On Chemical Communication and Pattern Formation in Living Organisms: Some Roles of Size and Growth Rate

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by

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Abstract

Sensing (measurement) of the size of its own body is a common phenomenon in living organisms. This work discusses possible reasonable ways of performing such measurements via hormones. It is argued that there are three general methods for size measurement, which may be used by organisms in numerous implementations. Possibilities for experimental distinction between different methods are discussed. Roles of particular hormones in the context of maturing plants are suggested.

Different aspects of the impact of an increase in the growth rate of plants on hormonal communications are discussed. The potential importance of periodic signals is stressed. Application to signals involved in the transition to flowering is suggested, with particular reference to the inhibitory influence of young leaves on the transition from vegetative to reproductive growth.

A new model for the establishment of a pattern in a developing organism by means of positional differentiation is proposed. The model is capable of preserving a differentiation pattern of two cell types in cutting experiments. Important properties of the model are: (i) the processes of establishment of chemical and differentiated patterns proceed simultaneously; (ii) memory is included; (iii) there is no direct interaction between morphogens; (iv) small initial polarity is assumed. Formulation of the model is in terms of linear differential equations, which are ordinary in the one dimensional case. Application to the slime mold Dictyostelium discoideum is discussed.
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General Introduction

One of the parameters that characterize any given living organism is size. During their life cycle organisms pass through an orderly sequence of developmental patterns—combinations of forms and functions characteristic for each developmental stage that are transferred genetically from generation to generation. In some cases size is an important parameter and some specific patterns cannot be formed until the organism in question acquires sufficient size. In other cases the same pattern is formed in organisms of a very wide range of sizes and an important parameter is the ratio between parts of an organism.

It would be fair to say that in general for most patterns there is some range of permissible sizes, with a minimal and a maximal size. Taking humans as an example one can see that the smallest normal individuals are about 80cm tall while the largest are just under 3 meters, but it is difficult to imagine a human being 1cm or 10cm long. The question thus arises: how can an organism sense its own size to regulate pattern formation?

Formation of patterns in a living organism depends on communication between its various parts. Chemical-hormonal communication is one of the important types. One of the important factors that can influence hormonal communication is the size of an organism. In Chapter One of the present work we discuss possible ways of interaction between the size of an organism and hormonal communication between its parts. In other words we analyze how an organism can sense its own size by means of hormonal communication and develop patterns that depend on size.

There is an almost complete lack of theoretical work on this subject, and not very much experimental data. Our analysis is thus mostly qualitative. We believe that it provides a basis for both deeper experimental inquiry into the problem and, subsequently, quantitative modelling.

Another parameter closely related to size is the growth rate of an organism. Growth rate also can influence hormonal communication and, through it, formation of new patterns. In Chapter Two we discuss an example of the impact of growth on hormonal communication.

Finally, in Chapter Three, we study size-independent pattern formation. We put forward a new model that has various advantages (discussed in the text) over earlier models of that kind.

As model systems we use juvenile maturing plants of certain species, prior to and under transition to flowering, in Chapters One and Two, and the slime mold Dictyostelium discoideum in Chapter Three. The former model system provides an example of strongly size-dependent behaviour so that a pattern (flowering) occurs only when the plant in question becomes large enough. Transition to flowering in many plant species is accompanied by sharp changes in growth rate. A special
advantage of plants as a model system for the study of size-related phenomena on hormonal communication is the immobility of plant cells (in contrast to their animal counterparts). In plants therefore one can observe the relevant phenomena in their purest form.

The slime mold *Dictyostelium discoideum* provides an extreme example of size independence. The same pattern is formed in organisms of an extremely wide range of sizes (see Chapter Three). Another major feature of this system is that growth can be neglected. We show that several important types of behaviour that are usually modelled by means of nonlinear models can be obtained using linear equations.

The results presented in Chapters One and Three have appeared in the *Journal of Theoretical Biology*. 
Chapter One

Hormonal Mechanisms for Size Measurement in Living Organisms
in the Context of Maturing Juvenile Plants
Introduction

Size sensing is a common phenomenon in living organisms. Bodies of animals (including human beings) serve as examples of complex systems with highly correlated sizes of their parts. The growth pattern of plants is much more flexible than that of animals, but even in plants there is a correlation between sizes of the shoot and the root (see e.g. Isawa & Roughgarden 1984). Another phenomenon that provides an example of size sensing (measurement) in plants is juvenility.

The concept of juvenility in botany arises from the fact that regardless of environmental conditions, most plants grow vegetatively for some time after sowing. While in the juvenile phase, a plant is insensitive to stimuli that later promote flowering. The duration of a typical juvenile phase may vary from a few days to several months for herbaceous annuals or biennials, while for woody perennials this stage may last for years.

The tip of the plant, more precisely the shoot apical meristem, generally produces some leafy nodes below the earliest flower or inflorescence. This observation has led to the concept of minimal leaf number, the irreducible vegetative growth produced prior to flower initiation in plants that are held in conditions optimal for flowering from the very beginning of germination. The number of nodes to first flower may be used as a measure of the duration of the juvenile phase (Bernier, Kinet & Sachs 1981).

Of the factors put forward as explanations for the juvenility phenomenon, one is insufficient physiological size (length) of the shoot, which means relative closeness of the apex to the root system of the plant in question (Bernier et al 1981). By the term physiological length one usually means the distance of the shoot apex of the plant from its root system, measured in the number of leaf nodes along the shoot.

The importance of the size factor has been confirmed repeatedly (e.g. Zimmerman [1971], Schwabe & Al-Doori [1973], McDaniel [1978]). It has been shown in experiments with various species that graftings of the mature apex onto its own roots prevents maturation of the apex. In experiments with certain species the juvenile state of the apex is indefinitely prolonged by continuous rootings when the plant apex is excised and rooted each time before the plant has reached mature size (McDaniel (1980), Wareing & Phillips (1981)).

The question thus arises as to how the distance between the root and the shoot apex influences the behavior of the latter. More frivolously one may ask how the apex senses the size of the plant? Below we deal with this question. We believe that the suggested answers are relevant not only to juvenility, but also to the analysis of size sensing in other aspects of plant and animal behaviour.

Our modelling approach will be to try to study all reasonable possibilities in a semiquantitative way. This approach provides a basis for more detailed models for specific experimental results obtained from work with various species, and
suggests new experiments to distinguish between different possibilities. We believe that asking "what are all the possibilities" is most helpful in the development of paradigms in biology in general and in plant physiology in particular (Burström 1979, Tzvi Sachs private communication).

Size measurement — general remarks

What type of mechanism in plants may carry out a size measurement? In the strictest sense it may be said that a plant measures its size if there is a parameter characterizing the behaviour of its apex that is in one-to-one correspondence with its size. The requirement of a one-to-one correspondence leads to the above parameter being either an increasing or a decreasing function of the distance between the shoot apex and the roots.

