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From observations to paradigms; the importance of theories and models

An interview with Hans Meinhardt

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Hans Meinhardt received his PhD in physics from the University of Cologne at 1966. For a postdoctoral fellowship, he went to the European High Energy Laboratory CERN in Geneva where he joined a group working on the leptonic decay of the Xi-minus particle. One of his duties was to perform computer simulations to optimize the complex experimental setup - a skill which turned out to be helpful later on. In 1969 he switched to biology and joined the department of Alfred Gierer at the Max Planck Institute for Developmental Biology (formerly Virus Research) in Tübingen. His interest was focused on mechanisms of biological pattern formation. Using computer simulations as a tool, he developed models for essential steps in development. Most fascinating for him was the possibility to recapitulate and to reconstruct–using the computer the genesis of structures where no structures were before and to see how these emerging structures become subsequently further refined. In addition to the interaction with Alfred Gierer and his group working on hydra development, the Max-Planck Institute as a whole provided a very stimulating environment. In the seventies, the work of Klaus Sander on gradients in early insect development was highly influential. Collaboration with Martin Klinger in the eighties revealed that the pigmentation patterns on tropical sea shells are convenient to study highly dynamic patterning processes. The variability and the asthetic beauty of these patterns turned out to result from the chaotic nature of the underlying reactions. Mechanisms deduced from shell patterns became a key to understand other developing systems such as orientation of chemotactic cells or phyllotaxis. Officially Hans Meinhardt retired at the end of 2003. At present he works on refinements and extensions of models which account for the different modes of embryonic axis formation in different phyla from an evolutionary point of view.

As far as I know, you are a physicist (or mathematician?) by your background. How did you become so involved in biological problems? Aren't you disappointed by your contacts with this somehow non-precise science?

Indeed, I was trained as an experimental physicist. My PhD work dealt with a problem of the so-called weak interaction, a force that is involved in β-decay. Afterwards I joined a group at the European High Energy Research Institute CERN in Geneva involved in the determination of the leptonic decay rate of the Xi-minus particle. Although this was an exiting time for me, it was not satisfactory. The experiments were so work-intensive that no time remained to go deeply into the underlying theories. Without a full understanding of the theory, however, the insights I could gain

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The authors of the activator-inhibitor model: Hans Meinhardt (to the left) and Alfred Gierer.

from our experimental results were limited. This lead to my decision to change my field of research.

To find a new one, I visited many labs where former colleagues were working. I expected more enthusiasm then I could build up for high-energy physics. To my surprise I found a lot of frustration, even by those who went into biology. (" I know that sugar can enter an *E. coli* cell, but why should I spend so much of my life time to find out how"). During this search I came by accident to Tübingen. First Kuno Kirschfeld explained to me how a fly sees the world and his fascination for his research was infecting. At this time, however, there was no open opportunity in his group. His recommendation to cross the road to visit Alfred Gierer turned out to be decisive for my further life.

Alfred Gierer was interested in how pattern formation is achieved. The freshwater polyp Hydra served in his group as a model system. His intention was to explore in addition another new field: what distinguishes differently determined cells - at those times a completely open problem. It was clear that the genetic information is the same in all cells and that differentiation must be accomplished by regulatory proteins. The involvement of histones could be ruled out since these are also (more or less) the same in all cells. So, I started 1969 with the isolation of non-histone proteins of lymphocytes obtained from freshly slaughtered cows. This work, however, was again frustrating. Harsh methods where needed to separate the proteins from the DNA. The presumably denatured proteins I recovered from my columns where unable to discriminate between vertebrate and *E.coli* DNA. Appropriate methods such as footprinting were not available at those times.

In this period during the early seventies Günter Gerisch gave a fascinating seminar on *Dictyostelium* and how these cells find each other by coordinated oscillations. Oscillations are very common in physics. In my work at CERN, I learned how to perform complex computer simulations. So, the idea came up to simulate the aggregation of *Dictyostelium*, more for curiosity than as a new working field. On the very next day I met Alfred Gierer and proposed such an approach. His response was unexpected for me. He told me that he had also something that should be simulated. It turned out that he had a theory of biological pattern formation almost ready in his mind. By means of the simulations we performed over the next months, it became evident that all his ideas worked extremely well. Such simulations where at those times an entering into unknown territory, at least for me. All the numerical approximations and the graphic representations had to be newly developed. Computers were foreign in a biological institute. Each run of a program required a visit to the downtown computer centre of the university with piles of punched cards. A single error in the code had the consequence that one had to wait for another day to run the program once again.

Thus, the reasons to change eventually to biology were more emotional than rational. Personalities played a more important role than the future subject in itself. This decision I never regret. The possibility to contribute to the basic problems of how structures emerge from apparently structure-less initial situations was most exiting for me. In this way, I found my field.

How did you encounter Turing's work on morphogenesis, a work that was so popular in the 60s and even in the 70s? What were your impressions and how did this interact with your own line of thinking?

