2.5* is expressed at very anterior positions (e.g., Tonissen *et al.*, 1994) and is responsible for heart formation. The hydra foot and the vertebrate heart, both involved in pumping, have a common ancestry (Shimizu and Fujisawa, 2003). (C) *Otx*, a gene typical for the fore- and midbrain in higher organisms, is expressed throughout the hydra except in the terminal parts (Smith *et al.*, 1999). The posterior *Otx* border marks in vertebrates the midbrain–hindbrain border (reviewed in Rhinn and Brand, 2001), in hydra the border between the tentacle zone and the hypostome. (D) These expressions suggest that the body pattern of an ancestral organism—the *gastrea* in terms of Haeckel (Haeckel, 1874)—evolved into the brain of higher organisms. The so-called hydra head is in fact the posterior end. The vertebrate gastrula can be regarded as a remnant of this ancestral organism. The midline and the trunk (see Figs. 12–14) are two major later evolutionary inventions. For instance, the trunk–typical Hox gene clusters are absent in Cnidarians (Kamm *et al.*, 2006).

Meinhardt and Gierer, 2000). This condition, not inherent in Turing's paper, is essential for a homogeneous distribution to become unstable. We have derived a general criterion for which interactions lead to a stable pattern and which do not. Pattern formation from more or less homogeneous initial situations is also common in the inorganic world. Examples are the formation of sand dunes, cumulus clouds, stars or lightning. Pattern formation is also common in social interactions. These processes are based on the same principle (Meinhardt, 1982).

A prototype of such a pattern-forming reaction consists of a short-ranging substance, to be called the activator, which promotes its own production directly or indirectly. It also regulates the synthesis of its rapidly diffusing antagonist, the inhibitor. The latter slows down the autocatalytic activator production (Fig. 4; Gierer and Meinhardt, 1972) or catalyzes the activator decay. A homogeneous distribution is unstable since, for example, a small local elevation of the activator will increase fur-

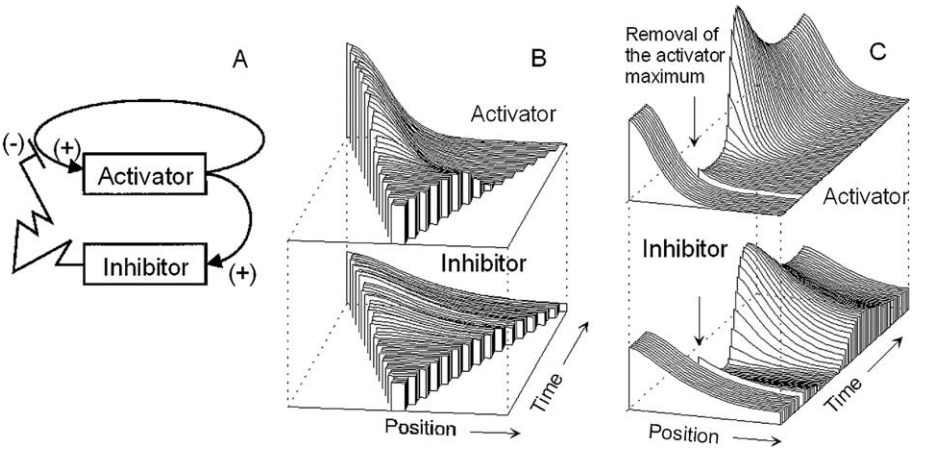

Figure 4 Pattern formation by an activator–inhibitor interaction. (A) Reaction scheme: The activator catalyses its own production. The production of its rapidly spreading antagonist, the inhibitor, is also under activator control (Eqs. (1a) and (1b); Gierer and Meinhardt, 1972). In such a reaction, the homogeneous distribution of both substances is unstable. (B) The simulation illustrates pattern formation in a growing chain of cells as a function of time. Whenever a certain size is exceeded, random fluctuations are sufficient to initiate patterning. A high concentration appears at a marginal position. Thus, although the genetic information is the same in all cells, such a system is able to generate a reproducible polar pattern, appropriate to accomplish space-dependent cell differentiation (see Figs. 19 and 20). (C) Regeneration. After removal of the activated region, the inhibitor is no longer produced. After decay of the remnant inhibitor, a new activation is triggered. The graded profiles are restored as long as the remaining fragment is still large enough (see also Fig. 10).

ther due to autocatalysis despite the fact that a surplus of the inhibitor is also produced at the same position. The latter, however, dilutes rapidly by a fast diffusion into the surroundings of this incipient maximum, slowing down the autocatalysis there. Therefore, a local rise is intimately connected with a down-regulation at larger distances. A new patterned steady state is reached when a local high activator concentration is in a dynamic equilibrium with the surrounding cloud of the inhibitor (Fig. 4). Both the more localized activator and the more smoothly distributed inhibitor can be used as a morphogenetic signal. Thus, pattern formation depends critically on the spatial distribution of signals. Although diffusion is a good approximation, the real process is usually much more complex requiring a chain of several molecules: secreted ligands, receptors and components that transmit the signal from the cell surface to the nucleus. The term ‘diffusion’ is only used as shorthand for a long-ranging signaling. Other modes of redistribution of molecules are conceivable as well. In plants, for instance, active transport of auxin plays a major role.

At the time the theory was proposed (1972), activator–inhibitor systems were completely hypothetical. Since then several systems have been found that correspond to this scheme. For instance, *Nodal* is a secreted factor that has a positive feedback on

its own production; *Lefty2* is under the same control as *Nodal* and acts as an antagonist. *Lefty2* cannot dimerize and blocks in this way the *Nodal* receptor. This system is involved in mesoderm and midline formation as well as in left–right patterning (Chen and Schier, 2002; Nakamura *et al.*, 2006). Another example is the specification of heterocyst cells in the blue-green alga *Anabaena* (see Fig. 7). In hydra, *Wnt* and β-catenin are expressed in the hypostome, suggesting that the canonical *Wnt* pathway is involved in the formation of the hydra organizer (Fig. 2; Hobmayer *et al.*, 2000; Broun *et al.*, 2005). However, the molecular basis of the self-enhancement and of the long-ranging inhibition is not yet clear.

A. A Mathematical Description

Since all biological processes are assumed to be accomplished by the interaction of molecules, a theory of biological pattern formation has to describe the changes of concentrations in space and time as function of the local concentration of the relevant substances involved. The following set of equations describes the local change of the activator $a(x)$ and inhibitor concentration $h(x)$ per time unit (Gierer and Meinhardt, 1972), for simplicity written here for a one-dimensional array of cells:

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

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

Such equations are easy to read. Equation (1a) states that the concentration change of the activator a per unit time ($\partial a/\partial t$) is proportional to a nonlinear autocatalytic production term (a^2). The autocatalysis is slowed down by the action of the inhibitor $1/h$. The second term, $-\mu a$, describes the degradation. The number of activator molecules that disappear per time unit is proportional to the number of activator molecules present (like the number of people dying per year in a city is on average proportional to the number of inhabitants). The autocatalysis must be nonlinear (a^2) since it must overcome disappearance by linear decay ($-\mu a$). This condition is satisfied if the active component is not the activator itself but a dimer of two activator molecules. An example is the dimerization of the activator that leads to heterocyst formation in *Anabaena* (see Fig. 7). The factor ρ, the *source density*, describes the general ability of the cells to perform the autocatalytic reaction. Its function is close to what is described as 'competence' in the biological literature. Slight asymmetries in the source density can have a strong influence on the *orientation* of the emergent pattern. The concentration change of a and h also depends on the exchange of molecules with neighboring cells. This exchange is assumed to occur by simple diffusion but other mechanisms are conceivable as well.

Equation (1b) can be read in an analogous manner. A necessary condition to enable spatial pattern formation is that the inhibitor spreads more rapidly than the activator, i.e., the condition $D_h \gg D_a$ must be satisfied. In addition, the inhibitor must have a

more rapid turnover ($\nu > \mu$), otherwise the system will have the tendency to oscillate (see Fig. 9).

For many simulations Eqs. (1a) and (1b) are used with a few extensions:

$$\frac{\partial a}{\partial t} = \frac{\rho a^2}{h(1+\kappa a^2)} - \mu a + D_a \frac{\partial^2 a}{\partial x^2} + \rho_a, \tag{2a}$$

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

The term κ leads to a saturation of the self-enhancing reaction at higher activator concentrations and thus to an upper limit of activator production. This allows, for instance, the formation of stripes (see Fig. 6). The last term in Eq. (2a) is a small activator-independent activator production. This term ensures that the concentration of the activator never sinks to zero and enables the reformation of an activator maximum after removal of an established maximum. It is important for the initiation of the autocatalytic reaction at low activator concentrations as required for regeneration (Fig. 4) and for oscillations (see Fig. 9). The last term in Eq. (2b) is a small activator-independent inhibitor production. It has the consequence that a uniform low concentration of activator can be a semistable situation. This baseline inhibitor level can suppress a spontaneous trigger. In this way the pattern-forming system can be "asleep" until a trigger occurs that raises the activator concentration above a threshold from which the patterning proceeds further due to the self-enhancement. Such a trigger can be supplied, for example, by adjacent activated cells during the spread of traveling waves (see Fig. 9C).

For simulations, the concentration changes are calculated for small but discrete time steps and the space is subdivided into discrete units or 'cells.' Starting with initial distributions of both substances, these equations allow the computation of their changes over a short time interval. Adding these changes to the given concentrations leads to new concentrations. By repeating this computation the total time course can be calculated in an iterative way. The exchange of molecules between adjacent cells requires special conditions at the boundaries of the field of cells. Usually it is assumed that the boundaries are impermeable. Simple and well commented programs that can be compiled and executed on a PC are available on our website.

B. Polar Patterns, Gradients, and Organizing Regions

For the generation of a primary body axis, it is essential that one side of the developing organism becomes different from the other. Pattern formation requires a certain field size such that the different diffusion rates can come into play. If an activator–inhibitor mechanism is involved, a high concentration emerges at one and a low concentration at the opposite side whenever a certain size of tissue is exceeded (Fig. 4). The generation of such a polar pattern is a most important step: Although the genetic information is the same in all the cells, different genetic information can be activated in a position-dependent manner.

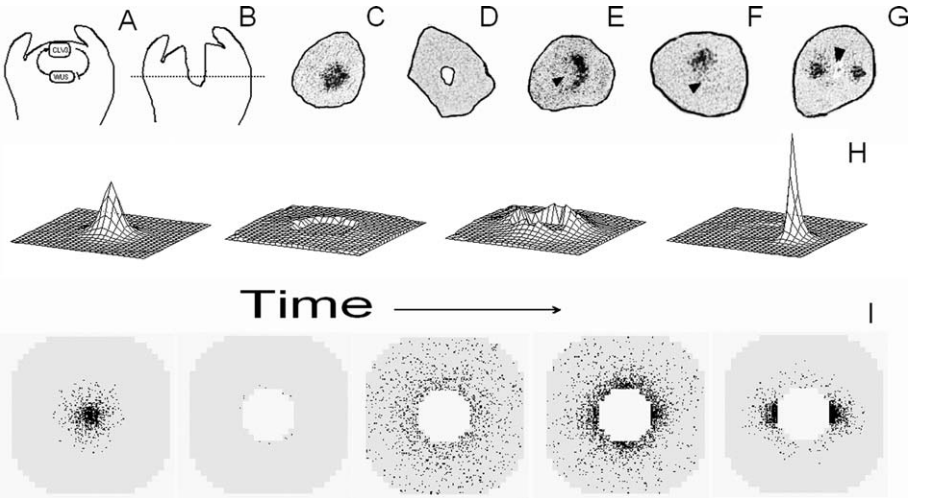

Figure 5 Regeneration of the organizer in the shoot apical meristem. (A) *Wuschel (Wus)* is a crucial component in the maintenance of the shoot apical meristem (Mayer *et al.*, 1998). The size of *Wus* expression is controlled by a negative feedback via *Clavata3 (Cl3)* (reviewed in Clark, 2001; Tsiantis and Hay, 2003). The autocatalytic component expected from the model is not yet known. (B–G) Restoration of *Wus* activity after laser-ablation of the *Wus* expressing cells (Reinhardt *et al.*, 2003; hole in B). *Wus* activity in the plane indicated in B before (C) and after (D) ablation. *Wus* expression reappears in cells that were previously not expressing *Wus*; first in a rather diffuse way. After two days the expression has a ring- or crescent-like distribution (E). Eventually one (F) or two maxima (G) emerge. The latter case leads to a split of the meristem. (H, I). Simulations: killing the cells that produce the activator leads to a decline of the inhibitor and to a new trigger of the activator in the surrounding competent cells. The activation is first more diffuse. The concomitantly produced inhibitor (not shown) leads to a competition and peak sharpening. Either one (H) or two (I) maxima survive. In (I) the activator concentrations are plotted as a pixel density. To localize the new activations near the center of the meristem, a graded competence that decreases towards the periphery has to be assumed (see Fig. 10). (B–G is drawn after Reinhardt *et al.*, 2003.)

The model accounts not only for the generation of a pattern but also for pattern regulation (Figs. 4 and 5). With the removal of an area of high activator concentration, the area of inhibitor production is also removed. After the decay of the remnant inhibitor the formation of a new activator maximum is triggered in the remaining cells, starting from a low level activator production [ρ_a in Eq. (2a)]. The pattern becomes restored in a self-regulatory way.

C. Formation of Periodic Patterns

In many developmental situations, periodic structures are formed. Examples are the spacing of the leaves on a growing plant, the bristles on insects or feathers of birds. In terms of the model, periodic structures can be formed if the field is or becomes larger

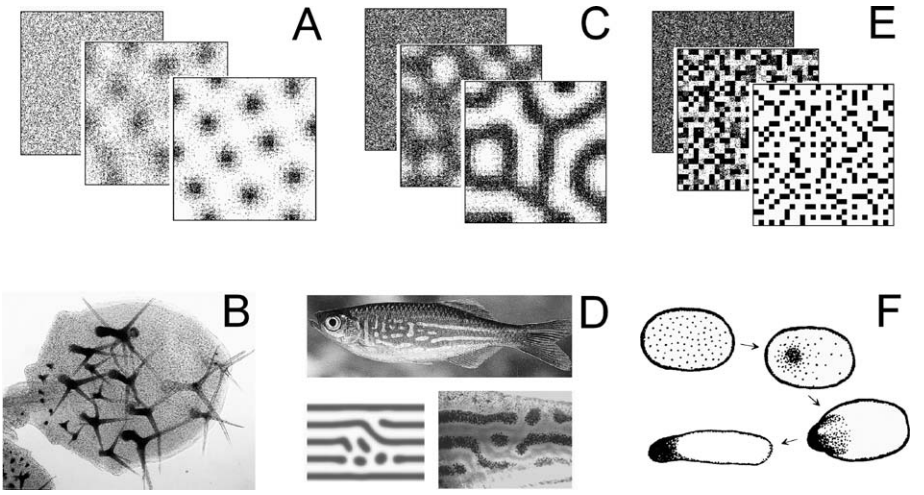

Figure 6 Generation of elementary periodic patterns in a cell sheet. Assumed is an activator–inhibitor system (Eq. (2a) and (2b); only the activator distributions are shown). (A) Several maxima emerge if the size of the field is larger than the range of the inhibitor. When initiated by random fluctuations, the spacing is somewhat irregular but a maximum and minimum distance is maintained. (B) Biological example: Trichome formation in *Arabidopsis* (reviewed in Pesch and Hülskamp, 2004). The three spines of each trichome are produced by a single cell. The gene *GL2* (dark) is involved in trichome activation (see Szymanski *et al.*, 2000). The genes *TRY* and *CPC* (not shown) are involved in the inhibition. Although *TRY* and *CPC* inhibit trichome formation, they are only expressed in the future trichome cells, as expected from our model. (C) Stripe-like distributions emerge if the activator production cannot surpass a certain level, e.g., due to a saturation [$\kappa > 0$ in Eq. (2a)]. Stripe formation requires some spread of the activator. (D) Examples: The stripes of pigment cells in zebrafish. Although the molecular details are not yet clear (reviewed in Parichy, 2006), the reappearance of the stripes after laser ablation occurs as expected by our theory (Yamaguchi *et al.*, 2007). (E) Without spread of the activator, a segregation into two different cell types occurs. Activated and nonactivated cells appear in a certain ratio in a salt-and-pepper distribution. (F) Such a pattern is characteristic for the early prestalk/prestalk patterning in *Dictyostelium discoideum* (Maeda and Maeda, 1974; see Zhukovskaya *et al.*, 2006; for modeling see Meinhardt, 1983cc). (B kindly supplied by Martina Pesch and Martin Hülskamp; D—by Shigeru Kondo.)

than the range of the inhibitor (the range is the mean distance a molecule can travel between its production and degradation). When the pattern is formed in a field that has already a substantial extension, the resulting pattern will be somewhat irregular; only a maximum and minimum distance will be maintained (Fig. 6). The cilia on the surface of *Xenopus* embryo (Deblandre *et al.*, 1998, 1999) or the trichomes of leaves (Hülskamp, 2004; Fig. 6B) are biological examples of this type of pattern. In contrast, in growing fields new maxima will be formed whenever the distance to existing maxima becomes too large. Then the inhibitor concentration between the maxima can become so low that autocatalysis is no longer repressed. New maxima are inserted at the maximum distance of existing peaks. The resulting pattern will be more regular.

