Space-dependent Cell Determination Under the Control of a Morphogen Gradient

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A model is proposed for space-dependent cell determination under the influence of a morphogen gradient. It provides an explanation of how groups of cells can be programmed in a particular direction and how a jump from one determination stage to the next can occur between them even though the controlling signal is of a smoothly graded morphogen concentration. Together with an earlier proposed mechanism for pattern formation, these models offer a complete system for the generation and interpretation of positional information. Each member of a set of structure-controlling genes is assumed to feed back onto its own activation such that a gene, once activated, remains in the activated state. A repressor, however, is produced by any activated gene of this set. This assures that only one gene of this set is active in one cell at any one time. A selective activation of a particular gene is possible if (i) the morphogen competes with the gene-produced, non-diffusible repressor, (ii) the feedback loops have some overlap and (iii) a hierarchy exists among the structure-controlling genes. The kinetics of this determination have all the properties demanded earlier from a study of the early insect development: It proceeds stepwise from determination for more anterior to more posterior structures until the gene that is activated corresponds to the local gradient level. A more anterior structure will be formed if the gradient is destroyed before the final determination level is reached. A more posterior structure will be formed after an additional increase of the morphogen concentration.

After completion of the determination, the repressor concentration in each cell depends on which gene has become activated and it can be made roughly proportional to the morphogen concentration which the cell has seen. Therefore, a stable parameter (positional value) becomes available which can be used for further developmental decisions.

1. Introduction

A most fascinating aspect of higher organisms is the reproducible generation of their structures in all their fantastic complexity and precise spatial arrangement during embryonic development. There are indications that not every
structure is determined independently, but that sets of structures are formed under a common control. Several "organizers" (Spemann, 1938; Child, 1946) are known which must be present for the determination of the surrounding cells. For instance, if the posterior tenth of the egg of a dragon fly is removed, none of the normal structures are formed (Seidel, 1929), or, a small area, the zone of polarizing activity (ZPA) at the posterior boundary of the chick wing bud is necessary for the development of the wing digits (Saunders & Gasseling, 1968; Tickle, Summerbell & Wolpert, 1975).

A classical model for the determination of several structures under a common control assumes a graded distribution of a substance—the morphogen—and that the local concentration of this morphogen controls the further pathway of development of each particular cell. For the above-mentioned antero-posterior organization of a chicken wing, many transplantation experiments (Tickle et al., 1975) can be explained under the assumption that the "positional information" (Wolpert, 1969, 1971) for the digits consists of a graded distribution of a substance which is produced at the posterior boundary, at the ZPA. The gradient was assumed to be made by a very local production of the morphogen, by its diffusion to the surrounding, and by its decay.

The gradient hypothesis requires that the cell can sense a particular morphogen concentration with quite high precision. Over a small range of a particular morphogen concentration, a particular control gene has to become activated; whereas, if the morphogen concentration is only slightly higher, another control gene must become active and the former one must presumably be repressed. The question is: what mechanism can be responsible for such precise activation and repression of genes. In the following article, we propose a mechanism, something like, say, a biochemical analogue to digital converter, in which the activation of a particular gene becomes possible by an interference of the morphogen with a gene-activator-repressor system.

Some information as to how the cell determination proceeds under the influence of a morphogen gradient we have derived from the analysis of segment formation after transverse ligations of insect eggs which has forced the following conclusions (Meinhardt, 1977):

(1) Initially, all cells or nuclei together with their plasma environment are programmed to form the most anterior structure.

(2) The interpretation of the gradient is not a one-step process, but rather the cells proceed stepwise to more posterior determination levels until their determination corresponds to the local morphogen concentration. The process of stepping through all determination levels is time-consuming. The higher the morphogen concentration, the more posterior the structure formed will be and the later the final determination will be achieved.
(3) This process is essentially irreversible. After removal of the morphogen, the cells will remain in the determination state they have already attained. In contrast, after an additional increase of the morphogen concentration, the cells will follow and form a more posterior structure.

