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A Theory of Biological Pattern Formation

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Abstract

One of the elementary processes in morphogenesis is the formation of a spatial pattern of tissue structures, starting from almost homogeneous tissue. It will be shown that relatively simple molecular mechanisms based on auto- and cross catalysis can account for a primary pattern of morphogens to determine pattern formation of the tissue. The theory is based on short range activation, long range inhibition, and a distinction between activator and inhibitor concentrations on one hand, and the densities of their sources on the other. While source density is expected to change slowly, e.g. as an effect of cell differentiation, the concentration of activators and inhibitors can change rapidly to establish the primary pattern; this results from auto- and cross catalytic effects on the sources, spreading by diffusion or other mechanisms, and degradation.

Employing an approximative equation, a criterium is derived for models, which lead to a striking pattern, starting from an even distribution of morphogens, and assuming a shallow source gradient. The polarity of the pattern depends on the direction of the source gradient, but can be rather independent of other features of source distribution. Models are proposed which explain size regulation (constant proportion of the parts of the pattern irrespective of total size). Depending on the choice of constants, aperiodic patterns, implying a one-to-one correlation between morphogen concentration and position in the tissue, or nearly periodic patterns can be obtained. The theory can be applied not only to multicellular tissues, but also to intracellular differentiation, e.g. of polar cells.

The theory permits various molecular interpretations. One of the simplest models involves bimolecular activation and monomolecular inhibition. Source gradients may be substituted by, or added to, sink gradients, e.g. of degrading enzymes. Inhibitors can be substituted by substances required for, and depleted by activation.

Sources may be either synthesizing systems or particulate structures releasing activators and inhibitors.

Calculations by computer are presented to exemplify the main features of the theory proposed. The theory is applied to quantitative data on hydra — a suitable one-dimensional model for pattern formation — and is shown to account for activation and inhibition of secondary head formation.

Introduction

The development of an organism is a complex phenomenon involving a set of more elementary processes such as gene regulation, alteration of cell shapes and cell to cell interaction, cell proliferation, growth and cell movement. One of these elementary processes in embryology and regeneration is the formation of a spatial pattern of tissue structures. Starting from almost homogeneous tissue, different areas develop strikingly different structures. In some cases, their proportions are regulated to be independent of total size. The pattern may be aperiodic or periodic.

The formation of a morphological pattern is generally assumed to result from a primary pattern (Child, 1941; Waddington, 1962) of morphogen concentrations, or other physical parameters varying in space, often called gradients or fields. Several types of theories have been proposed for this primary pattern: A patterned morphogen distribution can result from auto- and cross catalysis (Turing, 1952). Polar cells may be assumed to pump morphogens in one direction, leading to a graded distribution (Lawrence, 1966). Two periodic events of different wavelengths have been postulated, where the phase difference, which varies in space, is assumed to determine morphogenesis (Goodwin and Cohen, 1969). This paper is concerned with mechanisms of auto- and cross catalysis which are most closely related to known biochemical processes and cellular properties.

Models of differentiation can be constructed by postulating two substances, with mutual interaction on their respective rates of production (or degradation). Depending on initial conditions, this may lead to different stable states, which may represent states of differentiation (e.g. Delbrück, 1949). Spatial differentiation can be achieved by postulating, in addition, different modes or rates of distribution of these substances in space, e.g. by linear equations employing different diffusion terms as proposed by Turing (1952). However, the solutions of the linear system are generally unstable. Non-linear reaction kinetics, on the other hand, are too general to permit simple and straightforward interpretations in terms of molecular biology unless restrictions are imposed by biological considerations.

These restrictions will be introduced by basing the theory on three postulates suggested by fairly general

embryological phenomena: Short range activation, longer range inhibition and a conceptual distinction between effective concentrations of activator and inhibitor, on one hand, and the density of their sources on the other.

Many aspects of morphogenesis can be explained in terms of activating and inhibiting substances. For example, the induction of organs by small transplants can be interpreted as short range activation. The inhibiting action of an organ on the formation of another similar organ in its vicinity can be attributed to a long range inhibition. In most cases, long range inhibition has implications very similar to depletion of a substance (Barth, 1940; Spiegelman, 1945), derived from a wider area, which is necessary for activation.

Short range activation and long range inhibition are not only useful to explain, qualitatively, the *effect* of morphogens, but also the *formation* of morphogenetic patterns, which is somewhat analogous to lateral inhibition in biological cybernetics (Hartline, Wagner and Ratcliff, 1956). Therefore, the hypothesis is being stated that the elementary process in pattern formation may be the formation of a primary pattern of two morphogens, one acting as activator, and one with inhibitory effect, the inhibition being derived from, and extending into a wider area. Activator and inhibitor react auto- and cross catalytically on their sources. Since linear relations will not suffice, non-linear equations have to be postulated.

There is an additional, essential feature of the theory proposed: The densities of morphogen sources (for instance, the concentrations of certain cell types releasing morphogens) are conceptually distinguished from the effective concentrations of the morphogens. There is empirical evidence to distinguish, in morphogenesis, fast processes establishing a primary pattern (for instance head determination in a regenerating hydra occurs within a few hours [Webster and Wolpert, 1966]) from slower processes such as cell differentiation and organ formation. Therefore, densities of sources of activators and inhibitors are assumed to be due to the distribution of cell types, or subcellular structures, which change at a slower rate, probably as a result of differentiation processes, than the production or release of effective activators and inhibitors from established sources which might proceed even in the absence of differentiation. As shown in the following sections, this fast process can lead to striking patterns of activators even with shallow source gradients. While source density is expected to survive sectioning and/or transplantation of a tissue, source activation will be quickly changed. It will be shown that the source density distribution is the main determinant of polarity of a tissue. On the basis of the three postulates, a simple approximative equation will be derived which permits the generation of a variety of theories leading to pattern formation. Simple examples will be given and applied to the results of transplantation experiments on hydra. Finally, the common aspects and the different molecular interpretations of such models are described.

