Theoretical models in development

Butterfly wing patterns: how good a determining mechanism is the simple diffusion of a single morphogen?

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The generation of the wing patterns of three species of butterflies has been simulated using a set of biologically-plausible assumptions. First, that there are morphogen sources and sinks on wings; second, that the morphogen, itself unstable, moves through the wing by simple diffusion; and, third, that the overt pattern derives from cells interpreting the local morphogen concentration. The computations were done on wing-shaped arrays of hexagonally-packed cells of approximately one quarter the length of the original wing. With this system, we have been able to simulate the patterns of rings, ellipses and bands, in some cases with asymmetric distribution, that characterise species of Tenaris, Callicore and Ragadia. The simulations also show that, with reasonable values of the diffusion constant, the formation of a stable pattern readily takes place within the time available to the pupa (~2 days). The simulated patterns are not, however, robust and cannot match the fine detail of those on the wing. There are, moreover, other classes of pattern that the system outlined above cannot generate. It thus seems that the simple diffusion of a single morphogen is not the mechanism that operates in vivo. However, as the model can generate the essential features of many butterfly wing patterns, it can be considered as a degenerate case of that mechanism.

A model and a computer simulation of the induction of the chick neural plate

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A model explaining the observed morphogenetic role of Hensen's node during neurulation has been examined by computer simulation. The neural plate is induced in the chick blastoderm by a hypothetical substance, called the evocator. The evocator is produced by the node of Hensen at a rate which keeps the concentration in the node constant. The evocator diffuses into the extracellular matrix of the ectodermal layer with a diffusion constant of about 2·10^-9 cm²/sec and a degradation rate of about 1·10^-5 1/sec. Hensen's node moves through the blastoderm at a changing speed as observed in vitro. Induction of the ectodermal cells into neural plate cells is triggered when the local evocator concentration reaches a fixed level.

The model is supported by in vitro grafting experiments. Grafting the node of Hensen in a host blastoderm induces a second neural plate in the host. A circular plate is induced if one prevents movement of the grafted node by interposition of a Micropore film.

The computer simulation showed that the model had predictive value: it could generate the keyhole shape of the normal neural plate and the shapes of neural plates induced by grafting a node into a host blastoderm. The simulation showed that in this model the speed of Hensen's node is the only input function needed to explain the shape of the neural plate.

The height of the ectodermal layer was measured in a Stage 6 chick embryo (i.e. before neurulation) by digitizing photomicrographs of transverse sections. A remarkable correspondence between the height pattern and the proposed evocator concentration pattern was found.
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Axonal guidance and generation of projections in neural tissue: some theoretical considerations

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Connectivity in the nervous system depends on the subdivision of developing neural tissue into physico-chemically and functionally different parts, and the establishment of spatially specific connections between them. Presumably, self-organization of tissues requires positional information encoded in spatial concentration patterns. Short range autocatalytic processes coupled to longer range inhibition effects, are capable of the de novo generation of spatial distributions, showing self-regulatory properties characteristic of developmental biology. Such mechanisms are suggested to determine pattern formation in the two dimensions of cell sheets, as it occurs in the developing nervous system of higher organisms, generating segments with sharp boundaries as well as graded distributions. On the other hand, the third dimension – the arrangement of layers in multilayered sheet – results from contact mediated cell to cell interactions, cell migration, and, possibly, the time course of differentiation of various cell types.

With respect to neural connectivity, the generation of projections (such as the retino-tectal projection) poses a challenging problem because, logically, few positional markers (and, therefore, few genes) suffice to specify connections for a very large number of neurons, and nature probably makes use of this logical possibility. Its analysis, however, is mathematically more involved than simple lock-and-key theories of connections. According to transplantation studies, positional markers on the target are essential; directional cues operating across considerably distances indicate that graded distributions are involved. Theoretical analysis has shown that two antagonistic graded effects on the target area are required in each dimension to specify internal target positions in the tectum and that retinal positional markers must exert a modulating influence, possibly by influencing the relative contribution of the two antagonistic markers in target tissue. Mechanisms by which gradients guide axonal growth could but need not be confined to forces of adhesion: any directional cue, however slight, may be enhanced within the axonal growth cone (in analogy to chemotactic guidance of cells), leading to growth in a defined direction. It is postulated that graded distributions are an essential cue in primary path finding, though undoubtedly further effects (involving direct or indirect fiber-fiber interactions and, at later stages, functional sharpening) also contribute to projections and their regulation.

