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A Cell-Contact Model for Cellular Position Determination in Development

(differentiation/plasma membrane/*Dictyostelium discoideum*/cyclic AMP)

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ABSTRACT **The fate of a cell in a developing organism is a function of its position within the organism. The molecular mechanism that cells use to determine their position and to convert positional information into a form that can be used to regulate the expression of genes is not understood. This paper presents a model in which contacts between complementary molecules on plasma membranes of adjacent cells regulate the concentration of a morphogenetic substance and transmit positional information. This model is described by four equations that, when solved with realistic parameters, demonstrated that this mechanism will produce discrete populations of cells within a few hours from an initially undifferentiated array of cells. The model suggests an explanation for several phenomena that have been observed and suggests experiments to test it and to clearly differentiate it from other models for position determination.**

The many kinds of cells that compose a multicellular organism are arranged with striking precision. Classic studies have investigated the processes by which undifferentiated cells are organized into the tissues and organs of the adult. The concept of the morphogenetic field, a group of initially identical cells that produces at least two different kinds of cells as output, has arisen from many of these investigations. The position of a cell within the field decides its developmental fate. Morphogenetic fields have the following general properties: (*i*) Acting cells cannot be distinguished from reacting cells; (*ii*) a constant ratio of output cell types is produced from a very wide range in the absolute number of input cells; and (*iii*) the field is often regulative (i.e., removal of some cells can result in a reorganization of the field to produce the correct proportions of output cell types).

Two kinds of theories have been offered for the molecular mechanism of position determination in a regulative, size-invariant field. One type suggests that a gradient of diffusible substance(s) provides cells with positional information (1, 2). A concentration gradient of a substance of low molecular weight can be established by diffusion in the time available for the commitment to differentiation of the cells in a field (3). To specify the relative position of a cell in a chain of cells of variable length, two opposing gradients [with their ratio specifying position (1)] or a single gradient with a fixed concentration of the gradient substance at either end are necessary (2). According to another model proposed by Goodwin and Cohen (4), positional information is specified by the phase-angle difference between two periodic signals. Their theory specifies that a pacemaker cell generates two signals of equal frequency but that one of these is transmitted from cell to cell more slowly than the other. As the signals move through the field, the phase-angle difference between them increases linearly and this difference specifies the positional information. An additional signal is necessary to make the system invariant with change in size (4). L. Wolpert has done much to organize and identify the processes suggested to be important in organization of developmental fields (5).

I would like to suggest another perspective on this problem, initially developed to suggest an explanation for the mechanism used by cells in the pseudoplasmodium of the social amoeba *Dictyostelium discoideum* to determine their position. This idea will be discussed and other theories criticized largely in terms of this organism. However, the implications are more general.

Much of the information about the development of *D. discoideum* has been reviewed (6, 56), so only a few salient points will be presented. Starvation of the amoebae stimulates them to secrete large amounts of cyclic 3′:5′-adenosine monophosphate (cAMP) and to become chemotactic towards it. As a result, up to 10^5 cells aggregate into groups and then form a pseudoplasmodium. The pseudoplasmodium may migrate or may rapidly complete differentiation without migration. In either case it finally constructs a sorocarp composed of a stalk and a ball of spores. The proportion of spore cells to stalk cells appears to be constant in sorocarps from 12 to 10^5 input cells (7, 8). Several other features of the development are important in the current context. Isolated cells do not differentiate (9), although high concentrations of exogenous cAMP may induce formation of stalk cells (10). The cells become committed to their final developmental fate on the basis of their position in the pseudoplasmodium (11). It has been suggested (12, 13) and disputed (14) that the position of a cell in the pseudoplasmodium may be the result of sorting of cells based upon some predisposition of the amoebae. Sorting could hardly be the principal mechanism of determination, since K. Raper showed that a bisected pseudoplasmodium can regulate and produce normal sorocarps (11). Bonner *et al.* (15) and Gregg (16) have shown that the committed cells show biochemical differentiation associated with commitment and that this differentiation is also reversible in a bisected pseudoplasmodium. Fig. 1 (from ref. 6) shows that the discrete cell types are located in two areas separated by a sharp dividing line and that determination of position must occur with an accuracy of $\pm 5\%$ in each row of cells.

