MODELS OF BIOLOGICAL PATTERN FORMATION

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Abstract

The development of a higher organism is controlled by a complex network of biochemical reactions that are under genetic control. In the following a short overview is given for some of the models we have proposed to describe essential steps in this process. Many of these models have found meanwhile direct support by molecular-genetic experiments. By computer simulations it has been shown that the models describe many of the observed phenomena (for details and animated simulations see http://www.eb.tuebingen.mpg.de/meinhardt)

1. Basic mechanisms of biological pattern formation

For the generation of patterns in originally more ore less homogeneous tissues we have proposed that local self-enhancement and long-range inhibition is the driving force [1, 4]. Basic types of patterns can be generated in this way: organizing regions, gradients, periodic structures and stripe-like patterns. The simulations in Fig. 1 provide some examples.

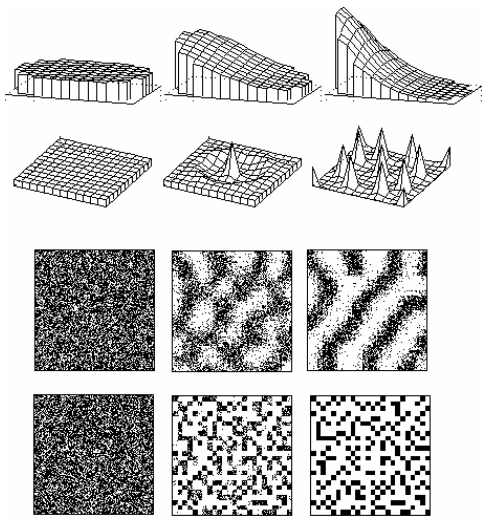

Fig. 1: Elementary patterns generated by local self-enhancement and long-ranging inhibition. Left is the initial situation, at right the final stable pattern. An intermediate pattern is at the centre. If the range of the activator is compatible with the field size, graded distributions emerge. Such a pattern is able to generate polarity in an initially homogeneous field (top). If the size is larger than the range of the inhibitor, periodic patterns are formed. If the autoregulation saturates at high activator concentrations, stripe-like patterns are formed [15]. In the absence of activator diffusion, activated and non-activated cells are distributed in a salt-and-pepper fashion.

The following set of equations describes a prototype of an interaction between an autocatalytic activator *a* and the inhibitor *b* that allows pattern formation:

$$\frac{\partial a}{\partial t} = s\,\frac{a^2 + b_a}{b} - r_a\,a + D_a\,\frac{\partial^2 a}{\partial x^2} \qquad (1a)$$

$$\frac{\partial b}{\partial t} = s\,a^2 - r_b\,b + D_b\,\frac{\partial^2 b}{\partial x^2} + b_b \qquad (1b)$$

Eq. 1a can be read as follows: the change of the activator concentration per time unit is given by the production, by the decay and by the exchange with neighbouring cells due to diffusion. The non-linear self-activation of the production of the activator *a* is crucial. This production is slowed down by the inhibitor *b*. The production is proportional to the source density *s*, which describes the ability of the cells to perform the autocatalytic reaction. In order that pattern formation can occur the inhibitor has to diffuse much faster *($D_b >> D_a$)*. The pattern will be stable in time if the decay rate of the inhibitor is higher than that of the activator *($r_b > r_a$)*, otherwise oscillations will occur. b_a describes a small activator-independent production rate of the activator that is necessary to initiate the autocatalytic activator production at low levels of *a*, e.g. during regeneration. In most simulations *s* is assumed to be uniformly distributed except some small random fluctuations that initiate the patterns and that remain constant during the simulation (except simulation shown in Fig. 3 that includes changes in the source density).

Biological pattern formation shows in many situations a high degree of pattern regulation. This is a feature of the reaction described above. For instance, after the removal of the activated region, the inhibitor decays until the autocatalysis triggers again, restoring the original pattern.

