Chapter 11

The activation and maintenance of determined states

The geometry of a morphogenetic field is quite restricted if pattern formation mechanisms are involved which depend critically on diffusion. Since the time required for communication amongst all the cells of a field increases quadratically with the mean dimension of the field, pattern formation must take place within small assemblies of cells. As has been pointed out by Wolpert (1969, 1971), embryonic fields are indeed small, of the order of 1 mm or 100 cells across, a size in which communication via diffusion can take place in a few hours. As this initially small field grows to attain its final size, it becomes necessary that either the pattern forming mechanism or the response of the cells is turned off. Otherwise, as the field of cells enlarges, periodic prepatterns may emerge, which would destroy the normal relationship between body parts. By the instructions a cell obtains during its early developmental history the cell becomes determined for a particular developmental pathway. Determination would be a long-term memory for the developmental signals to which the cell was exposed. This memory should be maintained even when the cells come into contact with cells of different determination, for instance, due to folding of cell sheets or due to migration of individual cells (such as neural crest cells) through the organism.

11.1 Biochemical switches

The maintenance of a particular determined state is a dynamic process. For instance, imaginal disk cells can maintain their state of determination over many generations in tissue culture. However, transdetermination - abrupt changes in determination - can occur (Hadorn, 1967; Gehring and Nöthiger, 1973), indicating that determination involves stabilization of a particular state and suppression of alternative pathways. A particular determined state is presumably characterized by activation of a characteristic set of genes. Such a state can be maintained by feedback of the activated genes upon their own activity. This would occur if a gene is transcribed in the nucleus, the associated mRNA is then transported into the cytoplasm and directs there the synthesis of a protein which activates the further
transcription of that gene. Non-linear feedback-loops enable the formation of two or more stable states which can be selected by external signals and in which the cell would remain even after a removal of these signals.

The following simple reaction has two stable states (Meinhardt, 1976; Lewis, Slack and Wolpert, 1977)

$$\frac{dg}{dt} = \frac{g^2}{1 + fg^2} - eg + m$$

(11.1)

The substance $g$ has a non-linear, saturating feedback on its own production and a normal first-order decay. At low $g$ concentration, the negative linear decay term dominates, $dg/dt$ is negative, and the $g$ concentration decreases further (Fig.11.1). At higher $g$ concentration, the quadratic production term becomes important and the concentration increases until saturation is reached. A well-defined threshold exists and only two stable states are possible (Fig.11.1).

An externally supplied morphogen, $m$, can cause a transition from one such stable state to the other. If $m$ contributes to the production of $g$, the $g$ concentration can be pushed above the threshold and the cells made to switch irreversibly to the state of high $g$ concentration. Even a shallow gradient of the morphogen distribution can cause an unequivocal separation of an area into distinct sub-areas with cells of high and low $g$ concentration, respectively (Fig.11.1b). Alternatively, developmental decisions could be characterized by a restriction of possible pathways, involving the switching off of sets of genes rather than the activation of additional genes. This is possible if the morphogen interferes with the feedback, bringing the $g$-concentration temporarily below the threshold. In both cases, the cells would remain in the new state even after withdrawal of the morphogen.
11.2 Alternative states

Determination can also consist of the selection of alternative pathways (in contrast to the possible activation of one feedback loop). For instance, either gene 1 or gene 2 becomes activated but the cell must decide between these alternatives since both gene activities are mutually exclusive within one cell. A simple interaction with such a switching behavior is described by the following equations.

\[
\frac{dg_1}{dt} = \frac{1}{a + g_2^2} - g_1 \quad (11.2a)
\]

\[
\frac{dg_2}{dt} = \frac{1}{b + g_1^2} - g_2 \quad (11.2b)
\]

(production and decay rates have been set arbitrarily to unity, \(a\) and \(b\) are introduced to give a Michaelis-Menten kinetics). It is easy to see that the switching behavior of Eq.11.2 has a similar formal basis as that of Eq.11.1. Calculating the steady state of \(g_2\) \((dg_2/dt = 0)\) and neglecting \(b\), we find \(g_2 = 1/g_1^2\). Inserting this into Eq.11.2a leads to

\[
\frac{dg_1}{dt} = \frac{g_1^2}{1 + fg_1^2} - eg_1 + m
\]

which is identical with Eq.11.1.

A biological system in which the selection of two alternative pathways depends on the mutual repression of two genes is the \(\lambda\)-phage. The DNA of the phage can either be integrated into the \(E\.coli\) chromosome and replicate accordingly (lysogenic mode) or can replicate independently and thereby eventually kill the host cell (lytic mode). A simplified reaction scheme is given in Fig.11.2. This reaction has an even higher non-linearity than given in Eq.11.2, since both repressing substances are active as dimers and, in addition, the \(\lambda\)-repressor has an autocatalytic feedback on its own mRNA transcription (Ptashne et al., 1980).
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Figure 11.3: Alternative stable states. Determination requires that a cell can remain stable in one of several alternative states. (a) A molecular interaction on the level of the genes which can accomplish this. Each of the alternative genes \( i (i = 1, 2, ... n) \) feeds back on their own activation via a gene activator \( g_i \); but competes with the others via the common repressor \( R \) (wavy line = repressor coding site). (b) Computer-simulation (Eq.11.3). Only one of the feedback loops (gene 1, 2...) is stable within a given cell. Artificial activation of another loop will either decay or win the competition, suppressing the previously active gene.

11.3 Similarities between pattern formation and the selective activation of genes

For many developmental decisions, presumably more than two alternatives must to be envisaged. How can one stabilize one out of, let us say, ten genes and suppress all the others? The selective activation of genes has many formal similarities with the formation of a pattern. In pattern formation, only a few cells (those in a given region) become activated, the others are inhibited. Similarly during determination, only a few particular genes become activated, the others are repressed. Determination is, so to speak, pattern formation in gene space and it is tempting to assume formally similar mechanisms.

An interaction analogous to the Eq.3.1 for pattern formation is given in Eq.11.3.

\[
\frac{dg_i}{dt} = \frac{c_i g_i^2}{r} - \alpha g_i \quad (11.3a)
\]

\[
\frac{dr}{dt} = \sum_i c_i g_i^2 - \beta r \quad (11.3b)
\]

Each gene \( i (i = 1, 2...n) \) of the set receives feedback from its own activity via a gene activator \( g_i \) (Fig. 11.3a). This has the consequence that a gene, once activated, remains activated. An antagonistic reaction is required in addition, otherwise every gene would be activated. This can be brought about by a repressor, \( r \), which is produced by every active gene and which acts upon every gene belonging to the set of alternative genes (Fig.11.3b). We have seen that differing diffusion rates play an important role in pattern formation. The corresponding parameter in the selective activation of genes is the specificity of the activating and inhibiting molecules. The low diffusion rate of the activator molecules corresponds to a weak cross-reaction of the different \( g_i \) molecules with
one another. The autocatalysis is either position-specific as in pattern formation or gene-specific as in gene activation. In contrast, the inhibitor acts due to its redistribution by diffusion, upon every cell of a field. Correspondingly, the repressor has to act upon every gene belonging to the set of alternative genes. In such a system, two active genes of the same set within one cell would create an unstable situation. Each would compete with the other via the common repressor (Fig. 11.3b): the dominating gene attains a stable equilibrium with the repressor. A decrease of a $g_i$ concentration, for instance, would lead to an overproportional reduction in the $r$ concentration, enabling a readjustment. Instead of utilizing a repressor, the mutual exclusion of the (autocatalytic) genes can also be obtained by competition for a common precursor molecule, in analogy to the pattern formation equation 5.1.

Taken together, the simple reactions described in Eqs.11.3 have two essential properties: a gene, once activated, remains active; and activation of different genes is mutually exclusive; only one of the several alternative genes can be active within one cell. The formalism developed is general and can be applied to many self-stabilizing systems. The activation of particular genes is only one of the more straightforward interpretations. The major question that remains is how a system chooses the correct state from several alternatives. As mentioned, experimental evidence suggests that there are two possibilities: either mutual induction of such locally exclusive states (see chapter 13), or control by a morphogen gradient (see below).
11.4 Interpretation of positional information

Evidence has been discussed that the local concentration of a substance, the morphogen, is used to select a particular developmental pathway. The graded distribution of the morphogen controls, therefore, the formation of an ordered sequence of structures. The question then is how this positional information is to be interpreted: how to convert the labile morphogen concentration, which would be sensitive to any change in the geometry of the developmental field, into a stable state of determination. A biochemical analogue-to-digital converter is required. There are several ways that the local concentration of a morphogen can serve to selectively activate genes. From ligation experiments with insect embryos, it has been deduced that the cells do not measure the local concentration all at once but rather that they are “promoted” step by step, switching from a more anterior (or proximal) to more posterior (distal) state until the state achieved corresponds to the local morphogen concentration (see Fig. 8.7). This narrows down the possibilities of interpreting positional information considerably. A straightforward mechanism consisting of the direct sequential activation of the genes until the activated state matches the local morphogen concentration will be described in this section. Another possibility, combining a sequential with a periodic mechanism to activate genes will be developed later (see chapter 14).