A limit cycle oscillator concept of the cell cycle

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Cell replication is a fundamental feature of developmental biology. Any valid concept of proliferation processes should enable the mechanism of the interdependence between the two phenomena to be discerned (if only in general terms) and, moreover, it should explain, or be consistent with all available experimental observations on cell proliferation and not merely one facet thereof. An outline will be given of a concept of the nature of the cell cycle which is based on the view that it reflects the behaviour of a metabolic control system involving the synthesis, degradation and interconversion of coenzyme, or coenzyme like, components (e.g. thiols/disulphides) which can regulate a wide range of cellular reactions. It will be shown that, depending on the rates/kinetics of the control reactions, and their modulation by deterministic and random processes, it is possible to explain all of the major facets of cell replicative behaviour in some detail. These include the mechanism of action and interaction of regulators, cell generation times, cell cycle variability and the effect of the malignant transformation on cell replication characteristics. The basic assumption is that replicative quiescence reflects stationary levels of the coenzymes and that proliferation occurs when the control system reactions are modulated such that the coenzymes undergo a cycle of oscillatory change in levels and thereby initiate, suppress and co-ordinate other processes. It is proposed that, since the control system reactions do not exist purely for the process of replication but form steps in various metabolic pathways, the interdependence between replication and development results from modulation of reactions common to cell cycle and differentiation development processes.

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The bithorax phenocopy and prepatterning formation

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The spatio-temporal characteristics of the phenocopy response suggest the involvement of continuous, diffusion-like processes in the prepatterning event affected by ether (Ho et al. 1983a). A phenomenological model accounts for the data in terms of the propagation of two wave fronts in sequence and in approximately orthogonal directions (Ho et al. 1983b).

We present the mathematical justification of the model and a computer simulation which predicts the different combinations of compartments transformed. These predictions are in close agreement with the observations, in contrast to those based on the binary switch-genes model of Garcia-Bellido et al. (1976), which are not. The effect of temperature on the spatio-temporal characteristics of the bithorax response is also consistent with the involvement of diffusion-like processes in prepatterning.

A simple model capable of producing the two waves in the correct sequence and directions is described elsewhere (Saunders and Ho, 1984).


Head regeneration in hydra: biological studies and a model

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The study of head regeneration in hydra should lead to insights into the more general mechanisms of pattern formation. From the tissue of hydra, two morphogenetic substances have been isolated, the head activator and an inhibitor, which seem to have high significance for the regulation of such processes. Studies of the changes of the concentrations of both substances during hydra head regeneration have shown that free inhibitor blocks its own release from sources and that of head activator as well. On the basis of this feedback mechanism a system of differential equations was formulated which describes the changes of the free and bound portions of the substances during regeneration in a computer simulation. Removal of the head as depicted by the simulation results in a stable distribution of the free substances during the first hours of regeneration, which is the necessary prerequisite for head formation. Using the stimulating property of the head activator on the production of nerve cells which in turn are activator and inhibitor producing cells, restoration of the gradient of the sources in the model is assured. The application of the model on morphogenetic mutants of hydra will be discussed.
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Turing's model and the interpretation of time scale in pattern formation: an example from algal morphogenesis

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Direct observation of developing organisms provides only two quantities for comparison with morphogenetic theory: (1) pattern spacing and (2) the time scale of pattern formation. Both are interpretable using reaction-diffusion theory (i.e. Turing's model) because of the severe constraints on parameter values inherent in such theory. We derive here the mathematical expressions needed to interpret data on time and spatial scale and show how the effective diffusivities for Turing's morphogens can be calculated from these. Such calculations are important in establishing Turing's model as a realistic one, and reasonable values for the diffusivities are obtained. In unicellular morphogenesis, these values serve also to indicate the probable site of pattern formation (e.g. whether cell membrane or cytoplasm) because the main candidates have such markedly different viscosities.

We use the example of lobe branching in *Micrasterias* to illustrate our interpretive methods. The results implicate membrane as the site of pattern formation and correspond precisely with results obtained by one of us (L.G.H.) for another example of algal morphogenesis, hair initiation in *Acetabularia*.

Models of pattern formation in insects: the determination of segments and appendages

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Segmentation or metamerization is a basic feature of almost all higher organisms. In recent years genes have been identified in *Drosophila* (Nüsslein-Volhard and Wieschaus, 1980) which, when mutated, alter segmentation in a predictable way. These observations have enabled us to construct molecularly feasible models.