If we attempt to apply the gradient or phase-shift theories to the specification of cell position in *D. discoideum*, we are confronted with problems that decrease their attractiveness. Both theories generate continuous gradients of positional information but the process of development generates discrete cell types. Therefore a threshold mechanism for transduction of the positional information must be postulated. Both models also contain unspecified mechanisms that determine bound-

aries (and polarity) of the field and specify the source(s) or sink(s) of the gradient(s) (or the pacemaker in the theory of Goodwin and Cohen). The complexity necessary for a biochemical realization of the phase-shift model is disconcerting. The authors use 20 reactions or interactions of molecules to specify the control circuits for signal generation in a nonregulative field, and additional reactions are necessary to produce a regulative field (4). This complexity makes it refractory to biochemical or quantitative analysis.

RESULTS AND DISCUSSION

General properties of the model

Cellular contact is necessary for development of normal cells of the social amoeba and for synergism between developmental mutants (17). I suggest that positional information is transmitted from cell to cell on the basis of contacts between complementary molecules on their surfaces. A general feature of this mechanism is the fact that it can generate a step distribution of morphogenetic substance along a line of cells without postulating unusual biochemical mechanisms. Each cell regulates its internal concentration of morphogenetic substance through negative feedback, but this regulation is defeated for the cells at the ends of the field. The extent of the field is determined by the number of contiguous cells with complementary "contact-sensing" molecules. The net effect is transfer of information from cell to cell in a manner and at a time that a directly related to its relative position in the field.

The model is based on the following propositions:

(*1*) There are "contact-sensing" molecules on the plasma membranes of cells in a morphogenetic field. They are activated by making contact with their complementary molecule on an adjacent cell. The activated molecules regulate the concentration of a morphogenetic substance, A.

(*2*) One kind of contact-sensing molecule, when activated, increases the concentration of A, while its complement, when activated, decreases the concentration of A. For example, molecules on the front of the cell, F, might increase the rate of destruction of A and those on the rear of the cell, R, might stimulate production of A.

(*3*) Polarity of morphogenetic fields is determined by polarization of the distribution of contact-sensing molecules on the plasma membranes of the cells in the field or on cells or extracellular material at the boundaries of the field.

(*4*) The concentration of A is regulated by negative feedback. A inhibits formation or activity of those contact-sensing molecules that increase its concentration. It stimulates formation (or activity) of those contact-sensing molecules that decrease its concentration.

Propositions *1*, *2*, and *4* can be discussed in terms of a known biochemical system that will be used throughout the rest of this paper as an example of the kind of control system suggested by the model. Cyclic AMP is the product of one enzymatic reaction and the substrate of another. Its isolation from the mainstream of metabolism makes it an ideal molecule for regulating differentiation. Adenylate cyclase, the enzyme that produces it, is associated with the plasma membrane in eukaryotes, as are at least some forms of the phosphodiesterase that degrades cAMP (see ref. 21 for review). The activity of adenylate cyclase is regulated by peptide hormones that bind to the plasma membrane, and cAMP functions as the "second messenger" of many hormones (22). Lectins, molecules that bind to carbohydrates on the plasma membrane, in some cases also produce significant changes in the internal concentrations of cyclic nucleotides (23, 24) and considerable changes in the biochemistry and "differentiation" of the lymphocyte. These follow in time the changes in concentration of cyclic nucleotides (23–26). The effects of hormones and lectins are clearly the result of interaction of these molecules with receptors on the plasma membrane and not of their uptake into the cell (25–28). No equivalent regulation of phosphodiesterase by peptide hormones has been demonstrated, but its activity is regulated by several small molecules (29); Cheung has demonstrated a 10-fold stimulation of the activity of a phosphodiesterase from brain by a protein activator (30).

Cyclic AMP can produce many changes in the cell that resemble those accompanying differentiation. It regulates the activity of enzymes, transcription of genes, and cellular permeability (21). It has been reported to regulate translation (31, 32) and to stimulate the phosphorylation of chromosomal proteins (33, 34), effects that may also be relevant to regulation of the expression of genes. Increases in its concentration inhibit the movement of cells (35) and change cellular morphology (36). Induction of mitosis in lymphocytes by lectins is preceded by considerable alterations in the internal concentration of cyclic nucleotides (23, 24).

There is some evidence that synthesis of cAMP is regulated in the manner suggested in postulate *4*. Cyclic AMP induces cAMP phosphodiesterase when added to 3T3 cells (51), and it has been suggested that norepinephrine induces a cAMP phosphodiesterase by activating adenylate cyclase (52). Finally the suggestion has been made that adenylate cyclase of the social amoeba may be partially inactivated in the process of making cAMP (37).