There may be a parameter which is a function of the plant length that is one-to-one for some limited range of length. In particular, taking into account a bounded range of plant chemical sensitivity, which in the final analysis performs the size measurement, the dependence of the measuring parameter on length may change for some range of lengths and be constant above and below this range (Fig. 1a). Another modelling possibility takes into account the supposition that after reaching a certain size, the apex does not "feel" the influence of roots of the plant on its maturation behaviour. This situation may be simulated by a model in which the length of the plant, as the apex "feels" it, grows to infinity when the actual size of the plant approaches the critical value (Fig. 1b).

One can imagine numerous specific ways for implementing size measurement in plants. We believe that all these ways may be grouped and separated into a small number of classes, each based on a single mechanism. (Of course, there is a possibility of coupling between different mechanisms).

Our knowledge of plant physiology has not reached the level where we can discard some of the possible variants of size measurement out of hand. Consequently we feel that it is worth mentioning even those possibilities that have no experimental evidence up to now but have some interesting specific features.

We proceed from the assumption that measurement is chemical in nature. Measurement occurs at the shoot apex. The measuring procedure consists of two stages: interaction of the measuring chemical with the plant and report of this interaction to the apex. Consideration of a given mode of measurement usually suggests how the report might reach the apex. A measuring mediator is produced either by the apex itself or by other parts of the plant (in particular by the roots). In order to sense its size the measuring mediator should reach the entire plant. (E.g. if the subject of measurement is the number of leaf nodes it should reach all of them.) The apex should receive information concerning the interaction between the measuring mediator and the plant and should translate this information into length-dependent behaviour. Our purpose is to catalogue the possible ways of apex-plant communication that result in the sensitivity of the plant shoot apex to
Caption for Figure 1

Fig. 1. Modelling qualitative possibilities for size sensitivity.

$L$ – actual length of a plant measured either in physical or in physiological (e.g. number of leaf nodes) units. $L^*$ – length of a plant as sensed by the shoot apex.

a. Taking into account a bounded range of plant chemical sensitivity one envisage that the dependence of the plant behaviour on length may, as depicted in the graph, change for some range of lengths and be constant above and below this range.

b. Another modelling possibility stresses the supposition that after reaching a certain size the apex does not “feel” the influence of roots on its maturation behaviour. This situation may be simulated by a model in which, as illustrated, the length of the plant as the apex “feels” it, grows to infinity when the actual size of the plant approaches the critical value.
the physiological length of the plant.

What can happen to the measuring mediator at a leaf node? One way to approach the problem is to try to imagine what may be changed if one more node is added to the existing nodes. We envisage three possibilities:

I. There are changes in the chemical composition of the mediator or its concentration. While the possibility of a change in the chemical composition of the mediator by leaves or buds cannot be ruled out altogether, we know of no report that disbudding or removal of lower leaves leads to markedly different results in the maturation-related behaviour of plants. An alternative possibility is partial loss of the measuring substance at the added node.

II. The concentration of the mediator diminishes because it has more volume to fill. We do not know the exact meaning that one should ascribe to the notion of physiological volume but the general idea is that the larger a part of the plant is, the greater the amount of the chemical in question it can absorb.

III. There is an increase in the transportation time of the mediator between the plant ends. The delay occurs because the mediator has a longer way to go owning to the increase in the size of the plant. There is also a possibility of added delay (of unknown relative importance) if the measuring substance is arrested at leaf nodes or if it slackens in its pace through the nodes.

These three possibilities lead to three different ways of length measurement. To be specific we use plant terminology in the analysis below. To apply our results to other relevant systems one should change the jargon of the discussion.

Methods of size measurement

(A) Toll method. (Fig. 2a).

Suppose that roots produce a certain hormone or several hormones that are transported through the shoot towards the apex. At each leaf node a certain amount of substance is lost (as if a toll is paid). When the signal at last arrives at its destination at the apex, the size of the plant (the number of leaf nodes) can be estimated by measuring the hormone concentrations.

One can suggest many methods of such estimation. The simplest variant employs just one hormone. The plant apex may compare the current concentration of this hormone arriving from below with some built-in value (e.g. by receptor sensitivity or through a critical value needed for some chemical reaction).

Measurement of the shoot size should be independent of the pattern of root growth. If the amount of the hormone entering the shoot depends on its production by the roots, one would have to assume some complicated regulation mechanism between different branches of the root to ensure the correctness of the measurement. Otherwise, if each root branch independently produces the measuring mediator, the amount of the chemical reaching the apex will depend on the root size. In this case shoot size measurement will be disturbed. (Fig. 2b).
Fig. 2

Diagram a: Apex, Leaf nodes
Diagram b: Shoot, Roots
Diagram c: Branching point, Towards roots
Fig. 2. Aspects of the toll method.

a. The roots produce a hormone that is transported throughout the shoot towards the apex. At each leaf node a certain amount of the substance is lost. The situation may be pictured as if there were holes in a tube throughout which the hormone is transported.

When the hormone arrives at its destination at the apex, the size of the plant (a number of leaf nodes) is estimated by the apex sensing the hormone concentrations.

b. To ensure that the measurement of shoot size is independent of the pattern of root growth the amount of the hormone entering the shoot must be controlled by the transportation capabilities of the shoot and not by the hormone production by the roots. To model this situation one can imagine a pool filled with hormone under the shoot base with a tube of small radius emerging from this pool in the direction of shoot apex.

c. At the shoot branching point the hormone influx will split unless each branch establishes its own communication link with the roots.
To ensure that the measurement is independent of the pattern of the root growth (in particular to ensure independence of measurement from root branching) one can assume that the amount of the hormone entering the shoot is controlled by the shoot transportation capabilities. This argument does not help in dealing with shoot branching. At the branching point the hormone influx will split so that at the apex of each branch a smaller amount of the hormone will be registered. The amount of the measuring mediator (the hormone in question) that reaches the apex is translated by the apex into behaviour characteristic of the distance of the apex from the roots. Branching will consequently be sensed at the apex as additional effective length. If such sensing is regarded as inappropriate, then one has to assume that each branch establishes its own communication link with the roots (e.g. by inducing the emergence of additional veins as discussed by Sachs (1978) and modelled by Mitchison (1980)). (Fig. 2c).

Another way of size estimation using a single hormone can be based on measuring decreases in hormone concentrations rather than their absolute value. The apex may accommodate to the initial concentration of the hormone. As the concentration decreases the apex measures the time derivative of this concentration and the degree of the decrease will control maturation. Here one does not need an assumption of some programmed value of concentration against which the current one is to be compared. This method permits more flexibility in adapting to different conditions that may influence the production rate of the hormone (and its initial concentration at the apex against which a decrease is sensed) and the transportation capability of the shoot.