The antagonistic reaction can also be based on the depletion of a substrate or co-factor that is required for activator autocatalysis. The following equation provides an example:

$$\frac{\partial a}{\partial t} = s\,b\,a^2 - r_a\,a + D_a\,\frac{\partial^2 a}{\partial x^2} \qquad (2a)$$

$$\frac{\partial b}{\partial t} = b_b - s\,b\,a^2 - r_b\,b + D_b\,\frac{\partial^2 b}{\partial x^2} \qquad (2b)$$

In this interaction, the substrate or co-factor *b* is produced by all cells of the field in which the reaction takes place with the constant rate b_b and removed during the autocatalytic activator production. As shown in the simulation below, in growing fields such an interaction leads to maxima that have the tendency to split. A maximum has the tendency to shift towards regions where higher substrate concentration is available. This will play a role in the formation of net-like structures (see Fig. 7)

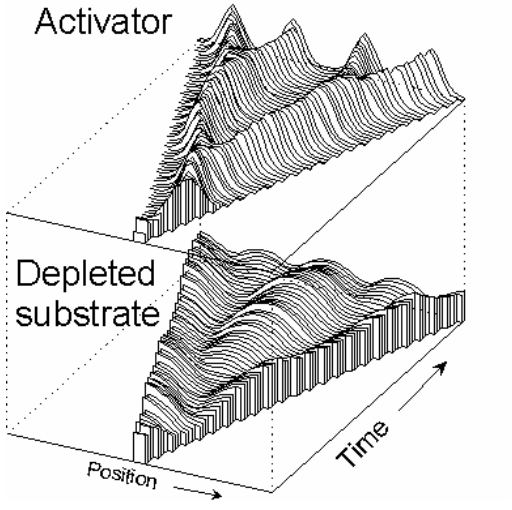

Fig. 2: Pattern formation in which a production of an autocatalytic activator (top) proceeds on the expense of a factor (bottom) that becomes depleted during activator synthesis (Equation 2). Again, the co-factor must be long-ranging. Shown is the activator and co-factor (depleted substrate) distribution as function of time in a growing linear field of cells. Whenever the distance between maxima exceeds a certain level, the maxima will split. In this way, the distance between maxima remains essentially constant.

2. How to maintain a graded distribution

In a simple pattern forming reaction a graded concentration profile can be maintained only over a range of about a factor two. With a further increase of the field size, the range of the antagonistic reaction becomes insufficient. Transitions from the polar into symmetric and ultimately into periodic distributions would occur, either by insertion of new or by splitting of existing maxima. This is inappropriate if the graded concentration profile should be used in the growing embryo as positional information for the determination of the primary body axes. Multiple maxima could lead to severe malformations such as the formation of several partially fused embryos instead of one. Observations clearly demonstrate that nature was able to solve this problem. Again Hydra is a good example. Its polar nature is maintained over a wide range of sizes.

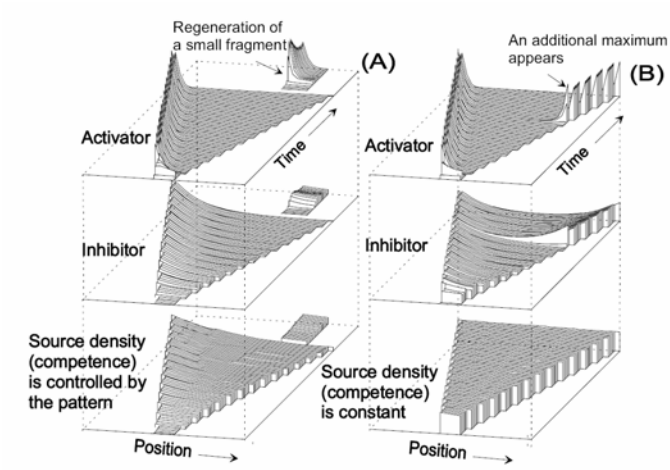

Fig. 3: The maintenance of a polar pattern during growth: the solution of the wavelength problem. (A) An activator - inhibitor system with an additional feedback on the ability of the cells to perform the pattern forming reaction (*s* in Eq 1 and 3; source density, bottom distribution). At small field size, only a marginal maximum can be formed. This leads on a long time scale to a graded *s* distribution. Due to the reduced *s* level in regions distant to the activated (organizing) region, a further activation is efficiently suppressed. After removal of the head and thus of the inhibitor-producing region, pattern regeneration is possible and occurs due to the ***s*** gradient according to the original polarity, in agreement with observations in many systems. Since the wavelength of the activator-inhibitor system is much smaller that the field size, also small fragments are able to regenerate, in agreement with the observation. (B) Without this feedback, i.e., if the source density remains unchanged, secondary maxima can arise.