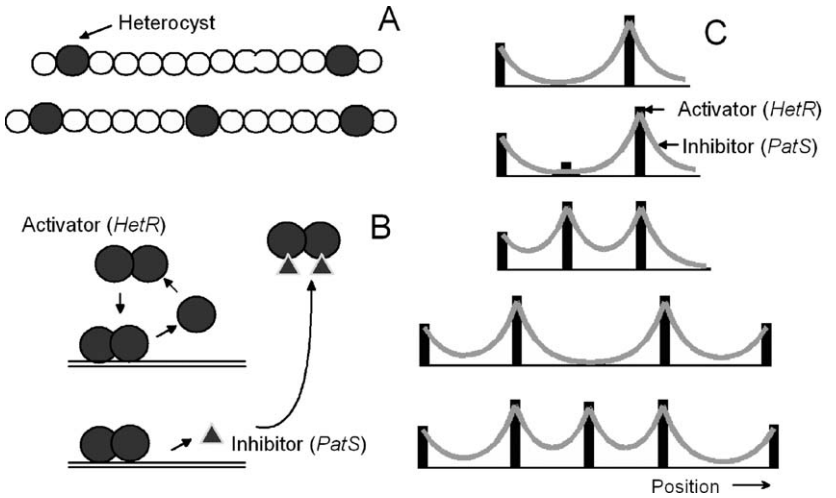

Figure 7 Insertion of new maxima during growth. (A) Biological example: the insertion of new nitrogen-fixating cells, so-called heterocysts, in the blue green algae *Anabaena*. Whenever the distance between two heterocysts (dark circles) in the linear chain of cells becomes larger then ca. 12–14 cell, a normal cell differentiates into a larger, nondividing heterocyst. It is the cell that has the largest distance from the existing heterocysts. (B) Heterocyst formation is under control of the transcription factor *HetR*. *HetR* form dimers that directly activate *HetR* transcription (Huang *et al.*, 2004), Dimerization satisfies our prediction that the autocatalysis must be nonlinear [Eqs. (2a), (2b)]. *HetR* also activates the formation of a small peptide, *PatS* (triangles) that can spread through intercellular junctions (Yoon and Golden, 1998) and that can bind to *HetR*. If *PatS* is bound to *HetR* DNA-binding of *HetR* is no longer possible. Thus, *PatS* inhibits the activator autocatalysis, as predicted. (C) Simulation: only the inhibitor is diffusible across the cells. Therefore, activation occur in isolated cells. Whenever the inhibitor drops below a threshold level, a new autocatalysis of the activator is triggered from a baseline activation [ρ_a in Eq. (2a)]. Since the inhibitor distribution around a minimum is shallow, initially more than one cell can start this activation process. Due to competition only one isolated cell eventually becomes activated. In agreement with the expectation from our theory, if *HetR* is mutated, no heterocysts are formed. In contrast, if *PatS* is mutated, most cells form heterocysts (Buikema and Haselkorn, 2001).

Examples are the insertion of new leaves at a growing shoot, new heterocyst cells in *Anabaena* (Fig. 7), new trichomes (Fig. 6B) or new bristles in insects (Wigglesworth, 1940).

D. Stripe-Like Patterns and the Role of Saturation in the Self-Enhancement

Stripe-like patterns, i.e., structures with a long extension in one dimension and a short extension in the other, are formed in many instances during embryogenesis. Proverbial are the stripes of zebras. Stripe-like distributions can emerge if activator production has an upper bound (Meinhardt, 1989, 1995). If activator autocatalysis saturates at a relatively low concentration [$\kappa > 0$ in Eq. (2a)] the inhibitor production is limited too and the mutual competition between neighboring cells is reduced. Due to the sat-

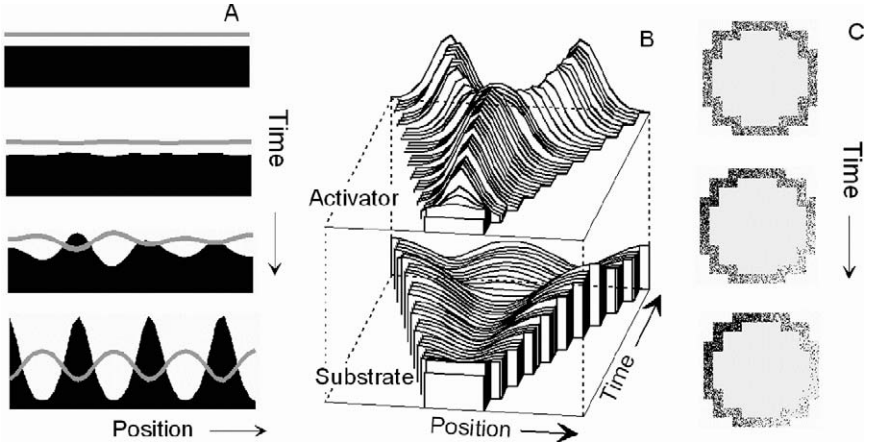

Figure 8 Pattern formation by an activator-depletion mechanism. (A) Autocatalysis proceeds at the expense of a rapidly spreading substrate or co-factor (Eqs. (3a), (3b); Gierer and Meinhardt, 1972). The concentration of the antagonist is lowest in regions of high activator concentration, in contrast to the situation in an activator–inhibitor system (Figs. 4 and 7). (B) During growth, activator maxima have the tendency to split due to the inherent saturation of the activator autocatalysis in this system. Since the substrate supply is higher at the flanks than in the center, activator production can become higher in the flanks, which leads to a deactivation in the center and a shift of the maxima towards higher substrate levels. This is in contrast to the behavior of activator–inhibitor systems without saturation in self-enhancement (Fig. 7). Splitting activator maxima are crucial for dichotomous branching during filament elongation (Fig. 27). (C) Such a system is appropriate for *intracellular* pattern formation. In this simulation the self-enhancing reaction is assumed to proceed by a cooperative aggregation of molecules at the membrane. This aggregation proceeds at the expense of freely diffusible monomers that can spread rapidly in the cytoplasm (not shown). Local high concentrations emerge at a particular part of the cell membrane. Corresponding mechanisms are discussed for the *Dictyostelium discoideum* (Charest and Firtel, 2006; see also Fig. 11).

uration more cells remain activated although at a lower level. Thus, activated cells tolerate activated cells in their neighborhood, independent of the range of inhibition. In addition to saturation, a further condition for stripe formation is a modest diffusion of the activator. Due to this diffusion, activated regions tend to occur in large coherent patches since activated cells tend to activate adjacent cells. On the other hand, pattern formation requires that activated cells are close to nonactivated cells into which the inhibitor can diffuse. These two seemingly contradictory features, coherent patches and proximity of nonactivated cells, are characteristic for stripe-like patterns (Fig. 6C). If initiated by random fluctuations, the stripes have random orientations too. It is a feature of this mechanism that the width of the stripe and the interstripe-region is of the same order. Therefore, the formation of a single straight, nonbranching stripe as necessary for midline formation in higher organisms requires additional constraints. As shown further below, different mechanisms evolved in different phyla to solve this intricate patterning problem.

In growing tissues that are patterned by systems with saturating activator production [$\kappa > 0$ in Eq. (2a)] periodic structures can emerge by splitting of existing maxima, in contrast to the insertion of new maxima in the absence of saturation (see Fig. 7). Saturation leads to a plateau-like widening of the maxima. If the area into which the inhibitor can escape enlarges in the course of growth, the plateau-like activation enlarges too, i.e., the pattern is size-regulated. From a certain extension onwards, however, the activator production at the center of a maximum can be lower than that at the flanks due to the rising inhibitor level at the center. This leads to a deactivation in the center (see also Fig. 8B). Splitting of existing maxima is the basis for branching in the lung and in tracheae (see Fig. 27).

E. The Antagonistic Reaction Can Result from a Depletion of a Substrate or Co-Factor

Instead of an inhibition that is produced at an activator maximum, the antagonistic effect can also result from the depletion of a substrate $s(x)$ which is a prerequisite for the self-enhancing reaction and which is consumed during the autocatalytic activator production (Fig. 8; Gierer and Meinhardt, 1972):

$$\frac{\partial a}{\partial t} = \rho s a^2 - \mu a + D_a \frac{\partial^2 a}{\partial x^2} + \rho_a, \tag{3a}$$

$$\frac{\partial s}{\partial t} = \delta - \rho s a^2 - \nu s + D_s \frac{\partial^2 s}{\partial x^2}. \tag{3b}$$

According to Eq. (3b), the factor s is produced everywhere with constant rate δ; s is removed by the autocatalytic reaction at the same rate as the activator is produced. The activator-depletion mechanism has an inherent upper bound of the activator production since the production comes to a halt if most of the substrate is consumed. As mentioned above, such saturation can lead in growing systems to new maxima that result from the splitting of existing maxima (Fig. 8B). Thus, an activator-depletion mechanism is unsuitable for the formation of organizing regions, i.e., of isolated signaling centers that are surrounded by large regions that are devoid of signaling centers.

F. Pattern Formation within a Cell

Pattern formation does not only occur between cells but also within a cell. Intracellular pattern formation is often the first pattern that is generated in development. Pattern formation in the egg of the brown alga *Fucus* is an example of an unstable system where almost any external asymmetry can orient the emerging pattern (Jaffe, 1968; Leonetti *et al.*, 2004). In the absence of such asymmetries, a polar pattern will nevertheless arise, although with a random orientation. The pattern consists of a localized influx and efflux of calcium ions.

To satisfy our general conditions for pattern formation within a cell, the self-enhancing reaction is expected to be restricted to parts of the cell cortex while the antagonistic reaction spreads more rapidly within the entire cytoplasm. Activator-depleted substrate mechanisms appear as especially suitable for such intercellular patterning. Activation can occur by a cooperative aggregation of molecules at the cell cortex, i.e., aggregation proceeds more rapidly at positions where some of these molecules are already present. This aggregation is antagonized by the depletion of unbound molecules diffusing freely in the cytoplasm. In such a system the condition for different ranges of the autocatalytic and antagonistic reactions is satisfied in a straightforward manner. In intracellular patterning 'long range' denotes a communication over the entire cell while 'short range' indicates a cooperative process that covers only a part of the cell cortex.

G. Oscillations and Traveling Waves

The discussion so far has considered only patterns that are stable at least for a certain period of time. Stable patterns can result if the antagonistic reaction has a shorter time constant than the activator. Under this condition any deviation from the steady state concentrations is rapidly back-regulated. In contrast, if in an activator–inhibitor system, the inhibitor has a longer half-life than the activator (i.e., if in Eq. (1) $\nu < \mu$), oscillations will occur (Fig. 9A). Since the inhibitor follows too slowly, activation proceeds in a burst-like manner. In the course of time, however, more and more inhibitor

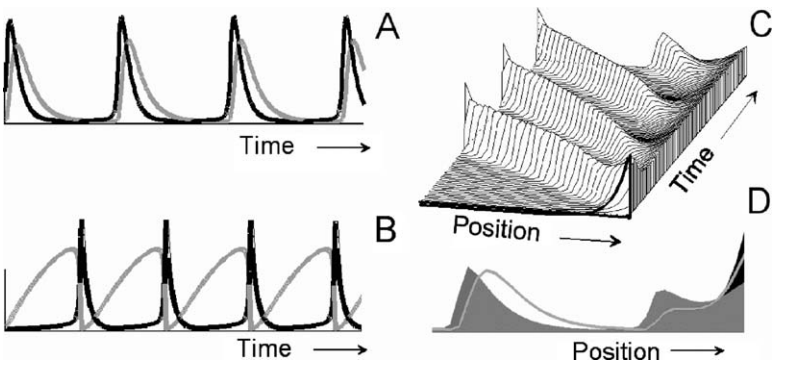

Figure 9 Oscillations and traveling waves. Oscillations can occur in single cells if the antagonist reacts too slowly to a change in the activator concentration. (A) In an activator–inhibitor system oscillations occur if the inhibitor half-life is longer than that of the activator. (B) In an activator–substrate system, oscillations occur if the production rate of the substrate is lower than the removal rate of the activator. (C) Traveling waves can occur if the activator but not the antagonist spreads. An activated cell 'infects' its neighbor. Such wave formation needs an initiation site, a pacemaker region. In this simulation a stable pattern is assumed (black) that causes a high baseline activator production in the oscillating system. Waves spread into the surrounding cells at regular time intervals. (D) A snapshot of a distribution as shown in C. A new wave is just detaching from the pacemaker region (see also somite formation, Fig. 24).

accumulates until the activator production breaks down suddenly. The slow decay of the inhibitor leads to a refractory period until the next trigger occurs that starts from a baseline activator production ρ_a. In the activator–substrate mechanism, oscillations occur if substrate production is insufficient to maintain a steady state [$\delta < \mu$ in Eq. (3)]. Substrate concentration increases until a threshold is reached. The burst-like activation leads to a collapse in substrate concentration and thus to a switching off of activator production. Substrate can accumulate again until the next activation is triggered, and so on (Fig. 9B). Depending on the spread of the components, global oscillations or traveling waves can emerge (Fig. 9C). Thus, the same reaction can generate patterns in space or in time, depending on the spread and the time constants of the components involved. Oscillations and traveling waves will play an important role in the discussion of somite formation (see Fig. 24).

H. How to Avoid Supernumerary Organizers: Feedback on the Competence

The generation of more and more signaling centers during growth is characteristic of the formation of periodic patterns (Fig. 7). For the generation of embryonic axes, however, a single organizer has to be maintained despite growth, otherwise supernumerary and possibly partially fused embryos will result. As mentioned, a fragment of the chicken blastodisc can regenerate a complete embryo even if it does not contain the original organizing region (Fig. 1). This capability for pattern regulation, however, is lost at later stages. Obviously, cells distant to the organizer lose their competence to form an organizer and become unable to trigger a secondary organizer even when the inhibitor drops to very low levels due to growth. Such fading of the competence to form an organizing region is a process of primary importance in making development reproducible. One way to suppress the trigger of new organizing regions is an elevated baseline inhibitor production [ρ_h in Eq. (2b)]. Under such a regime, a nonactivated fragment cannot regenerate a new organizer.

Experiments in hydra, however, suggest a different mechanism since fragments from all positions remain able to regenerate a new polyp. In fragments the regeneration of a head always occurs at the side pointing towards the original head. Thus, the tissue has a systematic polarity; the competence is graded. It is the *relative* position of a group of cells within the fragment that is decisive as to whether they will form a head, a foot, or something in between (Fig. 10). Dissociation and reaggregation experiments have shown that this polarity is based on a systematic change of the tissue composition and not on the orientation of the individual cells (Gierer *et al.*, 1972). This polarity is a very stable tissue property. After head removal, it takes about 1 hour to reform a β-catenin and *Wnt* signal, indicative for a regenerating organizer (Hobmayer *et al.*, 2000). In contrast, as revealed by grafting experiments, reversal of polarity requires about two days (Wilby and Webster, 1970a, 1970b).

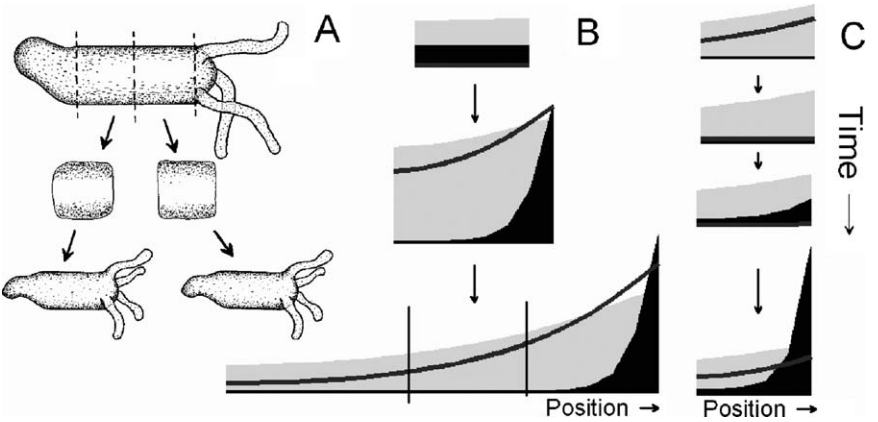

Figure 10 Feedback of the organizer on the competence to avoid secondary organizing regions. (A) Evidence for a graded competence: fragments of a hydra regenerate a new head always at the side pointing towards the original head. (B) Model: if the activator (black) has a long-ranging and long-lasting feedback on the ability of the cells to perform the autocatalysis (source density; gray distribution, ρ in Eqs. (2a), (2b) and (4); the black line is the inhibitor), cells distant to the organizer become unable to compete with the primary organizer for activation. Despite substantial growth, a single organizer and thus a monotonic gradient is maintained, although the range of the activator can be very small (compare with the periodic pattern formed in Fig. 7). (C) Due to the graded competence, regeneration can be a rapid process within the competent region since no time-consuming competition is required for one region to win. Since the source density (gray area) changes only slowly, it remains nearly unchanged during regeneration. The new activation occurs at a predictable position as suggested by the experiment (A). Due to the graded competence, the activator maximum appears at a marginal position. Due to the short range of the activator relative to the field size, regeneration can occur in small fragments.