Turing's work (Turing, 1952) was not very popular at the beginning of the seventies. We became aware of this now famous paper only by a comment of a critical reader to our first paper, i.e.,

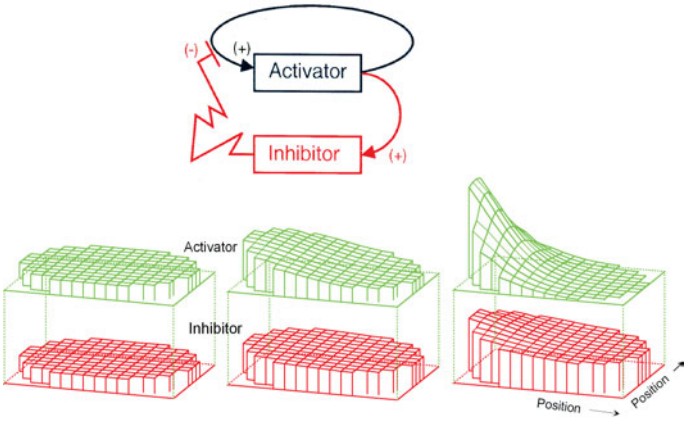


Fig. 1. The interaction of a short-ranging autocatalytic activator with a long-ranging inhibitor can lead to stable patterns in space *(Gierer and Meinhardt, 1972; Meinhardt, 1982; for animated simulations see http://www.eb.tuebingen.mpg.de/meinhardt). The simulations show an initial, an intermediate and the finally stable distribution. Random fluctuations are sufficient for initiation. If the range of the inhibitor covers the whole field, only a single maximum can appear, appropriate to generate an organizing region and graded distributions. Thus, although all cells have the same genetic information, this mechanism generates the prerequisite that different genes become activated in different parts of the developing embryo (see also Fig. 5).*

after finishing our work. Turing's approach was entirely mathematical; investigating under which condition a small perturbation can grow and obtain a new stable steady state. He did not provide an intuitive explanation of how the mechanism works. The concept of lateral inhibition does not occur in his paper. We came from a biological point of view. Lateral inhibition was postulated long before, e.g., for the spacing of leaves or for hydra patterning. The work of the sister institute of Biological Cybernetics on the campus dealt with insect vision, in which lateral inhibition also plays a substantial role. The crucial question was then: how can a structure suppress the formation of a similar structure in its neighbourhood without inhibiting itself, being in the centre of this inhibition? We have shown that lateral inhibition has to be complemented by a non-linear local self-enhancement. Both components together make a uniform distribution unstable and a new self-regulating patterned steady state is reached when the self-enhancing reaction has attained an equilibrium with the long-ranging antagonistic component (Fig. 1). With the knowledge that local autocatalysis and long-range inhibition is the driving force in pattern formation, it is easy to see that the example equation provided by Turing satisfies our condition. Knowing the generative principle allowed us to propose appropriate non-linear reaction schemes. These are required for interactions based on a reasonable molecular kinetics.

Do you think that the main problems of biology (of morphogenesis) can be adequately formulated and solved within the framework of a physicalistic (model) approach? Or, on the contrary, do you see some fundamental biological problems which go beyond the scopes of this approach?

Crucial in development is intercellular signalling and the corresponding response of the cells by activating particular genes, which in turn, could change the signalling. Models that describe the production, spread and removal of molecules as a function of space and time can adequately reproduce these processes. These models are necessarily minimum models. This is, however their strength. In this way the models can reveal the core of a process. Frequently it is not necessary to consider all the details. For instance, signalling between cells requires the production and secretion of ligands, their reception by another cell and a signalling cascade to the nucleus. The assumption of a simple diffusion, however, is in most cases a sufficiently good approximation. Thus, modelling can reveal the logic behind a process. In my view it is an essential supplement to the presently prevailing approach in which an attempt is made to find all components involved in a particular process. A complex system has many new properties not inherent in their parts. Therefore, even if all parts are found, their functioning together may remain nevertheless unclear, unless complemented by a theoretical approach addressing the system as a whole. For instance, it was found in *E.coli* bacteria that the determination of the position where the next cell division takes place depends on a pole-to-pole oscillation. The corresponding components, the *MinCD/MinE* proteins, were well-known. The underlying mechanism, however, remained a mystery (Shapiro and Losick, 2000). By modelling, a mechanism has been found that is now widely accepted (Meinhardt and De Boer, 2001).

In my own modelling, essential processes are so far not considered. Examples are the mutual rearrangement of cells as occurring, for instance, during gastrulation. 'My cells' were mostly elements of a rigid grid. For a more complete picture one has also to include the pattern formation within a cell in which, as we believe, the same principles are at work. Inclusion of intracellular patterning is required, for instance, to describe the formation of cell sheets and their bending. This adds more challenges to the programming but does not call for a search for as yet unknown basic principles. Back to your question: I don't expect to find components that are beyond molecular physics and simulations which are based on molecular interactions and movements only should be ultimately feasible.

What do you consider as the most actual problems of morphogenesis, pattern formation studies and the related biological problems? What might be the impact of the model approach in solving these problems?