A single maximum can be stabilized if cells distant to an established maximum loose the capability to perform the pattern forming reaction [7]. This capability of the cells we have called source density (bottom distribution in Fig. 3), corresponding to the observable feature of competence. Cells at larger distance become unable to compete with the primary maximum. To achieve a smoothly graded competence it is assumed that either the activator or the inhibitor has a positive feedback on the competence. Together with Eq.1a,b the feedback of the pattern forming system on the ability to perform the autocatalysis can have the following form (with $r_s << r_a$)

$$\frac{\partial s}{\partial t} = r_s\, a - r_s\, s + D_s \frac{\partial^2 s}{\partial x^2} + b_s \qquad (3)$$

In a region distant to the primary activation, the initiation of secondary maxima becomes less likely due to the reduced competence. Thus, the maximum that has been formed at a small size becomes dominant during further outgrowth.

In many systems, regeneration occurs in such a way that the polarity is maintained. Again, Hydra is a well-known example. This is a straightforward consequence of the model. The graded source density keeps track of the polarity. A small fragment regenerates a pattern according to the origi-

nal polarity since the graded source density is always higher in those cells originally closer to the organizing region (Fig.3). These cells have a head start in the competition to form the new organizing region. Regeneration can proceed faster since no symmetry breaking is required.

According to this model, an organizing region has two opposing influences on the surrounding tissue. The long-ranging inhibitory effect prevents the formation of additional organizing regions in the surrounding tissues. A positive feedback keeps only the surrounding cells competent. Why both effects do not simply cancel each other? The two effects have different time constants. After (partial) removal of an organizing region, the inhibition decays rapidly in order that regulation can occur. In contrast, competence should have a much longer time constant. It has to remain almost unchanged at the time scale required for pattern regulation (Fig. 3). Thus, not only the formation of a pattern is an important step. To make development reproducible and to suppress malformations it is also essential that the capability to form a particular pattern fades away at the correct later stage.

Hydra has only a single axis. It can be regarded as a living fossil that tells us about evolutionary inventions necessary to achieve bilateral symmetry. The crucial step was the formation of a midline of the body. As shown in Fig. 1, stripe like patterns result by a saturation of autocatalysis. However, this leads to many stripes. The formation of a single stripe requires a cooperation of a patch-forming and a stripe-forming system. The patch-forming system makes sure that only a single stripe is formed while the stripe forming system is responsible for the formation a high concentration in a continuous line that is stable against decay into individual patches. We have shown that insects and vertebrates use different strategies [9, 16]. In vertebrates, a patch-like system, the node, *elongates* a stripe like system, e.g. the notochord. In insects, a dorsal organizer *repels* the midline to the ventral side.

3. How to generate structures close to each other, how at a distance: head, foot and tentacle formation in Hydra

The complexity of the patterns in higher organisms requires a hierarchical linkage of many pattern forming reactions. One or more patterns generate the precondition for a subsequent pattern. For instance, by an appropriate coupling it can be achieved that two pattern forming systems (anteroposterior, dorsoventral) emerge perpendicular to each other [16]. The combinatorial possibilities are very large, making modelling very difficult. That nevertheless the modelling of complex patterning processes is possible should be illustrated with a model for the freshwater polyp Hydra (Fig . 4).

Hydra is under control of two organizing regions located at opposite ends of the tissue, the head and the foot. This is common in many morphogenetic fields. How can it be achieved that two organizing centres reliably appear at opposite positions of an extended field? For Hydra a simple cross-inhibition is not appropriate since in small (young) animals head and foot must appear very close together. If at such short distances a mutual inhibition between the head and the foot system would be at work, this would lead to a suppression of the foot by the nearby head or vice versa. This problem disappears when the spacing between the head and foot system is achieved by an interaction by an employing of the source density. As mentioned above, the head activation appears at the position of the highest source density and elevates the source density further. If the foot system has the opposite behaviour, i.e., it appears at the lowest source density, the foot is formed at the maximum distance from the head.

