Cell Determination Boundaries as Organizing Regions for Secondary Embryonic Fields

HANS MEINHARDT
Max-Planck-Institut für Verhaltensphysiologie, D-74 Tübingen, West Germany

Received July 12, 1982; accepted in revised form November 17, 1982

A model is proposed for pattern formation in secondary embryonic fields. It is stipulated that the boundaries, resulting from the primary embryonic organization of a developing organism, act as organizing regions for secondary embryonic fields, e.g., imaginal discs in insects. This boundary mechanism would allow very reliable pattern formation in the course of development: Primary positional information leads to cells of different determination, separated by sharp borders. At these borders, in turn, positional information would be generated for the next finer subdivision, and so on. This occurs if two or more differently determined cell types (e.g., compartments) cooperate for the production of a morphogenetic substance. A high concentration of the morphogen would appear at the common boundary of the cell types involved. Many experiments reported in the literature, for instance, the formation of duplicated and triplicated insect legs and the regeneration—duplication phenomenon of imaginal disc fragments can be explained under this assumption. The proposed boundary mechanism provides a molecularly feasible basis for the polar coordinate model.

INTRODUCTION

The question of how particular structures, for instance legs, wings or eyes are initiated at a particular location during development of higher organisms is a major problem of development. In addition to a reproducible asymmetry (left or right), a particular orientation with respect to the main axis of the embryo and a complex fine structure. The primary spatial organization (head to tail) of a developing organism can be understood to a large extent on the basis of positional information and its interpretation (Wolpert, 1969, 1971). For instance, the results of many experiments concerned with early insect development (Sander, 1975) are explicable under the assumption that a graded morphogen distribution is generated within the egg by a local morphogen source at the posterior egg pole. The local concentration of the resulting morphogen gradient would determine which segment is to be formed (Meinhardt, 1977). The final structure of a higher organism is, of course, much more complex than achievable by the interpretation of just one or two orthogonal gradients. In secondary fields, further pattern-forming systems organize the formation of substructures. Well-investigated secondary fields are the limb field of vertebrates (for review see Hinckliffe and Johnson, 1980) and the imaginal discs (Gehring and Nöthiger, 1973; Bryant, 1978) of certain insects which form legs, wings, and other appendages during metamorphosis.

The primary subdivision of a developing organism into patches of different determinations implies that borders are formed between the differently determined cells. We propose that these borders can act as organizing regions for the determination of substructures. This would occur if a cooperative interaction between two or more differently determined cell types is required to produce a morphogen, which provides positional information for a particular substructure. For instance, one cell type could produce a precursor, another convert it into the active morphogen. Morphogen production would be restricted to the area where both cell types are close to each other, i.e., to the corresponding boundary region. The local morphogen concentration would provide a measure for the distance of a cell from the boundary. In this way, a secondary field would be created. The primarily formed borders would become boundary regions in the sense used by Wolpert (1969, 1971) i.e., the high points in a positional information scheme. Since the secondary fields are created by the primarily formed borders, the substructures necessarily have the correct spatial relation to the already determined cells even if the primary subdivision is not very precise.

The postulated morphogenetic substances are still hypothetical. Support for such a model can only be obtained from the demonstration that the predicted regulatory features agree precisely with the modes of pattern regulation observed after experimental interference. The model proposed predicts that supernumerary
structures can develop whenever a new confrontation between the required cell types is experimentally provoked. For the proximodistal axis of imaginal discs I have shown (Meinhardt, 1980) that many experimental observations reported in the literature are explicable under the assumption that the intersection of the major compartment borders acts as organizing region and further that this mechanism provides a molecular basis for the polar coordinate model (French et al., 1976; Bryant et al., 1981) since the requirement of two intersecting borders (i.e., a meeting point between three sectors or four quadrants) is equivalent to a requirement of a complete circle with three or four positional values. In the present paper, I will discuss with respect to the proximodistal axis of imaginal discs only a recent experimental observation which provides new support for the model. In the main part of the paper I will propose a model about how the compartment borders control the circumferential pattern of a disc. This model suggests a basis for the regeneration–duplication phenomenon.

