A boundary model for pattern formation in vertebrate limbs

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SUMMARY

We postulate that positional information for secondary embryonic fields is generated by a cooperative interaction between two pairs of differently determined cell types. Positional information is thus generated at the boundaries between cells of different determination. The latter are assumed to result from the primary pattern formation in the embryo. The application of this model to vertebrate limbs accounts for the pairwise determination of limbs at a particular location, with a particular handedness and alignment to the main body axes of the embryo. It accounts further for the gross difference in the regeneration of double anterior and double posterior amphibian limbs as well as for the formation of supernumerary limbs after certain graft experiments including supernumeraries in which the dorsoventral polarity changes or which consist of two anterior or two posterior halves. Our model provides a feasible molecular basis for the polar coordinate model and successfully handles recently found violations, for instance formation of supernumerary limbs after ipsilateral grafting with 90° rotation. The most frequent types of developmental malformations become explicable. The models allow specific predictions which are fully supported by recent experiments (see the accompanying paper of M. Maden).

INTRODUCTION

Pattern formation in vertebrate limbs has been the subject of intensive investigation in recent years (for review see Hinchliffe & Johnson, 1980; Stocum & Fallon, 1982). It is regarded as a model system to study pattern formation in secondary embryonic fields. The question is how a particular structure can be determined at a particular location, with a particular asymmetry (handedness) and with a predictable orientation with respect to the main axes of the developing embryo. Two major models are presently under discussion:

(i) The gradient hypothesis. Assumed is a localized source of a morphogenetic substance (Tickle, Summerbell & Wolpert, 1975). By diffusion into the surrounding cells, a graded morphogen distribution arises. The sequence of the digits becomes determined by an interpretation of this ‘positional information’ (Wolpert, 1969, 1971).

(ii) The polar coordinate model (PCM). It is stipulated that a set of 12 positional values is arranged similar to the numbers on a clock face (French, Bryant &
Bryant, 1976; Bryant, French & Bryant, 1981). Distal transformation is assumed to take place whenever a complete circle of positional values is present.

Both models have contributed substantially to our understanding of limb development, both have their merits and drawbacks. Many transplantation experiments in the chicken wing bud have been explained by the gradient model in a quantitative way (Tickle et al. 1975; Summerbell, 1979). However, it is a one-dimensional model, the dorsoventral dimension is neglected. Thus, the gradient model cannot make statements about the handedness of the limb. From a local source one would expect a cone-shaped morphogen distribution and thus a concentric arrangement of the primordia. In contrast, the digits are arranged along a line. The proximodistal pattern formation is assumed to be under a separate control of a progress zone (Summerbell, Lewis & Wolpert, 1973) while experimental observations indicate that the induction of a second anteroposterior (AP) axis is intimately coupled with an induction of a second proximodistal axis (Summerbell, 1974).

The polar coordinate model (PCM) was very successful in describing the formation and handedness of supernumerary limbs which occur after certain graft experiments (Bryant & Iten, 1976). However, several recent observations are difficult to integrate into the PCM. Examples are the formation of supernumerary limbs after 90° rotation (Maden & Turner, 1978; Wallace & Watson, 1979), the regeneration of limbs which consist of two posterior halves (Slack & Savage, 1978a,b) and the absence of intercalation in supernumerary limbs in which a change of the dorsoventral (DV) polarity occurs (Maden, 1980, 1982, 1983a; Maden & Mustafa, 1982b). In both models it remains an open question as to how a local source or a complete circle is formed initially in the embryo at a particular position, i.e. how pattern formation in secondary fields is linked to the primary pattern formation in the embryo.

Determination of a substructure such as a limb requires a preceding primary pattern formation events along the main axes of the embryo (anteroposterior, dorsoventral). This process can be understood to a large extent on the basis of positional information and its interpretation (Wolpert, 1969, 1971). As a result of this primary pattern formation we expect that areas with different determination arise which are separated from each other by well-defined borders. We have proposed that cell determination boundaries can act as organizing regions for pattern formation in developmental subfields (Meinhardt, 1980b, 1982a,b, 1983b). It is stipulated that by a cooperative interaction of at least two differently determined cell types a morphogen is produced (Fig. 1). Due to the required cooperation this production is restricted to an area which is close to the common border of the involved cell types. The local morphogen concentration is a measure for the distance of a cell from such a boundary and therefore convenient to supply positional information (Fig. 2). The general principle should be illustrated by a molecularly feasible example. Let us assume that one cell type produces a precursor molecule, the adjacent cells of the other type are able to
Fig. 1. Two possibilities for the generation of secondary embryonic fields. (A) A particular area (dashed) becomes determined by the interpretation of two primary gradients (anteroposterior, AP, and dorsoventral, DV). Within this field, a similar mechanism of pattern formation as during primary pattern formation is recapitulated (for instance, by autocatalysis and lateral inhibition, see Gierer & Meinhardt, 1972; Meinhardt, 1982a). This mechanism, looking straightforward, would require an evolved system of cell permeabilities. The boundaries of the secondary field must be impermeable for the substances which generate the secondary pattern, but the substances which generate the primary pattern must penetrate into the secondary field. Otherwise the secondary pattern would not obtain a reliable orientation with respect to the primary pattern. Further, the pattern generated in the secondary field would depend sensitively on the geometry of the secondary field. (B) Two pairs of cell patches with particular determination - one in the AP and the other in the DV dimension - cooperate in the production of two morphogens, one for each cardinal axis. The two boundaries and especially their intersection become the organizing regions for the secondary field. Symmetrical morphogen profiles result. If only one cell type can respond, monotonic gradients are effective (see Fig. 2). The pattern is to a large extent independent of the geometry of the secondary field. Such a secondary field has necessarily the correct spatial relation to the already determined structures and a handedness (e.g. anterior-posterior-ventral would be arranged counterclockwise in a right leg). In contrast to mechanism (A), no special permeabilities at the boundaries are required.

