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Some Physical, Mathematical and Evolutionary Aspects of Biological Pattern Formation

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Some physical, mathematical and evolutionary aspects of biological pattern formation

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An important mechanism in the generation of biological structures is the production of defined spatial patterns within initially near-uniform cells and tissues. This process can be modelled on the basis of conventional molecular kinetics if there is a short-range activating effect in conjunction with depletion or inhibition extending over a wider range ('lateral inhibition'). Such pattern-generating systems exhibit simple self-regulatory properties empirically observed in developmental biology such as polarity effects, proportion regulation and the inducibility of secondary centres.

Autocatalysis and lateral inhibition have been shown to be mathematically necessary for the simplest two-factor case. Certain generalizations of these conditions to multi-component systems are possible; they are suitable for modelling intercalary regeneration.

The evolution of higher organisms seems to be determined to a considerable extent by many small changes of patterns and proportions. While evolution proceeds at varying rates in the course of time, the rate-limiting steps may be due to mutations of low selection pressure. A semi-quantitative argument suggests that there might be an upper limit of evolutionarily effective genetic complexity.

1. Types and features of biological pattern formation

One of the most interesting aspects of higher organisms, aside from behaviour, is their specific spatial structure, which is produced anew in each generation. Several quite different mechanisms contribute to the generation of structure during development, including self-assembly (the movement and interaction of cells or cell constituents until a defined energetically favourable configuration is attained) and the conversion of order in time into order in space (as in the consecutive formation of a linear array of structures). Of particular importance, however, is the 'morpholactic' generation of defined spatial structures *within* originally nearly uniform cells or tissues. This latter mechanism often shows impressive self-regulatory properties, including the capacity of parts to form wholes at reduced size, such as a complete organism from half of an early embryo; and the inducibility of secondary centres, such as an embryonic axis leading to a second head. To explain such pattern generation one has to postulate the formation of morphogenetic fields (that is, spatial distributions of some physical parameters, possibly though not necessarily the concentrations of morphogenetic substances) that precede and elicit local responses of cells, giving rise to visible pattern and form (Child 1929; Wolpert 1971).

A classical model system for the demonstration of the existence and regulation of morphogenetic fields is the regeneration of the coelenterate hydra from sections cut from the gastric column; the initially near-uniform tissue regenerates a new animal with head and foot (figure 1). The part of the regenerate that was closest to the original head acquires head-activating potential in a rapid process long before the actual head is produced (Webster & Wolpert 1966), thus indicating that a morphogenetic field is formed that precedes and directs head formation. The orientation of the field is determined by some asymmetric pre-existing property in hydra

tissue, probably by a polarity-defining gradient extending from head to foot (Gierer *et al.* 1972). The pre-existing polarity-defining gradient, however, cannot itself act as morphogenetic field, because the same subarea of the animal can form either head or foot upon regeneration depending on how the section was cut (see figure 1). Rather the morphogenetic field must be formed anew after the onset of regeneration in a rapid process involving cell-communication within the regenerating tissue. Despite its simplicity, the scheme in figure 1 embodies a number of elementary features that a consistent theory of biological pattern formation must incorporate.

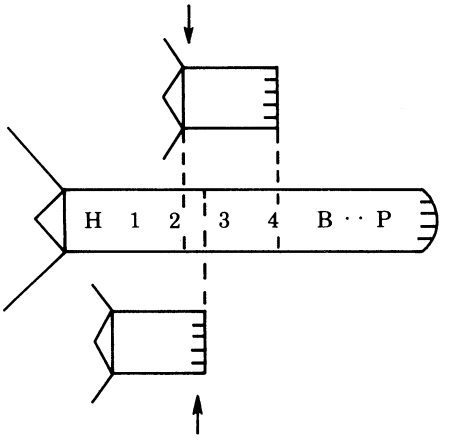

FIGURE 1. Some elementary properties of pattern formation, exemplified, schematically, by regenerating hydra tissue. Hydra is a polar animal, with head (H), gastric column (1...4), budding region (B) and peduncle (P). Any section cut from the gastric column (if it exceeds a minimal size) is able to regenerate an animal with head and foot. Heads form at the area closest to the original head. The same area of the original animal (arrow) can form a head or foot depending on the position of the cuts. It follows that the pattern is formed anew during regeneration and that the position of the head cannot be determined by a local cell property or the existence of a cut, of both; rather, a morphogenetic field is newly and rapidly formed at the onset of regeneration, activating the future head area. Its orientation, but not its form, is determined by a pre-existing slightly asymmetric distribution in the gastric column of the polar animal.

