A bootstrap model for the proximodistal pattern formation in vertebrate limbs

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SUMMARY

For the sequential determination of proximodistal structures during the outgrowth of vertebrate limbs, a 'bootstrap'-mechanism is proposed: by increasing feedback of more distally determined cells onto the production of a morphogen at the apical ectodermal ridge a successive increase of the morphogen concentration is achieved during outgrowth. The model accounts for the formation of a progress-zone at the limb tip, for the correct regeneration after truncation, for the presence and absence of proximodistal intercalation after certain graft experiments in amphibian limbs, for the tendency with which distal structures form in proximal position after certain experimental manipulations and for the intimate coupling of the anteroposterior and the proximodistal axes.

INTRODUCTION

In the preceding paper (Meinhardt, 1983) we have shown that many experiments reported in the literature concerning limb development in vertebrates are explicable under the assumption that an intersection of two borders gives rise to a limb field. The first border separates the polarizing and the competent (P and A) zones. The second border separates dorsal and ventral (D and V) tissue and coincides with the apical ectodermal ridge (AER) in the future limb. A condition for limb outgrowth (i.e. the induction of an AER and of a new proximodistal axis) would be a DV-border in the competent region which is flanked on one side by polarizing tissue. This model predicts a strong coupling between the anteroposterior (AP) and the proximodistal (PD) axis of a limb: the formation of a new PD axis would be strongly correlated with the formation of the posteriormost structure of the AP pattern. The formation of supernumerary and reduplicated limbs has been discussed under this aspect in the preceding paper. The coupling between two perpendicular axes (circumferential versus PD) is also an essential element of the polar coordinate model (French, Bryant & Bryant, 1976; Bryant, French & Bryant, 1981) and is possibly the most important point procuring the models predictive success.

During normal development the proximodistal elements of a limb (e.g. humerus, ulna/radius, wrist etc.) are determined one after another during outgrowth.

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of the limb bud (Saunders, 1948, Fig. 1). Such sequential pattern formation in
time cannot be understood on the basis of a simple gradient generated, for
instance, by a constant morphogen production at the tip of the outgrowing limb
(e.g. at the AER) since newly added cells would be exposed to the same con-
centration that other cells have seen before. No new structures would emerge,
since the maximum morphogen concentration would remain the same during
outgrowth. Summerbell, Lewis & Wolpert (1973) have therefore proposed that
PD pattern formation does not proceed under control of a morphogen gradient
but that cells in a ‘progress-zone’ at the limb tip – possibly coupled to the number
of cell divisions – acquire more and more distal determinations while cells leaving
this progress zone are fixed in their determination. This model is adequate for
the description of chicken wing development, but it neglects the coupling be-
tween the AP and PD axes (Summerbell, 1974a) and it cannot account for
pattern regulation as observed in amphibian limbs (see below).

The differently determined cells which give rise to a limb field represent
essentially a two-dimensional pattern. The intersection of the two boundaries
determines the location at which proximodistal outgrowth should be initiated.
The question remains how the proper pattern formation along this new axis is
achieved. In the present paper, I would like to propose a mechanism for the
generation of positional information in outgrowing systems. The basic idea is
that due to a feedback by the previously determined PD structures onto the
strength of a source at the limb tip, the morphogen concentration at the tip
increases during outgrowth. Thus, a new structure will be added in the PD
dimension after a certain amount of outgrowth. The term ‘bootstrap’ is borrowed
from programming terminology and describes the initiation of a second, usually
more complex program by a primitive program. The second program can give
rise to a third program and so on. An outline of this model has been published
(Meinhardt, 1980, 1982).

The model

In extending the hypothesis of Saunders (1969) and Zwilling (1961) I would
like to make the following assumptions:

(i) A morphogen responsible for the PD axis is produced at the AP/DV
intersection or especially the apical ectodermal ridge (AER), the future limb tip.
The PD morphogen induces the structures in the underlying mesodermal cells.
Higher concentrations cause more distal determinations.

(ii) The mesodermal cells produce an apical ectodermal maintenance factor
(AEMF). This diffusible factor controls the PD-morphogen production of the
AER. More distally determined cells produce more AEMF.

(iii) Once obtained, a distal determination is irreversible in the sense that cells
do not change their determination after a decrease of the morphogen. If, how-
ever, the morphogen concentration (positional information) becomes higher
than that required for the original cell determination, a blastema is formed in
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which proliferation takes place and in which the cells obtain more distal determinations (distal transformation, Rose, 1962).