convert it into the active morphogen. An efficient morphogen synthesis would be possible only at the common boundary since only there converting cells become exposed to precursor molecules. The morphogen distribution resulting
Fig. 2. Generation of positional information at the boundary between two differently determined tissues (to be called A and P). (A) If a cooperative interaction between A and P is required for the production of a morphogen m, the m-production is restricted to the boundary region between A and P. A symmetrical distribution, centred over the boundary will emerge. The resulting pattern may be asymmetric (I–IV versus I–IV) since the same positional information may be differently interpreted in A and P. (B) If only one cell type is competent (c) to respond to the morphogen, only a monotonic gradient would remain effective and an ordered sequence of structures is formed. The local m-concentration is a measure for the distance from the boundary. The boundary region between two differently determined cells would become the boundary region in the sense used by Wolpert (1969, 1971), i.e. a high point in a positional information scheme. (C) An alternative possibility would be that all polarizing cells produce the morphogen and the gradient emerges by diffusion into the competent zone. (This possibility is one of the explanations offered by Slack (1977a) for his experiments shown in Fig. 3.) A decision between possibilities (B) and (C) could be made on the basis of how many polarizing cells are required to induce a new AP axis. If cooperation is involved (B) we expect that very few polarizing cells can form a new AP axis upon transplantation since independent of the size of the transplanted tissue, only the cells located directly at the boundary contribute to the m-production. In contrast, if all P-cells produce m, we expect that transplanted P tissue must have at least one half of the size of the competent tissue participating in limb formation to induce a complete AP axis. Corresponding experiments have been done only for chicken wing development with the result that very few (ca. 100) cells are sufficient to induce a complete new gradient system (Tickle, 1981) arguing in favour of mechanism (B). The formation of the most posterior digit after 90° rotation (see Fig. 9) indicate also that few posterior cells are sufficient.
from such a cooperative interaction would have a more or less symmetrical profile. The highest concentration is centred over the common boundary. The resulting pattern must not necessarily be symmetric since the gradient can be differently interpreted in both cell types (Fig. 2A). The most extreme case would be that only one of the two cell types is competent to respond to the gradient. These competent cells would be exposed to a monotonic gradient with the highest concentration at the boundary (Fig. 2B).

The boundary between two cell types has a linear extension. If three cell types (or two pairs of cell types; Fig. 1) are involved, the source region is almost a point since three patches are close to each other only at a particular location, at the intersections of the borders. Such an arrangement of three or four cell types has necessarily a handedness, it is different from its mirror image.

We have shown for imaginal discs of insects that many experiments reported in the literature can be explained under the assumption that the compartment borders act as organizing regions (Meinhardt, 1980a, 1983b). In the present

![Figure 3](image-url)

Fig. 3. Explanation of Slack's transplantation experiments (Slack, 1976, 1977a,b) in the context of the boundary model. (A) normal limb field: We suppose that by cooperation between the competent (A, anterior, dashed) and polarizing (P, posterior, crossed) cells, a symmetrical morphogen concentration, centred over the common boundary is produced (Fig. 2). The A-cells are exposed to an exponential gradient which determines the anteroposterior pattern of the limb, e.g. the sequence of the digits. (B) After grafting P-tissue in front of A-tissue, A-tissue is bordered on both sides with P-tissue. A symmetrical U-shaped morphogen profile and therefore a symmetrical double-posterior (PP) limb results. (C) Grafting A-tissue posterior to P-tissue leads to two gradients with opposite polarity and correspondingly to two limbs of opposite AP polarity. After the same operation Slack (1977a) found also supernumerary symmetrical (PP) limbs and limbs with normal AP-polarity. In terms of the model, if the graft includes some polarizing cells, the graft would be flanked on both sides by P cells and a PP limb would result. If such a graft is implanted so far posterior that it does not have a common border with the polarizing tissue of the host, a limb with normal AP polarity results.
paper I would like to present an application of the boundary model to pattern formation in vertebrate limbs. Our model reconciles the gradient and the polar coordinate model under a straightforward hypothesis. It handles correctly recently observed violations of the PCM and predictions of the model are fully supported by recent experiments of Maden (see the accompanying paper of Maden, 1983a). An outline of this model has been published already (Meinhardt, 1980b, 1982a,c). A model for the pattern formation in the proximodistal axis is presented in a subsequent paper (Meinhardt, 1983a).