Although the detailed chemical basis of morphogenetic fields is still unknown, one may nevertheless study requirements and properties of pattern-generating systems on the basis of a rather general and non-committal assumption about the type of physics involved, namely that morphogenetic fields are generated by molecular interactions and movements. Physical laws applicable to a wide variety of these processes are such that concentrations of compounds change in time as function of local concentrations (to account for interaction) and of the spatial distribution of substances (to account for movements due to diffusion, convection, etc.). This form imposes stringent constraints on the construction of theories and models. Can spatial concentration patterns be formed at all on this basis? This was first shown by Turing (1952) who found that, if there are at least two components interacting auto- and cross-catalytically, patterns can be generated. Several groups have since then further developed the mathematics of this process, especially with respect to stability analysis (see Prigogine & Nicolis 1971).

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2. Pattern formation by autocatalysis and lateral inhibition

Is molecular kinetics adequate for the explanation of biological patterns? To study this question, H. Meinhardt and I have searched for properties of molecular systems required for the formation of distinct spatial patterns from near-uniform conditions and for the self-regulation found experimentally in developing organisms (Gierer & Meinhardt 1972, 1974). The set of conditions listed in table 1 emerged from these studies: out of two components, one must be

Table 1. Conditions for pattern formation based on autocatalysis and lateral inhibition (for two-component systems)

(*a*) One of the two components A, B (say A) must be self-enhancing.
(*b*) The other component (B) must be cross-inhibiting; inhibition can be replaced by depletion of a substrate required for, and consumed by activation.
(*c*) The inhibitory effect must be sufficiently strong to ensure stability of the uniform solution.
(*d*) The inhibitory effect must be relatively fast compared to the activating effect.
(*e*) The range of activation must be below a limit of the order of total field size.
(*f*) The range of inhibition must be sufficiently large in relation to the range of activation.

activating in the sense of self-enhancement, the other cross-inhibiting, either directly or indirectly via depletion of a substrate required for and consumed by activation. The inhibitory effect must be sufficiently fast and strong to prevent an overall autocatalytic explosion of the system. An essential concept of the theory is range, defined as the mean distance between production and decay of molecules. (Range can be expressed in terms of physico-chemical properties, such as diffusion constants and decay rates.) The range of activation must be small in relation to total field size, and the range of inhibition must be large in relation to the range of activation (the latter condition has been called 'lateral inhibition' by analogy with the use of the term in neurophysiology and the field of pattern recognition). If these conditions are met, a small deviation from an initially near-uniform distribution will be self-enhancing; however, activation at one site leads to deactivation elsewhere, because of the long-range inhibitory effect. The local increase and spatial confinement of activation will proceed until saturation or diffusion effects stabilize the pattern. The form of the pattern is eventually determined by the ranges of activation and inhibition; the simplest form is a gradient, but symmetric and periodic distributions in one or several dimensions can also be generated.

These conditions can be given a mathematical form, which is particularly simple if power terms dominate expressions for production and decay rates of molecules, and which allows the assessment of models for pattern formation. All the models that we have studied were generated in this way. One example is a model in which an activator and an inhibitor are produced by enzymes that undergo an allosteric transition to the active state by association with two activator molecules, this process being responsible for autocatalysis; the inhibitor is assumed to inhibit the activating enzyme and has a wider range, owing to diffusion, than the activator. This is only one of many possible models, and only biochemistry can provide proof for a particular mechanism. Some nonlinear reaction is generally required, but only well known features of molecular biology are necessary, though in some special combinations.

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3. Pattern regulation, including proportion regulation by adaptation of the metric

Computer calculations demonstrate that such pattern-forming systems exhibit self-regulatory features experimentally observed in biological systems (figure 2). A graded distribution can be formed, starting from near-uniform initial conditions with the orientation defined by some pre-existing cues to asymmetry, however slight, whereas the form is invariant to details of the initial conditions. In larger fields, symmetric and periodic patterns can arise. Induction of secondary centres can occur, depending both on the strength of an inducing stimulus and its distance from the primary centre. Aside from modelling for qualitative self-regulatory features of development, quantitative aspects have been simulated for several systems, including hydra regeneration (Gierer & Meinhardt 1972; Meinhardt & Gierer 1974).

