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Models of biological pattern formation: common mechanism in plant and animal development

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ABSTRACT Earlier proposed models for primary pattern formation, for gene activation and for segmentation are summarized and compared with recent molecular-genetic observations. A model for head, foot, tentacle and bud formation in Hydra illustrates that complex patterns can be reliably generated. Stable cell determination requires autocatalytic (autoregulatory) genes. Segmentation in insects has been proposed to result from a reiteration of (at least three) cell states. Their patterning is achieved by a mutual activation of cell states that locally exclude each other. A model for accretion of new segments by proliferation at the posterior pole is proposed that accounts for the generation of a periodic and a sequential pattern in register with each other. The assumption of a process analogous to segmentation in plants can account for the initiation of leaves with an intrinsic polarity that eventually leads to the upper and lower leaf surfaces. The model accounts also for the formation of axillary buds in correct relation to a leaf and for the much smaller spacing of leaves within a whorl when compared with the spacing between two successive whorls along the shoot. It is concluded that the generation of complex structures in distantly related organisms may be based on similar mechanisms.

KEY WORDS: *pattern formation, models, segmentation, phyllotaxis, hydra, gene activation*

Introduction

A central issue in developmental biology is how the complex structure of a higher organisms is generated from a single cell in a reproducible way. Basic concepts, such as *positional information* (Wolpert, 1969) or the *embryonic organizer* (Spemann and Mangold, 1924) have been derived from experiments involving perturbations of normal development. From the observed regulatory phenomena one cannot directly deduce the molecular mechanism on which development is based. We have used such observations to develop specific models for different developmental situations. By computer simulation we have shown that the regulatory features of the models correspond closely to the experimental observations. In the meantime, several of these models have been directly confirmed by molecular-genetic investigations. In the present article, I would like to discuss some of these models and compare them with the observations on the molecular level.

Generation of primary organizing regions

A most striking feature of some developmental systems is their capability to generate patterns from a more ore less structure-less initial situation. For instance, in amphibians dissociation and re-aggregation of animal and vegetal cells and a subsequent co-culture leads to distinct organizing regions and dorsoventral patterning (Nieuwkoop, 1973, 1992) although any asymmetry imposed by the sperm entry is certainly wiped out by such a procedure. Similarly, in Hydra complete and normal organisms can be formed from dissociated and re-aggregated cells (Gierer *et al.*, 1972). So far, for none of the systems that are able to generate *de novo* patterns, the molecular basis has been worked out since genetic tools like those in *Drosophila* are not yet available.

Formation of patterns from almost homogeneous initial conditions is not unique to living systems. The formation of high sand dunes or of sharply contoured rivers are examples. Common in all these inorganic pattern formations is that small deviations from a homogeneous distribution have a strong feedback such that the deviations grow further. We have proposed that primary embryonic pattern formation is accomplished analogously by the coupling of a short range self-enhancing (autocatalytic) process with a long range reaction that acts antagonistically to the self-enhancement (Gierer and Meinhardt, 1972; Gierer, 1981; Meinhardt, 1982, 1992). A simple molecular realization would consist of an activator molecule whose autocatalysis is antagonized by a rapidly diffusing inhibitor. Figure 1 shows computer simulations demonstrating that the model accounts for the generation of elementary patterns frequently encountered in development. Depending on kinetics of the interaction and the ranges of the activator and the inhibitor molecules, the generation of graded distributions (Fig. 1A), of more

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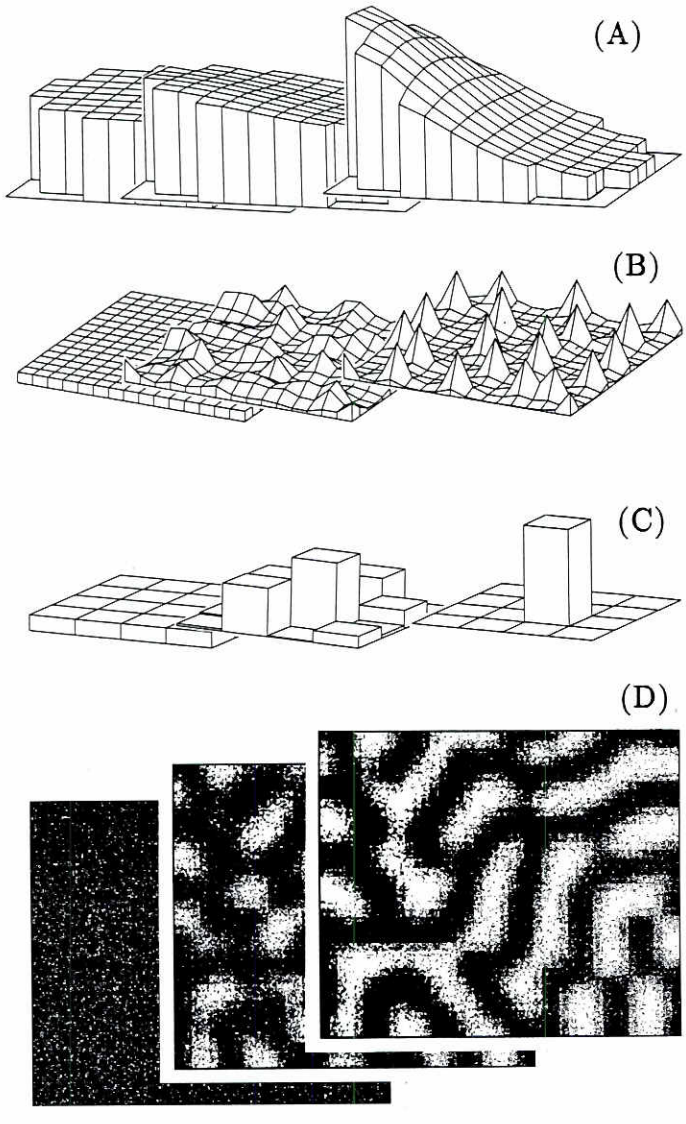

Fig. 1. Stages in the generation of elementary patterns by local self-enhancement and long ranging inhibition. *Shown are the initial, an intermediate and the final activator distributions.* **(A)** *Monotonic gradients are formed if the range of the activator is comparable to the size of the field. The pattern orients itself along the longest extension of the field.* **(B)** *A more or less regular arrangement of peaks results in fields that are large compared to the range of the inhibitor.* **(C)** *If the activator is non-diffusible and the range of the inhibitor is sufficiently large, only a single cell remains activated. This may be the mechanism that separates the oocyte and fifteen nurse cells during Drosophila oogenesis.* **(D)** *Stripe-like distributions result if the autocatalysis saturates (Meinhardt, 1989).*

or less regularly spaced peaks (Fig. 1B), of single activated cells in a non-activated surrounding (Fig. 1C) or of stripe-like distributions (Fig. 1D) are possible. For instance, a single source region at one boundary of the field and graded concentration profile emerges if the range of the activator is comparable to the size of the field. Such a distribution is convenient to provide positional information (Wolpert, 1969). Pre-existing asymmetries can orient the emerging pattern but unavoidable fluctuations or minute external perturbations (as demonstrated for brown algae *Fucus*, see Jaffe, 1968, Goodner and Quatrano, 1993) are sufficient for pattern initiation. The pattern is self-regulating and, except for the orientation, in a wide range independent of the mode of initiation. With other parameters, the same interaction can generate oscillation and travelling waves that play a role in other developmental systems (see Fig. 7).

The autocatalysis need not to be direct. It can result from a chain of interactions. For instance, a nucleus-restricted transcription factor controls the production of a small molecule. The latter is able to diffuse between cells and activates, in turn, the transcription factor. In *Xenopus* a corresponding system may be realized. The gene *goosecoid* becomes activated in those cells that form the Spemann-Organizer. Artificial activation of this transcription factor at the ventral side causes a second embryonic axis (Cho *et al.*, 1991). Similarly, by treatment of cells with activin, a small TGFß-related molecule (Smith and Harland, 1991; Christian and Moon, 1993), ventral cells also obtain organizing capability and the *goosecoid* gene becomes activated (Cho *et al.*, 1991). The missing link would be the demonstration that the activation of the *goosecoid* gene (or any other gene of that group) leads, in turn, to the synthesis of activin.

The best-known gradient system controlling gene activation in a position-dependent manner is the *bicoid* gradient in *Drosophila* (Driever and Nüsslein-Volhard, 1988a,b, 1989). Its generation appears to be quite different from the model proposed. The mRNA required for the proper morphogen, the *bicoid* protein, is produced by the nurse cells and deposited at the anterior pole of the oocyte. Its translation leads to the *bicoid* gradient: Gradient formation by a local source and diffusion. Such a mechanism, however, depends on a preceding patterning step. The fifteen nurse cells and the oocyte are derived from a single cell, the primordial germ cell. Although the number of cytoplasmatic bridges remaining between the sixteen cells are decisive of which cell can form the oocyte, two cells are in an identical situation and a choice has to be made between the two (see Sander, 1976). As shown in the simulation depicted in Figure 1C, the model accounts for the activation of a single cell in a non-activated surrounding. The condition is that the activator is non-diffusible and the range of the inhibitor is sufficiently large.