**The Intersection of Compartment Borders as Organizing Regions for Imaginal Discs**

The model predicts that the primary event in the generation of a secondary field is the formation of a border and that secondarily the substructure proper is determined in cells adjacent to this border. The observed sequence of events which leads to imaginal discs follow this scheme. Garcia-Bellido et al. (1973, 1976) have shown that a clonal separation of the thoracic segments into an anterior (A) and a posterior (P) compartment occurs in *Drosophila* almost simultaneously with the separation of the segments at the blastoderm stage. The anterior and posterior cells are separated by a sharp and well-defined compartment border. However, at the time of A–P border formation, the separation into the leg and wing disc is not yet achieved since the progeny of a single cell, genetically marked at that time, can contribute to wing and leg formation (Wieschaus and Gehring, 1976; Steiner, 1976). This rules out the notion that there is initially a uniform imaginal disc which becomes subdivided subsequently into an anterior and posterior compartment. Rather, the primary process is the formation of a border between anterior and posterior. Only later cells from both sides of the border are allocated to form the disc proper.

The common border between the anterior and posterior compartment is a line. To define the position of a disc along this border, a subdivision of the D–V dimension is required which should lead, among other determinations, to two pairs of dorsal (D) and ventral (V) determinations, one for the future wing and another for the future leg disc. The intersection of the AP and a DV border then defines a unique point, the center of the disc to be formed. Topologically, three compartments are sufficient to specify the center of a disc since they meet each other at a single point. As a result of the cooperation, the intersection of the AP and DV border would become a local morphogen source; diffusion into the surrounding sheet of cells leads to a cone-shaped morphogen distribution. Experimentally, it has been shown that segments in a leg disc are arranged as a series of concentric rings (Schubiger, 1968). A cone-shaped morphogen distribution is appropriate to specify this pattern. The highest concentration would cause the determination of the most distal structures (Fig. 1). These are expected at the location where cells of three compartments are close to each other, in agreement with the experimental observation (Steiner, 1976). The lower concentrations at greater distances from this intersection would lead to the determination of the more proximal leg structures. The formation of the (roughly circular) imaginal disc within the larval ectoderm could be specified by the same cone-shaped signal if a certain threshold concentration is required for disc determination. In this view, the disc is laid down around the primarily formed intersection of borders. No separate positional information system is required for disc determination.

According to our model, a high morphogen concentration and therefore distal transformation (Rose, 1962) is expected whenever cells of the compartments involved become close to each other. For instance, small marginal wing fragments usually do not regenerate distal structures while two such fragments derived from opposite sides of the wing disc do so (Haynie and Bryant, 1976). Only in the latter case, cells of the three (or four) compartmental specifications become juxtaposed. Or, to give another example, dissociation and reaggregation of imaginal discs should lead to very strong distal transformation since, after this procedure, new confrontations of the compartments are present everywhere in the reaggregated disc and this would lead to a greatly enhanced morphogen production. Strong distal transformation in reaggregated discs has been observed by Strub (1977).

**Evidence for the Involvement of Compartments in Leg Duplications and Triplications**

Bateson (1894) has collected a number of animals in which spontaneously three legs were formed instead of a single leg. He found that the following rules were always obeyed: (i) The three legs lie in a plane (they are not arranged like the legs of a tripod). (ii) The two outer legs have the expected handedness of the normal
limb while the central leg has opposite handedness. (iii) Occasionally, two of the three legs are fused over the entire length. The fused legs are symmetric, consisting, for instance, of two anterior leg halves. Such limbs are frequently distally incomplete. These rules will find a straightforward explanation by the proposed mechanism.