One problem in gaining more insights into morphogenesis is a strategic one. By means of the new molecular-genetic tools, many, many new components and details have been uncovered; indeed, so many that for an individual a specialization to particular model systems is unavoidable. Consequently, there are specialists for particular sub-systems such as limb formation, somites,

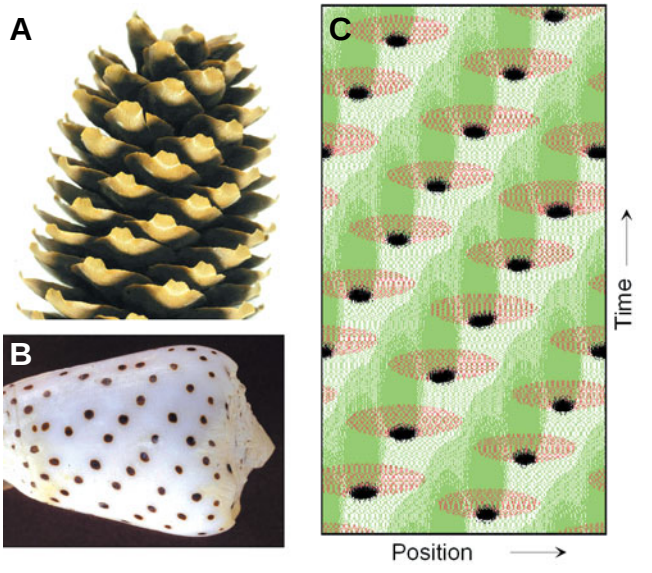


Fig. 2. A common principle behind very different-appearing patterns. (A, B) *The seedlings of a fir cone and the pigment patterns on the shell of a mollusc emerge in the course of time in a growth zone. Both patterns are therefore time records of a one-dimensional patterning process that takes place either in the ring-shaped zone next to the meristem of the plant or in the mantle gland along the growing edge of the mollusc shell. Both patterns indicate a regular displacement of local signals in the course of time.* **(C)** *Model: an activator is antagonized by* two *inhibitors. One inhibitor has a long range but a short time constant (red), responsible for the sharp localization of the signal. The other inhibitor has a short range but a long time constant (green), which extinguishes the activation (black) shortly after it is triggered. In this way localized activations flash up, disappear shortly later and reappear at displaced positions where both inhibitions are low (white regions) (Meinhardt and Klingler, 1987, Meinhardt, 2003 a,b).*

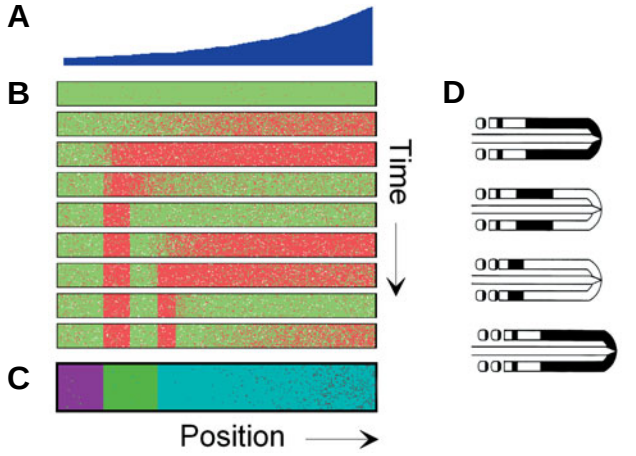


Fig. 3. Somite formation was predicted to depend on a sequential conversion of an oscillating pattern into a pattern which is stable in time *(Meinhardt, 1982).* **(A,B)** *Oscillations between two states (red/green in B) are possible only at a posterior region where the concentration of a graded substance (blue in A and now identified as* FGF, *Dubrulle* et al., *2001) is above a certain threshold. Signals appear first at the highest gradient level, i.e., at posterior. They move towards anterior and come to rest at a position where the level of the gradient is too low to drive the oscillation. The resulting pair of stable activations is assumed to cause the formation of the next pair of half-somites. Each full cycle adds one pair* **(C)**. *One reason for the predicted oscillation was that this enables a precise sequential activation of new AP-specifying genes (now HOX-genes; violet, green, blue… in C). Like the back-and-forth movement of the pendulum of a grandfather's clock which allows a controlled advancement of the pointer, the oscillation was assumed to drive a sequential activation of AP-specifying genes. Both patterns are precisely in register. This mechanism allows a counting of somites or segments on the gene level.* **(D)** *The observation of the* c-hairy *oscillation (Palmeirim* et al, *1997, drawn after Pourqui, 2003) provided the first support for the predicted oscillation. In the model, both the oscillation and the spatial pattern formation were assumed to be based on the same molecular circuit. Consistently, it has been observed that the* Notch *pathway is involved both in the oscillation and in the formation of the somite borders. However, the long-lasting antagonistic effect required for the oscillation seems to be realized by a mechanism that differs from the pathway usually involved in* Notch*-mediated lateral inhibition, by a transcriptional delay (Lewis, 2003). It was further predicted (Meinhardt, 1982) that within certain limits, the steepness of the gradient determines the size of the somites, in agreement with more recent observations (Dubrulle* et al., *2001). Evidence for the coupling of the oscillation and Hox-gene activation has also been obtained (Dubrulle* et al., *2001; Zákány* et al., *2001).*