Many primary pattern systems regulate after an experimental interference. For instance, each fragment of a bisected sea urchin embryo may form a complete larva. The model accounts for this feature. For instance, after removal of the activated region, the remnant inhibitor decays until a new maximum is formed *via* autocatalysis, thus restoring the gradient. Similarly, an unspecific lowering of the inhibitor at the non-activated side may trigger the onset of a new activation that mimics the natural organizing region. The many unspecific procedures that cause a second embryonic axis in amphibians (Waddington *et al.*, 1936) may have ultimately this basis.

Reproducible generation of complex patterns: head, tentacle, bud and foot formation in Hydra as example

The complexity of the patterns in higher organisms requires a hierarchical linkage of many pattern forming reactions. One or more patterns generate the precondition for a subsequent pattern. For instance, by an appropriate coupling, two pattern form-

ing systems (anteroposterior, dorsoventral) can emerge perpendicular to each other (Meinhardt, 1989). The combinatorial possibilities are very large, making modelling very difficult. A model for patterning of the freshwater polyp Hydra (Fig. 2; Meinhardt, 1993) should illustrate that the theory provides a tool to understand complex regulatory phenomena as well as unexpected experimental details.

Regenerating fragments of Hydra always maintain the original polarity. The tissue is obviously not uniform. This can be accounted for by a feedback of a primary pattern (see Fig. 1A) on *the* source density, i.e., on the ability of the cells to perform the pattern forming reaction. Due to this feedback cells closer to the maximum will obtain a higher source density. After removal of the activated (organizing) region, the cells that were originally closest to the maximum have the highest source density, and thus an advantage in the competition to form the new maximum (Fig. 2B,C). In other words, a fragment regenerates according to the original polarity. The graded source density provides the required asymmetry. It has a long time constant and remains essentially unchanged during regeneration. Regeneration can be fast since no time-consuming competition is required as to which group of cell will form the regenerating head.

Hydra is under control of two organizing regions, the head and the foot. This is a common feature of many morphogenetic fields. Planarians are another example (Chandebois, 1976). How can it be achieved that two structures reliably appear at opposite positions, for instance, during regeneration? For Hydra a simple cross-inhibition is not appropriate since in small (young) animals, this would lead to the suppression of a foot *by the near*-by head or vice versa. We have shown that the spacing between the head and foot sy*stem* must be achieved via the source density. As mentioned above, the head activation appears at the position of the highest source density. If the foot system has the opposite behavior and causes a lowering of the source density, the foot appears at the lowest source density and thus at the maximum distance from the head (Fig. 2A). Nevertheless, head and foot system can coexist at a close neighborhood in small animals since no direct inhibition is involved. The graded source density only generates a preference.

Many structures emerge during development close to each other in a precise arrangement. We have shown that a controlled neighborhood of structures is enforced if one structure activates the other on long range but excludes it locally (Meinhardt and Gierer, 1980). In Hydra, the tentacles appear around the hypostome. Many experiments can be accounted for by the assumption that tentacles are under control of a separate activator-inhibitor system that also depends on the source density. Since the source density increases under the influence of the primary head system, the latter generates the precondition for tentacle initiation. Locally, however, the head signal suppresses tentacle formation. Thus, tentacle formation is possible only at a sub-hypostomal position. (Fig. 2A,D).

The model accounts for a strange-appearing observation. With tentacle-specific antibodies Bode *et al.* (1988) have shown that after head removal, tentacle activation first reappears at the very tip of the gastric column. It is only later that this activation becomes shifted to the position where the tentacles eventually appear. In terms of the model, the tentacle inhibitor can have a short half life (since the maxima appear close together, the tentacle inhibitor need not to diffuse very far). After head and tentacle removal, the tentacle inhibitor fades away faster than the head inhibitor. Therefore, the tentacle activator can reappear

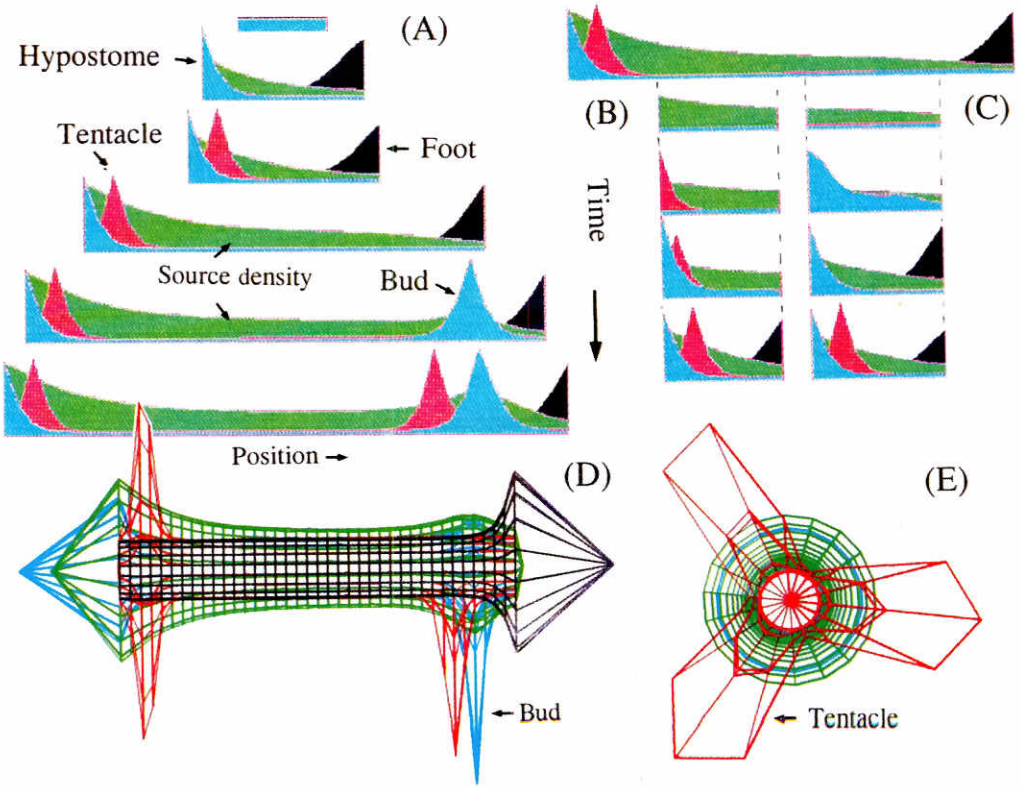

Fig. 2. Simulation of hypostome, tentacle, bud and foot formation in Hydra. (A) *Primary head (blue) and foot activation (black) appear at opposite end of the field due a coupling via the source density (green). Tentacle activation (red) appears close to the hypostome since it requires a high source density but is locally suppressed by head activation. Budding results from a second head activation. Due to the long-ranging head inhibitor, this can occur only at a large distance from the original head.* **(B)** *In regenerating near-head fragments tentacle activation precedes head activation. Tentacle activation emerges first at the tip and shifts later on, in agreement with the experimental observations.* **(C)** *In more basal fragments head activation appears first and tentacle activation takes place at the final position.* **(D,E)** *Lateral and top view of a Hydra simulated as a cylinder: the periodic arrangement of the tentacles (red) around the hypostome and the lateral localization of the bud (blue) is correctly described (after Meinhardt, 1993).*

sooner than the head activator. Since no suppressing head activator is present, this happens at the highest possible source density, i.e., at the tip (Fig. 2B). After the trigger of the primary head activation, tentacle activation becomes shifted to the final position. The prediction that the sequence of events is the reverse in a more basal fragment (Fig. 2C or in buds (Fig. 2A) has meanwhile found direct experimental support (Technau and Holstein, 1995).

An analogous pattern formation takes place in plants. Secondary structures such as leaves or flower elements (petals, sepals etc.) are formed at regular distances from a dominating region, the primary meristem, and they keep distance from each other. Frequently they are arranged in whorls like the tentacles in Hydra (see Coen and Meyerowitz, 1991). We expect that basically similar mechanisms are at work: one pattern forming system generates the primary meristem, a second one the periodic structures. The activation of the second system is restricted to the narrow zone that surrounds the primary system (see also Fig. 6).

Cell determination requires autocatalytic genes

Signals generated by diffusible molecules are necessarily transient since the communication between different parts in the enlarging tissue would require more and more time. At an appropriate developmental stage, the cells have to make use of position-specific signals, i.e. they become determined for a particular pathway by activating particular genes. Afterwards the cells may maintain this determination even if the evoking signal is no longer present. The activation of a particular gene has formal similarities with the formation of a pattern in space. In pattern formation, a morphogenetic substance has to be produced at a particular location but this production must be suppressed at other locations. Correspondingly, determination requires the activation of a particular gene and the suppression of the alternative genes of a given developmental situation. Based on this analogy I have predicted that genes exist that have a non-linear autocatalytic feedback on their own transcription (Meinhardt, 1978, 1982). In addition, genes responsible for alternative pathways compete with each other such that only one of the alternative genes can be active within a cell. Fig. 3A shows a reaction scheme.