In a heat-sensitive mutant of Drosophila such triplications as well as duplications can be induced with high probability (Figs. 2a–c). The mutation causes cell-autonomous cell death at the elevated temperature (Russell et al., 1977; Postlethwait, 1978; Girton and Russell, 1980, 1981). After induced cell death or after a surgical interference, patches of cells can change their compartmental specification (Szabad et al., 1979). Such a change can lead to new intersections of compartment boundaries and—according to our model—to additional legs. The locations of experimentally observed additional legs (Postlethwait, 1978) indicate that the cells of the anterior compartment are especially prone to switch to posterior specification. We have proposed elsewhere a model for compartmentalization and segmental specification during primary pattern formation in insect embryos (Meinhardt, 1982a,b) according to which the different compartmental specification locally exclude each other but stabilise each other on long range. Surgical removal of one compartment or hindrance of cell–cell communication by killed cells can lower this stabilization to such a degree that a switch of some cells from one compartmental specification into the other becomes possible, leading to a new stable situation. The model provides also an explanation why an A–P transition is more frequent than the reverse transition. Killed cells need not to appear in patches to cause this destabilization. Even the increased temperature may be sufficient and compartmental specification could take place without cell death as observed by Jürgens and Gateff (1979).

According to our boundary model, if the patch of posterior cells appear at the anterior margin of a leg disc, one new intersection of anterior dorsal (A), anterior ventral (V), and posterior (P) compartments can appear which would lead to an additional set of positional information and thus to a duplicated leg (Fig. 2e). Depending on the distance between the two intersections, the gradient systems overlap to a greater or lesser degree. If the distance is small, the two maxima are separated only at high concentrations. Only the distal structures are separated. We expect that the more proximal parts consist of two fused posterior leg halves and that proximal anterior leg structures are lost, in agreement with the observation of Girton and Russell (1980). According to our model, this loss of structures results from the overlap of the two gradient systems and not because these particular structures are removed by cell death.

If an “island” of posterior cells appears in the anterior compartment, two new intersections can occur, leading to leg triplication. As can be seen from Fig. 2f, the two outer intersections have normal handedness (A, P, and V clockwise in a left leg) while in the central leg the handedness is reversed, in agreement with Bate son’s rule. Moreover, the model predicts correctly that the three legs appear in a plane since they arise on the straight A–V border.

If the patch of newly formed P cells is close to but does not touch the A–V boundary (Fig. 2g), one would expect inefficient cooperation; the maximum morphogen concentration necessary for the determination of the distalmost structures would not be achieved. Distally incomplete symmetrical (fused) legs are expected. The model makes a firm prediction: Distally incomplete legs should not contain cells of the ventral compartment (Fig. 2g), at least not in their distal portion. This prediction has proven to be true. Girton (1981) has determined the structures present at the base of distally complete and incomplete triplicated legs. Without exception, all distally complete triplications contain ventral cells (Fig. 2b) while distally incomplete don’t (Fig.
2) As Figs. 2h, i show, the critical parameter which is decisive whether distally complete legs are formed or not is whether cells of the three compartments are available, in agreement with the model proposed. It is clearly not the completeness of the circumferential structures (the length of the curved lines in Figs. 2h, i). The latter would be expected from a recent version of the polar coordinate model (Bryant et al., 1981).

Pattern duplications with other planes of symmetry can also emerge. For instance, Jürgens and Gateff (1979) found leg duplications in the temperature-sensitive mutation mad in which supernumerary legs appear on the ventral side of normal legs. The anterior–posterior axis is not affected and the A–P border is not transgressed. The mad duplications produced by early heat shocks (40–80 hr) can be explained under the assumption that a second dorsal compartment is formed at the ventral side of a leg disc. Later heat shocks (80–110 hr) lead to pattern alterations of the type shown in Fig. 2f.

According to the polar coordinate model, the cells maintain their determinations (positional values). After induced cell death and wound closure, the physical contact of normally nonadjacent cells stimulates cell proliferation and intercalation. In contrast, in our model, juxtaposition of normally nonadjacent structures results from a compartmental respecification and not from closing wounds. Our model is supported by several recent observations. Girton and Russell (1981) found that a new A–P border is established before the onset of cell division and further that the new border is a necessary step toward the formation of a supernumerary leg. Similarly, Abbott et al. (1981) found in disc fragments the onset of cell proliferation before completion of wound closure. We expect that cell proliferation starts around the newly formed intersections of compartment borders to bring the sequence of proximodistal structures, determined by the gradient at a minute scale, to its appropriate size. After an A–P respecification, we expect...
the center of cell proliferation at the former AD–AV border, in agreement with the observation of Abbott et al. (1981) and Karpen and Schubiger (1981).