RESULTS AND DISCUSSION

The polarizing and competent zone in amphibian limb determination

The assumption of two zones which collaborate to produce a morphogen allows an explanation of grafting experiments with axolotl tissue (Slack, 1976, 1977a,b). Extending classical experiments of Harrison (1921), Slack has found two zones – the competent and the polarizing zone. The competent zone forms the limb proper. The adjacent polarizing zone determines the anteroposterior polarity of the limb. Both zones behave differently upon transplantation (Fig. 3). If polarizing tissue is transplanted anterior to the competent tissue, the competent tissue is flanked on both sides by polarizing tissue. A symmetrical limb grows out which consists of two posterior halves. In contrast, if competent tissue is transplanted posterior to the polarizing tissue, in addition to the normal limb a supernumerary limb can grow out which has a reversed anteroposterior polarity. An explanation of these results in terms of the boundary model is given in Fig. 3.

The extension of the competent zone is significantly larger than the area from which the limb proper is derived (Slack, 1980). In terms of the model, only a fraction of the gradient is used. Competent cells at larger distances from the boundary, exposed to very low morphogen concentrations do not participate in

Fig. 4. Proposed steps in the formation of vertebrate limb fields. (A) A primary anteroposterior gradient can serve to subdivide an embryo into bands of distinct determinations. Among these are the polarizing (P, crossed) and the competent (anterior, A, blank) zones. (B) To locate the position of the outgrowing limb a global dorsoventral (DV) subdivision of the embryo is required in addition. The interpretation of a DV gradient should lead to two pairs of D (==) and V (blank) stripes (one pair on each side of the body). (C) The area around the intersection (O) of the AP and DV border (framed in A and B) defines the position of the limb field (ellipse). The dotted line marks the expected position of the apical ectodermal ridge (AER) on the DV border in A (competent) tissue near a P region. (D) The positional information generated in this way (indicated by the triangle in C) provide a measure for the distances of a cell from the borders. The ridge-like morphogen distribution centred over the DV boundary determines the positional information for the dorsal and ventral side of the limb bud. Along this ridge the digits are formed. (E) Geometry of the AP and DV-stripes in an outgrowing right limb bud, viewed from a posterior-dorsal position. (F) An outgrowing chick wing bud. The apical ectodermal ridge is clearly visible (drawn after Hinchcliffe & Johnson, 1980).
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Fig. 4
limb formation. This tissue may become the anterior necrotic zone. The utilization of only a portion of the competent zone makes a separate size-regulation mechanism for the limb-forming gradient superfluous since the required amount of competent tissue will be available even if the competent tissue is somewhat larger or smaller than normal.

Normal pattern formation can occur after a wide variety of experimental manipulations (see Stocum & Fallon, 1982 for review). This is expected from the boundary model. Not only the unused competent tissue distant to the boundary can be removed without damaging normal pattern formation. After removal of the boundary region and wound closure, a new juxtaposition of the two cell types and thus a reconstitution of the gradient should occur. The induction of a new AP-axis is expected to be independent of the number of transplanted polarizing cells over a wide range since in any case only very few cells, the cells of the proper boundary region, contribute to the morphogen production.

An intersection of two boundaries is required to define a limb field

The border between the polarizing and competent (P and A) zones is not sufficient to define a limb field; the border would surround the embryo in a belt-like manner. The dorsoventral (DV) position of the limb outgrowth as well as the DV pattern and the handedness of the limb itself remain to be determined. This could be achieved by a global dorsoventral pattern formation of the embryo which should lead, among others, to cells of ‘dorsal’ (D) and ‘ventral’ (V) determination. The condition for a limb field would be a DV border in competent A tissue which is flanked on one side by polarizing P cells (Fig. 4). A primary dorsoventral gradient would have the appropriate concentrations for the D and V stripes on both sides of the body. This accounts for the pairwise determination of the limbs. Both limbs have necessarily opposite handedness. The expected position of the DV border on the outgrowing limb is the same as that of the apical ectodermal ridge (AER, Fig. 4F) in chickens and we will assume that a DV juxtaposition is a precondition to form an apical ectodermal ridge.

The fact that removed distal parts of an amphibian limb can regenerate suggest that the limb field can be reestablished after truncation. To explain this, our model requires that the limb bud does not consist entirely of competent A tissue but that at least a narrow stripe of polarizing P tissue is carried along with the outgrowing limb (Fig. 4E). After truncation and closure of the wound, a new intersection of the AP and of the DV border is created which leads to regeneration of the limb.