Mutations affecting segment polarity can be explained under the assumption that segmentation arises from a repetition of three subunits (. . . P/SAP/SAP/S . . .). A segment border is formed whenever P and S cells are juxtaposed while the A-P confrontation is a precondition for the formation of limbs and wings. This threefold subdivision guarantees polar development within each segment. A loss of a gene for one of the three regions leads to a symmetrical pattern, e.g. after A-removal, to . . . S/P/S/P . . . with twice as many normal segment borders (corresponding to the mutation *patch*).

The pair rule mutations indicate an intermediate formation of double segments. An explanation of these mutations can be given under the assumption that in the wild-type double segments are determined by an iteration of four cell states - the two extreme positional values in two successive segments. If one of these cell states is lost due to a mutation, the three remaining states allow the development of half the number of normal segments. The model predicts specific denticle patterns which are in agreement with the experimental observation.

Gap mutations indicate a primary subdivision into a few cardinal regions (three internal and two marginal regions). I propose that the border between two cardinal regions organizes two double segments. Thus, segmentation seems to proceed under the control of a hierarchical series of pattern forming events: a primary positional information controls the formation of few cardinal regions, each of which forms in turn four double segments. Each segment forms three compartments (of which only two seem to contribute to the adult organism).

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Morphogenesis of epithelia: the shaping of a fluid elastic shell

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In the development of animal embryos one of the most important processes is the shaping of sheets of cells. Cell sheets, or epithelia, thicken and fold in early stages of development of the central nervous system, limbs, special sensory organs, and many internal organs.

To understand the shaping of an epithelium one needs a mechanical model, to relate molecular and cellular processes to the forces they generate and to the deformations they produce. Our model treats an epithelium as a thin shell of material. The shell is fluid-like, in that cells in a small multicellular element can exchange neighbors to relieve shear stress. The element responds to bending, or to isotropic in-plane tension or compression, as an elastic medium. Each element is in mechanical equilibrium with forces and bending moments external to it. The support within the element for these forces and moments comes from edge tensions – interfacial tensions between lateral surfaces of cells which differ in their adhesive affinities – and from the stiffness of the epithelium, associated with the intracellular cytoskeleton and with intercellular junctions. These properties of an element, with the conditions of mechanical equilibrium and boundary conditions, yield the apico-basal thickness and the principal curvatures everywhere in the epithelium.

An epithelium underlies the integument in arthropods. Predictions of the model agree with observations in three tests on arthropod integument. (1) After a patch of integument is grafted to an ectopic host site, the shape of a normal arthropod leg segment can be used to predict the shape of a triplicated segment which regenerates after contralateral grafting. (2) A pattern of edge tensions compatible with the shape of a normal arthropod leg segment can be used to predict the shape of a triplicated segment which regenerates after contralateral grafting. (3) The model can predict how the shapes of leg segments of different size should differ. These confirmed predictions show that the model represents a useful step toward a biomechanics of development.

Activation of maternal RNA

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The possible mechanism of maternal mRNA activation is discussed. In unfertilized eggs mRNAs are stored as translationally inactive mRNPs. After fertilization or parthenogenetic activation most of these mRNAs are found on polysomes and become involved in protein synthesis. The following steps may be involved in the transfer of mRNA from a maternal inactive mRNP compartment into the active polysomes: 1) Disaggregation of the heavy aggregates of different levels of complexity, containing maternal mRNA (cortical granules?) into simple mRNPs, probably as a result of postfertilization release of Ca2+ (Mano Y., Nagano H. (1970) J. Bioch. Tokyo, 67, 611–628) and/or other physico-chemical changes. 2) Proteolytic activity of the enzymes released from the cortical granules upon fertilization (Mano Y., Nagano H. ibid.; Fodor et al. (1975) Biochemistry, 14, 4923–4927) inducing degradation and/or dissociation of the maternal set of proteins from the coding sequences and polyA tail of mRNA. 3) Digestion of polyA tail which becomes accessible for the enzymatic action, by polyA ribonuclease (ribonuclease IV, which, as yet, has only been found in somatic nuclei – Muller W. (1976) Eur. J. Bioch. 70, 241–248; Muller et al. Euro. J. Bioch. 70, 249–258). Frequent periodic dissolution of nuclear membrane during cleavage would enable the enzyme to get out into cytoplasm and digest maternal polyA tracts. 4) Resynthesis of polyA tails in the process of cytoplasmic polyadenylation after the reconstruction of nuclear membranes. The two alternate and opposite processes (polyA degradation and cytoplasmic polyadenylation) would account for a quick turnover of polyA in the early embryos and de novo polyadenylation of maternal mRNA (Slater et al. (1972) Nature, 240, 333–337; Wilt F. (1977) Cell, 11, 673–681; Olszanska et al. (1984) J. Embry. Expt. Morph. 79, 11–24). 5) Exchange of the set of proteins specific for maternal stored mRNP for the set specific for polysomal and/or free cytoplasmic mRNPs, including exchange of proteins complexed with polyA in an egg and zygote (Peters C., Jeffery W. (1978) Differentiation, 12, 91–97) for a protein complexed with polyA in polysomal mRNP which is probably responsible for a specific conformation of translationally active form of mRNP (Goldenberg et al. (1980) Nucl. Acid Res. 8, 5057–5070; Vincent et al. (1981) Eur. J. Bioch. 114, 179–193).