In the model the competence corresponds to the ability of the cells to perform the autocatalytic reaction, a property we have called 'source density' [ρ in Eq. (1)]. Activator production is assumed to depend not only on the presence of activator molecules itself but also on the presence of other factors necessary to accomplish this autocatalysis. If these other factors are missing, an activation would be impossible even at low inhibitor levels. If the organizer exerts a positive feedback on these prerequisites, the ability to perform the self-enhancing reaction is preferentially maintained in the region closer to the organizer. In contrast, regions distant to a once established organizer become unable to generate secondary maxima. In this way, a single maximum can be maintained even in fields that grow substantially. Equation (4) gives a possible interaction:

$$\frac{\partial \rho}{\partial t} = \gamma a - \mu_\rho \rho + D_\rho \frac{\partial^2 \rho}{\partial x^2}. \tag{4}$$

A simulation using Eqs. (2a), (2b), and (4) is given in Fig. 10. This model is in agreement with the observation in *Xenopus*, zebrafish and chick that development is found

to proceed normally after removal of the proper organizer as long as cells next to the organizer remain present (Steward and Gerhart, 1990; Yuan and Schoenwolf, 1998; Saùde *et al.*, 2000). As discussed further below, maternally provided factors may restrict from the beginning the competence to a small part of the developing embryo, counteracting in this way the formation of secondary organizing regions. Such a head start also shortens the time needed for one region to win the competition with all other regions to become the organizing region.

An organizing region thus exerts two seemingly conflicting effects. On the one hand, it inhibits the formation of other organizing regions. On the other hand, it promotes organizer formation in the first place. Why do both effects not cancel each other? This is because inhibition and promotion have different time constants. To allow stable patterns and pattern regulation, the inhibitor must have a rapid turnover such that a new organizer can reappear shortly after removal of the original organizer. In contrast, the competence has a long time constant such that within the time scale required for pattern regulation it remains almost unchanged (Fig. 10C).

While organizer formation is a local event, the graded competence extends over a much larger region. This suggests that some agent spreads from the organizer that, in turn controls directly or indirectly, the competence. The molecular basis of this graded source density (or head activation gradient as it is frequently called in the experimental literature on hydra regeneration) is not yet clear. After treatment of hydra with Alsterpaullone, a drug that stabilizes β-catenin, the whole polyp obtains properties that are normally restricted to the tissue near the genuine organizer (Broun *et al.*, 2005). In terms of the model, the source density becomes high everywhere. This effect, however, does not allow the conclusion that β-catenin as such acts as source density. β-Catenin reappears about 1 hour after head removal (Hobmayer *et al.*, 2000), indicating that it has a short time constant, suggesting that β-catenin belongs to the activator loop. This 1 hour is short compared with the 2 days required to change the intrinsic polarity. An ectopic elevation of the activator concentration would also lead to an overall increase of the source density [Eq. (4)].

Since the complementary influence of organizing regions is crucial for explaining many biological observations, it is worth illustrating the situation with an anthropomorphic analogy. A king, president or any other figure in power usually has a strong tendency to suppress others from taking over—a long-range inhibition. On the other hand, he promotes individuals among his courtiers to obtain a higher ranking, to become ministers, etc. In this way, the center of power generates a hierarchy. Inhibition and promotion are two closely interwoven processes. If the top position becomes vacant, due to this nonuniformity, a fight will set in only between the few who have high ranking in the hierarchy. Usually proximity to the former center is an advantage. In the short time interval until a new hero is selected, the ranking in the hierarchical pyramid remains essential unchanged. This analogy also illustrates what can happen if the whole hierarchy is eliminated, for instance, in a revolutionary situation. Many rivaling centers and civil-war like situations could emerge, with all their unpredictable consequences.

I. A Graded Competence Allows Small Organizing Regions

As shown earlier (Fig. 4), if the competence is homogeneously distributed, the range of the activator must be comparable to the size of the field if a polar pattern with a terminal maximum should emerge. In such a situation a small fragment cannot regenerate since its size would be smaller than the range of the activator. However, hydra fragments of 1/10 of the body length can regenerate perfectly. Again, this problem disappears if the competence is graded since the maximum will appear at the highest level of the graded source density, i.e., at a terminal position even if the range of the activator is small (Fig. 10).

J. An Inhibition in Space *and* in Time: The Generation of Highly Dynamic Patterns

In the preceding section it has been shown that a positive feedback of an activator maximum on its own sources, i.e., on the ability of the system to perform autocatalysis, can stabilize an existing maximum and can enhance its dominance over more distant regions. The opposite interference, a *destabilization* of established maxima by a second local-acting antagonistic reaction, is also a frequently used strategy in development. It can lead to highly dynamic systems that never reach a stable state. Imagine a conventional two-component system as described above. On its own it would produce a stable pattern. Imagine further a second antagonist that has the opposite properties to the normal antagonist: a short range and a long time constant. Shortly after the generation of a maximum, this maximum will be 'poisoned' by the second antagonistic reaction and thus locally quenched. Depending on the parameters, the system can respond in two ways. (i) The maximum shifts into an adjacent position, only to become quenched there too: traveling waves result. These waves can have unusual properties, e.g., they can emerge without a pacemaker region and can penetrate each other. (ii) The maxima disappear and reappear somewhat later at a displaced position, only to become quenched there again. Either regular out-of-phase oscillations between adjacent regions occur or maxima appear and disappear at somewhat irregular positions. Examples of both modes are given in Fig. 11. We came across this reaction type by searching for mechanisms that account for the pigmentation pattern on some tropical sea shells (Meinhardt and Klingler, 1987; Meinhardt, 2003). Subsequently it has turned out that such three-component systems—an activator–inhibitor system coupled to a quenching component—are appropriate to describe a wide range of biological phenomena. Examples are the pole-to-pole oscillations in the bacterium *E. coli* for the determination of the division plane (Meinhardt and de Boer, 2001), the separation of the barbs of an avian feather (Figs. 11A–11E; Harris *et al.*, 2005), the highly sensitive orientation of chemotactic cells and growth cones by minute external cues (Figs. 11F–11K; Meinhardt, 1999), and the initiation of new leaves around a growing shoot with a displacement of the golden angle (Meinhardt, 2004b). A detailed discussion of these systems is beyond the scope of the present article.

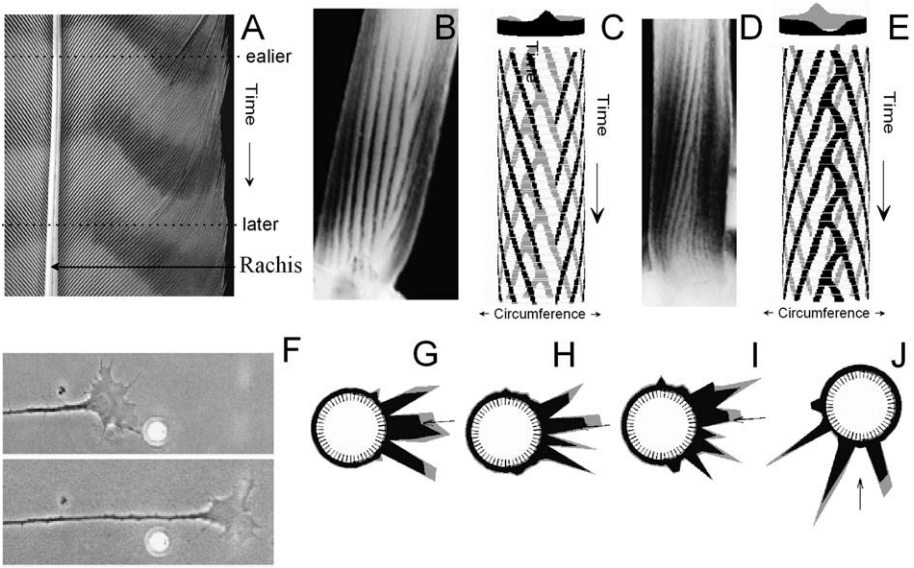

Figure 11 Patterning in avian feathers and orientation of chemotactic cells as examples for the role of destabilization by a second antagonist. (A–E) Formation of feather filaments (barbs) by traveling waves. Feathers are formed by proliferation of stem cells at the base of feather buds. Therefore, the tip of a feather is the oldest part (in contrast, for example, to the situation of a tree!). Also the cells forming the tip of the barbs are born earlier than those forming the connection to the rachis. A permanent regional cell death along the ventral side (the side opposite to the rachis; dark branching line in D and E) allows an opening of the cylindrical sheet into a plane. The signal is formed by *Shh* (B and D)/*BMP2* that acts as an activator–inhibitor system (Harris *et al.*, 2005). Like cutting with scissors, traveling waves of high *Shh* expression (dark lines) separate the individual barbs (white oblique stripes). These waves run from the "cut-open" region on the ventral side towards the rachis at the dorsal side. For the simulation C and E a second, short-ranging but long lasting inhibitor was assumed that locally quenches the maxima, enforcing traveling waves by a permanent shift of the maxima. This cutting comes to rest near the future rachis (wave-free region in B and C), otherwise the filaments would detach from the feather. (F–K) Orientation of growth cones (F) and other chemotactic sensitive cells by minute external asymmetries. The problem is to maintain sensitivity for the minute external asymmetries although a strong internal amplification is involved to generate a pronounced cell-internal pattern. In the model (Meinhardt, 1999), isolated signals for filopods (black) are generated by a saturating self-enhancing reaction together with an inhibition that covers the whole cell (not shown). They appear preferentially at the side where the guiding signal (arrow) is slightly higher. A second antagonistic component (gray) quenches locally the signal after a certain time interval. Thus, signals for filopods appear and disappear permanently at the cell surface (G–I). After a change in the direction of the guiding signal, the internal signals emerge at the new side (J), although the guiding asymmetries are minute (2% across the cell plus 1% random fluctuation between cell surface elements).

III. The Two Main Body Axes

In vertebrates the famous Spemann organizer and its relatives such as Hensen's node play a crucial role in axes formation. Many of the molecular components involved are

known (reviewed in Harland and Gerhart, 1997; De Robertis and Kuroda, 2004; Stern, 2001; Boettger *et al.*, 2001; Niehrs, 2004; Schier and Talbot, 2005). However, which axes the organizer controls—AP, DV or both—remained remarkably fuzzy. How can a single organizer organize two axes that are oriented perpendicular to each other? In amphibians even the orientation of the main body axes in the early embryo relative to the animal–vegetal axis of the egg is controversial (Gerhart, 2002). What is the relation of the hydra-type organizer of ancestral organisms and the Spemann-type organizer? Is there a hidden organizer for the second axis? How it is achieved that the two axes are so rigidly coupled (Fig. 1)?

The finding that β-catenin and *Wnt* are expressed in the hydra organizer (Fig. 2A; Hobmayer *et al.*, 2000) was very exciting since it was well known that the same pathway also plays a crucial role in the formation of the Spemann-type organizer. Indeed, hydra-derived β-catenin mRNA injected into an early amphibian embryo can induce a second embryonic axis, as would a graft of the Spemann organizer. However, as shown below in more detail, in vertebrates, control for the AP axis does not reside in a Spemann-type organizer but in the equivalent of the hydra-type organizer, the blastoporal ring. The Spemann-type organizer, located on this ring, initiates the formation of a stripe-shaped midline organizer. The DV (or better mediolateral) specification of a cell depends on its distance to this midline rather than on the distance to the original organizer. Midline formation is realized with different structures in the brain and in the trunk, with the prechordal plate and the notochord, respectively. Thus, in the early gastrula there are two separate organizers, one for the primary AP axis and one for the secondary DV axes. Both have a stripe-like extension with orthogonal orientation, convenient for generating a near-Cartesian coordinate system (Figs. 12A and 12B). The actual positional information for the mediolateral axis is presumably a reversed gradient since the midline acts as sink for BMP (Dosch *et al.*, 1997).

A. The Blastopore (Marginal Zone) as Organizer for the AP Axis

As mentioned above, the small hydra organizer located around the gastric opening became in vertebrates a large ring (Figs. 3 and 12A). The canonical *Wnt* pathway is a crucial component of the hydra organizer (Hobmayer *et al.*, 2000). Likewise in the vertebrates, *Wnt-8* expression is high in the marginal zone/germ ring (Christian and Moon, 1993) and evidence has accumulated that a gradient of *Wnt* controls the AP pattern of the brain (Kiecker and Niehrs, 2001a; Nordström *et al.*, 2002; Dorsky *et al.*, 2003). This suggests that the signaling center required for the AP patterning is the blastopore itself, i.e., the ancestral organizing region of radially-symmetric organisms. Cells distant to the blastopore are exposed to low *Wnt* levels and form the forebrain; cells closer to the blastopore form the midbrain. In agreement with recent observations this early AP specification is essentially independent of the Spemann-type organizer. For instance, by removal of maternal determinants from the fish embryo it was possible to suppress the formation of the organizer completely. By

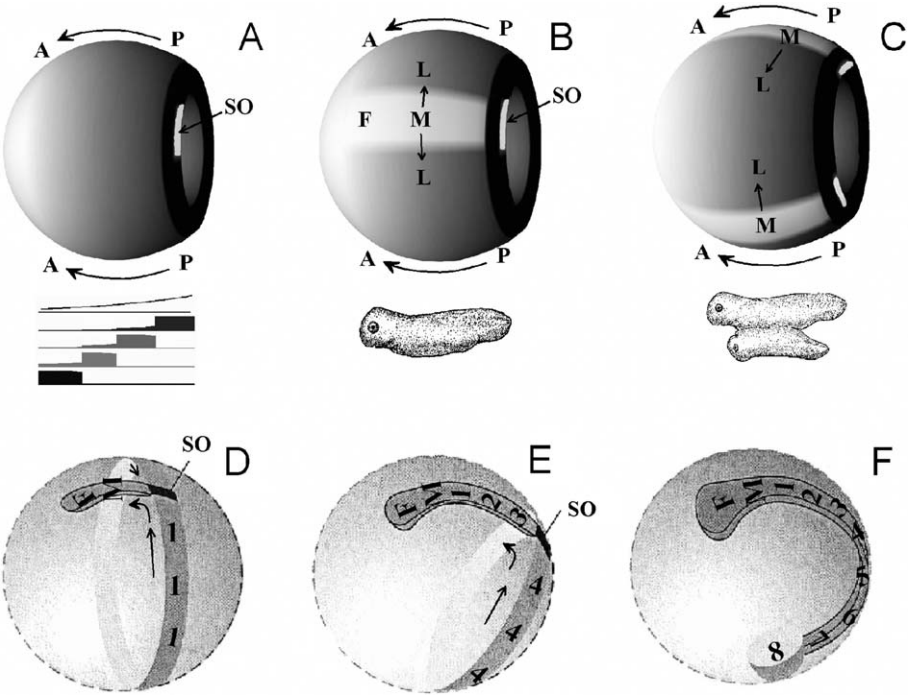

Figure 12 Model for the generation of a near-Cartesian coordinate system in two steps—the amphibian embryo as example. (A) AP-patterning of the early gastrula is assumed to be accomplished by an ancestral system (see Fig. 3). The marginal zone (black) is assumed to be equivalent to the hydra organizer and controls the posterior-to-anterior pattern in a gradient-based manner (fading gray), a process that does not require an organizer. (B) The Spemann organizer (SO, white) forms on the blastoporal ring. The ingressing organizer-derived mesodermal cells form the prechordal plate (light gray), which acts as the midline organizer for the mediolateral (L ← M → L) pattern of the brain and induces neuronal development in the overlying ectoderm. The distance from this midline determines the mediolateral specification. Both signaling sources have a stripe-like extension and provide a near-Cartesian positional information system determining the pattern of the fore- (F) and midbrain (M). (C) Induction of a second organizer (see Fig. 17) leads to two embryos that are fused at the ventral side. (D–F) AP patterning of the trunk. The cells at the blastopore obtain in a time-autonomous process more and more posterior determinations (1, 2, 3 . . .). The pace of this process is given by an oscillation that leads also to the periodic patterning of the somites (see Fig. 24). Cells near the blastoporal ring move towards the organizer and the incipient midline, forming the mediolateral pattern along the emerging AP axis. When cells obtained a certain distance from the organizer, the somitic oscillation stops (see Fig. 24), somites are formed and the cells obtain their final AP determination. Cells antipodal to the organizer have to move further to reach the region near the organizer/midline and are later integrated into the axial structures. Due to the prolonged posteriorization these cells form more posterior structures. Thus, cells antipodal to the dorsal organizer form posterior and not primarily ventral structures. In this schematic drawing, the animal–vegetal axis is fixed and the shape changes due to the conversion–extension mechanism are ignored (partially after Meinhardt, 2006).

a simultaneous suppression of *BMP* signaling due to the *swirl* (*ZBmp-2b*) mutation, neuronal development was enabled all around the circumference of the early embryo (Ober and Schulte-Merker, 1999). Most remarkable, the expression of neuronal markers such as *Otx* and *Krox 20* appeared in the normal order although no organizing region was present, supporting the view that the Spemann-type organizer does not play a decisive role in AP patterning. Similar results were observed in *Xenopus* (Reversade *et al.*, 2005). Also during later development, newly expressed Hox genes, which are required to specify more posterior structures, become activated exclusively near and around the marginal zone but not in the organizer itself (Wacker *et al.*, 2004a, 2004b, see below).

B. The Spemann-Type Organizer Induces Midline Formation

In principle, any second signaling source with an-off-axis localization would lead to symmetry breaking and bilaterality. However, to pattern the DV or mediolateral axis of an animal with a long-extended AP axis, a spot-like signaling center is inappropriate. A reasonable, i.e., a near-Cartesian combinatorial patterning requires for the DV axis an organizing region with a stripe-like geometry. Only then, for instance, can floor plate, notochord, somites, neural crest cells or particular nerve cells be formed at the midline or at a certain distance from it, independent of the AP level.