In the wing disc the situation is very similar. The distalmost structure, the wing blade, is formed around the intersection of the DV and AP compartment border. Fragments which contain only the ventral compartment do not show distal transformation except if, after prolonged culture, cells with dorsal specification suddenly appear ( Kirby et al., 1982). Determination of distal structures as well as the onset of cell proliferation seem to start only after this ventral–dorsal respecification, in agreement with our model. Wing duplications have also been observed in certain genotypes (Whittle, 1976). The position of the supernumerary wing indicates an anterior–posterior respecification. The line of symmetry is perpendicular to the DV boundary, in agreement with our model.

**Interpretation of Positional Information**

In all developmental systems in which pattern formation appears to be under the control of a graded morphogen concentration, the experiments can be interpreted under the assumption that the cells respond to the morphogen in the same way: they are “promoted” step by step from proximal (or anterior) determinations to more distal (or more posterior) determinations. This process continues until the achieved level of determination corresponds to the local morphogen concentration. Each step is irreversible. After removal of the morphogen by removal of the organizing center, the cell determination is stable in the sense that it does not relapse to more proximal or anterior determinations; it is labile in the sense that a subsequent increase in the morphogen can lead to further changes in the distal (or posterior) direction (distal transformation). The determination of the basic body pattern of insects ( Meinhardt, 1977, 1978), of the pattern of vertebrate digits ( Tickle et al., 1975) and of the proximodistal sequence of segments in insect appendages ( Meinhardt, 1980) are in agreement with this assumption. This mode of interpretation is important for systems which show substantial growth after the determination of structures is completed. Since the slope of the morphogen gradient is assumed to be established by diffusion and decay, the slope remains unchanged during growth as long as these parameters remain unchanged. In a leg of a cockroach, for instance, the gradient will be restricted to a small area near the tip where cells of all compartments are close to each other (assuming a similar compartmentalization as in a Drosophila leg). The proximal leg structures—far away from any intersection—are exposed to a very low morphogen concentration and re-

![Fig. 3. Positional information, regeneration, and absence of intercalation. (a) After truncation of a cockroach leg, a complete leg regenerate. According to the model, a new intersection of compartments is formed during wound healing. This leads to a new morphogen gradient which causes distal transformations of all those cells which are exposed to a morphogen concentration higher than corresponding to their own determination. Since the range of the morphogen is small (corresponding to the dimension of an imaginal disc), the new distal leg part is laid down at minute scale, in agreement with the experimental observation (drawn after Bullière, 1972). A well-proportioned leg is formed after corresponding growth of these initially minute leg parts. (b) A femur–tibia (F, Ti) joint is not replaced after its removal (Bohn, 1970). According to the model, the morphogen is produced only at the intersection of “compartments” at the tip. Its concentration is too low at the host–graft junction to allow any respecification. No disparity of intrasegmental pattern occurs which could initiate intercalation. (c) However, after incomplete wound healing of a grafted tibia fragment, three new tarsi can be formed (French, 1976). According to our model, the tube-shaped ectoderm may be closed at the host/graft junction and the compartments may come into touch. Distal transformation occurs on both sides as well as at the terminal end of the graft.](image-url)
explains the strange-appearing absence of intercalary regeneration after removal of a femur–tibia joint, a result which is difficult to account for by any model based on intercalation between normally nonadjacent cell types.

