

# Cooperation of Compartments for the Generation of Positional Information

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A mechanism is proposed for pattern formation in developmental subfields. In application to imaginal disks, the compartmentalization appears as a prerequisite for the generation of positional information in the proximo-distal dimension. Cooperation of three or four compartments in the production of a morphogen leads to a cone-shaped morphogen distribution, since a high production rate of the morphogen is possible only at the intersection of compartment borders. The local concentration of the cone-shaped distribution is a measure for the distance from this center and can be used as positional information. In agreement with the experimental observations, the model predicts that (i) the distalmost structures are formed at the intersection of compartment borders; (ii) distal transformation occurs whenever cells of all compartments come close to each other; (iii) distal transformation does not require a complete set of circumferential structures; (iv) mutants exist in which the positional information and not the response of the cells is altered and (v) no distal to proximal intercalation of missing leg segments occur. Regeneration and formation of supernumerary insect legs can be explained as well. Simple molecular reaction can account for this type of pattern formation. The "complete circle rule" of French, Bryant and Bryant (Science **193**, 969–981 (1976)) for distal transformation may be simplified by a "cooperation of compartments" rule.

## Introduction

The spatial pattern of an organism can be assumed to be specified by positional information and its interpretation [1, 2]. A primary morphogenetic gradient can accomplish a subdivision of an area into defined subareas. Further subdivisions require secondary gradients and their interpretation. A primary gradient may specify the sequence of the body segments while a secondary gradient can specify e.g. the sequence of legs. The generation of primary gradients are explainable on the basis of autocatalysis and lateral inhibition [3–5]. Secondary gradients may be generated in the same way. It is conceivable, however, that, once a primary subdivision has been achieved, the boundary region between at least two subareas (compartments) obtains special properties by a cooperation of these subareas, enabling the generation of secondary gradients which can be used as positional information. A corresponding mechanism will be proposed.

A type of subfield for which many experimental data are available are the imaginal disks of *Drosophila*. Most of the discussion of the model to be

proposed will be therefore restricted to this system. Two elements in the spatial differentiation of imaginal disks have received much attention during recent years: The progressive compartmentalization [6–8] on the one hand and the features of pattern regulation on the other, especially with respect to whether or not pieces of tissue regenerate distal structures. The rules for pattern regeneration have been successfully described in the formal polar coordinate model of French *et al.* [9]. However, there seems to be very little connection between these conceptions. Compartments are therefore not an element of the polar coordinate model. The impression that both processes are independent from each other presumably results from the observation that small fragments duplicate whereas larger fragments regenerate the missing structures but the borderline between regeneration and duplication appears to be independent of the compartment boundaries [10–13]. The orientation of the major compartment borders of the leg and the wing disk indicates a cartesian coordinate system while the regenerative behaviour of imaginal disk fragment suggests a polar coordinate systems [9]. Further suggestive evidence for a polar coordinate system is the circular arrangement of structures in the fate map. For instance, the primordia of leg segments are arranged in

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concentric rings. The distal structures, tarsi and claws are formed from the more central rings, the proximal structures such as coxa and femur are formed from the outer rings of the disk [14].

The analysis of the early insect development has suggested that the sequence of the body segments is specified by a graded morphogen distribution in the egg [5]. The model proposed provides a mechanism for the generation of a cone-shaped morphogen distribution in imaginal disks, leading to the circular arrangement of *e.g.* leg segment primordia in a straightforward manner. The initial subdivision of the imaginal disks into compartments is a prerequisite for this type of pattern formation and the model provides therefore a link between compartmentalization and pattern formation in the proximo-distal dimension (the third dimension in addition to the antero-posterior and the dorso-ventral extension of the blastoderm).