A Method for Generating Simple Theories of Pattern Formation

The approximative equation should lead to patterns of morphogen concentrations even with shallow gradients of source distributions, and nearly even initial distributions of activating and inhibiting substances. Source densities $\varrho(x)$ for activators, and $\varrho'(x)$ for inhibitors, are introduced. Activator concentration is given by $a(x,t)$, and inhibitor concentration by $h(x,t)$. (Instead of inhibitor, the inhibiting effect of depletion of a substance of concentration $s(x,t)$ can be introduced). Slow changes of source density resulting, e.g., from cell differentiation, are neglected in the establishment of the primary pattern of activators and inhibitors. The primary pattern of activators and inhibitors results from synthesis or release by sources, from spreading, and from degradation (or other modes of removal such as leakage into the environment). If synthesis or release of inhibitor depends on local activator concentration as a result of cross catalysis and if inhibitor is assumed to spread and equilibrate fast within a wider area, inhibitor concentration can be approximated as a function of activator concentration, averaged over the mean area from which the inhibitor is derived. It also depends on the mean inhibitor source density $\bar{\varrho}'$ averaged over the area in which inhibitor is produced.

Activator concentration changes according to a rate given by the difference between production and destruction terms. Cross catalytic effects of inhibitor concentration are indirectly described as functions of $\bar{a}$. As a result of auto- and cross catalysis, both the production and the destruction terms are assumed to be dependent on some powers of a and $\bar{a}$. Production rate is considered as proportional to the local activator source density ϱ. These considerations suggest an equation of the following type:

$$\frac{\partial a}{\partial t} \approx \gamma \varrho \frac{a^k}{\bar{a}^l}\left(1 - \frac{\beta}{\varrho}\frac{\bar{a}^n}{a^m}\right) \qquad (1\,a)$$

The effect of $\bar{\varrho}'$ is subsumed by γ and β. To avoid negative values of a, $k > m$.

This equation is useful in generating theories because it can be shown to meet the main conditions of pattern formation if the following inequality holds:

$$n > m > 0. \tag{1b}$$

Assuming a near-even distribution of ϱ and starting with a near-even distribution of a

$$\varrho \approx \bar{\varrho}, \tag{2a}$$

$$a \approx \bar{a} \tag{2b}$$

a will regulate up or down until a pseudo-equilibrium is reached if $n > m$

$$\bar{a} = a_0 = \sqrt[n-m]{\frac{\bar{\varrho}}{\beta}}. \tag{3}$$

However, this solution is neither exact nor stable. If, in a particular region, ϱ and/or a is slightly above average, a will increase. If

$$\varrho = \bar{\varrho} + \Delta\varrho, \tag{4a}$$

$$a = \bar{a} + \Delta a \tag{4b}$$

one obtains

$$\frac{\partial a}{\partial t} \approx \gamma \bar{\varrho} \bar{a}^{k-1} \left(\frac{\Delta\varrho}{\varrho} + m \frac{\Delta a}{a} \right). \tag{5}$$

Starting from an even distribution of a ($\Delta a = 0$), regions of high source density ($\Delta\varrho > 0$) cause an increase of activator concentration $\left(\frac{\partial a}{\partial t} > 0\right)$, which leads to further increase of activator concentration if $m > 0$. Even from an even source distribution ($\Delta\varrho = 0$), a slight local peak of activator concentration ($\Delta a > 0$) would lead to further increase of a. Where a is below average, it will decrease. This mechanism, which will be called the "firing" of a gradient, will alter $\bar{a}$ which codetermines the area of further increase or decrease. After some time, a will be mainly confined to some fraction p of the total area, where a has an average value a^*, whereas a in the remaining area will be small. The site of this region of high activation, and whether it is coherent or distributed, depend on the source distributions, on initial and boundary conditions and on the mode of distribution of activating and inhibiting substances by diffusion or other mechanisms. If the activator source density ϱ forms a gradient, if a is evenly distributed initially and inhibition extends over the entire area, the region of high source density will enhance the production of a and thus "fire" the gradient. In this way a shallow gradient of source density can determine the polarity of the pattern of morphogens.