**Intercalary Regeneration within Insect Leg Segments**

The complete proximodistal pattern of an insect leg results from the superposition of at least two pattern-forming systems: One determines the sequence of segments, the other the sequence of structures within the segments. Both have different regulatory properties, indicating that different pattern-forming mechanisms are at work. While in the respecification of segments only proximodistal changes occur (Strub, 1979), the intercalary regeneration of structures within leg segments proceeds frequently in a distal–proximal sequence (Fig. 4) (Bohn, 1971). Gaps between segments are frequently not repaired (Fig. 3b) while discontinuities in the intrasegmental pattern are smoothed out by intercalation (Fig. 4a). We assume that the morphogen distribution controls only the sequence of segments. Pattern formation within a segment and its intercalary regeneration can be explained by assuming that the pattern consists of a sequence of discrete cell states which mutually activate each other over longer range but exclude each other locally (Fig. 4) (Meinhardt and Gierer, 1980). Thus, the pattern within a particular segment presumably does not result from the generation of a morphogen gradient and its interpretation but consists from the beginning of a spatial sequence of different but related qualities.

**The Regeneration-Duplication Phenomenon**

Large fragments of imaginal discs regenerate the missing structures while smaller disc fragments duplicate the existing structures (Bryant, 1975a,b). In the context of the polar coordinate model (French et al., 1976), this observation is accounted for by the rule of intercalation according to the shortest route.
(1978, 1980) showed that intercalary regeneration can also take place around the circumference of cockroach legs. In this process polarity reversal can take place analogous to that observed in the intercalation of proximal-distal discontinuities (Fig. 4a). The assumption of cell states which activate each other on long range and exclude each other locally would also be appropriate for the circumferential pattern and its intercalation. With this scheme, there is in a sequence of, for instance, 12 positional values no need for a discontinuity between 12 and 1, as would be necessary if the circumferential pattern is organized by a gradient of a single substance. Structure 12 can support structure 1 in the same way as 1 supports 2, etc. The question remains, however, how such a sequence of circumferential structures is laid down initially during development. How, during regeneration of fragments, do the cells “know” what the shortest route is?

We have argued above that the primary stimulus for an imaginal disc is an intersection of compartment borders. In this model, the disc begins with a coarse circumferential determination—namely, the three sectors or four quadrants of the major compartments. The compartment borders must act as a frame for the finer circumferential subdivision similarly as the segment borders provide a frame for the intrasegmental patterning. Again, the distance from a particular compartment border could be measured by a diffusible morphogen. For the organization within a wing disc, Crick and Lawrence (1976) have proposed already that a symmetrical positional information is centered over the A–P compartment border. Alternatively, to bring a set of, for instance, 12 circumferential cell states into register with three compartment borders it would be sufficient that each border induces a particular state. Intercalation could then produce intervening states (Fig. 5). This mode of organization would provide a basis for the regeneration–duplication phenomenon. A small disc fragment contains, as the rule, only two compartments. Wound closure creates the same compartmental confrontation that already exists in the fragment. This leads to duplication (Figs. 5e–g). A fragment only slightly larger can contain three compartments and regeneration will follow. The decisive factor that controls whether regeneration or duplication occurs is assumed therefore whether or not all compartments are represented in the fragment. If all compartments involved are present, regeneration will follow, otherwise duplication will occur. Karlsson (1981a,b) has shown for the wing disc that a border line between regeneration and duplication is located very close to the A–P compartment border. Evidence for the induction of a particular circumferential structure at a compartment border has been provided by Lawrence et al. (1979). They have found that in Droso-

**Fig. 5.** The regeneration–duplication phenomenon. (a–d) Postulated events which lead to normal pattern formation in the leg disc. The primary event is the formation of the compartment borders (a). By cooperation of compartments a cone-shaped morphogen concentration is produced (see Fig. 1) which lead to the separation of the disc cells from the surrounding larval ectoderm and to the circular arrangement of the future leg segments (b). The compartments represent a rough circumferential pattern. The compartment borders have to direct the organization of the circumferential fine structures, for instance by inducing the structures 1, 5, and 8 (c). Remaining structures are filled in by intercalation (d). (e–g) Duplication: a small fragment contains, as the rule, cells of only two compartments (e). After wound closure, the same compartmental confrontation takes place as it is already present in the fragment. In this example, structure 5 (encircled) is induced at the new A–P border (f) and this leads, after intercalation, to a duplication (g). (h–j) A somewhat larger fragment can contain all three compartments and, after induction of structure 1 at the new A–P border, the missing circumferential structures regenerate. It has to be taken into consideration that missing compartmental specifications can reappear by partial compartmental reprogramming in the fragment (after Meinhardt, 1982a).

sophila, a particular tarsal bristle row coincides with the AP boundary, and interpreted this as an induction by cooperation of these compartments.

