APPLICATIONS OF A THEORY OF BIOLOGICAL PATTERN FORMATION BASED ON LATERAL INHIBITION

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SUMMARY
Model calculations are presented for various problems of development on the basis of a theory of primary pattern formation which we previously proposed. The theory involves short-range autocatalytic activation and longer-range inhibition (lateral inhibition). When a certain criterion is satisfied, self-regulating patterns are generated.

The autocatalytic features of the theory are demonstrated by simulations of the determination of polarity in the Xenopus retina. General conditions for marginal and internal activation, and corresponding effects of symmetry are discussed. Special molecular mechanisms of pattern formation are proposed in which activator is chemically converted into inhibitor, or an activator precursor is depleted by conversion into activator.

The (slow) effects of primary patterns on differentiation can be included into the formalism in a straightforward manner. In conjunction with growth, this can lead to asymmetric steady states of cell types, cell differentiation and proliferation as found, for instance, in growing and budding hydra. In 2 dimensions, 2 different types of patterns can be obtained. Under some assumptions, a single pattern-forming system produces a 'bristle' type pattern of peaks of activity with rather regular spacings on a surface. Budding of hydra is treated on this basis.

If, however, gradients develop under the influence of a weak external or marginal asymmetry, a monotonic gradient can be formed across the entire field, and 2 such gradient-forming systems can specify 'positional information' in 2 dimensions.

If inhibitor equilibrates slowly, a spatial pattern may oscillate, as observed with regard to the intracellular activation of cellular slime moulds.

The applications are intended to demonstrate the ability of the proposed theory to explain properties frequently encountered in developing systems.

INTRODUCTION
In the course of development, spatial order (pattern) appears, reproducibly, within initially uniform-appearing tissues (or cells). Visible patterns may often be the expression of previously formed invisible 'primary patterns' of physical parameters such as morphogen concentrations. Primary patterns may be established by auto- and cross-catalytic systems involving two or more substances (Turing, 1952; Gmitro & Scriven, 1966). Linear reaction kinetics can lead, for instance, to periodic patterns. However, instabilities and other properties of linear systems indicate that non-linear effects have to be implicated. Non-linear kinetics, on the other hand, are too general to be applicable unless restrictions are imposed by biological considerations.

We have proposed a non-linear theory based on properties frequently encountered in development (Gierer & Meinhardt, 1972). Short-range activation as observed,
e.g. if a piece of tissue derived from one organizing centre is transplanted to cause the formation of a new centre; longer-range inhibition by which such secondary centres can be inhibited if the primary centre is too close in the vicinity; and polarity which often is a fairly stable tissue property determining the orientation of a developing structure. It can be accounted for by an asymmetric distribution (e.g. a shallow gradient) of activator or inhibitor sources such as enzyme systems, or particulate structures. Whereas primary patterns can be established within a few hours, source distributions are expected to change more slowly as a result of cell differentiation, growth, etc.

When the kinetics of production and removal of morphogens fit a certain criterion, self-regulating stable primary patterns are generated starting from nearly uniform initial conditions. Slightly asymmetric source distributions are able to determine polarity. According to particular assumptions, the pattern may be size-regulating, or lead to activated areas independent of total size. The auto- and cross-catalytic effects which are assumed are easily interpretable in molecular terms, suggesting that such mechanisms actually occur in biological pattern formation. Bimolecular activation and a variety of inhibitory effects such as monomolecular inhibition, or monomolecular depletion fit the criterion. For example, the following equations suffice for pattern formation:

\[
\begin{align*}
\frac{\partial a}{\partial t} &= \rho_0 \rho + c_\rho a^2 - \mu a + D_a \frac{\partial^2 a}{\partial x^2}, \\
\frac{\partial h}{\partial t} &= c'_h a^2 - \nu h + D_h \frac{\partial^2 h}{\partial x^2}.
\end{align*}
\] (1a, 1b)

(\(\rho\) and \(\rho'\) source concentration for activator, and inhibitor, respectively, \(a\) activator-, \(h\) inhibitor-concentration, each generally depending on position \(x\); \(\rho_0\) basic production of activator; \((ca^2/h)\) autocatalytic production of activator, the inhibitory effect being approximated by the term \(1/h\); \(\mu a\) activator degradation, and \(D_a\) diffusion constant of activator. Similarly, \(c', \nu,\) and \(D_h\) are concerned with inhibitor production, degradation and diffusion.) Stable patterns of \(a\) and \(h\) are formed if \(\rho_0\) is sufficiently small, and \(\nu\) and \(D_a\) are sufficiently large.

Pattern formation can also be achieved if long-range inhibition is not due to an inhibitor, but to depletion of a rapidly diffusing substance of concentration \(s\), consumed by the process of activation. An example of this type is given by the following equations:

\[
\begin{align*}
\frac{\partial a}{\partial t} &= \rho_0 \rho + c_\rho a^2 s - \mu a + D_a \frac{\partial^2 a}{\partial x^2}, \\
\frac{\partial s}{\partial t} &= c_0 - c'_0 a^2 s - \nu s + D_s \frac{\partial^2 s}{\partial x^2}.
\end{align*}
\] (2a, 2b)

Properties of such equations have been discussed in detail previously (Gierer & Meinhardt, 1972).