Regeneration of double posterior limbs and the failure of double anterior symmetrical limbs to regenerate

By surgical operations symmetrical limbs can be produced which consist either of two anterior (AA) or two posterior (PP) halves (Fig. 5). The two types of limbs show a remarkable difference in regeneration after truncation. While PP limbs
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Fig. 5. Explanation of regeneration of double posterior (PP) limbs and absence of regeneration of double anterior (AA) limbs. (A, B) The operation: reciprocal exchange of tissue (A) followed by truncation (B) leads to AA and PP limbs (drawn after Holder, Tank & Bryant, 1980). After truncation, a PP limb regenerates a PP limb (C) while an AA limb shows little or no regeneration (Tank, 1978; Stocum, 1978). (D–F) Model: after truncation of a normal limb (D) and wound closure, one intersection of the AP and DV border results. This leads to a new limb field which enables regeneration. Truncation of a PP limb leads to two intersections (E) and therefore to the regeneration of a PP limb. In contrast, after truncation of an AA limb, only a DV border but no intersection of the AP and DV border remains (F), which is insufficient to initiate regeneration (after Meinhardt, 1982a). The observed asymmetry in the regeneration of symmetrical limbs is a straightforward consequence of the proposed asymmetry of the limb field in relation to the two boundaries by which it is generated (see Fig. 3). VV limbs and DD limbs show also very little regeneration (Tank, 1978). We would expect that the regeneration of such limbs depends sharply on whether a DV boundary remains after transplantation. Other factors influencing the regeneration frequency of symmetrical limbs are less well understood. The frequency of regeneration decreases with increasing time between the surgical production of the symmetrical limbs and truncation (Stocum, 1978; Holder et al. 1980). While upper arm AA and PP limbs do not regenerate, lower arm AA stumps regenerate almost as well as PP stumps (Bryant, 1976; Krasner & Bryant, 1980). For recent discussion of possible reasons see Slack (1983).

Regenerate, AA limbs show very little if any regeneration (Slack & Savage, 1978a, b; Stocum, 1978). This difference is a straightforward consequence of the proposed mode of limb field generation. A double posterior limb would carry a stripe of polarizing tissue at the posterior as well as at the anterior side of the limb (Fig. 5). In contrast, an AA stump contains no polarizing tissue at the wound.
Skin graft experiments show a similar asymmetry (Slack, 1980; Maden & Mustafa, 1982a). Striking differences exist also in the capability for regeneration of limbs in which either the anterior or the posterior side has been heavily irradiated (Maden, 1979). Regeneration occurs only if the posterior side remains intact, in obvious agreement with the proposed model.

Formation of supernumerary legs after rotation or contralateral graft of a limb tip

Transplantation of amphibian limb buds or regeneration blastemas either to a stump of the same side after rotation or to a stump of the contralateral side can lead to outgrowth of supernumerary limbs at the graft–host junction (see Bryant & Iten, 1976). However, strikingly different supernumerary limbs are produced in the two operations. After ipsilateral rotations, one obtains 'normal' supernumeraries and three additional major types: limbs in which the AP axis is normal but the DV polarity changes within a limb (DV–VD limbs; see accompanying paper by Maden, 1983a); symmetrical limbs which consist of two posterior halves (PP-limbs, Fig. 7D; frequent especially in Rana) and double anterior limbs (AA-limbs, see Fig. 9C). In contrast, after grafting a blastema to the contralateral side, only normal supernumerary limbs are formed.

Our model predicts the difference between ipsi- and contralateral grafts as well as the abnormal types of supernumeraries. To clarify which type of AP and DV intersections occur after both operations, in Fig. 6 and 7 the leg cylinder is shown in an unrolled and flattened form at the graft–host junction. According to the model, the two intersections resulting after contralateral grafting always correspond to normal limb fields (Fig. 6D, E, compare with Fig. 4C). The model predicts the difference between ipsi- and contralateral grafts as well as the abnormal types of supernumeraries. To clarify which type of AP and DV intersections occur after both operations, in Fig. 6 and 7 the leg cylinder is shown in an unrolled and flattened form at the graft–host junction. According to the model, the two intersections resulting after contralateral grafting always correspond to normal limb fields (Fig. 6D, E, compare with Fig. 4C). The model predicts that after an AP confrontation (D) the AP axes of the supernumeraries are oriented perpendicular at the stump–graft border. The supernumerary limb which grows out at the posterior host side is essentially graft derived (at least in its anterior portion, arrow) while that of the anterior host side is essentially host derived. According to Maden (1982) the stump–graft border crosses the AP axis at variable positions. This results presumably from the movement of a superficial cell layer in respect to the underlying mesoderm (see Fig. 8). In contrast, after DV juxtaposition (E), the AP axis and the stump–graft border should be parallel. The supernumerary limb which grows out at the posterior ventral host side should be in its ventral side host derived (arrow), in its dorsal side graft derived.
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predicts the graft and stump contribution to the supernumerary limbs as well as their orientation in relation to the stump axes. These predictions are essentially in agreement with Maden's (1982) observation.