2. How to Generate Positional Information

The assumption of a localized morphogen source for the explanation of pattern formation would contain a circular argument as long as no explanation for its formation can be provided. We have proposed (Gierer & Meinhardt, 1972, 1974) a mechanism for pattern formation which is based on the autocatalytic feedback of a substance, the activator $a$, on its own production and in which this autocatalysis is acted upon by a more rapidly diffusing antagonist, the inhibitor $h$. According to the general criterion, such a pattern-forming reaction can consist of the following interaction (Fig. 1):

$$\frac{\partial a}{\partial t} = c \frac{a^2}{h} - \mu a + D_a \frac{\partial^2 a}{\partial x^2} + \rho_0$$  \hspace{1cm} (1a)

$$\frac{\partial h}{\partial t} = c a^2 - v h + D_h \frac{\partial^2 h}{\partial x^2} + \rho_1.$$  \hspace{1cm} (1b)

By the inclusion of these aspects—autocatalysis and lateral inhibition—a quantitative simulation of many experiments concerning early insect development is possible (Meinhardt, 1977), for review of the experiments see Sander (1976) or Counce (1973). Further, this dynamic regulation of the source provides a natural explanation (Meinhardt, 1978) for a rather unexpected observation in the determination of digits in the chick wing bud. Tickle et al. (1975) have found that two organizing centres (ZPA’s) grafted close together, surprisingly leads to fewer digits than found in normal development. This result indicates that two sources in close proximity produce only a lower morphogen concentration compared to that produced by one source alone. This observation contradicts the assumption of a static gradient formation. In contrast, the mutual inhibition of two sources in close proximity is a necessary consequence if the pattern formation is based on autocatalysis and lateral inhibition. The activated area has many properties of an organizer (Meinhardt, 1978) and, at least for early insect development, the experiments are compatible with the assumption that the long-ranging inhibitor acts as the morphogen and provides the positional information for the cells surrounding the organizer.

If the field has to be subdivided into only two portions, a reaction according to equation (1) is sufficient to separate the cells unequivocally into “on”
Fig. 1. Pattern formation by autocatalysis and lateral inhibition (Gierer & Meinhardt, 1972, 1974). (a), (b): Generation of a graded concentration profile of an autocatalytic substance, the activator (a), and its more diffusible antagonist, the inhibitor, according to equation (1). Concentrations of both substances are plotted as function of space and time. A growing linear array of cells with impermeable margins is assumed. In a small area, both substances are in a stable equilibrium since any increase of the activator concentration leads to a corresponding increase in the inhibitor concentration which in turn regulates back the activator concentration. Pattern formation starts quite abruptly after a size of the order of the activator range has been exceeded. The homogeneous distribution is then no longer stable since a small local activator increase, caused even by random fluctuation can increase further by the autocatalysis because the additionally produced inhibitor diffuses rapidly into the surroundings, regulating down the activator production there. In growing systems, the high concentration must appear at one end and can also remain there during further growth. The result is a stable, graded distribution of both substances which can be used for the supply of “positional information” (Wolpert, 1969, 1971). (c), (d): Zones with high or low concentrations and with a discontinuous jump in between are formed if, after the formation of the pattern, the diffusion is restricted. This may be achieved by the closure of intercellular junctions. Cells which contain at least a certain activator concentration will return to the average concentration, the others will remain stable at very low concentration. Whether or not such a threshold exists, depends on $p_0$ and $p_1$ in equation (1). Therefore, if the field has to be separated into only two parts, the mechanism for the pattern formation and interpretation may be identical.

and “off” cells should diffusion become restricted [Fig. 1(c), (d)]. In such a case, the mechanism of pattern formation and interpretation may be identical. Binary decision processes (Kauffman, Shymko & Trabert, 1978) seem to occur in the first subdivision of imaginal discs (Garcia-Bellido, Ripoll & Morata, 1973). In the model, the activated portion is usually smaller than the
non-activated portion. Therefore, such model would explain the different sizes of both portions as is experimentally observed (Garcia-Bellido et al., 1973).

3. The Activation of One Gene out of a Set of Genes

The process of pattern formation and the selective activation of a particular gene share many properties. In pattern formation, a substance is produced at a particular location and this production is inhibited at other locations. Similarly, the determination of cells in a particular direction would correspond to the activation of a particular control gene and the repression of others. It is thus tempting to explain the selective activation of a gene with principles similar to those we have used to explain pattern formation, namely autocatalysis and inhibition. In pattern formation, the inhibition has a spatial component hindering other cells from becoming autocatalytic. In gene activation, the inhibition (repression) takes place within cells, in "gene space", to assure that only one gene of the set becomes active.