(Fig. 4). Nevertheless, head and foot system can coexist at a close neighbourhood in small animals since no direct inhibition is involved. The graded source density only generates a preference. Experimental evidence indicates that the foot also lowers the source density, contributing in this way to the maintenance of the source density gradient. Evidence for the involvement of a self-enhancing Reaction in foot formation has been found by the Bosch-Group [20]

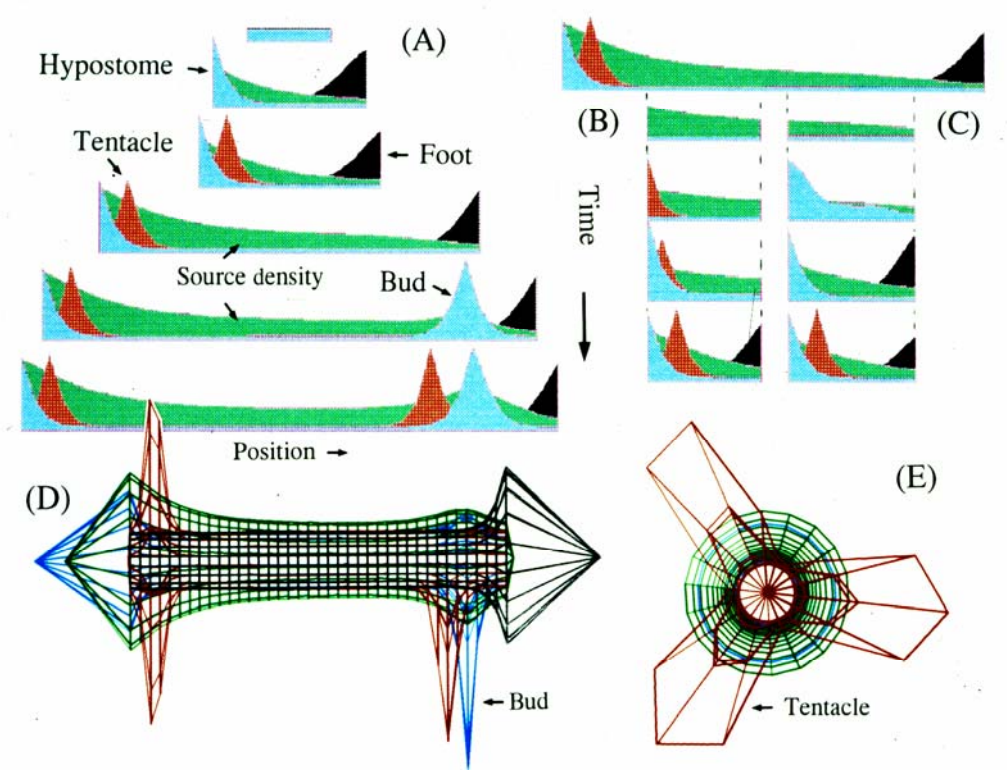

Fig. 4: Generation of complex patterns by linkage of several pattern forming reaction. (a) The fresh water polyp Hydra. (b-f) Simulation of hypostome, tentacle, bud and foot formation in Hydra. (b) Primary head (blue) and foot activation (black) appear at opposite end of the field due a coupling via the source density (green). Tentacle activation (red) appears close to the hypostome since it requires a high source density but it is locally suppressed by head activation. Budding results from a second head activation. Due to the long-ranging head inhibitor, this can occur only at a large distance from the original head. (c) In regenerating near-head fragments tentacle activation precedes head activation. It occurs first at the tip and shifts later on, in agreement with the experimental observations. (d) In more basal fragments head activation appears first. Tentacle activation takes place later at the final position after the source density has obtained a threshold value. (e, f) Lateral and top view of a Hydra simulated as a cylinder: the periodic arrangement of the tentacles (red) around the hypostome and the lateral localization of the bud (blue) is correctly described. (The simulations describe only pattern of the signalling molecules on a cylinder, but not the subsequent shape changes of the tissue; after [7]).