Generally, $\bar{a}$ will be proportional to a^*, and to a function increasing with p.

$$\bar{a} = \varphi(p)\, a^*. \tag{6}$$

Without any limitation of either a^* or p, the entire activation will concentrate in an infinitely small area, reaching an infinitely large value. With a limitation of either a^* or p, a stationary stage can be reached for the activated area. The relation between a^* and p will be given approximately by $\frac{\partial a}{\partial t} = 0$, Eq. (1):

$$\frac{\beta(\varrho'^*)}{\varrho^*} [\varphi(p)]^n a^{*n-m} = 1 \tag{7}$$

ϱ^* and ϱ'^* are the mean source densities in the activated area. Except for very steep source distributions, ϱ^* and ϱ'^* will be nearly independent of p for $p \ll 1$, rendering β/ϱ^* nearly constant. Thus, Eq. (7) is essentially a relation between p and a^*. Several mechanisms can be proposed to limit p or a^* effectively. If a is subject to diffusion and degradation, limiting its mean area of distribution to d_0, this will generally limit p to some minimum value $\frac{d_0}{L}$ (L total length). This in turn limits a^* [Eq. (7)]. On this assumption, the size of the area of high activator concentration is nearly independent of total size, whereas the amount of activator increases with size as long as total size is within the range of inhibitor. On the other hand, if we postulate a mechanism directly limiting a^* to a_{max} (say by a maximal production rate, or by inhibition of high order), this a_{max} may define p as a constant, thus regulating the area of activation as a *constant* proportion of *total* size (as long as total size does not exceed the area over which inhibition extends). Both assumptions make sense in biological terms for different cases. Other, more indirect limitations of a or p can also be introduced but their effectiveness has to be tested in each case.

Specific Models

On the basis of these considerations, one may construct molecular models, which, for limiting values of their parameters, correspond to the Eq. (1) described above. There should be suitable finite ranges of the parameters, which lead to pattern formation. One-dimensional examples will be calculated in the following section.

a) Depletion Model

One model may be constructed by assuming that the sources of distribution $\varrho(x)$, are activated by

$a(x,t)$, and, in addition, by some substance of concentration, $s(x,t)$, which is consumed by activation or some indirect effect of activation. Further, a basal production of activator proportional to ϱ is introduced. s may be derived from a larger area, being produced everywhere at a constant rate c_0. a and s are removed according to first order kinetics, e.g. by enzymatic degradation. In one dimension this leads to the following equations, where ϱ_0, c and c' are numerical constants, and $f(s)$ is a function increasing with s.

$$\frac{\partial a}{\partial t} = \varrho_0 \varrho + c \varrho a^k f(s) - \mu a + D_a \frac{\partial^2 a}{\partial x^2}, \qquad (8a)$$

$$\frac{\partial s}{\partial t} = c_0 - c' \varrho a^k f(s) - \nu s + D_s \frac{\partial^2 s}{\partial x^2}. \qquad (8b)$$

We now assume that ϱ_0, D_a, ν are small, that s always reaches near-equilibrium values $\left(\frac{\partial s}{\partial t} \to 0\right)$ and that D_s is large enough for s to spread out over the entire area. If ϱ forms a shallow gradient ($\varrho \approx \bar{\varrho}$),

$$f(s) \approx \frac{c_0}{c' \bar{\varrho} \bar{a}^k}. \qquad (9)$$

One obtains

$$\frac{\partial a}{\partial t} \approx \frac{\varrho c c_0 a^k}{\bar{\varrho} c' \bar{a}^k}\left(1 - \frac{c' \mu \bar{\varrho} \bar{a}^k}{c c_0 \varrho a^{k-1}}\right) \qquad (10)$$

which meets the condition of Eq. (1), $k > k-1 > 0$, assuming k to be an integer, if $k \geqq 2$. The resulting activator pattern is nearly independent of absolute source density.

The simplest version of this equation would be

$$\frac{\partial a}{\partial t} = \varrho_0 \varrho + c \varrho a^2 s - \mu a + D_a \frac{\partial^2 a}{\partial x^2}, \qquad (11a)$$

$$\frac{\partial s}{\partial t} = c_0 - c' \varrho a^2 s - \nu s + D_s \frac{\partial^2 s}{\partial x^2}. \qquad (11b)$$

b) Activator – Inhibitor Models

Another model rests on the assumption that there is an activator, $a(x,t)$ and an inhibitor, $h(x,t)$, acting on sources of activators and inhibitors having distributions $\varrho(x)$ and $\varrho'(x)$, respectively. For the sake of simplicity we assume that activation and inhibition of sources are functions of some powers of a and h. This approximation is consistent with many types of reaction kinetics for suitable ranges of parameters. In addition, we assume that a and h are removed by first order kinetics either by enzyme degradation, or leakage, or re-uptake by the source, or by any combination of such mechanisms, and that h diffuses faster than a (a and h having diffusion constants D_a and D_h, respectively). To initiate the system, we postulate a basal production of activator proportional to ϱ.

Thus we obtain:

$$\frac{\partial a}{\partial t} = \varrho_0 \varrho + c \varrho \frac{a^r}{h^s} - \mu a + D_a \frac{\partial^2 a}{\partial x^2}, \qquad (12a)$$

$$\frac{\partial h}{\partial t} = c' \varrho' \frac{a^t}{h^u} - \nu h + D_h \frac{\partial^2 h}{\partial x^2}. \qquad (12b)$$

With the approximation that ϱ_0 and D_a are small; that h equilibrates fast; and that D_h is big enough to ensure nearly equal distribution of h over the entire area, Eq. (12) leads approximately to the following relation:

$$\frac{\partial a}{\partial t} \sim \varrho \frac{a^r}{\bar{a}^{\frac{st}{u+1}}}\left(1 - \frac{\beta}{\varrho} \frac{\bar{a}^{\frac{st}{u+1}}}{a^{r-1}}\right). \qquad (13a)$$

This again is the standard Eq. (1) which will "fire" a gradient if

$$\frac{st}{u+1} > r - 1 > 0. \qquad (13b)$$

Thus, r must be at least 2 if it is an integer.