A similar mechanism, i.e. proteolytic release of mRNAs from the masked maternal mRNP structures, might also be operating in the process of embryonic induction. In this case any factor releasing mRNA from its masking proteins – e.g. by proteolytic digestion or dissociation of proteins from mRNA – might be acting as an inducing factor. The postulated mechanism would necessitate unequal distribution of different mRNA in the egg and early blastomeres and such cases have already been reported.
Forces and pattern in limb morphogenesis


Prior to cartilage and bone formation in the limb bud chondroblasts condense into foci which provide the pattern for subsequent bone development. Formation of these condensations is, finally, a mechanical event, and so it is natural to ask what are the forces responsible for creating them.

We have constructed a model for the process of cell aggregation during chondrogenesis which involves the following forces: (1) the passive elasticity of the extracellular matrix (ECM), (2) the osmotic swelling pressure of the ECM, which is generated principally by the hyaluronate (HA) component, (3) the active cell tractions developed by the chondroblasts. By examining the balance of forces between the cells and matrix, we find that patterns of cell aggregation can spontaneously arise by an instability mechanism analogous to that which occurs in chemical pattern formation models.

According to our model, the following scenario creates the chondrogenic pattern, (a) Cells emerge from the progress zone and commence to manufacture the HA component of the ECM. (b) Because of its high fixed density, the HA component generates a powerful swelling pressure which inflates the limb bud. (c) At the time of condensation the cells commence to produce hyaluronidase (HAase). This initiates a partial osmotic collapse of limb bud core which draws the cells closer together. (d) With the collapse of the HA barrier, which keeps cells apart, cells are brought into close apposition and intercellular contacts increase in number and strength. (e) At this point cell traction forces commence to become effective, and the final condensation pattern emerges.

Following aggregation the cells begin to resecrete HA and the core of the condensations rehydrates and swells. This creates a stress pattern which generates the perichondrium.

Pattern regulation in one- and two-dimensional morphogenetic fields

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In the framework of reaction-diffusion theory a gradient $S$ can be formed which is approximately a homogeneous function of the positional value $x$ and the total linear length $L$ of the morphogenetic field. The profile of $S$ is the following (Papageorgiou, 1980):

$$S(x, L) = L^p f(x/L)$$  (1)

The scaling function $f$ depends only on the relative distance $x/L$ while the scaling factor $L^p$ is position-independent. A paradigm of reaction-diffusion systems in one-dimensional fields has been worked out (Papageorgiou and Venieratos, 1983) leading to gradients of the form (1) where $p = -1$. It is then straightforward to form a new gradient (Papageorgiou, 1980) which depends only on $f(x/L)$ thus achieving pattern regulation on a one-dimensional field. The model can account for the regulation as observed quantitatively by Cooke (1981) on early amphibian embryos.

From dimensional considerations we expect that $[S] = [\text{length}]^{-d}$ where $d$ is the space-dimensionality. For a linear gradient the above value of $p$ is consistent with the canonical dimension of $S(d=1)$. Going over to two dimensions ($d=2$) and with the same reaction-diffusion equations, it turns out that $p = -1$. Therefore $S$ develops ‘anomalous’ dimensions as in the critical phenomena of phase transitions (Wilson, 1983).


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Primary and secondary waves in prepattern formation

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We suppose that determination is initiated by a signal which passes through a region and sets off chemical reactions which then proceed more or less autonomously to completion. There will then be, in the terminology of Zeeman (1974), a primary wave of the reaction beginning and a secondary wave of the system returning to equilibrium. Between the passage of the two waves the system is sensitive to perturbations.

Only if the time required for the reaction to be completed is the same at all points in the region will the secondary wave follow in the path of the primary wave and at the same speed. A numerical simulation based on a model of Lewis et al. (1977) demonstrates that even with a simple underlying dynamic the waves can be almost at right angles to each other. This provides support for the two-wave model of prepattern formation put forward by Ho et al. (1983) and Ho & Saunders (1984).