The set of alternative states of determination may be compared with the steps of a staircase; a particular state would correspond to a wooden barrel resting on a particular step. It will remain stable on each of the steps but no intermediate levels are possible. The interpretation of positional information would correspond to a positioning of the barrel on a particular step under the influence of an external signal. A possible mechanism, by analogy, is that the barrel can be lifted up by a flood and the level at which the barrel comes to a rest after the flood has diminished is a measure of the highest level of the flood (Fig. 11.4). In a system based upon a morphogen gradient the height of the flood is position-dependent and therefore, successively higher final stable states are attained at defined spatial intervals.

11.5 Molecular mechanisms enabling the controlled activation of particular genes

The selection of one particular state out of several alternative states requires competing feedback loops (Fig. 11.3). Genes and gene activators are the most obvious candidates for the required feedback-loops but it should be kept in mind that feedback can take place at other levels, e.g. in the control of translation or of RNA-processing and transport into the cytoplasm. In terms of genes, each gene $i$ ($i = 1, 2, ..., n$) has to have an autocatalytic feedback on its own activity and must compete with the others to assure that only a particular gene of the set can be active in any particular cell. To make such a system useful for the interpretation of positional information we have to arrange that the particular gene which becomes
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Figure 11.5: Stages in interpretation of positional information. A set of feedback loops ("genes") is assumed which compete with each other via a common repressor (Eq.11.4). A sufficient morphogen concentration ($m$, positional information) enables the activation of the next control gene. This transition is connected with an increase in the repressor concentration ($r$, positional value). Since the positional information provokes the transition, the positional value slows down the transition, the stepping through comes to a rest if the achieved determination corresponds to the local morphogen concentration. (a) Initially, gene 1 is active in every cell. The activity of control genes is indicated by the density of dots. (b) Intermediate and (c) final stable state. In groups of cells, the same control gene is active and an abrupt switch from one gene to the next takes place between neighboring cells. The pattern of gene activity would remain stable even after removal of the morphogen.

activated depends on the external signal, the local morphogen concentration. The following properties are required:

(1) Each gene $i$ must have the tendency to activate the following gene in the sequence, $i + 1$. In other words, a particular gene activity is triggered by the activity of the gene preceding it in the sequence.

(2) Transition to the next gene is possible only under the influence of the morphogen.

(3) The process of stepping through a sequence of genes must stop when the gene which is activated corresponds to the local morphogen concentration. Each step must require a progressively higher morphogen concentration. At a particular state of determination, the local morphogen concentration becomes insufficient to induce a further step. This requires some sort of hierarchy, similar to the levels of the steps in the staircase analogy given above.

The general principle can be molecularly realized in different ways (Meinhardt, 1978a). The following extension of Eq.11.3 (which describes the mutually exclusive activation of genes) should serve as an example. It allows a controlled activation of a particular gene under the influence of a morphogen:

$$\frac{d g_i}{dt} = \frac{c_i g_i^2}{r} - \alpha g_i \quad \text{with} \quad g_i = g_i + \frac{\delta m}{r} g_{i-1} \quad (11.4a)$$

$$\frac{d r}{dt} = \sum_i c_i g_i^2 - \beta r \quad (11.4b)$$

The first term describes the autocatalysis. A gene controlling a particular structure becomes slightly activated if the gene $i - 1$, the gene controlling the
Figure 11.6: Genealogical tree of determinations in systems controlled by positional information. (a) Under the influence of the morphogen, the cell switches from one state of determination to the next (1,2,3,4,...) controlling, for instance, which type of segment is to be formed in an insect. This suggests a ladder-like decision tree. The cells belonging to a particular specification can be further subdivided by a second positional information system, controlling, for instance, which part of an insect leg is formed (a,b,c,...). (b) In contrast, a sequence of binary decisions, such as proposed by Kauffman et al. (1978). The sequence of decisions shown in (c) is formally equivalent to that shown in (a). Such a representation is used to characterize one type of cell lineages in Nematodes (Ehrenstein and Schierenberg, 1980; Kimble, 1981).

anterior or proximal neighboring structure, is active. The cross-activation from the gene \( i - 1 \) is enhanced by the morphogen \( m \) and inhibited by the repressor \( r \). The factor \( c_i \) describes the efficiency of the feedback. Arbitrarily, we will assume a hierarchy \( c_{i+1} > c_i \). It is a property of the reaction Eq.11.4 that in the steady state \( (dg_i/dt = 0) \) the concentration of the gene activator depends only on the decay rates \( (g_i = \beta/\alpha) \) while the repressor concentration depends on which gene is active \( (r = c_i\beta/\alpha^2) \). With the hierarchy chosen \( c_{i+1} > c_i \), the repressor concentration increases with each transition to a higher gene. Since the repressor undermines the activation of the following control gene, a higher morphogen concentration is required, after each successful step for a further step. The stepping forward will come to rest at a particular control gene which is determined by the \( m \)-concentration. Fig.11.5 shows a simulation of Eq.11.4 for a linear array of cells. The positional information is a smoothly graded function of the position of the cells. Nevertheless, the cells respond in an unequivocal way. In groups of neighboring cells, the same gene is active and a switch from one gene to the next occurs without a zone of transition. Since the repressor concentration depends on which gene is active (Fig.11.5) it can be used as a stable indicator for the achieved state of determination of the cell, as positional value. A control gene once activated remains active in a homeostatic manner even after a decrease of the morphogen concentration. The cell has a long term memory with respect to the morphogen concentration to which it was once exposed. If, however, the morphogen concentration increases, the cells can switch to higher genes. In other words, if the positional information (morphogen concentration) is higher than the positional value (achieved state of determination, measured by the repressor concentration), the cells switch to higher states. If the positional
information becomes lower than the positional value, a cell remains stable in its present state. This interpretation of positional information is a strictly local process. Cell communication is required only for the generation of the positional information, not for the response. If cells not normally neighbors are juxtaposed, missing structures of the gap are not intercalated as long as no new positional information is generated. Gaps which are not repaired can occur between insect segments (Fig.8.3). The sequential switch from one state of determination to the next suggests a ladder-like sequence of decisions and not a series of binary decisions (Fig.11.6).

11.6 Positional information in systems with marginal growth - the proximo-distal axis of the vertebrate limb

Pattern formation by reaction-diffusion mechanisms can occur with or without growth. In discussing systems in which cell determination is controlled in a sequential fashion by threshold effects of the morphogen concentration, we have assumed that no substantial growth occurs before the interpretation of positional information is completed. This assumption seems valid for the insect egg, and the anteroposterior organization of the vertebrate limb. In contrast, the proximo-distal axis of the vertebrate limb becomes determined during a period of substantial growth. The elements are layed down in a proximo-distal sequence (Saunders, 1948, Summerbell et al., 1973, for review see Hinchliffe and Johnson, 1980). If cells are determined by a local morphogen concentration, a source of a morphogen at the tip of the limb as such would be insufficient to account for the sequential formation of new structures, because newly formed cells at the tip would be exposed to the same morphogen concentration as previously formed cells. One may question whether a positional information scheme is realized at all in such outgrowing systems and what type of interaction would eventually allow the accretion of new structures during outgrowth. We have shown that a possible mechanism for the sequential formation of structures consists in the increase of the maximum morphogen concentration during outgrowth (Meinhardt and Gierer, 1980). Such increase can be accomplished by feedback of the achieved determination onto the morphogen production. In addition to sequential determination, the model provides an explanation for regeneration, for presence and absence of intercalary regeneration, and for the instability to form the most distal structures, the digits, without all proximal structures being present.

The bud of a vertebrate limb consists of mesodermal cells in an ectodermal jacket with a thickening at the tip, the so-called apical ectodermal ridge (AER, see also Fig.10.7b). The AER is essential for limb outgrowth. Removal of the AER of the chicken wing bud leads to termination of further outgrowth. In this case only those structures which are already determined are formed. The later the AER is removed, the more distally complete the wing will be (Fig.11.7). At a very early stage, limb bud mesoderm from the chicken can induce an AER even in an
ectopic ectodermal cell layer (Kieny, 1960). Different models emphasize different aspects of limb development which should be part of an integrated explanation. The Saunders-Zwilling hypothesis (Saunders, 1969; Zwilling, 1961) stresses the mutual dependence of the limb bud mesoderm and the apical ectodermal ridge (AER). On the one hand, mesodermal cells are required to maintain the AER. The mesodermal cells presumably produce a substance required by the AER, the so-called apical ectodermal maintenance factor (AEMF). On the other hand, the AER induces the underlying mesodermal cells. To account for sequential specification along the proximo-distal axis, Summerbell et al. (1973) proposed that the cells in a so-called progress zone at the limb tip obtain, in the course of time, perhaps coupled to the cell divisions, a more and more distal determination. Cells leaving this labile zone are assumed to be fixed in their positional values. Faber (1976) proposed that a morphogen source is located at the tip but that the slope of the gradient close to the tip is so steep that it can be interpreted only after further outgrowth.