The generation of a solitary straight stripe-like organizing region is an intricate patterning problem. As mentioned above (Fig. 6), under certain conditions—saturation of autocatalysis—stripe-like patterns can be generated. Stripe formation requires a reduced lateral inhibition because otherwise the stripes would disintegrate into isolated patches. The reduced lateral inhibition, however, has the consequence that an existing stripe can suppress other stripes only up to a moderate distance. The width of the stripe and the width of the interstripe regions are of the same order. Nevertheless, the formation of a single straight organizing region is possible by an appropriate interaction of a spot-forming and a stripe-forming system whereby the spot-forming system makes sure that only one stripe can emerge. A surprising diversity of such midline-generating mechanisms has evolved (Meinhardt, 2004a). In vertebrates midline formation occurs by sequential elongation under the driving force of the organizer. The midline appears on the *same* dorsal side as the organizer (Fig. 12). In insects and spiders, a dorsal organizer repels the midline (Moussian and Roth, 2005; Akiyama-Oda and Oda, 2006). The midline and thus the central nervous system appear on the *opposite* side, i.e., ventrally (Fig. 13A). The well-known DV–VD inversion between insects and vertebrates is proposed to have this origin. While in vertebrates the midline becomes sequentially elongated, in insects the midline was predicted to have from the beginning the full AP extension but becomes narrower in the course of time (Meinhardt, 1989, 2004a), in agreement with recent observations (Fig. 13B) (Chen *et al.*, 2000). Thus, neither a moving node nor a moving prechordal plate is involved in midline formation in insects. For planarians the situation is very different. A primary DV confrontation is the precondition for generating the secondary AP axis (Chandebois, 1979;

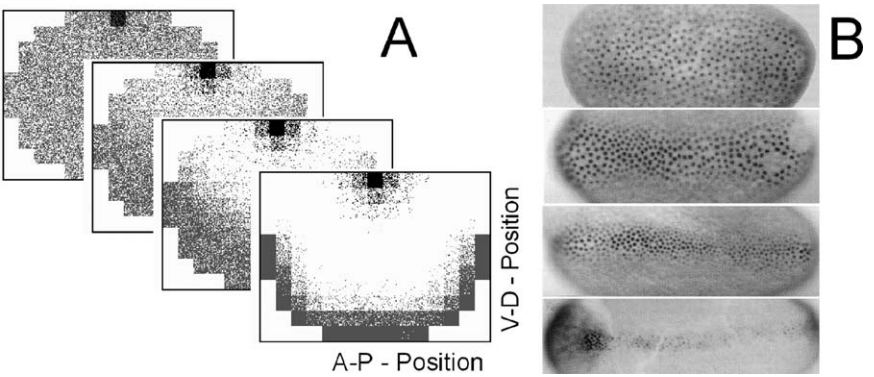

Figure 13 Model for midline formation in insects by an inhibition from a dorsal organizer. (A) Predicted mechanism: A patch-like dorsal organizer repels the midline; the midline forms at the ventral side (Meinhardt, 1989, 2004a). Two pattern-forming systems are assumed, one with a patch-forming (black) and one with a stripe-forming characteristic (gray). The first has an inhibitory influence on the second. A high activation of the stripe-forming system is possible only at a distance from the patch-shaped organizer. The midline appears simultaneously along the whole AP axis but sharpens in the course of time to a narrow ventral midline, in contrast to the sequential elongation in vertebrates (Fig. 12). (B) Such sharpening has been found in *Tribolium*. Shown is the *Dorsal* expression on the ventral side at successive stages (Chen *et al.*, 2000). (Figure kindly supplied by Siegfried Roth.)

Kato *et al.*, 2001). This suggests that the DV pattern is primary and that a DV border is the precondition for forming the terminal structures of the AP axis. The DV border represents an organizing line that circumvents the organisms (Fig. 14).

The temporary expression of *Noggin* at this DV-border (Ogawa *et al.*, 2002) suggests that what in vertebrates is the single dorsal midline corresponds in planarians to *two* lateral organizing stripes that extend from the anterior to the posterior pole. This corresponds well with the *two* lateral nerve cords that are typical for planarians, one at each side. The assumption of a repulsion from the circumventing organizing borders explains the formation of a single dorsal midline (Fig. 14), realized by a high *BMP* expression (Orii *et al.*, 1998). These very different modes of generating a bilateral body plan suggest that different ancestral bilateral organisms evolved in a different manner from radially-symmetric ancestors without a common urbilaterian.

C. The Orthogonal Orientation of the Main Body Axes in Vertebrates

To obtain a near-Cartesian coordinate system in vertebrates, it is crucial that the midline has an orientation parallel to the AP axis, i.e., perpendicular to the blastopore. In the early gastrula the blastopore and thus the organizer is posterior to the brain anlage while the trunk does not yet exist. Therefore, from the initial position of the organizer,

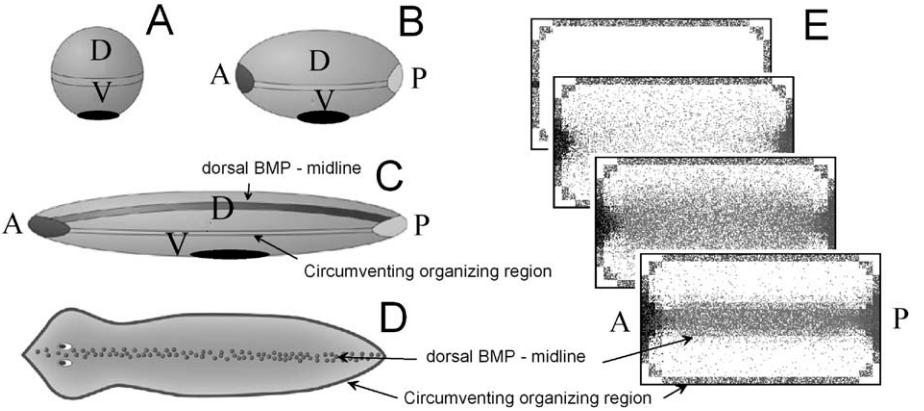

Figure 14 Model for axes formation in planarians. (A) Primary is a DV subdivision. The DV border obtains organizing function. The single opening is ventrally located, in contrast to the posterior localization in vertebrates and insects. (B) The formation of the terminal A and P regions depend on this DV border. (C, D) The expression of *Noggin* (Ogawa *et al.*, 2002) suggests that these two lateral organizing lines are equivalent to the single dorsal midline in vertebrates. The inhibitory influence of these borders allows *BMP* expression only at the central dorsal midline as observed (Orii *et al.*, 1998). (E) Simulation: A stripe forming system (*BMP*) is repelled from the two circumventing lateral borders and forms the midline. The terminal regions A and P are assumed to have an activating influence of midline formation in order that the midline can reach the anterior and posterior pole (Meinhardt, 2004a).

the midline has to be extended both towards the anterior to organize the brain and towards the posterior together with the sequential elongation of the trunk.

Mesodermal cells all around the marginal zone ingress. During early ingression, these cells move necessarily away from the blastopore, i.e., towards the anterior. Cells of the organizer, being a small part of the large mesodermal ring, populate with this ingression a narrow stripe, the prechordal plate (Kiecker and Niehrs, 2001b; Gritsman *et al.*, 2000). This stripe has necessarily an orientation perpendicular to the blastopore and parallel to the AP axis as required (Fig. 12). Most interestingly, although this polarized cell movement occurs normally in a cell sheet, this ingression can be accomplished by single cells. Single wildtype cells transplanted into a mutant in which ingression is no longer possible move in the correct way (Carmany-Rampey and Schier, 2001), suggesting that some chemotaxis-like orientation of the cell movement away from the blastopore is involved (see also Fig. 11F–11J).

D. The Hydra-Type Organizer (Marginal Zone) Provides the Prerequisites to Generate the Spemann-Type Organizer

Based on the considerations above, it is expected that an ancestral molecular system is involved in blastopore formation. To localize the organizer on the blastopore, it

is expected that this ancestral system provides the precondition for a second pattern-forming system that generates the proper Spemann-type organizer. The canonical *Wnt*-pathway including β-catenin is a good candidate to provide this precondition since it marks the blastopore already in cnidarians and in *Amphioxus* (see Fig. 2; Hobmayer *et al.*, 2000; Holland *et al.*, 2000, 2005; Yu *et al.*, 2007). Indeed, during early stages the *Wnt* pathway is crucial for the formation of dorsal structures in all vertebrates (see Fagotto *et al.*, 1997; De Robertis *et al.*, 2000).

E. The Spemann-Type Organizer: The *Chordin*/*BMP*/*ADMP* System as a Pattern-Forming Reaction

The Spemann-type organizer displays many regulatory features typical of a genuine pattern-forming process. For instance, after organizer ablation a more or less normal development can follow in many systems (see Harland and Gerhart, 1997; Yuan and Schoenwolf, 1998; Saùde *et al.*, 2000). As mentioned, complete chicken embryos can be formed after early fragmentation of the blastodisc (Fig. 1). In *Xenopus*, co-culture of dissociated animal and vegetal cells leads not only to mesoderm induction but also to the formation of axial structures such as notochord and neural tube (Nieuwkoop, 1992), although such a procedure certainly wipes out any maternally imposed asymmetries. Thus, it is expected that the formation of the Spemann-type organizer is based on a pattern-forming process in which both self-amplification and long-range inhibition play an important role. Evidence for this mechanism has accumulated recently (Chen and Schier, 2002; Lee *et al.*, 2006; Reversade and De Robertis, 2005).

Central in organizer formation is the mutual inhibition of *BMP* and *Chordin* (reviewed in Harland and Gerhart, 1997; Niehrs, 2004; De Robertis and Kuroda, 2004; Stern, 2001; Boettger *et al.*, 2001; Schier and Talbot, 2005). Two components that mutually inhibit each other produce as a system a positive autoregulation. For instance, an increase of the first component leads to an enforced repression of the second, which, in turn, leads to a further increase of the first as if this substance would be directly autoregulating. To obtain a balanced activation of *BMP* and *Chordin* at opposite positions, a third component is anticipated that acts antagonistically on one of these indirectly self-enhancing reactions. A candidate is the *Anti-Dorsalizing Morphogenetic Protein* (*ADMP*) (Moos *et al.*, 1995). Its properties have been frequently regarded as counterintuitive: being expressed in the organizer, but functioning by reducing organizer activity. However, as its acts over a longer range (Lele *et al.*, 2001; Willot *et al.*, 2002; Reversade and De Robertis, 2005), it satisfies the theoretical expectations: being produced in the organizer region, and yet antagonizing a self-enhancing reaction. Equations (5a)–(5c) describe this simplified *Chordin* (c)–*BMP* (b)–*ADMP* (a) interaction and Fig. 13 shows a simulation of pattern formation based

on this model system.

$$\frac{\partial c}{\partial t} = \frac{\rho}{\gamma_c + b^2/a^2} - \mu_c c + D_c \frac{\partial^2 c}{\partial x^2} + \rho_c, \tag{5a}$$

$$\frac{\partial b}{\partial t} = \frac{\rho}{(\gamma_b + c^2)} - \mu_b b + D_b \frac{\partial^2 a}{\partial x^2} + \rho_b, \tag{5b}$$

$$\frac{\partial a}{\partial t} = \gamma_a c - \mu_a a + D_a \frac{\partial^2 a}{\partial x^2}. \tag{5c}$$

In this model equation c (*Chordin*) is inhibited by b (*BMP*) and *vice versa*; a (*ADMP*), under control of the organizer c, undermines the c-inhibition by b. This model is certainly a simplification. The mutual interference also depends on a competitive binding to receptors. Further, *Tolloid* plays an important role in degradation and transport. The side antipodal to the organizer might harbor a complete pattern-forming system in itself since *BMP* autoregulation (Hild *et al.*, 1999) and long-ranging antagonistic component (*swirl* and *sizzled*) have been found (Martyn and Schulte-Merker, 2003; Lee *et al.*, 2006). Thus, more complex interactions are to be envisaged. Nevertheless, the simplified scheme given in Eqs. (5a)–(5c) accounts for many observations, such as broadening and shrinking of expression regions if *BMP*, *Chordin* or *ADMP* are misexpressed or for the regeneration of an organizer after ablation. The more complex system encompasses the fact that residual pattern formation can take place even if some components of are nonfunctional.

F. The Role of Maternal Determinants

Frequently in the literature the formation of the Spemann-type organizer is regarded more as a chain of inductions rather than as a self-organizing process since organizer formation depends on the correct placement of maternally derived determinants. This is the case in amphibians and in fish (Harland and Gerhart, 1997; Ober and Schulte-Merker, 1999). After separation of the blastomeres of a frog embryo at the two-cell state, the cell not containing the future Spemann organizer is unable to form dorsal structures. Only unstructured tissue, 'Bauchstücke' in terms of Spemann, results (see Sander and Faessler, 2001).

As shown above, if pattern formation starts from a nearly homogeneous initial distribution, a single organizing region can be generated only in a field small enough that the range of the antagonistic reaction is sufficient to suppress secondary organizing regions (Fig. 4). In contrast, for instance, the early amphibian gastrula is very large. Even if organizer formation is restricted to the marginal zone, the inhibitor would be further diluted by spreading into the whole embryo, making inhibition of a secondary activation at the antipodal side difficult (Fig. 16). A way to ensure the formation of only a single organizing region in a large embryo is to make only a small portion of the marginal zone competent. Since the *Wnt* pathway provides the precondition for

organizer formation, an asymmetric distribution of components of the *Wnt* pathway can provide a strong bias for the positioning of the organizer up to the point that the disadvantaged side becomes incompetent to form the organizer. Indeed, by cortical rotation, components of the *Wnt* pathways are displaced from the vegetal pole to the marginal zone, making the cells competent for organizer formation. The model also provides a rationale for why strong initial asymmetries are especially common in large embryos such as amphibians but less important for small ones such as the mouse.

Wnt signaling is not required to maintain the organizer. Axis formation can be induced by injection of *Chordin* RNA even if the appropriate localization of components of the *Wnt* pathway was suppressed by UV irradiation (Smith and Harland, 1992). Moreover, although required for organizer initiation, *Wnt* synthesis is switched off in the organizer as soon as the organizer is formed (Christian and Moon, 1993). In terms of the model proposed, this is required since *Wnt* is the signal for posteriorization. When organizer-derived cells move anteriorly to form the prechordal plate, this posterior signaling has to be switched off; otherwise no anterior brain structures could be formed. *Goosecoid*, a downstream target in the organizer, directly suppresses *Wnt8* (Yao and Kessler, 2001).

G. Pattern Regulation and Unspecific Induction: How Dead Tissue Can Induce a Second Embryonic Axis

Many classical transplantation experiments have shown that an existing organizer has a strong inhibiting effect on the formation of a secondary organizer. This has been shown for the hydra (Wilby and Webster, 1970a, 1970b) and for the chick organizer (Khaner and Eyal-Giladi, 1989). The simulations in Fig. 15 show that our model describes these regulations correctly.

One of the problems in the early search for molecules involved in organizer formation was that very unspecific manipulations, such as implantation of denatured tissue or injury, can trigger the formation of a secondary embryonic axis. Waddington *et al.* (1936) proposed that this nonspecificity results from the removal of an inhibitor. The tendency for unspecific induction is species-dependent. It is low in *Xenopus* but high in *Triturus*, the model system most studied in the early days. 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 a leakage through an injury or by the activation of degrading enzymes. At larger distances from an existing activator maximum, the inhibitor concentration is low anyway. Any further decrease may result in the onset of autocatalysis (Fig. 17D). A second activator maximum will be triggered that becomes 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.

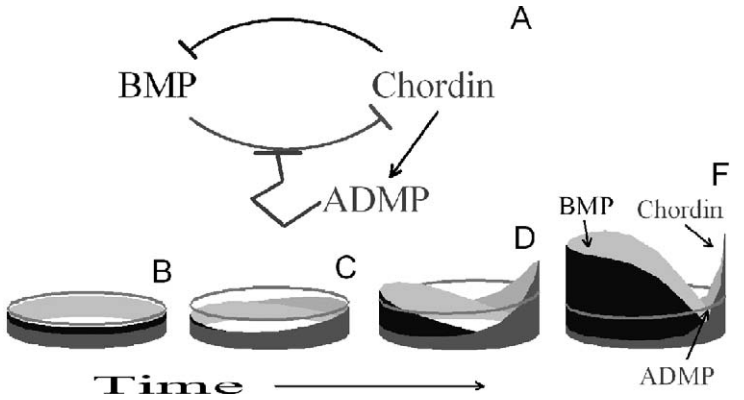

Figure 15 Self-enhancement realized by in inhibition of an inhibition—a simplified model for pattern formation in the Spemann organizer. (A) *Bmp* and *Chordin* mutually repress each other. Such an interaction is equivalent to a self-enhancement. On its own, such a system behaves as a switch (a classical example is the switching between the lytic and lysogenic stage of the Lambda phage). A balanced ratio between the regions in which either the *Bmp* or the *Chordin* level is high is achieved by a diffusible substance, *ADMP*, which is under the same control as *Chordin*. Thus, *ADMP* is expressed in the organizer although its function is to down-regulate the organizer-generating components, as observed (Moos *et al.*, 1995; Lele *et al.*, 2001). The balance between the three components is easily understandable. For instance, an elevation of *Chordin* production would lead to an elevation of *ADMP* and thus to a back-regulation. Or an elevation of *BMP* would lead to a down-regulation of *Chordin* thus to a down-regulation of *ADMP* thus to an increase of *Chordin* causing a return to the steady state. (B–F) Simulation of *BMP*/*Chordin*/*ADMP* patterning using Eqs. (5a)–(5c). The *Chordin* distribution is sharp since it also gives rise to the inhibitory component, *ADMP*. In contrast, *BMP* is complementary to *Chordin* and has, therefore, a more plateau-like distribution.