If we postulate similar or common sources for a and h ($\varrho = \varrho'$, $r = t$ and $s = u$), one of many possibilities would be $r = t = 2$; $s = u = 4$, leading to an

Activator – Inhibitor Model with Common Sources:

$$\frac{\partial a}{\partial t} = \varrho_0 \varrho + c \varrho \frac{a^2}{h^4} - \mu a + D_a \frac{\partial^2 a}{\partial x^2}, \qquad (14a)$$

$$\frac{\partial h}{\partial t} = c' \varrho \frac{a^2}{h^4} - \nu h - D_h \frac{\partial^2 h}{\partial x^2}. \qquad (14b)$$

If we permit activator and inhibitor sources to be different ($s \neq u$), there are particularly simple versions with $u = 0$, such as $r = s = 2$; $t = 1$; or $r = t = 2$; $s = 1$. The latter case will be given explicitly as an example of an

Activator – Inhibitor Model with Different Sources:

$$\frac{\partial a}{\partial t} = \varrho_0 \varrho + c \varrho \frac{a^2}{h} - \mu a + D_a \frac{\partial^2 a}{\partial x^2}, \qquad (15a)$$

$$\frac{\partial h}{\partial t} = c' \varrho' a^2 - \nu h + D_h \frac{\partial^2 h}{\partial x^2}. \qquad (15b)$$

The activator activates both the sources of activators and inhibitors, whereas the inhibitor inhibits only the activator sources. Two molecules of activator are necessary to activate, and one to inhibit a source. Instead of two molecules of the same type, a minor

extension of the formalism would lead to closely similar results if two different activating molecules were required. It can easily be shown that the activator pattern is nearly independent of absolute source density if ϱ' is proportional to ϱ.

This example will also be chosen to introduce an explicit limitation of a in order to obtain a nearly constant activated proportion p [Eq. (7)] within a given size range. This can be done, for instance, by assuming a saturation of activator production, substituting a^2 by $\frac{a^2}{1+\kappa a^2}$; in this case, a will saturate if $a^2 \approx \kappa$. The inhibiting effect is taken as non-competitive. We may write:

$$\frac{\partial a}{\partial t} = \varrho_0 \varrho + \frac{c \varrho a^2}{h(1+\kappa a^2)} - \mu a + D_a \frac{\partial^2 a}{\partial x^2}, \quad (16a)$$

$$\frac{\partial h}{\partial t} = c' \varrho' a^2 - \nu h + D_h \frac{\partial^2 h}{\partial x^2}. \quad (16b)$$

Similar or different limitations can be introduced into the other equations as well, depending on the molecular mechanism assumed.

In all cases mentioned, the source gradients $\varrho(x)$ and $\varrho'(x)$ can be substituted by, or added to, graded distributions of sinks $\mu(x)$ and $\nu(x)$ to "fire" the pattern.

While the theory is mainly proposed with regard to morphogenesis of multicellular organisms, it may be applied as well to intracellular morphogenesis, e.g. the production of polar cells, and to the responses of cells to external gradients by orientation or directed movement. Any of the equations mentioned can be used for this purpose, such as the bimolecular activation/monomolecular depletion mechanism described by Eq. (11) except that the one-dimensional approximation will not usually suffice. In this way, activation can be confined to part of the cell or its membrane, to explain for example polar differentiation. Further, the location of the activated area can be strongly influenced by external gradients, to account for cell orientation or directed cell movement.

These examples may suffice to illustrate the method of constructing molecular models. It must be emphasized that regulative properties can be expected only for certain choices of the range of parameters, and each case must be tested for whether the orders of magnitude are reasonable in molecular terms.

Examples of Basic Properties of Pattern Formation

To demonstrate basic properties of pattern formation which can be obtained with the equations given in the preceding sections, examples have been calculated by computer for a number of situations. A shallow gradient of source distribution can lead to a striking pattern of activator concentration, with a maximum at the terminal of high source density, for the depletion model Eq. (11) (Fig. 1 a), the activator – inhibitor model assuming common sources Eq. (14) (Fig. 1 b), and the simple activator – inhibitor model with different sources Eq. (15) (Fig. 1 c). Thus, the source density distribution determines the polarity of the pattern. The last model [Eq. (15), Fig. 1 c] has been used for the following calculations. For the sake of simplicity, ϱ' is taken as proportional to ϱ.

Subsections of (c) give patterns which retain polarity (Fig. 1 d, e). Slight random fluctuations in source density are smoothed out by the diffusion mechanisms and do not strongly influence the activator pattern (Fig. 1 f). Even if source gradient and initial activator gradient have opposite signs, the source gradient shifts the activated area to regions of higher source density (Fig. 1 g) (a shift to the margin of highest source density occurs if steeper source gradients are chosen). A small step at one end suffices to determine the polarity of the pattern (Fig. 1 h). Comparison of Fig. 1 c, f, and h shows that the activator pattern is not strongly dependent on mean source density. If the range of inhibition is reduced, nearly periodic patterns may be obtained (Fig. 1 i). In these calculations, boundaries were assumed inpermeable to activators and inhibitors. For uniform or shallow source distributions, other boundary conditions would influence the results, in particular the preference for internal or terminal positions of activated regions.