In this article, we will apply the theory to a variety of problems of development. An application to a problem of neural differentiation exemplifies the autocatalytic
features of the theory. Examples for marginal and internal positions of activated areas will be given. The effects of morphogens on differentiation and source densities, the establishment of secondary centres, pattern formation in 2 dimensions, and an extension of the theory to oscillatory processes are discussed. Biological examples to be considered are growing and budding hydra, the developing frog retina, and cellular slime moulds.

**Autocatalytic features in pattern formation**

Investigations on the amphibian retina have elucidated basic properties of developmental systems (Stone, 1960; Jacobson, 1968; Gaze, 1970). In this paper, we will discuss the determination of the axes of the developing retina. Jacobson (1968) and Hunt & Jacobson (1972) transplanted the developing Xenopus retina at different stages in various orientations. The subsequently developing retinotectal connexions indicate the final polarity of the retina along 2 perpendicular axes. The internal polarity of a retinal axis is not reversed following 180° rotation of the retina if the rotation is performed after a critical time \( t_0 \) (this stage is reached at different times for the 2 axes). If rotated before this stage, the host would cause the polarity to revert. This experiment can be simulated by computer on the basis of the theory (Fig. 1A--C); it is assumed that the surrounding tissue imposes on the retina a very slight asymmetry such as a shallow gradient of inhibitor (possibly by indirect mechanisms).

In addition, Hunt & Jacobson (1973a) have demonstrated that determination of an axis is not to be considered an all-or-none event occurring at time \( t_0 \); if, at a time before \( t_0 \), the developing retina is placed in tissue culture, from which it is removed some time after \( t_0 \) and transplanted in reverse orientation, the transplant then retains its polarity despite its new environment. This effect can also be simulated (Fig. 1D). Once the surrounding tissue has imposed a slight asymmetry on the retina, its influence is no longer necessary, for the asymmetry is autocatalytically amplified thereafter. Eventually, the magnitude of the internally generated gradient exceeds the magnitude of the asymmetry a surrounding tissue can impose. At this point, the polarity can no longer be reversed by the influence of the surrounding tissue, and the retinal polarity therefore appears determined upon transplantation. The effect and its simulation indicate that the autocatalytic features involved in the theory are likely to occur in development.

The time \( t_0 \) after which the axis appears determined is relatively insensitive to assumptions on the external asymmetry. A 10-times shallower external inhibitor gradient or an external gradient of activator rather than inhibitor does not significantly change the time \( t_0 \). On the other hand, the model predicts that rotation by 180° delays the time \( t_0 \).

In the calculation (Fig. 1) growth of retinal tissue during the period of gradient formation has been neglected. Similar results have been obtained with growth included into the formalism. The complex connexions resulting from compound eyes (Gaze, Jacobson & Székely, 1965; Hunt & Jacobson, 1973b) probably require a 2-dimensional treatment of the problem, including perhaps effects of growth, of the injuries produced during transplantation, and of mutual orientation of the gradients.
Fig. 1. Computer simulation of experiments on the determination of an axis of the *Xenopus retina* (Jacobson, 1968; Hunt & Jacobson, 1972, 1973a). Since the retina is initially unpolarized, the source distribution is taken to be uniform. The polarizing influence of the surrounding tissue is modelled as an external source of inhibitor. The initial activator distribution is taken to be uniform and the developing activator distribution is plotted after each 100 iterations. The axis of the host tissue is the same in the figures A-D; the arrow indicates orientation of the retina with respect to the surrounding tissue (left to right: original orientation, right to left: 180° rotation). The moment of eye rotation is indicated by a cleft with an arrow marking the new direction.

A, control without transplantation.

B, after a critical time t₀ (600 iterations, assumed to correspond to embryonic stage 31) a 180° rotation of the tissue does not change the polarity of the activator pattern within the (rotated) tissue.
C, if the rotation takes place earlier (300 iterations, embryonic stage 28), the surrounding tissue reorients the gradient.

D, after 300 iterations, the eye is removed to culture (no external influence). After 300 further iterations 'in culture', the gradient is not reversible upon inverted implantation in the host.

Equation (1) is used in the following form:

\[ da_i = 0.1 a_i^2 h_i - 0.005 a_i + 0.02 (a_{i-1} + a_{i+1} - 2 a_i) + 5 \times 10^{-5} \]

\[ dh_i = 0.5 a_i^2 - 0.007 h_i + 0.45 (h_{i-1} + h_{i+1} - 2 h_i) + 0.005 (1 + 0.001 i). \]

\( i = 1, \ldots, 15 \) is the number of element, the last term describes the external influence on the inhibitor. Control calculations (not shown) lead to very similar results if the growth of the developing tissue is included into the formalism.
Fig. 2. *Internal and marginal activation*. Influence of initial activator distribution (+ --- +) and boundary condition on the final state of activator (-----) and inhibitor (- - - -) distribution plotted in arbitrary units.

A, B, an activated area appears in the centre, if the initial activator distribution has some random fluctuations (A) or a non-marginal peak (B).