We have provided arguments to show that the limb field results from the cooperation of patches of differently determined cells (chapter 10). The AER marks presumably a boundary between “dorsal” and “ventral” cells (Fig.10.3). In attempting to integrate the different aspects - the role of the AER as well as the origin and effect of a progress-zone - let us assume that the AER is the source of a morphogen which controls the proximo-distal axis. It generates a morphogen gradient with the high point at the distal tip. At a very early stage of limb development, only very few structures are determined under the influence of this incipient gradient, let us say, structure 1 and 2. To achieve the increase of the morphogen concentration during outgrowth, we will assume a feedback of the achieved state of determination onto the source strength. The mesodermal cells are assumed to produce a substance - we will call it also AEMF - which controls morphogen production in the AER. Important is that a more distal structure produces more AEMF. With the addition of more state-2 cells at the growing tip, the AEMF concentration at the AER increases and therefore the
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source strength of the AER increases too. If a certain number of state-2 cells are present, the morphogen production of the AER becomes sufficient to switch some underlying mesodermal cells at the tip from state 2 to the state 3. Since AEMF is diffusible, its concentration depends on the average of the cell states, and the switch to state 4 is possible only after a significant proliferation of state 3 cells has taken place. Only at a region close to the tip can positional information be higher than the achieved determination (Fig.11.8). The model describes the progress-zone correctly. Cells at the tip acquire progressively a more distal determination while cells leaving this zone are fixed in their determination. Fig.11.8 shows a simulation of the pattern formation during outgrowth, in which the repressor concentration has been used as a measure of the achieved determination (see Fig.11.5). The production of AEMF is assumed to be proportional to the repressor concentration while the production of the morphogen, taking place in the terminal cell only, is proportional to the AEMF. It is an inherent property of the assumed switching mechanism that determination of a cell can only be changed towards more distal determination and then only if the positional information is higher than the positional value.

11.7 Regeneration of structures of the proximo-distal axis of the vertebrate limb

The model describes not only sequential determination during outgrowth but also the regeneration of parts removed. After removal of the distal part of a limb
Figure 11.9: Presence and absence of intercalary regeneration in amphibian limbs. If a distal fragment is transplanted onto a proximal limb stump (a, operation 1), intervening structures are reformed. The regenerate is entirely host-derived (b) as indicated by the pigment of the host (drawn after Pescitelli and Stocum, 1980). In contrast, if a larger distal fragment transplanted to a stump at a distal position (a, operation 2) no intercalation at the mismatching graft-host junction can be detected (c, drawn after Stocum, 1975b). Explanation in terms of the model: Morphogen concentration etc. drawn as in Fig. 11.8. (d,e) After removal of intervening structures (d), the distal tip still allows relatively high morphogen production. Cells in the stump become exposed to higher morphogen concentration (arrow in e) and acquire a more distal determination. The gap can be repaired (f). In contrast, if the graft-host junction is more remote from the AER, (g) the morphogen concentration is lower (arrow) than achieved determination and no respecification can take place.

Bud, regeneration can take place after formation of a new AER (in amphibians) or after implantation of a new AER (in chickens). Since the mesodermal cells of the stump determine via AEMF the source strength of the new AER, the morphogen concentration will be similar to the corresponding stage of outgrowth. The missing parts can regenerate in the same way as the original pattern was formed. In agreement with experimental observation, the age of the transplanted AER is without influence (Rubin and Saunders, 1972).

If a very distal tip (giving rise to the wrist and phalanges) of an amphibian limb bud is grafted on a proximal stump, the intervening structures can regenerate (Fig.11.9; Stocum, 1975a,b; Iten and Bryant, 1975). In more formal terms, if we denote the normal sequence of proximo-distal structures with 1,2...8, a gap of the type 123/67 will be repaired. In contrast, grafting a larger distal limb bud (3...7) onto another stump at a distal level (1...6) leads only to the structures expected from the fate map. In other words, the gap in the experimentally produced sequence 1...6/3...7 is not repaired (Fig.11.9; compare with a "real" intercalation, Fig.13.1). In both cases, the same structures (3 and 6) are juxtaposed. The difference in indicates that the decision whether intercalation takes place or not, is not a local process. Remarkably enough, in the first case (123/67), the new structures (45) are derived entirely from the stump. The cells of the stump are therefore
respecified towards a more distal determination. According to the model, the distal cells of the transplanted tip still allow relatively high morphogen production. The gradient extends from the AER into the stump region. The cells of the stump become exposed to a higher morphogen concentration in comparison with their own determination. In agreement with the unidirectional interpretation of positional information, they switch to higher (more distal) determination. Due to this, more AEMF is produced, and this leads to increased morphogen production and finally to repair of the gap. The respecification is caused by a high morphogen concentration and not by a juxtaposition of normally non-adjacent cells. The absence of intercalation in a sequence 1...6/3...7 strongly supports this view. According to the model, after this operation, the graft-host junction is too remote from the AER. The morphogen concentration is insufficient for any respecification (Fig. 11.9c,g). The regeneration of intervening structures after implantation of a very distal limb bud fragment on a proximal stump and the absence of repair of a gap at a larger distance from an AER, provides the best evidence available that interpretation of positional information and not a mutual induction of neighboring structures is involved in the determination of the proximo-distal sequence. In chicken wings, regeneration of intervening structures is possible only in very young wing buds, up to stage 22 (Summerbell, 1977). In terms of the model, whether internal deficiencies can be repaired depends on the range of the morphogen. If the range is small (low diffusion and/or short lifetime) the morphogen concentration declines rapidly with increasing distance from the AER (as shown in Fig. 11.8). After removal of an intervening structure the morphogen concentration in the stump area remains too small and no repair occurs.

A system of positional information with a feedback from achieved states has
an inherent instability. More distal determination leads to an increase in positional information which, in turn, causes even more distal determination. The system is normally stable since AEMF transmitting the feedback diffuses and depends therefore on the average determination, while an increase in positional information leads only to a local distalization in the region close to the AER. An increase in positional information therefore has only a limited effect on the average determination. However, certain parameter can lead to an unstable situation. Examples are: lowering of the AEMF diffusion rate or of the proliferation rate resulting, for instance, from killed cells. The result would be the formation of the most distal structures, the digits, at a premature position. Such a pattern has been observed in chicken limb buds after X-ray irradiation (Wolpert et al., 1979) and in children whose mothers have taken the drug Thalidomide during pregnancy (see Merker et al., 1980 for review).

A similar manifestation of the instability in the formation of the distalmost structure can be seen in the formation of digits if a small piece of a regeneration blastema is cultured in an ectopic position of the organism (Stocum, 1968). According to the model, cells at a distance from the AER must be available into which the increased AEMF can diffuse (Fig.11.10), otherwise its accumulation would lead to premature distal transformations.

In conclusion, feedback of achieved determination onto morphogen production and therefore onto positional information provides a positional information scheme in outgrowing systems. The lability of cells in the progress zone and the stability at more proximal positions is a necessary consequence. With such a model, the mechanism for determination of the antero-posterior (Fig.10.1, 10.7) and the proximodistal axes become very similar and both axes become fixed by the cooperation of patches of differently determined cells.
Chapter 12

Pattern formation by lateral activation of locally exclusive states

Several phenomena remain unexplained by the mechanisms of pattern formation discussed so far. These include the formation of a stripe-like pattern, the intercalary regeneration of missing elements in a sequence of structures, and the decision as to whether regeneration or duplication occur in imaginal disks. These phenomena are explicable under the assumption of a lateral activation of mutual exclusive states (Meinhardt and Gierer, 1980). An intuitive understanding of the mechanism envisaged may be provided by an analogy. Let us assume there are two families, A and B. At places where A is living, B cannot live and vice versa, they are locally exclusive. But both help each other and depend on the mutual help. A stable state is possible when areas populated by A are in close proximity to areas populated by B. The help has to be of a longer range, “across the street”. Due to the required help, both can exist only in a close but wellbalanced community. Due to the local exclusiveness, they belong either to A or to B but not to both and are therefore separated. The analogy is easily extended to more than two families, let us say 1,2,3...n and each needs the help of one or both neighbours. The most stable state is then a sequential order of the families in space.

The analogy can be used to illustrate the difference with respect to the mechanism of lateral inhibition discussed above which may be compared with the rise in power and wealth of one family, at the expense of the rest of the population. (For an adaptation of that model to socioeconomic problems see Gierer, 1981c.) The population plays merely a passive role, it is the necessary background on which a center of power develops in a self-enhancing manner. Such a family would engage all its power to suppress the rise in power of a second family with similar ambitions, especially in a close community. In contrast, in the lateral activation mechanism, the co-existence of two (or more) different families is favored since they need each other in a symbiotic manner. A mutual activation of cell types, a “cell sociology”, has also been proposed by Chandebois, 1976b.
CHAPTER 12. PATTERN FORMATION BY LATERAL ACTIVATION

Figure 12.1: Pattern formation by lateral activation of locally exclusive states. (a) general reaction scheme. Two (or more) autocatalytic feedback loops \( (g_1 \text{ and } g_2) \) compete with each other e.g. via a common repressor which leads to local exclusion. The long-ranging help ensures that both states are formed in a close and well balanced neighbourhood. (b) Simulation: \( g_1 \) and \( g_2 \) concentration is plotted as function of space and time. A homogeneous distribution of both substances is unstable, an area of high \( g_1 \) and of high \( g_2 \) concentration is formed. The system can show good size regulation. In this example, proliferation of the \( g_2 \)-cells leads to a corresponding enlargement of the \( g_1 \) area. Calculated with Eq.12.2.