neural crest cells, etc. The situation seems for me compatible with that described in the legend of the tower of Babel. The building came to an end since the worker could no longer find a common language. Models may be helpful in this situation. They can facilitate to memorize the many details if they provide a framework in which the details make sense. Ideally they reveal the core of a process. In this way, common principles can be found in systems that have overtly nothing in common with each other. For instance, by modelling it has turned out that an enforced displacement of signals shortly after their generation by a local quenching is a very general mechanism. The regular displacement of leaf-forming signals around an elongating shoot, the flashing up of signals to stretch out cell protrusions in chemotactic cells towards the target region or the pole-to-pole oscillation of the *Min* proteins in *E.coli* cells to localize the next cell division can be described in this way (Meinhardt and De Boer, 2001; Meinhardt, 2003a). This mechanism was found during an analysis of the never-identical pigment pattern on the shell of tropical sea shells (Fig. 2).

Among the processes for which I hope that I can still develop models are (i) planar polarity, i.e., the link of intra-cellular signalling with a signalling between adjacent cells to obtain a consistent overall polarity of the tissue; (ii) the finding of their final position by moving cells - neural crest or germ cells are examples and (iii) the generation of and the control of cell proliferation such that parts obtain their correct relation to an organ or to the whole organism. Another central interest is of how the different body plans evolved from a common ancestor, although the main body axes are organized by well-preserved pattern-forming mechanisms (see also Fig. 4).

To my mind, your main (together with Alfred Gierer) and a very fundamental contribution is the statement about the involvement of a short-range activation and a long-range inhibition (linked with "+, -" feedback) in pattern formation and morphogenesis. Do you think that these interactions can be mediated by chemical diffusible substances only, or do you consider that other physico-chemical agents participate in this feedback loop? Can you imagine any pattern formation process going on without either short-range activation, or long-range inhibition, or both?

We claim that local self-enhancement and long-range inhibition is involved whenever pattern formation, departing from an initially more or less homogeneous situation, takes place. If we look to inorganic pattern formation, e.g., the formation of sand dunes, patterns of erosion, formation of stars and galaxies, all these processes follow that scheme. Mechanisms other than diffusion might be involved in long-range communication, for instance, via the long filaments that extend from particular cells. But this would not change the general principle.

The mechanism can be different in systems which start from an initially patterned situation. For instance, I proposed in 1980 that new signalling substances are produced at borders which separate differently determined cells. New coordinate systems for substructures such as legs and wings in insects and vertebrates can be generated around the intersections of two such organizing borders (e.g., the compartment borders in *Drosophila*). This mechanism does not need short-range activation and long-range inhibition since the production of the new morphogen is from the start localized to a region close to borders that are generated in a preceding process. In view of the overwhelming evidence that now exists for this mechanism, this model seems at present to be straightforward if not trivial. At those times, however, it was very difficult to publish this idea. The paper was accepted only in the fourth journal to which it was submitted (Meinhardt, 1980). The prevailing model at those times was that first a homogeneous limb field is formed which becomes subsequently patterned in along the main body axes of the embryo. In contrast, in the model proposed, a homogeneous limb field never exists, since the preceding subdivision is the prerequisite. In retrospect, it seems difficult to understand the resistance against this model since it

provides a clue why development is so reproducible: the interpretation of a primary positional information (Fig. 1) leads to borders (see Fig. 5), which in turn give rise to new positional information that leads to a finer subdivision of the new parts and so on. Each newly formed structure has necessarily the precise relation to the patterning already accomplished.

Another question is whether autocatalysis and lateral inhibition can be realized by different means. For instance, a mechanism for gastrulation has been proposed in which self-amplification and the long-ranging antagonistic effect are realized by mechanical components such as stress and stress-release (Odell *et al.,* 1981). Such a model would predict that mechanical deformation could initiate gastrulation at the side of an experimental indentation. I am not aware of any unequivocal experiment in support of this view, but there are countless experiments where a secondary axis is induced by ectopic activation of specific genes. Thus, I believe that pattern formation is accomplished primarily by the generation of local signals based on the exchange of molecules. These signals, in turn, can lead to changes in mechanical properties, e.g., by the local modification of the cytoskeleton. This does not exclude that a cell can detect mechanical deformations, which could feed back to the signalling (for recent work see Bershadsky *et al.*, 2004; Tamada *et al.*, 2004).

Could you formulate the main experimental evidence for the existence of morphogenetic substances obeying the rules of a short-range activation – long-range inhibition?