C, a small initial peak of activator at one margin leads to a stable activation at the margin.

D, if one allows a slight diffusion of inhibitor across the margins (amounting to 10% of the internal diffusion rate) marginal activation occurs.

Equation (1) with $c = 0.01$, $\mu = 0.01$, $D_a = 0.015$, $c' = 0.005$, $v = 0.015$, $D_b = 0.4$, $\rho_0 = 10^{-4}$, $\rho = 1$ (homogeneous source distribution), 5000 iterations.
**Marginal and Internal Activation**

The position of an activated area within a tissue or cell generally depends on source distributions as well as initial and boundary conditions. If there is no graded source distribution, and the margins are impermeable to morphogens, activated areas preferentially arise internally because in this case inhibitors can diffuse away from activated areas in two directions (Fig. 2A, B). Source gradients, or initial activator distributions favouring a margin, can lead to marginal activation. Depending
on initial condition, the activation occurs at one end (Fig. 2c) or both. However, marginal activation can also be obtained by boundary conditions that permit a slight leakage of inhibitor (Fig. 2d). Thus, in the absence of a source gradient, internal vs. marginal activation depends strongly on special boundary conditions.

A possible example of the influence of marginal areas is provided by Kalthoff & Sander’s (1968) experiment on the effects of u.v. irradiation on developing insect eggs: Irradiation of eggs near the anterior margin can prevent normal anterior-to-posterior organization of the larvae, causing a symmetrical development, with an abdominal region at each end, interpretable on the basis of a symmetrical (Fig. 2a) instead of an asymmetrical (Fig. 2c) morphogen distribution.

Conversion Mechanisms

The generating criterion (see Gierer & Meinhardt, 1972, equations 1, 8, 12 and 13) permits special theories of pattern formation in which the activator and inhibiting agent are not independent of each other but are related by chemical conversion. One such mechanism involves the conversion of the activator into the inhibitor by a chemical alteration. For ranges of parameters consistent with the generating criterion, the following equations describing this type of process lead to pattern formation (Fig. 3a):

\[
\begin{align*}
\frac{\partial a}{\partial t} &= \rho_0 \rho + \rho c \frac{a^2}{h^2} - \mu a + D_a \frac{\partial^2 a}{\partial x^2}, \\
\frac{\partial h}{\partial t} &= \mu a - \nu h + D_h \frac{\partial^2 h}{\partial x^2}.
\end{align*}
\]

(3a)

The term \(\mu a\) describes the rate of conversion of \(a\) into \(h\). It is assumed that the activator activates its own production bimolecularly, e.g. by interaction with an allosteric enzyme, and that the activator can be converted chemically to an inhibitor which inhibits activation, possibly by competitive inhibition of the autocatalytic process. To achieve long range inhibition \((D_h \gg D_a)\), one has to assume that the conversion of activator to inhibitor is accompanied by an increase of the diffusion rate of the molecule (for instance, it may pass more readily through intercellular junctions).

Another conversion model can be obtained if a substance is depleted by conversion into the activator. The following mechanism, for instance, meets the generating criterion for ‘firing’ primary patterns. In activator production, a protein is involved, which has 4 sites at which an allosteric transition to an active state can be co-operatively induced, 2 sites for activator \(a\) and 2 sites for the substrate \(s\). The substrate is consumed by conversion into activator. This leads to the following approximate equations (Fig. 3b):

\[
\begin{align*}
\frac{\partial a}{\partial t} &= \rho_0 \rho + \rho c a^2 - \mu a + D_a \frac{\partial^2 a}{\partial x^2}, \\
\frac{\partial s}{\partial t} &= c_0 - \rho c a^2 s^2 - \nu s + D_s \frac{\partial^2 s}{\partial x^2}.
\end{align*}
\]

(4a)

\(c_0\) constant rate of replacement of substrate \(s\). One has to assume that \(a\) has a short
Fig. 3. Conversion models. Steady-state distributions for A the activator-to-inhibitor-conversion model (equation (3)) and B the depleted substance-to-activator-conversion model (equation (4)). The slight asymmetry in the source distribution $\mu (x)$ ($\Delta \Delta \Delta$) initiates the formation of a strongly asymmetric stable distribution of activator (-----) and inhibitor or depleted substance (- - - -). Total length is divided into 15 elements; the initial distributions of activator and inhibitor are homogeneous. The distribution is plotted after 4000 iterations when the steady state is reached within the limits of accuracy.

Fig. 3A: equation (3) with $c = 0.001, \mu = 0.005, D_a = 0.005, \rho_0 = 0.0, c' = 0.001, \nu = 0.01, D_b = 0.45$.

Fig. 3B: equation (4) with $c = 0.001, \mu = 0.01, D_a = 0.005, \rho_0 = 0.0001, c_0 = 0.01, c' = 0.0001, \nu = 10^{-6}, D_b = 0.45$. 
range due to diffusion and decay, whereas $D_s$ is large and $v$ is small; $s$ equilibrates fast, and derives from a wide area.

It is emphasized that such conversion models are only special cases of the general scheme. In view of their molecular simplicity, they are not unlikely to occur in biological systems.