There is a possibility of employment of a system of two hormones with different rates of losses on the nodes along their way to the top of a plant. The apex measures some relation (e.g. ratio, difference) between the concentrations of the two chemicals that will depend on the size of the plant.

Consider as an example the following system. The root produces equal amounts of the two hormones A and B. Suppose that the rates of losses of these hormones at the shoot are exponential with factors a and b respectively ($a > b$). Then the amounts of the hormones as sensed by the apex will be proportional to $e^{-aL}$, $e^{-bL}$ respectively, where $L$ is the physiological length of the shoot. If the apex registers the ratio of concentrations of the two hormones, it will measure the value of $e^{(b-a)L}$, which may characterize a level of decreasing (because $a > b$) root-governed inhibition of flowering capabilities of the shoot apex.

The important feature of the latter variant is that shoot branching does not disturb measurement if one assumes that both hormones travel through the same transportation system so that each branch receives equal proportions of both chemicals and the ratio between them does not change at a branching point.

Under certain conditions (e.g. when their distance from the shoot apex is sufficient) roots might switch to promoting maturation in a two-hormone system slightly different from the one discussed above.
One can suggest more sophisticated ways of length measurement with various features. In particular, the variants described above may be coupled with each other. There is no possibility of a priori evaluation of these methods. We cannot decide which one is the best because we do not know exactly what information the apex must receive. One can imagine that the size of the root is an important factor in the apex behaviour of certain species, or that it may be important for the apex to "know" about branching. These factors may influence the behaviour of the apex as an additional effective length. In this case sensing the root size or not distinguishing between additional nodes on the main axis and branches becomes valuable features.

All the above variants employ losses in the concentration of a mediating chemical, which moves from the roots that produce it to the apex. One cannot a priori rule out the possibility of an apex-produced chemical as a mediator in a toll method of length measurement. Such a chemical may move along the organism and return back through some circulation system. In this case the apex can directly compare the concentrations incoming and outgoing mediator. Circulation mechanism of size measurement may be used in systems other than maturing plants. The hormone produced at the apex may also "phone back" after passing through the plant by inducing the roots to send an appropriate signal to the apex (e.g. the root will produce another chemical whose concentration is proportional to that of the first mediator). This method may be of particular importance if coupled with other root-shoot interaction mechanisms when information on the shoot size modulates the root signal. (E.g. phase delay method below or mechanisms of joint maturation of the root and the shoot discussed elsewhere.)

(B) Communicating vessels method. (Fig. 3a).

Here we assume that a hormone is produced at some location on the plant (most probably by the shoot apex or the roots) and is distributed along the plant. The bigger the plant is, the smaller is the concentration of the hormone. To give an example, let us consider the simplest case of homogeneously distributed hormone on a plant without branches. The hormone is produced at one location and spreads along the plant. In the steady state the production of the hormone is balanced by elimination (e.g. break down). Thus the overall amount, $S$, of the chemical in the plant does not change. The amount of the substance per unit length is equal to $S/L$, where $L$ is the length of the plant. Thus the shoot apex that senses the concentration of the hormone feels the size of the plant.

Let us assume that the measuring hormone is produced by the apex. The requirement of preferentially downward (polar) movement of the hormone results in only a marginal influence of the shoot branching on propagation of the chemical. (Such a simple solution of the branching problem is unlikely if the hormone is produced by the roots). One should notice, however, that the polar movement of the mediating hormone puts some constraints (dependent on specific implementations of the method) on the nature of the communication between hypothetical vessels.
Caption for Figure 3

Fig. 3. Aspects of the communicating vessels method.

a. In the steady state the overall amount of the hormone in the plant does not change. Thus the amount of the substance per unit length decreases with increase in the length of the plant. This decrease may be sensed by the shoot apex.

b. Backing up of the hormone with intensified branching (see text).

c. The hormone can move between the leaf nodes throughout the veins of negligible volume compared to the nodes themselves. Then the apex will measure the size of the plant in terms of the number of nodes.

d. Another possibility for node counting is that there are gaps between the veins at nodes. Hormone leaks out through these gaps and fills internodes. The hormone can move freely in each internode but can escape into another internode only through the veins.
On the other hand, intensified branching may lead to filling of the lower volumes of the plant by hormone which has arrived from many branches. Consequently the hormone will be backed up into the upper parts of each branch, rendering an increase in the hormone concentrations at each apex. This increase will be interpreted by the apices as an effective decrease in the size of the plant (Fig. 3b). This is in contrast with the toll method described above, when the branching could be the cause of an effective increase in the plant size as sensed by the apices.

To limit the measurement only to the shoot, one can assume that the root concentration of the hormone is negligible. One may assume various distributions of effective volumes to account for the actual hormone concentrations at various loci in the plant. For example the chemical can move between the nodes in veins of negligible volume as compared to the nodes themselves. Then the apex will measure the size of the plant in terms of the number of nodes (Fig. 3c).

There is another possibility that also leads to counting the number of the leaf nodes by the shoot apex. Suppose that there are gaps between the veins at the nodes. The hormone leaks out through these gaps and fills internodes (Fig. 3d). The hormone can move freely in each internode but can escape into another internode only through the veins. It seems plausible that there is less permeability for hormones between internodes than inside them, taking into account the fact that each internode is produced by the leaf above and thus forms a separate unit that contacts other such units through the leaf nodes. In this variant, internodes rather than nodes play the roles of vessels.

A characteristic feature of this method is that the apex does not depend on the roots for the execution of measurement.

(C) Phase delay method. (Fig. 4).

It has long been known that living organisms have mechanisms for time measurement. Moreover, different organs of the same organism have their own clocks which usually interact, but may be decoupled by external intervention (Bünning 1967). Various aspects of cyclic behaviour have been explored theoretically (e.g. Cohen & Goodwin 1969). Concerning plants, the most conspicuous sign of this capability is the circadian cycle but there are also annual cycles in perennials and other cycles less connected with standard time units (Sweeney 1969).

It is plausible that when plant organs exchange signals, these signals characterize the state of the sender, which is a function of time. The signal arrives at its target organ with a delay of $L/v$, where $v$ is a transportation velocity and $L$ describes the distance between the source and the receiver. $L$ is a physiological length which depends on the comparative importance of different organs in the delay. In particular it may be equal to the geometrical length of the signal route or to the number of leaf nodes on its way. Using its own clocks, the target organ can detect the delay in phase of the signal and translate it into size-dependent
Fig. 4.

Phase delay method of size measurement based on the fact that hormone transport through the plant consumes time that characterizes the size of the plant. This time may, thus, be an indicator of the plant size for the shoot apex.
behaviour.