Many structures emerge during development close to each other in a precise arrangement. We have shown that a controlled neighbourhood of structures is enforced if one structure activates the other on long range but excludes it locally [19]. In Hydra, the tentacles appear around the hypostome, the opening of the gastric column. Many experiments can be accounted for by the assumption that tentacles are under control of a separate activator-inhibitor system that also depends on the source density. Since the source density increases under the influence of the primary head system, the latter generates the precondition for tentacle initiation. Locally, however, the head signal suppresses tentacle formation. Thus, tentacle formation is possible only at a subhypostomal position (Fig. 4B,D).

The model accounts for a strange-appearing observation. With tentacle-specific antibodies and with the expression patterns an *aristaless* homolog Bode et al. [18] have shown that after head removal, tentacle activation first reappears at the very tip of the gastric column. It is only later that this activation becomes shifted to the position where the tentacles eventually appear. Since the tentacles are formed close together, the tentacle inhibitor needs not to diffuse very far. In terms of the model, the tentacle inhibitor can have a short half life. Thus, after removal of the head and the tentacles, the tentacle inhibitor fades away more rapidly than the head inhibitor. Therefore, the tentacle activator can reappear sooner than the head activator. Since no suppressing head activator is present, this happens at the highest possible source density, at the front end of the remaining gastric column (Fig. 4B. After the trigger of the primary head activation at the same position, tentacle activation becomes shifted to the final location. The prediction that the sequence of events is the reverse in more basal fragments (Fig. 4C) or in buds has found meanwhile direct experimental support [17, 18].

4. Gene activation: a pattern formation among alternative genes

The temporary nature of signalling systems based on diffusion allows pattern formation only at a small scale. The formation of a large organism requires a permanent memory within the cells to which signals they have been exposed at earlier stages. Gene activation also requires self-enhancement and competition to allow the activation of only a particular gene among several alternative genes. Thus, cell differentiation can be regarded as a pattern formation in the gene space [4].

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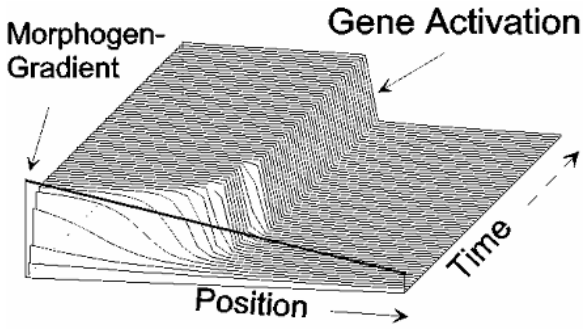

Fig. 5: A stable switch-like activation of a gene based on an autocatalytic feedback loop that saturates at high concentration (Equation 3). Only those cells that are exposed to a certain threshold concentration in the morphogen concentration *m* switch from a low into a high concentration.

The equation below shows a very simple case to illustrate that a positive feedback can lead to threshold behaviour. A gene is assumed whose gene product *g* has a non-linear feedback on the activation of its own gene [3].

$$\frac{\partial g}{\partial t} = \frac{r_g g^2}{1+\kappa g^2} + r_g g + m \qquad (4)$$

At low *g* concentrations, the negative term dominates and the g-level will decline further. From a certain threshold level onwards, the autoregulatory term dominates and the concentration increases further until the saturation level is reached (Fig. 5). A graded external signal m leads to stable the activation of the gene whenever *m* is above a certain threshold. This gene activation would remain unchanged in this sharply confined region even after switching off of the signal.