If the region of high activation is assumed to define the size of a tissue structure, there will be cases in biology where organ size is independent of total size, or is a constant fraction of total size. Intermediate cases may also occur. If the size of activated area is mainly determined by diffusion and decay of activator as in the case of Eq. (15), it is fairly independent of total size (Fig. 1 c, d, e). Of particular interest is the possibility of adapting the region of activation to total size. This size regulation can be obtained, as discussed in the preceding section, by limiting activator concentration to a maximal value, e.g. by Eq. (16). A simple molecular interpretation would be, for instance, a limited capacity of sources for activator production. A suitable choice of constants leads to "normalisation" of the pattern in subsections (Fig. 1 k, l, m), the activated area forming an approximately constant proportion of total size within certain limits. This relatively easy and straightforward explanation of what Wolpert (1969) called the "French flag problem" of morphogenesis is a feature of the theory proposed.

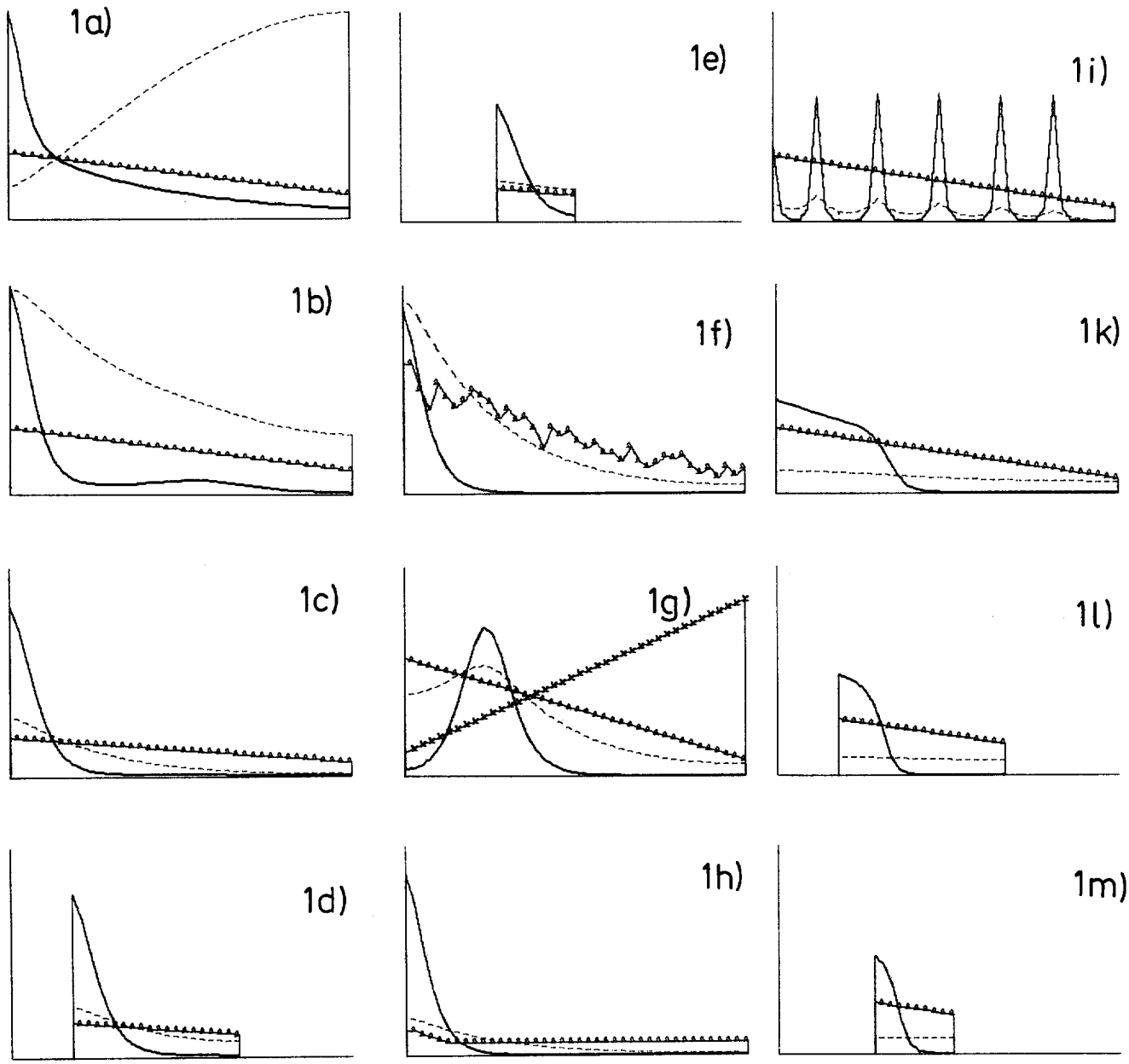

Fig. 1. Basic properties of pattern formation. Assuming a (shallow) gradient in source density, ΔΔΔ and starting with an uniform distribution of activator and inhibitor, one obtains a strongly polar pattern of activator ——, with high activator at the terminal of high source strength. ----- depleted substance (Fig. 1a) or inhibitor (Fig. 1 b–m). a Depletion model [Eq. (11)]. b Inhibition acts both on activator and inhibitor production or release [Eq. (14)]. c Simple inhibition model [Eq. (15)]: Inhibition acts on activator but not on inhibitor production or release. The following calculations are based on Eq. (15) (activator-inhibitor model) unless stated otherwise. d, e Subsections derived from (c) develop a pattern of the same polarity as (c). f Small random fluctuations of source gradient do not change the pattern significantly. g Source gradient shifts activated area to regions of higher source density even if initial activator gradient ×–×–× has opposite sign. h A small source density peak at one end suffices to determine the polarity of the pattern. i Reducing the range of inhibition can lead to a nearly periodic pattern. k–m Size normalisation can be achieved by limiting activator production, Eq. (16): k Distribution of activator and inhibitor in total area; l, m distribution in subsections. Activated area is approximately proportional to total size. Calculations: Total length was divided into 40 segments. Source distributions as plotted ($\varrho' = \varrho$, full scale corresponds to $\varrho = 3.2$). The following constants were used: (a) $\varrho_0 = 0.01$; $c = 5 \times 10^{-5}$; $\mu = \nu = 0.0025$; $D_a = 0.001$; $c_0 = 0.02$; $c' = 1.5 \times 10^{-4}$; $D_s = 0.45$. (b) $\varrho_0 = 6 \times 10^{-4}$; $c = 0.05$; $\mu = 0.005$; $D_a = 0.01$; $c' = 0.025$; $\nu = 0.001$; $D_h = 0.45$. (c)–(h) as (b), except: $\mu = 0.0035$; $\nu = 0.0045$. (i) as (b), except: $\mu = 0.01$; $D_a = 0.001$; $\nu = 0.01$; $D_h = 0.04$. (k)–(m) as (b), except: $\nu = 0.0075$; $D_h = 4.5$; $\kappa = 0.1$. Initial conditions $h = 5$; (a)–(h): $a = 0.1$; (i)–(m): $a = 1.5$; (a)–(h): 2000 iterations, (i)–(m): 3000 iterations were calculated to reach a stable distribution

An Application to the Primary Gradient of Hydra

To apply the theory to quantitative data, a particularly instructive set of transplantation experiments on hydra will be chosen which have been performed by Wolpert, Hicklin and Hornbruch (1971) and by Wilby and Webster (1970). Hydra is an animal a few mm in length, consisting of about 100000 cells of about 15 different types, with a polar structure which can be described, distal to proximal, by tentacles, hypostome (*H*), gastric regions 1–4, budding area (*B*), peduncle (*P*), and basal disk (*D*) (Fig. 2a). Gastric regions usually regenerate a new animal having the same polarity. The area forming the new head in a regenerate is determined within a few hours. On the other hand, reversal of tissue polarity by transplanting a head from the distal to the proximal end is a very slow process. Even after days, stem sections mostly regenerate according to their original polarity (Wilby and Webster, 1970). According to the theory outlined, the polarity of the tissue H1234 is described by a source density distribution which is high in the head, and extends into the gastric region. The particular shape of the assumed source distribution is not critical. The assumed values (Fig. 2) are consistent with evidence that polarity is related to the nerve cell distribution (Bode *et al.