Let us give an example of a possible implementation of a phase delay mechanism for size measurement. Suppose that the time for some cycle is measured by the change in parameters $D$ and $N$ in the root; where $D = \cos t$, $N = \sin t$. Parameters $D$ and $N$ are internal characteristics of cells (e.g. they may describe oscillations in concentrations of certain enzymes around average values). Suppose that $D$ and $N$ are associated with the production of hormones $D^+$, $D^-$, $N^+$, $N^-$. That is, hormones $D^+$ and $N^+$ are produced when parameters $D$ and $N$ (respectively) are positive while $D^-$ and $N^-$ are produced when $D$ and $N$ are negative (e.g. $D^+$ and $N^+$ are produced if the corresponding enzymes $D$ and $N$ are above their average values, otherwise $D^-$ and $N^-$ are produced). These chemicals are transported along the plant axis towards the shoot apex. Suppose now, that the sensitivity of the apex receptors $D_a$, $N_a$ also depends on the same clocks (that is on the clocks synchronized with the clocks at the roots that govern production of the hormones). Then the summed signal received by the apex will be equal to

$$S = D_a D + N_a N = \cos t \cos(t - \frac{L}{v}) + \sin t \sin(t - \frac{L}{v}) = \cos \frac{L}{v}. \quad (1)$$

(The importance of variable sensitivity of the target organ to arriving hormone as a key factor in plant behaviour was stressed by Trewavas (1982).) If for example $S$ acts as an inhibitor to flowering it might be that inhibition will be eliminated when $L/v \rightarrow \pi/2$ (measured in suitable time units) and will be converted to induction.

Another way of size measurement by the phase delay method is by exploiting differences in transport velocities. Suppose that two hormones $A$ and $B$ are produced in simultaneous pulses of some frequency. Suppose that they are transported to the apex with velocities $v_A$, $v_B$ ($v_A > v_B$). Then to estimate the size of the plant, the apex should measure the time interval $\Delta t$ between arrival of the two signals. This interval is proportional to the size of the plant in question.

$$\Delta t = \left( \frac{1}{v_B} - \frac{1}{v_A} \right) L.$$ 

One does not need periodic phenomenon to measure the size of plant by the phase delay method. (But one needs some time measurement.) Suppose that the root produces two hormones $C_1$, $C_2$ and that strength of the source decreases exponentially with time. (Actually any reasonable monotonic function of time may be used.) The signals that the root sends to the apex (by means of these hormones) are of strengths $C_1 = C_2 = e^{-kt}$. If the transport velocities of $C_1$ and $C_2$ are equal to $v_1$ and $v_2$ one expects that at time $t$ the apex will register signals of

$$C_1 = e^{-k(t-\frac{L}{v_1})}, \quad C_2 = e^{-k(t-\frac{L}{v_2})}.$$ 

If the apex actually detects the ratio $R$ of $C_1$ and $C_2$ then at time $t$ it will detect

$$R = e^{k_1 L},$$

$$-10-$$
\[ k_1 = k \left( \frac{1}{v_2} - \frac{1}{v_1} \right), \] which may describe the root influence on the shoot apex.

In various implementations of the present method, information on the size of the plant is carried by the structure of the signal and not by its strength. If at each branch the signal loses strength but does not change in structure (i.e. the amounts of all components of the signal diminish by the same proportions) then branching does not cause serious difficulties for the measurement.

The specificity of the phase delay method suggests a relatively simple non-interacting way of coupling between this and the toll method. Let us return to eq (1). Suppose that the hormones \( N^+, N^-, D^+, D^- \), that carry the message for the phase delay method, lose a certain (but equal for all four chemicals) part of their amount at each node, as discussed in the description of the toll method above. Then eq (1) will be transformed into

\[
S = f(L)D_aD + f(L)N_aN = f(L)\cos \frac{L}{v},
\]

where \( f \) is a function that characterizes the losses of the hormones while they are transported along the shoot. The apex can double-check the size of the plant using the modulation mechanism. Alternatively the plant may use modulation for measurement of different aspects of the plant size. For instance it might use phase delay to measure the physical length of the shoot, employing the superimposed toll method for counting the number of the leaf nodes. Then eq (2) should be replaced by

\[
S = f(L^*)\cos \frac{L^*}{v},
\]

where \( L^* \) and \( L \) characterize respectively physiological and physical lengths of the plant.

**Discussion**

**(A) Experimental distinction of different mechanisms**

The three mechanisms for length measurement exploit different effects of increasing size, so that one might expect to find experimental possibilities to reveal which system is used by a given plant. However, matters are not that simple because plants may use several measuring methods together and because each of these systems may have different effects on the usual experimental treatments when it comes to various implementations. Nevertheless let us see what may be some expected effects of different ways of measuring the size of a plant on experimental results.

The toll method is characterized by an active participation of the root system in the measuring process. Roots play the role of flowering inhibition, enforcing the juvenile state on the shoot apex (or under certain conditions promoting maturation). Thus cutting roots off will effect maturation behaviour of the shoot apex.
more than in the communicating vessels method where the roots do not play an important role.

Both promoting and inhibiting effects of the root system on capabilities of plants to flower were registered. Flowering of *Silene armeria* is promoted by removal of the roots. The opposite effect was observed with *Rudbeckia*, which cannot flower if deprived of its root system. (Bernier et al 1981).

It is difficult to predict the effect of root detachment on the phase delay measuring method. Assuming that each part of the plant has the same clock one expects that the signaling from below will be restored as fast as the new roots emerge. But it is unclear what will happen if the shoot is exposed to induction before the roots are reestablished, as in the experiments of Schwabe & Al-Doori (1973).

To test for the presence of the phase delay mechanism one may try to expose different parts of the plant to different regimes. The purpose of such manipulation would be to shift the phases of these parts in relation to one another and to disturb time measurement. On the other hand plant organs may communicate to synchronize their clocks, rendering this treatment ineffective. Classical experiments of induction of flowering, exposing a small part of one leaf to light, show the plants capability for intercommunication and do not leave much room for optimism concerning this line of investigation.

Another way to test the phase delay mechanism is to increase the size of the roots so that the signal will have a longer way to travel and, thus, its movement will consume more time. Hence the insufficient size of the shoot may be compensated to a certain degree.

Still another possibility to single out the phase delay method is to influence the hormone transport velocity. A constant relation between the clocks of the plant organs and measuring mediator transport velocity is presupposed for correct size measurement by the phase delay method. Therefore any increase or decrease in this velocity, presuming that the pace of two clocks is unchanged, will result in an effective decrease or increase (respectively) in the plant size as sensed by the shoot apex.

We do not see a way to find a characteristic and predictable influence of the communicating vessels system by surgical treatment. There is no clear way to change the hypothetical volumes which have to be filled because we do not know what these volumes are.

Another approach to the problem is to find specific hormones which behave in a way that suggest their use in one of the possible measuring systems. Here we tread on thin ice because of numerous incomplete and even contradictory data obtained from different species in different conditions by different methods.

