Models for positional signalling with application to the dorsoventral patterning of insects and segregation into different cell types

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Summary
Models of pattern formation and possible molecular realizations are discussed and compared with recent experimental observations. In application to the dorsoventral patterning of insects, it is shown that a superposition of two pattern-forming reactions is required. The first system generates the overall dorsoventral polarity of the oocyte, the second generates the positional information proper with a stripe-like region of high concentration along the ventral side of the embryo. A single reaction would be insufficient since the two reactions require different parameters. The model accounts for the orientation of the DV axes of the oocytes in the ovary of Musca domestica and Sarcophaga, independent of the DV axis of the mother, for the formation of several ventral furrows in the absence of the primary gurken/torpedo system in Drosophila, as well as for the good size regulation of the dorsoventral axis as observed in some insect species.

Segregation of a homogeneous cell population into different cell types requires autocatalytic processes that saturate at relatively low concentrations and nondiffusible substances responsible for the autocatalytic feedback loops. Thus, these loops can be realized directly on the gene level via their gene products, for instance, by the mutual repression of two genes. A balance of the two cell types is achieved by a long-ranging substance interfering with the self-enhancing process. This substance is expected to have a more or less homogeneous distribution. This model accounts for the reestablishment of the correct proportion after an experimental interference and the change of determination after transplantation. Applications to the segregation of pre-stalk and prespore cells in Dictyostelium and of neuroblast cells from the ventral ectoderm in Drosophila are provided.

Key words: models, pattern formation, morphogenetic gradients, cell determination, Drosophila, segregation.

Introduction
Pattern formation during development of higher organisms requires communication among the cells. We have proposed several models for pattern formation for different developmental situations. They are based on molecularly feasible interactions. Using the new tools of molecular biology, several molecules have been identified that play a decisive role in the control of pattern formation. In this article, I provide a comparison between these models and recent experimental observations.

The models proposed can be summarized as follows:
(i) Primary gradients as well as periodic structure can be generated by local selfenhancement and long-range inhibition (Gierer and Meinhardt, 1972).
(ii) Stable cell states result from genes that feed back directly or indirectly on their own activation (e.g. transcription) but which compete with alternative genes for activation. Positive feedback and competition has the consequence that only one of the alternative genes remains active within one cell. By appropriate coupling with gradients generated by mechanism i or iii a position-dependent gene activation results (Meinhardt, 1978).
(iii) Borders between regions in which different genes are active act as organizing regions for the determination of substructures. Pairwise substructures, such as legs and wings, are initiated at the intersections of two borders, one resulting from a patterning in the antero-posterior, the other from a patterning in the dorso-ventral dimension (Meinhardt, 1980, 1983a,b).
(iv) Ordered sequences of cell states result from a mutual long-range activation of cell states that locally exclude each other. This allows a controlled neighbourhood of cell states. Missing elements can be intercalated (Meinhardt and Gierer, 1980).

Pattern formation by local autocatalysis and long-range inhibition
The complexity of a higher organism cannot be present already within the egg in a hidden form since in many systems an early separation into two fragments leads to two complete embryos. If the egg contains prelocalized
determinants, the question remains how this prelocalization has been achieved. We have proposed that primary pattern formation proceeds via local autocatalysis and long-range inhibition (Gierer and Meinhardt, 1972; Gierer, 1981; Meinhardt, 1982). This does not require a molecule with direct autocatalytic regulation, but autocatalysis can be a property of the system as a whole. For instance, if two substances, A and B, exist and A inhibits B and vice versa, a small increase of A above an equilibrium leads to a stronger repression of the B production and thus to a further increase of A, and so on in the same way as if A were be autocatalytic. The same holds for B. A and B together form a switching system in which either A or B is high. (The switch of the lambda phage between the lytic and the lysogenic phase is based on such a mutual repression; see Ptashne et al. 1980). To allow pattern formation, a long-ranging signal is required which interferes with the mutual competition of the two substances. For instance, if A has won the A–B competition in a particular region, B must win in the surroundings. A possible realization would be that the A molecules control the production of a substance C which, in turn, either inhibits the A or promotes B production. These modes are equivalent since, in competing systems, a self-limitation is equivalent to the support of the competitor. A more symmetrical pattern arises if the long-ranging help is reciprocal. In Eq. 1a–c, an interaction is described in which the diffusible antagonistic substance C is produced under control of the A molecules. It undermines the repression of B production by the A molecules. No direct autocatalytic interaction is assumed.

\[
\begin{align*}
\frac{\partial a}{\partial t} & = \frac{\rho}{\kappa + b^2} a - \mu_a + D_a \frac{\partial^2 a}{\partial x^2} + \rho_0. \tag{Eq. 1a} \\
\frac{\partial b}{\partial t} & = \frac{\rho}{\kappa + a^2/c^2} b - \mu_b + D_b \frac{\partial^2 b}{\partial x^2} + \rho_0. \tag{Eq. 1b} \\
\frac{\partial c}{\partial t} & = \gamma a - \mu_c c + D_c \frac{\partial^2 c}{\partial x^2}. \tag{Eq. 1c}
\end{align*}
\]

Fig. 1 shows the formation of graded distributions by such interaction. A high A concentration is formed at one side, a high B concentration at the other with countergradients in-between. If one of the components is missing due to a mutation, the remaining one will have a high concentration everywhere. Modifications of this mechanism that account for the formation of stripes or for segregating cell populations will be discussed further below.

