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A MORPHOGEN GRADIENT MODEL FOR PATTERN REGULATION. I. FORMATION OF NON-REPETITIVE AND REPETITIVE STRUCTURES

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A model for pattern formation is proposed based on two concentration gradients S and Σ. S is a local morphogen generated by a reaction–diffusion mechanism while Σ is a by-product of the S-decomposition. Under certain conditions S is well approximated by $S(x, L) = \alpha(L) f(x/L)$, where $\alpha(L)$ is a scaling function of the length L and $f(x/L)$ is a monotonic function of the relative distance x/L from the origin. Σ degradates and diffuses in the field, reaching a stable L-dependent homogeneous distribution. An allosteric protein P with several active sites reacts with S and Σ and separates the field into segments. To every segment a corresponding active state of P is dominant. Pattern regulation is automatically achieved since the compartmental separation depends explicitly only on x/L. For the case of repetitive patterns, a supplementary Gierer–Meinhardt mechanism is introduced for activator X and inhibitor Y. The level of Σ can affect the decomposition rate of X or Y, e.g. via a second order degradation reaction, hence making the chemical wavelength λ size-dependent. For a particular decay scheme of Y, a variation of L induces a change of λ so that finally the number of repetitive structures becomes independent of the field size.

1. Introduction

One central problem of Developmental Biology is to determine the mechanisms which control cell differentiation during Embryogenesis. The concept of a gradient initiating pattern formation is old enough and constitutes the working hypothesis for the understanding of many features of Morphogenesis. (For a survey, see Cooke [1].)

How a gradient is created remained an unanswered question until the pioneering theoretical work of Turing [2], where it was shown that concentration inhomogeneities can be established in a initially homogeneous medium. Some hypothetical substances that he named morphogens are evenly distributed in the field in a state of unstable equilibrium. If these morphogens react between themselves in an appropriate way and at the same time diffuse in the medium with suitable speeds, the system ceases being homogeneous and can approach a stable state where the morphogens are unevenly distributed. Turing gave analytic solutions for the possible final states, but in order to do that, he made simplifying assumptions concerning the chemical reactions involved. In the set of coupled differential equations he approximated the kinetics term with linear expressions of the morphogen concentrations.

In the framework of Turing's theory, Gierer and Meinhardt have put forward a model with chemical kinetics inspired by well established biochemical mechanisms [3]. Analytic solutions cannot be written down anymore and only with computer simulations one estimates the form of the final morphogen distributions. These distributions possess however the stability (not shared by the analytic solutions of Turing's work) which is necessary for the onset of molecular differentiation [4]. Reaction–diffusion theory can explain a wide variety of phenomena in Embryogenesis (see e.g. the applications of Gierer–Meinhardt model [5] or the sequential formation of compartments in Drosophila according to Turing's linear model [6]).

Regulation is a characteristic feature of Morphogenesis. In normal development as well as in many regeneration experiments, cell-to-cell communication leads to the formation of the same pattern adapted to the size of

the morphogenetic field [1]. Reaction–diffusion theory fails to produce inhomogeneities (monotonic or periodic) which vary with size in a manner consistent with regulation [7–9]. This severe limitation of the theory is due to the inherent scale imposed by the diffusivities of the morphogens. In view of this weakness and despite of the striking successes of Turing's theory, several other morphogenetic models have been proposed [7,8]. With regulation built-in, these models are based on quite different mechanisms the nature and origin of which remain unclear. (For a critical review of some models see [9,10].) We think that it would be of some value if, without abandoning the well founded and successful reaction-diffusion theory, one could invent some procedure to be associated with the theory and finally obtain pattern regulation. In the present work we formulate such a mechanism for both non-repetitive and repetitive structures.

In section 2 we start with a Gierer–Meinhardt model and establish a global morphogen distribution whose uniform level depends on the length of the field. We then indicate how a size-independent monotonic gradient is formed.

In section 3 a biochemical scheme is introduced which adapts a pattern to the size of the field.

In section 4 we extend the model so that it can be applied to repetitive patterns and produce constant number of identical structures in fields of variable size.

Finally some general conclusions are drawn in section 5.