In these considerations, one has to take into account that compartmental specifications are not irreversibly fixed in disc fragments. Missing compartmental specifications can be regenerated. Then, two complementary fragments would both have the complete set of compartmental specifications and both would regenerate, as observed by Kaufman and Ling (1981) and Kirby et al. (1982). Compartmental reprogramming can be a fast process occurring within hours after the experimental interference (Szabad et al., 1979; Girton and Russell, 1981), indicating that reprogramming is the primary event and does not result consequent to the progressing intercalation of missing structures. If, for instance, a leg
disc fragment contains only anterior cells and regenerates the complete circumference, we assume that the initial step is a regeneration of the missing compartments. The new compartment borders would organize the circumferential fine structure as well as the distal transformation of the cells close to the intersection of the borders. In agreement, Schübiger and Schübiger (1978) found that whenever an anterior–proximal leg disc fragment regenerates distal structures, this is always accompanied by a regeneration of structures belonging to the posterior compartment. Similarly, Kirby et al. (1982) found after prolonged culture of ventral wing fragments spontaneous formation of proximodorsal wing structures, indicating a V-D respecification. Afterwards, a more or less complete regeneration is possible by an intercalation of the pattern discontinuities generated by this respecification. Thus, compartmental respecification is presumably the reason why the borders between regeneration and duplication (Bryant, 1975a,b) do not coincide precisely with the compartment borders.

The mechanism envisaged for the fine structuring—the mutual activation of locally exclusive states—has pattern-forming capabilities. Thus, not only intercalation (12/67 → 1234567) but also elongation of a partial sequence (12 → 1234567) should be possible (Fig. 4). Experimental evidence for such an elongation of an incipient sequence of structures has been obtained by Karlsson and Smith (1981), who found partial regeneration around the circumference of otherwise duplicating wing disc fragments. Partial regeneration is restricted to structures of a particular compartment, no compartment borders are crossed. According to the model, whenever some compartmental specifications are absent in a fragment, duplication will follow (Fig. 5). Thus, duplicating fragments are not expected to show regeneration of distal structures, even if partial regeneration around the circumference takes place, in agreement with the observation by Karlsson and Smith (1981).

Relation of the Proposed Boundary Model to the Polar Coordinate Model

In their polar coordinate model, French et al. (1976) have uncovered rules which have been very successful in describing pattern regulation of insect and vertebrate legs as well as imaginal discs after an experimental interference. Our model provides a molecularly feasible basis for the polar coordinate model since there is no difference in principle between a complete circle of 12 values and a circle of 3 or 4. Since the handedness is independent whether 3, 4, or 12 positional values are assumed, the model proposes makes the same predictions about the handedness of supernumerary limbs as the polar coordinate model. In our model, the circumferential values are precisely localizable. They correspond to groups of cells (compartments) whose particular determination has been shown by independent methods. This makes the model inherently testable. In the polar coordinate model, both the number of the positional values and their assignment to particular structures are arbitrary.

The polar coordinate model only provides rules according to which an already existing structure regenerates; no explanation is given how a complete circle is formed initially during development. The compartment borders—certainly the primary developmental decisions toward an insect leg or wing—do not play a role in the polar coordinate model. In contrast, our model links the formation of secondary embryonic fields with the primary organization of a developing embryo. The initiation of a supernumerary structure would have the same basis as the initiation of the normal structure during normal development. This is supported by the observation of Girton and Russell (1981) according to which number of precursor cells and growth rate in duplicated legs is the same as during normal leg determination.