Various observations imply that the cells of *D. discoideum* are polarized before aggregation. Time-lapse photography by myself and others (18) demonstrates that aggregating cells are elongated, with a front that is the locus of pseudopodial movement and that attaches quite specifically to the rear end of another cell, producing chains of cells (19). These front to rear attachments are specifically inhibited by antibodies against aggregating cells (53). John T. Bonner has shown that polarity exists also in the pseudoplasmodium and its constituent cells (54). Polarization of developing cells is not unique to *D. discoideum* (20).

Quantitative analysis

We will consider the quantitative consequences of the model in terms of the cAMP-mediated system. This choice has one minor effect on the form of the equations. Since the concentration of ATP in the cell [about 1 mM (38)] is considerably above the K_m of most adenylate cyclases (39–41), I will assume that the rate of synthesis of A (e.g., cAMP) is independent of the concentration of precursor A (e.g., ATP). The concentration of cAMP is probably below the K_m for the social amoeba's membrane-bound phosphodiesterase (42, 43), and so the term representing breakdown of A is proportional to the concentrations of A (44) and of the molecules that effect its degradation.

For each cell i in a line of cells

$$d(A)_i/dt = k_2R_i - k_1F_i(A)_i \quad [1]$$

$$dF^*_i/dt = l_1(A)_i - m_1 \quad [2]$$

$$dR^*_i/dt = (dR^*/dt)_0 - l_2(A)_i - m_2 \quad [3]$$

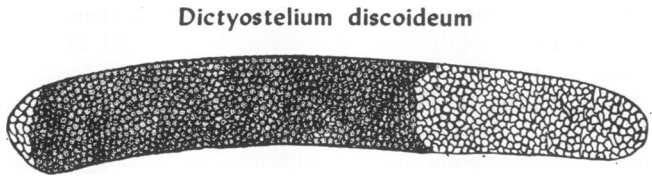

FIG. 1. Commitment of cells in the pseudoplasmodium stage of *D. discoideum*. In this drawing, taken from ref. 6, the prestalk cells form a wide zone at the front of the pseudoplasmodium (on the right) and are followed by a zone of prespore cells, which have been stained with periodic acid-Shiff's reagent. The thin zone of cells at the rear will become the basal disc.

$$F_i = R_{i-1} = \min [F^*_i, R^*_{i-1}] \quad [4]$$

where:

$(A)_i$ is the concentration of cAMP in cell i. It is assumed to be uniform within the cell. F_i and R_i are the front and rear *contacts* of that cell. F^*_i and R^*_i are the front and rear contact-sensing *molecules*. $(dR^*/dt)_0$ is the rate of production (or activation) of R^* in the absence of A. l_1 is the rate constant for production of F^*, while l_2 is the rate constant for inhibition of production of R^*. m_1 and m_2 are the rates of inactivation of F^* and R^*. The choice of a rate of destruction of F^* and R^*, which is independent of their concentration, is somewhat unusual. It is based upon the apparent rates of destruction of developmentally controlled enzmes in *D. discoideum* (49, 55). If the rate of destruction is made proportional to the concentration of F^* and R^*, no major change in conclusions results. k_1 is the rate constant for destruction of A, while k_2 is the pseudo-first order rate constant for its production.

Since these equations cannot be solved analytically, they have been solved by numerical integration by the Runge–Kutta method. No negative values for F^* and R^* were allowed in the computer program. The parameters used were estimated from the experimentally determined quantities that are presented in Table 1. In general, rates were estimated to be only a fraction of the possible rates indicated by the data in Table 1 to produce a conservative estimate of the minimum time necessary for commitment. The general form of the solution is quite resistant to changes in the values of the parameters used to solve the equations. Each curve in Fig. 2 presents cAMP concentration as a function of the position of a cell in the one-dimensional field at a given time after the process of position determination has begun. This zero time occurs after the time of aggregation for *D. discoideum*. Waves of changing cAMP concentration move from either end of the line of cells and are extinguished at the dividing line between the two cell types. The final stable solution has several satisfying features. The two populations of cells are present in about the right proportions; they are separated by a sharp dividing line (solutions with a single cell at the rear with a high concentration of cAMP can be produced under some conditions); and they differ by 10- to 20-fold in their concentration of cAMP. The process takes about 3 hr and is in agreement with the time available for commitment in the social amoeba (6). The time necessary to produce commitment and the accuracy of commitment (in this case, the number of cells with intermediate concentrations of cAMP) is better than that which Wolpert suggests is required for fields produced in a wide variety of organisms (5). The ratio of cell types produced by a given set of parameters is independent of the number of cells, but the time necessary for the system to reach its final steady state is directly proportional to the length of the field in cells.