12.1 Molecular interactions enabling lateral activation

In the preceding section, molecular reactions have been discussed which lead to mutually exclusive states. The lateral help can be introduced via diffusible substances in a straightforward manner; several examples will be given. One is based on the reaction scheme drawn in Fig.12.1; \( g_1 \) and \( g_2 \) are the (autocatalytic) substances required for the self-stabilization. The local mutual exclusion of the two states can be brought about by a common repressor (see Eq.11.3). The diffusible substances \( s_1 \) and \( s_2 \) provide the long-ranging help of one feedback system to the other.

\[
\frac{\partial g_1}{\partial t} = \frac{cs_2 g_1^2}{r} - \alpha g_1 + D_g \frac{\partial^2 g_1}{\partial x^2} + \rho_0 \quad (12.1a)
\]

\[
\frac{\partial g_2}{\partial t} = \frac{cs_1 g_2^2}{r} - \alpha g_2 + D_g \frac{\partial^2 g_2}{\partial x^2} + \rho_0 \quad (12.1b)
\]

\[
\frac{\partial r}{\partial t} = cs_2 g_1^2 + cs_1 g_2^2 - \beta r \left( + D_r \frac{\partial r}{\partial x^2} \right) \quad (12.1c)
\]

\[
\frac{\partial s_1}{\partial t} = \gamma (g_1 - s_1) + D_s \frac{\partial^2 s_1}{\partial x^2} + \rho_1 \quad (12.1d)
\]

\[
\frac{\partial s_2}{\partial t} = \gamma (g_2 - s_2) + D_s \frac{\partial^2 s_2}{\partial x^2} + \rho_1 \quad (12.1e)
\]

Since all molecules \( g_i \) compete with each other, a disadvantage for one feedback loop is an advantage for the other. Therefore the lateral activation can be of a hidden form in which each of the feedback loops is subjected to a long-ranging self-inhibition:
12.1. MOLECULAR INTERACTIONS

\[
\frac{\partial g_1}{\partial t} = \frac{c g_1^2}{r s_1} - \alpha g_1 + D_g \frac{\partial^2 g_1}{\partial x^2} + \rho_0 \tag{12.2a}
\]

\[
\frac{\partial g_2}{\partial t} = \frac{c g_2^2}{r s_2} - \alpha g_2 + D_g \frac{\partial^2 g_2}{\partial x^2} + \rho_0 \tag{12.2b}
\]

\[
\frac{\partial r}{\partial t} = \frac{c g_1^2}{s_1} + \frac{c g_2^2}{s_2} - \beta r \left( + D_r \frac{\partial^2 r}{\partial x^2} \right) \tag{12.2c}
\]

(The equations for \(s_1\) and \(s_2\) are the same as Eq.12.1d,e.) The mutual help may be achieved by only one substance \(s\). For instance, \(s\) can be produced by \(g_1\) to which it is “poisonous” and can be destroyed by \(g_2\) which needs it; \(g_1\) needs \(g_2\) for the removal of the poison whilst \(g_2\) needs \(g_1\) for the supply of \(s\). Similarly, two stable states can be generated by two molecules repressing each other (Eq.11.2) and can be mutually stabilized by substances of a high diffusion range. A symmetrical form would be:

\[
\frac{\partial g_1}{\partial t} = \frac{c s_2^2}{a + g_2^2} - \alpha g_1 + D_g \frac{\partial^2 g_1}{\partial x^2} \tag{12.3a}
\]

\[
\frac{\partial g_2}{\partial t} = \frac{c s_1^2}{a + g_1^2} - \alpha g_1 + D_g \frac{\partial^2 g_2}{\partial x^2} \tag{12.3b}
\]

(For the Eq. of \(s_1\) and \(s_2\) see Eq.12.1d,e).

In all these examples a homogeneous spatial distribution is unstable since, for example, a local \(g_1\) elevation increases further due to the direct (Eq.12.1 and 12.2) or indirect (Eq.12.3) autocatalysis; the local \(g_1\) increase is connected with a corresponding \(g_2\) decrease (locally exclusive). Outside of this incipient \(g_1\) maximum, \(g_2\) wins the competition with \(g_1\) due to the direct (Eq.12.1) or indirect (Eq.12.2) help via \(s_1\). The result is an area of high \(g_1\) (low \(g_2\)) and an area of high \(g_2\) (low \(g_1\)) (Fig.12.1).

These systems have features desirable for the explanation of properties of different developmental systems. The interactions can subdivide a field into two or more parts with very good size regulation. For instance, if the \(g_2\) area is relatively large in comparison with the \(g_1\) area, \(g_1\) is strongly cross-activated. Cells at the boundary are converted from the high \(g_2\) into the high \(g_1\) state until the correct proportion is restored (Fig.12.1). The size regulation works only over a certain range, determined essentially by the range of the lateral help. If a field of cells has a much larger extension, a periodic alteration of \(g_1\) and \(g_2\) patches will be formed. Further, the mechanism allows the formation of a stable pattern in the short extension of the field, and of a stripe-like pattern. The observation of these features in isotropic developmental fields is a first indication that lateral activation may be involved.
12.2 Formation of stripes

Stripes - structures with a long extension in one dimension and a short extension in the other - are frequently encountered in development. A very obvious example is the coloration of many animals (see Murray, 1981), the stripes of a zebra being proverbial. In the visual cortex of vertebrates, areas connected with the right eye and with the left eye respectively are arranged in a stripe-like manner (Fig.12.2). The thoracic segments of insects are subdivided into the stripe-shaped anterior and posterior compartments. Similarly, transplantation experiments with epidermal tissue of insect abdomen (Locke, 1959) suggest that the pattern elements have a very narrow extension in antero-posterior dimension but a large extension in dorso-ventral dimension. The pattern formation mechanism of lateral activation has the capability to form stripes. Since both feedback loops need each
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Figure 12.3: Formation of stripes. Mutual activation favours long common boundaries between areas of high $g_1$ and $g_2$ concentration. A checkerboard-like arrangement is disfavoured since it requires a high spatial resolution at the corners. To form larger coherent patches, some $g_1$ and $g_2$ diffusion is required, which blurs the resolution. A smoothing of the edges in the direction of the arrows (a) leads to the more stable stripes (b). Small asymmetries decide the orientation of the stripes. (c) Stripes in inorganic pattern formation: The Bénard-instability in a layer of liquid which is heated from below. The warmed up lower layer becomes lighter; long rolls of upstreams and downstreams are formed. Both exclude each other locally but enforce each other in a neighbourhood, satisfying our condition for stripe-formation.

other in close proximity, a long common boundary between both regions is favored. Fig.12.2 shows a computer simulation of Eq.12.3. If the pattern formation is initiated by random fluctuations, the orientation of the stripes is somewhat irregular. Nevertheless, they consist of long narrow ridges. Perfect stripes are formed if some initial spatial cues are present, e.g. if the pattern formation starts at one side of the field. The two types of stripes can have a different width if the strength of the autocatalysis or of the mutual help is different in the two feedback loops. Then, the equilibrium between the $g_1$ or $g_2$ cells would be shifted in favor of one or the other leading to a corresponding change in the number of high $g_1$ and high $g_2$ cells. In the ocular dominance columns mentioned above, visual deprivation of one eye leads to a narrowing of the corresponding stripes (Hubel et al., 1977). The question may arise why a stripe-like pattern emerges and not a checkerboard-like arrangement. In the latter case, even more boundary regions between ”black” and “white” fields are created. Characteristic of a checkerboard pattern are sharp corners which would require a high spatial resolution for their formation. Any diffusion of $g_1$ and $g_2$ would blur this resolution. In contrast, a stripe-like pattern has no such corners (Fig.12.3).

A non-biological example for such “stripe” formation is the arrangement of upstreams and downstreams in layers of liquids after the onset of the Bénard instability (see Velarde and Normand, 1980). Heated from below, the lower layer becomes lighter and tends to stream upwards, while the upper cooler layer tends to stream downwards (Fig.12.3c). At a particular location either upstreams or downstreams are possible, but not both. They are locally exclusive. However, an upstream enforces a downstream in its surroundings and vice versa - it is obviously impossible to have only upstreams. Our formal conditions for stripe formation are therefore met in this example from physics, too.

In the following, the regulatory behavior of some developmental systems is
summarized and compared with that of the lateral activation mechanism, in particular with respect to size-regulation and the formation of striped pattern.

12.3 The dorso-ventral organization of the insect embryo

The dorso-ventral (DV) extension of an insect egg is only about one third of the antero-posterior extension. The possibility of stripe formation inherent in the mechanism of lateral activation, permits the formation of a stable high “dorsal” and a high “ventral” concentration along the whole antero-posterior axis and a graded concentration in-between. After a longitudinal ligation parallel to the A-P axis of a leaf hopper egg, a complete embryo is formed in both the dorsal and the ventral half of the egg (Fig. 12.4b). Each half produces many more structures when compared with the corresponding part of the non-operated egg. As shown in the simulations Fig. 12.4d-f, the mechanism of lateral activation is able to reform the terminal concentrations across the small (D-V) extension of the field even if, due to an experimental interference, it becomes even more narrow. In contrast, an activator-inhibitor mechanism would orient the pattern along the longest extension of the field. The employment of an activator-inhibitor mechanism for the DV-axis would require a primary organization of the A-P axis, e.g. a subdivision into segments as noted in Fig. 4.3.