**Effects of Morphogens on Differentiation, Especially for Steady States in Growing Tissues**

In the theory discussed in this paper, the primary pattern is assumed to be established in a shorter time than that required for effects on source density changes and cell differentiation. This assumption is in accordance with experimental data. In hydra, for instance, it takes only a few hours to determine the position of a new head in a regenerate, whereas polarity reversal of the tissue, which is presumably due to changes in source density, takes much longer (Webster & Wolpert, 1966; Wilby & Webster, 1970). These different time scales justify neglecting, in a first approximation, changes of source density during the firing of the primary pattern. Generally, the primary pattern once fired may cause changes in source density. In some cases, the primary pattern as it first appears may exert its determining influence in a short time interval. In other cases the determining activity may last long, until a steady state is reached with respect to source densities and morphogen distribution.

The (slow) changes of source concentrations can be included into the formalism by adding a corresponding equation, e.g. to equation (1). The simplest relation, assuming $\rho' = \rho$ and neglecting growth as well as any time lapse between morphogenetic effects caused by the activator, and the establishment of new sources, would be:

$$\frac{\partial \rho}{\partial t} = K f(a) - \epsilon \rho,$$

where $\epsilon$ measures the decay of the sources. If a steady state is reached, $\rho = K f(a)/\epsilon$. If $f(a) = a$, $\rho$ would be proportional to the (continuous) activator distribution in the steady state. If $f(a)$ saturates, $\rho$ is more evenly distributed than $a$. If $f(a)$ is a step function, a discontinuous distribution of $\rho$ can result. Molecular models on the gene level for a discontinuous cell response to morphogen concentrations have been proposed elsewhere (Gierer, 1973). A continuous morphogen distribution in a developing tissue can lead to different sections with sharp boundaries.

In cases as described by equation 1 or 2, where the distribution of $a$ is nearly independent of $\rho$, the steady-state distribution of $\rho$ is approximately determined by the initial primary pattern of the activator.

For many cases, growth has to be included into the scheme. An instructive quantitative example of a continuously growing tissue with a patterned steady state of proliferation and differentiation is given by the body column (head and gastric column) of well-fed asexually budding hydra (Campbell, 1967; David & Gierer, in preparation). In a 'mature' animal, the epithelial cells, making up most of the volume in the animal, proliferate at nearly equal rates everywhere in the column. Interstitial
cells, which are stem cells to nerve cells and nematocytes, form mainly nerve cells in the head region, and mainly nematocytes in the gastric column. The interstitial cell pool maintains itself because its rate of proliferation is higher than that of epithelial cells. Interstitial cells are partially depleted in the head area. In the mature animal, about one fifth of the cell production is exported into the tentacles, and four fifths into buds (Campbell, 1967).

Growth is included in the following equation describing the change in source density:

$$\frac{dp}{dt} = K \cdot f(a) - \gamma \rho - \gamma' \cdot (x-x_s) \frac{dp}{dx}.$$  \hspace{1cm} (6)

$x-x_s$ is the distance from the centre of cell movement, one fifth of the total length from the distal end of the column; $-\gamma \rho$ describes the dilution (and possibly decay) of sources during growth of the tissue; the term including $\frac{dp}{dx}$ describes the movement of tissue due to uniform expansion by cell proliferation.

A calculated example for a steady-state distribution of $a$, $h$ and $\rho$ is given in Fig. 4a. $f(a)$ has been taken as proportional to $a$, and $\gamma = \gamma'$. As a result of tissue movement, gradients of $\rho$ can extend far into the gastric region although the activator is nearly completely confined to the head area.

A further refinement of the model is to consider the proliferation and depletion of stem cells. Stem cells of concentration $C$ are assumed to be capable of producing (a) new stem cells $C$; (b) source cells $\rho$, presumably nerve cells in the case of hydra (Schaller, 1973; Schaller & Gierer, 1973), and (c) other differentiated cells such as nematoblasts $n$, according to the following equations:

$$\frac{dC}{dt} = \alpha C \left[ 1 - \frac{\beta_1 C}{1 + \beta_2 C} \left( \frac{a + \beta_4}{1 + \beta_3 a} \right) \right] - \gamma C - \gamma' (x-x_s) \frac{dC}{dx},$$  \hspace{1cm} (7a)

$$\frac{dp}{dt} = \frac{\alpha \beta_1 C^2}{1 + \beta_2 C} \frac{a}{1 + \beta_3 a} - \gamma \rho - \gamma' (x-x_s) \frac{dp}{dx},$$  \hspace{1cm} (7b)

$$\frac{dn}{dt} = \frac{\alpha \beta_2 C^2}{1 + \beta_3 C} \frac{\beta_4}{1 + \beta_3 a} - \gamma n - \gamma' (x-x_s) \frac{dn}{dx}.$$  \hspace{1cm} (7c)