2. Local and global morphogen gradients

The pattern regulating method we are going to present can be incorporated into the general reaction-diffusion theory. We restrict ourselves however to the formulation of Gierer and Meinhardt because of the extensive and successful application of this model to diverse developing systems [3,5]. According to the more common version of the model, in a linear field of length L at every point x morphogens A and S are involved in an auto- and cross-catalytic set of interactions. Taking also into account the morphogen diffusion in the field, the rate equations for A and S take the following form:

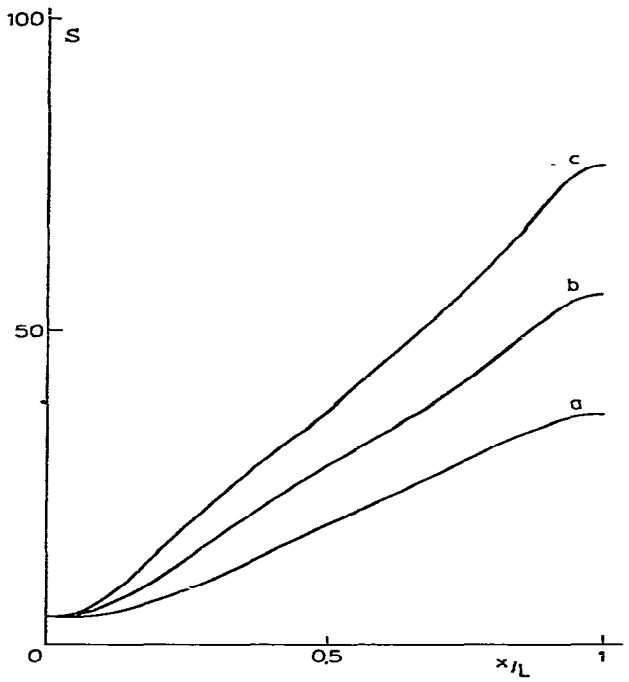

Fig.1 . Final concentrations of S (in arbitrary units) as derived from eqs. (1) for different field lengths L: a) $L = 40$ cells, b) $L = 60$ cells, c) $L = 80$ cells. The parameter values are: $c_1 = 0.05$ $c_2 = 0.0035$, $c_3 = 0.0006$, $c_4 = 0.025$, $c_5 = 0.0045$, $D_A = 0.05$, $D_S = 0.10$, $\rho(x) = 0.1 + 0.02x$. Initial values: $A = 0.1$, $S = 5.0$. On the average, stable distributions are reached if 6.000 iterations are performed.

$$\frac{\partial A}{\partial t} = c_1 \frac{A^2}{S}\rho(x) - c_2 A + c_3\rho(x) + D_A \frac{\partial^2 A}{\partial x^2},$$
$$\frac{\partial S}{\partial t} = c_4 A^2 \rho(x) - c_5 S + D_S \frac{\partial^2 S}{\partial x^2}. \qquad (1)$$

c_1 and c_4 are parameters for the auto- and cross-catalytic production of A and S while c_2 and c_5 determine the first order kinetics for the degradation of A and S. Morphogen A is independently produced by a shallow source $\rho(x)$ with a rate constant c_3 while D_A and D_S are the diffusion constants for A and S respectively. The solutions of eqs. (1) can be derived numerically only. Their qualitative asymptotic behaviour depends critically on the values of parameters involved. We are interested in the formation of stationary waves with characteristic wavelength λ. Without making any assump

tions about preexisting uneven source distributions, Babloyantz and Hiernaux [11] have analyzed the stability properties of the solutions of eqs. (1). They derived morphogen distributions similar to the ones obtained by Gierer and Meinhardt.

For the moment we consider the case where only monotonic gradients are formed along the field. In section 4 the case is considered where $L \gg \lambda$ and several concentrations peaks are contained in the field. Because we always assume $D_A < D_S$, the range of A is smaller than the range of S. When all other parameters are kept fixed, we find qualitatively that λ becomes bigger when D_A is increased (but always kept smaller than D_S).