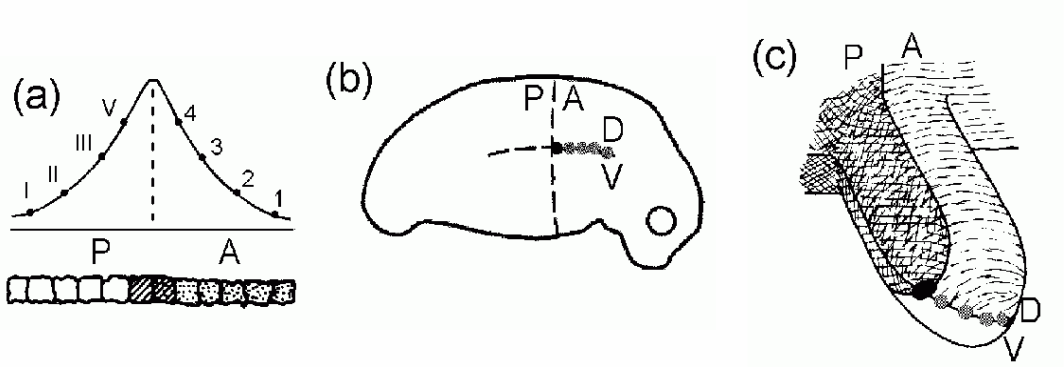

Fig. 6: Initiation of limbs at the intersection of two differentiation borders [5]. (a) If two cell types, e.g., anterior (A) and posterior (P) have to cooperate to produce of a new signaling molecule, its production is restricted to the region close to common border (hatched). (b) The intersections of two borders separating A / P and D / V cells, one along the anteroposterior and on along the dorsoventral (back-to-belly) axis defines two unique points, one on the left and one of the right side of the organism. (c) Such interactions define new coordinate systems used for initiation substructures such as legs and wings. In vertebrate limb and wing formation only the A-cells are competent to respond to the signal. The A-region is exposed to graded signal concentrations. The type of the digit depends local signal concentration and thus on the distance of the cell to the AP border; the little finger requires the highest concentration. The digits are formed along a border that separates dorsal and ventral (D/V) regions. Therefore, the digits emerge in a plane.

5. Initiation of substructures such as legs and wings

How it is achieved that wings and legs are always formed at particular positions of an organism, with a particular handedness and a particular orientation relative to the main body axes of the embryo? The complex structure of a higher organism requires the reproducible generation of new coordinate systems for sub-patterns. Such a new coordinates system can be generated if differently determined cells cooperate with each other to produce a new set of signalling substances [5, 6]. New signalling regions emerge that are centred over differentiation borders. The formation of a new coordinate system for a leg or a wing requires the intersection of two differentiation borders, one with A/P, and the other with a DV orientation (Fig. 6). This model has found meanwhile much support in insects and vertebrates [14, 2].

6. Generation of net-like structures

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

As shown above, the activator/inhibitor systems allow the generation of local maxima (Fig. 1). Such signals can be used to trigger stable gene activation when a threshold is exceeded (Fig. 5). The exposed cells differentiate and become, e.g., part of a vascular system. It is assumed further that differentiated cells repel the signal. The signal will be shifted into a neighbouring cell that

will differentiate too. In this way, it becomes a part of the vascular system. A repetition of this process - differentiation, shift of the signal, differentiation - leads to a strand of differentiated cells behind a wandering activator maximum (Fig. 7).

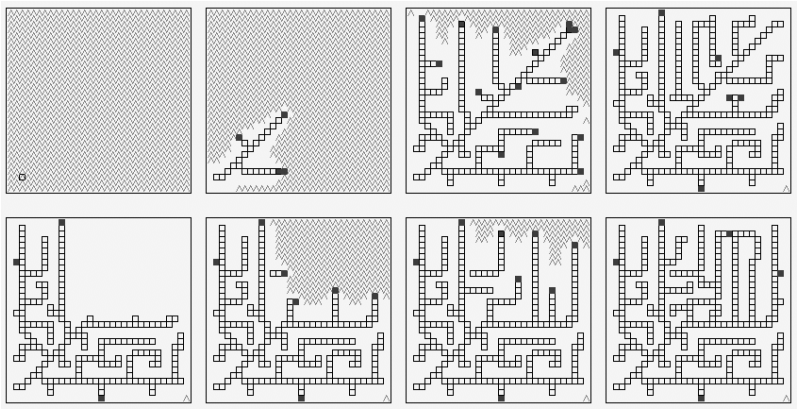

Fig. 7: Formation of a net-like structure. The interaction of four substances is sufficient to generate a structure with branching filaments (Equation 5a-d). A signal for the local elongation of the filament is generated by an activator *a* (black dots) / inhibitor system. In this simulation the signal is used to differentiate cells (squares). Differentiated cells remove a substrate *c* (wavy lines). Since the activator/inhibitor system depends on this substrate, the activator maximum is shifted to that neighbouring cell which has the largest distance from other differentiated cells. This is usually the cell in front of tip of the filament. The patterning process comes to rest if a certain density of the filaments is reached [3]. As shown in the lower panel, the model describes the regeneration of a net-like structure after partial removal of filaments.