Meanwhile, many genes with autocatalytic properties (autoregulation) have been found. Examples are the genes *engrailed* (Condie and Brower, 1989), *even-skipped* (Jiang *et al.*, 1991), *fushi tarazu* (Schier and Gehring, 1992), *twist* (Leptin, 1991) and *Deformed* (Regulski *et al.*, 1991). Examples for autoregulatory plant genes are *deficiens* and *globosa* (Zachgo *et al.*, 1995). Based on this autoregulation, a short activation of the Deformed gene under heat-shock control is sufficient for a long-lasting activation of this gene (Kuziora and McGinnis, 1988). On theoretical grounds it is expected that the autocatalysis is non-linear. This can result from a dimerization of the activating molecules or by multiple binding sites on the DNA. The *Deformed* gene is an example for the latter possibility. Taking together, the predicted principle, i.e., the maintenance of the determined state by feedback of a gene on its own activity combined with a repression of alternative genes has turned out to be a general mechanism to generate stable determined states.

Several possibilities exist for a coupling between the gene switching system and the morphogen gradient. For instance, at lower concentrations the morphogen has an activating, at higher concentrations an inhibiting influence on the gene activation. This leads to an optimum morphogen concentration for gene activation that is different for different genes. Depending on the local concentration different genes win the mutual competition. Although the gradient is shallow, regions of particular gene activation emerge that are separated by sharp boundaries (Fig. 3C). This sharpness does not result from the precision by which the morphogen concentration can be measured by a cell but has its origin in the non-linear self-activation and mutual repression of the genes, allowing only one gene of the set to be active in a given cell. If a gene is missing due to a mutation, the neighboring genes expand their territory of activation since the competitor is absent (Fig. 3D), a feature frequently observed in position-dependent gene regulation.

Alternatively, under the influence of the morphogen the cells switch from one activated gene to the next. The number of steps depends on the morphogen concentration. Due to the autocatalysis the cells would remain in a once achieved state even when the morphogen is removed later on. Such a dynamics has been recently observed by Gurdon *et al.* (1975) for the activation of

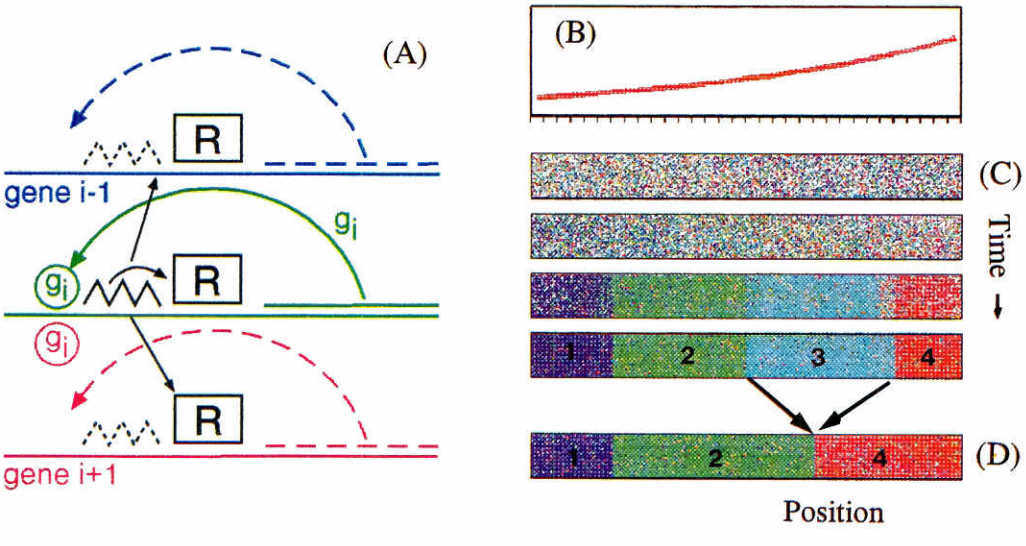

Fig. 3. (A) *Proposed reaction scheme. The gene products have a feedback on the activation of their own genes. They compete with alternative genes either directly or by a common repressor R.* **(B-D)** *Space-dependent gene activation under control of a morphogen gradient. Gene activation is indicated by the density of coloured dots (like an autoradiography). The morphogen has an activating and, at high concentrations, an inhibitory influence on gene activation. Different genes have an optimum at different concentrations. Depending on local morphogen concentration, sharply confined regions arise in which gene 1, 2, 3 or 4 is active.* **(D)** *Gene 3 is missing due to a mutation. Gene 2 and 4 expand their territory.*

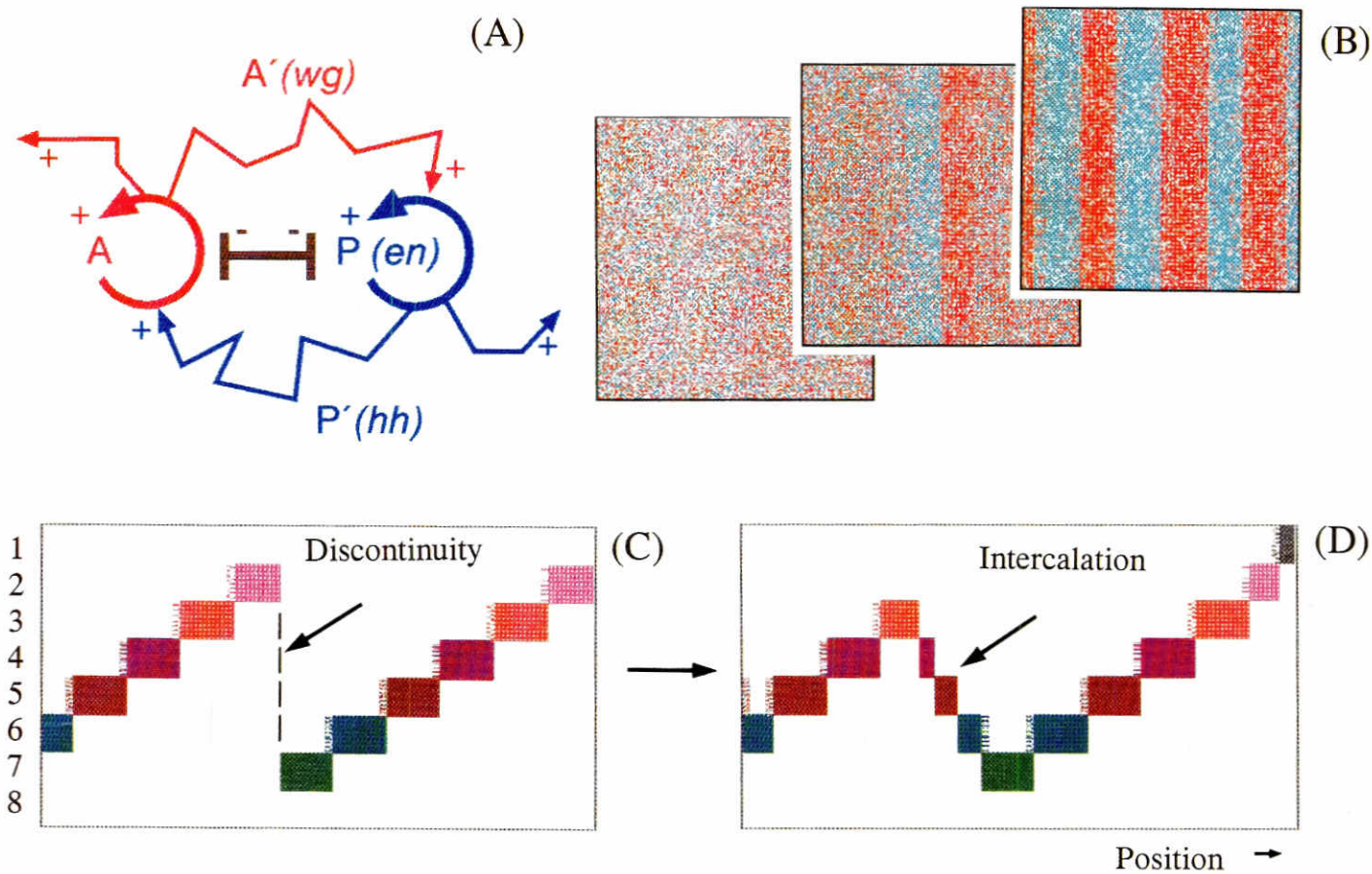

Fig. 4. Segmentation by mutual long range activation and short range exclusion of cell states. (A) *The proposed reaction scheme correspond closely to genes identified in Drosophila.* **(B)** *Generation of stripes by this mechanism.* **(C,D)** *Intercalation: an artificial discontinuity in a sequence of cell states becomes repaired. Not the normal pattern but the correct neighbourhood of structures becomes restored. The activity of a particular gene is indicated by the colour density; for computational details, see Meinhardt and Gierer, 1980).*

Xbra-gene under the influence of *activin*. As predicted (Meinhardt, 1978, 1982a), the activated gene remains stable even after transfer into morphogen-free medium but a later increase of the morphogen leads to the activation of a higher gene.