A particularly interesting regulatory property is proportion regulation, the capacity of parts to develop all structures at reduced size. A simple model is to assume that activator production cannot exceed a saturation level; in this case the area of near-maximal activation adapts in proportion to total size. While this leads to proportion regulation of a substructure within a given area, it does not give rise to exact proportion regulation of a morphogenetic gradient specifying positional information at intermediate levels.

Whether exact proportion regulation of morphogenetic gradients occurs in biology is not yet known, but this property can also be incorporated into models of the lateral inhibition type. The approach is based on the idea that the primary formation of a gradient itself determines the metric of the system (Gierer 1981). Let us assume that we start with a high degree of cell communication, owing to many open intercellular junctions, leading to a mean range of activators above the upper limit consistent with pattern formation within the size of the field (see condition (*e*), table 1). If cell communication is gradually reduced so that the range of activation drops below the upper limit for pattern formation, a gradient will be formed with high activation in part of the field. If we assume that this activation then induces a wave across the entire tissue that stops further closure of junctions, a stable morphogenetic gradient results. The gradient exhibits good size-regulating properties in that smaller fields form, at a state of more reduced cell communication, a steeper proportion-regulated gradient. An interesting feature of this type of model is that the degree of cell communication, and thus the *metric* of the tissue with respect to ranges of molecules, is now adapted to total size. This may lead to proportion regulation of any aspect of pattern formation, including proportion regulation of repeating structures: if, after formation of the primary gradient, a secondary periodic pattern is initiated, which forms, say, eight peaks, then the same eight peaks can be produced in the smaller section with reduced distances. Possibly proportion regulation of somite spacing (Cooke 1975) might be of this type.

4. Two-dimensional patterns

The theory can be extended to more than one dimension. Figure 3 shows some types of patterns in two-dimensional fields such as cell sheets. A graded distribution (figure 3*a*) may define positional information in one dimension, and a second pattern-forming system of this type can then lead to a Cartesian specification of a two-dimensional field. If the ratio of field size to activator range is higher, symmetric patterns (figure 3*b*) or multiple peaks can be formed.

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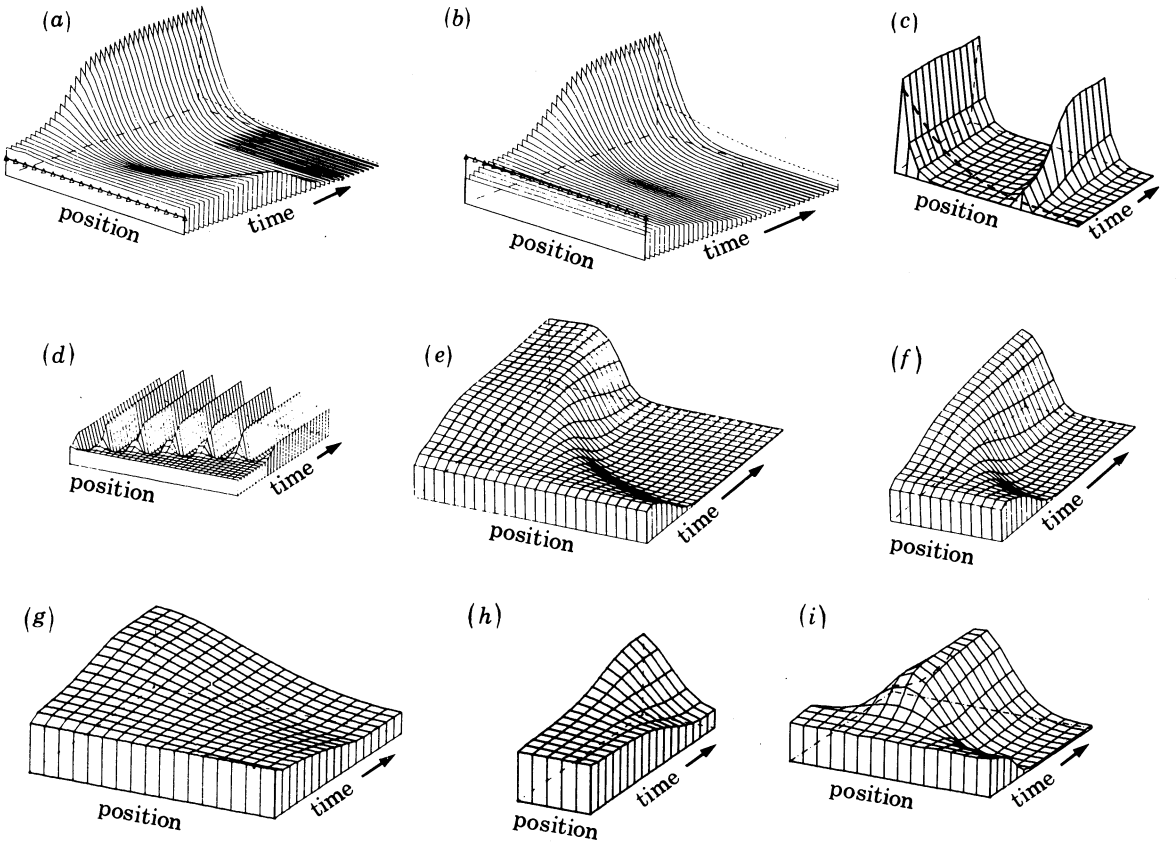