The steady state is plotted in Fig. 4b. The formalism and constants are chosen to lead to preferential differentiation of source cells in the head area and of other cell types such as nematocytes in the gastric column, with a partial depletion of stem cells in the head area; a gradient of source density extends far into the gastric column as required to explain that small sections derived from regions distant from the head retain their polarity upon regeneration. The formalism implies that differentiation of stem cells is regulated by the activator as well as by a compound produced by the stem cells themselves; the ratio of nerve to nematoblast production is regulated by the activator. We emphasize that more data are necessary to choose the optimal formalism for hydra, and that the inclusion of other properties is probably required, such as differences within the nerve cell population, or stem cell migration which would lead to a higher peak of source density in the head area. The example is mainly intended
Fig. 4. Steady state of growth, differentiation and proliferation (hydra). The centre of cell movement is assumed to be the sixth element (arrow); activator (---) and inhibitor (- - -) distributions are plotted after 4000 iterations. A, the rate of source differentiation is assumed proportional to the activator concentration. This, together with growth (equation (6)), leads to a steady-state distribution of sources (X X X X), which is shallower than the activator distribution. Equation (1) with \( c = \circ \text{05} \), \( \mu = \circ \text{0035} \), \( D_A = \circ \text{03} \), \( \rho_2 = \circ \text{00025} \), \( c' = \circ \text{05} \), \( \nu = \circ \text{0045} \), \( D_A = \circ \text{45} \) and equation (6) with \( f(a) = a \), \( K = \circ \text{0005} \), \( \gamma = \gamma' = \circ \text{0005} \). B, the activator is assumed to cause differentiation of stem cells (X X X X) into source cells (X X X X) (probably nerve cells) while inhibiting differentiation into nematocytes (X X X X). On the basis of equation (7), this leads to a depletion of stem cells in the region of high activator concentration. Particularly shallow gradients of \( \rho \) are obtained in the gastric column, because constants are chosen to permit most of the new sources to be produced locally. Equation (1) with \( c = \circ \text{05} \), \( \mu = \circ \text{0035} \), \( D_A = \circ \text{006} \), \( c = \circ \text{05} \), \( \nu = \circ \text{0045} \), \( D_A = \circ \text{45} \), \( \rho_0 = \circ \text{0003} \) and equation (8) with \( x = \circ \text{0025} \), \( \beta_1 = \gamma' = \circ \text{0005} \).

To show how the theory proposed can account for a steady state of growth, differentiation into several pathways, and proliferation, in which a spatial pattern is continuously maintained.

**SECONDARY CENTRES**

If the size of the area of pattern formation exceeds the range of inhibitor, a second region of high activator concentration can occur. In case of tissue growth, a new activator peak will arise at some stage at a distance from the primary activated area; eventually, a periodic structure can arise (Fig. 5). Many examples of such pattern formation are known in biology, e.g. regularly spaced patterns in the blue-green alga *Anabaena*, which have been interpreted on the basis of activation and inhibition by Wilcox, Mitchison & Smith (1973); other examples are leaf formation and branching phenomena in plant development. We will exemplify the principle again with reference to hydra.

In growing hydra, secondary centres occur as buds. Though budding is different from head formation, the 2 structures may have one determinant in common, namely high activator concentration. We assume that a bud is initiated when in a non-terminal part of the gastric region, \( a \) is above a minimal value. The activation in the bud tip
Pattern formation by lateral inhibition

remains at the tip during outgrowth so that the new head can be formed. The activated
tip establishes new sources, and produces inhibitor which contributes to inhibit the
next bud. In this way, one obtains a scheme for bud outgrowth of the type given in
Fig. 6. The first bud occurs closer to the head than the following ones, because it is
inhibited only by the primary head but not by other buds; eventually a steady state of
growth is obtained which defines a budding zone. (See p. 335.)

Fig. 5. Periodic pattern in an outgrowing array of elements. The system is initiated with
2 elements with different activator concentration. Growth is achieved by adding on
each end an element identical to the existing terminal element after each 500 iterations.
The distribution (--- activator, - - - - inhibitor) after 14,000 iterations is plotted.
Equation (1) is applied with \( c = 0.01 \), \( \mu = 0.01 \), \( D_a = 0.001 \), \( \rho_a = 0.0005 \), \( c' = 0.01 \),
\( \nu = 0.12 \), \( D_h = 0.3 \), \( \rho = 1 \).

Pattern formation in two dimensions

In 2 dimensions, one system of an activator and an inhibitor can give rise to a pattern
of peaks of activity nearly equally spaced on a plane, resembling the distribution of
bristles, stomates, etc. A growing field exceeding the range of inhibition will acquire
fairly regular patterns of local peaks of activator in a 2-dimensional field if fluctuations
of source density are introduced, or if the outgrowth of the field is not radially
symmetric (Fig. 7A). Such patterns develop in uniformly growing fields, and in fields
in which growth is restricted to the margins. Under other conditions (e.g. statistical
fluctuations without growth) the pattern of activator peaks is less regular, although
still not totally random, since a certain minimum peak spacing is observed (Fig. 7B).
No gradient of source density is required for these processes.