Segmentation: mutual activation of cell states that locally exclude each other

Many biological systems suggest that the subject of regulation is not the natural pattern but the correct neighborhood of structures. The pattern within insect segments is a well-investigated example (Locke, 1959; Bohn, 1971). For instance, if the natural pattern could be described by a sequence of the structures 12345....9, a graft of the type 1234567+3456789 leads to the intercalation of structures *654* such that the discontinuity 7/3 is removed: 12345676543456789. The polarity reversal in the intercalary regenerate is frequently visible by a reversal of the orientation of hairs, bristles, etc.

In earlier works pattern formation within the body or leg segments of insects has been assumed to be under control of sawtooth-shaped gradients (Lawrence, 1973). However, the formation of graded distributions with a discontinuity that also shows the correct regulatory behaviour is difficult (or perhaps impossible) to achieve by molecular realistic interactions. In contrast, we have proposed that segmentation results primarily from *qualitative* and not from *quantitative* differences (Meinhardt and Gierer, 1980). According to this view, the internal pattern of a segment consists of a sequence of different cell states. As mentioned above, stable cell states are generated by self-activation and mutual competition of genes. If two (or more) such states not only exclude each other locally but activate each other over longer ranges, these cell states depend on a close neighborhood. The local exclusiveness assures that the two states do not merge.

According to this model, segmentation requires the following molecular ingredients (Fig. 4A): (i) genes (or more general feedback loops) must exist that have a direct or indirect positive feedback on their own activation. (ii) These activities are locally exclusive; only one of the alternative genes can be active in a given cell. (iii) Long ranging molecules provide a mutual activation of those cell states that eventually become neighbors. Each cell state in a given cell depends on the help from different cell states in neighboring regions.

Recent molecular-genetic investigations (see Ingham, 1991) have provided direct support for this scheme (Fig. 4A). As mentioned, *engrailed (en)*, a key gene for segmentation, is autocat-

alytic. It activates a neighboring cell state *via* a diffusible molecule *hedgehog (hh)* (Ingham and Hidalgo, 1993). This cell state is characterized by the activation of the gene *wingless (wg)*. The wg protein can also diffuse into neighboring cells (Baker, 1987; van den Heuvel *et al.,* 1989) and stabilizes *en*. As expected from the theory, the *en* gene activity requires a functional *wg* gene in its neighborhood and *vice versa*, although both genes are transcribed in non-overlapping regions. The prediction of such a complex molecular interaction by a theory could hardly be more precise. From the theory we would expect that the wingless gene is under transcriptional control of a second (directly or indirectly) autocatalytic gene. The gene *cuD* (Eaton and Kornberg, 1990) on which *wingless*-expression depends is perhaps a part of the missing system.

It is of obvious importance for *Drosophila* segmentation is that this type of pattern formation can generate stripe-like distributions of high activation (Fig. 4B). Long common borders between different cell states allows a most effective mutual support. At least three members are required to generate a sequence of cell states with an internal polarity. This led to the prediction (Meinhardt, 1982) that in addition to the anterior *(A)* and posterior compartment *(P)* in the early *Drosophila* embryo at least one additional element must be present. Now it is generally assumed that each of the four founder cell of a (para)segment represent a different cell state (Ingham, 1991). If more than two cell states are involved, the mutual help can be cyclic. There is no need for a discontinuity. By computer simulations we have shown that the resulting sequences of cell states are self-regulating. Missing structures become intercalated, if necessary with polarity reversal (Fig. 4C,D), in full agreement with the experimental observation (Bohn, 1971).

Formation of a precise number of different segments during terminal outgrowth

For the segmentation of *Drosophila*, I have proposed a hierarchical model for the linkage of maternal positional information, and the activation of the gap, pair-rule and segment polarity genes (Meinhardt, 1986a). The model accounts for the basic phenotypes of embryonic lethal mutations (Nüsslein-Volhard and Wieschaus, 1980). Crucial in the model is that the positional information for the hierarchically lower level is generated at the borders between different gap- or pair-rule genes, in agreement with more recent observations (Stanojevic *et al.,* 1989; Pankratz *et al.,* 1990).

The simultaneous formation of the segments by a cascade of pattern forming events, such as occurring in *Drosophila* is a later evolutionary invention. In lower arthropods, annelids, and in short germ insects, such as grasshoppers (Fig. 5A-C), a sequential addition of segments takes place during outgrowth at the posterior end of the embryo until the correct number is reached. The sequential formation of the 32 segments of the leech is another example.

The lateral activation scheme accounts for the addition of segments during localized cell proliferation. Let us assume, as mentioned above, that segmentation results from the reiteration of three cell states, *S, A,* and *P* (the minimum number to have a polar structure). By proliferation at the posterior end, cells of the same specificity are added. Whenever, for instance, too much *A* cells have been formed, at a distance from the *S/A* border the support of the *A* cells by the *S* cells will become low, but the support of the *P* state by the many *A* cells will be high and the most posterior cells will switch from the *A* state to the *P* state and so on. The result will be a periodic ...*P/SAP/SA*.. pattern (Fig. 5B). The *P/S* confrontation is assumed to generate a segment border while the *A/P* confrontation acts as the precondition to form appendages (see below)

In the leech, more than the final 32 segments are initially formed. A few surplus segments are later removed by programmed cell death (Fernandez and Stent, 1982; Shankland, 1991). The number $32=2^5$ may suggest a digital counting mechanism. This is misleading since, for instance, the polychaete *Clymenelly torquata* has 22 segments. It regenerates removed segments such that the number of 22 segments will be restored independent of the number of segments removed (Moment, 1951; Goss, 1969). These observations suggest some sort of counting mechanism. Its molecular basis is not yet clear. From the phenotypes of mutations in the *bithorax* complex (Lewis, 1978) one can deduce that the formation of the periodic pattern is the primary event and that the genes responsible for segment identity are under its control (Meinhardt, 1982b). The simulation in Figure 5B demonstrates the generation of a sequential pattern of selector gene activation *(1,2,3...)* that is precisely in register with periodic reiteration of cell states (parasegmental PSA pattern). The periodic alternation between the cell states at the growing posterior end is used in a mechanism analogous to the escape-mechanism of a grandfather's clock. There, the periodic movement of the pendulum controls the switch mechanism that leads to a sequential advancement of the pointer in a controlled manner. In terms of gene switching, the transition from one gene to the next is *prepared* in one state *(P)* but the actual transition is blocked. In another state *(A)*, the transition takes place but no further transition is prepared. Thus, with each full parasegmental *PSA* cycle, there will be one and only one transition from one gene that controls segmental identity to the next. Recent experiments suggest that the periodic pattern generated during outgrowth can but need not is accomplished on the level of the pair rule genes (Sommer and Tautz, 1993; Patel *et al.,* 1994).

Elements of such a counting mechanism may still be present in the *Drosophila* genome. Gyurkovics *et al.* (1990) found a dominant mutation (deletion) causing a transformation of parasegment 11 in parasegment 12. In terms of the model, the deletion of a region on the DNA that is involved in block of the transition from one gene to the next will cause premature transition. The loss of that region on one chromosome would be sufficient. Thus, it is expected that such a loss leads to a dominant mutation, as observed. Such a counting by a stop and go mechanism, driven by a cyclic alternation between two or tree cell states, may provide a rationale why the selector genes are frequently arranged on the chromosome in the same order as the corresponding pattern in the real organism.

The initiation of legs and wings at the intersection of compartment (differentiation) borders

A higher organism is much to complex to be generated by a single morphogenetic gradient. Based on his experiments with limb initiation in axolotl, Harrison (1918, 1921) has proposed that first cells are set aside that will form eventually the future the

limb. At later stages an anteroposterior and subsequently a dorsoventral axis becomes determined in these cells. This model for the organization of secondary embryonic fields, assuming first a homogeneous patch of founder cells and a subsequent sequential patterning along both axes, was very influential over many decades.

In an attempt to perform corresponding computer simulations it has turned out that such a scheme is difficult to realize. To avoid several problems I have proposed that borders between different cell determinations become new organizing regions for the initiation of substructures such as legs and wings (Meinhardt, 1980, 1983a,b, 1986b). As discussed below in detail, this model has found meanwhile direct experimental support (see also Vincent and Laurence, 1994; Martin, 1995).

Let us first regard only one axis. Imagine that a primary pattern forming process leads to a subdivision into several discrete regions by region-specific gene activation (see Figs. 3 and 4). Among them are the adjacent regions *A* and *P*. If, for instance, in the *P* region a co-factor is produced that is required in the *A* cells to produce a new morphogen, its synthesis is restricted to a position close to the *A/P* border. The concentration of this morphogen provides a measure for the distance from the border and is therefore suitable for the internal organization of the *A* and the *P* region. Although the positional information is symmetric, the resulting pattern can be asymmetric since *A* and *P* cells can respond differently. In the extreme case, only one cell type may respond at all.

A border that separates two cell types along the anteroposterior axis surrounds an embryo in a belt-like fashion. To determine the position of a limb along this line the cooperation of another pair of cell types is required. This restricts secondary fields to regions around the intersection of two borders. Assuming that the embryo has a cylindrical shape, any reasonable subdivision of the embryo along the anteroposterior and the dorsoventral axis leads to intersections that occur in pairs, one at the right and one at the left side of the embryo. They have opposite handedness, a feature of obvious importance for the formation of legs, wings, eyes etc. Many classical observations of insect and vertebrate appendages become explicable under this assumption (Meinhardt 1983a,b).