Let us assume that a set of control genes no. 1, 2, ..., i, ... exist. An active control gene is characterized by a high concentration of the corresponding gene activator $g_i$. To achieve a state in which an activated gene maintains its activity, each gene activator has to feed back on its own production. In analogy with our previous pattern-forming equation (1a) and (1b) the change of a particular gene activator concentration can be written

$$\frac{dg_i}{dt} = \frac{c_i g_i^2}{r} - \alpha g_i,$$

where $c_i g_i^2$ describes the autocatalytic feedback ($c_i$ is a constant) and $-\alpha g_i$ the decay. The feedback is counterbalanced by the action of the repressor $r$. The repressor $r$ is assumed to act on all genes of the set and each activated gene will produce this repressor:

$$\frac{dr}{dt} = c_i g_i + \ldots c_l g_l + \ldots - \beta r.$$

In such a system, only one gene can be active in one cell. Two active genes would compete with each other via the repressor. This creates an unstable situation in which one gene would finally suppress the other [Fig. 2(a)]. On the other hand, the single gene which is active is in a stable equilibrium with the repressor since, for instance, after a reduction of $g_i$, a more pronounced reduction of the repressor will follow [equation (3)]. The system will then, due to the autocatalysis, return to the stable equilibrium.
Fig. 2. (a): Only one of several non-linear feedback loops (gene 1, 2,...) is stable if they compete via a common repressor ($R$), equations (2) and (3). An artificial activation of another loop will either disappear or will win the competition, suppressing the previously activated one. (b): If the feedback loops show some overlap and saturation [equation (5)] and a hierarchy [equation (4)], a controlled sequential activation is possible under the influence of an interfering morphogen ($M$). To demonstrate the abrupt switch from one gene to the next, a slight, smooth increase of the morphogen is assumed. The morphogen competes with the repressor, which leads to an increase in the concentration of the gene activators. Since the activated gene is in any case at its upper saturation level, the gene next higher in the hierarchy which is slightly activated by the overlap of the feedback loops mainly profits from this. Ultimately it becomes autocatalytic and represses the previously active gene. (c), (d): The activation of a particular gene under the influence of a lower or higher morphogen concentration. After passing through all intermediate states, a certain either lower or higher (more posterior) gene will become activated. With each step, the repressor concentration increases and the stepping through will come to rest if the morphogen concentration is insufficient to replace the increased repressor concentration. Therefore, after the completion of the determination the repressor concentration is approximately proportional to the morphogen concentration the cell has seen. (e): After reduction of the morphogen concentration, the cell will remain in the already obtained determination stage. If the removal happens before the final state is obtained, the determination level will be lower (more anterior). (f): After an additional increase of the morphogen, the determination will follow to a higher level. Constants used for the computer simulations with equations (3) and (5): $c_1 = 0.05$, $c_{i+1}/c_i = 1.35$; $\delta = 0.05$, $\kappa = 2$, $m = 0.5$ [Fig. 2(c)] or 2 [see (d)], $\alpha = 0.2$, $\beta = 0.3$. 
4. Stepwise, Sequential Activation of the Feedback Loops

To make this system useful, we must arrange it so that the particular gene which becomes activated is controlled by the local morphogen concentration. This can be achieved in several ways. To give an example, we make the following assumptions:

(1) Genes have an overlap; gene activators of neighbouring structures have a certain physical similarity to each other. The gene activator $g_i$ can bind also to gene $i+1$ and $i-1$, but less effectively than to gene $i$. Thus, if, for instance, the first gene is active, the $g_1$ molecule will activate also the gene 2, but only to an extent that is normally insufficient to overcome the repression arising from gene 1.

(2) The genes have a hidden hierarchy in the efficiency of the autocatalysis. In terms of equation (2), we will assume that

$$c_{i+1} > c_i. \quad (4)$$

Such a hierarchy might arise from ordered differences in the rate or the firmness of binding of the activators to their corresponding genes.