To see how such a system can form a sequential pattern during outgrowth, let us regard the situation at the beginning of limb development. At first the morphogen production is low since the source strength of the AER is low. Only the first elements of the proximodistal sequence can be determined, let us say 1 and 2. Due to its diffusion, the AEMF concentration is a measure of the average proximodistal determination. Since initially only the most proximal structures are laid down, the AEMF concentration is low. Due to preferential cell proliferation near the tip (the most distal cells existing at that stage, in the example cells of type 2) the AEMF concentration increases since, on average, the proportion of cells with more distal determination increases. This has the consequence that the PD-morphogen production of the AER increases until the subsequent element in the PD sequence becomes determined (type-3 cells). The next further element can be determined only after substantial proliferation of the cells bearing this new most distal determination since only then the AEMF concentration, which depends on the average PD level, is sufficiently increased to allow the subsequent step. Fig. 1 shows a computer simulation of this mechanism. This model predicts an AEMF increase towards more posterior structures. Zwilling (1974) has obtained direct experimental evidence for such an AEMF gradient. According to this model, pattern formation of the PD axis and the AP axis would be very similar. Both would depend on positional information and its interpretation (Wolpert, 1969).

DISCUSSION

The progress-zone

The bootstrap mechanism explains the formation of a progress zone. Only close to the AER can the morphogen concentration (positional information) be higher than the actual determination (positional value) and thus only here can distal transformation take place (Fig. 1). At larger distances from the tip, the morphogen concentration is low and the cells remain stable in their state of determination. (However, a treatment with vitamin A can lead to a reversion to more proximal determination, see Maden, 1982, 1983.)

Regeneration

The model describes correctly the regeneration of truncated limbs. After implantation of a new AER in chicken or simply by wound closure in amphibians, a new source area is formed at the tip. Its strength depends on the AEMF production and thus on the proximodistal (PD) level of the stump. In agreement with the model, the regeneration is independent of the age of a transplanted AER (Rubin & Saunders, 1972). Due to the overall growth of the
Fig. 1. Sequential pattern formation in the proximodistal (PD) dimension of vertebrate limbs. (A) After removal of the apical ectodermal ridge (AER) further outgrowth stops. (B) The later the AER is removed, the more distally complete is the resulting leg (Saunders, 1948; drawn after Summerbell, 1974b). (C–F) Model for the PD determination: A morphogen (m, ———) is produced at the distal tip of the limb, the AER. Its source strength is determined by the concentration of the diffusible Apical Ectodermal Maintenance Factor (AEMF, ——), produced by the mesodermal cells. More distal structures produce more AEMF. (C) In a small limb bud, the incipient gradient only allows the determination of few proximal structures, e.g. 1 and 2. The activity of corresponding 'control genes' is shown in the lower half of each subpicture. The step-like curve (P) is measure for the achieved determination, in terms of Wolpert (1969), the positional value. (For a more complete description of how a morphogen gradient can lead to the activation of particular genes, see Meinhardt, 1978, 1982.) With proliferation of the relatively more distal cells at the tip, the AEMF concentration and in turn, the morphogen concentration increases. Structure 3 is laid down (D) and so on until the complete sequence of structure is formed (E). (F) shows a superposition at different stages. The model provides a molecular realization of the progress-zone model of Summerbell et al. (1973). Only at the tip, the positional information can be high enough to allow a switch to a more distal determination. (This occurs if the positional information m is larger than the positional value P.) In the remaining limb, the positional information is low, and the cells remain stably in the state of determination they have once achieved (after Meinhardt, 1982).

Limb before amputation, the distalmost structures remaining in the stump are larger than during the corresponding stage of outgrowth. This has the consequence that the AEMF concentration and thus the morphogen production is higher than during the corresponding stage of outgrowth. Therefore, cells even at some distance from the AER become exposed to a higher morphogen concentration than the one which gave rise to their original determination. These cells would be distally transformed. Reprogramming these cells would be expected to result in formation of a regeneration blastema. But in principle, according to the model, regeneration is based on the same mechanism as the primary PD pattern formation.
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Presence and absence of intercalary regeneration