FIGURE 2. Pattern formation and regulation on the basis of autocatalysis and lateral inhibition. ——, Activator distribution. Dimension left to right describes position within tissue; development of the pattern in the course of time is plotted in the dimension front to rear. (*a*) A monotonic gradient is generated starting from near-uniform initial distribution; a slight fluctuation (left) suffices for initiation. Activation (left) proceeds at the expense of the deactivation (right), leading to a stable gradient. (*b*) Its form is nearly independent of initial conditions: a shallow gradient (△— △— △) of sources (e.g. an enzyme synthesizing activators and inhibitors) can also determine the orientation of the pattern generated, though its form is almost independent of the source distribution. (*c*) Model for induction: a small stimulus can give rise to the formation of a secondary peak of activation if the stimulus exceeds threshold (which, in turn, decreases with distance from the pre-existing head). – – –, Inhibitor distribution extending from the head at the onset of induction. (*d*) If the range of activator is small compared with the total field size, a periodic pattern can be obtained if initiated by a stimulus at one margin. (*e*, *f*) Model for size regulation by activator saturation; activated area adapts nearly proportionally to total size of the field. (*g*, *h*) A more precise proportional regulation of a graded distribution (and of any secondary pattern initiated in the field including periodic patterns) could be obtained by regulation of the metric: starting from a state with a strong cell communication and thus with large diffusion ranges of activator above the limit consistent with pattern formation, cell communication is assumed to be gradually reduced, e.g. by closure of intercellular junctions; at some stage of this reduction, pattern formation occurs, leading to a graded distribution. If the corresponding activation triggers a signal that causes the reduction of cell communication to stop in the entire area, a stable gradient is obtained (*g*). This mechanism is size-regulating, leading, in smaller sections, to correspondingly steeper gradients formed at a stage of more reduced cell communication (*h*). (*i*) An example of a symmetric pattern initiated by random fluctuation superimposed on a graded distribution.

Recursive initiation of multiple peaks starting at the centre or a margin leads to regular spacing (figure 3*c*, *e*, *f*), whereas random initiation produces a distribution that is less regular (figure 3*d*), but which shows, owing to lateral inhibition, second-order statistics excluding small distances.