Pattern formation by autocatalysis and long-range inhibition has many regulatory properties known to exist in real systems. In fragments, the pattern can regenerate. Small external asymmetries can be amplified and therefore used to orient the emerging pattern. The pattern itself, however, is, except for the orientation, to a large degree independent of the triggering asymmetry.

However, the actual mechanism found in a developing system can be more complicated. The equations contain, for instance, simple diffusion terms. However, the passage of larger molecules from one cell to the

Fig. 1. Realization of autocatalysis by an inhibition of an inhibition. If two substances, A and B, inhibit each other’s production in a nonlinear way (Eq. 1), a small increase of one of the substances above the steady state leads to its further increase. Both substances together have the property of ‘selfenhancement’, such as required for pattern formation (part A and part B of the activator). The long-ranging inhibitor (c in Eq. 1) is assumed to antagonize the repression of B-production by the A-molecules. Thus, only inhibitory interactions are involved in this scheme. (A) Generation of graded distributions in a linear array of cells as function of time. The pattern is initiated by random fluctuations. (B) Concentrations at the final stable steady state. A and B are distributed in countergradients, the inhibitor distribution has the same polarity as the A distribution but is shallower due to the higher diffusion rate.
Models for positional signalling

other can be a complex process, requiring active transport mechanisms through the membrane, not just leakage. Further, a particular developmental step involving pattern formation is embedded in a series of events and this requires a coupling with other pattern-forming systems. For instance, the organization of a particular embryonic axis requires a precise orientation in relation to the other axes. Or, size-regulation becomes much improved by a superposition of two such reactions, one determining the position, the other the size of the structures. Other examples will be discussed below in more detail.

Stabilization of apical dominance by a feedback on the source density

Usually the size of morphogenetic fields increases during the growth of the embryo. A graded concentration profile can be maintained only over a range of about a factor two. Growth to larger extensions bears the danger that the pattern flips over into symmetric or periodic distributions (Fig. 2A). A possible solution would be a conversion of the dynamically regulated pattern into a more stable pattern at an early stage, for instance, by position-dependent gene activation. The initial patterning mechanism would subsequently become suppressed. However, such a mechanism is inappropriate for systems that show regulation over a large range of sizes. For instance, a hydra maintains its polar structure over substantial growth but, nevertheless, a fragment of 1/10 of the normal body size is still able to regenerate.

The range of dominance of the activated region, the apical dominance, can be increased by an order of magnitude if a feedback of the activator on the source density exists (Meinhardt and Gierer, 1974). Usually the sources of the activator and inhibitor synthesis are assumed to be homogeneously distributed, except for small random fluctuations. If, however, an increased activator concentration leads to an increase of the source density, it becomes unlikely that, in a region at a distance from the activated region, i.e. in a region of lower source density, the inhibition can be overcome and a secondary maximum appears. Thus, the apical dominance is stabilized (Fig. 2B). In addition, the graded source density distribution provides information about the polarity of the tissue. Small fragments will regenerate an activator maximum in the region of relatively highest source density, i.e. according to the

Fig. 2. Stabilization of polar distributions by a feedback of the activator on the source density – a model for the maintenance of apical dominance in Hydra. In a growing field, a system based on autocatalysis and long-range inhibition forms a pattern if a critical size is exceeded. However, with further growth, a tendency exists to switch into a symmetrical and later into a periodic pattern, a pattern which is inappropriate to supply unique positional information. (B) If the activator (or the inhibitor) has a feedback on the source density, the source density becomes graded. The graded source density stabilizes the polar distribution since at a region of low source density, the initiation of secondary maxima is unlikely. The graded source density provides the long-lasting information about the polarity of the system. A small fragment regenerates a pattern according to the original polarity.
original polarity. This requires that the source density has a longer time constant in comparison with the activator. The simulation in Fig. 2B shows the maintenance of polarity during a period of substantial growth as well as the regeneration of a small fragment.

The source density can be, for instance, the density of a certain cell type to which the activator and inhibitor synthesis is restricted. An increased activator concentration could lead to an increased proliferation or differentiation, such that more cells of this type are formed. The head activator isolated from hydra and other tissues (see Schaller, this volume) may be a factor involved in the generation of a graded source density. Addition of head activator leads to the differentiation of more nerve cells from stem cells while, in turn, under normal conditions, the head activator is mostly produced by the nerve cells. The source gradient would be a morphogenetic gradient in the terms of Morgan (1904), a gradient in the tendency to form a particular structure. The tissue in the field that bears the highest tendency will form the structure.

**A model for the dorsoventral organization of insects**

In *Drosophila*, the dorsoventral (DV) axis is organized by at least two pattern-forming systems. One, the *torpedo*/gurken system, acts during early oogenesis and requires an interaction between genes expressed in the soma and in the germline (Schüpbach, 1987). A second one, the *dorsal*/Toll system, provides the proper positional information and acts during early embryogenesis (Anderson and Nüsslein-Volhard, 1984a,b; Anderson, 1987). According to the model outlined below, the first system generates an orienting asymmetry for the second. The model provides an explanation why such a superposition of two systems is required, how the region of high concentration of the dorsoventral gradient achieves a stripe-like shape along the whole antero-posterior extension, how the DV axis becomes oriented in the ovary and why in many systems this axis shows a good size regulation.