Our model—according to which boundaries act as organizing regions for secondary fields—accounts successfully for experimental observations which are difficult to reconcile with the polar coordinate model. One such observation is that triplicated legs lie in a single plane (Fig. 2). According to our model, this is because they arise along the AD–AV compartment border. In the polar coordinate model, there is no reason why these limbs should be coplanar. To resolve some recently discovered violation of the complete circle rule it has been postulated that a partially complete circle is sufficient for distal transformation (Bryant et al., 1981). Then, distal transformation is expected to be the more complete the more circumferential fine structures are present. The experiments of Girton (1981, 1982) show, however, that distally incomplete legs can contain even more circumferential structures than distally complete legs (Figs. 2h, i). But in all distally incomplete legs one finds that a specific region is missing—the ventral compartment—in agreement with the model proposed. Nevertheless, in a more complete circumferential pattern the chance is higher that cells of all compartments are present. Therefore, our model leads one to expect that, as the rule, the predictions of the polar coordinate model will be correct.

According to the model we propose, the proximodistal pattern in insect legs is generated in two steps: first the subdivision into the sequence of segments (coxa, femur . . . ) and second, into the sequence of structures within each segment. In contrast, in the polar coordinate model,
a single continuous sequence of positional values is assumed. The different regulatory features of both pattern-forming systems strongly support the two-step mechanism. For instance, the presence and absence of gap repair as shown in Figs. 3b and 4a are hardly understandable on the basis of a single sequence of positional values as assumed by the polar coordinate model.

Recently, it has been pointed out be several authors (Winfree, 1980; Lewis, 1981; Mittenthal, 1981) that polar coordinates are not necessarily required to account for the regeneration and intercalation of structures such as cockroach legs, especially with regard to distal transformations. The leg can be regarded as a two-dimensional sheet of cells where the most distal part is located at the center of the sheet. Truncation of a leg would correspond to a removal of the central part of this cell sheet and regeneration would correspond to an intercalary regeneration across this central hole. However, intercalation allows only a smoothing out of pattern discontinuities. Models based on intercalation implicitly require mechanisms to create initially the extreme values of the pattern. Intercalation models are insufficient to give a complete description of pattern formation in secondary fields. Further, on the basis of intercalation models, the failure of intercalation after operations such as shown in Fig. 3b is hard to understand. All these problems do not exist in the model we propose since it describes both: how the extreme pattern elements are laid down, and how they are restored after an experimental interference.

DISCUSSION

To date, it has been a generally accepted view that secondary fields are initiated as a patch of cells with a particular but uniform determination. For instance, Kauffman et al. (1978) have proposed a model for compartmentalization of imaginal discs according to which gradients are generated in the (existing) disc of elliptical shape in a sequential order. (No explanation is provided how a disc and its shape are formed initially.) According to the model I propose, the sequence of events is the reverse: Secondary fields are created around differentiation boundaries resulting from the primary subdivision of the embryo. Secondary fields would have therefore from the beginning a pattern. The mechanism proposed would enable a reliable fine structuring since the newly determined structures have necessarily the correct spatial relationship to the already determined structures. Since at least three different cell types are required to determine, for instance, a limb field, the handedness of such limb is unequivocally fixed. If anterior, posterior, and ventral cells are arranged clockwise, a left leg will result. Since the morphogenetic substances are assumed to be produced only in those cells which are close to the boundary regions, their local concentrations provide a measure about how distant a cell is located from the borders. Thus, the cooperative interaction of compartments (in the broadest sense) could determine not only the precise location of, for instance, a limb but also provide the positional information of its fine structuring.

Boundaries between differently determined cells seem to play an important role also in the organization of other developmental systems. In amphibians, the diencephalon determines the polarity of the neighboring brain structure, the optic tectum (Chung and Cooke, 1975). This seems to be basically similar to the way in which the polarizing zone determines the polarity of an amphibian limb (Slack, 1976, 1977a,b). Recent rotation experiments have shown that the eye anlage in Xenopus, as early as it is detectable during development, has a fixed anteroposterior polarity (Gaze et al., 1979). The eye results from an inductive interaction of an outgrowing part of the forebrain and the ectoderm. Our model would suggest that the position of the outgrowth is—similar to position of the outgrowth of insect legs—determined by a hidden boundary. Again, a border would precede the structure to be formed.