*, 1972) and with quantitative data suggesting that particulate structures in nerve cells contain substances, which, at very low concentrations, stimulate head formation and may be involved in the primary morphogenetic gradient (Schaller, 1972; Schaller and Gierer, 1972).

Eq. (15) was chosen for computer calculations because of its simplicity. A set of constants have been chosen to give the following set of results (Fig. 2), interpreting high activator concentrations as leading to head formation:

A striking pattern with a coherent region of high activator concentration in and near the head region is obtained (Fig. 2a). Subsections of the gastric region give rise to a similar striking distribution of activator in spite of the shallow source gradient, the polarity being retained (Fig. 2b).

The following results are in agreement with the transplantation experiments by Wolpert *et al.* (1971) and Wilby and Webster (1970). A transplant 1/1234 gives only one area of high activation (head) at the distal end (Fig. 2c). A transplant 12/1234 rise to two heads (Fig. 2d). The second head is inhibited if the head at the distal end is present: H 12/1234 does not develop a second head (Fig. 2e). However, the head at the distal end does not succeed in inhibiting the formation of a second head if the transplanted Section 1 is further away: H 123/1234 can develop a second head (Fig. 2f).

Transplantation of head from distal to proximal end 1234/H gives rise to a head at the 1 end (Fig. 2g). This does not happen if a head is transplanted to the 4 end (Fig. 2h) before the original head is removed from the 1 end. In this case, inhibition has had sufficient time to spread from the transplanted head to the 1 area to inhibit formation of a second head (Fig. 2i). Original polarity is retained if section 234 is excised and allowed to regenerate (Fig. 2k).

The theory, thus far, accounts for the decision whether or not a secondary head is formed upon transplantation. Calculations on other aspects of hydra morphogenesis, like the slow changes in source distribution, the effects of growth and two dimensional applications to the process of budding, are in progress.

Concerning the interpretation of Eq. (15), the formal diffusion rate D_h for the inhibitor has been assumed to be about 2.5×10^{-6} cm^2/sec in these calculations (Fig. 2). This is the same order of magnitude as estimated by Crick (1970) (0.8×10^{-6} cm^2/sec), and leaves open the question whether molecular diffusion suffices to account for the spread of inhibition, or whether other mechanisms like convection, active transport etc. have to be inferred.

The theory of this section is consistent with the concept that activation proceeds by the release of small amounts of activating substances from particulate structures (Schaller and Gierer, 1972) by mechanisms in which the released substances themselves influence further release. Since the amount of activating substance in a hydra is about 1000 times greater than that required for activation (Schaller, 1972), and the decay time is of the order of 1% of a generation time, sources are not strongly depleted during the short time required to establish a morphogenetic pattern of the activator.

A molecular interpretation of Eq. (15) would be that two (equal or different) molecules are required, directly or indirectly, to cause the (discrete and continuous) release of activators, and inhibitors, from particulate structures. For instance, two activators may interact with receptor molecules in an allosteric manner to render vesicles permeable to activators or inhibitors contained in them. One molecule of inhibitor may be assumed to prevent this release as far as the activator is concerned.