The model unifies the generation of cartesian and of polar coordinate systems in secondary fields. On the one hand, it provides information for the distance from two orthogonal lines. On the other, a measure for the radial (proximodistal) distance from the centre, the point of intersection, is available. The four quadrants or three sectors provide a coarse information about the angular position. Therefore, the model provides a molecular feasible basis for the formal polar coordinate model (French *et al.*, 1976). The complete circle rule of the polar coordinate model is to be substituted by a complete compartment rule. While the polar coordinate model accounts only for pattern regulation after perturbations of an existing structure, the boundary model describes in addition the initiation of these structures during early development.

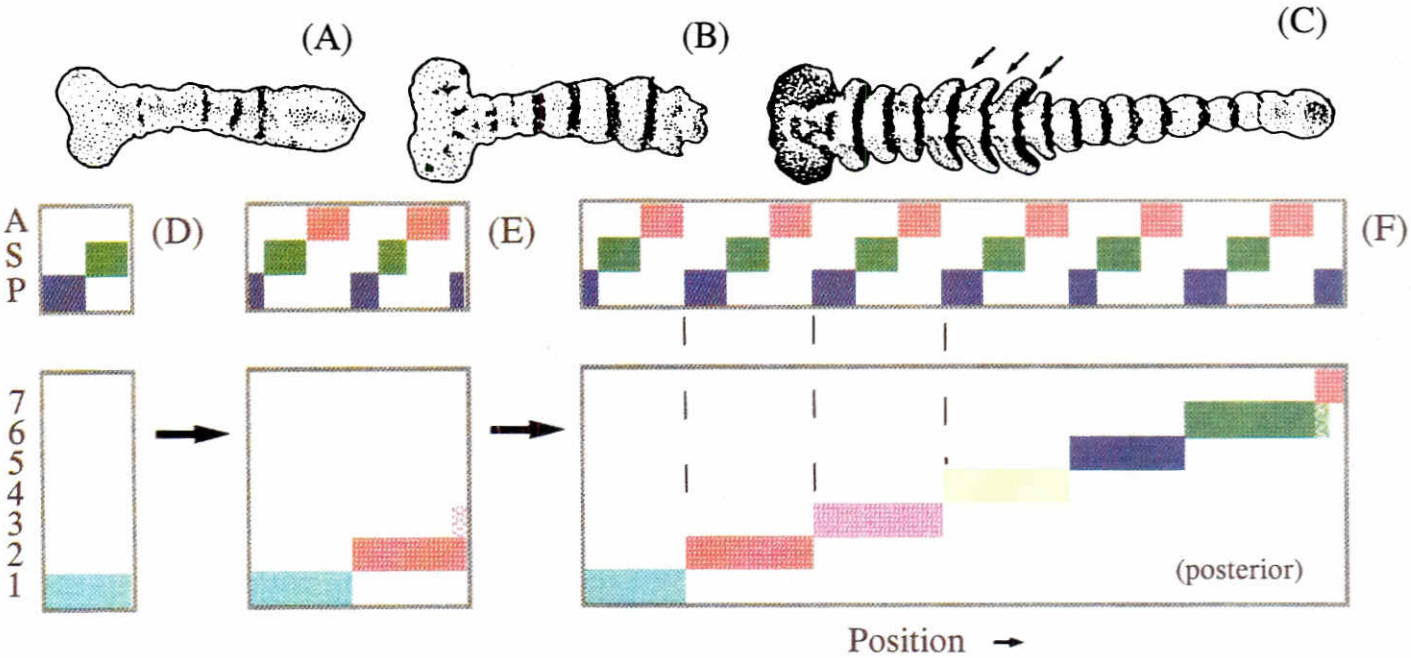

Fig. 5. Formation of a periodic and sequential pattern in register by marginal growth. (A-C) *Stages in the development of a grasshopper embryo. At the posterior end, cells proliferate at a high rate. In the course of development, more and more segments are added. In the posterior part of each segment the gene engrailed (black stripes) is transcribed (Patel et al., 1989). The segments are different from each other. Legs (arrows) are formed only in the three thoracic segments at the anterior/posterior compartment border (see Meinhardt, 1983a, 1986b).* **(D-E)** *Model: By addition of cells at the posterior side (right), the A, S or P regions become enlarged until the subsequent cell state becomes activated. A periodic pattern with polarity results. Further, if cells are in the P-stage, the subsequent element of the sequential pattern (1, 2..) becomes activated but the transition its blocked. Only after switch from the P to the A state, this block is released and the activation of the subsequent gene takes place. The resulting 1, 2, 3... pattern is in precise register with the parasegmental ../PSA/PSA/.. pattern. The generation of the periodic pattern may occur on the level of the pair-rule or of the segment polarity genes.*

In *Drosophila*, the genes *engrailed* and *wingless* are required for the initiation of leg and wing disks and their internal patterning. These genes belong to the class segment polarity genes (Nüsslein-Volhard and Wieschaus, 1980) and become activated during segment determination. Therefore, the prediction that the formation of the border precedes formation of secondary fields is at least for the A-P border clearly satisfied.

The gene *decapentaplegic (dpp)* is required for the formation of the proximodistal axis in wings and legs. The *dpp* gene can only be activated in the *wingless*-region. Required for this is the *hedgehog* protein, produced in the neighboring *engrailed* region Thus, the *dpp* gene becomes activated in a narrow stripe in the anterior compartment along the *A/P* compartment border (Posakony *et al.*, 1991). In other words, a cooperation of the *en* and the *wg* region is required for *dpp* activation, in agreement with the model proposed. Moreover, the same molecule that stabilizes the *wingless* gene (see Fig. 4) is also involved in the cooperation of the two compartments to produce precondition for the proximodistal axis. An artificial activation of the hedgehog gene in the anterior compartment leads to ectopic *dpp* activation (Basler and Struhl, 1994). If (and only if) this occurs close to the D-V border a complete additional proximodistal axis is formed, in complete agreement with the prediction.

Also most of the ingredients for the cooperation across the dorsoventral border are known. At the dorsal but not at the ventral side of the future wing blade the gene *apterous* is expressed (Cohen *et al.*, 1992; Diaz-Benjumea and Cohen, 1993). A border of *apterous* expressing and non-expressing cells is the precondition to activate *fringe* (Irvine and Wieschaus, 1994; Kim *et al.*, 1995) and *vestigal* (Williams *et al.*, 1994), The two genes are required for the generation of the proximodistal axis. This suggest two genes, *vestigal* and *decapentaplegic*, that are specifically activated at two orthogonal borders have to cooperate to generate the proximodistal axis proper, in full agreement with the model proposed.

The boundary model and the initiation of vertebrate limbs

In developing vertebrate limbs, the zone of polarizing activity exhibits features of a local morphogen source (Tickle *et al.*, 1975). However, a local source by itself would lead to a concentric fate map while the digits are arranged in a plane. This discrepancy can be resolved by assuming that two intersecting borders are required for limb initiation (Meinhardt, 1983b) The digits can only appear in the competent A-region along a DV-border that determines the position of the apical ectodermal ridge. The boundary model accounts for many classical observations, including that of Harrison mentioned above.

Experimentally two locally exclusive homeobox genes, XlHbox 1 and Hox 5.2 with a common border have been observed (Oliver *et al.*, 1989). In the early limb bud stage this border is located at the same position as the ZPA. Even the signalling molecule is preserved. The product of a gene homologous to *hedgehog*, *sonic hedgehog*, fulfils all requirements for the corresponding morphogen (Riddle *et al.*, 1993). Also a first indication for a D-V border has been found. A molecule related to *wingless*, wnt-7a is expressed only in the dorsal but not in the ventral ectoderm of the limb bud. A related molecule, wnt-5a is restricted to the apical ectodermal ridge (Dealy *et al.*, 1993). In their distribution these molecules correspond to the *apterous* and *vestigal* molecules mentioned above.