**Generation of dorsoventral polarity requires selforganizing mechanisms**

In *Musca domestica* and *Sarcophaga*, the orientations of the DV axes of the developing oocytes in the ovary provide a strong indication that a mechanism with selforganizing capabilities is at work. These orientations can be detected by the eccentric position of the germinal vesicle. In *Musca domestica* (Kleine-Schonnefeld and Engels, 1981) and in *Sarcophaga* (Geysen et al. 1988), the dorsal sides are oriented towards a point within the ovary (Fig. 3A,B). Thus, the DV polarity of an oocyte can have any orientation indicating that the DV axes are not under the control of the DV axis of the mother.

As mentioned above, pattern formation and especially the generation of polar patterns within a cell or a field of cells can be accomplished by local autocatalysis and long-range inhibition. If the inhibitory molecule (or one of several inhibitory molecule species) diffuses in a restricted manner from one oocyte or follicle to a neighbour, a mutual orientation results. Let us assume, for reasons that will become clearer below, that the dorsal side is the activated region of this primary system. If in one oocyte an activator maximum has been formed, the dorsal side is fixed. In a neighbouring cell, due to the restricted diffusion of the inhibitor, the dorsal side will be formed at the largest possible distance from the first. Thus, its dorsal side will point in the same direction. Fig. 3C shows a simulation of this process. In performing such computer simulations, it is very difficult to avoid influences of the boundary. For instance, if inhibitor penetrates the envelope of the ovary less easily than the oocytes or follicles themselves, those sides that point towards the boundary of the ovary will become the nonactivated or ventral sides. The orientation of the outermost layer of oocytes directs the orientation of the next inner layer and so on. In agreement with this model is the finding by Geyser *et al.* (1988) that the regularity of the outside-inside orientation is much higher in oocytes close to the outer boundary. This effect is reproduced in the simulation. According to the model, the regularity at more central positions is diminished by two facts. First, random fluctuations can lead to a random selection of an orientation before that oocyte is reached by the orienting wave. Second, a spot at which many dorsal sides point towards each other leads to a less stable situation due to the local accumulation of the inhibitor. In contrast, a misalignment of some oocytes leads to a more stable situation.

In summary, these experiments and their simulations indicate that the dorsoventral polarity is not only a strong system and minor influences either from the outer margin of the ovary or from nearby oocytes or follicles are sufficient for an orientation. The irregularities indicate that a slavish transmission of a strong polarizing signal is not involved but that an oocyte is capable of generating a DV polarity on its own.

**Orientation of the dorsoventral axis perpendicular to the anterior-posterior axis**

The formation of a local high concentration creating dorsoventral polarity must be restricted to an equatorial zone in relation to the anterior and posterior pole. The latter poles appear to be predetermined by the position of the follicle within the ovary. The following model is able to produce such pattern. The anterior and the posterior pole of an oocyte is marked by a maximum of either the same or two different activator-inhibitor systems (Fig. 4). If the inhibitor of the DV system has some cross-reaction with that of the AP-system, the high point of the former system will appear at a maximum distance from the terminal poles, i.e. at an equatorial position, as it is required.

The use of the same signal at the anterior and the posterior pole has the advantage that the pattern is necessarily symmetrical, providing more safety the prerequisites to localize the future dorsal side. Exper-
Fig. 3. Alignment of the dorsoventral (DV) axes of developing oocytes in the ovary. (A) Observations of Kleine-Schonnefeld and Engels (1981) in Musca domestica and (B) of Geysen et al. (1988) in Sarcophaga. Shown is a cross-section through an ovary. Each arrowhead indicates an oocyte with its DV-polarity. The tips of the arrowheads indicate the dorsal sides. They are oriented towards a point within the ovary but not aligned with the DV-axis of the mother. (C) Model: It is assumed that the DV-polarity of the oocytes or follicles is generated by an activator–inhibitor system, that the inhibitor can diffuse in a restricted manner from one oocyte or follicle (small square) to the neighbouring oocytes and that the outer envelope is impermeable. The position of the activated region is assumed to determine the future dorsal side. Shown are stages in the generation of the DV-pattern. The activator concentration is indicated by the density of dots. The oocytes close to the outer envelope become polarized first due to their asymmetric environment. The activated regions point away from the outer envelope due to the accumulation of the inhibitor there. The DV-polarity of the remaining oocytes become aligned due to the restricted diffusion of the inhibitor. Each dorsal side tends to keep distance from other dorsal sides. The model reproduces the preferential orientation of the dorsal sides towards the center as well as the higher degree of misalignment at more central positions. (Fig. A redrawn from Kleine-Schonnefeld, 1981; Fig. B from Geysen et al. 1988).

mentally it has been shown by P-element insertions and β-galactosidase staining that the follicle cells surrounding the anterior and the posterior pole are marked very early during oocyte development (Fasano and Kerridge, 1988). Some of these insertions mark the anterior, some the posterior and some mark both poles simultaneously. Similarly, a staining of a pair of cells at both poles with the same antibody has been described by Brower, Smith and Wilcox (1980). Therefore, a symmetrical pattern as expected by the model seems to be available.