The situation is very different after ipsilateral grafting and 180° rotation. As
Fig. 7. Intersection of borders resulting from ipsilateral 180° grafts. (A) Expected arrangement of A, P, D and V cells after 180° rotation and formation of normal supernumerary limbs. The dotted line indicates a potential symmetrical PP limb field. (B) Cell specifications around the leg cylinder at the graft–host junction. Two limb fields with normal AP axis (triangles; enlarged in E, F) and two double posterior (PP) limb fields (M-shaped morphogen distribution; enlarged in C) are created. (D) An experimentally observed PP limb in *Rana* (after Maden, 1981). The symmetrical limb is easily recognized by the bifurcated central digit. In limb fields with normal AP polarity, the D and V tissues are diagonally arranged at the intersections (A, B, E, F) which can lead to a DV polarity change within a supernumerary limb (see Fig. 8). In PP-limb fields, the A–P boundary only touches but does not cross the AD–AV boundary. This may reduce the probability that such limb fields lead to a limb outgrowth. Depending on the circumference of the leg cylinder and the size of the P-region, a PP limb field may give rise to two separated limbs. Variation in these parameters may account for the different frequency of PP limbs in axolotl and *Rana*.

shown in Fig. 7, the model predicts two pairs of intersections which correspond to two symmetrical PP-limbs. Such PP limbs have been observed by Maden (1981) in *Rana* (Fig. 7D) and by Maden & Turner (1978) in axolotl. They occur only after ipsilateral grafts. Moreover, the model predicts two intersections in which a normal AP polarity is given but where the D and V specifications appear in diagonal positions (not on the same side as in a normal limb field, see Fig. 4 and 6). This can lead to a DV polarity reversal in a supernumerary limb (Fig. 8),
in agreement with Maden's (1982, 1983a) observation. The observed variability of the location of the DV–VD polarity change in relation to the A–P axis indicates that some movement of the tissue which bears the DV border is possible in relation to that which contains the AP border. The DV organization resides presumably in a superficial cell layer; in the ectoderm in chickens (MacCabe, Errick & Saunders, 1974), in the total skin in amphibians (Maden & Mustafa, 1982a). Some of the experimentally observed 180°-supernumeraries consist partially or completely on both sides of D or of V tissue. This will occur if the DV tissues, diagonally arranged after the graft, slide against each other due to a higher adhesion among the D or among V cells compared with that between D and V cells. The assumption of such a tissue sliding is reasonable since, after 90° rotation, frequently a back rotation has been observed (Maden & Turner, 1978; Wallace & Watson, 1979) which must similarly have its base in a difference of adhesion between cells of the same and of different determinations. Fig. 8 shows examples of possible patterns with DV polarity change. They should be compared with the experimentally found patterns (Fig. 1 of the accompanying paper of Maden, 1983a). A change of the DV polarity has never observed in supernumerary limbs resulting from contralateral grafts, in agreement with the model we propose.

Limbs with a high degree of symmetry in the DV axis regenerate as well as normal limbs (Maden, 1982); the DV pattern is maintained in the regenerate. This is in sharp contrast to surgically produced symmetrical DD or VV limbs which do not regenerate (Tank, 1978). Our model explains this difference. Even in very symmetrical DD or VV limbs formed after rotation we expect that an AP–DV intersection remains (Fig. 8C, D), in contrast to DD or VV limbs produced surgically (see Fig. 5). The DV border may be difficult to detect in the outgrowing limb since it could be located very close to the unused polarizing tissue.

The model predicts further that two supernumerary limbs formed either after 180° rotation (APDV confrontation) or after contralateral grafting (AP confrontation) do not grow out at the same proximodistal level, but that the supernumerary at the anterior host side arises more proximal than that of the posterior host side (Fig. 7A). This shift is expected because, according to the model, a leg is formed predominantly of anterior tissue. This prediction is fully supported by experimental observations (Maden, 1983a).

**Supernumerary limb formation after 90° or 270° rotations**

Supernumeraries can be formed even after a much smaller misalignment of the AP and DV axes (Maden & Turner, 1978; Wallace & Watson, 1979). This result is surprising in the view of the polar coordinate model since no 'complete circle' is generated by such an operation. In our model, new intersections do occur even after 90° or 270° rotations (Fig. 9A). Depending on the precise alignment of graft and stump, expected are either two limbs which grow out very close
Fig. 9. Supernumerary limb formation after 90° rotation and ipsilateral grafting. (A) Intersections occur already after rotation of about 90°. They depend heavily on the actual alignment between graft and host. At the encircled regions a polarizing region touches two DV borders in competent regions (triangles). (B) Expected is the formation of positional information for double anterior (AA) limbs. An example is given in (C). The numbers indicate the digits in the AA-supernumerary limb. The remaining digits belong to the graft (after Maden & Mustafa, 1982b). This AA leg has been actually observed after a 180° rotation. We assume that some back-rotation was occurring before determination of the supernumerary limb. We expect that (at least originally) both parts of an AA limb have a 90° orientation towards each other. Two AA-limb fields may fuse (A) which would explain complex limbs with more than eight digits such as observed by Wallace & Watson (1979).