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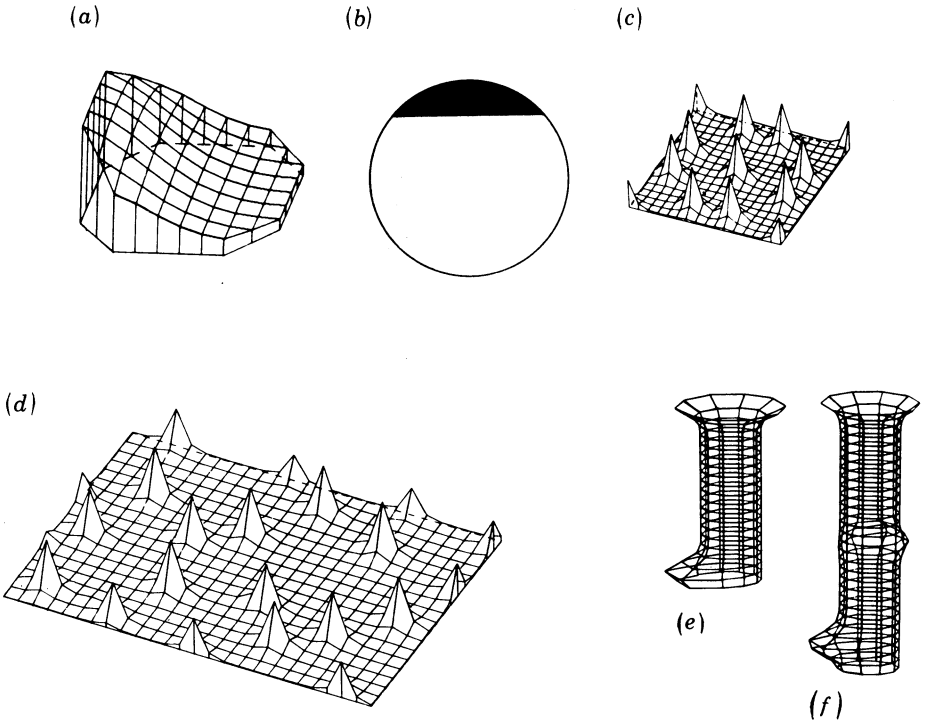

FIGURE 3. Pattern formation in two dimensions, e.g. within cell sheets. Final distributions are plotted. (*a*) Monotonic gradient. (*b*) Single peak of activity calculated on a sphere, simulating the activation of an area of a cell sheet; the model may apply to closed cell sheets but also to intracellular and intramembrane patterns generating polar cells. Their orientation can be determined by some shallow external gradient initiating the intracellular pattern. (*c*) Multi-peak pattern initiated at one point leading to near-regular distances. (*d*) Randomly initiated multi-peak pattern. Positions of peaks are random, but small distances are systematically avoided. (*e*,*f*) On a growing cylinder, peaks of activity can be produced on alternating sites. This spacing is found, for example, for buds in hydra, and for leaf rudiments in many plants.

5. EFFECTS OF MORPHOGENETIC FIELDS ON CELL DIFFERENTIATION AND REAL FORM

Morphogenetic fields are expected to exert effects on determination, proliferation, movement, form and death of cells. Determination may occur with probabilities proportional to field values giving rise to smooth distributions; or, above certain thresholds of morphogenetic fields, thus dividing an area into subareas with different states of differentiation separated by distinct boundaries. It is expected that the same or similar field-forming mechanisms can give rise to subpatterns in subsystems. In this way a complex pattern could be laid down in a combinatorial fashion (Kauffmann 1973; Gierer 1973; Garcia-Bellido 1975).

Perhaps the most interesting effect of morphogenetic fields is in the generation of real form. Form is determined by the spatial distribution of curvatures that define the contour of organs and organisms. Biological form can result from a large variety of mechanisms. A simple prototype is the activation of areas within initially nearly flat cell sheets, caused by internal morphogenetic fields or external induction by neighbouring tissues; this local activation then generates bending moments leading to curvature and form. The self-regulatory feature of such processes, for example effects of reversible inhibition, suggests that in the generation of curvature and form a steady state is approached that is describable as a state minimum general potential;

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this, in turn, is a function of cell form and interactions within the cell sheet that are modified by morphogenetic fields. Certain non-trivial features of cell interactions are required for a cell sheet to be stable against clumping or decay. Local activation of a subarea of a stable cell sheet by a morphogenetic field or by induction leads to evagination or invagination if one further condition is met: that the cell sheet is anisotropic in the inside–outside dimension; this property is often obvious by a different appearance of inside and outside boundary regions of cell layers. Processes of evagination and invagination, as well as the generation of a elongated structures of various types, have been modelled (figure 4) by using shell theory for the simulation of real form (Gierer 1977).