On the basis of available experiments, it is difficult to assess whether the
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fine structure within the dorso-ventral dimension results from a concentration
gradient, specifying positional information and leading, directly, to a position-
dependent cell determination as discussed above for the A-P axis. Other mech-
isms are conceivable. The DV-gradient can orient the sequence of dorso-
ventral structures while this sequence itself is formed in a self-regulatory way
(see Fig.13.7). Or, the primary D-V pattern could determine only the terminal,
most dorsal and most ventral structures whereas the other structures in between
are formed by intercalary “regeneration” (see Fig.13.3). An indication in favor
of a positional information scheme comes from a maternal effect mutation \( dl \)
of \textit{Drosophila}, isolated by Nüüsslein-Volhard (1979). If heterozygoteous, the most
ventral structure, the mesoderm, is missing to a greater or lesser degree. The
more dorsal structures are shifted and stretched towards the ventral midline. In
a positional information scheme, missing structures are expected whenever the
maximum concentration is not reached.

## 12.4 Compartmentalization and the reestablishment of compartment borders after experimental interference

The subdivision of the thoracic segments of insects into compartments (Garcia-
Bellido et al., 1973, 1976; Steiner, 1976, Wieschaus and Gehring, 1976; Crick and
Lawrence, 1975) is presumably a key paradigm for understanding progressive
subdivision of a developing embryo (see also chapter 9 and 14). In recent years,
much experimental effort has been concentrated on this subject and we would
like to show that some basic regulatory features of compartmentalization can be
explained by the lateral activation mechanism.

The thoracic segments have, at the time when they are determined, at the
blastoderm stage, an antero-posterior extension of only 3 - 4 cells (Lohs-Schardin
et al., 1979). Almost simultaneously a clonal separation into anterior and poste-
rior compartments takes place. These compartments have therefore the geometry
of narrow stripes, 1 - 2 cells wide, which extend presumably in a belt-like manner
around the blastoderm. As mentioned, the mechanism of lateral activation is able
to account for the stripe-like arrangement of two differently determined states.
The connection of this compartmentalization and the segmental determination
will become obvious in chapter 14.

The compartments are characterized by the following features:

1. **Clonal restriction:** A cell, once specified to participate in the formation of the
   anterior compartment will usually not be reprogrammed to form structures
   belonging to the posterior compartment.

2. **Transgression of compartment borders:** After a severe experimental interfer-
   ence with an imaginal disk, the compartment boundary can appear at a new
   location. This shows that the normally fixed border does not result from
   an irreversible determination but that it is maintained by a dynamic pro-
   cess. Szabad et al. (1979) found after an incision of the wing disk that the
Figure 12.5: Transgression of compartment boundaries in the wing (after Szabad et al., 1979). Clones (hatched) are induced in a wing disk. The genetically marked cells are the offspring of a single cell. One day later (day 6), either an incision is made in the disk or cell death is induced. Due to these manipulations, the progeny of the marked cells can populate different compartments. This would never occur without the experimental interference. (a) A clone crossing the A/P boundary, (b) a clone crossing the D/V boundary, populating the dorsal and the ventral wing surface. (c) A wing with a bubble-like extrusion, crossing the A-P boundary. Such a distal structure is expected if dorsal specification appears in the ventral compartment (or vice versa) close to the A-P boundary. It would be analogous to leg duplication, Fig.9.4.

(3) 
Compartmental respecifications: After other types of experimental interferences, the overall pattern is altered dramatically. For instance, a heat shock leads in a mutant of *Drosophila* to some cell death and this causes duplications or triplications of legs. Compartmental respecification (and with that, the formation of new compartment borders) is presumably the primary event in this malformation (see Fig.9.4). The location of the additional legs indicates that the cells of the outer anterior margin are especially labile with respect to a switch into a posterior specification.

These three phenomena - the normally fixed boundary, the possibility of a slight shift of the boundary, and a switch of some cells at a distance from the boundary into another compartmental specification - are easily explained by mutual activation of two locally exclusive feedback loops, A and P ($g_1$ and $g_2$ in Eq.12.1-12.3). The compartment border would be the transition between cells
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Figure 12.6: Behaviour of a “compartment border” after an experimental interference. Assumed are two mutual exclusive states ($g_1$, top, and $g_2$, below), corresponding, for instance, to an anterior and posterior specification. The simulation shows a cross-section through a disk as function of time. (a) If the diffusion of $g_1$ and $g_2$ is low, the border between high $g_1$ and $g_2$ remains stable even after a substantial fraction of e.g. $g_1$-area is removed, the remaining $g_1$ cells stabilizes the $g_2$ cells. (b) However, a complete removal of the $g_1$-area triggers the pattern formation again and a new compartment border is formed. (c) A somewhat larger diffusion of $g_1$ and $g_2$, the border is less sharp and a partial removal leads to a shift of the border, and to a new partition into two areas. Whether a boundary can be shifted or not could depend on small variation in the diffusion rate.

of high A and high P. The transition will be a sharp step if the substances accomplishing the self-stabilization, A and P, show very little or no diffusion. After removal of a large part of, for example, the high P area, the border is not shifted, because the help of the remaining P cells is sufficient to stabilize the A cells (Fig.12.6a). However, after almost complete removal of the P area, the pattern formation process starts anew, leading to a new border at a different position (Fig.12.6b). A higher diffusion rate of A and P leads to different behavior (Fig.12.6c). The compartment border is not as sharp and can be shifted if one compartment is too large in relation to the other resulting in proportion regulation. Whether or not a sharp boundary between patches of differently determined cells exists may thus depend only on a difference in a diffusion rate and not in the underlying mechanism. The sharpness of the compartments in Drosophila and therefore their clonal restriction is presumably dictated by the small number of founder cells of a compartment. If many more founder cells were be involved, a reasonable diffusion of A and P would be required to maintain these “compartments” as a contiguous patch of cells. Such diffusion would lead to a loss of clonal restriction. The possible absence of clonal restriction in other developmental systems does not indicate that different mechanisms are involved. Therefore, whether or not compartments are involved in development of vertebrates is presumably only a semantic question.

The possible reason for compartmental respecification after heat shock (and cell death) may be that the killed cells do not participate in the cell communication via diffusion. With that, the support of one cell type by the other may become insufficient and a switch to the alternative compartmental specification occurs. Fig.12.7 shows some altered pattern after induced cell death. The probability of respecification increases with distance from the border since these cells
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Figure 12.7: The change of compartmental specification by cell death. (a) two compartmental specifications (== and :::) are stable (see Fig. 12.6). (b-d) killed cells (x) are assumed to participate no longer in the cell communication via diffusion. Hence, the mutual support of one state by the other may be reduced which can lead to a partial change in the compartmental specification. The switch from one determination to the other may occur in one compartment (b,c) or both (d), and at a position close or distant to the border, (c,b). A new compartmental specification can lead to a new system of positional information, causing a new proximo-distal axis. (see Fig. 9.4)

become less and less supported by cells of the other compartment. The details of this switch mechanism depend on how the lateral activation is molecularly realized (see Eq.12.1-12.3). For instance, if the basis was a mutual inhibition of competing feedback loops (Eq.12.2), any lowering of this inhibition could lead to a compartmental respecification. Such a process would be similar to the unspecific induction (Fig.8.4). An increase in the basic production ($\rho_0$ in Eq.12.1 and 12.2), for instance, due to an elevated temperature, could also lead to a switch. On the other hand, the number of cells with A and P specification increases dramatically between the clonal separation (ca. 20) and the mature disk (ca. 50,000). This leads also to an increased distance of the cells from the border and, with that, from the other stabilizing cell type. The cells can be stabilized in the A or P determination if the stabilizing substances $s_1$ and $s_2$ are produced at a constant minimum rate ($\rho_1$ in Eq.12.1 and 12.2). A reason why A-cells are more easily reprogrammed to form P-cells then vice versa will be given on p. 155.

12.5 Systems with an organizing region at each end - regeneration of planarians

In discussing the regulatory features of hydra (chapter 6), we have not considered that hydra has in addition to the head a second organizing area with similar properties, the foot. Similarly, the head and the foot of planarians are two boundary regions which organize the field in between (see Chandebois, 1973, 1976a). In hydats as well as in planarians, a head, a foot or both regenerate even in very small tissue fragments, indicating that systems which bear an organizing center at each end are stable over an enormous range of size. The head field and the foot field cannot be independent from each other, otherwise they would not appear at
12.5. REGENERATION OF PLANARIANS

Figure 12.8: Regeneration of planarians as example of pattern regulation in a bipolar field. Bipolar fields require two organizing regions, one at each end. It can be created by two activator maxima, one controlling, for instance, the head formation, the other the foot formation. A common inhibitor assures that both maxima appear at the largest possible distance from each other, at the opposite ends of the field. It is assumed further that on long range, both systems help each other either by a direct help (Eq.12.1) or by a specific self-inhibition (Eq.12.2). This assures that no system can suppress the other. (a) Pattern formation and growth: such pattern is stable over an enormous variation of sizes. (b) Removal of the foot leads to a regeneration of the foot activator despite of the proximity of the head. (c) Simultaneous regeneration of the head and the foot activator. The result is independent of the precise position of the fragment.

opposite sides. The mechanism of mutual activation of two feedback loops suggests an appropriate coupling of a head-forming and a foot-forming system which assures that both structures are present in the system and that they appear at maximum distance from each other.