The model requires that the range of the inhibitor covers the whole field in order to obtain a single activator maximum. Diffusion of the activator facilitates the localization of the maximum between the two terminal poles at an optimum position. The resulting maximum is expected to have a patch-like shape with similar extensions in the AP as well as in the DV axis. It is assumed that this pattern formation occurs early in oocyte development, in Drosophila under control of genes such as K10, gurken and torpedo (Schüpbach, 1987). Due to its patch-like shape, it is inadequate to supply positional information for the DV axis, but, as shown below, it can orient a second pattern-forming system which generates an activator maximum with a stripe-like extension.

Generation of positional information for the dorsoventral axis

In Drosophila, the positional information for the DV-axis is generated by a different system in which about 11 genes are involved (Anderson and Nüsslein-Volhard, 1984a,b; Anderson, 1987). The proper signal is provided by the dorsal protein which has, in the early embryo, a graded distribution (Steward et al. 1988; Nüsslein-Volhard and Roth, 1989). In agreement with the prediction of genetic observations (Nüsslein-Volhard, 1979) the ventral side carries the high dorsal concentration. In contrast, the distribution of the dorsal mRNA is nearly homogeneous. Two proteins involved
in the generation of the dorsal gradient, snake (DeLotto and Spierer, 1986) and easter (Chasan and Anderson, 1989), have homologies to serine proteases. (Such enzymatic activities play a decisive role in cascade-like amplification processes in the blood coagulation, see Furie and Furie, 1988). Another key gene is Toll. Injection of wild-type cytoplasm into Toll-mutants can initiate the formation of a new ventral region at the point of injection independent of the original DV polarity of the egg (Anderson, Bokla and Nüsslein-Volhard, 1985).

**Formation of stripe-like distributions requires saturation of selfenhancement and activator diffusion**

To provide positional information for the DV-axis, a region of high concentration must be generated which has different extensions along the main body axes. It must extend over the whole anteroposterior axis but must have a short extension along the dorsoventral axis. According to the model, such a stripe-like distribution requires a limitation in the mutual competition between neighbouring cells (Meinhardt, 1988). If activator autocatalysis saturates at relatively low activator concentration, more cells remain activated although at a lower level. Stripe formation requires, in addition, a modest diffusion of the activator. Due to this diffusion, activated regions tend to occur in large coherent patches since, if a cell is activated, the probability is high that the neighbouring cell becomes activated too. On the other hand, it is necessary that activated cells are close to nonactivated cells into which the inhibitor can diffuse, otherwise no activation above average would be possible. The two seemingly contradictory requirements, coherent patches and proximity of nonactivated cells are satisfied if a stripe-like pattern is formed (Fig. 5). Each activated cell is bordered by other activated cells but nonactivated cells are not too far away.

Fig. 6 shows a simulation of the emergence of the dorsal pattern as function of time under the orienting influence of the primary DV-system mentioned above. The stripe-like extension from pole to pole is clearly visible. Due to the saturation, neighbouring elements can remain activated without downregulating competition. The activator–inhibitor mechanism used for this simulation is, of course, only an example. An inhibition of an inhibition mechanism may be involved as well (see Figs 1, 7E).

Without activator diffusion, the decision to become activated would be a cell-autonomous process and a sprinkled pattern of activated cells would result (Fig. 6D, see also Fig. 7). The requirement of activator diffusion is compatible with experimental observations. The Toll gene product appears to be a transmembrane protein (Hashimoto, Hudson and Anderson, 1988). The snake protein is presumably an extracellular serine protease (DeLotto and Spierer, 1986). Thus, a substantial part of the molecular interactions leading to the dorsal gradient presumably takes place outside of the cells or can pass from one cell to the next.

Saturation of autocatalysis leads, in addition to stripe formation, to a good size regulation (Gierer and Meinhardt, 1972). The number of activated cells becomes a certain proportion of the nonactivated cells as long as the range of the inhibitor is comparable with the size of the morphogenetic field. This accounts for the fact that some insect species show complete pattern regulation after a longitudinal ligation (Sander, 1971). A complete embryo is formed in each fragment, of course with an even shorter dorsoventral extension.