### The Model

As a possibility for the generation of positional information in a subpattern I propose that adjacent patches of differently determined cells (compartments) collaborate in the production of a morphogen which specifies positional information. Two cooperating compartments will form a ridge-like distribution of the morphogen along the compartment boundary (Fig. 1). If three or four compartments have to cooperate, a morphogen production is possible only at the center where cells of all compartmental specifications are close to each other. The intersection of the compartment borders becomes the source region of the morphogen. By diffusion and decay, a cone-shaped morphogen distribution is formed with the highest concentration at the intersection of the compartment borders. The local concentration is a measure for the distance from the intersection and can be used as positional information for the proximo-distal dimension. The most distal structures are formed at the intersection of the compartments and the interpretation of the cone-shaped morphogen distribution leads in a straightforward manner to the circular arrangement of structures. The cone-shaped distribution may be generated in two steps. By the cooperation of each pair of the major compartments two ridge-like distributions are formed and a product of both has then the cone-shaped distribution (Fig. 1).

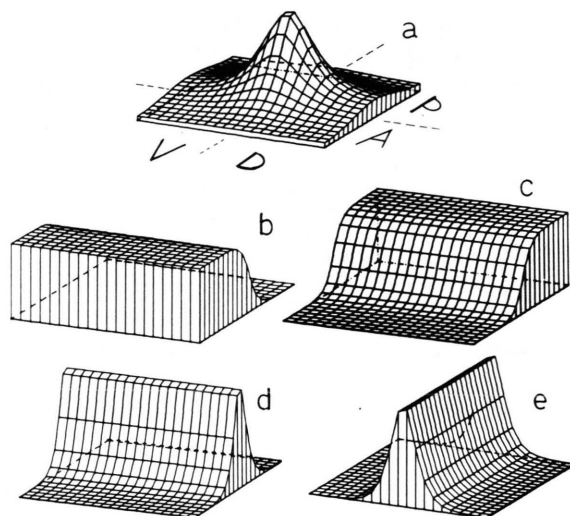


Fig. 1. Formation of a cone-shaped morphogen concentration by cooperation of compartments. The local concentration is a measure for the distance from the center and can be used as positional information (a). One possibility for its generation consists of the following steps: Two oppositely oriented gradients (b, c) are formed in a two-dimensional field by mutual activation of locally exclusive states [15]. The high concentrations could be the signal to form the anterior or posterior compartment of an imaginal disk. Cooperation requires the presence of both substances for the synthesis of a third substance (d) which is therefore synthesized only at the border, a ridge-like distribution emerges. To define a center, a similar system rotated by  $90^\circ$  is assumed (specifying, for instance, the dorsal and ventral compartments). The product of their cooperation is shown in (e). Cooperation of d) and e) leads to the cone-shaped morphogen distribution (a).

Molecular mechanisms for such a cooperation are easily constructed. For instance, each compartment may be responsible for a particular step in the synthesis of the morphogen or each compartment may produce a diffusible co-factor which is required for morphogen production.

In analogy with the unidirectional, stepwise interpretation of positional information in early insect embryogenesis [5, 16], distal determination, once obtained under the influence of the local morphogen concentration, is assumed to be irreversible. Furthermore the local morphogen concentration determines only for instance which leg segment a group of cell has to form. Details of the pattern *within* a segment is assumed to depend on a different process which is treated elsewhere [15].

The "complete circle rule" for distal transformation of French *et al.* [9] which is difficult to interpret

in molecular terms is simplified to the straightforward "cooperation of compartment" mechanism. The achievements of that model such as the explanation of supernumerary appendages remain valid.

## Discussion

The model links early compartmentalization and the generation of positional information for the proximo-distal dimension. The model stipulates two elements: the cooperation of compartments in the formation of a cone-shaped morphogen distribution, and the response of the cells in a stepwise, uni-directional manner.

### *Evidence for the cooperation of compartments in the generation of positional information*

1. The most distal structures, *e.g.* the wing tip [6, 7] or tarsus and claws of the leg [9] are formed at the intersections of the major compartment borders. These are not trivially the geometrical centers of the disk since the compartment borders are, as the rule, asymmetrically located.

2. Distal transformation of leg fragments require a close neighborhood of all compartmental specifications. As can be seen from experiments of Schubiger and Schubiger [17] and Strub [18] the upper lateral quarter of a leg disk fragment (Fig. 2 f) does not regenerate the removed distal primordia (center of the disk). It does not contain the ventral compartment. Similarly, the lower medial quarter (Fig. 2 b) contains the anterior-dorsal compartment only marginally and shows a low frequency of distal transformation. In contrast, a fragment which contains cells of all compartmental specifications shows distal transformations very frequently (Fig. 2 d).