In the application of lateral activation discussed so far, the common repressor which causes the local exclusivity has been assumed to be non-diffusible \((D_r = 0)\) in Eq.12.1 and 12.2). This has the consequence that in each cell one (and only one) of the feedback loops is active otherwise the repressor concentration would drop to such low values that one of the loops would become autocatalytic. This was appropriate to describe, for instance, the compartmentalization where a cell must be either anterior or posterior. In contrast, if the common repressor is diffusible, the autocatalysis of the two feedback loops \(g_1\) and \(g_2\) (the head and the foot activators) will be restricted to small patches of cells. In the rest of the cells, neither \(g_1\) nor \(g_2\) is produced. They are suppressed by the diffusible repressor. Since both loops produce the same repressor, they repel each other and the autocatalytic areas appear therefore at opposite ends of the field. Neither the head system can dominate over the foot system nor the other way round because of the required mutual support of the two systems on a very long range (Eq.12.1). The same would be achieved if both systems, in addition to the common inhibitor, employed each a head- and a foot-specific inhibitor (Eq.12.2). Then, for instance, after removal of the foot, the foot inhibitor will drop until a new area of a high foot activator is induced. It appears at the end opposite to the head since this process is also sensitive to the common inhibitor. In Fig.12.8 and 12.9, these regulatory features are compared with those of planarian regeneration. The insensitivity with respect to size, and the ability to regenerate independent of whether one terminal structure remains present or not is in agreement with the experimental observation. Formation of a new head or foot does not require a complete separa-
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Figure 12.9: (a,b) An incision into a planaria can be sufficient to initiate the formation of a new head and foot. (c,d) Model: In a two-dimensional field, a system with a head activator at one side and a foot activator at the other side would be stable. An incision, however, provides a diffusion barrier. The substances providing the mutual help ($s_1$ and $s_2$ in Eq.12.1 and 12.2) becomes so low that new maxima appear in the vicinity of the incision.

The same basic mechanism has been successfully applied to explain regulatory features of developmental systems which are so different as planarians and imaginal disks. In both cases, the common repressor (or inhibitor) keeps the two systems separate from each other and the long range help assures that both systems coexist with each other. Minor changes in the parameters - the substance bringing about local exclusion is diffusible or not - can account for the differences in these systems. In one case, the cells are either anterior or posterior and a sharp boundary exists inbetween. In the other case, the head and the foot areas are restricted to the opposite ends of the field and both areas are separated by a region which is neither head nor foot. The similarities of both systems become apparent in the similar reaction upon the same experimental interference, an incision. In an imaginal disk this can lead to a compartmental respecification, in planarian to the formation of new heads and feet. This example demonstrates that by minor changes of a basic mechanism, an adaptation of its properties for different requirements of developmental systems can be achieved.
Chapter 13

Generation of sequences of structures by mutual induction of locally exclusive states

13.1 A biological example: pattern regulation within a segment of an insect leg

In insects, the internal organization of a particular segment of a leg or of the abdomen has properties which differ essentially from the control of the overall sequence of segments in the body or leg. To have a firm basis for what a theory has to explain, the main results of intercalary regeneration in cockroach legs (Bohn, 1965, 1970a,b; French, 1976a,b, 1978) should be summarized. For the propose of a brief description of the experimental results, the normal proximodistal sequence of structures within a leg segment will be called 123456789. This assignment is somewhat arbitrary since clear demarcation lines such as the segment borders between different segments do not exist between the internal structures. It has turned out that: i) internal parts removed from a sequence of structures by surgical interference are replaced. For instance, an experimentally produced sequence 123/89 would intercalate the missing structures after one or two molts and the normal sequence 123456789 (intercalated structures are in bold italic) is restored (Fig.13.1); ii) surplus parts are duplicated in an inverted form, e.g. an artificially produced sequence ...45678/456789 would form the structure 45678765456789 (Fig.13.1c); iii) a confrontation of the type ..678/345.. can also lead to an intercalary regeneration of the type ..678912345.. with an additional articulation in the intercalated 1-region (French, 1976a); iv) the elements within the segments are specified in a repetitive manner. Confrontation of different segments at the same internal level, e.g. a sequence 1234 of the femur (bold) and a sequence 5789 from the tibia would not intercalate the missing elements 567891234 (see Fig.9.6), while a confrontation 12/89 does lead to an intercalation of the type 123456789; v) intercalary regeneration is possible also in the circumferential direction, removed longitudinal stripes being replaced
13.2 Possible mechanisms

Several mechanisms can be ruled out by just realizing that a sequence of the type ...56787654567... (Fig. 13.1) is stable. For instance, the sequence cannot be controlled by a concentration gradient, generated by a source at one end and a sink at the other end of the segment, since such a gradient would be always monotonic. The intermediate peak would disappear. Is it possible to stabilize the intermediate peak, e.g., by an autocatalytic reaction as discussed above? Autocatalysis can compensate the loss by diffusion but would have the tendency to form the maximum concentration and thus to form the most terminal structures. We have seen such a behavior in the *Euscelis* egg where three abdomina (Fig. 8.2) can be formed. During intercalary regeneration, the elements forming the initial graft-host junction (8 and 4 in the example ...56787654567...) are stable. An autocatalytic maintenance of the intermediate “maximum” is therefore unlikely. The same argument holds if one assumes that the gradient is stabilized by an active transport against the steepest slope (Lawrence, 1966a). The element 7 in a sequence ...56765... would profit from both sides and increase to 8 etc. until the terminal structure are formed.

An assumption which fits the observations more closely is that different quali-
ties and not different quantities are characteristic for the particular element of the sequence. The homeostatic property of the elements requires a self-stabilization. We will assume therefore that the sequence of structures consists of a sequence of differently determined (though perhaps closely related) structures, characterized for instance by the activation of particular genes out of a set of closely related control genes. The control of the correct neighborhood of structures as indicated by the experiments summarized above would require a mutual activation of the self-stabilizing states. Confrontation of cells which are usually not neighbors leads at the mismatching junction to a respecification of some cells into that of the missing structures (and presumably to an increased rate cell division, though proliferation is no logical requirement for the proposed mechanism).

The absence of intercalary regeneration when a proximal part of a femur is combined with the distal part of a tibia (123456789 with 1234 from the femur, 56789 from the tibia) indicates that the same feedback loops are used repetitively for the determination of a particular levels within different segments. Therefore only one set of few control genes would be required for the internal specification of different segments.

The ability to rebuild a removed part of an organism has a clear selective advantage. However, the removal of only an internal fraction from a leg or from an abdominal segment will never occur under natural circumstances. The presence of intercalary regeneration suggests that this process is not primarily invented to regenerate lost internal parts, but may be a normal process in the formation of the diversities of structures. For instance, during normal development, the terminal elements of the sequence could be determined and the sequence is then completed by the filling in of the missing structures.

### 13.3 Chains of induction

In the last chapter we have seen how two different structures can stabilize each other. The essential ingredients of the model are autocatalytic feedback loops which exclude each other locally but which help each other via diffusing substances. This mechanism can be extended to more than two structures (feedback loops) in a straightforward manner. The generalization of Eq.12.1 to many loops is

\[
\frac{\partial g_i}{\partial t} = \frac{c_i g_i^2}{r} - \alpha g_i + D_g \frac{\partial^2 g_i}{\partial x^2} 
\]

(13.1a)

with \( g_i' = g_i + \delta^- s_{i-1} + \delta^+ s_{i+1} \)

\[
\frac{\partial s_i}{\partial t} = \gamma (g_i - s_i) + D_s \frac{\partial^2 s_i}{\partial x^2} 
\]

(13.1b)

\[
\frac{\partial r}{\partial t} = \sum_i c_i g_i^2 - \beta r 
\]

(13.1c)
CHAPTER 13. MUTUAL INDUCTION OF LOCALLY EXCLUSIVE STATES

Figure 13.2: Generation of sequences of structures by lateral activation. (a) Molecular interactions which allow the generation of self-stabilizing sequences of structures in space. Each state of the sequence 1,2,... has (i) feedback on its own, for instance via an autocatalytic gene activator, (ii) a long range activation of its neighbors and (iii) it produces and reacts upon a common repressor (black double arrow). This three interactions lead to a self-stabilization and to correct neighborhoods. (b-d) Simulation with Eq. 13.1. Growth is assumed at the right margin. The concentration of the gene-activator molecules (shown as density of the dots) is plotted as a function of gene number and position. Initial separation of the field into two parts is accomplished as shown in Fig. 12.1. With increasing number of gene-2 cells, the concentration of the cross-activator of gene-3 ($s_2$, not shown) reaches a level which induces a transition of the gene-2 state into the gene-3 state etc. Long sequences of structures can be formed which are able to intercalate missing parts (see Fig. 13.3). (Equation 13.1 with $c_1 = 0.01, c_{i+1}/c_i = 0.74, \alpha = 0.1, D_g = 0.009, \beta = 0.15, \gamma = 0.1, D_s = 0.3, \delta^- = 0.4, \delta^+ = 0.12$. (after Meinhardt and Gierer, 1980)

In this example, the lateral help is introduced as a strong additional help and not as a necessary requirement (multiplicative factor). For instance, $\delta^- s_{i-1}$ describes the long range help from a lower neighbor. This enables stability of a particular state on its own, while in an interaction according to Eq. 12.1 a state without a supporting neighbourhood state would oscillate between the different states. The interaction according to Eq. 13.1 has the capability for pattern formation, no external positional information is necessary to initiate the sequence. The formation of a sequence is shown in Fig. 13.2. The orientation of the emerging sequence depends on small asymmetries, e.g. any slight preference for the location of the second element in relation to the first is sufficient (see also Fig. 13.7). After the first two elements, No.1 and No.2, of the sequence have been laid down, the next state, No.3, has to be triggered and not No.1 again, otherwise only an alternation of two stages would emerge. A sequence will be formed if a state exerts a stronger help on the following state than on the preceding one. In terms of Eq. 13.1, $\delta^- > \delta^+$. In fact, a term $\delta^-$ would be sufficient to activate each following state and therefore the whole sequence. As shown below, the term $\delta^+$ facilitates intercalary regeneration.