Some characteristic gradients of S are plotted in fig. 1 as derived from eqs. (1). The size of each cell is fixed in arbitrary units and the length of the field varies according to the number of cells along the line. Concentration S is plotted in arbitrary units against relative distance x/L from the origin, for lengths of 40, 60 and 80 cells on the line. For all three lengths the S-distributions are smooth sigmoid-like curves multiplied by an overall size-dependent factor. We are therefore justified to suggest that, for an appropriate choice of parameters, S is well described by an analytic expression of the form

$$S(x, L) = \alpha(L) f(x/L) . \tag{2}$$

Here $f(x/L)$ is a monotonic function of x/L and $\alpha(L)$ a scaling factor depending on L. In this description, S is a *homogeneous* function of x and L in which case $\alpha(L)$ is not arbitrary but it is of a power form [12]

$$S(x, L) = L^p f(x/L) . \tag{3}$$

From fig. 1 it turns out that the exponent p in (3) is close to 1.

In the framework of positional information theory [13], a concentration gradient can naturally be used as a positional signal for every cell. For the formation of a faithful pattern, it is necessary however that, when scaled to the total length, the shape of the gradient should be identical for all fields independently of the field size. From fig. 1 or the analytic expression (3), it is clear that S cannot reliably serve this purpose. The failure is characteristic of the theory as explained in the Introduction. Gierer and Meinhardt [3] obtain a kind of regulation with a version of their model based on saturation of activator production. The field is separated roughly by a step-like gradient into two domains. Cells can then differentiate to one between two distinct states. A gradient of this form however, is inadequate to inititate differentiation towards a state among a set of more than two final cell-types. For such a multiple choice it is indispensable for the gradient to be smooth enough and resolvable into several discrete ranges of concentration.

Since S cannot provide an unambiguous positional information signal, a supplementary information is needed. Suppose that substance Σ is some product of the S-degradation by a first order reaction with rate constant k_1. From eqs. (1) it is clear that k_1 is related to c_5. In its turn Σ is also decomposed by first order kinetics with rate constant k_2. Because of the inhomogeneous production of Σ from S, diffusion will tend to equalize concentration differences in the field. The total rate of change of Σ will then be:

$$\partial\Sigma/\partial t = k_1 S - k_2 \Sigma + D\, \partial^2\Sigma/\partial x^2 , \tag{4}$$

where D is the Σ-diffusion constant in the medium of the field. The solution of eq. (4) is derived in detail in [15]. For asymptotic values of time, Σ approaches a stable distribution represented by a power series of the dimensionless parameter $\omega^2 = L^2 k_2/D$. When $\omega^2 < 1$, the x-independent first term is dominant in the series expansion. Intuitively we see this if we consider the simple case of instant diffusion ($D \to \infty$, therefore $\omega^2 \to 0$). We easily then estimate the steady-state of Σ from eqs. (2) and (4):

$$\int_0^L k_1 S(x,L)\, \mathrm{d}x - k_2 \Sigma L = 0 ,$$

or

$$\Sigma = (k_1/k_2)\,[F(1) - F(0)]\alpha(L) . \tag{5}$$

Here $F(z)$ stands for the integral of $f(z)$

$$F(1) - F(0) = \int_0^1 f(z)\, \mathrm{d}z ,$$

and $F(1) - F(0)$ is a constant number since $f(z)$ is a known function.

The steady-state distribution of Σ as approximated by eq. (5), is independent of x and depends only on $\alpha(L)$. From embryological data we can estimate that ω^2 is so small that the x-dependent higher terms can be neglected [15]. The Σ distribution therefore is well

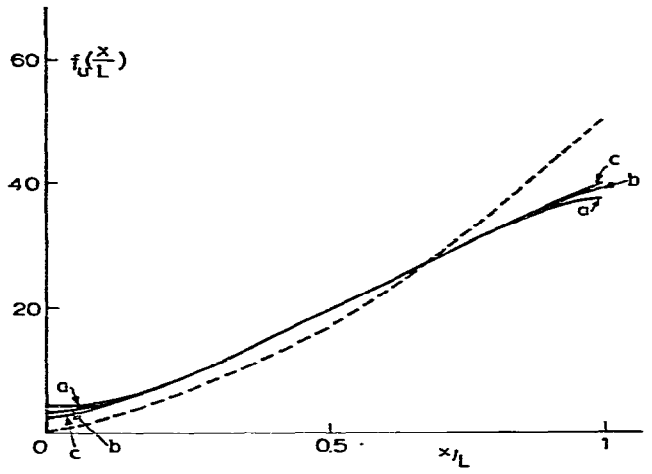

Fig. 2. Universal distribution $f_u(x/L)$ for different lengths L. a) $L = 40$ cells. Normalization is fixed so that this curve is identical to fig. 1a. b) $L = 60$ cells, c) $L = 80$ cells. Curves b and c are plotted from fig. 1b, c respectively with the help of eq. (6). The dashed line corresponds to $L = 400$ cells.

described by the homogeneous expression (5) as derived in the instant diffusion limit.