3. A complete set of circumferential structures is not required for distal transformation. Distal transformation of leg disks and of the wing disk is possible without an initial regeneration of all proximal structures around the circumference [17, 19]. Schubiger and Schubiger, for instance, have found distal transformation in a fragment as shown in Fig. 3 d without an preceding circumferential regeneration of the missing proximal structures [17]. Our model is consistent with violations of the complete circle rule found experimentally since only a close neighbourhood of all major compartments is required.

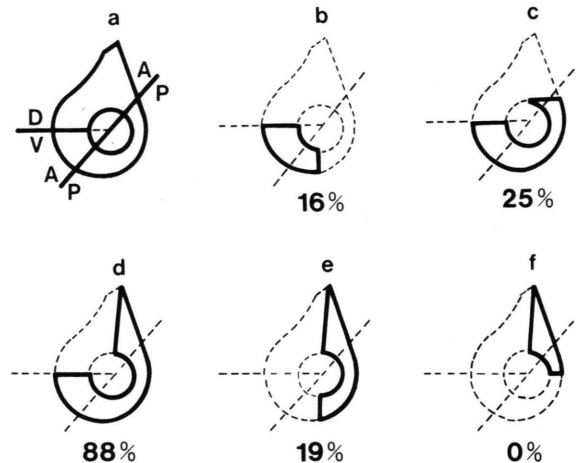


Fig. 2. Distal transformation occurs frequently if a leg fragment contains cells of all compartmental specifications. a: Leg disk with compartment borders according to Steiner [8]. The distal elements (tarsi and claws) are determined in the central part of the disk. (b–f) Frequency of distal regeneration of proximal leg fragments, according to Schubiger and Schubiger [17] and Strub [18]. The fragments b, c, e, f contain essentially only two compartmental specifications and show low frequency of distal transformation. In contrast, a fragment containing cells from all three compartments (d) frequently show distal transformation in agreement with the proposed model.

4. Distal regeneration of a fragment derived exclusively from the anterior leg compartment [13, 9] seems to contradict the model. However, compartment borders can be reformed during regeneration of fragments. According to Schubiger and Schubiger [17], distal transformation of an anterior fragment is always connected with the regeneration of structures from the posterior compartment. The reformation of new positional information for the proximo-distal dimension in such a fragment is assumed to be a two-step process. The first step is the regeneration of parts of the missing compartment(s). The second step is the formation of a new morphogen distribution, centered over the new intersection of compartment boundaries. In the polar coordinate model the ability of anterior leg fragment to regenerate the missing members of the major compartments is accounted for by a clustered assignment of positional values [9].

For the wing disk, the data are somewhat controversial to what extent the compartment borders can be transgressed [20, 21]. The antero-posterior compartment border seems to be more rigidly fixed

while the dorso-ventral compartment border is more labile [20]. From these data, it is expected that a wing fragment has to include the antero-posterior compartment border while the dorso-ventral specification is of less importance, in agreement with the experimental observations of Karlsson [19] and Wilcox and Smith [22].

5. Small marginal wing fragments usually do not show distal transformation by themselves [11, 12] because they contain at most cells of only two compartments. Two marginal wing fragments derived from opposite positions of the disk frequently show regeneration of the missing distal structures, since these fragments together, as the rule, contain cells from all major compartments. The same is valid also for an outer ring fragment of a disk [9, 24].

6. The abdomen, a structures without proximo-distal dimension is not compartmentalized [25]. According to the proposed view, subdivision into compartments is a condition for the generation of structures with a proximo-distal axis, for instance of wings and legs, and is therefore not required in the abdomen.