13.4 Conditions for intercalary regeneration

Imagine a mismatching junction, for instance 12/678. Each structure has the tendency to induce its neighbours, especially the more distal neighbours. In the
13.4. CONDITIONS FOR INTERCALARY REGENERATION

Figure 13.3: Simulation of intercalary regeneration. Assumed is a mutual activation of locally exclusive states (Fig.13.2). The repair of a gap is possible if the lower states dominate. (a-c) The area in which genes 3, 4 and 5 are active is removed (a). (b) Close to the mismatching junction, the activity of gene 6 will cease in favor of either state 2 or 3. (c) The missing structures are then reformed by cross-activation similar as in Fig. 13.2. (d-f) Intercalation of excessive parts in reversed polarity. The distally programmed cells become reprogrammed to form the proximal structures, in agreement with the experimental observation Fig. 13.1c. The experimentally observed stimulation of cell proliferation at the mismatching junction is not taken into consideration (after Meinhardt and Gierer, 1980).

example, structure 2 tends to induce 3, structure 6 induces 7. Both structures cannot be formed at the border between 2 and 6 since the mechanism assures that the structures are locally exclusive. To achieve a correct regeneration of the sequence, the structure 3 has to be formed, that means, the lower, more proximal structure must be dominating over a more distal structure. That signifies that a hierarchy exists among the feedback loops, or in terms of Eq.13.1 that $c_i > c_{i+1}$. For the communication between the cells a small diffusion of the $g_i$ molecules is important. At the mismatching junction, $g_2$ and $g_6$ molecules are exchanged between the cells. Since the lower state 2 dominates, the $g_6$ production ceases in cells at the junction in favour of $g_2$. If structure 2 is sufficiently extended, structure 3 is induced (similar as during the original formation of the sequence) and the first step in the intercalary regeneration is completed. This process repeats itself until the correct neighbourhood of structures is restored. The mechanism is in agreement with the fact (Fig.13.1) that both sides of the mismatching junctions contribute in the formation of the intercalate since the distal elements are reprogrammed by the contact with the more proximal structures and proximal structures form the more distal ones by the long-range induction. The mechanism has also the property of duplication of surplus structures as shown in the simulation Fig.13.3d-f since only the correct neighbourhood between adjacent cells is controlled and directional (vectorial) cell properties are not involved; the polar-
Figure 13.4: The femur (FE) and tibia (TI) of a cockroach leg. The scanning electron micrograph show the different structures of (a) the outer (anterior and posterior) and (b) the inner (ventral) face of the femur.

ity of the sequence may be reversed during the correction of the neighbourhoods. The mechanism predicts that a particular element can only induce the neighboring element. No averaging mechanism should occur. For instance a sequence 12/89 should regenerate via an intermediate state 123789 and not via 12589.

13.5 Organization of imaginal disks and insect legs around their circumference

A leg segment of an insect has a fine structure not only in its proximo-distal dimension, but also around its circumference. From the very careful and detailed experiments of French (1978, 1980) we know that the pattern around the circumference is able to intercalate missing structures or to duplicate excessive structures analogous to the pattern regulation in the proximo-distal axis. French et al. (1976) have proposed a “polar coordinate model” postulating that the circumferential pattern consists of a continuous sequence of structures to which they assigned arbitrarily the positional values 1,2...12. They are arranged like the numbers on a clock face. Missing structures are assumed to regenerate according to the rule of shortest route. Thus confrontation of the type 12/78... would lead to the regeneration of the missing structures 3456 while a confrontation ...2345/234... would lead to the insertion of the structures 43, restoring in both cases normal neighborhoods. This rule accounts for the regeneration-duplication phenomenon observed in imaginal disks (Bryant, 1975a,b). Small disk fragments duplicate the remaining structures while larger fragments regenerate the complete structure. For instance, a small disk fragment 2345 duplicates during the closing up and wound healing. The terminal structures 5 and 2 become juxtaposed and the structures 4 and 3 are intercalated. This leads to the (circular) duplication 2345432. A larger fragment consisting for instance, of the structures 23456789 would intercalate, according to the rule of the shortest route, the missing structures 10 11 12 1, leading to the regeneration of the complete sequence.
13.5. ORGANIZATION OF IMAGINAL DISKS AND INSECT LEGS

Figure 13.5: Steps in the subdivision of an imaginal disk. (a) The primary event is assumed to be the formation of compartment boundaries. (b) By cooperation of three or four compartments a positional information system is formed (see Fig. 9.1, 9.2). The distance from the intersection of borders is decisive for the proximo-distal determination of the cells and whether the cells will form an imaginal disc or not. (c) Particular structures out of the set of circumferential structures are induced along the border between two compartments. (d) The missing structures are filled in by intercalation.

The polar co-ordinate model provides formal rules. What could be the molecular mechanisms on which this regulatory behavior is based? How do the cells recognize what the shortest route is? In principle, the maintenance of the correct neighborhoods of structures around the circumference can be achieved in the same way as described above for the proximo-distal sequence. Long range activation of states which locally exclude each other leads to sequences of structures in which the correct neighborhood is maintained in a self-regulatory manner. Since the circumference is assumed to consist of different qualities (not quantities as in a gradient system) each structure can support their neighboring structure: no special discontinuity occurs, for instance, between structure 12 and 1. Several questions are to be answered: How is such a sequence of structures initially formed in development? How is the circumferential pattern aligned with respect to the primary body axes? Why are left and right legs mirror images of each other? As we have seen (chapter 9), the subdivision into compartments is the precondition that an imaginal disk and therewith a leg or any other appendage can be formed. Thus, in disks or in legs a coarse subdivision is given from the beginning. In the leg, these compartments are long narrow stripes running in proximodistal direction. The compartments must be, so to say, the frame for the finer subdivision around the circumference similar as the segment borders are the frame for the finer subdivision within the segments. In Drosophila, a particular tarsal bristle row coincides with the A-P compartment border (Lawrence et al., 1979). The bristles are made irregularly from both compartments, indicating that along the border a signal is created (see Fig.9.1b-d) which enables bristle formation. On the other hand, the symmetrical tarsal structures have no obvious relation to the non-symmetric pattern of compartments. Direct evidence exists that intercalary regeneration has something to do with boundaries. During intercalary regeneration of cockroach legs, boundaries of clonal restriction are maintained, suggesting a similar compartmentalization in cockroaches and Drosophila (French, 1980). For instance, cells of the posterior compartment can force cells of the anterior compartment to eventually regenerate missing structures up to the border, but
the anterior cells will not give rise to posterior structures and vice versa. The
question is then how the fine structured pattern of the circumference (e.g. 1 -
12) falls into register with a coarse subdivision into the anterior (A), posterior
(P) and ventral (V) compartment? A possible mechanism consists of a strong
inducing influence of a particular compartment border on a particular structure,
for instance the structure 1 may be induced by an A-P border, structure 5 by a
P-V border and structure 8 by V-A border, followed by the intercalation of the
missing structures (Fig.13.5). This mechanism of co-ordinating the fine structure
with the compartmental subdivision provides also a molecularly feasible basis for
the regeneration-duplication phenomenon (Fig.13.6). Small fragments contain,
as a rule, only cells of two compartments. If, for instance, cells of anterior com-
partmental specification are missing in a fragment of a disk, they will remain
missing. After closing the wound, a second confrontation of the remaining poste-
rior and ventral compartment occurs and this leads to a duplication (Fig.13.6a-c).
In contrast, if some anterior cells remain in the fragment, a regeneration of the
complete circumference would follow (Fig.13.6d-f). However, the possibility of
compartmental respecification has to be taken into consideration. A missing
compartmental specification may be restored by a partial respecification of re-
mainning cells (Fig.12.5, 12.6). In such a case, both complementary fragments
resulting from a partition of a disk can regenerate the complete set of circumfer-
ential structures such as observed by Kauffman and Ling (1981). In contrast, a
duplication of both complementary fragments is less likely and would occur only
after massive cell death.