For the numerical estimates of S plotted in fig. 1 we establish the proportionality relation:

$$\Sigma_a : \Sigma_b : \Sigma_c = 19.77 : 28.87 : 38.45 , \qquad (6)$$

Σ_a, Σ_b and Σ_c are the Σ-levels for fieldlengths of 40, 60 and 80 cells respectively.

Local morphogen S and global morphogen Σ can now provide *complete* positional information for the emergence of a size-independent pattern. One way to achieve this is the following: In a given cell of the field, S and Σ have constant values. Another substance P, whose concentration is kept the same in all cells, interacts reversibly with S and Σ:

$$P + S \rightleftharpoons Q + \Sigma .$$

At equilibrium, the Q concentration will be proportional to $P \cdot S/\Sigma$. Taking into account eqs. (2) and (5) we find finally that Q is a universal function f_u proportional to $f(x/L)$. (In the following section a more detailed version will be elaborated). The value of Q in every cell unequivocally furnishes the complete positional information. Therefore Q must be directly involved in the process of differentiation while all other substances play probably only a supporting role.

It is interesting to test the above "regulating" procedure for gradients S of fig. 1. We normalize the universal function f_u to the S distribution when $L = 40$ cells. In fig. 2 are plotted the $f_u(x/L)$ distributions for different lengths L. Apart from some small deviations at the boundaries of the field, sigmoid curve (a) remains almost invariant when the size of the field increases up to $L = 80$ cells and even higher values. The dashed line corresponds to $L = 400$ cells in which case the Σ-level is: $\Sigma = 281.74$ (in the normalization of eq. (6)). The deviation from the universal gradient (a) is apparent, indicating that expression (2) for S is no more a good approximation.This discrepancy is expected because the field size of $L = 400$ cells is close to the critical length which admitts peaks for the S-concentration at both boundaries of the field.

We presented heuristically how regulating gradient Q is established. We can easily incorporate the consecutive steps for its formation in a unified reaction-diffusion scheme. Gradient S with a shape represented by eq. (2) is indirectly involved in the morphogenetic procedure. Q is the final product which provides positional information for a scaled pattern fromation. Global morphogen Σ is important for the emergence of Q, and it can prove necessary for other processes where the knowledge of the fieldsize is crucial, an example being given in section 4.

It is important to note, that in our model Σ is automatically obtained without making any additional assumptions. In contrast, other regulating theories are based on the assumption of local predifferentiation, as for instance in the source and sink model. There, it is a priori assumed that the boundary cells behave like morphogen sources and sinks [9,10]. Clearly, such presuppositions weaken these models since in this way the pattern formation problem is merely reduced to the establishment of a pre-pattern.

3. Regularized compartments in the field

We propose now a biochemical mechanism making use of both local and global signals in order to divide the field into size-independent domains of molecular differentiation.

Suppose that an allosteric protein P is produced and maintained at a fixed level in all cells of the field. We

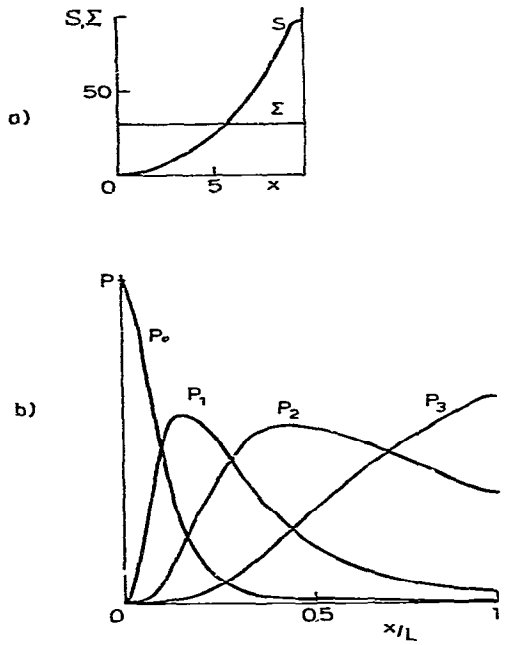

Fig. 3. a) Concentrations of S and Σ in arbitrary units. b) Equilibrium concentrations P_0, P_1, P_2 and P_3 following eqs. (9). Equilibrium constants are: $K_{01} = 100, K_{12} = 12, K_{23} = 2$,