Fig. 8. Explanation of DV-VD polarity change in supernumerary limbs generated by 180° rotation. The expected types are exemplified by supernumerary limb fields formed at the posterior side of a right stump (Fig. 7A, F). (A) At the intersection of the AP and DV border (which leads to the outgrowth of the supernumerary limb), the D (dashed) and V (blank) specifications are diagonally arranged (see Fig. 7). Since only the competent tissue (A, blank) forms the limb proper, the limb can have a normal DV polarity (class 1 in terms of Maden, 1983a). It would be a left limb (AV-AD-P clockwise). (B) A shift of the DV specifications resident in a superficial cell layer (Ectoderm or Dermis) relative to the AP specifications (in the underlying Mesoderm) can bring the DV-VD polarity change to variable positions in the competent region. This would lead to a DV-VD polarity change within the outgrowing limb. (C-G) The cell adhesion between D cells or between V cells may be larger than between D and V cells. Then, tissue movement would lead to symmetrical structures. Depending on the extent of the shift (length of the arrows), either completely symmetrical limbs are formed (C, D), a symmetrical part is located in the posterior (E, F) or in the anterior (G) portion of the limb. The model predicts that if the anterior part has a DV-polarity (B, E, F), it has the expected handedness (opposite to the stump, AV-AD-P clockwise, i.e. a left limb). If the posterior part is asymmetric, it has stump handedness (B, G). These expected patterns agree with the pattern experimentally observed (see the accompanying paper of Maden, 1983a). According to the model in all these patterns an intersection of the AP with the DV border remains present. It is independent of the degree of symmetry. This allows outgrowth and regeneration even of very symmetrical limbs in agreement with the experimental observation (Maden, 1982). (H) An arrangement which is, according to the model, impossible: the posterior part is a left limb while the anterior part is a right limb. Such an arrangement would require two diagonal D-V confrontations. However, such a limb with a left handed posterior part and with a right handed anterior part can arise in a supernumerary limb which grows out at the anterior stump side of a right leg (see Fig. 7E).
together or double anterior (AA) limbs. The latter are caused by two DV borders in anterior (competent) regions which meet each other at a corner of polarizing tissue (circles in Fig. 9A). Such AA limbs contain therefore P tissue and are expected to regenerate. This is in contrast to surgically produced symmetrical (AA) limbs (Fig. 5). Whether this prediction is true is not known. The experiment has not yet been done. AA limbs are relatively rare. As can be seen from comparison of Fig. 9A and Fig. 7B, we expect that slight variations in host–graft alignment or in the extension of the polarizing-competent zone will have a dramatic influence on the resulting intersections. Thus, our model accounts for the variability of the types of observed supernumeraries.

**Predictions of the model for ipsilateral rotations**

The assumption that a limb field is generated by the intersection of two borders has enabled us to account for the many patterns of supernumerary limbs. This may lead to the impression that the model could be adapted to predict any pattern. However, the model makes firm predictions which makes the model inherently testable. For 180° supernumeraries these predictions are:

(i) A supernumerary which grows out at the posterior host side has the opposite handedness of the host (Fig. 7B, F).

(ii) A supernumerary limb which grows out at the anterior host side has host handedness (Fig. 7B, E).

(iii) In supernumeraries with normal A–P polarity but changing DV polarity, the anterior part has the expected handedness as listed under (i) and (ii). In the posterior part, it is the reverse (Fig. 8).

(iv) Partial symmetric limbs can occur in the anterior or in the posterior part of a supernumerary limb. If the posterior part is symmetric, the anterior part should have a handedness as listed under (i) and (ii). If the anterior part is symmetric, the posterior part should have the opposite handedness as listed under (i) and (ii).

The predictions (ii)–(iv) are fully supported by Maden’s observation. Unexpected from prediction (i) is that single supernumeraries with host handedness can grow out at any location (including posterior). Maybe in these cases a stump regeneration takes place independent of a host–graft interaction (see the accompanying paper of Maden, 1983a).

The model predicts further that AA and PP limbs are not just reciprocal pattern. In PP limbs we expect that both halves have frequently less digits than normal. Due to the overlap of the two gradient systems, the lowest concentration and thus the anterior-most digits could be missing (see also Fig. 3B). In contrast, we expect that AA limbs comprise, as the rule, two almost complete limbs which are fused at their posterior side. This is because AA-limbs need a wedge of P-tissue for their creation (Fig. 9). Whenever the number of P-cells is high enough, the maximum concentration and therefore the most posterior structures will be formed. Low concentrations result automatically. The published figures
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of AA and PP legs seem to support this prediction (see Fig. 9C and 7D).

Frequently, fewer supernumerary limbs grow out than expected from the model proposed. An intersection of the two borders seems to be only a precondition for limb formation but other factors appear to be involved. A model for the proper proximodistal pattern formation is provided in a subsequent paper (Meinhardt, 1983a).