#### *Evidence for a morphogen and a stepwise unidirectional determination*

1. No distal to proximal intercalary regeneration occurs. Confrontation of proximal and distal fragments either of wing or of leg disks never leads to a respecification of distal segments into more proximal segment [26, 27], in agreement with the proposed model since a once obtained distal determination is assumed to be irreversible. (This is in sharp contrast to the distal-proximal regeneration within *e.g.* a leg segment [28, 29] and emphasizes once more that different mechanisms are involved in both types of pattern formation.)

2. Mutations are known which alter the positional information within the imaginal disks. If the positional information is formed by the cooperation of many cells, a mutant in which the positional information is altered should not be cell autonomous. *Drosophila* flies carrying the mutation *wingless* fail to form a wing blade. However, clones of *wingless* cells participate in wing formation [30]. According to the model, either the compartmentalization or the production of the morphogen by the cooperation of compartments may be affected.

Jürgens and Gateff [31] found partial and complete duplication of legs in a temperature-sensitive mutant of *Drosophila*. Mosaic studies revealed that both the mutant and the wild type cells participate in the formation of the duplicated leg and the authors concluded that the positional information and not the response to an unaltered gradient is changed. The duplications found are explainable under the assumption that a second dorsal compartment is formed at the ventral side of the disk (Fig. 3), leading to two points where all compartments are in touch with each other. The distance between the two centers would be decisive in how many elements are missing at the ventral side due to the overlap of the two gradients (Fig. 3).

3. Distally incomplete structures occur in the leg-duplicating mutant mentioned above [31] and in cockroach legs after an injury (see Fig. 4). Since only the local concentration of the morphogen is interpreted, distally incomplete structures are expected if the normal maximum concentration is not achieved. This can be caused by a restricted collaboration of the compartments, *e.g.* if too few cells of a particular compartmental specification are available or if they are not close enough.

#### *Application to pattern regulation in insect and vertebrate legs*

Many experiments have been done with cockroach legs [28, 29, 32, 33]. Nothing is known about

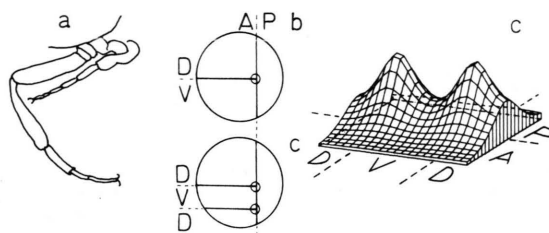


Fig. 3. a) Duplication of a leg of *Drosophila*, carrying the mutation *su(f)mad ts* (after Jürgens and Gateff, [31]). Either two complete legs (as drawn) or duplications of the distal parts are formed. b–d: Proposed explanation: assumed compartmentalization (b) in the normal and (c) in the mutant disk; a second dorsal compartment is formed at the ventral side, two points of confrontations (small circles) are present in which cell of all three major compartments are close to each other, generating there positional information for the proximo-distal axis. (d) Expected morphogen distribution. The separation of the two high points determines the overlap of the two gradients and therefore the level from which on duplications occur and the extent of proximal ventral structures which are missing.



compartmentalization in this system but as a working hypotheses, we will assume an analogous compartmentalization as in *Drosophila* [8, 34]. Under this assumption, the model describes also the regenerative behaviour of the leg. After amputation, cells of the three compartments come into close contact when the wound is closing. This leads to the formation of a new gradient which respecifies distal parts of the remaining structures into the missing structures. Leg regeneration can therefore be assumed to be a morphallactic process. The experiments of Bulliere [35] support this view, showing that during regeneration cell division starts only after the reformed segments are already clearly visible. The occurrence of supernumerary distal parts after incomplete wound healing [29, 33] can be explained in a similar way.