can further assume for P that it is indiffusible and has the structure of a polymer with N active sites reversibly occupied according to the sequential scheme:

$$\begin{aligned} &P_0 + S \rightleftharpoons P_1 + \Sigma \quad (K_{01}) \\ &P_1 + S \rightleftharpoons P_2 + \Sigma \quad (K_{12}) \\ &\vdots \quad \vdots \quad \vdots \quad \vdots \\ &P_{N-1} + S \rightleftharpoons P_N + \Sigma \quad (K_{N-1,N}) \end{aligned} \tag{7}$$

$P_0, P_1, P_2, \ldots, P_N$ correspond to states of the protein with none site, one, two, ... up to N sites occupied respectively, while $K_{01}, K_{12}, \ldots, K_{N-1,N}$ are the equilibrium constants in the sequence of reactions (7). In every cell the total amount of protein P is fixed and given by:

$$P = P_0 + P_1 + P_2 + \ldots + P_N \,. \tag{8}$$

Since in a given cell S and Σ have constant values, the system reaches an equilibrium where the distributions $P_0, P_1, \ldots, P_N$ are found in a straight forward calculation:

$$\begin{aligned} P_0 &= P\left[1 + K_{01}\left(\frac{S}{\Sigma}\right) + K_{01}K_{12}\left(\frac{S}{\Sigma}\right)^2 + \ldots \right. \\ &\quad \left. + K_{01}K_{12}\ldots K_{N-1,N}\left(\frac{S}{\Sigma}\right)^N\right]^{-1} \\ P_1 &= P\left[\frac{1}{K_{01}}\left(\frac{\Sigma}{S}\right) + 1 + K_{12}\left(\frac{S}{\Sigma}\right) + \ldots \right. \\ &\quad \left. + K_{12}K_{23}\ldots K_{N-1,N}\left(\frac{S}{\Sigma}\right)^{N-1}\right]^{-1}, \\ &\vdots \\ P_N &= P\left[\frac{1}{K_{01}K_{12}\ldots K_{N-1,N}}\left(\frac{\Sigma}{S}\right)^N + \ldots \right. \\ &\quad \left. + \frac{1}{K_{N-1,N}}\left(\frac{\Sigma}{S}\right) + 1\right]^{-1}. \end{aligned} \tag{9}$$

Details for the time course until the system reaches equilibrium are found in [15]. This biochemical scheme has some common features with the model proposed by Kiger [16] and MacWilliams and Papageorgiou [14].

In fig. 3a are plotted some characteristic gradients S and Σ, while in fig. 3b equilibrium distributions are shown of the active states of a protein P with 3 active sites.

By inspection it is evident that the field is separated into domains $R_0, R_1, \ldots, R_N$. In domain R_i, active state P_i dominates as compared to all other states of protein P. Without any further specification, we can assume at this stage that, in every compartment R_i a P_i-specific molecular process is triggered leading to spatial differentiation of the field. From eqs. (9) we can draw the following conclusions:

a) S and Σ never appear separately but always combine to form the ratio S/Σ (or Σ/S). Taking into account expressions (2) and (5) for S and Σ we note that S/Σ is proportional to $f(x/L)$. Since $P_0, P_1, \ldots, P_N$ depend only on x/L, the compartmentalization of the field is explicitly regularized.

b) As long as S and Σ behave according to (2) and (5) respectively, scaling function $\alpha(L)$ drops out in all expressions (9). As a result, the same pattern emerges independently of how $\alpha(L)$ varies with the size of the field (or any other dynamical variable).

c) Although $\alpha(L)$ does not affect the final pattern, it is essential for the whole procedure since it deter-