It must be emphasized that Eq. (15) has been selected for its simplicity and straightforward interpretations. More parameters may be necessary for a further development of the theory. Other sets of equations consistent with Eq. (1) might fit the data equally well. Therefore, any particular model can be proven only by biochemical methods, and not by kinetic considerations per se.

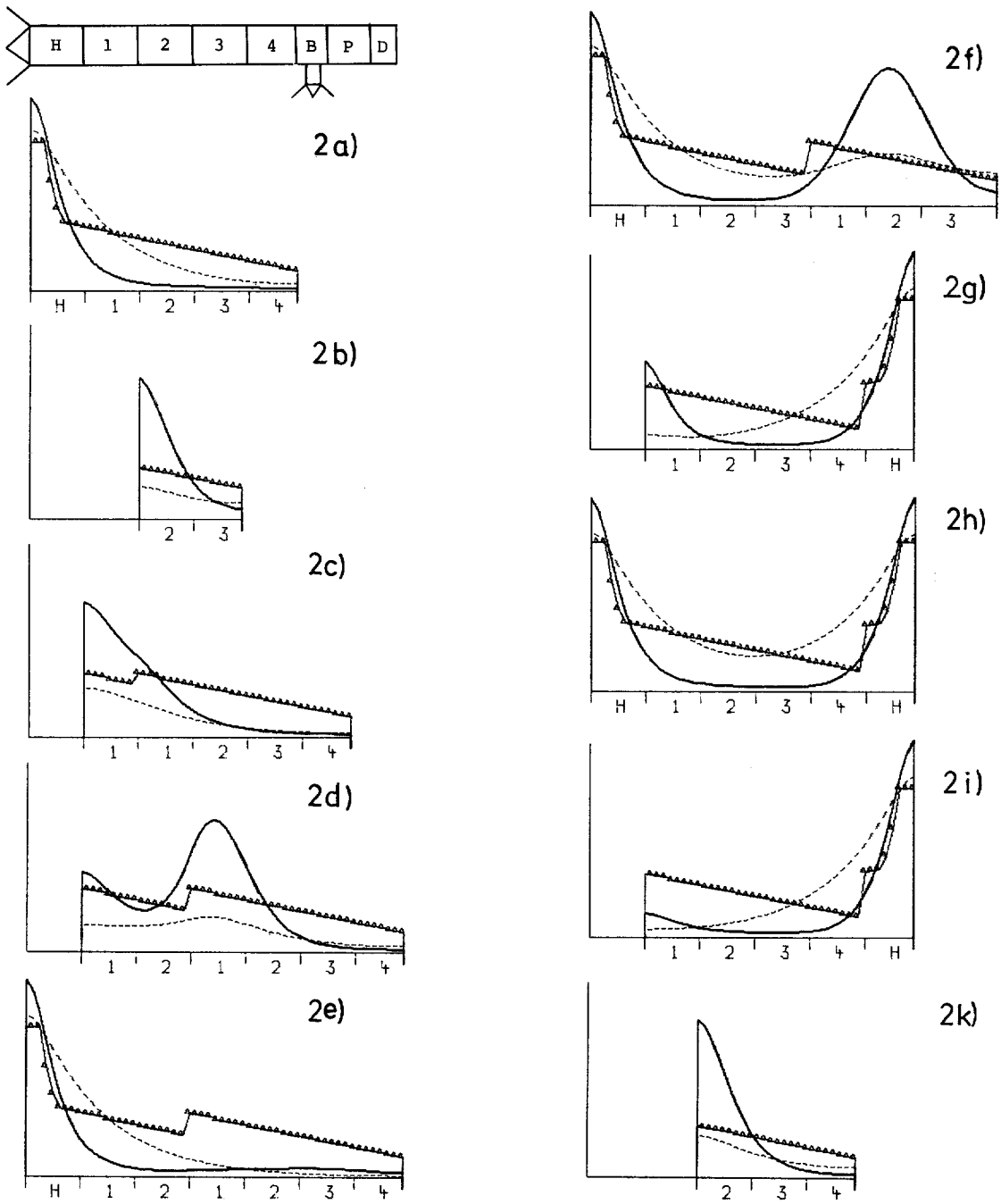

Fig. 2. Head activation upon regeneration and transplantation of hydra sections. a Schematic representation of hydra; notation of sections H 1234; assumed source distribution ^^^^. Activator ——— and inhibitor – – – – concentration according to Eq. (15). In Fig. b–k, high activation is taken as inducing head formation. b "Regenerating" cut section 23; distal area 2 forms a head. c Graft 1/1234: Only distal area forms a head. d Graft 12/1234: A secondary head is formed. e Graft H 12/1234: H inhibits secondary head formation. f Graft H 123/1234: H does not inhibit secondary head formation. g Grafting head from 1 to 4 terminal: A second head develops. h Second head is transplanted to 4 terminal. i Original head (Fig. h) is cut after 10 hours: No second head develops at 1 terminal. k Cut section 234 (Fig. i) develops head at the (distal) 2 terminal: Polarity is retained. Calculations: Region H 1234, approximately 4 mm in length, is represented by 40 segments. After the cut 23 (Fig. 2b), about 800 iterations were necessary to fire the gradient of the activator. This presumably corresponds to the time required for head determination in hydra, — in this case about 4 hours. Thus, one iteration corresponds to approximately 0.3 min. 2000 iterations were calculated to reach the plotted nearly stable distributions. The following constants were used: $\varrho_0 = 7.5 \times 10^{-4}$; $c = 0.05$; $\mu = 0.0035$; $D_a = 0.03$; $c' = 0.025$; $\nu = 0.0045$; $D_h = 0.45$. This value of D_h corresponds to 2.5×10^{-6} cm^2/sec. Source distributions as plotted ($\varrho = \varrho'$, full scale corresponds to $\varrho = 3.2$)