Pattern formation in the chicken wing bud

Another well-investigated model system of vertebrate limb development is the chicken wing. There, a small nest of mesodermal cells, the so-called zone of polarizing activity (ZPA) is located at the posterior margin of the bud (Gasseling & Saunders, 1964). Upon transplantation, these cells behave as a source of a morphogenetic substance which organizes the A–P axis of the wing bud (Tickle et al. 1975). In terms of our model, it could be that in chickens, the confrontation between A and P tissue does not lead to morphogen production directly but to the induction of the source region, the ZPA, which, in turn, produce the morphogen. Further experiments suggest (Wolpert, Lewis & Summerbell, 1975) that the ZPA is only effective as morphogen source if transplanted close to the apical ectodermal ridge (AER). In our model, the AER represents the DV border and again, an intersection of two borders has to be present for limb field formation.

The cells can maintain their developmental program even after removal of the ZPA (Fallon & Crosby, 1975) or after their separation from the ZPA by a barrier (Summerbell, 1979). Thus, the interpretation of the morphogen gradient seems to proceed in the same stepwise irreversible fashion that we deduced from the insect system (Meinhardt, 1977, 1978, 1982a,b): the cells are stepwise 'promoted' from more anterior determination to more posterior determination until the actual determination corresponds to the local morphogen concentration. Once it is obtained a cell determination is stable in the sense that it does not relapse towards more anterior determinations after removal of the morphogen. However, it is labile in the sense that cells can obtain more posterior determinations after an increase of the morphogen. The latter happens, for instance, after the implantation of a ZPA at the anterior margin of a wing bud and the induction of additional posterior digits. After a very early removal of the ZPA one might also expect that the newly formed confrontation between A and P cells induces a new ZPA. Then, the normal pattern would be maintained by a regeneration of the positional information and not by the maintenance of the already achieved cell determination.

Developmental malformations

From the model we expect the formation of symmetrical or supernumerary limbs whenever additional A, P, D or V stripes are formed. This would lead to new intersections and thus to additional outgrowth. Our model makes specific predictions about the number, the handedness and orientation of supernumerary
limbs. The predictions agree with the malformations most frequently observed. To see what types of errors can occur in the formation of AP and DV stripes, one has to realize which types of interaction can give rise to the normal AP or DV pattern. We have shown (Meinhardt & Gierer, 1980; Meinhardt, 1982a) that stable sequences of different determinations (for instance, ...A, P, ...) are formed if the different determinations locally exclude each other but, on long range, mutually support each other and depend on this support. A stripe-like pattern of the different determinations is especially stable since the long common boundary between two different determinations enables a very effective mutual stabilization. Since the different determinations locally compete with each other, the long-range activation of adjacent determinations may be realized by a long-range selfinhibition since, in competing systems, a selflimitation of one system is equivalent to a support of the competitor.

This mechanism is inherently endangered because some of the cells of a particular specification, let us say, some of the P-cells may become respecified into the determination of adjacent stripe, in our example, into A cells. Very unspecific stimuli (e.g. lowering of the selfinhibition or closing of intracellular junctions, reducing in this way the range of the mutual support) or minor genetic variation can lead to such a destabilization. This respecification will take place preferentially at a distance from the AP boundary since at the boundary the mutual support is optimal.

A single new patch of A-cells in a P environment can lead to two new AP-DV intersections (Fig. 10) and thus to two supernumerary outgrowths. From the model we expect that the three limbs are formed in a plane (along the DV border). Further we predict that the most anterior and the most posterior limbs have the handedness expected at this position while the central limb is of opposite handedness. The model thus provides a rationale for Bateson's rule (1894). This is independent of whether an A-P or P-A respecification has caused the supernumeraries (Fig. 10B, C). Due to the asymmetry of the limb field, we expect that the two more posteriorly located outgrowths are frequently fused and form a double posterior (PP leg). Fig. 10E shows a spontaneously arisen triplication in *Rana*.

The sharpness of our prediction may be further illustrated by listing which pattern is not expected: A PP leg in front of a normal leg is very unlikely (even if such an arrangement could be compatible with Bateson's rule of alternating handedness and, as shown below, PP legs alone are expected from the model). If a fused leg appears anterior to a normal leg, it must be of the AA-type (similar to that shown in Fig. 9C).

If a patch of new P cells appears at the anterior margin of the competent A region, a symmetrical PP limb is expected (Fig. 10D). Then the same PAP pattern appears as that produced experimentally by Slack in his graft experiments (Fig. 3B). In chickens a mutation *symmetric* is known which leads to PP limbs with high frequency (Fig. 10F, drawn after Landauer, 1956). Maden
Fig. 10. Teratogenic formation of supernumerary limbs. (A) Normal limb field (see Fig. 4). (B, D) Very unspecific stimuli can lead to a lowering of the mutual stabilization of A and P tissue. This can lead to a switch of some P cells into A cells (A* in (B)) or vice versa (P* in (C)) at a distance from the existing boundary (see Meinhardt, 1982a). Two new intersections would be formed. Three limbs are therefore expected. Due to the asymmetry of the limb field, the two posterior most limbs are likely to be fused (PP-limbs). (D) If A–P respecified cells, P*, touch the limits of the competent A region, only one new intersection is formed which would lead to a PP-limb. (E–G) Biological examples: (E) *Rana temporaria* with an additional PP arm (drawn after an X-ray photograph of Rahmann, Engels & Grüter, 1962), (F) a symmetric PP wing in a fowl carrying the mutant *symmetric* (after Landauer, 1956) and (G) a human arm of the PP-type (after Bateson, 1894).