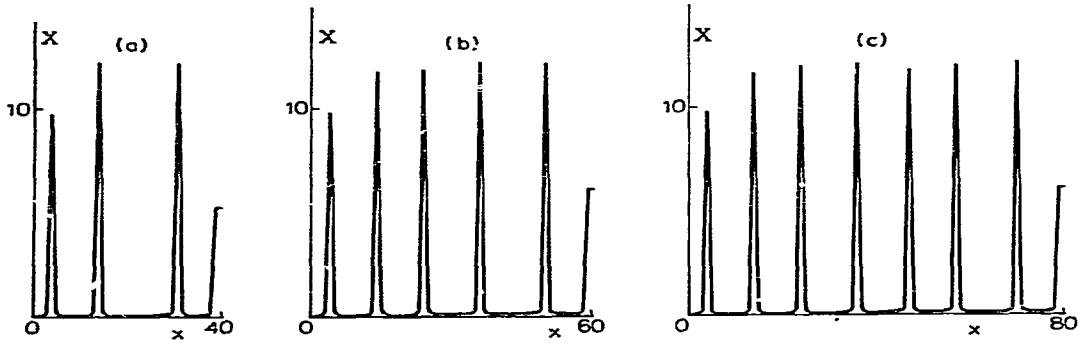

Fig. 4. Stable X concentration (in arbitrary units) obtained from eqs. (10) for different field lenghts L: a) $L = 40$, $n = 4$, b) $L = 60$, $n = 6$, c) $L = 80$, $n = 8$. The parameter values are: $c'_1 = 0.05$, $c'_2 = 0.01$, $c'_3 = 0.0006$, $c'_4 = 0.025$, $c'_5 = 0.01$, $D_X = 0.0001$, $D_Y = 0.1$, $\rho'(x) = 1 + 0.01x$. Initial values: $X = 1.5$, $Y = 5.0$. On the average, stability is reached if 4.000 iterations are performed.

mines the time needed for the establishment of total equilibrium [15].

4. Regulation of repetitive patterns

When the chemical wavelength λ is much smaller than L a pattern of stable repetitive peaks establishes itself.

In the linear approximation, Turing calculated λ in terms of the kinetic and diffusion constants [2,17]. L and λ are uncorrelated and as a result when L varies, the number of repetitive peaks changes accordingly. We conclude that, at least in its linearized version, reaction-diffusion theory fails to produce constant number of homologous structures when the fieldsize is not fixed. This is an undesirable result since it contradicts both observation [18] and excision experiments during early Embryogenesis [19]. In higher bifurcations a correlation between λ and L is eventually established, but in the present work this possibility is not examined.

Consider a Gierer–Meinhardt mechanism for activator X and inhibitor Y, for which the inherent wavelength is much smaller than L. The morphogen diffusion constants therefore should differ considerably ($D_X \ll D_Y$). The rate equations for X and Y are similar to eqs. (1):

$$\frac{\partial X}{\partial t} = c'_1 \frac{X^2}{Y}\rho'(x) - c'_2 X + c'_3\rho'(x) + D_X \frac{\partial^2 X}{\partial x^2}\,,$$
$$\frac{\partial Y}{\partial t} = c'_4 X^2\rho'(x) - c'_5 Y + D_Y \frac{\partial^2 Y}{\partial x^2}\,. \qquad (10)$$

In fig. 4 are plotted final X-distributions for different field lengths. The number of peaks n varies in correlation with L.

Suppose now that, through an independent process similar to the one introduced in section 2, a stable global morphogen Σ is established in the field providing a signal for the length L. It is possible to incorporate the size-sensing morphogen Σ into eqs. (10) by adjusting the rate constants [20,21]. We propose two simple chemical schemes leading to direct and inverse proportionality of the rate constants to the Σ-level.

Assume that a substance Z, instead of being decomposed by a first order reaction, decomposes by second order kinetics.

A) If Σ is maintained at a constant concentration and Z decomposes according to:

$$Z + \Sigma \rightarrow \text{decay products}\,,$$

the effective rate constant for the Z-decomposition becomes proportional to Σ.