(3) We will further assume that an activated gene operates at its upper saturation level: Thus, a decrease in the repressor concentration will not lead to a significant increase in the gene activator production in the activated gene.

(4) The differentiation-controlling morphogen has an activating influence on the gene-activator production. For instance, the morphogen can have some physical similarities with the repressor molecule such that it can block the binding sites of the repressor (competitive inhibition of the repression).

With these additions, the change of the gene activator per time unit can be written

$$\frac{dg_i}{dt} = \frac{c_i(g_i+\delta g_{i+1}+\delta g_{i-1})^2}{1+\kappa g_i^2} \cdot \frac{1}{r/[(1+m/r)]} - \alpha g_i. \quad (5)$$

The first term describes the autocatalysis. Genes neighbouring in the hierarchy of an activated gene become also activated, but only slightly, since the overlap $\delta$ is small. The denominator of the first term describes the saturation at high $g_i$ concentration. The second term describes the repression by $r$ and the competitive inhibition of the repression by the morphogen $m$. For instance, if $m = r$, the effective repressor concentration is reduced by a factor two.

To see how gene activation in this system can be controlled by the morphogen, let us assume that initially gene 1 is active. Gene 2 is therefore also active, but only to a limited extent. If increasing amounts of morphogen molecules displace the repressor from the binding sites, the general effectiveness of the
repressor is undermined and the concentration of all the gene activators will increase. Since gene 1 is already operating at its upper saturation level, it is mainly the neighbour of the activated gene, in this case gene 2, which profit from this activation due to the morphogen. If the morphogen concentration reaches a sufficient level, the fact that gene 2 is more effective than gene 1 in the autocatalysis will become important: gene 2 becomes autocatalytic and the repressor produced will suppress gene 1. A sudden change from one activated gene to the next has occurred and the next determination level has been reached [Fig. 2(b)].

An interaction according to equations (2) and (3) has a property which is important for the ordered activation of a sequence of genes: The concentration of a gene activator in the steady state is independent of which gene is activated and only determined by the decay rates: \( g_i = \beta/\alpha \), whereas the repressor concentration depends on which gene is active: \( r = c_i\beta/\alpha^2 \). Since \( c_{i+1} > c_i \), this signifies that the repressor concentration increases if the next gene in sequence has been activated [Fig. 2(b)]. Consequently, for each additional switch to the next gene, a higher morphogen concentration is necessary to displace the increased amount of repressor. Therefore, the sequential activation of one gene after the other will come to rest if a gene is activated whose repressor concentration corresponds to the local morphogen concentration.

The stepwise determination in a single cell to different levels under the influence of different morphogen concentrations is shown in Fig. 2(c, d). The system has also the properties deduced earlier from the nature of determination of insect segments: If the morphogen is removed before the determination process is completed [Fig. 2(e)], the cell will remain stable at the determination level which has already been obtained and which can be lower than that which would eventually have been achieved. On the other hand, if the morphogen concentration is increased at a later time, the determination can proceed to a much higher level [Fig. 2(f)]. Figure 3(a)–3(c) shows the determination process in a linear array of cells under the control of an activator-inhibitor (morphogen) system. Under the influence of the smoothly graded morphogen concentration, the determination level remains the same in a number of cells but then changes discontinuously to the next higher level. Figure 3(d) shows the resulting determination if a ligation (diffusion barrier) is introduced. The terminal segments are formed as long as the ligation is not too close to the source-containing end. However, some levels are absent around the ligation. This may correspond to the gap phenomenon observed after the ligation of an insect egg (Sander, 1976).

The assumed interaction of the morphogen and the repressor is of course only one of several possibilities. For instance, the morphogen could also
Fig. 3. Stages in the formation and interpretation of pattern in a linear array of cells. Positional information is generated by an activator-inhibitor system (see Fig. 1); the inhibitor is assumed to act as morphogen.