(1983b) has found that when amputated animals of the species *Rana temporaria* are treated with vitamin A, the regenerating leg is frequently of the PP type. He interpreted his result in a similar way.

Extra digits (and missing digits) could result if the positional information is essentially normal but the response of the cells towards this positional information is changed. Explanations of these malformations require a model for the
interpretation of positional information which accounts especially for the periodic arrangement of similar but not identical structures. This is beyond the scope of the present paper (see Meinhardt, 1982a, b).

Open questions

Despite the successful explanation of so many phenomena by a simple hypothesis, some questions remain open. One problem is how the formation of the AP stripes is coupled to the primary embryonic pattern formation. In early axolotl embryos, the width of each stripe corresponds to the extension of two to three somites (Slack, 1976). Either several somites give rise to a particular stripe or the stripes are the more primary structure which become subsequently subdivided into several somites or both structures are formed independently under the control of the primary positional information.

Relation of the boundary model to the polar coordinate model (PCM)

A requirement for two intersecting borders is equivalent to a requirement for a common point of three sectors or four quadrants. In terms of the PCM (French et al. 1976; Bryant et al. 1981), we require for vertebrate limbs a ‘complete circle’ of four positional values (or, better, of two pairs of positional values). Since the handedness is independent of whether 3, 4 or 12 positional values are involved, our molecularly feasible model makes the same prediction with respect to the handedness as the formal PCM. In another respect, our model is very different. In the PCM, the complete circle of positional values is the fine structure. In our model positional information is created at the boundaries separating different positional values. The interpretation of this positional information, i.e. the generation of the limb’s fine structure is a secondary process. Thus, distal transformation can take place even if some fine structures are missing as long as an intersection of the boundaries remains present (as it is the case in PP legs, Fig. 5).

According to the PCM, only local cell–cell interaction are involved (Bryant et al. 1981). We expect that the interactions at the boundaries are more or less local processes, but that the resulting products have a long range. Long-ranging influences have been demonstrated by Honig (1981). Our model handles correctly experimental observations which are difficult to understand under the PCM. For instance, from the PCM, one would expect that supernumerary limbs produced after 90° rotation result from an incomplete set of positional values. Thus, they are expected to be frequently distally incomplete and have in the AP dimension fewer structures than normal. This is in contrast to the distally complete and very complex supernumeraries observed experimentally after such operation (Wallace & Watson, 1979). According to our model, intersections occur already after 90° rotations (Fig. 9) and we expect complete outgrowth. Further, the DV polarity change in 180° supernumeraries is very unexpected under the PCM since one would expect intercalation at these pattern discontinuities (Maden, 1982). In our
model, DV confrontations are nothing special but occur also in normal limb fields (they are even necessary for their creation, Fig. 4 and 6). The abnormality only results from the unusual (diagonal) type of DV confrontations in these legs (Fig. 7 and 8).

**CONCLUSIONS**

The straightforward hypothesis that positional information for secondary embryonic fields is generated at particular pre-formed boundaries has enabled us to account for many observations, including formation of limbs at a particular position in a defined orientation and with a particular handedness as well as the formation of supernumerary limbs with diverse abnormalities of the AP or DV axis. The asymmetry in the regeneration of double anterior or double posterior limb blastemas becomes understandable. The observed intimate coupling between the AP and the PD axes (see following paper; Meinhardt, 1983a) is a consequence of our model. The model we propose requires only standard biochemical interactions. The postulated cooperation in the morphogen synthesis could result from co-factors produced by the different cell types.

The primary positional information must be generated by a different mechanism since in the fertilized egg presumably no borders are present. Primary pattern formation can be explained assuming local autocatalysis and long-range inhibition (Gierer & Meinhardt, 1972; Meinhardt, 1982a). The same mechanism is presumably also involved in the determination of multiple periodic substructures such as feathers or leaves.

The boundary mechanism seems to be widely used in the generation of secondary embryonic fields: in the generation of appendages in as distantly related organisms as insects (Meinhardt, 1980a, 1983b) and vertebrates as well as in the generation of positional information in parts of the brain (the diencephalon polarizes the optic tectum, see Chung & Cooke, 1975).

The mechanism we propose for pattern formation in secondary embryonic fields accounts for the high reliability of embryonic development. By interpretation of a primary positional information, areas with different determination emerges which are separated by borders. At these borders, in turn, positional information is generated for the next finer developmental subdivisions, and so on. The determination of the location of a structure and generation of positional information for the fine structure is accomplished by one and the same process. Thus, relatively simple molecular interactions may be responsible for the chain of pattern-forming events occurring during development of an embryo.

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