Clonal analysis of intestinal crypt populations in mouse aggregation chimaeras

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We have shown, using H2 antigens and a carbohydrate polymorphism recognised by the lectin Dolichos biflorus agglutinin as chimaeric markers, that the epithelium of individual crypts in intestine of mouse aggregation chimaeras is always composed of cells of a single parental genotype. Applying the lectin marker to entire sheets of intestinal mucosa, we have analysed the two-dimensional mosaic patterns of discrete patches of crypts of like genotype in the intestinal epithelium.

The results show that: (1) the relative proportions of each genotype are not homogeneous along the length of the intestine, because the patches of each genotype are not randomly distributed. (2) In chimaeras with highly unbalanced proportions, isolated groups of patches of the minority type are probably members of a single descendant clone, and may therefore provide an estimate of the number of progenitors from which the intestinal epithelium is derived. (3) Patches of single or a few crypts are most frequent, but a smaller number of much larger patches is always found. Consequently, the size frequency distribution of patches is not symmetrical. The small and large patches are often widely separated from one another. This suggests that, in the chimaeric strains examined, intestinal crypts proliferate at different rates: the majority never or rarely divide while a minority proliferate to a much greater extent.

These results are in contrast to assumptions made in theoretical accounts and in previous statistical analyses of sectioned chimaeric tissues. Qualitative data from other published studies are consistent with our findings, and indicate that differential proliferation and non-random distribution of clones may occur widely in chimaeric tissues.
The production of extra limbs (supernumeraries) after ipsilateral rotation of the regeneration blastema of amphibian limbs has presented modellers with serious difficulties. This has led to a rather careful experimental elucidation of the characteristics of these limbs and their production. We present here a quantitative model which can account for many of the observations and which, unlike a recent model of Meinhardt (J. Embryol. exp. Morph. 76, 115–137 (1983)), does not require special polarizing regions. In our model supernumerary limbs appear as symmetry-breaking bifurcations in the limb field. The limb field is represented by a vector field and its dynamics is represented by certain rotationally invariant nonlinear field equations of reaction-diffusion type. The model we propose has been designed to be as simple as possible and yet capable of accounting for the production of supernumeraries in a quantitative fashion. We discuss how the underlying difference in symmetry makes the ipsilateral blastema graft inherently more difficult than the contralateral graft. We have found a series of steady state solutions with 0, 2 and 4 supernumeraries as the amount of intercalary growth is varied. Also supernumeraries appear as the angle of rotation is varied. The steady state solutions can account for the generation of supernumeraries at angles of rotation less than 180 degrees, the occurrence of supernumeraries anywhere about the limb, the regeneration of bisymmetric limbs, the probabilistic aspects of the phenomenon and the relative size independence of the pattern formation mechanism. Furthermore we expect that the non-steady state solutions of the pattern forming fields, frozen in as outgrowth occurs, will produce limbs with discontinuities as are observed. In addition to predictions concerning the number of supernumeraries possible with varying amounts of intercalary growth, the model makes possible a prediction on the likelihood of supernumerary production at different proximal-distal levels. Our approach is compatible with the observed effects of vitamin A on amphibian limb regeneration.

Mathematical analysis of regenerative growth curves

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Although the regeneration of parts of an organism is governed by biochemical, physiological, developmental, and many more mechanisms, the increase in volume or length of a regenerating part, on the whole, follows a rather smooth curve. Because such a curve summarizes the effects of all underlying mechanisms, its analysis may help understand better some of the details of regeneration.

Regenerative growth curves are analyzed with a new mathematical function. The function includes known regenerative growth laws as special cases but also accounts for the simultaneous normal growth of the organism. The function is embedded in a general system of growth descriptions that was derived earlier from underlying mechanisms. The major advantage of the function is that the regenerative growth can be mathematically separated from the normal growth. The separation is shown to be biologically meaningful.

The regenerative growth function is used to analyze experimental data on limb regeneration in salamanders (Triturus salamandra). The function describes adult regeneration equally well and larval regeneration significantly better than the regenerative growth functions used before. The mathematical analysis shows objectively that regeneration rate and capacity differ in larvae and adults. Our data do not yet allow us to derive the exact functional dependence of the regeneration rate on an animal's age, but the mathematical model suggests which experiments would have to be performed.

The method of analysis can be used to characterize quantitatively the influence of experimental or environmental conditions like temperature, light/dark régime, or concentrations of chemicals that affect regeneration.