Fig. 5. (A–E) Stages in the formation of a stripe-like pattern. Assumed is an activator–inhibitor system and a saturation of autocatalysis at low activator concentration which limits the competition among neighbouring cells. More cells remain activated, although at a lower level. Small diffusion of the activator leads to the tendency to form coherent activated regions. Stripes are the most stable pattern since activated cells have activated cells in the neighbourhood but, nevertheless, nonactivated cells are nearby into which the inhibitor can escape. (F) For comparison, without saturation but otherwise the same parameters and initial conditions, a bristle-like pattern results. The maximum concentration is about ten times higher than in Fig. E.
Fig. 4. Formation of orthogonal coordinate systems. (A) Generation of an anterior, a posterior and a dorsal pole by three activator-inhibitor systems coupled by a cross-reaction of the inhibitors. Due to the cross-reaction, the high activator concentrations determining the anterior (blue) and posterior pole (red) appear at opposite ends of the field. Due to a longer time constant, the high concentration determining the dorsal side (green) develops with some delay. It keeps maximum distance from the anterior as well as from the posterior pole because its activator is also inhibited by the inhibitor of the anterior and posterior system. The calculation is made with a two-dimensional array of cells which represents a cross-section through an oocyte or follicle. (B) A system of two pattern-forming reactions is sufficient if the dorsoventral pattern is oriented by a symmetrical pattern with a high concentration of the same activator (red) at both the anterior and the posterior pole. (For the generation of a symmetrical pattern, see Fig. 2A). Again, the high activation determining the dorsal side (green) appears at maximum distance from both poles. Shown is the initial, an intermediate and the final stable pattern. The density of dots is a measure for the concentration. The high concentration at the dorsal side is assumed to orient a second pattern-forming reaction that provides the proper positional information for the dorsoventral axis (see Fig. 6).
Fig. 7. Model for the segregation of a homogeneous cell population into two cell types. Assumed are two genes, A and B, which repress each other in a nonlinear way (Eq. 1). This creates an unstable situation in which either A (red) or B (blue) becomes activated. Via a long-ranging substance, a balanced ratio of both cell types is achieved (see also Fig. 1). Since the gene products are assumed to be nondiffusible, the choice of a particular pathway in a particular cell is largely independent of the choice in a neighbouring cell. No tendency exists to form coherent patches. Due to a saturation of the self-enhancement in the A–B system (high \( k \) in Eq. 1), no zone of inhibition is formed around a cell with a particular differentiation. (A) Stages in the segregation. (B) Determination is reversible. For instance, a homogeneous population of B-cells is unstable. The cells return first to the semistable steady state followed by a repetition of the patterning process, restoring in this way the correct ratio. (C) Change of the ratio due to a mutation. In this example, a reduced production of the substance \( C \) leads to an overproduction of A-cells. (D) Superposition of a graded pattern can lead to a preferential appearance of one cell type at a particular position. (E) For comparison, with diffusion of the A and B products, a stripe-like pattern would be formed.
Fig. 6. Model for the generation of positional information for the dorsoventral axis. Assumed is an activator–inhibitor system with stripe-forming capabilities (Fig. 5; a system based on an inhibition of an inhibition, such as shown in Fig. 1 and 7E, would work as well). (A–C) Stages in the generation of a stripe-like high concentration along the ventral side connecting the anterior and posterior pole. (D) Without diffusion of the activator, the high ventral stripe would disintegrate into separated spots. (E,F) For reproducible pattern formation an orienting influence is required. Due to an inhibitory interference from the primary DV-system (Fig. 4) the effective source density (E) is reduced at the dorsal side. The outcoming pattern is, due to the selforganizing properties, to a large degree independent of the orienting pattern. However, without this orienting influence several high points can appear at arbitrary positions, in agreement with the observation that often several ventral furrows are formed in the case of a nonfunctional gurken/torpedo system (Schüpbach, 1987).

**Coupling with the primary DV-system**

In the absence of additional cues, the high dorsal stripe could have any orientation within the embryo. Even the formation of several high points or stripes would be possible (Fig. 6F). As mentioned, this cue is assumed to be provided by the primary DV-system (green in Fig. 4). The lowest concentration of this pattern has a stripe-like extension in the egg which connects the two poles. Thus, it is conceivable that the primary DV-pattern has an inhibitory influence on the second DV-pattern.

If the torpedo/gurken system, the assumed primary system, is nonfunctional, a general ventralization takes place (Schüpbach, 1987). This is compatible with the view that it exerts an inhibitory influence spreading out from the dorsal side on the dorsal/Toll system. It could be that such negative influence is responsible for the remaining polarity in dominant Toll-alleles. In embryos developing from eggs laid by mothers without a functional gurken/torpedo system, very often two sites of invagination are formed (Schüpbach, 1987). In the model, in the absence of the orienting influence of the primary system, several high ventral points can emerge (Fig. 6F). If this interpretation turns out to be correct, this would be strong support for the suggestion that dorsal/Toll system on its own is able to generate a pattern and that the primary system provides the information only where the high dorsal concentration should appear.

The question may arise whether the dorsal/Toll system is a pattern-forming system at all. According to the model, the region with the least repression of the primary system would have a shape quite close to the high dorsal distribution. The advantage of having a pattern-forming system would be its capacity for self-regulation. For instance, the precise shape of the high dorsal stripe would be independent of the contour lines of the inhibition exerted by the primary system and, thus, it would be independent of the egg shape. The possibility that a new ventral side can be induced in a Toll mutant at a dorsal position of the egg, despite the fact that a functional primary system was present, is a further indication that the primary system provides only an asymmetry and does not dictate the fate.

**Segregation of cell populations**

A very common patterning process is the segregation of an originally homogeneous cell population into two different cell types. The segregation of neuroectodermal cells into neuroblasts and in ventral ectodermal cells in *Drosophila* (see Campos-Ortega, 1988; Artavanis-Tsakonas, 1988; Hartley, this volume) or the forma-
tion of prestalk and prespore cells in Dictyostelium (see Williams, this volume) may serve as examples. Common to both systems is a very good regulation of the proportion of the two cell types. Removal of either prespore or prestalk cells leads to the restoration of the correct ratio due to reprogramming. In the insect system, ablation of neuroblast cells causes ectodermal cells to take over the fate of the deleted cell (Doe and Goodman, 1985). Transplantation of marked ectodermal cells from the neuroectodermal region into a younger host can cause a switch into the neuronal pathway (Technau and Campos-Ortega, 1985).