Solutions tend toward a stable one in A when all intercellular contacts have disappeared as a result of the disappearance of one type of contact-sensing molecule (either R^* or F^*) on each cell. This can be seen by solving equation **1** at steady state ($dA/dt = 0$). The term, $-k_1F_1(A)_1$, is zero for the first cell. Since k_2 is not zero, R_1 must be zero when $dA/dt = 0$. Then F_2 is zero by equation **4**, etc. The cell is then irreversibly determined. Fields remain at least partially regulatable and reversible as long as $dA/dt = 0$, even if the rate of change of A is small. The model does not depend on strict adherence to

TABLE 1. *Experimental determinations of quantities useful for numerical solution of the equations describing this model*

Property determined	Value
Protein per cell[a]	9 pg
Median molecular weight of peptides in plasma membrane[b]	10^5
Rate of synthesis of a protein[c]	9 fg/hr per cell
Rate of degradation of a protein[d]	22.5 fg/hr per cell
Maximum rate of cAMP synthesis[e]	3.2 attomol per cell per min
Maximum rate of cAMP destruction[f]	0.12 attomol per cell per min
Molecular parameters of adenyl cyclase[g]	V_{max} = 15 μmol/min per mg Molecular weight = 2×10^5
Molecular parameters of phosphodiesterase[h]	V_{max} = 1 μmol/min per mg of protein Molecular weight = 1.3–7.5 × 10^5
Cellular concentration of cAMP[i]	0.6–3.4 μM

[a] Determined by the Lowry method (48).

[b] Unpublished observations on *D. discoideum*. Used as an estimate of the size of F^* and R^*.

[c] Calculated from the data on uridine diphosphoglucose pyrophosphorylase in ref. 49; an estimate of the terms l_1 $(A)_{max}$, l_2 $(A)_{max}$, $(dR^*/dt)_0$. This would produce 900 molecules per min of protein of molecular weight 10^5 in each cell.

[d] Also estimated from the data in ref. 49 and is an estimate of m_1 and m_2. For a protein of 10^5 daltons, it would be 2250 per min per cell.

[e] From ref. 37 and the protein content of a cell. This provides an estimate of k_2R_{max}.

[f] From the value for late aggregation cells in ref. 42, assuming that the pelleted fraction is about 20% of the cellular protein. Used to estimate $k_1F_{max}(A)_{max}$.

[g] Assuming complete purity for the enzyme from *Brevibacterium liquifaciens* (50), the cAMP-synthesizing activity of a *D. discoideum* cell could be explained by 850 molecules per cell.

[h] Assuming a molecular weight of 4×10^5 and complete purity for this enzyme from brain (30), 9000 molecules per cell would produce the observed activity of phosphodiesterase.

[i] Determined on various organisms and tissues (51). The intracellular content of cAMP has not been estimated for *D. discoideum*. The concentration has been converted to molecules per cell by assuming that each cell is a sphere with a diameter of 10 μm.

proposition 4. Even if the activity of the enzymes making and degrading cAMP is partially resistant to negative feedback regulation, a wave of changing cAMP concentration moves into the pseudoplasmodium from each tip. These waves could trigger differentiation.

The solution of the equations describing the cell-contact model for a single file of cells that was presented above is probably somewhat too simple when applied to a large pseudoplasmodium with multiple files of cells. Two observations suggest that this is true. Although the front of the pseudoplasmodium is slightly convex, the boundary between cell types is slightly concave; in addition Bonner has shown that the ratio of lengths of prestalk zones to prespore zones is greater in thick than in thin pseudoplasmodia (57). Several minor lateral interactions between cells can explain these observations. An attractive explanation is that some slight diffusion of A occurs from and into cells. The effects of this would be in the appropriate direction for both observations. Diffusion of cAMP from the pseudoplasmodium has been observed (56). Another application of the mechanism presented here, using another contact-sensing system with different kinetic parameters, could explain the production of the zone of basal cells at the rear of the pseudoplasmodium.