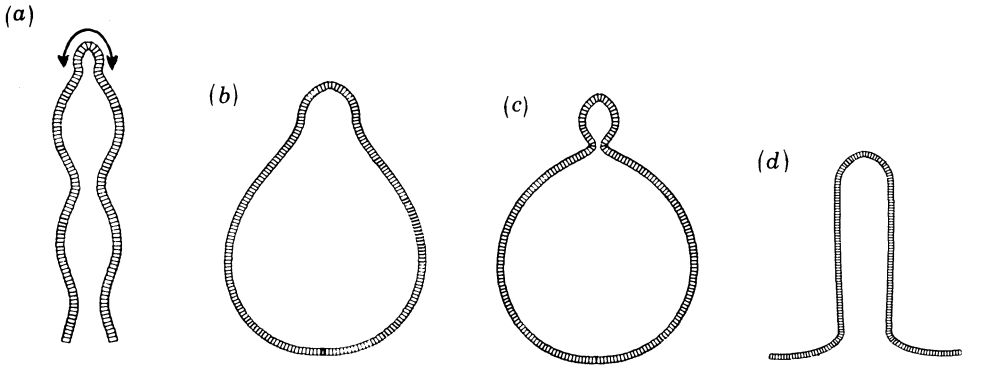

FIGURE 4. Rotationally symmetric structures formed by local activation of an area (centre-top of the structures drawn) generating excess bending moments. Pictures represent sections through (three-dimensional) cell sheets. The axis of rotation is vertical. Calculations were made on the basis of shell theory. (*a*) A single circular area of activation (top) can lead to a complex structure owing to the interaction of curvatures in two dimensions. (*b*, *c*) Evagination from a closed sphere, resulting from activation of a small area on the top. Such models can be applied to cell sheets to model tissue form as well as to membranes and boundaries of single cells to model cell form. (*d*) If there are two degrees of activation, a strong one in a small circular area (top) and a weaker one extending into the surrounding area, an elongated structure such as a protruding bud can be produced.

6. MATHEMATICAL REQUIREMENT FOR AUTOCATALYSIS AND LATERAL INHIBITION IN TWO-FACTOR PATTERN-FORMING SYSTEMS

The conditions for pattern formation by autocatalysis and lateral inhibition have been derived from considerations of the qualitative behaviour of two-component systems. Patterns cannot arise starting from near-uniform distributions with stable average values if any one of the conditions described in table 1 is extremely violated and must evolve if all of them are met asymptotically. How does this approach relate to the analytical methods of general stability theory (Prigogine & Nicolis 1971; Babloyantz & Hiernaux 1975)? Granero *et al.* (1977) have applied this theory to the equations that we have used in most simulations of biological patterns, and confirmed the conditions of autocatalysis and lateral inhibition. As has been shown recently (Gierer 1981), the correspondence of the conditions of autocatalysis and lateral inhibition to the theory of destabilization is a much more general one. The linear approximations of the equations for deviations from the uniform solution can be transformed in such a way that they are expressed in the concepts of the 'lateral inhibition' theory: rates as reciprocal mean lifetimes of molecules; ranges as mean distances between production and decay; orders of

reactions in terms of logarithmic derivatives of production and decay rates of molecules. To the equations thus derived, a classification of models according to signs of parameters can be applied, taking into account that the effect of diffusion and other modes of redistribution is always to counteract distortions. On this basis, conditions (a)–(f) in table 1 can be derived point by point irrespective of details of models. No principally different models, say with long-range activation and short-range inhibition, can lead to pattern formation for the two-factor case. The conditions apply to mechanisms involving redistribution by diffusion, convection or other processes. Inclusion of vectorial effects such as orientation of ion pumps as proposed by Jaffe (1968) would require an extended formalism. These mechanisms are an attractive possibility for the explanation of polar cells. On the other hand, it seems unlikely that the regulatory properties of tissue morphogenesis are explicable by such pumping mechanisms.

7. Extension of the 'lateral inhibition' theory to more than two components

Can one extend the scheme of lateral inhibition to more than two components? The principal difficulty is to define activation and inhibition unambiguously in multi-component schemes; for instance, activation may result from inhibition of inhibition. A generalization is possible, however, in cases in which two subsets of components can be distinguished from the outset according to the range of redistribution (Gierer 1981). If one subset, characterized by short ranges, has activating properties (defined by the existence of at least one positive diagonal term after diagonalization of this subset) and if the other subset prevents, by cross-inhibition, an overall autocatalytic explosion, then sufficient redistribution of the components of the cross-inhibitory subset always leads to destabilization of uniform distributions and thus to spatial patterns. In this case, short-range activation and long-range inhibition represent features of subsystems of several components rather than properties of individual substances.