Possibly, the budding pattern in hydra can be accounted for by the same system of
an activator and an inhibitor which we hypothesize to control head formation. On this
assumption, 2-dimensional model calculations have been made for activator and inhi-
bitor concentrations on the cylindrical surface of a growing hydra. The body column
of hydra has a head on one end covering all angles of the column, with a high con-
centration of activator and inhibitor sources. If, at some distance, local activation
overrides the inhibition which arises mainly from the head, a new activator peak is
formed. For reasons of symmetry, this activation will occur simultaneously at all
angles unless asymmetries are introduced into the model. If there are random fluctua-
tions of local source density \( \rho \) or basic activator production \( \rho_0 \), some random angular
position obtains an advantage in initiating activation, and this can give rise to a local
peak of activity (Fig. 8A, B) to cause outgrowth of the bud in this direction. The inhibition arising from this peak of activity will compel the following bud to arise mainly in the opposite (180°) angular position (Fig. 8C, D), even if no fluctuation exists. Occasionally, 2 buds occur in a zone simultaneously; if this is the case, they are usually opposite, and the next bud will arise at an angle of about 90° (Fig. 8E, F). These theoretical expectations agree with observations.

In contrast to 'bristle' type patterns involving peaks of activity with regularities in their spacing on a plane, other developmental patterns require a distinctly different pattern-forming system for each dimension. For instance, determinations of the 2 axes of the *Xenopus* retina occur independently at different stages of development (Jacobson, 1968). If we define the morphogen patterns as specifying positional information (Wolpert, 1969), each point on a plane should correspond to a unique pair of 2 morphogen concentrations, e.g. activators. In the simplest case, 2 gradients may be formed at approximately right angles. More generally, the concentration of each of 2 morphogens should increase monotonically across the entire field in the dimension defined by constant concentration of the other morphogen.

On the basis of our theory, such separate gradients for each dimension in a 2- (or 3-) dimensional field can be obtained. If the ranges of activator and inhibitor are sufficiently large, so that only one peak of activity develops in the field, a slight asymmetry such as a shallow gradient from a marginal or external source or sink is able to 'fire' a monotonic gradient of activator, resembling a slope (Fig. 9). This type of gradient can define positional information for one dimension in a 2-dimensional field. Further, it may direct processes like cell orientation, cell movement, fibre outgrowth, etc. No internal source gradient is required, but fluctuations of source density are small. Obviously, a second pattern-forming system of this type can produce another gradient specifying positional information in the second dimension. These gradients (Fig. 9A, B) as they appear soon after firing do not change significantly after longer time intervals, even if the initiating asymmetry is removed subsequently. On the other hand, if the range of activator is small, or the geometry of the field more asymmetric, the pattern as it is first fired under an external influence (Fig. 7C) may undergo subsequent changes to reach a stable configuration (Fig. 7D).

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Fig. 6. A simplified model of budding for hydra. Growth is simulated by doubling the fifth element (arrow) after each 50 iterations. The condition for bud initiation is high activator concentration ([3][]) and an inhibitor concentration lower than a critical level. This condition is fulfilled after a second centre of high activator concentration appears in the body column. The corresponding element and subsequently the adjacent tissue is shifted into the bud with a rate corresponding to the growth rate of the body column. This continues until the inhibitor concentration has increased to the critical level. Then growth leads to increase in length until a new centre develops, etc.

Equation (1) with \( c = 0.02, \mu = 0.01, D_a = 0.005, \rho_h = 0.0012, c' = 0.005, \nu = 0.06, \) \( D_a = 0.1 \) and equation (5) with \( f(a) = a, K = 0.002; \epsilon = 0.002; \) numerals on the figure indicate numbers of iterations.
Pattern formation by lateral inhibition

100

150

200

250

300

600

900

1100

1350

Fig. 6. For legend see opposite.
Fig. 7. Peaks of activity in a 2-dimensional field ('bristle' type pattern). A, 2-dimensional array of elements, growing on its margins, develops a regular pattern of peaks of activation. The calculation begins with 4 elements; statistical fluctuation of activator concentrations fires the central peak of activity; the others develop following outgrowth. Growth is simulated by adding new marginal elements after each 400 iterations. The activator and inhibitor concentrations in each new element are set identical to those in the adjacent, previously marginal, elements.

B, peaks of activation developing at a distance from each other, but less regularly spaced as compared to A, are obtained in a non-growing field with initial small statistical fluctuations in the activator concentration.

c, d, in a 2-dimensional field (similar to that in B) the system is initiated by an excess of activator in one element (arrow). At a distance greater than the range of the inhibitor, an activated ring appears (shown in c). This ring is not stable but segregates into stable individual peaks (as in d). If the morphogen acts only during a critical period, it is possible that the unstable ring could induce a ring of differentiated cells. Equation (1) with diffusion term generalized for 2 dimensions with \( c = 0.01 \), \( \mu = 0.015 \), \( D_a = 0.003 \), \( c' = 0.01 \), \( \nu = 0.02 \), \( D_b = 0.15 \), \( \rho_b = 0.003 \).
Pattern formation by lateral inhibition

Fig. 7c and d. For legend see opposite.

SINGLE CELLS AND OSCILLATORY SYSTEMS

The theory of pattern formation discussed here can be applied to single cells as well as to cell systems. In single cells, reactions such as those described by equations 1–4 may occur in the plasma or on the membrane. A slight external or internal gradient may fire a pattern leading to a focus of activity in one part of the cell which in turn may give rise to polar cell development, chemotactic movement in the direction of the external gradient, etc.