Initially the gene no. 1 is assumed to be active in all cells (a), the repressor concentration is correspondingly low. Under the influence of the morphogen, one feedback loop after the other of the higher genes becomes activated (b) until (c) the system achieves a stable state in which groups of adjacent cells are in a certain determination state and in which a switch from one gene to the next occurs without a zone of transition. The repressor concentration in this final state is approximately proportional to the morphogen concentration the cell has seen. Since the repressor concentration (in contrast to the morphogen concentration) is a stable cell property even if the cell is isolated from its neighbours it can be used as “positional value”. (d) If, by a ligation, a diffusion barrier is introduced [at the time shown in fig. (b)] in the non-activated part the morphogen concentration drops to very low concentration. Here, the cell maintains the already achieved determination level. In the activated part, the inhibitor accumulates at the barrier and the result is a gap in the sequence of activated genes, similar to what is observed after ligations of insect eggs. Constants used for the simulation: equation (1a) and (1b): $\epsilon = 0.01, \mu = 0.007, D_a = 0.01, \rho_0 = 0, \nu = 0.009, D_h = 0.4, \rho_1 = 0.02$; equations (3) and (5): see Fig. 2 and $m = 1.2 \times h$.

substitute (unspecifically) for one of the two gene-activator molecules $g_i$ which are necessary for the activation. The example chosen should merely illustrate the general principle.

A gradient and its interpretation can accomplish a subdivision along one axis only. However, the subdivision generates the prerequisite condition for an orthogonally oriented subpattern. This is because the pattern has the
tendency to orient itself in the direction in which there is the most space in which the diffusion of the molecules in question can take place. This would be a reason why subdivisions in perpendicular orientation occur mostly one after another in discrete steps. In insects, the second field to be organized would consist of a narrow ring of blastodermal cells. A high concentration at one point has to decrease symmetrically on both sides. Any particular concentration will be presented twice and the resulting structures would be formed in pairs, like eyes, wings or legs.

5. Some Points About a Possible Molecular Interpretation

In the following we will attempt to demonstrate that simple and biochemically reasonable interactions are compatible with the proposed mechanism.

Let us first assume that a control gene contains a recognition site for the gene activator. Since the reaction equation (2) is co-operative \( \left(g^2\right) \), two gene-activator molecules or a dimer have to bind to allow transcription. The control gene must further contain a repressor coding site and a repressor binding site; both would be the same in each gene of the set. To account for the autocatalysis, a control gene must also contain the sequence which codes for its own gene activator.

It seems to be worthwhile to consider the possibility that the gene-activator molecules are RNAs (Britten & Davidson, 1969; Davidson, Klein & Britten, 1977). If, indeed, base-pairing between RNA and DNA can lead to the initiation of transcription, the autocatalytic feature of such reaction—as required by the model proposed—is evident. Since we require at least two activating molecules to initiate the transcription, the possibility arises that both DNA strands have to be paired with the corresponding RNA sequence. However, if transcription proceeds only in one direction, a base-pairing to both DNA strands is only possible if the site to be recognized has a reverse complementary (palindromic) arrangement of base pairs. This would facilitate the DNA–RNA base-pairing and would also guarantee that not every RNA–DNA homology, but only that of very special sites, leads to an activation of transcription. Palindromic sequences are known to exist in the chromosomes of higher organisms and it has been shown that they are transcribed (Schmid, Manning & Davidson, 1975).

The required similarities between the recognition sites (see also Gierer, 1973) of neighbouring structures would be given if only a few base pairs are different. The hierarchical aspect could be realized in that a gene higher in the hierarchy contains more copies of the recognition sequence in a repetitive manner. Since the size of the steps in the hierarchy is decisive in
determining how much more morphogen is required for the next switch, this would allow a direct control of the size of the individual structures.

A direct regulative role by RNA is not known to exist in prokaryonts, but strong autocatalytic elements for the maintenance of a particular pattern of gene activities independent of changes in the cell environment, are necessary and to be expected in higher organisms only. However, the general model would work as well if the regulatory RNA sequences are first translated into regulatory proteins which then in turn recognize the corresponding sites on the DNA.

It is easy to imagine how such a system has been evolved. If a control gene duplicates and the binding is slightly changed by a mutation, the required similarity between the $g_i$ molecules on one hand and the $c_i$ constants on the other is still very likely to be present and a new and similar structure would have been added or inserted into the set. Also the strong similarity of the pattern formation system and the pattern interpretation system, including the physical similarity of both the inhibitor and the repressor suggest a common evolutionary origin.