For the simulation, the following set of equations has been used [3]; it is a combination of the equation given above).

$$\frac{\partial a}{\partial t} = \frac{sca^2}{b} - r_a a + D_a \Delta_a + b_a d \quad (5a); \qquad \frac{\partial b}{\partial t} = sca^2 - r_b b + D_b \Delta b + b_b d \quad (5b)$$

$$\frac{\partial c}{\partial t} = b_c - r_c c - c_c c\, d + D_c \Delta c \quad (5c); \qquad \frac{\partial d}{\partial t} = \frac{r_d\, d^2}{1 + s_d d} - r_d d + b_d a \quad (5d)$$

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

This simple example shows that by superposition of several reactions leads to pattern-forming systems that have a far richer repertoire then the components. This allows a tailoring of systems such that requirements for specific developmental situations are met.

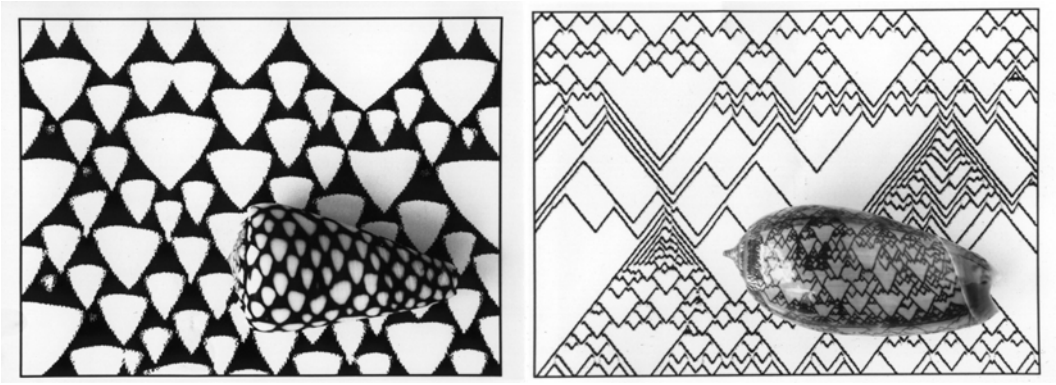

Fig. 8: Pigment pattern on tropical sea shell. The pattern on *Conus marmoreus* (left) results from a permanent change between a widening and breakdown of a steady state pigment reaction The pattern on *Oliva porphyria* result from travelling waves that generate backwards-running waves whenever the number of waves drop below a critical level [12, 10].

7. Pigment patterns on shells of tropical sea shells and centre finding in bacteria: Highly dynamic pattern emerge by destabilization of a once established pattern.

Since a mollusc can enlarge it shell only by accretion of new material at the margin, the patterns on the shells are a time record of a one-dimensional patterning process. They provide a natural picture book to study dynamic systems. Travelling waves play a crucial role therein, which have unusual properties. For instance, they can penetrate each other in a soliton-like fashion (crossing lines on the shells) or waves can split, forming spontaneously backwards-running waves. The complex patterns result from several superimposed patterning systems. Thus, since shells preserve a time record of the pattern along the growing edge, they provide a unique opportunity to decipher the logic of the underlying mechanism.

Central in shell patterning is that a once generated pattern becomes destabilized shortly after their generation. Other biological systems use the same trick. The initiation of new leaves in Phyllotaxis [11, 15], the orientation of cells or growth cones by minute external signals [8] or the determination of the division plane in an E.coli bacterium [13] are examples. These mechanisms allow a dynamic adaptation to changing conditions although non-linear reactions have normally a substantial hysteresis. Fig. 7 shows two shells in front of corresponding simulations.

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