Multicomponent pattern-forming systems allow us to model for features of developing systems that generate a spatial sequence of structures by mutual or consecutive induction (Meinhardt & Gierer 1980). The simplest example is the symmetric subdivision of an area into two stripes by mutual lateral activation. Let us assume that two neighbouring regions acquire different activated states, with each region enhancing its own activation locally and supporting the alternative activation of the neighbouring region by a more diffusible substance. Thus there are two short-range autocatalytic substrates and two more diffusible components involved in mutual lateral activation. In terms of the multicomponent analysis mentioned, this locally exclusive lateral activation can be shown to be formally isomorphous to lateral inhibition. Further generalizations along these lines lead to sequences of induction as they may be involved in intercalary regeneration in insects (Bohn 1970).

The analysis of destabilization as such does not permit us to derive the stability or the form of the spatial distributions produced; these depend on the nonlinear characteristics of the system. An analytic treatment is difficult and has been possible only for the most simple case of monotonic gradients (Mimura & Nishiura 1979). However, assessment by heuristic principles can often be confirmed by computer simulation: ranges of activator are the main determinants of width of activated areas, and ranges of inhibitor determine distances of, or exclusion areas for, multiple centres of activation.

In conclusion, it appears that short-range autocatalytic activation, in conjunction with long-

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range inhibitory effects, provides a rather general basis for the formation of concentration patterns showing regulatory properties of developing biological systems. A wide variety of different mechanisms is consistent with this scheme, and only biochemical evidence can decide on a particular mechanism. Spreading and redistribution mechanisms can, but need not, be due to molecular diffusion, and parameters of the theory need not be concentrations of individual substances but may subsume system features of several components.

8. Some remarks on the relation of biological facts to analytical mathematics

The assumption of the approach that I have discussed here is that understanding of biological patterns on the basis of physical laws and processes requires both formal mathematical analysis and structural biochemical studies. Biochemistry alone does not suffice, because we cannot infer the structure of an animal from the structures of molecules involved in its generation without phenomenological and systems theories. On the other hand, a purely formal explanation is also unsatisfactory, because experimental confirmation requires biochemical studies. Since this symposium is focusing on mathematical aspects of biological development, I should like to add some thoughts on the prospects and pitfalls of mathematics in this field. The main pitfall is perhaps the temptation to discard or distort basic biological facts for the sake of analytical mathematics. In extreme cases this can produce rather artificial problems far removed from the main issues of developmental biology. A few cautioning examples will be given.

1. The orientation of most substructures arising in embryonic development, such as the tusk or the leg of an elephant, are strictly determined by pre-existing asymmetries. The mathematical formalism of symmetry breaking instabilities is adaptable to certain problems of biological pattern formation; conceptually, however, it is a *distinguishing* feature of biological development, as compared with the formation of many inorganic structures, that true random symmetry breaking is *not* involved in the more interesting aspects.

2. The self-regulatory properties of pattern-generating systems in biology suggest that morphogenetic fields reach near-stable states; but life as such is finite. Morphogenetic fields exert their influence on development only in a limited time span. Nonlinear equations often lead to quasi-stable solutions. It follows that absolute stability of solutions is no valid criterion for the quality of mathematical models for pattern formation.

3. Diffusion-coupled differential equations, which are characteristic of the theory of autocatalysis and lateral inhibition, have many solutions if field size is large enough to allow for multi-peak patterns to arise. The distribution produced may then depend sensitively on boundary conditions and the mode of initiation, and would not reproducibly be formed upon random initiation. Its form and reproducibility may seem to present extremely intricate mathematical problems. However, there is as yet no biological evidence that reproducibility, multiple-peak patterns, and random initiation occur together. The evidence on genetic mosaics (Stern 1968) suggests that morphogenetic fields leading to reproducible structures are simple and do not depend sensitively on boundary conditions. The complexity of biological structures appears to result from the complexity of cell responses to simple fields (perhaps gradients, see figure 3*a*) and from the combinatorial formation of patterns, subpatterns, sub-subpatterns, and so on. In cases in which regularly spaced periodic patterns are reproducibly formed, as for instance in the formation of leaf rudiments, they are not randomly initiated but produced

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sequentially in a recursive manner, the position of the youngest rudiment being defined by the range of inhibition extending from the preceding one (see figure 3*e*, *f*). Certain multi-peak patterns, such as that of stomata in plant leaves, appear to be randomly initiated, but in this case the pattern is not reproducible. Density of peaks and a tendency to avoid small distances is conserved, but otherwise each leaf on a tree is different.