Molecules have been identified that play a decisive role in the formation of these patterns. In the slime mould, a molecule called DIF is required for prestalk formation (see Kay, this volume). In the insect system, about six genes are required for the formation of ectodermal cells (see Campos-Ortega, 1988; Artavanis-Tsakonas, 1988), among them the genes Notch and Delta.

It has been regarded as a surprise, and counterintuitive for a morphogenetically active substance, that both molecules, DIF and the Notch product, appear to be almost homogeneously distributed. However, according to the model discussed below substances with such distribution are a necessary component.

A model for segregation must have the following features: (i) In a certain proportion of the cells, a particular gene becomes activated. The remaining cells remain either in a ground state or activate an alternative gene. (ii) The proportion of the two cell types is regulated. Removal of one cell type leads to a reprogramming of some of the remaining cells, such that the correct ratio becomes restored. (iii) The two cell types appear more or less at intermingled positions.

With the appropriate parameters, the activator–inhibitor mechanism can reproduce these features and the known experimental and genetic data are compatible with such an interpretation. As already discussed above, if the activator autocatalysis saturates at low activator concentrations, the number of activated cells reaches a certain proportion of the nonactivated cells. Since the local activator increase is limited due to the saturation, the degree of competition between neighbouring cells is limited too. Thus, neighbouring cells can remain activated independent of the range of the inhibitor. The system is able to regulate: For instance, if relatively too few cells are activated, the level of the inhibitor is lower than that required for an equilibrium. More and more cells switch from the nonactivated into the activated state. This leads to an increase of the inhibitor concentration until no further switching is possible. At this stage, the correct ratio is obtained. The reverse argument is valid if too many cells are activated.

If the activator is nondiffusible, the decision whether a cell will become activated is to a large extent independent of the decision made by a neighbouring cell. Only on average, the correct ratio must be maintained. The chance to become activated is not increased by an activated neighbour. As long as no other constraints are superimposed, random fluctuations are decisive. Activated and nonactivated cells emerge at intermingled positions.

A possible realization of the selfenhancement, which is especially appropriate for the segregation into two different cell types, is a mutual repression of two genes, A and B. Such a system has already introduced in connection with the generation of graded distributions (Fig. 1). Two genes that mutually repress each other in a nonlinear way resemble an unstable system. In a particular cell, one gene will remain active and the alternative gene suppressed. Again, a long-ranging substance is required to achieve a balanced ratio of the two cell types. The essential differences to the version used for Fig. 1 are (i) the molecules involved in the selfenhancement are nondiffusible and (ii) the selfenhancement saturates.

The simulation Fig. 7, performed with Eq. 1 under these conditions, shows the segregation into two types, the restoration of the correct ratio and the overrepresentation of one cell type in a mutant (caused at this example by a lower production rate of C, Eq. 1c). The latter simulation shows also that the ratio of cells at which either A or B becomes activated can be easily adapted to the needs of the organism by the change of a parameter such as production (or decay) rates, parameters that can be easily encoded in the genetic network.

The model predicts that the mutual repression must be nonlinear, a requirement that is satisfied if the active agents are dimers of the gene products. The importance of the saturation has been mentioned. In Eq.1 the degree of saturation is given by the Michaelis–Menten constants k since they determine the maximum production rate if the alternative gene is completely repressed, i.e. if the concentration of their gene product would be zero.

In Drosophila embryos, the density of neuroblasts is higher at more ventral positions. In Dictyostelium, more prestalk cells are formed initially at the top of the aggregates. According to the model, small spatial inhomogeneities can change the local cell density while the overall ratio is maintained (Fig. 7D).

Alternative molecular realization and comparison with available experiments

The reaction schemes outlined above are only examples to illustrate the general principle envisaged. Alternative molecular realizations are conceivable. For instance, if all the cells produce a substrate S and the activated cells remove that substrate, the resulting S-concentration depends on the ratio of the activated cells, the consumers, to the total number of cells, the producers. Such a model has been proposed for the regulation of prestalk–prespore cells in Dictyostelium (Fig. 8; Meinhardt 1985c).

The gene Notch is transcribed in many cell types including neuroblasts and ectodermal cells (Hartley et al. 1987). This is compatible with the substrate-depletion scheme if ventral ectodermal cells remove the
Fig. 8. Role of homogeneously distributed substances in balancing the ratio of two cell types, exemplified by a model for prestalk/prespore cell differentiation in the slime mould Dictyostelium discoideum. Assumed is a pattern-forming system consisting of an autocatalytic activator and a substrate (possibly DIF, see Kay, this volume) which is removed in the process of autocatalysis. 

(A) Activated cells appear at intermingled positions (see Williams, this volume). They are assumed to sort out by an independent process (Meinhardt, 1983c). The substrate concentration does not change during this process since substrate removal by all cells at the steady state is the same as that by the fewer, but fully activated, cells. Due to the assumed high diffusion rate, the substrate has a near-homogeneous distribution. 

(B) Removal of the activated cells (the prestalk cells, the substrate-consumer) leads to a dramatic increase of the substrate. A new activation occurs in the remaining cell population and the patterning process is repeated. Eventually, the correct ratio of activated versus nonactivated cells becomes restored. 