The boundary model also suggests a mechanism for the generation of positional information for vertebrate limbs. The different capabilities of double anterior and double posterior amphibian legs and the formation of double posterior supernumerary legs after 180° rotation become explicable under this view (Meinhardt, 1982a,c).

Since the model has, on the one hand, the regulatory features of the polar coordinate model (French et al., 1976), and provides, on the other hand, a mechanism for the generation of positional information (Wolpert, 1969, 1971), it bridges the gap between the two concepts. With minor modifications, the model can account for either the circular arrangement of structure in the imaginal disc (three- or four-compartment cooperation, producing a cone-shaped morphogen distribution) or the linear arrangement of digits in a vertebrate hand (Meinhardt, 1982a,c).

Only the cooperation of very specifically determined cells is expected to produce new positional information. Thus, presumably, many borders formed in a developing organism will remain without organizer function. A cell determination boundary must not necessarily be a border of clonal restriction since, by regulation, a particular determination may be altered. Even in compartments, respecification is possible.

Together with the mechanisms we have previously proposed, a rather complete picture emerges about how development of higher organism could be controlled (see Meinhardt, 1982a). Local self-amplification coupled with
long ranging inhibitory effects enables the formation of stable self-regulating patterns even in initially almost homogeneous eggs and tissues (Gierer and Meinhardt, 1972; Gierer, 1981). The graded concentration profiles can provide positional information for the primary embryonic axis. Its interpretation, i.e., the conversion of the local concentrations into a sequence of stable states of particular determinations, may be in fact an oscillatory process. Cells alternate between two (or three) states, for instance “anterior” and “posterior” and this alternation controls the transition from one control gene to the next in a manner analogous to the way in which the periodic movement of pendulum and escapement control the stepwise movement of the hands of a clock (Meinhardt, 1982a,b). The formation of the periodic pattern of anterior, posterior, anterior

. . . compartments and, precisely in register with that, the sequential pattern of segmental specification can be explained in this way as well as a class of homoetic transformations. After this interpretation is completed in the primary field, boundaries are present and the secondary fields can be produced and subdivided as described in the present paper. In principle, this mechanism could be repeated many times. Boundaries create new positional information, and its interpretation leads to new boundaries, etc. Thus, our model suggests a chain of relatively simple molecular interactions which could provide a basis for the reliable generation of structures during embryonic development.

I thank A. Gierer and H. MacWilliams for helpful discussions and comments on the manuscript. I thank Academic Press for the permission to use some figures.

REFERENCES


BONH, H. (1970). Intekalare Regeneration und segmentale Gradien-


BONH, H. (1971). Intekalare Regeneration und segmentale Gradien-


CHRISTIAN, S. H., and COOK, J. (1970). Polarity of structure and of or-

CHRISTIAN, J., and LAWRENCE, P. A. (1975). Compartments and poly-

FRENCH, V. (1976). Regeneration in the cockroach, Blattella germ-

ansi. I. Regeneration from a congruent tibial graft/host junction. Wilhelm Roux’s Arch. 179, 57–76.


FRENCH, V., BRYANT, P. J., and BRYANT, S. V. (1976). Pattern regu-

lation in epimorphic fields. Science 193, 969–981.

GARCIA-BELDINO, A., RIPOLL, P., and MORATA, G. (1973). Develop-

GARCIA-BELDINO, A., RIPOLL, P., and MORATA, G. (1976). Develop-


GERRING, W. J., and NOUTHEGEN, R. (1973). The imaginary disc of Dro-


GIBSON, J. R., and RUSSELL, M. A. (1981). An analysis of compartmentalization in pattern duplications induced by a cell-lethal muta-


KARPEN, G. H., and SCHUERGER, G. (1981). Extensive regulatory ca-