9. Some evolutionary aspects of morphogenesis

Despite the cautioning considerations of the preceding section, there are many real problems of great general interest in developmental biology that could be elucidated by analytical mathematical studies. One of the challenging problems to be solved is the dynamics of the evolution of higher organisms, especially in relation to morphogenesis. The evolution of life started with the emphasis on molecular evolution, particularly the invention of new and better enzymes; the evolution of higher organisms, on the other hand, is mainly due to mutations affecting regulation, which alter patterns and proportions of structures, or tendencies of behaviour as indirect consequences of the generation of the neural network. The impressive evolution of higher organisms occurred with population numbers much lower and generation times much higher by many orders of magnitude compared with microorganisms.

While the morphological evolution of higher organisms proceeds at varying rates in the course of time, it is not unlikely that a stage of relatively rapid changes is preceded by an initial phase of mutations of low (positive) selective pressure such that the latter determine, or at least co-determine, the overall rate of evolution. For instance, if new genes and structures are generated by gene duplication, one expects low selection pressures in the decisive initial phase of development of new functions. If effects of mutations at different genetic loci interact in generating selection pressure, the resulting selection pressures are also expected to be low at the beginning of the evolution of properties resulting from the combined effects. Possibly a statistical theory of mutants of low selection pressure affecting patterns (and, possibly, behaviour as well) can be constructed that may contribute to the understanding of evolution. Increases in complexity of animals in the course of evolution are broadly correlated with decreases in population size and rate of reproduction. Further, the rate-limiting values of low selection pressures in the initial phases of evolution of new functions are likely to decrease as the complexity of the pre-existing genome increases. According to population genetics, decreases of population size, rate of reproduction, and selection pressure all reduce the probability of mutants of low selection pressure to succeed in the population. These correlations may determine an upper limit of evolutionarily useful genetic complexity, and large higher animals as well as man might be close to this upper limit.

The following considerations along these lines are only to suggest the *existence* of a limit of complexity without claiming that the presumptions of the numerical examples are adequate. According to population genetics, the probability that a mutant of small positive selective value s eventually succeeds in the population is of the order of s (see Jacquard 1973). If population size is N individuals and the geological timescale for major changes of evolutionary characteristics of species is G generations, whereas the number of functional nucleotides per genome is n, these values could be related to selection pressures s required for evolution if we introduce assumptions on the orders of magnitudes m of the number of mutations per generation and individual, and on the probability p for a mutation at a given site of the genome to succeed in

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the population in G generations, as requirement for evolution of new properties to proceed. According to population genetics, this probability is approximately given by

$$p \approx GN(m/n)\, s. \tag{1}$$

It is not known whether point mutations or other types of mutation such as excisions, insertions and recombinations are rate-limiting. Effective values of p and m are difficult to estimate on the basis of current knowledge. For the present discussion of general characteristics, we may assume both values to be of the order of 1. Then

$$s \approx n/GN. \tag{2}$$

In higher animals, estimates of the number of genes per genome and of average number of nucleotides per gene are both of the order of 10^4–10^5. While the reasons for the large number of nucleotides per gene are not yet known, and part of them may be without functions, it is not unlikely that many of them are involved in the regulation of gene activity; they may be sites of mutations of low selection pressure, resulting from point mutations, excisions or insertions. Requirements for regulation are expected to increase with increasing complexity of the patterns formed, whereas the evolutionary efficiency per mutation is likely to decrease. It is therefore conceivable that network theories could establish a reverse relation between rate-limiting selection pressure s and the complexity of the genome. A very simple assumption would be that there are some $\sqrt{n}$ genes per genome with some $\sqrt{n}$ functional nucleotides per gene, and that an approximate reverse relation holds between s on one hand and the number of genes, or the complexity of the regulatory part of the genes on the other, leading to $s \approx 1/\sqrt{n}$. In conjunction with (2), the upper limit of evolutionary useful genetic complexity, n, would then be of the order of $(GN)^{\frac{2}{3}}$. If both N and G are of the order of some millions, the number of functional nucleotides per genome n would be of the order of 10^8–10^9 and the genome would consist of several times 10^4 genes. The number n can be below the total number of nucleotides per genome because part of the latter may be without function.

While the crude quantitative assumptions introduced above still need theoretical justification, and may prove to be inadequate, the qualitative line of thought on correlations and anticorrelations indicates that a limit of complexity of the genome may exist and that its order of magnitude might be estimated if a suitable statistical theory of mutations of low selection pressure affecting patterns, proportions and behaviour could be developed.

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