6. Advantages of Overlapping, Competing Feedback Loops for the Control and Maintenance of Determination

The proposed mechanism is particularly simple in that the molecules which maintain a cell in a particular determination state and the molecules which allow a transition from one determination to the next are identical. The sequential activation of the genes depends in this model on the idea that the specificity of these molecules is not absolute. On the other hand, the stability and the low spill over from one gene to the next results from the non-linear feedback. A major simplification is achieved in having only one gene of the set active in a cell. On the contrary, in a pure threshold model (Lewis, Slack & Wolpert, 1977) an increased morphogen concentration will activate an additional gene without the repression of the other lower genes. However, it could be undesirable to have, for instance, also the genes 1, 2 and 3 active if the structure 4 were to be formed. An additional switching system would be necessary to select only the desired gene.

The translation of the morphogen gradient is very reliable since it does not depend on a single measurement at a particular time. In contrast, a particular structure can only be determined if previously a determination into a state corresponding to that of its lower (anterior) neighbour has been achieved. If, as in many insect species, only a fraction of the gradient is used, a change in one parameter would only shift the entire structure in a more posterior or anterior direction without endangering the structure itself. Also a separate size regulation mechanism would be superfluous. The dependence of the
reactions proposed on other parameters, such as temperature or nutrition, can be kept small since both pattern formation and interpretation depend on antagonistic reactions in a way which allows such influences to be compensated for.

As mentioned before, the repressor concentration in a particular cell depends on which gene is active. Moreover, the repressor concentration is roughly proportional to the morphogen concentration the cell has seen [Figs 2(c), (2d) and 3(c)]: therefore, the positional information is maintained as if in a frozen form and remains also available if the morphogen should disappear. Therefore, after the interpretation of the positional information, a quantitative parameter, a “positional value” (Wolpert, 1969, 1971) is available which records which gene is active. This can be used for the communication of a cell with its neighbours, as is necessary for growing systems (see below). The existence of such stable positional value has been deduced from experimental observations in several systems (Lawrence, Crick & Munro, 1972; Nardi & Kafatos, 1976). The synthesis of a surface protein may be controlled by the repressor such that each cell obtains a stable quantitative label according to its state of determination. This could enable a sorting out of cells, for instance, at the compartment borders (Lawrence & Green, 1975).

An alternative model for the spatial differentiation has been proposed by Kauffman et al. (1978) in which it is assumed that a sequence of different patterns of the same substances of a reaction–diffusion system appears, e.g. by the shrinkage of the diffusion ranges. These patterns are assumed to set the elements of a binary epigenetic code. A cell, subjected to a high concentration would be transferred to the corresponding “1”-state, otherwise it would remain in the “0”-stage. A major achievement is that this model can account very precisely for the observed transdetermination frequencies. However, a binary-code model would also require an additional decoding mechanism for the translation of the binary code word into the activation of the corresponding gene. Further, a cell which has seen a zero-concentration twice must be in a different state (00) compared with a cell which has been exposed to “nothing, but only once”. This would require, for example, an additional counting or clock mechanism synchronized with the pattern formation process. The relatively long time interval in which a “double abdomen” can be induced in an insect (Kalthoff, Kandler-Singer, Schmidt, Zissler & Versen, 1973) or a second dorsal axis in amphibians (Spemann, 1938; Spemann & Mangold, 1924) argues against a clock mechanism or against early irreversibly binary decisions.

A further possibility to distinguish on the basis of experimental observation between these two alternative models may be as follows: according to the binary-code model, the subdivision separates the field first into two precise
geometric halves, then further into quarters and so on. In contrast, no such geometric restrictions are present in a gradient model. The interpretation would be especially reliable if only a fraction of the gradient is used. In early insect development, species are known which use almost the total egg-like Drosophila. However, in others, like Tachycinis or Acheta, the basic body pattern is laid down only in a very small fraction of the egg, near the posterior pole (see Sander, 1976). This flexibility can be easily explained with a gradient model.