(C) Removal of nonactivated (prespore) cells leads to a reduction of substrate concentration which causes the activator concentration to decrease below the saturation level and the competition to start again. Some of the previously activated (prestalk) cells switch to nonactivated cells. Although the substrate concentration appears to have no pattern of interest and although concentration changes take place only under certain experimental conditions, the substrate (or more general, the antagonist of selfenhancement) plays the decisive role in the balancing of cell types (other modes of action of the DIF-molecule are conceivable as well. For instance, it can be an inhibitor for prespore development, especially if ammonia acts as inhibitor for prestalk development, see Meinhardt, 1983c).

Notch product, an EGF-like factor. The Notch concentration would provide a measure for the ratio of ventral ectodermal cells, the consumer, to the total number of Notch-producing cells. Thus, the function of Notch could be to accomplish a precise regulation of this ratio.

The product of the gene Delta has been interpreted as a diffusible inhibitor of the neuronal pathway (Vässin et al. 1987). It is produced by the neuroblasts. If Delta is such an inhibitor, why is this pathway not blocked in those cells exposed to the highest Delta concentration, i.e. in the neuroblasts that produce Delta? The answer in terms of the model is that the local selfenhancement is so strong that it overcomes the selfproduced antagonistic effect due to the diffusion of the latter into the surroundings. In addition, as mentioned, this inhibition may be indirect and may result from an activation of genes required for the ectodermal pathway that locally exclude the activation of neuroblast-specific genes.
Hartley et al. (1988) have reported that the gene Enhancer of split, E(spl), is transcribed in cells of the neurogenic pathway (N), is cell-autonomous and thus does not code for a diffusible factor, such as Notch or Delta, but nevertheless is required for the ectodermal pathway (E). If this observation is correct, according to the model, E(spl) could be involved in the reaction chain active in the N-cells leading to a diffusible molecule (Delta?) which inhibits the N-pathway, thus providing the E-pathway with a chance to win the competition in a certain fraction of cells. If E(spl) were involved in receiving such a signal, the expression of E(spl) would be also expected in the E-cells since the N-pathway must be suppressed in these cells. On the other hand, from the models, genes are expected that are active in cells of the E-pathway only. If mutant, the E-genes are incapable to compete with the N-genes and all cells would follow the N-pathway.

The role of homogeneously distributed substances in the segregation of cell populations

As mentioned, the Notch product in Drosophila and the differentiation-inducing-factor DIF in slime moulds are more or less homogeneously distributed. This may be in contrast to the expectation that morphogenetic substances must have position-dependent, for instance, graded or spike-like distributions to allow region-specific gene activation. According to the model, homogeneously distributed substances play a decisive role for segregating cell populations since they provide a measure of how many cells of a particular type are present. The system does not require that a separate prepattern provides the signal for one or the other gene to be activated. The regulation of the ratio of cell types proceeds via diffusible and thus more or less homogeneously distributed substance(s). This is illustrated in the simulation of the prespore/prespore regulation in Dictyostelium (Fig. 8). The distribution of the substrate (e.g. DIF) remains almost homogeneous and constant during the segregation process. However, after removal of the prespore cells (the substrate consumer), a dramatic increase of the substrate (Fig. 8B) takes place until a new activation is fired, enabling a repetition of the segregation process.

In the slime moulds, a high diffusion rate of the proportion-controlling substance is especially important since the prespore cells sort out at the tip of the slug. Thus, distances between the different cell types that should obtain a balanced ratio can be very high, but nevertheless, removal of prespore cells at the rear end of the slug should lead to a reprogramming of prespore cells at the tip (see Fig. 8C). In contrast, if the differentiated cell types remain in intermingled positions, as long as regulation is required, the range of the diffusible substance can be small to maintain everywhere a locally balanced ratio of both cell types.

Since, in such systems, the cell states are stable only if the correct ratio is given, an isolated cell will lose its commitment and return to the semistable steady state.

In the example given above, in an isolated cell, both gene A and gene B will become moderately activated, independent of whether originally A or B was active since otherwise too much or too little of the antagonist would be present (see also Fig. 7B). Therefore, it is not surprising that transplantation of a single cell can cause a change of its commitment (Technau, Becker and Campos-Ortega, 1988; Campos-Ortega, 1988) since it was isolated for a certain time during the experiment.

Conclusion: Expected function of molecules involved in positional signalling

From the point of view of the models proposed for positional signalling, molecules with specific roles and properties are expected. In the following, their expected general purpose as well as more specific properties, such as required for particular developmental situations, are summarized.

Substances or systems of substances with selfenhancing properties, called activators, are required if de novo patterns are to be generated. Preexisting asymmetries can be used to orient the pattern. The range of the activator determines the size of coherent patches. If this range is of the order of the field size, polar distributions emerge (Fig. 1). If the autocatalysis saturates, a certain proportion of the total number of cells will become activated independent of the total number of cells. If in such a case the activator is nondiffusible, a homogeneous cell population segregates into different cell types (Fig. 7). They appear at intermingled positions. In contrast, if the activator is diffusible, stripes are preferentially formed (Fig. 5). The precise localization requires an orienting influence by other pattern-forming systems (Fig. 6).

Antagonistic substances limit the spread of the autocatalytic reaction. They can work by a direct inhibitory effect (Fig. 2), by depletion of a substrate required for autocatalysis or by the long-range activation of a feedback loop which locally excludes the first. The range of the antagonist determines the distance at which secondary maxima can be formed. If the size of the field is larger than this range, periodic pattern will emerge (Fig. 2A). In the systems with saturating autocatalysis mentioned above, the antagonist has a more homogeneous distribution and controls the ratio of the activated versus nonactivated cells. The range of the antagonist can determine the range over which size regulation is effective.