A good example is the interaction of *Nodal* and *lefty2*. *Nodal* regulates its own activation and that of *lefty2* (Chen and Schier, 2002; Solnica-Krezel, 2003 for review). The latter, in turn, blocks the *Nodal* receptors and in this way self-enhancement. This reaction plays a role in the early patterning of the endo- and mesoderm and later in left-right patterning. *Nodal* is also required to initiate the oral field in sea urchins (Duboc *et al.*, 2004). Most interesting, in this case there are obviously no maternal determinants as to where the *Nodal* maximum has to appear – a rare case of true symmetry breaking. As expected from the model, minor external manipulations such as a unilateral reduction of oxygen are sufficient to bring the maximum to a predictable position. In the case of the sea urchin, the inhibitor seems to be *BMP2/4*. It is produced under *Nodal* control and *BMP* suppression leads to an overall activation of *Nodal* (Duboc *et al.*, 2004), as expected for an activator-inhibitor system.

An anti-dorsalizing protein involved in organizer formation has been found in the chick and in the fish (Moos *et al.*, 1995; Lele *et al.*, 2001). Its suppression or overexpression leads to an enlargement or shrinking of the organizer. At the time of its discovery it appeared counter-intuitive that a substance is produced in the organizer which down-regulates the organizer. However, this behaviour corresponds exactly to our prediction for an inhibitor.

In hydra the *β-catenin-Wnt* pathway has many properties we expected for an activator (Hobmayer *et al.*, 2000). About 1 h after head removal, the *β-catenin* and *Tcf* signals reappear. In cell aggregates, the emerging signals have initially a more smooth distribution which sharpens in the course of time, as theoretically expected. However, the precise mechanism of the autoregulation is unknown. Also the nature of the inhibitor and the mode of its spread are unknown. A possibility would be that secreted *Wnt* itself has an inhibitory influence, in contrast to intracellular *Wnt*, but this is speculation.

Self-enhancement as well as long-range inhibition can be based on indirect interactions. Then, the involvement of these essential ingredients appears less obvious and may be overlooked. An example is the *wingless (wg) / engrailed (en)* interaction in *Drosophila* segmentation: *en* has a direct positive feedback on the activation of its own gene. In contrast, long-ranging inhibition occurs indirectly by the long-range activation (via *hedgehog*) of a second feedback loop, the *wg* pathway, which locally inhibits the *en* pathway. Thus, in this case lateral inhibition does not occur by long-range self-inhibition, but by promoting a competing feedback loop. This interaction is reciprocal. The *wg* pathway has also an autoregulatory element via *sloppy paired* and secreted *wg* molecules are required to maintain *en* activity in

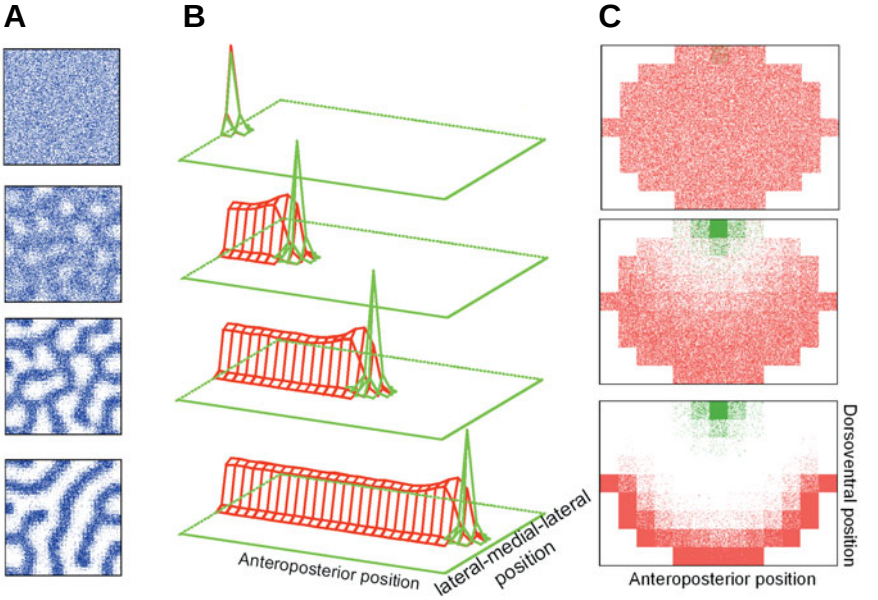


Fig. 4. The problem of midline formation. *An organizing region appropriate to pattern the dorsoventral (or mediolateral) axis of a long extended bilateral-symmetric organism must have the geometry of a single straight stripe.* **(A)** *Stripe-like distributions emerge if the autocatalysis saturates at higher activator concentrations. These stripes, however are bended and multiple stripes are formed.* **(B,C)** *Single stripe-shaped organizing regions can be formed by an interaction of a spot-forming (green) and a stripe-forming patterning system (red). In vertebrates* **(B)***, a local organizer (e.g. Hensen's node) triggers and elongates the midline sequentially towards posterior. The midline appears dorsal. In insects* **(C)***, a dorsal organizer has an inhibiting influence on the midline. The midline appears at the largest possible distance to the dorsal organizer, i.e., ventral. It has from the beginning the full AP extension but becomes more refined along the mediolateral extension (Meinhardt, 2004). This is in agreement with recent observations in* Tribolium *(Chen* et al.*, 2000). Thus, these models account for the different dynamics of midline formation in both phyla.*