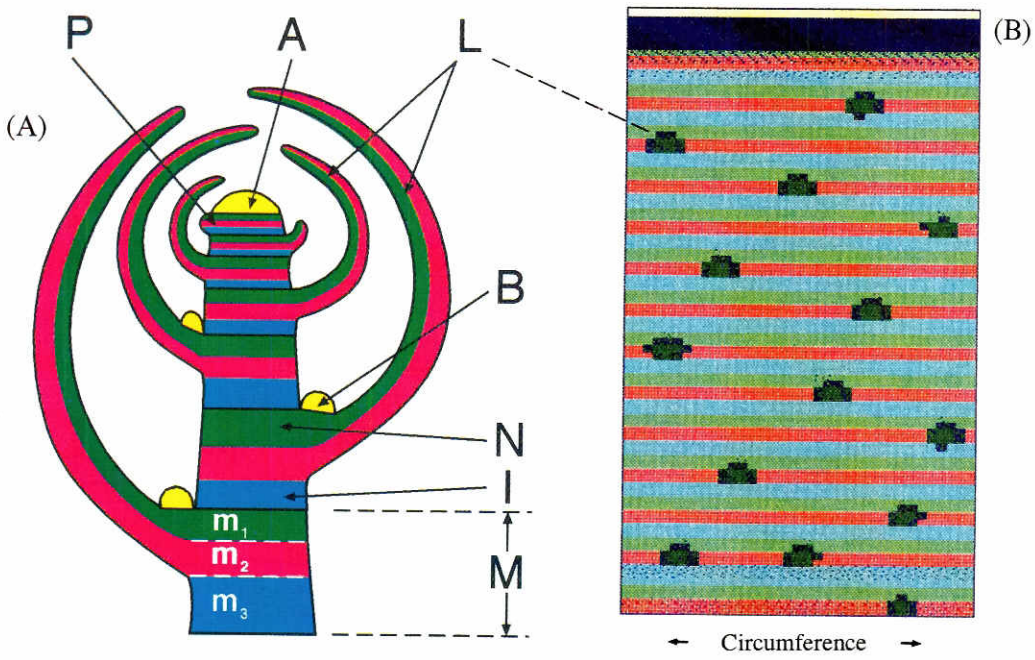

Fig. 6. The modular construction of a plant and its simulation. (A) *Schematic cross-section through the growing tip of a shoot. The apical shoot meristem A is a tissue in which rapid cell division occurs. At its periphery the primordia P appear that will grow into leaves L. Axillary buds B differentiate somewhat later, in proximity of a leaf. The shoot can be regarded as a periodic repetition of an "elementary module" M formed by a node N and internode I region. Every nodal-internodal segment bears a leaf L and an axillary bud B (see Lyndon, 1990). In our model it is assumed that each module M results from the iteration of (at least three) subunits, m_1, m_2 and m_3. It obtains in this way an intrinsic polarity.* **(B)** *Simulation of plant growth. The stem of the plant is idealized as a cylinder that is represented here unwrapped. The apical meristem A contributes to the stem elongation by addition on new cells. These differentiate so as to produce the repetitive sequence ...m_1, m_2, m_3, m_1... (... green, red, blue, green, ...). The m_1/m_2 border acts as a precondition for the activator - inhibitor system causing leaf initiation. Due to the lateral inhibition they are placed along spirals with a 2/3 phyllotaxis (the azimuthal distance between two successive primordia is approximately equal to 2/3 of the stem perimeter (after Koch and Meinhardt, 1994). The m_1 (green) region may correspond to the region of expression of the phantastica gene in Antirrhinum majus (Waites and Hudson, 1995)*

Segmentation in plants and the formation of upper and lower surfaces of leaves

Segmentation, the reiteration of polar units along the body axis, is usually regarded to be involved only in animal development. In contrast, the spacing of leaves is mostly assumed to result from a long-ranging inhibitory effect of one leaf primordia onto the formation of the subsequent primordia (Schoute, 1913). However, there are several features in leaf initiation that cannot be explained by such a simple spacing model. Shortly after initiation the polar structure of leaves becomes obvious and the leaves become flat. The upper and lower surfaces obtain distinctly different features. This polarity is always correctly oriented in respect to the axis of the growing shoot. Moreover, in many plants axillary buds are initiated close to a leaf at a position pointing towards the tip of the shoot. How is this achieved? In most models of phyllotaxis, these features are not considered.

Recently, we have shown that a mechanism analogous to segmentation in animals would resolve these problems (Koch and Meinhardt, 1994). In the simulation shown in Figure 6 it is assumed that during outgrowth a periodic sequence of (at least three) cell states is generated, to be called m_1, m_2, and m_3 (see also Fig. 5). They are arranged like belts around the shoot. The leaf primordium is generated by an activator-inhibitor mechanism as described above but an additional condition is a particular border, for instance, m_1/m_2. The resulting leaves necessarily consist of two different tissue types, m_1 and m_2, and both cell types have necessarily the correct orientation in respect to the apical meristem. The correct initiation of the axillary bud can be easily integrated into this model by assuming that the bud-inducing signal consists of the leaf signal plus a m_1 specification.

The restriction of leaf initiation to a differentiation border accounts in addition for several features that remain unsolved in other models of phyllotaxis. For instance, many plants form whorls. In whorls, the individual leaves (see also Fig. 2E) have a small distance from each other around the circumference while the distance between the whorls can be large. Thus, the different spacing cannot result from a simple lateral inhibition mechanism. According to the mechanism outlined above the leaves can only appear along the m_1/m_2 border. This determines where a whorl can be initiated. The distance from one whorl to the next is given by the repeat length of the nodal organization of the shoot, the $...m_3/m_1,m_2,m_3/m_1...$ pattern. In contrast, the spacing of the leaves within the whorls is given by the range of the inhibition, and this can be very short.

In monocotyledons, the width of a leaf may be a large fraction of the circumference of the stem while it has only a small thickness. A signal generated by an activator-inhibitor mechanism would have a more or less circular shape. According to the boundary model however, the thickness is given by the leaf formation mechanism at the border while the extension around the circumference depends on the pattern forming system. Since both processes are independent, the different extensions in both directions are easily described.

Many plants form leaves that consists of leaflets along a central stem. The acacia is an example. According to the model, the m_1/m_2 border would be maintained in the outgrowing leaf stem. New signals (activator maxima) can be generated along the stem on the m_1/m_2 border. Therefore, the leaflets necessarily appear in the same plane as a leaf would be formed.

Recently, Waites and Hudson (1995) described the gene *phantastica* in *Antirrhinum majus* that is required for dorsoventrality of leaves. In its absence, outgrowth still takes place but needles are formed instead of leaves. They proposed an early dorsoventral subdivision shortly after the determination of the primordial leaf cells. In our view, the sequence of events is the reverse. The activation of *phantastica* corresponds to the activation of the m_1 belt and is expected to precede leaf initiation. This sequence corresponds to the primary formation of the anterior/posterior compartment border in insects that precedes the formation of imaginal disks. The similarity to the insect system is especially striking in short germ insects with their zone of proliferation at the posterior pole (corresponding to the apical meristem): one *A/P* border appears after the other and their orientation is perpendicular to the direction of growth (see Fig. 5). Different in both systems is the actual positioning of the initiation site along the crucial border. According to our view, in leaves this is determined by the autocatalysis/lateral inhibition mechanism. As described above, in imaginal disks this is accomplished by the intersection with a second border at a particular dorsoventral position. Therefore, while leaves may appear in a spiral arrangement (Fig. 6), the imaginal disks emerge at a particular dorsoventral level.

The model proposed is related to the node-internode concept (Lyndon, 1990) according to which the leaves are derived only from the nodal regions. Different plants use different strategies to generate this periodic pattern. While in *Sambucus* a single cell layer gives rise to the one or the other structure (Zobel, 1989a,b), in *Silene* four layers of cells are associated with each leaf, two form the nodal and two the internodal cells (see Lyndon, 1990). The main difference of the model we propose is that an alternation of three elements is required and that the polar character of one of the resulting boundaries is used to generate a polar structure of the leaf, analogous to the compartment borders that generate the precondition for a polar limb.

Patterns on tropical sea shells

The pigment pattern on tropical sea shells represent a very exceptional but very interesting patterning system. A mollusc can enlarge its shell only at the growing edge. The two-dimensional pigment pattern represent therefore a time record of a one-dimensional process. Since these patterns are obviously without functional significance, an incredible variety of pattern has emerged during diversification of the species. Nature was able to play. We have shown that the basic patterns, lines perpendicular, parallel or oblique to the direction of growth can be accounted for by the same mechanism as described above: by autocatalysis coupled with an antagonistic reaction (Meinhardt and Klinger, 1987). The main difference to the normal pattern formation during embryogenesis is that also non-stationary patterns, i.e., oscillations, short bursts and travelling waves play an important role. For instance, in the time record travelling waves of pigment production in the shell-producing mantle gland at the growing edge give rise oblique lines on the shell. All these