One expects that hormones employed in different methods of the length measurement have different patterns of distribution in the plant and different modes of change with time.
Fig. 5
Caption for Figure 5

Fig. 5. Concentration of the measuring mediator.

a. Concentration of the measuring mediator (in the toll method decreases with increase in the distance from the root but does not change with time at any particular location. The distribution of the hormone at time $t_1$ when the length of the plant is equal to $L_1$, will be represented by the same graph as the one at time $t_0$, when the length of the plant was $L_0$, with addition of the increment.

b. The concentration of the hormone employed in the method of communicating vessels decreases with time, as the size of plant increases.
The concentration of the mediator of the toll method decreases with increase in distance from the root but does not change with time at any particular location. (Fig. 5a). In contrast the concentration of the hormone employed in the method of communicating vessels decreases with time, while at least in the simplest case it may be fairly constant along the plant at any given moment (Fig. 5b). The chemicals of the phase delay method are expected to change both in time and in space according to specific implementations. One has to identify all the components of the signal to find actual phase delay with increased size.

(B) Roles of specific hormones in maturing plants

The analysis above is not limited to the problem of juvenility and may be applied to the size measurement in living organisms in general. Here we make several suggestions on possible roles of particular phytohormones in various methods of size measurement in juvenile maturing plants.

The most conspicuous correspondence between important plant hormones and features we ascribe to our hypothetical size-measuring mediators is that between auxin and the hypothesized communicating vessels hormone. Auxin is produced by the uppermost leaves, it moves downwards in polar fashion and thus is probably only marginally deflected at the shoot branching points. Concentrations of auxin in the root system are reported to be several magnitudes smaller than in the shoot, while its distribution along the shoot is fairly homogeneous except for the lowest part where the concentration decreases (Scott & Briggs 1960). Finally auxin is known to be a flowering inhibitor, which is in accord with the need of plants to become large in order to diminish concentration of auxin at the apex.

Cytokinlin seems to be the chemical that, at least in some of the tested species, carries the root signal which inhibits flowering (Miginiac 1978) and maintains plant juvenility. The influence of root inhibition decreases with increase in the plant size. It would be interesting to try to associate cytokinin with the hypothetical hormone of the toll method.

Very little is known concerning the modes of the hormone production in plants. In many cases even the production loci of important plant hormones such as the gibberellins is unknown (Crozier 1980). However oscillatory behaviour in hormonal transport (Zajączkowski, Wodzicki, Bruinsma 1983) as well as in receptivity of the apex to induction (Fontaine 1972, Miginiac 1978) were reported. Oscillations in growth of various parts of plants even in constant conditions are also known (List 1969). Taking into account that maturation is associated with meristematic growth (Brink 1962), one hypothesizes that maturation-related hormone production, being growth-dependent, also reveals oscillating patterns compatible with various implementations of the phase delay method.

Concluding remarks

In the present work we discussed possible ways of size measurement by shoot
apical meristems of plants in the context of their maturation process. Sensing the size of a developing organism (or its parts) by various of its organs is important for many other processes (e.g. correlating growth rates of different parts of the organism). It is important for plants as well as for animals where one observes maintenance of (developmental stage-dependent) proportions between parts of the organism throughout the developmental process.

We believe that the methods of size measurement may be studied along the lines presented above in all developmental systems.

References

Chapter Two

The Impact of Growth on Hormonal Communication
Introduction

(A) General remarks

Communication by means of signals is an important tool in the development of living organisms throughout their life cycle. It is especially significant for plants. Because of the immobility of higher plant cells, signal communication is the only way of interaction between spatially separated organs. Hormonal messengers are an important means of such communication. In the present work we discuss possible types of hormonal signals in plants and mechanisms of their translocation.

An important feature of higher plants that distinguishes them from many other forms of life is the existence of embryonic regions of continuous growth throughout most of the plant's life cycle. Consequently various changes in plant behavior proceed simultaneously with the growth process. It is thus only natural to expect a correlation between growth and other processes. In particular one can study the impact of growth on hormonal communication. In other words one can ask what is the possible impact of changes in the plant growth pattern on the reception of hormonal signals by target organs.

One of the characteristic changes in the growth pattern of an organism is the change in its growth rate. We discuss below how such a change can influence the reception of hormonal signals. We discuss this question on three different levels of specificity. The suggested mechanisms of interaction between the growth rate of an organism and its development are applicable to any relevant developmental problem as far as it satisfies the conditions mentioned in our discussion. Nevertheless, in order not to be too abstract we discuss the particular application of our ideas to signals involved in transition of higher plants to flowering. In the Discussion we attempt to be even more specific and to point out which individual phytohormones could play various roles in the proposed mechanisms. The more general ideas put forward in the present work do not depend on the more specific applications.

(B) Transition to flowering

An increase of the growth rate at subapical regions of plant stems seems to be one of the earliest signs of the reproductive transition. The change is most spectacular in plants that possess the rosette mode of growth. These plants, which are characterized by an almost stemless "rosette" of leaves at their vegetative phase of development, proceed through a rapid elongation of stem (known as bolting) prior to flowering. Acceleration of growth, although less drastic, can be observed in many other plants at the initial stage of reproductive growth. In most cases stem elongation starts before the formation of any reproductive structure. (Bernier, Kinet & Sachs 1981).

The transition to flowering is governed by signals sent by leaves of plants to the shoot apex. (There also may be signals produced by other organs—e.g. roots—but
we do not deal with this question here.) Signals associated with flowering could be either promotive or inhibitive. Promotive signals (at least for photoperiodic plants) are known to come from leaves under appropriate light conditions. Young developing leaves (that were not subjected to flowering-related induction) were found to be inhibitive to the transition. In some species plants could be led to flower simply by continuous removal of all leaves (together with appropriate feeding). (Bernier, Kinet & Sachs 1981).

For specificity our discussion is limited to the interaction of the growth rate with inhibitive signals sent to the apex by uninduced leaves. We concentrate on the influence of growth on the level above that of individual cells, leaving aside the question of the possible impact of an increased rate of cell division (that is associated with accelerated stem growth) on the processes within cells.

Types of signals

Before proceeding further in our discussion of the impact of growth rate on the transition to flowering let us see what kinds of hormonal signals could be involved in plant communication.

Fifty years ago Chalakhian (1936) suggested the existence of a chemical flowering signal. He associated this signal with a hypothetical hormone — florigen. This notion has dominated the field ever since. Later on, experimental evidence led to the postulation of the existence of another hormone, one that inhibits flowering. In spite of considerable efforts neither of these hypothetical hormones has been found. Consequently more complicated schemes of flowering evocation were put forward. These later models assumed the existence of signals composed of several hormones or the sequential action of several hormones. (For reviews see Vince-Prue 1975; Bernier, Kinet & Sachs 1981).