For the generation of patterns that are stable in time, the antagonist must have a shorter time constant, otherwise oscillations will occur (Meinhardt and Gierer, 1974). An example of the latter case is the periodic secretion of cAMP by aggregating amoeba of Dictyostelium discoideum, a system in which the molecular mechanism of selfenhancement and the antagonist reaction is fully understood (see Devreotes, this volume).

According to the model, long-range activation of alternative feedback loops which exclude each other.
locally plays an important role in systems in which the neighbourhood of structures is controlled. An example is the intrasegmental pattern of insects or insect appendages. Such a mechanism is able to generate an ordered sequence of structures in space without global control by a morphogen gradient (Meinhardt and Gierer, 1980). This mechanism allows the repair of discontinuities by intercalation, if necessary with polarity reversal. This mutual-activation mechanism has obtained strong support by a recent observation of Martinez-Arias, Baker and Ingham (1988). They found that the product of the wingless-gene is required for normal expression of the engrailed gene, although engrailed is expressed in a neighbouring region (Baker, 1988).

In the models, communication between cells has been regarded as a simple diffusion, as a leakage. The actual mechanism can be more complicated, involving transmembrane proteins, cleavage of molecules that stick out of the cells, as well as receptors on the cell surface that receive the signal. For instance, the product of the wingless-gene mentioned appears to be a transmembrane protein. If such more complicated mechanisms are involved in the exchange of molecules between the cells, a pattern-forming system can appear to be more complex.

The feedback loops required for segregation into different cell types can be realized directly on the gene level since the corresponding substances should not diffuse. Such loops can consist, for instance, of a mutual repression of genes. If, on the other hand, a graded distribution of molecules is generated and used as positional information (Wolpert, 1969, and this volume), an interpretation by a concentration-dependent gene activation is required. This is possible if the decisive genes have properties formally similar to the pattern-forming reactions mentioned above, in that the genes have a direct or indirect feedback on their own activation but compete with each other. Sharp borders between regions of different gene activities are formed due to this autoregulation and competition. The autoregulation leads to a permanent activation of the genes, independent whether the positional signal is present or not. Meanwhile, positive autoregulation has been demonstrated for many genes (see, for instance, Kuziora and McGinnis, 1988; Bienz and Tremml, 1988; Hiromi and Gehring, 1987).

The term ‘morphogenetic gradient’ is used in the literature for two very different concepts. (i) Morphogenetic gradients as a graded ability of a tissue to form a particular structure upon regeneration (Morgan, 1904). The tissue with the relatively highest ability will form that structure. This concept has been introduced to account for the regeneration of fragments of tubularia with predictable polarity. In the models proposed, this graded ability corresponds to the graded source density (Fig. 2B). (ii) Morphogenetic gradients as substances that provide positional information (see Wolpert, this volume) by their absolute concentration via a concentration-dependent gene activation. In terms of the model, the activator, the inhibitor or any substance under their control or which spreads from a localized source can act as such a gradient. A clear distinction should be made between these two concepts.

For the generation of subfields such as limb fields, positional information can be created at the borders between regions of different determinations (Meinhardt, 1980; 1983a,b). According to this model, it is not that first a limb field is formed which later becomes subdivided into anterior and posterior as well as into dorsal and ventral parts; to the contrary, it is assumed that the structures are formed around borders generated in a preceding step. This model has found strong support, for instance by the finding that the gene responsible for the posterior compartment in Drosophila, engrailed, is expressed at the correct positions and with sharp borders already at the blastoderm stage (Kuner et al., 1985), long before any imaginal disk could be formed. The proximodistal dimension is assumed to be organized by a gradient generated by a cooperation of the anterior and posterior, as well as the dorsal and ventral compartment. The result would be a cone-shaped distribution, centered over the intersection of the two compartment borders. The gene decapentaplegic is presumably involved in this process (see Gelbart, this volume). A cone-shaped pattern is appropriate to organize, for instance, a leg disc with the concentrically arranged primordia of the leg segments as well as for the separation of the cells forming the imaginal disc from the cells forming the surrounding larval ectoderm.

According to this model, genes such as engrailed or wingless have two functions. Primarily, they are involved in the generation of a cell state (or in the mutual stabilization of two cell states) forming one element in a sequence of cell states which constitute together a segment with a defined polarity. In a second step, the juxtaposition of two such cell states provides a prerequisite to form a substructure such as a leg or a wing. The confrontation of other pairs of cell states provides the signal to form a segment border (Meinhardt, 1984; 1986).

The borders generating positional information for vertebrate limb development are less clear. Oliver et al. (1988a,b) found a large region of XIHbox1 expression in Xenopus and mouse with a posterior border within the forelimb, close to the predicted position of a border (Meinhardt, 1983a). An indication for the second predicted border coincident with the apical ectodermal ridge has not yet been obtained.

The models mentioned above have been proposed on the basis of regulatory properties of developing systems. The new tools of molecular biology provide an opportunity to isolate the molecules involved in the patterning process. I hope that both approaches provide a fertile interaction.

References