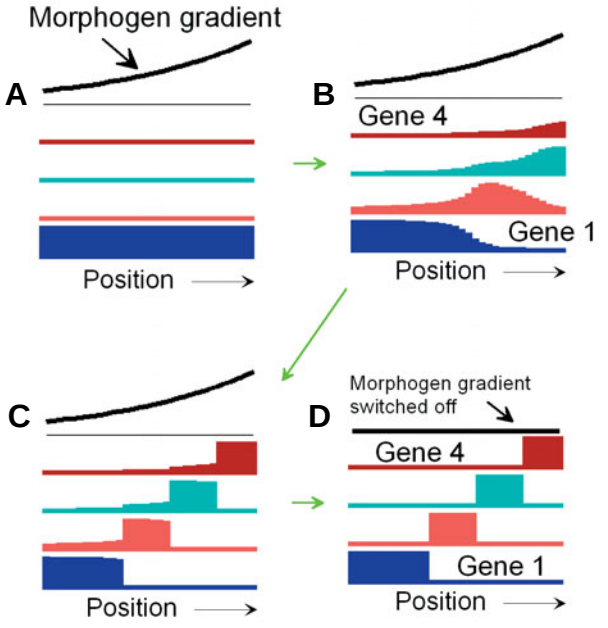


Fig. 5. Model for the space-dependent activation of genes. (A-C) *Assumed are genes (1, … 4) who's gene products feed back positively on activation of their own gene. Due to their mutual repression, within one cell only one gene of the set can be active. The morphogen is assumed to accomplish a stepwise and irreversible transition from one gene to the next. The transition from the default gene 1 to gene 2 (red) occurs first in the region of highest morphogen concentration* **(B)**. *The activation seems to spread in a wave-like manner since at lower concentrations this transition requires more time. Meanwhile cells exposed to a sufficiently high concentration switch from gene 2 to gene 3 and so on. The dynamics of this promotion agrees with the 'posterior transformation' as proposed by Nieuwkoop (1952). Eventually, particular genes are active in sharply confined regions. In adjacent cells different genes can be active although there are only minute concentration differences in the morphogen concentration. It is a property of such a system that a later increase of the signal can lead to a further promotion (posterior or distal transformation).* **(D)** *In contrast, after the signal is gone, the cells remain in their state of differentiation. 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. Modelling predicted that these less sensitive genes are better in the autoregulation, otherwise they could not win against the more sensitive feedback loops (Meinhardt, 1978, 1982).*

adjacent cells. Such a reaction type we have called "lateral activation of locally exclusive states". This process is able to generate a controlled neighbourhood of differently determined cell types (Meinhardt and Gierer, 1980, Meinhardt, 1982). Stripe-like distributions as seen in segmentation are a preferred mode since the long common border between two cell states allows an efficient mutual activation over short distances. Thus, although the mechanism for segmentation seems to be very different, in its core it depends also on self-enhancement and long-range inhibition.

Self-enhancement combined with an antagonistic reaction cannot only generate patterns in space but also patterns in time. This occurs if the time constant of the antagonistic reaction is longer than that of the activation. Pattern formation in time can be coupled to pattern formation in space. For instance, somite formation was predicted to proceed by a sequential conversion of a pattern in time into a pattern in space. Each full cycle of an oscillation leads to the addition of one pair of anterior and posterior half-somites (Fig. 3). In addition to the generation of the periodic pattern, the oscillation allows a precise activation of genes which specify the character of the segmental unit (Meinhardt, 1982). Meanwhile, the predictions found full support (see Fig. 3)

The list of examples could be extended. For periodic patterns, good evidence for an activator-inhibitor system exists for the initiation of leaf hairs (Hülskamp, 2004) and for the regularly spaced heterocysts in the blue-green alga Anabaena (Golden and Yoon, 2003). These examples make us confident that the mechanism we proposed will turn out to be an indispensable component of development.

Do you see any principal differences between the formation of a 2-dimensional "color" pattern and 3-dimensional shaping? Or, on the contrary, can they be described by the same model?

Although an organism is a three-dimensional object, surprisingly many developmental processes take place in two-dimensional cell sheets. Such sheets might close to form a tube and several such tubes can be nested into each other. The germ layers are an example. Even in organs in which the three-dimensional structure is essential, a two-dimensional pattern is frequently generated first and the pattern perpendicular to this sheet is generated by a different and independent process. An example is the formation of the layered structure of the brain. Steps towards a more complex three-dimensional structure are frequently connected with the formation of a new tube in an existing tube by infolding of tissue along a line that extends along the axis of the tube. The neural tube in vertebrates and mesoderm formation in insects are examples. In both cases it is evident that a signal is generated first, while the local change of the cell shape is a downstream event.