The model does not depend on an ‘averaging’ mechanism between different positional values as proposed by Maden (1977) but on a unidirectional ‘promotion’ mechanism where the degree of promotion is controlled by the local morphogen concentration. A critical test for such a model is whether it can account for the PD-intercalation of missing structures in amphibians (Fig. 2). To summarize the experimental results, let us call the elements of the PD sequence 1, 2… 7. If a distal part of a blastema (/67) is grafted to a proximal stump (123/) the missing elements (45) become intercalated and the complete sequence is restored (Pescitelli & Stocum, 1980). In contrast, if a larger limb blastema (/34567) is grafted onto a more distal level (123456/), the pattern discontinuity is not repaired (Stocum, 1975a,b; Iten & Bryant, 1975). This result is surprising since in both cases the same PD levels are confronted (3/6) or (6/3). This result indicates that whether intercalation takes place or not is not a strictly local decision. According to the model, after a graft of the type 123/67, proximal structures are located close to a source region. They are exposed to a high morphogen concentration and thus become distally transformed (Fig. 2). In agreement with this model is the observation of Pescitelli & Stocum (1980) that the intercalate is entirely stump derived. Since the distalizing morphogen spreads out from the tip, we expect that a normal tip can induce distal transformation in a double anterior (AA) stump despite the fact that an AA stump on its own would not show distal transformation (see fig. 5 of Meinhardt, 1983). In contrast, an AA tip should be unable to induce intercalation either in normal or in AA stumps because an AA tip would not contain an AP border. Thus it should be unable to produce the PD morphogen. These predictions are in full agreement with the observations of Stocum (1983). In the other case (123456/34567), the pattern discontinuity is far from the source region and no distal transformation can occur (Fig. 2). A discontinuity of the type 123456/34567 can also result from a vitamin A treatment (Maden, 1982, 1983) which is also not smoothed out by intercalation. In chickens, an intercalation after a 123/67 type graft is possible only at very young limb-bud stages (Kieny, 1964; Summerbell, 1977). In the model, the size a limb bud can have if intercalation is still to occur is determined by the range of the AER-produced morphogen. Thus, the model predicts a longer morphogen range in amphibians compared with that in chickens.

Developmental malformations

The proposed bootstrap mode of pattern formation for outgrowing systems has an inherent instability which can cause a premature formation of the distalmost structures. The result could be a discontinuity in the proximodistal sequence of structures. In the model, an increased concentration of the PD morphogen leads to more distal determinations which, in turn, cause an increased AEMF production
Fig. 2. Presence and absence of intercalation in amphibian limbs. Grafting a distal regeneration blastema on a stump at a proximal level ((A), operation 1) leads to formation of a complete limb (B, after Pescitelli & Stocum, 1980). According to the model, after this operation, the source at the limb tip is close to the stump (E). Stump cells are exposed to a higher morphogen concentration than that once required to reach their actual state of determination (m is larger than P, arrow). After their distal transformation, the gap is required (F, G). (C) In contrast, grafting a large fragment onto a stump at a distal level ((A), operation 2) leads only to a serial repetition of structures (after Stocum, 1975b) without intercalation of the pattern discontinuity between the distal radius/ulna and distal humerus (arrow). (D) The pattern expected on the basis of previous intercalation models (Maden, 1977). An additional radius and ulna (stipled) would be formed with reversed polarity, in contrast to the experimental result shown in (C). According to the model (H), the source is far away from the stump cells. The positional information is lower than the positional value (arrow in H). All cells remain stably in their state of determination (after Meinhardt, 1982).

and this leads to an increased morphogen concentration and so on. The situation is normally stable since an increased morphogen concentration can lead only locally (at the tip) to a more distal determination which has little effect on the average proximodistal limb determination and thus on the AEMF concentration. However, any reduction in the AEMF diffusion (the averaging) caused, for instance, by closing of intracellular junctions or by killed cells which no longer participate on substance exchange could lead to an AEMF accumulation close to the AER and thus to the unstable situation (for details and simulation see Meinhardt, 1982). This expected instability has its counterparts in experimental observations: distalmost structures are formed after grafting proximal blastemas
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to ectopic positions (Stocum, 1968). Discontinuities between proximal and distal structures occur after irradiation of limb buds (Wolpert, Tickle & Sampford, 1979) and after application of certain drugs (for instance, Thalidomide, see Merker, Nau & Neubert, 1980). These discontinuities are obviously not repaired by intercalation.

Spatial separation of two proximodistal axes

In secondary embryonic fields in which organizing regions are formed at the intersection of boundaries we expect that two such organizing regions can emerge very close together without competition arising between them. This is in agreement with the experimental observation. For instance, Slack (1977) found in reduplicated limbs a continuous spectrum of the separation of the two PD axes: ranging from a narrow duplication to a complete separation of the two limbs, each bearing, of course, its own PD axis. No indication of a dominance of one over the other can be seen. This is in contrast to primary embryonic pattern formation generated by autocatalysis and lateral inhibition (Gierer & Meinhardt, 1972; Meinhardt, 1982) where two organizing regions would compete with each other. This can be seen, for instance, in hydra where two heads can emerge only at a certain distance from each other (Hicklin, Hornbruch, Wolpert & Clarke, 1973).

In conclusion, the bootstrap model provides a simple mechanism for pattern formation in the proximodistal axis of vertebrate limbs. It could, in principle, be realized by two morphogenetic substances and a responsive genetic system. By minor changes of parameters, it accounts for different regulatory features such as those observed in limbs of the evolutionarily closely related birds and amphibians.

REFERENCES


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