All the above models are based on an assumption that both promotive and inhibiting signals are carried by chemical concentrations of hormones in such a way that some function of these concentrations governs the corresponding processes. However there also exists another way to carry signals: an oscillating signal might be generated and carried by a mediating hormone. Information could be carried by the frequencies of oscillations in the hormone concentrations. Usually modulated signals are associated with electrical media but oscillations in the production of chemicals and their translocation have been observed in numerous organisms. Chemicals that play the role of mediators for periodic signals well satisfy the classical definition of a hormone as being an organic substance produced at one part of an organism and acting in small quantities upon its transfer to another part, influencing specific physiological processes (Went & Thimann 1937; Thimann 1960). Models for the establishment of pattern by a periodic wavelike spread of hormones have been put forward (e.g. by Goodwin & Cohen 1969).

In the context of plants, periodicities have been found in many processes (Sweeny 1969). In particular oscillations of about 20–30 minutes in the transport
of the most studied phytohormone—auxin—have been reported (Newman 1963; Hertes & Flory 1968; Shen-Miller 1973; works of Zajączkowski, Wodzicki and associates reviewed in Zajączkowski, Wodzicki & Romberger 1984). The latter authors put forward a model for pattern formation by a hormone transported in a wavelike fashion. Khait (1986) pointed out implications of an assumption that a periodic signal is employed in the shoot size measurement by the apices of maturing juvenile plants. To the best of our knowledge the possibility that signals involved in flowering could be periodic rather than constant has not been considered.

The temporal factor in hormonal signalling in a pattern formation could be different. In some cases an organism (or a certain organ of an organism) can develop according to the chemical pattern with a capability to react to changes in this pattern virtually throughout the developmental process. Here the signal actually governs development. Alternatively, in other cases a temporally short signal plays a role of a switch. The process after initiation acts independently of the subsequent changes in environment.

There is still another possibility of intermediate type when an initially totipotent (in relation to a relevant set of developmental paths) group of cells gradually becomes committed to only one particular type of organization. Here the role of a signal decreases with the level of commitment of target cells so that it becomes progressively more and more difficult to change a specific path of the organ development by changes of chemical environment (that is by sending a new hormonal signal).

Concerning the transition to flowering it was observed (at least for some species) that in the initial stages of their development newly emerging reproductive organs do not appear to be different from their vegetative counterparts (except perhaps for different phyllotaxis) (Lyndon 1979). Hormonal signals thus seemingly control this development at least up to some advanced stage; they do not just switch on. However one cannot discard the possibility that signals of a switch type are also involved. It could happen that the initial changes in transition cannot be observed microscopically. One can expect that among the several signals that are associated with the transition process, various types of signals might be found. We consider this possibility below.

To return to our discussion of periodic signalling one should note that such a signal could be carried either by a single frequency or by slow informative oscillations modulated on a high carrier frequency. The latter possibility is usually implemented in communication via electromagnetic media. A signal of a high frequency (compared to characteristic times for growth and differentiation of organs in question) could play the role of a switch, initiating some development by several oscillations. In particular, such oscillations could be associated with the wavelike pattern of auxin transport mentioned above. Alternatively a high frequency signal can govern the developmental process (completely or up to a certain point). It could, for example, induce and maintain some periodic process that fades if it is not sustained.
Fig. 1
Caption to Figure 1

Changes in the distribution of sources of the hormone during the period of one plastochron.

a. Leaves $E$ and $F$ are major sources of a hormone for the apex. Leaves $B$, $C$, $D$ are gradually ceasing hormone synthesis while the oldest leaf, $A$, has finished its role as a source altogether. Young leaves $G$, $H$ and $I$ are in the process of increasing their hormone production.

b. One plastochron later. Leaf $B$ joins the leaf $A$ in the afterphase of hormone synthesis. Leaf $G$ substitutes for leaf $E$ as a major contributor (along with $F$) of the hormone. A new leaf ($J$) begins synthesis of the hormone.
A control role could also be assigned to a low frequency periodic signal. A naturally arising periodicity of this type is that of one plastochron (the time between the emergence of two successive leaves). The synthesis of hormones by leaves is a function of their age. Physical and physiological ages (the latter could depend on the number of leaves that have emerged above the leaf in question) could be taken into consideration. In any case the overall production of hormones changes to some extent during the duration of a plastochron. If the growth entered a steady phase or if the physiological age governs hormone production, appearance of a new leaf returns the situation to the one at the beginning of the previous plastochron. Each leaf takes the role of its predecessor with lower leaves gradually losing their roles as sources of hormone for the apex. (See Fig. 1 for illustration.)

To give an example (deliberately oversimplified) suppose that the mediating hormone is synthesized by the two uppermost leaves. The strength of each leaf as a source grows from zero to a maximum (assumed to be equal to 1, in suitable dimensionless units) during the first plastochron of its life (which is described by function $C_1(t)$ below) and consequently falls to zero during its second plastochron (described by $C_2(t)$).

$$
C(t) = \begin{cases} 
C_1(t) & 0 < t < T \\
C_2(t - T) & T < t < 2T \\
0 & 2T < t
\end{cases}
$$

(1)

describe the strength of a leaf as a source (assuming that the leaf in question emerges at $t = 0$) ($T$ – duration of one plastochron).

The constraints on $C_1$ and $C_2$ are

$$
C_1(0) = C_2(T) = 0; \\
C_1(T) = C_2(0)
$$

(2)

The overall signal received by the apex $s$ is a summation of the signals produced by two leaves, one in its first plastochron, the other in its second plastochron

$$
s = C_1 + C_2
$$

(3)

An example of the former case occurs if

$$
C(t) = \begin{cases} 
\frac{t}{T} & t \leq T \\
1 - \frac{t-T}{T} & T \leq t \leq 2T \\
0 & 2T < t.
\end{cases}
$$

(4a)

A periodic signal will appear with

$$
C_1(t) = \sin \frac{\pi t}{2T}, \quad C_2(t) = \cos \frac{\pi t}{2T}
$$

that is

$$
C(t) = \begin{cases} 
\sin \frac{\pi t}{2T} & 0 \leq t \leq 2T \\
0 & 2T < t.
\end{cases}
$$

(4b)
From eq (4a) the overall signal is equal to

\[ s = C_1 + C_2 = \frac{t}{T} + 1 - \frac{t-T}{T} = 2. \]  

(5a)

In the case of signal generation governed by eq (5b)

\[ s = \sin\left(\frac{\pi t}{2T}\right) + \cos\left(\frac{\pi t}{2T}\right) = \frac{\sqrt{2}}{2} \sin\left(\frac{\pi t}{2T} + \frac{\pi}{4}\right). \]  

(5b)

An organ that sends a periodic signal need not necessarily synthesize the hormone that carries the signal. Such a signal might be generated by the employment of hormones already present in a plant. Periodic changes connected with properties of cell membranes have long been suggested as means for time measurement in plants (Engelmann & Schrempf 1980). The possibility that a leaf that promotes flowering might act through its effect on endogeneous substances (by changing membrane permeability for particular hormones) has been mentioned in the literature. (See e.g. Mertz 1985). Phytochrome, the chemical associated with leaves that promote flowering, influences membrane properties (Marme 1977). However, this potentially important method of signal generation seems not to have been sufficiently explored.