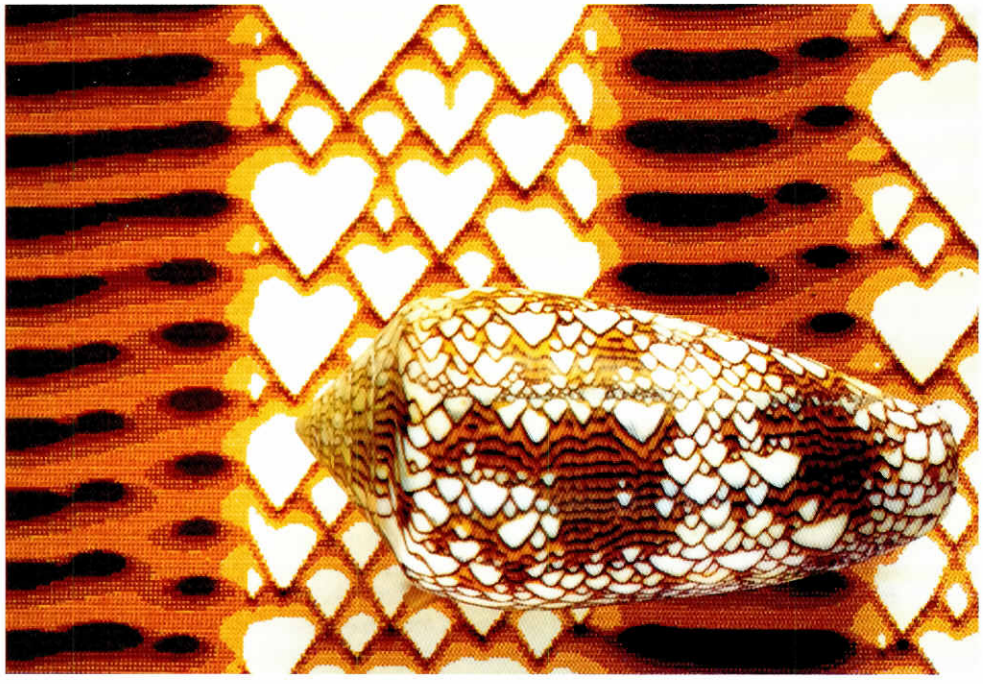

Fig. 7. Shell of *Conus textile*. *In the computer simulation, a pigmentation reaction is assumed that is enhanced by a second oscillating reaction. If the pigment reaction is in a steady state (regions with light brown background pigmentation), the oscillating system causes dark parallel lines. If, however, the supply of a substrate that is required for the pigmentation reaction is somewhat lower, only travelling waves are possible (oblique lines on non-pigmented background). In this regions, the enhancing reaction elongates pigment production such that backwards waves can be triggered. On the shell this leads to branching of these lines. Thus, according to the model, the dark parallel lines and the branching have the same origin. (For details and computer programs see Meinhardt, 1995).*

modes can be realized by the same basic mechanism and require only modifications in the time or diffusion constants.

More recently, a modelling of the more complex shell patterns has also been achieved (Meinhardt, 1995). They result from the modification of the pigment producing system by at least one other time-dependent system that has an enhancing and/or an extinguishing influence on the pigment system. As an example, Figure 7 provides a shell of *Conus textile* in front of a simulation. Very different patterns can be generated that fits the natural patterns rather closely by minor modifications of the underlying mechanism or even by using different parameters. The shell patterns are an example that complex patterning is hardly understandable by cloning the involved genes only since these are expected to be the same in all species.

Conclusion

Relatively simple molecular interactions can account for pattern formation during the development of higher organisms. The postulated main steps include the generation of positional information by a system of short range autocatalysis and long range inhibition and, under its control, the regional activation of different genes at particular locations. Segmentation requires cell states that locally exclude but on long range activate each other. This generates a self-regulating neighborhood. Differently determined cell types cooperate in the generation of new positional information at their borders at which, in turn, new positional information is generated. Thus, a cascade of simple molecular interactions allows reliable pattern formation in an iterative way. Closely related mechanisms may be involved in animals and plants.

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References

BAKER, N.E. (1987). Molecular cloning of sequences from wingless, a segment polarity gene in *Drosophila*: the spatial distribution of a transcript in embryos. *EMBO J. 6:* 1765-1774.

BASLER, K. and STRUHL, G. (1994). Compartment boundaries and the control of *Drosophila* limb pattern by *hedgehog* protein. *Nature 368:* 208-214.

DIAZ-BENJUMEA, F.J. and COHEN, S.M., (1993) Interaction between dorsal and ventral cells in the imaginal disc directs wing development in *Drosophila. Cell 75:* 741-752

BODE, P.M., AWAD, T.A., KOIZUMI, O., NAKASHIMA, Y., GRIMMELIKHUIJZEN, C.J.P. and BODE, H.R. (1988). Development of the two-part pattern during regeneration of the head in hydra. *Development 102:* 223-235.

BOHN, H. (1971). Interkalare Regeneration und segmentale Gradienten bei den Extremitäten von Leucophaea-Larven (Blattari). III. Die Herkunft des interkalaren Regenerats. *W. Roux Arch. 167:* 209-221.

CHANDEBOIS, R. (1976). *Histogenesis and Morphogenesis in Planarian Regeneration.* Monographs in Developmental Biology, Vol. XI. Karger, Basel.

CHO, K.W.Y., BLUMBERG, B., STEINBEISSER, H. and DEROBERTIS, E.M. (1991). Molecular nature of Spemann's organizer – the role of the *Xenopus* homeobox gene goosecoid. *Cell 67:* 1111-1120.

CHRISTIAN, J.L. and MOON, R.T. (1993). Interactions between xwnt-8 and Spemann organizer signaling pathways generate dorsoventral pattern in the embryonic mesoderm of *Xenopus. Genes Dev.* 7 13-28.

COEN, E.S. and MEYEROWITZ, E.M. (1991). The war of whorls: genetic interactions controlling flower development. *Nature 353:* 31-37.

COHEN, B., McGUFFIN, E.M., PFEIFLE, C., SEGAL, D. and COHEN, S.M. (1992). apterous, a gene required for imaginal disc development in *Drosophila* encodes a member of the LIM family of developmental regulatory proteins. *Genes Dev. 6:* 715-729.

CONDIE, J.M. and BROWER, D.L. (1989). Allelic interactions at the *engrailed* locus of *Drosophila* - engrailed protein expression in imaginal disks. *Dev. Biol. 135:* 31-42.

DEALY, C.N., ROTH, A., FERRARI, D., BROWN, A.M.C. and KOSHER, R.A. (1993). Wnt-5a and wnt-7a are expressed in the developing chick limb bud in a manner suggesting roles in pattern-formation along the proximodistal and dorsoventral axes. *Mech. Dev. 43:* 175-186.

DRIEVER, W. and NÜSSLEIN-VOLHARD, C. (1988a). A gradient of bicoid protein in *Drosophila* embryos. *Cell 54:* 83-93.

DRIEVER, W. and NÜSSLEIN-VOLHARD, C. (1988b). The bicoid protein determines position in the *Drosophila* embryo in a concentration dependent manner. *Cell 54:* 95-104.

DRIEVER, W. and NÜSSLEIN-VOLHARD, C. (1989). The *bicoid* protein is a positive regulator of hunchback transcription in the early *Drosophila* embryo. *Nature 337:* 138-143.

EATON, S. and KORNBERG, T. (1990). Repression of *cubitus interruptus Dominant* expression in the posterior compartment by *engrailed*. *Genes Dev. 4:* 1074-1083.

FERNANDEZ, J. and STENT, G.S. (1982). Embryonic development of the hirudinid leech *Hirudo medicinalis:* structure, development and segmentation of the germinal plate. *J. Embryol. Exp. Morphol. 72:* 71-96.

FRENCH, V., BRYANT, P.J. and BRYANT, S.V. (1976). Pattern regulation in epimorphic fields. *Science 193:* 969-981.

GIERER, A. (1981). Generation of biological patterns and form: some physical, mathematical, and logical aspects. *Prog. Biophys. Mol. Biol. 37:* 1-47.

GIERER, A. and MEINHARDT, H. (1972). A theory of biological pattern formation. *Kybernetik 12:* 30-39.

GIERER, A., BERKING, S., BODE, H., DAVID, C.N., FLICK, K., HANSMANN, G., SCHALLER, H. and TRENKNER, E. (1972). Regeneration of hydra from reaggregated cells. *Nature New Biol. 239:* 98-101.

GOODNER, B. and QUATRANO, R.S. (1993). Fucus embryogenesis – a model to study the establishment of polarity. *Plant Cell 5:* 1471-1481.

GOSS, J. (1969). *Principles of Regeneration.* Academic Press, New York

GURDON, J.B., MITCHEL, A. and MAHONY, D. (1995). Direct and continuous assessment by cells of their position in a morphogen gradient. *Nature 376:* 520-521.

GYURKOVICS, H., GAUSZ, J., KUMMER, J. and KARCH, F. (1990). A new homeotic mutation in the *Drosophia bithorax* complex removes a boundary separating two domains of regulation. *EMBO J. 9:* 2579-2585.

INGHAM, P.W. (1991). Segment polarity genes and cell patterning within the *Drosophila* body segment. *Curr. Opin. Genet. Dev. 1:* 261-267.

INGHAM, P. and HIDALGO, A. (1993). Regulation of *wingless* transcription in the *Drosophila* embryo. *Development 117:* 283-291.

IRWINE, D. and WIESCHAUS, E. (1994). *fringe*, a boundary-specific signaling molecule, mediates interactions between dorsal and ventral cells during *Drosophila* wing development. *Cell 79:* 595-606

JAFFE, F. (1968). Localization in the developing Fucus egg and the general role of localizing currents. *Adv. Morphogen. 7:* 295-328.

JIANG, J., HOEY, T. and LEVINE, M. (1991). Autoregulation of a segmentation gene in *Drosophila* – combinatorial interaction of the *even-skipped* homeo box protein with a distal enhancer element. *Genes Dev. 5:* 265-277.

KIM, J., IRVINE, K.D. and CARROLL, S.B. (1995). Cell recognition, signal induction, and symmetrical gene activation at the dorsal-ventral boundary of the developing *Drosophila* wing. *Cell 82:* 795-802.

KOCH, A.J. and MEINHARDT, H. (1994). Biological pattern formation: from basic mechanisms to complex structures. *Rev. Mod. Phys. 66:* 1481-1508.