H. The AP Patterning of the Trunk: A Time-Based Sequential Posterior Transformation and a Ring-to-Rod Conversion

Trunk formation in vertebrates occurs by the so-called convergence and extension mechanism (reviewed in Keller, 2005; Solnica-Krezel, 2005). Cells near the blastoporal ring move towards the organizer and the incipient midline, causing a conversion of the huge blastoporal ring perpendicular to the AP axis into the axial structures parallel to the AP axis. In this ring-to-rod conversion cells of the ring move towards the organizer and towards the organizer-derived midline-forming cells.

As mentioned, the blastoporal ring is the source of a posteriorizing signal for the patterning of the brain. Available evidence suggests that the acquisition of even more posterior determinations during trunk formation is a time-driven process. The sequential activation of new Hox genes occurs close to the blastopore excluding the organizer region, as shown for the chick (Gaunt and Strachan, 1996) and for *Xenopus* (Wacker *et al.*, 2004a, 2004b). Involved in this sequential posteriorization is an oscillation that leads also to the formation of the periodic structures (see Fig. 24). The vertebrate organizer is not required for the time-dependent posteriorization but it

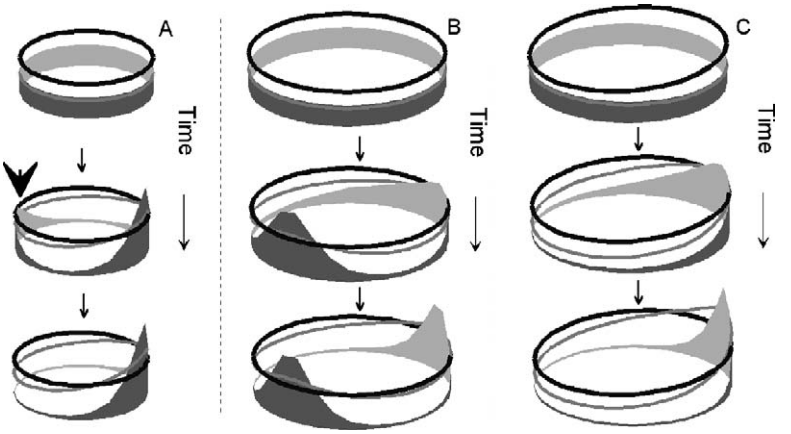

Figure 16 Maternal asymmetries as a mean to avoid supernumerary organizers. (A) In a small field only a single organizer will be formed even in the absence of maternal asymmetries (gray area: activator distribution, gray line: inhibitor distribution; black line: maternal influence, the source density ρ in terms of Eqs. (1a), (1b)); shown are the initial (top), an intermediate (middle) and the final stable state (bottom). Note that a transient activation (arrowhead) opposite to the more advanced maximum eventually disappears. (B) In a larger area, two maxima can coexist. (C) An asymmetry in the source density, e.g., due to maternally supplied determinants, makes sure that only a single maximum is formed. In even larger fields, stronger asymmetries are required to maintain a single maximum.

is crucially involved in the termination of posteriorization in the correct order. During convergence-extension movement, the cells obtain an increasing distance to the blastoporal ring and to the organizer (Fig. 12D–12F). Therewith they leave the zone in which posteriorization can take place. Cells originally closer to the organizer are the first to escape this posteriorization and form, therefore, anterior structures. This scheme accounts for the nontrivial fact that cells antipodal to the *dorsal* organizer form *posterior* structures (and not primarily *ventral* structures as it is frequently indicated in textbooks). The antipodal cells have to move the longest way to come close to the organizer and the midline. Therefore, they are exposed longest to the sequential activation of more posterior Hox genes.

After implantation of a secondary organizer, the new organizer attracts cells in the same way as does the normal organizer. Therefore, cells close to the newly implanted organizer are integrated much earlier into the supernumerary axial structure and are therewith in the same way protected from the sequential posteriorization at a much earlier stage (Meinhardt, 2001; Wacker *et al.*, 2004a, 2004b). Thus, cells that would form posterior structures in the unperturbed situation will form anterior structures instead. According to the model, this is not a posterior-to-anterior reprogramming but is based on an earlier relief from the posteriorization.

It should be kept in mind that this mode of axis formation holds only for vertebrates. In insects, for instance, formation of mesoderm occurs in a stripe that has from the

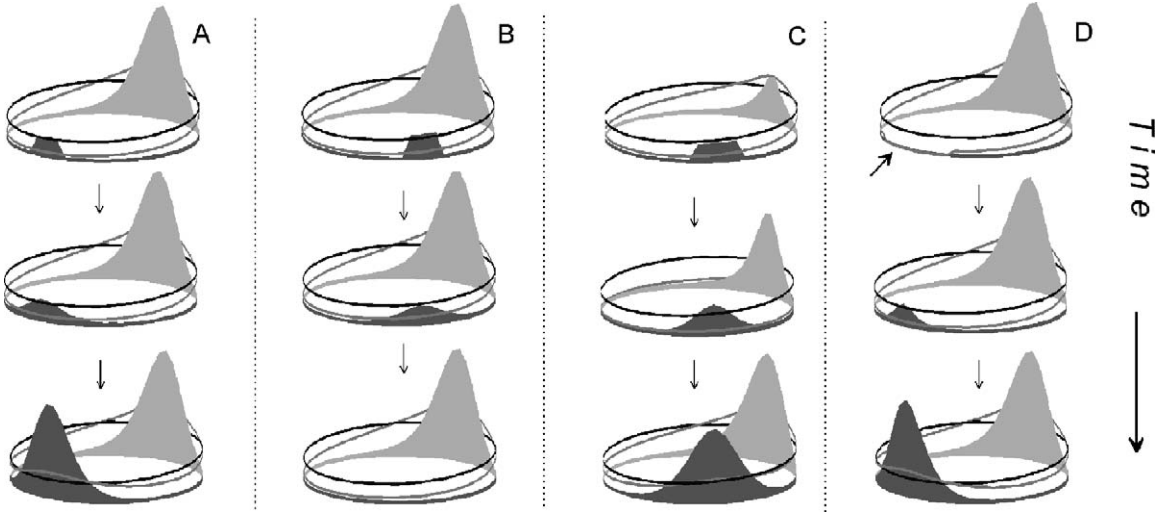

Figure 17 Simulations demonstrating regulatory properties of an organizing region. (A) Implantation of somewhat activated tissue at a position opposite to the endogenous organizer can induce a full activation. (B) When implanted closer to the organizing region, even a stronger activation will be down-regulated. (C) After partial removal of the existing organizer, the implanted activation has a better chance to survive. Whether one or two maxima survive depends on their distance and the total size of the field into which the inhibitor can escape. These simulations account for transplantation experiments in the early chick embryo (Khaner and Eyal-Giladi, 1989). (D) Unspecific induction. Any temporary reduction of the inhibitor (arrow) can lead to the trigger of a second organizing region that would have the same properties as the endogenous one. Thus, induction of secondary organizing regions is a straightforward consequence of our theory of pattern formation. That unspecific induction is frequent in some animals (as in *Triturus*) but rare in others (as in *Xenopus*) could depend on the competence of the tissue antipodal to the organizer, on the level of a baseline inhibitor production [ρ_b in Eq. (2b)] or on how rapidly the wound is closed. (Figure partially from Meinhardt, 2001.)

beginning an anteroposterior orientation, A ring-to-rod conversion as in vertebrates is not required (Meinhardt, 2004a, 2006).

I. The Left–Right Polarity: A Second Pattern Is Squeezed to the Side

Considerable progress has been made in understanding the generation of the left–right (LR) asymmetry of vertebrates (for review see Cooke, 2004; Raya and Izpisúa Belmonte, 2006). A key question is how a reliable bias is generated that determines left and right in a reproducible way (Levin and Palmer, 2007). However, even in the absence of this bias, an LR-type asymmetry emerges, although with a random orientation (Nonaka *et al.*, 1998). Many observations can be accounted for by assuming that the midline system induces on long range and represses on short range a second patterning system that marks the left side (Fig. 18) (Meinhardt, 2001). The 'left'-system again depends on the interplay between short-ranging activation and long-ranging inhibition. It is induced by the midline system, but becomes shifted to the side. Only in the presence of a systematic bias, is the side to which the LR-activator shifted non-random. This model has found recently support from the demonstration that *Nodal* acts as the activator and *Lefty2* as the inhibitor (Nakamura *et al.*, 2006). The repression of *Nodal* by the midline system, i.e., the notochord, has been shown (Lohr *et al.*, 1998; Bisgrove *et al.*, 1999). According to the model, if the activity of the midline activator is reduced, the shift may no longer work and the 'left'-signal can remain in the center. The model accounts for the observation that in a right fragment not only the midline signal but also the 'left'-signal regenerates (Fig. 18). In Siamese twins one of the embryos has frequently a *situs inversus* (Newman, 1928), an observation also made by Spemann with his artificially double embryos after organizer transplantation (see Sander and Faessler, 2001). According to the model, the mutual inhibition of the two 'left' signals can be stronger than the bias towards the left (Fig. 18F).

The left–right patterning is an obvious feature of vertebrates but not of insects (Cooke, 2004). In vertebrates, due to the local elongation of the midline by the node, a reliable signal can be generated next to the node (Fig. 18A). In contrast, the insect midline has from the beginning a long AP extension (Fig. 13). A squeezing out of the 'left'-signal by the midline could lead to signals on the left and on the right in an alternating sequence (Fig. 18B). Thus, the different mechanisms discussed above for midline formation provides a rationale as to why a LR-pattern is easier to realize in vertebrates.

IV. Subpatterns

The spatial complexity of higher organisms requires a finer and finer subdivision along the main body axes. There are several possibilities:

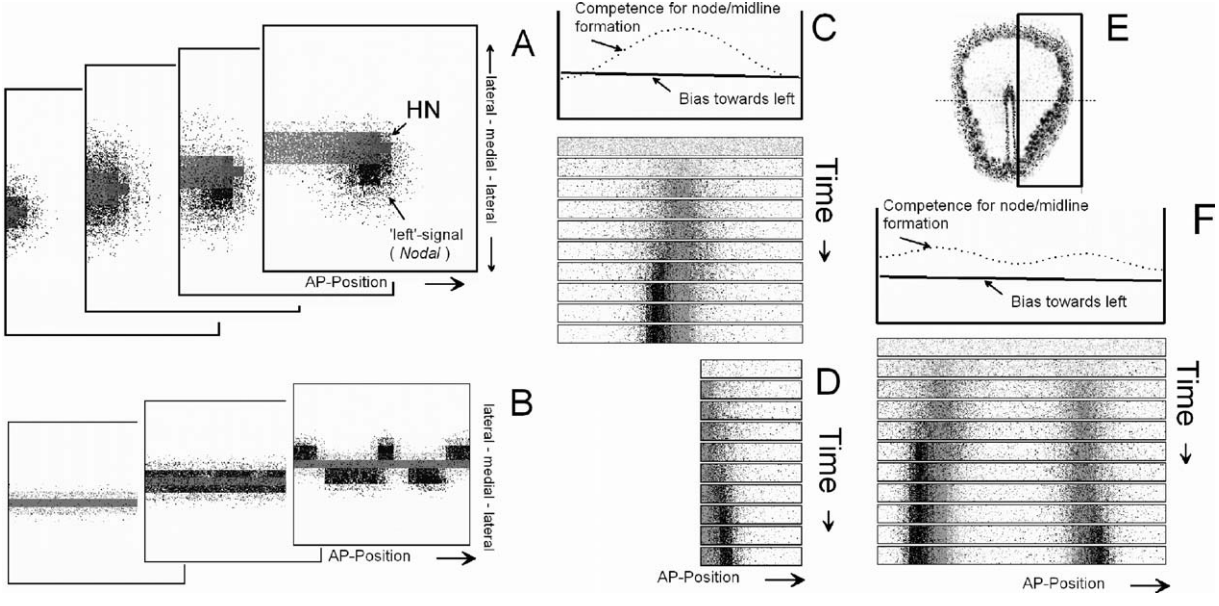

Figure 18 Model for left–right patterning. The midline/node (gray, HN) is assumed to induce on long range a second activator–inhibitor system that is responsible for the 'left'-signal (black; *Nodal/Lefty2*). (A) Originally centered on the midline the 'left' signal becomes shifted to a lateral position. (B) This mechanism would not work if the midline had the full AP extension from the beginning as it is the case in insects (see Fig. 13). The 'left' signal would appear at alternating positions on the left and the right side. (C) Simulation of the shift; shown is a cross-section (indicated by the dotted line in E). Midline formation (gray) triggers *Nodal* activation (black) and repels *Nodal* activation at high levels of the midline signal. A minor bias (oblique line) is sufficient for determining that *Nodal* displacement occurs reproducibly to the left. In the absence of this bias, the shift would occur at random to the left or to the right. (D) In a right fragment of the embryo (Levin and Mercola, 1998), first a new midline signal is reformed. This triggers again the 'left' signal (black) that normally appears on the left side. It is now shifted to the right since the left side no longer exists (Meinhardt, 2001). The bias no longer plays a role since a competition between the two sides is no longer possible. (E) Schematic drawing of the experiment (Levin and Mercola, 1998). The square indicates the right fragment. (F) Simulation of the generation of a *situs inversus* in one of a pair of Siamese twins. If two midlines are close to each other, the inhibitory effect of one 'left' signal onto the other could be stronger than the bias. The 'left' signal appears on the right side in the relatively delayed embryo.

(i) Concentration-dependent gene activation: sharply confined regions in which particular genes are active emerge under the influence of the graded distribution of a 'morphogen.' The gradients are generated as described above or by a 'cooperation of compartments' (see below).
(ii) Mutual induction of structures that locally exclude each other: cells of type A induce cells in their neighborhood to become cells of type B, which may, in turn, induce further cells to become C-cells, and so on. In such mechanisms there is no need for a global signal.

These mechanisms have different regulatory properties, and both are realized, in some cases even within the same organ. For instance, the sequence of leg segments in insects seems to be under the control of a gradient, while the internal structures *within* each leg segment is better described by the induction mechanism. The molecular ingredients required for both mechanisms and their different regulatory properties will be briefly discussed. Both mechanisms lead to sharply confined regions of gene expression. Borders between such regions can become new signaling centers that allow the generation of new coordinate systems, as required for the initiation of legs and wings. In the present paper, these mechanisms can be discussed only very briefly.

A. Switch-Like Gene Activation Requires Autocatalytic Genes

Signals generated by diffusible molecules are necessarily transient since in the enlarging tissue the communication between different parts would require more and more time. Moreover, the slope of a gradient depends on the half-life and the diffusion rate of the signaling molecule, and would not automatically adapt to natural changes in the field size. Therefore, signals generated at small scales have to be translated at a certain stage into more stable states of cell determination that can be maintained independent of the inducing signals. The obvious means is a concentration- (and thus space-) dependent activation of genes.

There is an interesting formal analogy between gene activation and pattern formation. Pattern formation requires an activation at a particular position and the inhibition in the remaining part. The selection of a particular pathway requires the activation of a particular gene and the suppression of the alternative genes. Thus, essential steps in development can be regarded as a sequence of pattern formation processes in real space coupled with a pattern formation among alternative genes. This formal analogy was the rationale for predicting autoregulatory gene activation underlying cell differentiation (Meinhardt, 1976, 1978). The long-range inhibition in pattern formation corresponds to the repression of the set of alternative genes in gene activation.

A stable switch-like activation of a single gene can result from a nonlinear autocatalytic feedback of a gene product on the activation of its own gene (Fig. 19). Equation (6) provides a possible interaction.

$$\frac{\partial g}{\partial t} = \frac{cg^2}{1+\kappa g^2} - rg + m. \tag{6}$$

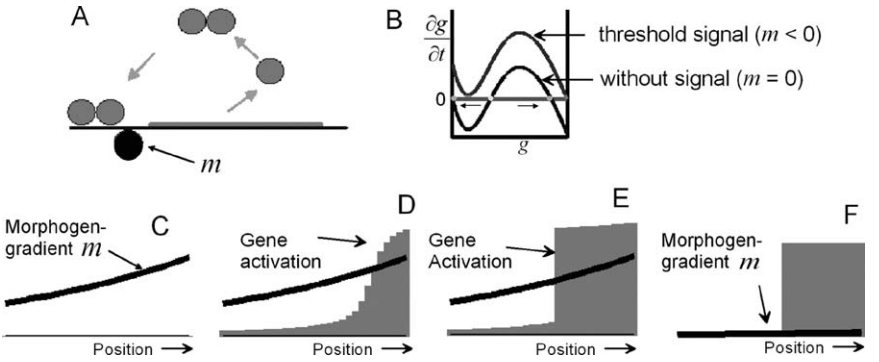

Figure 19 Model for a stable switch-like activation. (A) Assumed is a gene that codes for a gene product g that activates autocatalytically its own gene. Binding of g to the DNA requires a homo-dimerization. A signal m elicits an additional production of g. (B) In the absence the signal two stable steady states ($\partial g/\partial t = 0$; Eq. (6)) exist, one at a low and one at a high g level. With a signal m above the threshold level, cells in the low state switch to a high g-level. (C–E) Stages in gene activation. Cells exposed to a certain signal concentration m (black) make the switch; those exposed to higher m make this switch earlier. The region of high g concentration sharpens in the course of time. (F) After the signal is gone, the cells in which once the gene was activated maintain this activation due to the autoregulatory loop (Meinhardt, 1976).

The activation of this gene, i.e., the production rate of the self-activating gene product, $\partial g/\partial t$, depends on g-dimers (therefore g^2). At low g-levels the chance of finding a partner for building a dimer is low. Therefore, the linear decay term $-rg$ is dominating and the level of the gene product will remain low. The morphogen signal m is assumed to have an additional activating influence on this gene activation. Cells exposed to a certain threshold level of m obtain such a g-level that the autoactivation becomes larger than the decay rate. The gene activation switches from an OFF into an ON-state (Fig. 19). Due to the saturation term κ in the denominator the activation reaches a stable high level.