The proposed possibility leads to the need for a new interpretation of experiments that consist of external application of hormones to plants. The results of such experiments are usually discussed under the assumption that the concentration of the hormone in question (the externally applied part of which is controlled by a researcher) is the parameter that should be associated with the effects observed in the experiments.

The influence of the applied hormone will strongly depend on the site of application and on the timing of external interference. If a hormone that is a medium for a signal is applied to a signal receptor this will lead to a disturbance of the signal as it is perceived by the receptor. Because an informative signal will be summed with a noisy component, an application of the hormone will thus cause an effect opposite to that of the original signal. If for instance the signal was to induce flowering a natural conclusion from the application of the exogenous hormone will be that it inhibits flowering. This interpretation is certainly wrong under our hypothesis.

Suppose, on the other hand, that the hormone is applied at a location between its natural source and a signal generating organ (assuming that they are not the same). Suppose further that the applied hormone does not disturb natural production and that there is an additional transport capacity. Then the result of the application would be an increase in the amplitude of the natural signal so that in this case the application of the exogenous hormone would lead to an increase in the strength of a signal.
How an increased growth rate can influence signal reception

The change in the rate of growth can have an impact on the acceleration of the divergence between the apex and the leaves (mainly because of internode elongation) on the one hand and on the rates of primordia initiation and the growth of leaves on the other hand. (An increase in the rate of primordia succeeding initiation may or may not be matched by an increase in the rate of growth in leaves.) Both of these factors can influence hormonal communication.

In the previous section we noted that there are two types of signals that should be taken into account—constant and periodic. Inhibition could thus result either from inhibiting hormones synthesized by the leaves in sufficient quantity or from signals generated at inhibiting frequencies. We shall put forward strategies to overcome inhibition of both types.

(A) Escape from inhibition

Growth has various collateral results. In particular it can lead to an increase in the distance between different parts of a growing organism. An increase in growth rate brings about an increase in the velocity at which these parts move away from each other.

Suppose that a signal is generated at point A (Fig. 2a) at moment $t = 0$ and received at point B. Suppose that an interval AB is growing at each point inside this interval with rate

$$g = g(t, x).$$

Let us denote the length of AB by $\ell(t)$ ($\ell(0) = \ell_0$). The velocity of the movement of the apex away from the leaf below (for simplicity we shall refer to one sufficiently grown young leaf) is given by

$$V(t) = \int_{\ell(t)} g(x, t) \, dx.$$  

$\ell(t)$ signifies that the integral is taken over the region of length $\ell(t)$ between the leaf and the apex. The equation for the change in the distance of a travelling signal from the receiver at point B is

$$\frac{dz}{dt} = -v + \int_0^z g(t, x) \, dx, \quad z(0) = \ell_0. \quad (8)$$

$v$ is the velocity of a signal translocation.

In the present work we limit ourselves for simplicity to a constant uniform growth rate. In this case eq (8) yields

$$\frac{dz}{dt} = -v + g\ell, \quad z(0) = \ell_0. \quad (9)$$
The solution of eq (9) is

\[ z(t) = \frac{r}{g} - \left(\frac{v}{g} - \ell_0\right) \exp(gt). \]  

(10)

The signal approaches the receiver \((z \text{ decreases})\) only if

\[ v \geq g\ell_0. \]  

(11)

In this case the signal reaches \(B\) at time

\[ t = -\frac{1}{g} \ln\left(1 - \frac{g\ell_0}{v}\right). \]  

(12)

\[ (B) \text{ Doppler effect} \]

Suppose now that the signal is periodic. It is well known in physics (acoustics and optics) that the frequency of a perceived signal is different from the original frequency emitted by the source in accord with the relative velocity of the source and the receiver. This phenomenon is known as the Doppler effect. The formula that describes the change \((\Delta w)\) in frequency \((w)\) as a function of the relative velocity between the source and the receiver \((V)\) is

\[ \frac{\Delta w}{w} = \frac{V}{v}, \]  

(13)

\((v \text{ is the signal velocity})\). A derivation of this formula can be found in almost any standard physics text.

In the derivation of eq (13) one assumes that the receiver moves relative to the source of the signal through a motionless medium and in particular that the media remains static after the signal was emitted. In our case this assumption is invalid because the growth in the plant (the medium for signal propagation) persists even after the signal is emitted. Let us derive an equation alternative to eq (13) for growing media.

Suppose that the signal has frequency \(w\). One wave-length of a signal is emitted during a time interval \(r = \frac{1}{w}\). During the time \(r\) the distance \(AB\) (Fig 2b) increases so that

\[ \ell(r) = \ell_0 \exp(gT). \]  

(14)

(This result could be obtained from eq (10) putting \(v = 0\).)

From eqs (12) and (14) one can see that the last portion of the signal arrives at the receiver at time

\[ t^* = -\frac{1}{g} \ln\left(1 - \frac{g\ell_0 \exp(gT)}{v}\right) + r. \]  

(15)
The perceived frequency is

$$w^* = \frac{1}{t^* - t}.$$  \hfill (16)

From eqs (12) and (15)

$$w^* = \frac{w}{1 + \frac{w}{g} \ln \left[ \frac{1 - \frac{g\ell_0}{v}}{1 - \frac{g\ell_0}{v} \exp\left(\frac{\ell}{v}\right)} \right]}.$$  \hfill (17)

Thus

$$\frac{\Delta w}{w} = \frac{\ell n \left[ \frac{1 - \frac{g\ell_0}{v}}{1 - \frac{g\ell_0}{v} \exp\left(\frac{\ell}{v}\right)} \right]}{\frac{2g}{w} + \ell n \left[ \frac{1 - \frac{g\ell_0}{v}}{1 - \frac{g\ell_0}{v} \exp\left(\frac{\ell}{v}\right)} \right].}$$  \hfill (18)

Expression (18) is an alternative to formula (13) for the Doppler effect in a growing media. Note that the parameter $g\ell_0$ is the counterpart of $V$ from eq (13) in the growing medium since it describes the relative movement of the source and the receiver.