7. The Orientation of the Hierarchy

In the proposed form the model is based on the assumption that the undifferentiated cell is in a semistable equilibrium and that a small kick, applied in the form of the interfering morphogen, is sufficient to shift the cell stepwise into more stable (posterior) states (stepping down). But one can also envisage a reversed orientation of the hierarchy implying that the undifferentiated state is the most stable one and that the morphogen is able to lift the cell into an "excited" state (stepping up). Observable differences are to be expected from these alternatives. In the first case (stepping down) one would expect the general instability of the cell to proceed after a disturbance further in the direction which corresponds to the next differentiation level, that means, in the posterior direction. In the other case (stepping up) one expects that upon a disturbance the cell will fall back towards its original state, in more anterior direction.

Arguments can be found for both orientations. Homoeotic mutants (Morata & Lawrence, 1977) show frequently thoracic structures in the head region, an anterior to posterior transformation as expected from the orientation of the hierarchy as assumed in the paper. On the other hand, trans-determination (Gehring & Nöthiger, 1973) can lead—among other possibilities—to a transformation of genital or thoracic structures into head structures. Therefore, the orientation of the hierarchy must be regarded as an open question.

However, another possibility for a later change of a previously obtained determination should also be taken into consideration: the re-activation of the pattern formation system. The activator concentration of the gradient-forming system is very low outside the activated area. It can be kept at this low concentration by a small inhibitor production $\rho_1$ [equation (1b), see Fig. 1]. But if the inhibitor concentration becomes too low, maybe because $\rho_1$ is altered by a mutation or because a group of cells is kept in tissue culture and inhibitor diffuses into the environment, a new high activation can be generated via the basic activator production $\rho_0$ and autocatalysis. (According to the model
(Meinhardt, 1978), this process would correspond to an unspecific induction
(Spemann & Mangold, 1924) of a second organizer region.) If diffusion is
then restricted, only the average concentration which corresponds roughly to
a positional information for thoracic structures will be achieved [see Fig.
1(c), 1(d)]. This may be the reason why in homoeotic mutants a transformation
of head structure into thoracic structures is frequently observed, why in
transdetermination (Gehring & Nöthiger, 1973) thoracic structures appear
to be a developmental sink or why transdetermination can affect several
adjacent cells simultaneously (Gehring, 1967), despite the fact that gene
activators appear to be strictly non-diffusible (Garcia-Bellido, 1975).

8. Pattern Formation in Growing and Non-growing Systems

So far, we have dealt only with non-growing systems. However, frequently
determination takes place during either intercalary or marginal growth.
Imaginal discs (Garcia-Bellido, 1975; French, Bryant & Bryant, 1976) or
the proximo-distal organization of the vertebrate limb (Saunders, 1948;
Wolpert, 1975) are examples. In non-growing systems, as we have seen, it
suffices that a cell measures passively the local morphogen concentration.
No active communication is necessary between the cells as indicated by the
gap phenomenon in early insect development (Sander, 1976). However, it is
impossible in such a system to add new structures by growth, because the
new cells would see the same morphogen concentration as other cells have
seen before. Therefore, for growing systems, the passive interpretation is
insufficient. In contrast, the cells have to signal their state of determination
and this feedback-signal can modify the source strength of the organizer.
For instance, the source strength and therefore also the morphogen con-
centration can increase if higher genes are activated in the surroundings of
the source. This could have the consequence that even higher genes can
become activated after further growth, leading to a regular arrangement of
sequentially activated genes. In the proposed model, the concentration of
the repressor provides a measure of which structure is already present and,
therefore, it can be used to produce this feedback signal. The existence or
non-existence of this feedback may be the basis of the difference between
epipmorphosis and morphallaxis since the highest concentration of the
gradient either depends on the determination of the existing cells in the
neighbourhood of the organizer or it corresponds to the most posterior (or
most distal) structure to be formed, independent of the surrounding cells.

In growing systems, the first subdivisions may take place in the non-
growing mode also. For example, the first separations into anterior–posterior
and into dorsal–ventral compartments take place in a period without cell
proliferation (Garcia-Bellido, 1975). In fact, the feedback of the achieved determination on to the gradient-forming system, such as postulated for growing systems, would make sense only after some cells have obtained a different determination than others. This would signify that the determination in the non-growing mode, as proposed in this paper, is a first and essential step in the generation of the diversity of the differentiated cells.

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