Meanwhile many genes with positive autoregulation are known. The gene *deformed* in *Drosophila* is an example (Regulski *et al.*, 1991). Due to autoregulation, a short activation of the *deformed* gene under heat shock control is sufficient for a long-lasting activation of this gene (Kuziora and McGinnis, 1988). As predicted, *deformed* acts as a dimer. Other examples for autoregulatory genes are *hunchback* (Simpson-Brose *et al.*, 1994) and *twist* (Leptin, 1991); *deficiens* and *globosa* are examples from plants (Zachgo *et al.*, 1995).

B. Activation of Several Genes by a Morphogen Gradient: A Step-Wise Promotion

According to a classical view, graded 'morphogenetic' signals lead to a space-dependent determination due to a concentration-dependent response of the cells to

this 'positional information' (Wolpert, 1969). The question is then, however, how cells measure the local concentration with such precision. Imagine two adjacent cells. While the cell exposed to the lower concentration should activate, e.g., the gene 1, the neighboring cell, exposed to only a slightly higher signal concentration, should activate the gene 2. An analysis of ligation experiments with non-*Drosophila* insects suggested that cells do not measure different levels in a single step but that they compare their achieved state of determination with the strength of the external signal (Meinhardt, 1978). A sequential transition from one gene activation to the next will occur as long as the signal is still high enough. Each of the subsequent activations requires a higher concentration and each step requires a certain time (Fig. 20). According to this model the cells obtain a determination according to the highest concentration they were exposed to in their past. Therefore, a fading signaling strength due to the increasing distance between a cell and the signaling source has no effect.

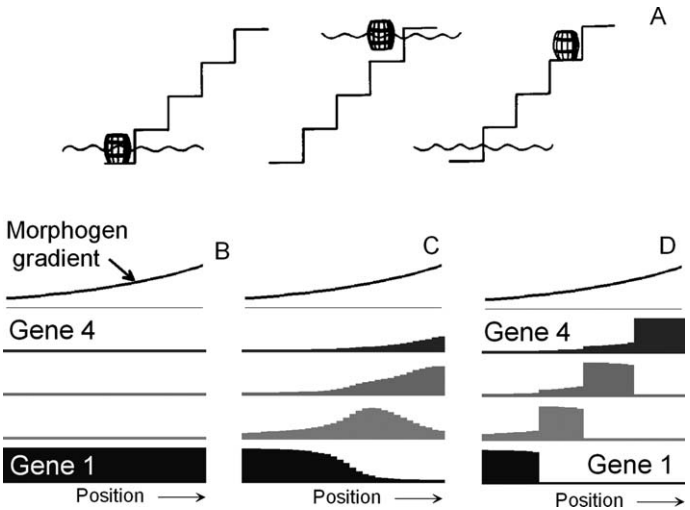

Figure 20 Model for the space-dependent activation of several genes. (A) An analogy: a barrel at the base of a staircase may be lifted up by a flood (morphogen signal). After lowering of the flood, the barrel can only remain at a few discrete levels (activation of particular genes). A later, higher flood can lift the barrel up even further; a second lower flood would have no effect. (B–D) Assumed are genes $(1, \ldots, 4)$ with their gene products feeding back positively on activation of their own gene. Due to their mutual repression, only one gene of the set can be active within one cell. The morphogen is assumed to accomplish a stepwise and irreversible transition from one gene to the next. Each step requires a higher signal concentration (Meinhardt, 1978, 1982). Note that the gene that becomes activated at the highest morphogen concentration (e.g., gene 4) is the gene that is least sensitive to the signal. Modeling predicted that these less sensitive genes are more effective in autoregulation, otherwise they could not win against the more sensitive feedback loops. The first gene that becomes active in the region of high signaling level is expressed later in a region of low signaling level, in agreement, for example, with the sequence of digit determination in vertebrates.

A characteristic feature of such systems is that determination can be changed only in a unidirectional way ('distal' or 'posterior' transformation). Therefore, cells transplanted from a region of high to a region of low concentration maintain their already achieved determination. In contrast, after a low-to-high transplantation, the cells change their determination according to the new level. Strong evidence for such a unidirectional promotion exists for the hindbrain (Gould *et al.*, 1998; Grapin-Botton *et al.*, 1998) and for the response to activin signaling in the early amphibian gastrula (Gurdon *et al.*, 1995). A stepwise posterior transformation was proposed for the AP-specification in the anterior neural tube (Nieuwkoop, 1952). In this mechanism there is no direct communication between adjacent structures. The correct neighborhood depends solely on the graded signal that evokes these structures. This has the consequence that mismatches caused by transplantation at later stages might be neither detected nor repaired.

Thus, the maintenance of the determined cell state by feedback of a gene on its own activity, combined with a repression of alternative genes, seems to be a widespread mechanism. It is, however, not the only one. Another mechanism is based on changes in the chromatin packaging, e.g., by DNA methylation. This leads to a different accessibility of particular genes in the chromatin—a mechanism that will be not considered there (see Schwartz and Pirrotta, 2007; Ringrose and Paro, 2007).

C. Mutual Induction

The statement '*A* induces *B*' seems to be straightforward. A closer look, however, shows that this is not that simple if *A* and *B* are derived from the same tissue. If *A* is the source of a *B*-inducing molecule, how it is achieved that *A* cells themselves do not become converted into *B* cells, although the *A* cells are exposed to the highest level of the inducing signal? A possibility is that *A* cells generate on long range the precondition for *B*, but a short-range exclusion makes sure that both structures do not merge. The long-ranging activation can be reciprocal in that *A* not only activates *B* but that, vice versa, *B* also activates *A*. Examples will be provided for both cases.

D. Hydra Tentacles as Example

Hydra tentacles appear in a ring around the primary organizer, the hypostome. This is a frequent occurrence. Leaves appear likewise at a certain distance from the organizer, the shoot apical meristem. The periodic nature of tentacle spacing indicates that their formation is under control of a separate pattern-forming system and does not occur by a simple gradient that is generated at the tip. This can be explained by the assumption that the primary organizer makes the upper part of the body column competent for tentacle activation. Locally, however, tentacle activation is excluded from the hypostome (Meinhardt, 1993).

After head removal, the tentacle signal appears first at the very tip and becomes subsequently displaced to the final subhypostomal position. In contrast, during bud formation, the tentacle signal appears directly in a ring at the correct distance from the tip. This large ring resolves later on into small rings that form the base of the tentacles (Fig. 21A and 21B) (Bode *et al.*, 1988; Smith *et al.*, 2000). The different dynamics in tentacle formation during head regeneration and during budding was predicted by early modeling (Fig. 21C and 21D) (Meinhardt, 1993).

These observations and their modeling allow several conclusions. (i) The primary organizer causes the tissue to become competent for the activation of a secondary organizer. (ii) The tentacle-competent zone is much larger than the zone in which tentacles are actually formed (since the formation of the tentacle signal can precede the formation of the head organizer, Fig. 21A and 21C). The fact that the remaining body column is free of tentacles results from the dominance of those tentacles that are formed in the region of relatively highest competence, not because the competence is restricted to a narrow zone (similar as the primary organizer obtains its 'apical dominance' by the graded competence, see Fig. 10). This model found support from experiments in which β-catenin was stabilized by treating animals with Alsterpaullone, which causes the entire body column to obtain the properties of the tissue that is located close to the head (Broun *et al.*, 2005). In terms of the model, Alsterpaullone causes the whole body column to become competent for tentacle formation. Indeed, after such treatment tentacle formation occurs with narrow spacing almost everywhere (Broun *et al.*, 2005).

E. Sequences of Structures and their Dynamic Control: Planarians as Examples

In planarians, the correct neighborhood of structures is maintained over the complete body axes in a dynamical way (for review see Saló and Baguñà, 2002; Newmark and Alvarado, 2002; Agata *et al.*, 2003; Saló, 2006). Planarians are well-known for an almost unlimited capability for regeneration. Any missing internal part becomes intercalated. In planarians, regeneration and the replacement of old cells occur by a population of stem-cells, the neoblasts. If all neoblasts are killed by irradiation in an anterior portion, the neoblasts that survived in the posterior part can regenerate the anterior part (Saló and Baguñà, 1985). The dynamic maintenance of the correct neighborhood led Chandebois to a comparison with a human community she termed 'cell sociology' (Chandebois, 1976). Although many genes are known to be involved, the molecular basis of this dynamic regulation as a whole is still unclear.

In the model for tentacle formation given above, a mechanism for the maintenance of two structures in the correct neighborhood was discussed. In the following this mechanism will be extended to several coupled pattern-forming reactions. Each system locally excludes the others. On longer range, however, each system activates the loops required to form the adjacent structures (Meinhardt and Gierer,

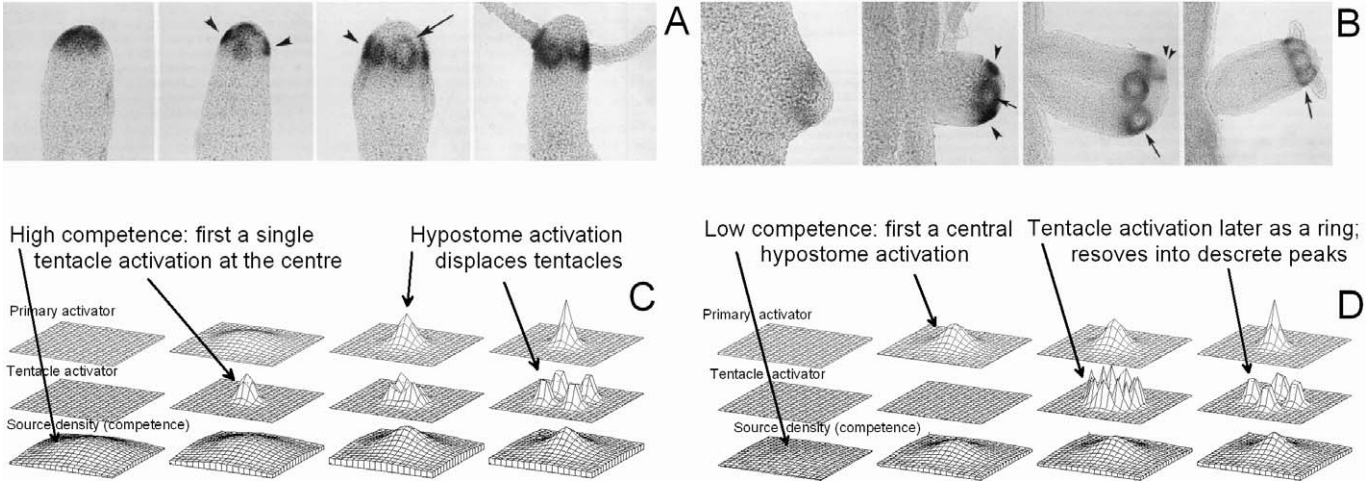

Figure 21 Dynamic regulation of a secondary organizing region: hydra tentacles as an example. The *hy-Alx* gene is a marker for tentacles (Smith *et al.*, 2000). In vertebrates, *Alx* is involved in brain patterning (for instance, Miura *et al.*, 1997). (A) After head removal, *Hy-Alx* first appears at the tip, to be later displaced to the final position where it disintegrates into separate signals. (B) In contrast, during bud formation *Hy-Alx* appears as a ring directly at the correct position that splits subsequently into individual rings that marks the base of tentacles. (C) Model for tentacle formation during regeneration: in a region of high source density (high competence for hypostome and tentacle activation, see Fig. 10), a single tentacle activation appears first at the position of highest source density. Only subsequently does the peak of the primary head activator form, displacing the tentacle activation. The tentacle signal widens to a ring that decays into individual spots. (D) Model for tentacle formation during bud formation: If the source density is low (as during bud formation), tentacles can be formed only after a sufficient increase of the source density under the influence of the primary head activation (Fig. 10). Since primary head (hypostome) and tentacle activator exclude each other, tentacle activation appears directly in a ring that surrounds head activation. Later this ring also resolves into isolated maxima (Meinhardt, 1993).

1980). Such a network is able to generate a dynamically stable sequence of structures in which the correct neighborhood is regulated. Mutual activation can also result, indirectly, from a self-inhibition since in competing systems the self-inhibition of one component is equivalent to promotion of the neighbors. While in the direct activator–inhibitor mechanisms discussed above there are few winners and many losers, in the mutual activation scheme in each cell one of the several alternative loops wins.

In the simulation in Fig. 22 it is assumed that the activators cross-react in such a way that each inhibits the self-enhancement of the other activators by competitive inhibition. The inhibitors also show competitive inhibition with the inhibitors that are responsible for the adjacent structures. Since an inhibition of an inhibition is in fact an activation, this leads to a long-range activation of the adjacent structures. To give an example, the type-2-inhibitor restricts the extension of the activity in which the system 2 is active, but simultaneously it has an activating influence on the structures 1 and 3 by undermining the action of the genuine 1 and 3 inhibitors. Since the inhibitors have a long range, the cross-activation is of long range too. The emerging neighborhoods are determined by these cross-reactions. Each of the feedback loops saturates at high levels. This leads to a plateau-like activation and enables size regulation such that each structure occupies an appropriate share. As shown in Fig. 22, the complete sequence is generated from any partial sequence, the polarity being maintained. If individual cells migrate into a zone in which a different subsystem is active, they adapt to their new surrounding. This respecification can go in both anterior and posterior directions; there is nothing like a unidirectional 'distal transformation.' This type of regulation also occurs in growing and budding hydra. Cells born in the middle of the animal eventually move either towards the head or to the foot, where they function according to their actual position.

Information of the underlying molecular network is sparse. Direct evidence for a reciprocal induction has been observed in the planarian head region (Bogdanova *et al.*, 1998). A hint could be the sequence of nested but overlapping expression domains of different *Wnt* genes in the sea anemone *Nematostella*, suggesting a *Wnt*-code as a possible candidate for such a system of linked pattern forming systems (Kusserow *et al.*, 2005).

F. Segmentation: A Superposition of a Periodic and a Sequential Pattern

Segmentation is a common theme in higher organisms with the trunk consisting of repetitive structures such as the alternation of anterior and posterior compartments in *Drosophila* or somites in vertebrates. Superimposed onto basically repetitive structures is a second pattern of Hox gene activation that causes the individual segments to undergo specific developments. Both patterns are precisely in register. Usually the border of Hox gene expression coincides with parasegmental organization (Zhang *et al.*, 2005; Damen, 2002) (a parasegment being definded as the overlapping unit ranging

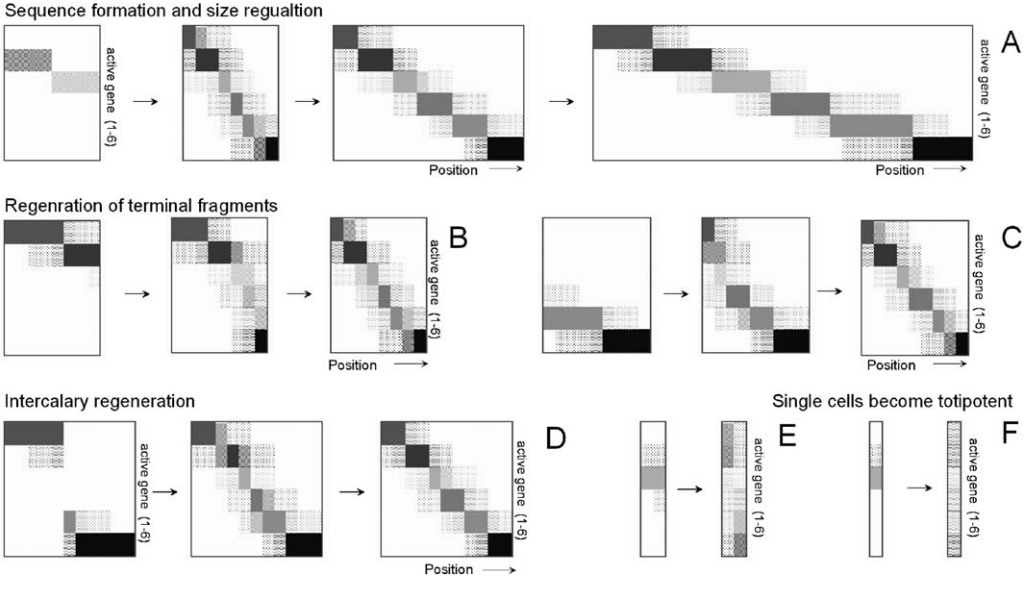

Figure 22 Generation of a dynamically stable sequence of structures by mutual activation and local exclusion of several pattern-forming systems. Arbitrarily six activator–inhibitor systems are assumed. Activity is mutually exclusive. Systems that are required for adjacent structures activate each other on a long range by a competitive interference of the inhibitors (only the activators are shown). (A) The complete sequence is generated from any partial sequence. Each region obtains a proportional share, maintained during further growth. Due to some activator diffusion, the regions have a substantial overlap. This overlap facilitates the transitions from one activation to the next and is required that each activation maintains its correct extension during growth or regeneration. (B, C) The sequence is restored after removal of terminal structures. Note that first the missing structures are formed in a compressed form, in agreement with observations (Saló, 2006). The regulation by which each part obtains its appropriate share requires a much longer period. (D) Replacement of internal structures. (E) In very small fragments positional specificity is lost, but a residual polarity is maintained (in starving planarians, the body size can shrink to a minute fraction of the normal body size, but after feeding the body pattern is reestablished with the original polarity). (F) An isolated cell activates all feedback loops, although at a low level. This is independent of the original activity. In other words, the cells are multipotent, although they obtain a position-specific activation during self-organizing patterning. In these simulations, all feedback loops have identical properties. The pattern-forming systems that generate the terminal structures may play in reality a more dominant role.

from the *P* compartment of one segment to the subsequent posterior *A* compartment, see Martinez Arias and Lawrence, 1985). In *Drosophila*, segmentation proceeds normally even in the absence of Hox genes (Lewis, 1978). This suggests that periodic patterning is the primary event and that the sequential activation of Hox genes is coupled to this.