B) In a slightly more complicated scheme, Σ is constant and it remains furthermore at equilibrium with another substance T of constant concentration according to the reversible reaction:

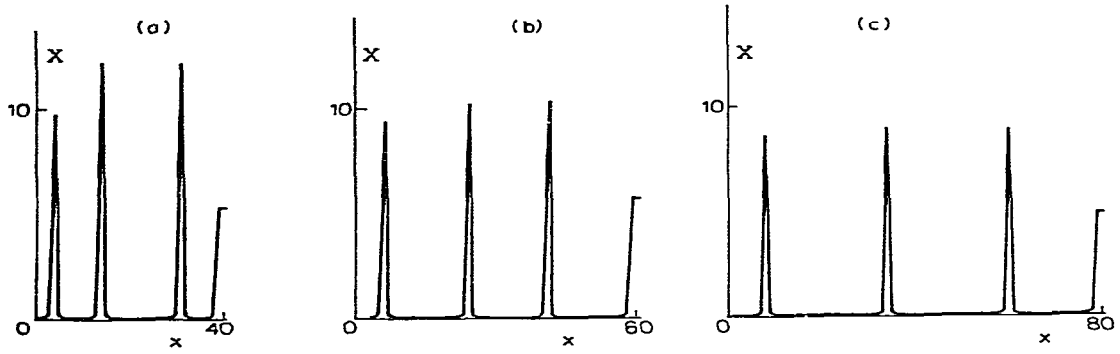

Fig. 5. The plots are similar to the plots of fig. 4. The same parameters are used except c'_5 for $L = 60$ and $L = 80$: b) $c'_5 = 0.0068$, c) $c'_5 = 0.0051$. In all plots $n = 4$.

$$\Sigma + R \rightleftharpoons T ,$$

If Z decomposes via a second order reaction like

$$Z + R \rightarrow \text{decay products} ,$$

the effective rate constant which determines the decomposition of Z is inversely proportional to the level of Σ. We have extensively applied both schemes A and B to modify the kinetic terms of eqs. (10).

When c'_2 is adjusted following scheme A, a correction is observed in the right direction, but still the number of peaks is not completely L-independent. If c'_5 is renormalized according to scheme B (becoming inversely proportional to Σ) the number of peaks is maintained fixed when the field size varies, as is seen in fig. 5. In fig. 5a $c'_5 = 0.01$, while in b) and c) c'_5 changes following the proportionality relation (6). The results above indicate that reaction–diffusion theory combined with a global morphogen can correctly provide a correlation between λ and L, so that a pattern with a given number of repetitive structures can emerge in a field of variable size.

5. Discusion

There is a wide variety of forms and patterns appearing in Development. For their establishment one expects that diverse mechanisms can be responsible. It is reassuring nevertheless that a positional information theory is formulated, as extracted from accumulated experimental evidence. In abstract form, this theory disentangles the complex phenomena of pattern formation, into a temporal sequence of discrete processes.

For the first step – the establishment of positional information, reaction–diffusion theory could be the generating mechanism. Founded on first principles of Physics and Chemistry, this theory provides a natural and convincing paradigm for the formation of a stable gradient. Recent experiments support the hypothesis of concentration gradients associated with morphogenetic activity [22].

The model presented in this work describes how size-independent patterns can be formed during normal development. For abnormal situations, as in regeneration experiments, we have made no predictions at this stage. In order to incorporate morphallaxis (or epimorphosis) into the model some complementary assumptions must be made. With some simple rules, the behaviour or Gierer–Meinhardt gradients is consistent with several data of surgical manipulations [3,5].

At the step of interpretation of positional information, an amplifying mechanism will be necessary to produce clearcut territories of specific geneactivity [14]. Another problem to be dealt with will be the reversibility (or not) to the initial cell state and the memory of the positional signal.

We have assumed throughout this work that once S and Σ have been established in every cell, their concentration will be fixed independently of any secondary reaction they participate. This is possible only if these reactions are causing in effect a small perturbation from the stability values of S and Σ. Consider for instance scheme A of section 4: In expression (5) for the level of Σ, the denominator k_2 should be substituted by $(k_2 + \bar{k}Z)$ where $\bar{k}$ is the rate constant for the second

order decomposition of Σ. The condition $k_2 \gg \bar{k}Z$ guarantees that the level of Σ does not change appreciably from its participation in scheme A. A natural extension of the model in two dimensions would include pattern formation in sheets of cells which is of great importance in Embryogenesis.

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