The cross-section of a cockroach leg has a triangular shape [32] with a long anterior and posterior side and a smaller ventral side. This form may reflect the anterior, posterior and ventral compartmentalization from which it has emerged. Bohn [33]

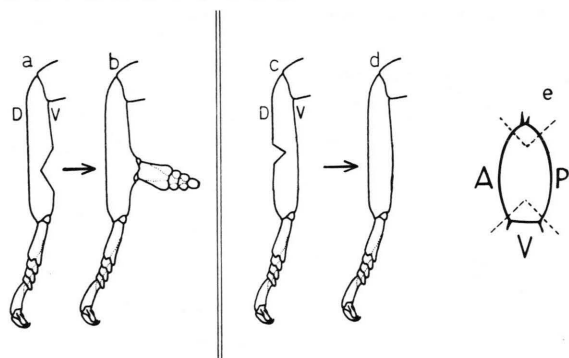


Fig. 4. Cutting a notch into the ventral (V) site of a cockroach leg (a, b) leads to outgrowth of additional leg-like structures [33] while (c, d) the same operation at the dorsal site (D) heals without much additional outgrowth. e: explanation based on "cooperation of compartments". A compartmentalization similar to that of the leg of *Drosophila* [8, 34] is assumed. The triangular cross-section of a leg [32] presumably reflects the original subdivision of the leg disk into three major compartments: anterior (A), posterior (P) and ventral (V). After removal of tissue at the ventral side, cells of anterior, posterior and ventral specifications become quite close to each other. Cooperation is possible and outgrowth of distal structures is expected on the basis of the proposed model. Since anterior and posterior cells could be separated by some ventral cells, the cooperation may be restricted and distally incomplete structures can be formed. After a similar incision at the dorsal site, ventral cells remain far away and cooperative of compartments is impossible.

has produced supernumerary appendages by cutting v-shaped notches into the ventral side of the leg (Fig. 4). In contrast, cutting on the dorsal side leads to very little, if any, outgrowth. In terms of the model, after an incision at the narrow ventral side, cells of the anterior, posterior and ventral compartment become quite close to each other. Therefore, distal regeneration is expected on the basis of the proposed mechanism. Since the anterior and posterior cells may be separated by some ventral cells, the cooperation may be restricted which accounts for the fact that the extra-appendages are usually distally incomplete. In contrast, after an incision at the dorsal site, only anterior and posterior cells come into touch with each other and the condition for generation of new positional information is not fulfilled.

Compartments have yet been found only in insect development. However, collaboration of patches of differently determined cells for the generation of new positional information is presumably also involved in the determination of vertebrate limbs. Slack [36–38] has found that a contact between a competent and a polarizing area is required for the generation of antero-posterior positional information in the limb buds of axolotls. The regeneration of double posterior limbs [39], the absence of regeneration in double anterior limbs [40] and the formation of supernumerary limbs after 180° rotation of a limb bud [41, 42] can be understood in terms of the model; details will be discussed elsewhere [43]. The proposed explanation becomes similar to that of the clock face model (9) if with respect to distal transformation only 3 or 4 elements around the circumference are assumed. However, cause and effect are different in both models. In the clock face model, initially all structures belonging to a particular proximo-distal level have to be formed if distal regeneration is to occur. In the model proposed, the confrontation of two cell types (competent and polarizing, see Slack [36]) creates the positional information for the antero-posterior dimension and the confrontation of four (or at least three) causes distal transformation. However, to what extent the interpretation of the antero-posterior pattern has proceeded or if structures thereof are missing is without importance for the occurrence of distal transformation. The proper pattern formation in the proximo-distal axis may be achieved by a progress-zone as proposed by Summerbell, Lewis and Wol-

pert [44], by lateral activation of locally exclusive states or by a feedback of the achieved determination onto the morphogen concentration [15].

## Conclusions

There are strong indication that the segments of the basic body pattern of insects are formed under the control of morphogenetic gradients [5, 45, 46]. With the proposed model, the developmental control of appendages becomes similar to that of the initial segmentation.

The positional specification around the circumference is much finer than that corresponding to the three or four compartments and is capable of inter-

calary regeneration [32]. We have shown, that intercalary regeneration can be explained by models based on lateral induction of locally exclusive neighbouring structures [15]. Such a mechanism could apply to intracompartamental specification around the circumference as well as to the intrasegmental specification along the proximo-distal axis. The question whether regeneration or duplication around the circumference is to occur is assumed to depend on this mechanism. However, the proximo-distal determination of segments themselves and the occurrence of distal transformation is explained by a gradient which is formed if, and only if, cells of the different compartments are close to each other.

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