French (1978) found a very striking absence of intercalation after confronta-
tion of particular circumferential structures of the anterior with those of the posterior
side of a cockroach leg. This situation is reminiscent of the absence of interca-
lation when, for instance, a mid-tibia is grafted onto a mid-femur. The missing
distal femur and proximal tibia remain missing (Bohn, 1970a, see Fig.9.7b). This
has led to the conclusion that the positional values for the internal proximo-distal
organization of segments are used in a repetitive manner within each segment and
that the overall proximo-distal subdivision is made in a combinatorial way. A
similar repetition of positional values within each compartment would explain
why the confrontation of a midanterior compartment and a mid-posterior com-
partment eventually heals without intercalation.

In conclusion, like an umbrella needs at least three spokes to put up the in-
terconnecting tissue, the three compartmental borders in the leg disk (or the two
intersecting borders in the wing disk) are required to unfold the circumferen-
tial pattern. The final stabilization of neighboring structures and intercalation of
missing structures can be accomplished by long range activation and short
range exclusion of different states. The control of the fine structure by the com-
partments assures its correct orientation in relation to the main body axes. In
connection with the model which describes the formation of compartments in the
first place (chapter 14) this mechanism accounts for the initial generation, the
maintenance during further development and regeneration of the circumferential
structures.
13.6 Sequence formation by induction and lateral inhibition

For the generation of a sequence of structures, we have assumed a long-range cross-activation of several competing feedback loops (Eq. 13.1). An alternative would be that the size of each element is limited by a long-range selfinhibitory substance. Equation 13.2, a generalization of Eq. 12.2, describes a possible interaction of substances.

\[
\frac{\partial g'_i}{\partial t} = \frac{c_i g_i'^2}{d_i r} - \alpha g_i + D_g \frac{\partial^2 g_i}{\partial x^2} \quad (13.2a)
\]

with \( g'_i = g_i + m \delta^- g_{i-1} + \delta^+ g_{i+1} \)

\[
\frac{\partial d_i}{\partial t} = \gamma (g_i - d_i) + D_d \frac{\partial^2 s_i}{\partial x^2} \quad (13.2b)
\]

\[
\frac{\partial r}{\partial t} = \sum_i \frac{c_i g_i'^2}{d_i} - \beta r \quad (13.2c)
\]
Figure 13.7: Orientation of a self-regulating sequence by a gradient. If the mechanism for generating a sequence of structures (sequence of activated feedback loops or “genes”) has the capability of pattern formation (reaction 13.2), a gradient can orient the sequence. (a,b) The emerging pattern is independent of the steepness or the absolute concentration of the gradient. The size regulation of the elements is a property of the sequence-generating mechanism, not of the gradient. (c) Initiation by a symmetrical distribution can lead to two complete sequences even if low concentrations are absent. Each element becomes correspondingly smaller. Such complete duplication is observed in the dorso-ventral pattern of amphibians (Fig. 13.8) and insects (Fig. 12.4). (d) If initiated by random fluctuation, the emerging sequence has an unpredictable orientation and gaps may occur but the mechanism Eq.13.2 assures that each element is present at least once in the field. From top to bottom in each subpicture: The orientating gradient (m in Eq.13.2), the initial, an intermediate and the final pattern of “gene activities” as function of position calculated with Eq.13.2 with $c = .01, \alpha = 0.03, D_g = .005, \beta = .05; \gamma = .02, D_d = .4, m\delta^+ < 0.1, \delta^+ = 0.0$

(m is a morphogen as shown at the top of Fig. 13.7) A sequence of elements, generated in this way, has a good size regulation of the elements. If one element is relatively too large, the larger self-inhibition provides a disadvantage for that particular feedback loop compared with the other competing loops and it will shrink. For similar reasons, a very strong tendency exists to form each element of the sequence at least once in the field. Should an element be missing, the self-inhibition of the missing structure would become so low that it would be induced via the cross-activation of the neighboring structures ($\delta^+$ and $\delta^-$ in Eq.13.2). Therefore, a sequence of the type 12/56 regenerates the missing elements 3 and 4. No hierarchy is required for this intercalation. However, the mechanism has no tendency to intercalate structures if this is connected with a duplication of existing structures as, for instance, in a sequence 2345/2345. The structures 4 and 3, missing at the gap, are already present twice in the field and the long-range selfinhibition emanating from the existing structures will suppress intercalation. The system has more the tendency to complete the two partial sequences. Without additional assumptions, this mechanism is not appropriate to explain intercalary regeneration within insect segments (Fig. 13.1). However, as shown below, it may be the way to lay down the dorso-ventral structures of vertebrates.
13.7 Orientation of a self-regulating sequence by a gradient - an alternative to the interpretation of positional information

If a mechanism is given which has the strong tendency to form a sequence of structures in space (Eq.13.2) a small and possibly unspecific stimulus is sufficient to orientate the sequence. The sequence itself is formed in a self-regulatory manner. This offers an alternative to the measuring of local concentrations as discussed earlier for the interpretation of positional information (Fig.11.5). Imagine a graded distribution of some substance or of a physical parameter which has, for instance, some influence on the cross-activation of the feedback loops (m in Eq.13.2). If initially the loop No.1 is active in all cells of a field, the cells on one side switch faster to loop 2 and so on, and the orientation of the emerging sequence is determined. Fig.13.7 shows that neither the steepness of the slope nor the absolute concentration but only the overall orientation of this gradient has an essential influence on the resulting pattern. No special thresholds exist for the particular structures. Therefore, the orienting gradient need not be size-regulated for an adaptation of the correct size of the individual elements in relation to the total size of the field. The size regulation is a property of the mechanism which generates the sequence. In short, not the signal but the response would be sizeregulated (Fig.13.7).

If the orientating stimulus is symmetric, two sequences, mirror-symmetric to each other, can result. Each element is present twice in the field but each is half as large. This type of pattern regulation is known to occur in amphibians. As shown by Spemann and Mangold (1924) in their classic experiment, the implantation of a dorsal lip of a blastopore into the ventral side of a blastula leads to a dorsal-ventral-dorsal duplication (Fig.13.8). Cooke (1981a) has shown that the duplicated structures are squeezed into the same total field, the structures are correspondingly smaller. No additional cell proliferation takes place. Especially in small duplications, the structure next to the plane of symmetry - the pro-nephros - is frequently absent. According to the model, both pro-nephros would be relatively close together and one may suppress the other. By removing portions of the egg, Cooke has also shown that the complete dorso-ventral (D-V) pattern can be formed in a much smaller field which demonstrates the size-regulating features of the DV-pattern. For amphibians, it can be ruled out that the size regulation of the D-V structures result from a size-regulated DV gradient which is produced by a source-sink mechanism. No organizing properties of the ventral side can be detected upon transplantation.

A complete DV duplication is also possible in insects (see Fig.12.4b). A comparison of these results with those obtained for the antero-posterior (A-P) pattern of insects (Fig.8.5 and 8.7) reveals basic differences between the two systems. If a symmetrical (A-P) pattern is formed in insects, each half forms fewer structures in comparison with normal development. This had forced the conclusion that the local morphogen concentration controls which particular structure is formed (Fig.8.5). In the D-V organization, each half forms many more structures - in fact
CHAPTER 13. MUTUAL INDUCTION OF LOCALLY EXCLUSIVE STATES

Figure 13.8: Pattern duplication in the dorso-ventral axis of amphibian embryos (after Cooke, 1981a). (a) An embryo with the plane of the dorso-ventral cross-section shown in (b). (c) If a dorsal lip of a blastopore, the organizer, is transplanted to the ventral side of a blastula (d), a symmetrical duplication of the dorso-ventral pattern results (e). Both halves contain the complete set of structures. The individual structures are correspondingly smaller. That is very different from the antero-posterior duplication in insects (Fig. 8.5). The gradient produced by the organizer is assumed to orient a self- regulating sequence (Fig. 13.7).

the complete set - suggesting that in this case it is not the absolute concentration which is measured but that a self-regulatory sequence is triggered. It is remarkable that - as far as we know - all systems in which the absolute concentration of a morphogen is measured (insect body segments, digits of vertebrates, segments of insect legs) the pattern to be formed consists of a repetition of similar but not identical subunits (see chapter 14).

In conclusion, feedback loops which support each other at long range, but compete with each other at short range can generate sequences of structures in space. These sequences are self-regulatory, missing structures can be added and gaps can be repaired by intercalary regeneration. Control genes could be, but need not to be involved in the generation of the feedback loops.

13.8 Other applications of equations describing mutual activation of locally exclusive processes

For the explanation of the early evolution of genetic information, Eigen and Schuster (1978) have proposed equations similar to Eq.12.1 and 13.1. This similarity is not accidental. In the evolution of genetic information as well as in the activation of control genes, autocatalytic loops are assumed. In one case genes feed back on their own activity, in the other case pieces of nucleic acids are self-replicating. In both cases, a diversity of such competing loops should co-exist with each other despite the “survival of the fittest”. The co-existence results from the mutual dependence of the feedback loops. The essential difference in the model we proposed lies in the spatial order of the feedback loops which arises from their short range exclusion and the long range support. Further, each group of dividing cells in an organism represents an autocatalytic system. Different groups compete with each other since they consume the same nutritional substances. Nevertheless, the faster-dividing cells should not overgrow the others. A balance between the different cell types requires mutual dependence. A cancerous cell may have escaped this dependence from other cell types. Eq.12.1 and 13.1 describe essentially a type of symbiosis and the applications are presumably more general.