KUZIORA, M.A. and McGINNIS, W. (1988). Autoregulation of a *Drosophila* homeotic selector gene. *Cell 55:* 477-48 .

LAWRENCE, P.A. (1973). A clonal analysis of segment development in *Oncopeltus* (Hemipter). *J. Embryol. Exp. Morphol. 30:* 681-699.

LEPTIN, M. (1991). *twist* and *snail* as positive and negative regulators during *Drosophila* mesoderm development. *Genes Dev. 5:* 1568-1576.

LEWIS, E.B. (1978). A gene complex controlling segmentation in *Drosophila*. *Nature 276:* 565-570.

LOCKE, M. (1959). The cuticular pattern in an insect, *Rhodnius prolixus*. Stal. *J. Exp. Biol. 36:* 459-477.

MARTIN, G.R. (1995). Why thumbs are up. *Nature 374:* 410-411.

LYNDON, R.F. (1990). *Plant Development – The Cellular Basis.* Unwin Hyman, Boston

MEINHARDT, H. (1978). Space-dependent cell determination under the control of a morphogen gradient. *J. Theor. Biol. 74:* 307-321.

MEINHARDT, H. (1982a). *Models of Biological Pattern Formation.* Academic Press, London

MEINHARDT, H. (1982b). The role of compartmentalization in the activation of particular control genes and in the generation of proximo-distal positional information in appendages. *Am. Zool. 22:* 209-220.

MEINHARDT, H. (1983a). Cell determination boundaries as organizing regions for secondary embryonic fields. *Dev. Biol. 96:* 375-385.

MEINHARDT, H. (1983b). A boundary model for pattern formation in vertebrate limbs. *J. Embryol Exp. Morphol. 76:* 115-137.

MEINHARDT, H. (1986a). Hierarchical inductions of cell states: a model for segmentation in *Drosophila*. *J. Cell Sci. 4 (Suppl.):* 357-381.

MEINHARDT, H. (1986b). The threefold subdivision of segments and the initiation of legs and wings in insects. *Trends Genet. 2:* 36-41.

MEINHARDT, H. (1989). Models for positional signalling with application to the dorsoventral patterning of insects and segregation into different cell types. *Development (Suppl.):* 169-180.

Meinhardt, H. (1992) Pattern-formation in biology – a comparison of models and experiments. *Rep. Progr. Physics 55:* 797-849

MEINHARDT, H. (1993). A model for pattern-formation of hypostome, tentacles, and foot in hydra: how to form structures close to each other, how to form them at a distance. *Dev. Biol. 157:* 321-333.

MEINHARDT, H. (1995). *The Algorithmic Beauty of Sea Shells.* Springer Verlag, Heidelberg.

MEINHARDT, H. and GIERER, A. (1980). Generation and regeneration of sequences of structures during morphogenesis. *J. Theor. Biol. 85:* 429-450.

MEINHARDT, H. and KLINGLER, M. (1987). A model for pattern formation on the shells of molluscs. *J. Theor. Biol. 126:* 63-89.

MOMENT, G.B. (1951). Simultaneous anterior and posterior regeneration and other growth phenomena in maldanid polychaetes. *J. Exp. Zool. 117:* 1-13.

NIEUWKOOP, P. (1973). The "organization centre" of the amphibian embryo, its origin, spatial organization and morphogenetic action. *Adv. Morphogen. 10:* 1-39.

NIEUWKOOP, P.D. (1992). The formation of the mesoderm in Urodelan amphibians. VI. The self-organizing capacity of the induced meso-endoderm. *Roux Arch. Dev. Biol. 201:* 18-29.

NÜSSLEIN-VOLHARD, C. and WIESCHAUS, E. (1980). Mutations affecting segment number and polarity in *Drosophila*. *Nature 287:* 795-801.

OLIVER, G., DeROBERTIS, E.M., HEINZMANN, C., SPARKES, R.S., SIDELL, N., FISKE, W. and MOHANDAS, T. (1989). Complementary homeo protein gradients in developing limb buds. *Genes Dev. 3:* 641-650.

PANKRATZ, E., SEIFERT, E., GERWIN, N., BILLI, B., NAUBER, N. and JÄCKLE, H. (1990). Gradients of *Krüppel* and *knirps* gene products direct pair rule gene stripe patterning in the posterior regions of the *Drosophila* embryo. *Cell 61:* 309-316.

PATEL, N.H., CONDRON, B.G. and ZINN, K. (1994). Pair-rule expression patterns of *even-skipped* are found in both short-germ and long-germ beetles. *Nature 367:* 429-434.

PATEL, N.H., KORNBERG, T.B. and GOODMAN, C.S. (1989). Expression of engrailed during segmentation in grasshopper and crayfish. *Development 107:* 201-212.

POSAKONY, L.G., RAFTERY, L.A. and GELBART, W.M. (1991). Wing formation in *Drosophila* melanogaster requires *decapentaplegic* gene function along the anterior-posterior compartment boundary. *Mech. Dev. 33:* 69-82.

REGULSKI, M., DESSAIN, S., MCGINNIS, N. and McGINNIS, W. (1991). High-affinity binding-sites for the deformed protein are required for the function of an autoregulatory enhancer of the deformed gene. *Genes Dev. 5:* 278-286.

RIDDLE, R.D., JOHNSON, R.L., LAUFER, E. and TABIN, C. (1993). *Sonic hedgehog* mediates the polarizing activity of the ZPA. *Cell 75:* 1401-1416.

SANDER, K. (1976). Formation of the basic body pattern in insect embryogenesis. *Adv. Insect Physiol. 12:* 125-238.

SCHIER, A.F. and GEHRING, W.J. (1992). Direct homeodomain-DNA interaction in the autoregulation of the *fushi tarazu* gene. *Nature 356:* 804-807.

SCHOUTE, J.C. (1913). Beiträge zur Blattstellung. *Rec. Trav. Bot. Neerl. 10:* 153-325.

SHANKLAND, M. (1991). Leech segmentation: cell lineage and the formation of complex body patterns. *Dev. Biol. 144:* 221-231.

SMITH, J.C. and HARLAND, M.R. (1991). Injected Xwnt-8 RNA acts early in *Xenopus* embryos to promote a vegetal dorsalizing center. *Cell 67:* 753-765.

SOMMER, R.J. and TAUTZ, D. (1993). Involvement of an orthologue of the *Drosophila* pair-rule gene *hairy* in segment formation of the short germ-band embryo of *Tribolium* (Coleoptera). *Nature 361:* 448-450.

SPEMANN, H. and MANGOLD, H. (1924). Über Induktion von Embryonalanlagen durch Implantation artfremder Organisatoren. *W. Roux Arch. Entw.Mech. Org. 100:* 599-638.

STANOJEVIC, D., LEVINE, M. and HOEY, T. (1989). Sequence-specific DNA-binding activities of the gap proteins encoded by *hunchback* and *Krüppel* in *Drosophila*. *Nature 341:* 331-335.

TECHNAU, U. and HOLSTEIN, T.W. (1995). Head regeneration is different in apical and basal fragments. *Development 121:* 1273-1282.

TICKLE, C., SUMMERBELL, D. and WOLPERT, L. (1975). Positional signalling and specification of digits in chick limb morphogenesis. *Nature 254:* 199-202.

VAN DEN HEUVEL, M., NUSSE, R., JONSTON, P. and LAWRENCE, P. (1989). Distribution of the wingless gene product in *Drosophila* embryos: a protein involved in cell-cell communication. *Cell 59:* 739-749.

VINCENT, J.P. and LAWRENCE, P.A. (1994). It takes three to distalize. *Nature 372:* 132-133.

WADDINGTON, C.H., NEEDHAM, J. and BRACHET, J. (1936). Studies on the nature of the amphibian organizing centre. III The activation of the evokator. *Proc. R. Soc. B 120:* 173-190.

WAITES, R. and HUDSON, A. (1995). *phantastica*: a gene required for dorsoventrality of leaves in *Antirrhinum majus*. *Development 121:* 2143-2154.

WILLIAMS, J.A., PADDOCK, S.W., VORWERK, K. and CARROLL, S.B. (1994). Organization of wing formation and induction of a wing- patterning gene at the dorsal/ventral compartment boundary. *Nature 368:* 299-305.

WOLPERT, L. (1969). Positional information and the spatial pattern of cellular differentiation. *J. Theor. Biol. 25:* 1-47.

ZACHGO, S.,SILVA, E.D., MOTTE, P., TRÖBNER, W., SAEDLER, H. and SCHWARZ-SOMMER, Z. (1995). Functional-analysis of the antirrhinum floral homeotic *deficiens* gene *in-vivo* and *in-vitro* by using a temperature-sensitive mutant. *Development 121:* 2861-2875.

ZOBEL, A.M. (1989a). Origin of nodes and internodes in plant shoots. I. Transverse zonation of apical parts of the shoot. *Ann. Botany 63:* 201-208.

ZOBEL, A.M. (1989b). Origin of nodes and internodes in plant shoots. II. Models of node and internode origin from one layer of cells. *Ann. Botany 63:* 209-220.