As revealed by classical transplantation experiments, the neighborhood of structures *within* insect segments is strictly regulated (Locke, 1959; Lawrence, 1970; Bohn, 1970). Our model of mutual induction of locally exclusive cell states was in fact developed to describe this controlled neighborhood, including situations in which intercalary regeneration occurs with a reversed polarity (Meinhardt and Gierer, 1980). Molecular details that lead to the fine structure within a segment are still not completely understood. It is unclear, for instance, whether this pattern is organized by one graded quantity or by a fine-grained activation of different genes, i.e., different qualities. However, experimental evidence shows that the basic machinery causing subdivision into compartments works by mutual long-range activation and local exclusion: The gene *engrailed (en)*, the key gene for posterior compartmental specification, is autocatalytically activated. Via the diffusible molecule *hedgehog (hh)*, *en* activates in addition the gene *wingless (wg)* that is crucial for the anterior compartment (Ingham and Hidalgo, 1993). The gene *sloppy paired* is involved in the *wg*-autoregulation. The *wg* protein can reach adjacent cells via vesicle transport and is required there to stabilize *en* (Baker, 1987; van den Heuvel *et al.*, 1989). As expected from the theory, the *en* gene activity requires a functional *wg* gene in its neighborhood and *vice versa*, although both genes are transcribed in non-overlapping regions. The gene *cuD* (Eaton and Kornberg, 1990), on which *wingless*-expression depends, is involved in the local exclusion of *en* and *wg* expression. The prediction of such a complex molecular interaction by a theory could hardly be more precise (Fig. 23A).

The mechanism of long-ranging activation and short-ranging exclusion is appropriate to generate stripe-like distributions. A long common border between narrow bands of different cell states allows a most effective mutual support. As mentioned, an exchange of the activator between cells facilitates stripe formation by counteracting decay into isolated patches. In this respect it is interesting that *engrailed* and some other homeobox genes can be actively exchanged between cells (Maizel *et al.*, 2002).

Segments have an internal polarity, in contrast to a periodic alternation of two compartments $\ldots APAPAP \ldots$. For this reason I have proposed that in addition to the anterior and posterior compartment at least one additional element termed *S* must be present, such that a sequence $\ldots SAP/SAP/SAP \ldots$ results. The region *S* separates one $A{-}P$ pair from the next (Meinhardt, 1982). Now it is generally assumed that the AP pattern of each parasegment is founded by four cells (see Ingham, 1991). Four cells also establish the parasegments in crustaceans (Scholtz *et al.*, 1994). Thus, there is only one A/P border per segment. This is important since the A/P border also generates the preconditions to form legs or wings (see Figs. 26E and 26F).

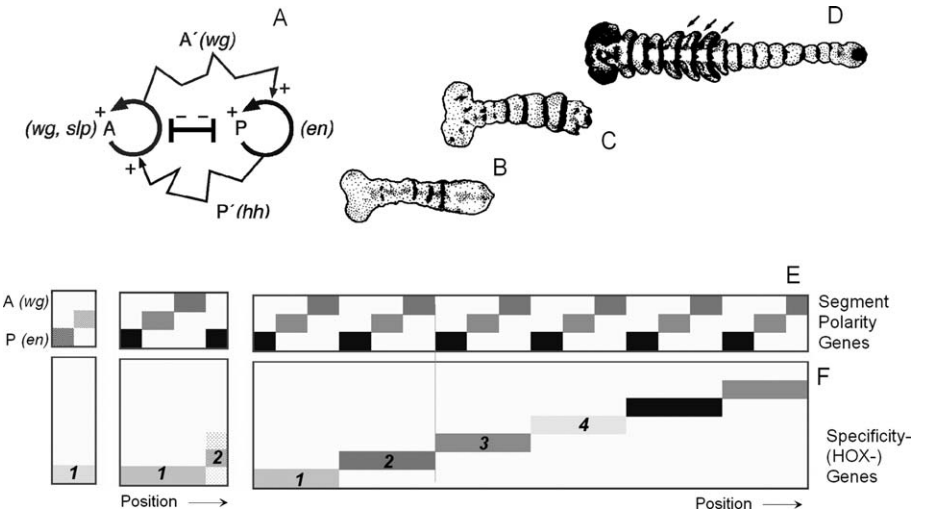

Figure 23 Segmentation—the formation of a periodic and a sequential pattern in register. (A) The basic network enabling segmentation. Proposed is that (at least two but presumably more) feedback loops activate each other on long range and exclude each other locally. This enforces a controlled neighborhood of structures (Meinhardt and Gierer, 1980; Meinhardt, 1982). (B–D) Stages in the development of a grasshopper embryo. In the course of development, more and more segments are added at the posterior pole. In the posterior part of each segment the gene *engrailed* (black) is transcribed Patel *et al.*, 1989. The segments are different from each other. Legs (arrows) are formed only in the three thoracic segments and are restricted to the *A*/*P* border (see Fig. 26). (E, F) Model: By proliferation of cells at the posterior pole, the respective terminal regions (*S*, *A*, or *P*) become enlarged until the long-ranging stabilization becomes insufficient and a transition into the subsequent cell state occurs. A periodic pattern with an intrinsic polarity results. Further, cells are in the *A*-stage produce a component that is required to activate the subsequent gene of the sequential pattern (1, 2, . . .). The actual transition, however, is blocked and can only occur after switch from the *A* to the *P* state. The resulting sequential (Hox-) gene activation 1, 2, 3, . . . is precisely in register with the parasegmental . . . *A*/*PSA*/*P* . . . pattern.

In short-germ insects, in contrast to *Drosophila*, new segments are added at the posterior pole. In the mutual activation scheme, the activation can be cyclic. For simplicity let us regard only two alternating components and an initial *AP* pattern. Whenever, due to growth, the *P*-region becomes too large, the support of the preceding *A*-cells will be too low (or the self-inhibition of the *P*-cells too high) and *P*-cells distant from the *AP* border will switch from *P* to *A*, elongating the *AP*- to an *APA*-pattern. A regular periodic pattern results (Fig. 23). This model predicted that the most posterior cells alternate between (at least) two states. This has been clearly demonstrated, e.g., by an ON/OFF alternation of *hairy* activation at the posterior pole of the spider embryo (Damen *et al.*, 2000). An oscillation of this type is crucial for somite formation discussed further below. The periodic pattern generated during outgrowth can be, but need not to be, accomplished on the level of the pair rule genes (Sommer and Tautz, 1993; Patel *et al.*, 1994).

G. Formation of a Precise Number of Different Segments during Terminal Outgrowth

In many cases the number of segments is precisely controlled. The polychaet *Clymenelly torquata*, for instance, has 22 segments. It regenerates removed segments such that the number of 22 segments will be restored independent of the number of segments removed (Moment, 1951). In the leech, more than the final 32 segments are initially formed. The surplus segments are later removed by programmed cell death (Fernandez and Stent, 1982; Shankland, 1991a, 1991b). These observations indicate that some sort of counting mechanism exists. As mentioned above, trunk formation requires a sequential step-wise posteriorization (Figs. 12D–12F). I have shown that the periodic alternation of cell states at the outgrowing posterior pole can be used as a counting mechanism on the gene level (Meinhardt, 1982). The mechanism is easily explained by an analogy. Imagine a grandfather's clock. The periodic movement of the pendulum drives the mechanism that allows a sequential advancement of the pointer with each full cycle by one and only one unit. In terms of gene switching, the transition from one *AP*-specifying gene to the next is prepared in one state (A). However, the actual transition is blocked. After switch to the other state (P), the prepared transition takes place but the preparation of an even further transition has to wait for a switch back to A. The simulation in Fig. 23 demonstrates the generation of a sequential pattern of (Hox-) gene activation $(1, 2, 3, \ldots)$ that is precisely in register with the periodic reiteration of cell states, the parasegmental ... *A*/*PSA*/*S* ... pattern. According to present knowledge, this early model has to be modified and extended because particular Hox genes are known to be active in several segments. In *Drosophila*, for instance, *Abdominal-B* is expressed in the abdominal segments 5–9. However, there are segment-specific *cis*-regulatory units on the chromosome, which are separated by chromosomal insulators (Drewell *et al.*, 2002; Estrada *et al.*, 2002). In terms of the model, each oscillatory cycle may cause the advancement from one such insulator to the next. Thus, a certain number of insulators have to be passed until the activation of the subsequent Hox gene takes place.

H. Somite Formation: The Conversion of a Periodic Pattern in Time into a Periodic Pattern in Space

Somites are the most conspicuous segmented structures in vertebrates. In *Amphioxus* (hemichordate) somites form in a similar way to the segments in short germ insects, in a growth zone at the posterior pole (Schubert et al., 2001a, 2001b). In contrast, in higher vertebrates, somite formation occurs at a considerable distance from the posterior pole by a sequential separation from a nonsegmented presomitic mesoderm in an anterior-to-posterior sequence. Fig. 24 shows chicken embryos at

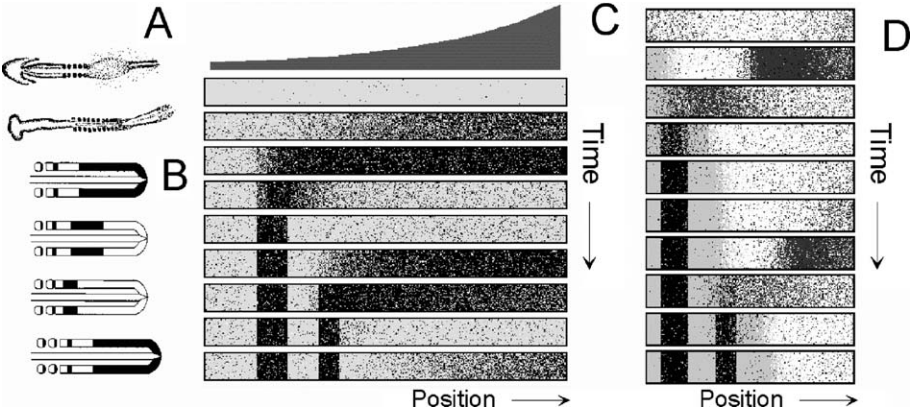

Figure 24 Somite formation. (A) Chicken embryos at 25 and 37 hour with 5 and 12 pairs of somites respectively. (B) Observed *c-hairy* pattern in the chick (Palmeirim *et al.*, 1997; Pourquie, 2004). (C) An early model (Meinhardt, 1982, 1986a). Predicted was that an oscillation between two cell-states takes place (gray and black) in the posterior presomitic part. Activity waves move towards the anterior. Each full cycle adds one pair of half-somites. Only cells that are exposed to a minimum level of a gradient (identified now as *FGF*, top) can participate in this oscillation. Cells below this level form a stable AP pattern in which *A* and *P* cells stabilize each other mutually. (D) A modified version based on *mesp2* expression (Takahashi *et al.*, 2000; Morimoto *et al.*, 2006). In each cycle, a somite-wide region of *mesp2* expression (light gray) is hit by a wave (dark gray), causing a suppression of *mesp2* in the posterior half of this region and its determination to a posterior half-somite (black). The remaining part forms the anterior half-somite. Subsequently *mesp2* is activated in a new somite-wide region. For this model, elementary networks as described in the text are employed involving gene activation with mutual exclusion (Fig. 20), and traveling waves (Fig. 9).

different stages. Somite formation has been reviewed extensively (Pourquie, 2004; Dubrulle and Pourquie, 2004a; Aulehla and Herrmann, 2004; Holley and Takeda, 2002; Takahashi *et al.*, 2000).

To be compatible with classical observations, the counter-intuitive prediction was made that segment formation occurs by an oscillation between two cell states at the posterior pole. The main reason to assume this oscillation was that, as in segmentation, the somites obtain with high precision different identities along the AP axis. As mentioned, an oscillation allows for the generation of periodic structures that are nevertheless different from each other by a counting mechanism on the gene level. According to the model for somite formation, these oscillating activities spread in a wave-like manner towards the anterior, coming to rest there and forming pairs of anterior and posterior half-somites (Fig. 24C). Each full cycle was predicted to add one pair of anterior and posterior half-somites: '*This model would obtain strong support if the postulated oscillations in the mesoderm before somite formation could be detected. One full cycle of this oscillation should take precisely the same time as that required for the formation of one somite*' (Meinhardt, 1982, 1986a). The prediction

was confirmed fifteen years later by the observation of the oscillation and wave-like spread of the *hairy1* gene in the chick (Palmeirim *et al.*, 1997) (Fig. 24B). At the positions where *c-hairy1* waves come to rest, new posterior half-somites are formed. At the time the model was proposed, the subdivision of somites into anterior and posterior half somites was still a prediction that has been verified only two years later (Keynes and Stern, 1984). The predicted coupling of the (Hox-) gene activation and oscillation has been, in the meantime, also established (Zákány *et al.*, 2001; Dubrulle *et al.*, 2001; Dubrulle and Pourquie, 2002).

It was assumed that the very same molecular interaction that leads to the periodic patterns in time is also responsible for the periodic pattern in space (see also Fig. 9). While, as explained above, *A*- and *P*-cells stabilize each other in the region of a common boundary, it is a property of such an interaction that groups of cells consisting of one type only (*A* or *P*) can oscillate back and forth between the two states. For instance, if all cells are in *A*-state, the *P*-state obtains substantial help from the activated *A* state while the *A*-state itself is not supported, much as the *engrailed* activation would not be maintained without *wg*-expressing cells in the neighborhood. Therefore, after a certain time, the cells switch from *A* to *P*. Later, for the same reason, the cells switch back to *A*, and so on. Such a spatially homogeneous oscillating system can be converted into a periodic pattern that is stable in time if an *A*–*P* border has been formed. For a reliable separation of the region in which the oscillation can take place from the region in which stable patterning can occur, a controlling gradient was assumed. The waves were assumed to come to rest whenever the gradient level became too low. The gradient is now identified as *FGF* (Dubrulle *et al.*, 2001; Dubrulle and Pourquie, 2004b).

Meanwhile it has turned out that the *Notch* pathway is crucial for the oscillation. *Notch* was known before only as a component involved in the formation of spatial patterns. If the very same reaction is assumed to generate the pattern in space *and* in time, the model works only in a narrow parameter range. This is presumably the reason why different components of the *Notch* pathway are involved in oscillation and in the stable patterning respectively. Further it was assumed that the oscillation is not only of an ON–OFF type but that an oscillation occurs between the activation of two different cell states. In addition to the oscillating *Notch* activation, an out-of-phase oscillation of the *Wnt*-pathway has been found (Aulehla *et al.*, 2003).

More recent observations revealed that each new somite is initiated by a somite-wide activation of a gene (*Mesp2*) (Takahashi *et al.*, 2000). The *Notch*-driven wave hits the posterior part of this region, causing its transformation into a posterior half-somite and a restriction of the *Mesp2* expression to the remaining anterior half. A simulation is given in Fig. 24D. There are many other features that have to be integrated into forthcoming models, e.g., the formation of the somitic clefts and the dramatic slowing down and sharpening of the waves before they come to rest (see also the contribution of Baker *et al.* in this volume).

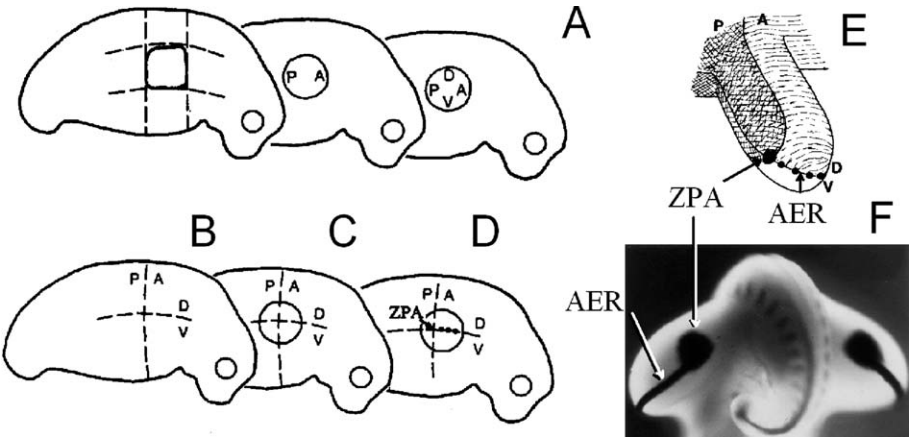

Figure 25 Models for vertebrate limb patterning. (A) The classical limb field model. First a nest of cells is determined for limb development that becomes subsequently patterned along the AP, and later along the DV axes (Harrison, 1918). (B, C) Boundary model (Meinhardt, 1982, 1983b): proposed was that the primary event is a subdivision along the axes. By cooperation of two pairs of differently determined cells (A/P and D/V) in the generation of new signals, two orthogonal borders become the organizing regions to set up the coordinate system for the substructure. Limbs are formed around previously established borders. (D, E) For vertebrate limbs only the anterior part is used. The intersection of both borders forms the organizing 'Zone of Polarizing Activity' (ZPA). The DV border forms the apical ectodermal ridge (AER), defining the plane along which the digits are formed. The outer and the inner face of the hand are different from each other since the former is formed from the dorsal and the latter from the ventral region next to the border (see Martin, 1995; Tanaka *et al.*, 1997; Martin, 2001; Niswander, 2003, for detailed experimental evidence and review). (F) View of a chick embryo from the tail onto the two wing buds (Grieshammer *et al.*, 1996). The thick black lines result from a staining of *FGF8* that is produced at the DV border (a *Wnt7-a/En-1* border) that forms the AER. The dark round spots mark high concentrations of *Sonic hedgehog* that is, according to the model, produced at an intersection of an A/P- and a D/V border. (F kindly supplied by Dr. Uta Grieshammer.)