Discussion

As shown in the preceding sections, models of – perhaps unexpected – simplicity based on auto- and cross catalysis can account for basic properties of pattern formation. Short range activators and long range inhibition acting on their sources lead to pattern formation if certain criteria, consistent with a wide range of molecular mechanisms, are met. Striking patterns arise from even slightly graded source distributions. The gradients are self-regulating. The polarity of the pattern is dependent on the direction of the source gradients, but the pattern itself can show little dependance on other details of source distribution. Patterns with constant sizes of one part irrespective of total size, or with constant size ratios of all parts can be obtained; patterns may be aperiodic or nearly periodic. Such properties are required to account for main aspects of biological pattern formation. The theory may be applied not only to multicellular tissues, but also to differentiation within cells such as the egg cell or polar cells, and to directed cell responses such as chemotactic movement.

The theory proposed permits different versions and interpretations. Source gradients may be replaced by, or added to, sink gradients. Inhibition may be substituted by depletion. Sources may be either synthesizing systems, or particulate structures releasing activators and inhibitors. Sinks may operate by enzymatic degradation, or leakage, or re-uptake by particulate structures. Spreading in space, formally described by a diffusion term, may be due to molecular diffusion or enhanced by other mechanisms like convection. It will be difficult to reach a decision between alternative models by kinetic analysis per se. In most cases, biochemical evidence will be required, such as the evidence supporting release models for an activating substance in the case of hydra. In no case is there a requirement for very complex model assumptions at this stage. A simple version of the release model, for instance, is bimolecular activation, and monomolecular inhibition of the release.

The theory proposed is related to concepts developed by Wolpert (1969), Lawrence (1966), and Crick (1971). Lawrence postulated a maximum slope for morphogen gradients. In the theory proposed here, the slopes of the gradients of free morphogens are limited by diffusion and decay. The stable property defining polarity and surviving transplantation is introduced in the pumping model (Lawrence, 1966) as direction of pumping and in the theory of Crick (1971) as a homoestatic process maintaining morphogen concentrations, whereas in our theory the stable property is source density. Only extensive calculations could decide whether theories of the type proposed here would be able to account for the very informative results on the insect cuticle (Lawrence, 1966), and which special features (e.g. sinks and secondary gradients) have to be introduced. Wolpert (1969) has interpreted the transplantation experiments on hydra by proposing a non-diffusible property P which is high in the head area, and an inhibitor derived from head areas. If P exceeds inhibition, it is adapted to reach maximal value. The theory proposed in this paper makes use of inhibition in a similar manner. "Firing" of the gradient if inhibitor is weak is an intrinsic consequence of our theory and requires no additional assumption. Burnett (1966) has proposed a model in which a long range activator is postulated. This parameter is not consistent with the free diffusible short range activator in the theory described in this paper, but it is related to the (non-diffusible) source density.

The theory outlined here produces a "primitive" gradient, leading to one-to-one or nearly periodic correlations between morphogen concentrations and relative position in a tissue. Embryonic development involves a long (branched) series of morphogenetic gradients implying that there are many such primitive gradients in time and space. A few remarks will be made on problems arising from the effects and the combinations of such gradients in the course of development.

Morphogens are expected to act on cell differentiation, cell migration, boundary formation, tissue evagination, growth etc. The result of these processes, which will generally be slower than the "firing" of the gradient, will alter the gradient itself by changing source and sink densities as well as distances and other parameters. As for the effects of morphogens, there is no reason why the inhibitor should not activate, or the activator inhibit some processes. If morphogens act on differentiation of multipotent cells, it is evident that activation of some processes implies inhibition of others. The terms activator and inhibitor refer only to their action on morphogen sources, and indirectly on activation or inhibition of the establishment of such sources, but not to their mode of action in general. It is possible that activated areas synthesize or release additional morphogens, with ranges differing for both activator and inhibitor. As long as they do not feed back on the sources, they do not affect the theory of formation of the primary gradient. Moreover, there could be more than one independent gradient system within one dimension. In hydra, for instance, foot formation seems to be based on an independent regulating system (MacWilliams, Kafatos and Bossert, 1970). It is possible, that a secondary gradient modifies the primary gradient,

for instance, by employing a different activator but the same inhibitor, or by causing a sink to be established for products of the primary gradient. Two or three dimensional patterns represent a special problem: A second dimension may use the same or a different set of activators and inhibitors, or the same inhibitor but a different activator, or vice versa.

Following the establishment of new sources and/or sinks in a pattern as a consequence of morphogen action, in conjunction with growth, new gradients may be fired in subsections, leading to more refined patterns. If one assumes that the same morphogens have different effects on different cell types, and can be fired and extinguished in the course of development, gradients need not be different biochemically if they occur at sufficiently different times, or locations. Therefore, a rather limited set of activators and inhibitors would suffice to form all gradients in the course of development of a complex organism.

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