In Fig. 10a, a model calculation is plotted in which a cell is represented as a ring; an activated area develops in the direction of an external gradient which causes a difference of only 1% in concentration of activator across the cell, either directly or by indirect chemical mechanisms. The focus of activity can be established within
Fig. 8A–D. For legend see opposite.
A--D, with fluctuations \( r = 0.5 \), an asymmetric secondary centre of high activator concentration appears, which is assumed to cause initiation of a bud. After further growth, a third centre at approximately 180° from the second is established (c). d is the same distribution as c, rotated 90° clockwise to show the arrangement of buds with another perspective.

E, F, lower fluctuations \( r = 0.2 \) can lead to 2 opposite centres. The third then appears perpendicular to the preceding ones. F, as in E, but rotated 90°. Constants used for equation (1): \( \varepsilon = 0.01 \), \( \mu = 0.02 \), \( D_a = 0.005 \), \( \epsilon' = 0.005 \), \( \nu = 0.06 \), \( D_b = 0.15 \), \( \rho_0' = 0.003 \). The calculations are intended to simulate angular relations between subsequent buds. Outgrowth and differentiation (see Fig. 6) are not treated in the 2-dimensional calculations.
Fig. 9. Gradient formation defining an axis (positional information in one dimension) in a 2-dimensional field. Under the influence of a small external source of activator, a 2-dimensional array of elements develops an activated edge. Activation is nearly constant in one dimension and decreases monotonically in the other. Equation (2) is used with $c = 0.002$, $\mu = 0.01$, $D_a = 0.02$, $e_0 = 0.02$, $c' = 0.004$, $\nu = 0.0$, $D_\omega = 0.10$, $\rho = 1.0$ with fluctuation of 0.1%0. The external influence is simulated by: 
$
\rho_a = 0.0012.(1 - 0.001.i); i \text{ is the number of the row (a) or column (b, c)}.
$
A, activator pattern initiated by a weak external gradient of activator derived from an external source behind the field.

B, a second gradient-forming system involving another activator and another depleted substance produces a gradient in the second dimension (left to right) under the influence of a slight initiating asymmetry derived from an external activator source to the left of the field.

C, distribution of the depleted substance in the dimension left to right, Fig. B.
fractions of 1 min with reasonable assumptions about the constants involved in the equation, including the diffusion constant \( D_h \) (0.2 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}) which is the most critical parameter in calculations of this type (Crick, 1970). Once the pattern is established, a change in the direction of the external gradient takes a relatively long time to alter the direction of activation of the cell, even if the steepness of the external gradient is increased 20-fold (Fig. 10B).

Fig. 10. Polar intracellular activation. A cell membrane is represented by a circular arrangement of elements (circle with radial lines). Under the influence of a small external activator gradient (arrow) differing by only 1\% across the cell, a pattern-forming system on the basis of equation (1) produces a highly asymmetric activator distribution with a maximum in the direction of the external gradient.

A, activator (----) and inhibitor (---) distribution around a circular arrangement of 16 elements, measured in each direction by the distance of the lines from the circular element, in this way the figure indicates the direction of the peak of activity.

B, after 4000 iterations, the direction of the external gradient (arrow) is changed, and the gradient enhanced by a factor of 20. The activator-distribution after 20000 iterations (△△△△) is shown to be similar to the original distribution (-----) of figure A. Equation (1) with \( c = 0.1, \mu = 0.001, D_a = 0.002, \epsilon = 0.5, \nu = 0.012, D_h = 0.4, \rho_0 = 10^{-4} \). The influence of the external gradient on each element \( i \) (\( i = 1, ..., 16 \)) is:

\[
d a_i = \rho_0 \left( 1 + 0.005 \cos \left( \frac{2\pi (i-k)}{16} \right) \right),
\]

where \( k \) is the direction of the external gradient (\( k = 1, ..., 16 \)).

In these calculations constants were selected such that inhibitor equilibrates rapidly by assuming high coefficients \( \nu \) for inhibitor degradation, leading to self-stabilizing distributions of morphogens. If \( \nu \) is small, and inhibitor equilibrates slowly, 'overshoots' of activation and, in some cases, periodic pulses of activation are obtained.

Such periodic activation is known to occur in aggregating cellular slime moulds (Bonner, 1947; Shaffer, 1962; Gerisch, 1965). The oscillation of the polar intracellular activation can be simulated (Fig. 11). Since inhibitor decays relatively slowly (Fig. 11 A), oscillations of this type show refractory periods with respect to external stimulation. Stimulation after the refractory period can trigger the next pulse earlier;
Fig. 11. Oscillating distribution of activator and inhibitor appears in the system described in Fig. 10, if the lifetime of the inhibitor exceeds that of the activator.

A, maximal activator (-----) and maximal inhibitor concentration (- - - -), as functions of time.