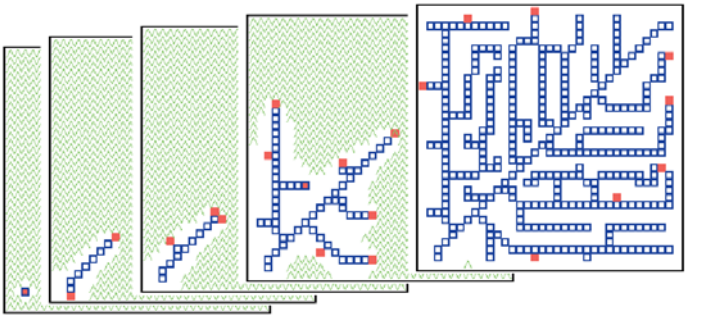
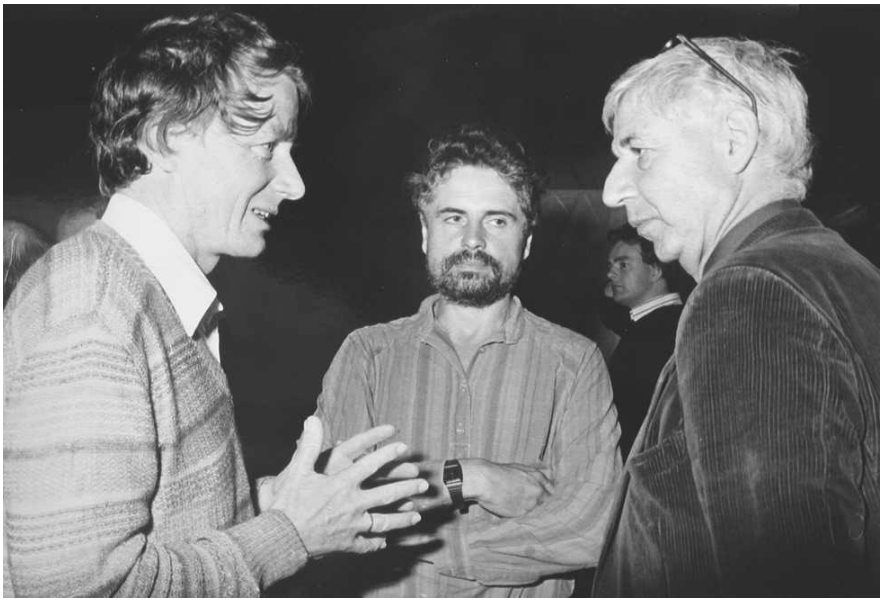


Fig. 6. Generation of filaments. *Branching filaments can be generated if a local signal (red) causes filament elongation at the tip, either by cell proliferation, differentiation or single cell extension (Meinhardt, 1976, 1982). Elongation occurs towards high concentrations of a guiding factor (green) that is produced either ubiquitously (as shown) or locally by a target region. It is removed by the filaments (blue). The filaments appear as a trace behind the moving signal. Branch formation either occurs by bifurcation or by lateral branching (as shown). Regions deprived from filaments obtain a high concentration of the guiding factor, which attracts filaments. The trachea of insects share many of the elements (Affolter and Shilo, 2000; Ghabrial* et al., *2003) and most of the postulated components have observed counterparts.*

The generation of a single stripe-like signal that extends along the long axis of a tube is crucial for the generation of bilateral-symmetric organisms and to pattern the mediolateral dimension. By modelling it has turned out that the formation of a single stripe-like organizing region is a highly non-trivial patterning problem which requires the coupling of a spot-forming with a stripe-forming system (Fig. 4). In vertebrates and in insects, very different modes are realized (Meinhardt, 2004). In vertebrates, a moving spot-like organizer (e.g. Hensen's node or the Spemann organizer) induces and elongates a stripe-forming system (signal for notochord formation), similar to the way in which a high-flying airplane leaves behind a stripe-like vapour trail. Since the organizer determines what is dorsal, the midline also appears on the dorsal side. In contrast, in *Drosophila* a dorsal organizer also exists, but it repels the midline. Thus, the single stripe-like organizing region is formed ventrally. This provides a new inroad into the well-known DV-VD conversion between insects and vertebrates. Other creatures such as flatworms found still other solutions. Together this leads to doubts whether an urbilaterian ever existed. An alternative would be that in ancestral radial-symmetric organisms the complete machinery for axes formation existed already and that during evolution, different mechanisms came about to realign the originally parallel axes, leading to the very different body plans we see in contemporary bilaterians.



Talking about pattern formation at a symposium of the Juan March foundation 1988 in Madrid: Hans Meinhardt, Jonathan Cooke and Lewis Wolpert (from left to right). *Among many other contributions Jonathan Cooke showed that in amphibians the size of somites are regulated in relation to the total size of the embryo. He proposed a clock-and-wavefront mechanism to account for this observation. Lewis Wolpert introduced the concept of positional information. Photograph kindly provided by Klaus Sander.*

Can you specify the role played by "genetic information" in your models? Do you suggest that the main role of genes is restricted to producing the morphogenetic substances with activator or inhibitor properties, or that they may affect structures and parameters of the feedback circuits in your model equations?