Experimental predictions

Only some of the model's predictions will be enumerated. It suggests that cells should acquire positional information (and could use it) in a wave moving from a tip of the field. The activity of alkaline phosphatase (an enzyme characteristic of prestalk cells) appears in exactly this way in the *D. discoideum* (15). The reversal of commitment that occurs in a bisected pseudoplasmodium also proceeds from the tip (15). Similar waves of developmental activity, for example, mitosis, occur in *D. discoideum* (45) and other systems (46). Dedifferentiation of cells should require cell contact, and this has been demonstrated by Gregg (9). There is one experiment that is most significant to the validation of the model. The model predicts that development of cells will be affected by exposure to pure plasma membranes and to molecules derived from them. This experiment clearly discriminates between this model and its alternatives. The receptors for the molecules derived from the membrane could be localized in small regions of the membrane. Postulate 4 suggests that the synthesis or activity of a contact-sensing molecule should be inhibited by treatment of the cell with the molecule's complement.

Mutations could alter this system in various ways. At least some mutants with altered ratios of cell types or altered speeds of development should have alterations in their contact-sensing molecules or plasma membranes. It should be possible to isolate "chain terminator" mutants that break the organization of a field by being unable to make effective cell contacts. These mutants could drastically change the ratio of spore to stalk cells even when mixed with a great excess of wild-type cells.

CONCLUSIONS

The model discussed here produces a regulative morphogenetic field that produces irreversible differentiation at steady state. With slight modification it produces reversible commitment to differentiation. It suggests the possibility that some physiological and developmental control systems might have a similar molecular basis. If this assumption is true, molecules

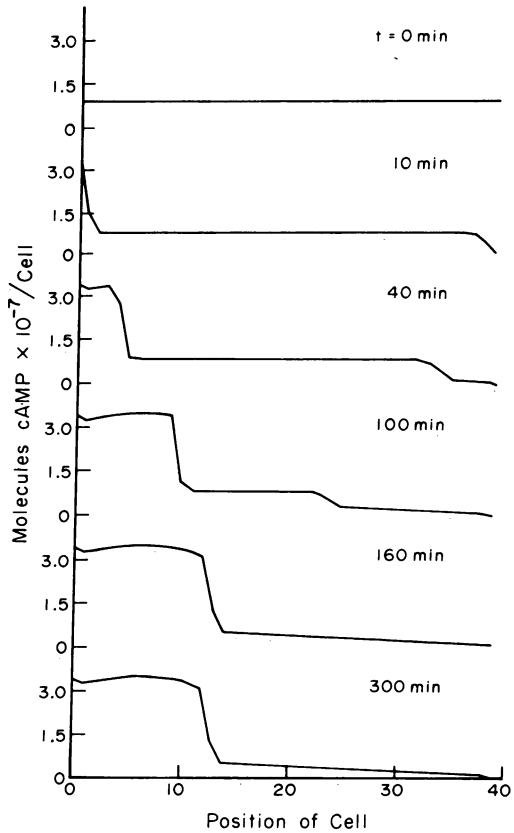

FIG. 2. Changes in cAMP concentration that occur with time and with position in a line of cells in a morphogenetic field. The parameters used to obtain this solution were: $k_1 = 2.1 \times 10^{-3}$ per min $\times F \times A$; $k_2 = 2 \times 10^3$ per min $\times R$; $l_1 = 2 \times 10^{-5}$ per min $\times A$; $l_2 = 1 \times 10^{-5}$ per min $\times A$; $m_1 = m_2 = 20$ per min; $(dR^*/dt)_0 = 30$ per min. The initial conditions were: $R = F = 200$ molecules per cell; $A = 10^7$ molecules per cell.

similar to the polypeptide hormones of higher organisms may have a long evolutionary history and a wide phylogenetic distribution as contact-sensing components of the plasma membrane. As multicellular organisms increased in size, cells could interact (or continue to interact) with cells at distance by exporting such molecules or soluble fragments derived from them into the circulation. Such considerations provide a possible explanation for the puzzling observation that the mammalian hormone, glucagon, can regulate the metabolism and stimulate the activity of adenylate cyclase of the fungus, *Neurospora crassa* (47). Although most developing systems are too complex to be adequately described by the simple model presented here, it is possible to extend it to suggest ways in which development in more than one dimension, bilateral symmetry, and neural specificity may be produced.

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