B, activator (-----) and inhibitor (- - - -) distribution at the indicated number of iterations (time). After 16,000 iterations, the direction of the external gradient (arrow) is changed; the activator distribution adapts to this new direction. Equation (1) is used with $c = 0.01, \mu = 0.0015, D_a = 0.0001, \rho_0 = 10^{-5}, c' = 0.01, \nu = 0.0005, D_b = 0.45$. The influence of the external gradient is taken as in Fig. 9.
there is a delay between stimulation and maximal activation. Cohen & Robertson (1971) have demonstrated that these properties are required for the propagation of waves of activation in cell systems as observed in aggregating cellular slime moulds. There is a functional advantage of periodic activation: the direction of activation of the individual cell can follow more easily a change of direction of an external gradient because each newly formed pulse occurs predominantly in the direction of the gradient shortly before the pulse is formed, while the cell 'remembers' very little of its past direction (Fig. 11B). These calculations are not intended to describe the slime mould system in detail, but to demonstrate oscillations of a polar pattern.

DISCUSSION

The applications of our pattern theory will be discussed separately for the systems considered to exemplify the principles involved, and for the principles themselves.

Hydra

In hydra, models based on the theory account for a steady state of growth and budding. The activator causes differentiation of new activator sources to balance source dilution by tissue growth. An asymmetric steady state of cell differentiation, proliferation and distribution, with a high source density in the head area, can be maintained indefinitely. Shallow gradients of sources extend into the gastric column as expected from studies on polarity upon regeneration. Budding can be described if high activator concentration is a condition to initiate a bud. Regularities of spacing of buds are obtained in agreement with experimental observations.

Xenopus retina

Properties of the developing Xenopus retina can be accounted for on the basis of the proposed theory. Two separate gradients in 2 different dimensions each involving an activator and an inhibitor can be fired separately and at different times to determine 2 axes. No internal source gradient is required if a slight external or marginal asymmetry of sources or sinks is present to fire the pattern, and determine polarity. There is a 'time of no return' after which a transplant is able to maintain its own axis against an opposite host gradient. Even if the explantation is performed before this time the transplant will eventually win if left for some time to itself so that its internal gradient can be enhanced by the autocatalytic mechanisms involved in pattern formation.

Slime moulds

In a single cell a local peak of activity can be obtained on that part of the cell surface pointing in the direction of an external gradient. This occurs even if the gradient shows only 1% concentration differences across the cell diameter. Local cell-surface activation may be important in the directional extensions of pseudopods, and chemotactic movement of cells like cellular slime moulds. If the inhibitory effects do not equilibrate fast, oscillating activation can be obtained. This periodic extinguish-
ing of activation permits rapid adaptation of the direction of cellular activation to changing directions of external gradients.

**CONCLUSION**

*Morphogen interconversions*

We have pointed out that the generating criterion, based on a short-range activation and longer-range inhibitory mechanism, subsumes mechanisms in which (*a*) the activator is chemically converted to inhibitor, the inhibitor having a diffusion rate and decay rate higher than the activator; or (*b*) the activator is produced at the expense of a depleted substance, the depletion constituting the inhibitory effect.

The kinetics of auto- and cross-catalysis these models require could be realized by allosteric enzymes.

*Cell differentiation and growth*

The slow changes of source concentration resulting from morphogen effects (e.g. differentiation) and tissue growth can be treated on the basis of the theory. In special cases, such as a continuously growing and budding hydra, the effects of morphogens on cell differentiation, in conjunction with growth, can render stable steady states involving patterned distributions of cell types, proliferation and differentiation.

*Secondary centres*

Secondary centres of activity can be produced continuously upon outgrowth of a tissue, whether it proceeds by growth in all parts, or at the margin. Periodic patterns may result.

*Multidimensional patterns*

In two dimensions, a pattern-forming system involving an activator and an inhibitor can produce peaks of activity on a plane, with regularities concerning their spacing, especially if local source density fluctuates somewhat, or if patterns develop during growth.

Another type of pattern formation involves separate gradients for different dimensions. They can be produced by separate pattern-forming systems each with one activator and one inhibitor. Each system may be fired independently under the influence of a slight asymmetry which may arise from a marginal or external source. If the range of activator is of the same order as the total size of the field, a monotonic gradient is produced, specifying positional information in one dimension. With 2 gradients of this type, a one-to-one correlation between position on a plane and a pair of morphogen concentrations is obtained.

*Oscillatory activation*

Activation can oscillate if the inhibitory effect does not equilibrate rapidly. The localization of the peak of activity is governed by similar considerations in pulsing
as in stable systems. Periodic extinguishing of activity permits the system to adapt more readily to changing external directional stimuli.

The considerations in this paper are intended to show the rather wide scope of possible applications of the theory of pattern formations based on auto- and cross-catalysis with lateral inhibition, as proposed in a previous paper. On the other hand, it is not claimed that all aspects of primary pattern formation are based on such principles. For instance, gradient formation as a direct consequence of growth as involved in a model by Summerbell, Lewis & Wolpert (1973) on limb formation, or mechanisms depending on the parallel array of polarized cells (Lawrence, 1966) may also give rise to pattern. In the latter case, however, the polarity of the cells themselves may be the result of catalytic mechanisms as discussed in this paper.

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