I. Borders and Intersections of Borders Become the New Organizing Region for Secondary Structures such as Legs and Wings

Substructures such as legs or wings have their own coordinate system, with an AP, a DV and a proximodistal axis. Based on experiments with limb initiation in axolotl, it was proposed that first a limb field forms (Harrison, 1918): Cells are set aside that eventually form the future limb. At later stages an anteroposterior and subsequently a dorsoventral axis becomes determined within this initially more or less uniform cell population (Fig. 25A). It was further assumed that cells have an intrinsic polarity that is aligned with the overall body axes of the embryo (like any fragment of a magnet retains the original polarity), and that these cell polarities are used to organize the final polarity in the limb field. This model was very influential over many decades. However, Harrison himself realized problems in his views. Transplantation of a limb field to a more posterior position without any rotation led to symmetrical limbs or to

limbs with AP polarity reversals, i.e., to results that were incompatible with his model (Harrison, 1921).

The observation that an organizing region is located at the posterior end of the limb field (Gasseling and Saunders, 1964) was a big step forwards. Many transplantation experiments were explicable by the assumption that this 'Zone of Polarizing Activity' (ZPA) generates a morphogen gradient that leads to the sequence of digits with the little finger close to the source (Tickle *et al.*, 1975). Although corresponding drawings were very convincing, important questions remained unanswered. How is the ZPA generated in the first place? Why are the digits formed along a line and not as a series of concentric rings, as expected if a local source generates a cone-shaped morphogen distribution? An alternative model was proposed shortly later, the 'Polar Coordinate' model (French *et al.*, 1976), postulating that positional identities are arranged in a circular fashion and that distal transformation occurs if this ring is complete. Though the model was completely formal and no explanation was given how such a ring was generated during early development, the model was surprisingly successful in predicting in detail the geometry and handedness of supernumerary limbs as they occur, e.g., after reimplantation of limbs with an angular mismatch. It was clear that any molecularly feasible model has to solve the problems inherent in the ZPA model and must be compatible with the handedness predicted by the Polar Coordinate model.

In 1980 I proposed that borders between differently determined cells can obtain organizing functions. (Meinhardt, 1980, 1983a, 1983b). Imagine that a primary pattern-forming process leads to a subdivision into several discrete regions by region-specific gene activation (see Figs. 19, 20, and 23). Among them are the adjacent regions A and P. If, for instance, in the P region a co-factor is produced that is required in the A cells to produce a new morphogen, its synthesis is restricted to a position close to the A/P border. The concentration of this morphogen provides a measure for the distance of a cell from the border and is therefore suitable for the internal organization of the A and the P region. Although the positional information is symmetric about both sides of the border, the resulting pattern can be asymmetric since A and P cells can respond differently. In vertebrate limb formation, for instance, only the A-cells are able to respond, which leads to the polar arrangement of the digits (Figs. 25D and 25E).

A border that separates two cell types along the anteroposterior axis surrounds an embryo in a belt-like fashion. To determine the DV-position of a limb, an intersection with a second border is required. Limbs are assumed to be initiated around the intersection of two borders (Fig. 25B and 25C). This explains the handedness. Since the embryo has essentially a cylindrical geometry, any subdivision of the embryo along the anteroposterior and the dorsoventral (or better mediolateral) axis leads to paired intersections, one at the right and one at the left side of the embryo. They have opposite handedness, a feature most characteristic for the formation of legs, wings, eyes etc. The four quadrants or three sectors placed around the intersection of two borders provide coarse information about the angular position. The proximodistal organization results from a cooperation of the two signals generated at each border. Therefore,

the 'Complete Circle' rule of the Polar Coordinate model is to be substituted by a 'Complete set of Compartment' rule, with at least three members of the set. The model was derived from classical observations with insect and vertebrate appendages (Meinhardt, 1983a, 1983b). It took about twelve years for a direct demonstration on the molecular-genetic level (reviewed in Vincent and Lawrence, 1994; Martin, 1995; Irvine and Rauskolb, 2000). Examples for vertebrate and insect appendages are given in Figs. 25 and 26.

According to this model a homogeneous limb field never exists since the preceding subdivision is the prerequisite of limb formation, much in contrast to Harrison's view. In view of the overwhelming evidence that now exists for this mechanism, this model seems at present to be straightforward if not trivial. In those times, however, it was very difficult to publish this idea. The paper was accepted only in the fourth journal tried (Meinhardt, 1980). In retrospect it seems difficult to understand the resistance against this model since it provides a clue to why development is so reproducible: the interpretation of a first positional information leads to borders, which, in turn give rise to new positional information that leads to a finer subdivision of the new parts. Such sequential formation of organizing borders is crucial, for instance, to regionalize the brain (reviewed in Joyner, 1996; Puelles, 2001; Prakash and Wurst, 2004; Rhinn and Brand, 2001).

J. Filamentous Branching Structures: Traces behind Moving Signals

Net-like structures are a common pattern element in all higher organisms. Blood vessels, lungs, tracheae of insects, axons or veins of leaves are examples. They are used to supply the tissue with nutrition, water, oxygen, information and drain the tissue. How can such complex patterns emerge? Local signals, generated e.g., by activator–inhibitor systems, may direct the elongation of the filament. In turn, the extending filaments cause a displacement of the signals and thus further elongation (Meinhardt, 1976, 1982). The orientation of the elongating filament is controlled by a tropic factor that is removed by the filaments, and which is required for signal generation. Its concentration is a measure of how urgently the cells need the approach of new filaments, for instance, to remove an oxygen deficiency. In the simulation Fig. 27, it is assumed that local signals are generated by an activator (gray squares)–inhibitor system. Cells respond by differentiating into members of the filament system. Differentiated cells are assumed to remove the tropic substance (hatched in Fig. 27), which is assumed to be a cofactor in the generation of the elongating signal. The resulting valleys of the factor around the filaments orients further extension, normally away from the existing filament, i.e., in the forward direction. Due to the lateral inhibition in the signal generation, the elongation is sensitive to minute concentration differences. Branch formation occurs in two ways. Either new elongating signals are triggered along the filaments behind the tip, causing lateral branching. Alternatively, the signal at the tip can split, causing a dichotomous branching.

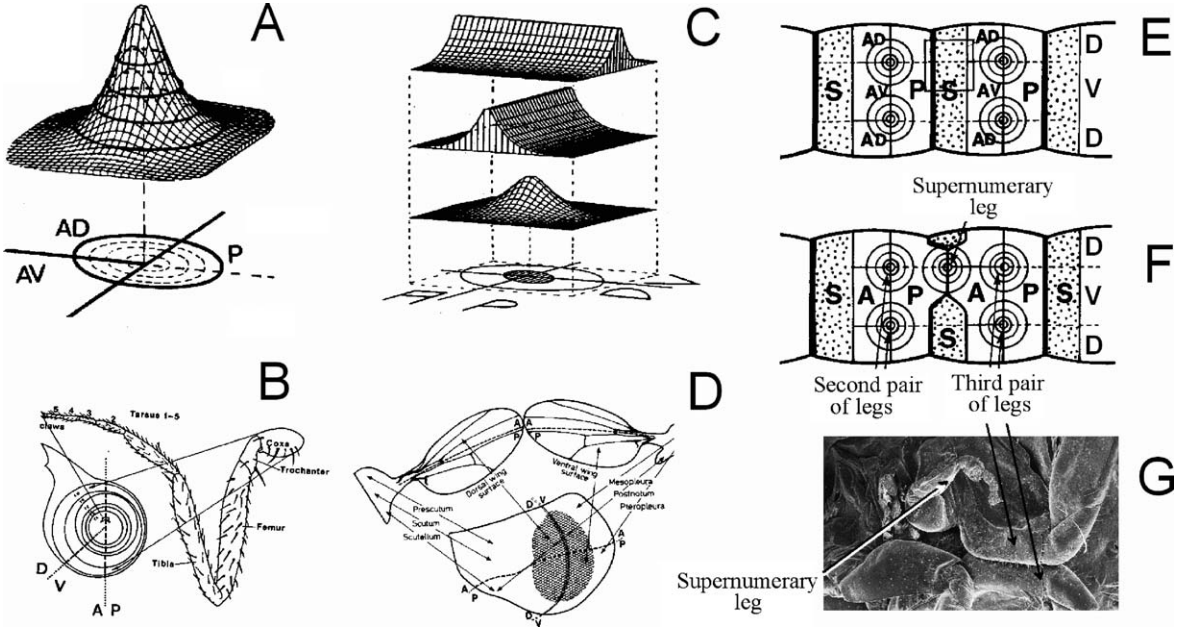

Figure 26 A boundary model for insect appendages (Meinhardt, 1982, 1983a, 1986b). The AP subdivision that occurs during primary segment formation (Fig. 23) is assumed to be the precondition for the formation of imaginal disks. (A, B) For the leg disk, a ventral compartment within the anterior compartment leads to a subdivision into three sectors. The morphogen for distal outgrowth is produced only at the region where the two borders intersect, i.e., where the three sectors are close to each other. The three sectors are characterized by *wingless* (AD), *dpp* (AV) and *en* (P). The cone-shaped distribution is appropriate for the circular fate map of the leg disk shown in B (for the molecular details see Diaz-Benjumena *et al.*, 1994; Irvine and Rauskolb, 2000). (C–D) For the wing disk, the posterior compartment also has a DV subdivision. The DV border gives rise to the wing margin. The cone-shaped proximodistal signal leads to the separation of the wing blade. (E–F) Induction of a seventh leg by removal of nonleg-forming epidermis (Bohn, 1974). In terms of the model, after removal of tissue (square in E) that separates one AP-boundary region from the next, a new AP confrontation is generated (F) that has the opposite polarity (PA). If the operation is done on the right side, a left-handed supernumerary limb emerges since the DV polarity remains unchanged. (G) A supernumerary limb generated in this way (specimen kindly provided by Horst Bohn) (Figures partially from Meinhardt, 1982, 1986b.)

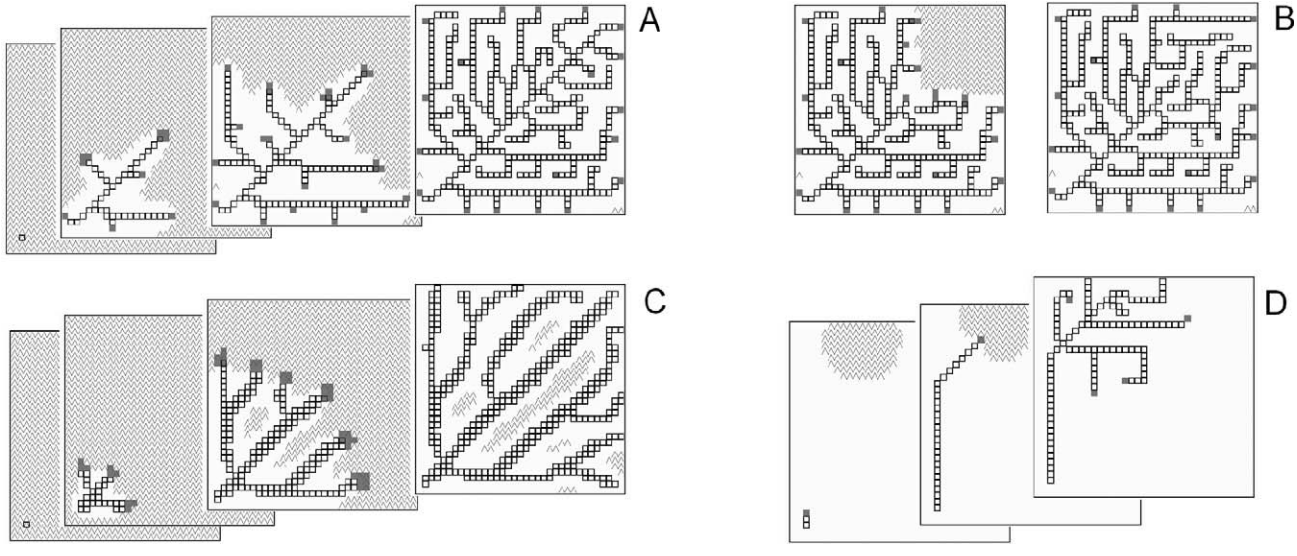

Figure 27 Formation of filamentous branched structures as a trace behind moving signals. Assumed is that local signals (filled gray squares) are generated by an activator–inhibitor mechanism. The local signal leads to cell differentiation (open squares; see Fig. 19). The differentiated cells remove a trophic substrate (hatched) that is required for signal generation. Thus, lowering of the substrate concentration by a newly differentiated cell leads to a destabilization of the signal and to its shift to that adjacent region that has the highest concentration of the trophic substrate. (A) Starting with a single cell, the filament becomes elongated towards a region of high substrate concentration, i.e., towards regions that are not sufficiently supplied by the veins. Branches can be formed by triggering new elongation signals along existing filaments. This occurs if the inhibition from active tips is low enough and the concentration of the tropic factor is sufficiently high. Branching occurs preferentially with a 90° angle. (B) Regeneration: After partial removal of a branched network, substrate accumulates again, causing new filaments to extend into the filament-deprived region. The regenerated pattern is similar but not identical to the original one. (C) If the activator autocatalysis saturates, branch formation can occur by peak splitting at the growing tip (see Fig. 8). (D) If the tropic substrate is only locally produced, filaments extend towards this region. Branching may be restricted to the region of high substrate production (Meinhardt, 1976, 1982). Addition of new cells is only one of the possible modes. Local signals also can elicit cell protrusions that lead to long filaments that consists of single cells (Fig. 11F).

The recruitment of new cells is one of the mechanisms for the elongation of filaments. Alternatively, single cells can form filaments by local extensions. A mechanism for the localization of signals on single cells under the influence of a trophic factor was given above (Fig. 11F–11J).

For the trachea of insects, most of the expected components now have been found. The following list compares the expected components and the corresponding genes so far isolated. An autoregulatory gene is expected, which allows cells to remember that they belong to the tracheal network. The corresponding gene is *trachealess* (Wilk *et al.*, 1996). A second autocatalytic loop is expected that is responsible for the generation of the local signal for filament elongation. A candidate is the transcription factor *drifter* (Anderson *et al.*, 1996). *branchless*, an *FGF*, is involved in the signal generation in front of the tracheae (Sutherland *et al.*, 1996). The corresponding receptor is *breathless* (Glazer and Shilo, 1991). In the generation of tracheae (Ghabrial and Krasnow, 2006) and of the vascular system (Hellström *et al.*, 2007; Siekmann and Lawson, 2007a, 2007b; Leslie *et al.*, 2007), the tip cells are selected by the Delta–Notch system, i.e., by a lateral inhibition mechanism as predicted. Only the selected cells send out protrusions for elongation. The trophic molecule in the formation of blood vessels is the vascular endothelial growth factor, VEGF (reviewed in Risau and Flamme, 1995; Coultas *et al.*, 2005). The secreted form of VEGF is synthesized by organs that attract blood vessels such as the brain or the kidney. Local overexpression causes hypervascularization. High amounts of this factor have been detected during embryonic and tumor angiogenesis. It orients chemotactically the elongation of the vessels.

Lung branching occurs at an epithelial–mesenchymal interface (for review see Cardoso and Lü, 2006; Chuang and McMahon, 2003). Local *FGF* maxima next to the tips attract the tips, which, in turn, cause a displacement of the *FGF* maxima. The tips are characterized by a high *Shh* level. Dichotomous and lateral branching does occur. *BMP4* seems to be one of the inhibitory components.

In leaf venation, the trophic factor seems to be auxin which is actively transported towards the roots. Veins extend towards higher auxin concentrations and local applications of auxin can attract new veins (Sachs, 1981), as shown in the simulation in Fig. 27D. The mutual competition of the cells for vein elongation seems to be based on a feedback loop that involves polarization of Auxin transport, not by a long-ranging inhibition as expected in the original model. This allows the formation of closed loops (Scarpella *et al.*, 2006). However, many details that would be crucial for more realistic modeling are still unknown, for instance, the auxin concentration within the veins. Thus, a satisfying model of the complex leaf venation pattern based on auxin transport is not yet available.

V. Conclusion

Modeling reveals the minimum requirements for essential steps in development. Self-enhancement and long-range inhibition were proposed to be the driving forces in

pattern formation and in the generation of organizing regions. Self-enhancement and competition were found also to be decisive for space-dependent gene activation. Boundaries generated on this basis organize emergent substructures. Hydra was originally chosen as a model system to get insights into basic mechanisms underlying biological pattern formation. More recent modeling suggests that this radially-symmetric animal can also provide key insights into the establishment of a secondary axis and the evolution of the bilateral body plan of higher animals. There are still many important aspects that wait for future modeling such as growth control, proportioning of the body parts, pattern formation within cells and organization of cell migration in the course of development. Mathematically based modeling combined with computer simulations is expected to contribute to uncovering more of the basic principles on which development is based and to keep the complex web of interactions comprehensible.

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