The production of signalling substances under genetic control is certainly an important step. Equally important is the interpretation of these signals that leads to a space-dependent activation of genes. The exchange of signals via diffusion can only take place at early stages when the fields to be organized are small. Therefore, to allow growth, a once obtained pattern of gene activation should become independent of the evoking signal. For this I proposed that stable gene activation is based on a non-linear self-activation of genes combined with a repression of the alternative genes (Fig. 5). The proposal for the mechanism for self-maintaining gene activation was based on the formal similarity to pattern formation in space. In spatial pattern formation, one region becomes activated, the rest suppressed. Similarly, a particular cell differentiation may require the activation of say gene 2 and the suppression of genes 1, 3 and 4 which could alternatively be activated in this situation. Thus, the activation of a particular gene can be regarded as a patterning process that takes place not in real space but in the 'space of alternative genes'. Meanwhile many autoregulatory genes have been found. Usually, if such a gene is lost, another gene will take over, indicating that the activity of the lost gene was repressing the activation of an alternative gene. Thus, space-dependent gene activation can be regarded as pattern formation in the space that is coupled to a pattern formation among alternative genes.

The stable activation of genes may exert a profound feedback on the signalling system. For instance, the formation of filamentous-branched structures can be accounted for by local signals which elongate filaments at their tips. This elongation has a strong feedback on the signal, causing its displacement. This, in turn, leads to a further elongation of the filament and so on (Fig. 6). Thus, the interplay of signalling, gene activation and modified signalling leads to an enormous repertoire of patterns that can be generated in a combinatorial way. These are well accessible to modelling.

In spite of considerable achievements of the model approaches in biology, they remain to be largely unknown and poorly understood by the main bulk of biology students in different countries. Do you think that the entire system of biological education should involve more mathematics and other elements of the model approach? Or, at least in the short term, would it be suitable to have a team of pure experimenters on the one hand and pure theorists on the other interacting only at conferences (as occurs, for example, at Hydra conferences)?

From its history, biology is certainly an experimental science. Many experiments can be done without any theory. For instance, searching for mutations that lead to an aberrant development or the determination of particular gene expression patterns do not require a theory. It may even be tempting to assume that a

complete understanding can be achieved just by measuring the distributions of all relevant molecules at all stages of development. However, as a rule, this would only shift the problem. If a local concentration of a particular substance elicits a particular structure, we need insights into why precisely at this position this concentration has piled up. For instance, the observation that an oscillation is involved in somite formation did not provide any explanation of *why* this is so (Palmeirim *et al*., 1997). In contrast, in the modelling which preceded this finding by fifteen years, this *"why"* was the starting point. The involvement of an oscillation was the only solution I found that allows the generation of a periodic pattern in which the individual metameric units are also different from each other (Fig. 3; Meinhardt, 1982). Thus, models are indispensable for the step from the observation to the paradigm.

Why do models have only a limited reputation? My own experience is that experimentalists are not very enthusiastic if it turns out that a process was correctly predicted. They worked hard to find the basic principles by themselves. Frequently the prediction is then handled more as a speculation, if not completely ignored. This is very different to the habit in physics where an experimental observation would be in no way diminished if it is preceded by a theoretical prediction, on the contrary. The reception of some of my models had a strange history. First they were regarded as unrealistic or misleading: "cannot be". More or less abruptly this changed later into: "that is trivial, how else should it be?". This switch had different time constants in different communities. Both attitudes provide the freedom to ignore the theoretical work.

Many biologists are presumably afraid of any mathematics and stop reading a paper after the first equation is encountered. Thus, equations are better put 'in quarantine', into an appendix or into supplementary material if the paper should appear in an experimentally oriented journal. This is a pity since an equation unambiguously shows within a few lines what the hypothesis really is. Moreover, they allow a verification of whether a proposed mechanism is free of internal contradictions and whether it has the postulated dynamic properties. Thus, educating students so that they can later approach and appreciate an equation without fear would be most helpful.

Theorists too have contributed to scepticism against theories. Frequently, new models list only what can be accounted for, but ignore phenomena which are difficult to integrate. The preconditions required for a model to work at all are frequently not discussed. Opaque mathematical treatments of particular aspects sometimes hide more than they elucidate. Often, new theories draw little attention to what preceding theories have achieved and where the differences are. This has had the consequence that the experimentally working community does not listen to the theorists, since they do not listen to each other. A major problem in this respect is that modellers usually came from other fields such as mathematics or physics (as I do). Due to the explosion of experimental facts, it is now increasingly difficult for them to obtain a profound overview of the experimental results. Usually experimentalists are very happy to find an error in the assumptions to have a good excuse to ignore the theory.

For me it has been very helpful and stimulating to work in the experimentally-inclined surrounding here at the Max-Planck Institute for Developmental Biology. The diversity of the lectures and the discussion with experimentally working colleagues were stimulating and indeed necessary to understand the biological background. And most of all, I would like to use this opportunity to thank Alfred Gierer for many years of a fruitful collaboration that is still vivid today. He settled the fundamentals of much of my own work.

KEY WORDS: *pattern formation, lateral inhibition, reaction-diffusion mechanism, Turing, gene activation*

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