If the growth rate is small compared to the frequency of the signal

$$\frac{g}{w} \ll 1,$$  \hfill (19a)

and if the relative velocity is not too close to the velocity of the signal

$$\frac{2g}{w} \ll \frac{r}{g\ell_0} - 1,$$  \hfill (19b)

then from eq (18) $\frac{\Delta w}{w}$ can be approximated by

$$\frac{\Delta w}{w} \approx \frac{g\ell_0}{v},$$  \hfill (20)

which is the equivalent of relation (13). If, however the growth rate is of the same order of magnitude as the frequency of a signal there is a remarkable difference between the divergence of a frequency as perceived by the receiver given by formula (18) from the result of (13).

To give an example, suppose that $g = w = 1\text{(day)}^{-1}$ (which describes vigorous growth), and that $v = 1\text{cm/hour}$. (We support the relevance of this example in the next section.) If $\ell_0 = 2\text{cm}$ then

$$\frac{g\ell_0}{v} \approx 0.1, \quad \frac{g}{w} = 1.$$

For these parameter values, formula (13) (that does not depend on $\frac{g}{w}$) yields $\frac{\Delta w}{w} = 0.1$, while from formula (18) $\frac{\Delta w}{w} = 0.2$. The discrepancy is even larger when $\frac{g}{w} > 1$. 
Caption to Figure 2

a. signal in the form of a point impulse produced at point A at $t = 0$ and moves towards point B with velocity $v$. The segment AB grows at each point with the growth rate $g$. The signal will reach point B only if $v > g\ell_0$.

b. When the signal has time structure, this structure is disturbed by the growth.
To return to our problem, one can see from relation (13) that an increase in the growth rate and the corresponding increase in velocity with which an apex moves from a source of inhibiting signal (formula (7)) leads to increased divergence of the perceived frequency from the emitted one. This might lead to a change of an inhibiting frequency to a neutral frequency or one that promotes flowering.

(C) Change in the rate of primordia initiation

and growth rates of leaves

The change in the rate of primordia initiation can also affect signals that move between the leaves and the apex. If the signal is associated with the frequency of emergence of new leaves (e.g. an example described by relations (1b) and (2b)) then a change in the duration of the plastochron leads to a change in this frequency.

Another effect of an increase in the growth rate at the apex could be observed if this increase is not followed by a corresponding increase in the growth rate of leaves, that is if an increase in initiation of leaf primordia is not matched by a simultaneous increase in the growth rate of individual leaves. In this case there is an increase in the distance between the apex and leaves that are sufficiently developed to exert an inhibiting influence. An apex finds itself farther away from the source of inhibition and, consequently, the effect of inhibition can diminish.

It is interesting to follow some implications of this effect on results of experiments. If there is another hormone that promotes the growth of leaves without influencing apical growth then its application will result in stronger inhibition of flowering because leaves will develop to acquire inhibiting capabilities closer to the apex. If the same hormone is effective in promoting the growth of both leaves and the apex then one expects confusing results of experiments when this hormone is applied to the apical region of plants. The results will depend on the exact final location of the hormone. Clear separation between close locations probably could not be achieved. Almost certainly the results will vary from species to species.

One should note that in contrast to the effects discussed in Sections A and B, all of the variants of the present possibility do not depend on the velocity of the mediating hormone.

On the velocities involved

Two velocities are important for the mechanisms presented above: the velocity of signal transport and the velocity of movement of the apex relative to the leaves on the stem (formulae (7), (11) and (18)). Let us consider these parameters in more detail.

The five possible routes for exchange of compounds in higher plants (Letham, et al., 1978) could be divided for our purposes into two groups. One such group includes active transport associated with a definite velocity while the other consists
of hormone translocation by means of diffusion. Usually long range translocation is associated with the more rapid ways of communication that belong to the first group while a slow (for nongaseous chemicals) diffusion transport is employed for short distance transportation. However, the mechanisms of phytohormone translocation is a subject of great uncertainty. Even for the most studied pattern of movement of a plant hormone—polar transport of auxin—many questions remain concerning the mechanisms of its translocation (Goldsmith 1977, Letham, Goodwin & Higgins 1978 v. 1; Jacobs 1979). There is evidence in support of both active and diffusive mechanisms of hormone transport. Concerning auxin it is also not clear if the mode of acropetal movement of this hormone is substantially different from its basipetal translocation or is essentially the same (McCready 1968; Keitt & Baker 1967, Goldsmith 1977, Jacobs 1979). To be specific we limit ourselves here to the possibility of an active transport.

Velocities associated with translocation of plant hormones over short distances (before they enter the main transportation routes of the plant) are reported to be very slow. Research concerning nondiffusive transport of auxin yields a velocity range between 1cm/hour and 1 mm/hour (Jacobs 1979). For gibberellins these figures are even lower (Graebe & Ropers, 1978). The slow rate of phytohormone transport over short distances actually served as one of the main reasons for suggesting diffusive mechanisms of translocation. The possibility that a vigorously growing piece of stem outgrows a slowly moving hormone (which is similar to our escape mechanism but without any signalling role assigned for the hormone) was put forward by Graebe & Ropers (1978) as an interpretation for experiments of Phillips & Hartung (1974) that aim (and fail) to detect gibberellin that was to pass through short sections of internodes.

There is a paucity of data on apical growth rates (Silk 1984). But data for the overall growth of bolting plants, usually considered to be one cm per several weeks, shows that the typical average growth rate of such plants is about 1 cm/day (e.g. Lang, Sandoval & Berdi, 1957; Cleland & Zeevaart, 1970). Some of this growth could be associated with the region of lower internodes that might be irrelevant for our discussion. On the other hand one cannot expect that there is a uniform growth rate through the period of several weeks. During some stages of the growth process there could be divergence of the growth rate from the average value to higher levels.

To conclude it seems that translocation rates of phytohormones through the short distances out of leaves and into the apex are of the same order of magnitude as growth rates of vigorously growing plants. This provides a basis for our hypothesis of the impact of growth rate on flowering (escape strategy and mechanism based on the Doppler effect).

Discussion

In the present chapter we did not discuss the question of the initiation of accelerated growth. Different mechanisms could be proposed. Acceleration of
growth is usually associated with the activity of gibberellin. External application of gibberellins to rosette plants that bolt upon transition to flowering (and even to the more general group of plants that exhibit some acceleration in growth at this stage) promotes the transition. Gibberellins mimic at least some of aspects of light induced flowering transition. In some cases application of gibberellin completely substitutes for induction by light so that a plant flowers in strictly uninductive light conditions. (Zeevaart 1978; Bernier, Kinet & Sachs 1981). Gibberellins seem to promote internode stem elongation. If they are synthesized by leaves (the sites of gibberellin synthesis are not fully established (Crozier, 1981)) they should not move to the apex (which is a long process taking into account their slow rate of translocation mentioned above) but they can act on internode tissue in the proximity of the source leaf.

An increase in activity of gibberellins could be a result of an additional signal that arises from the leaves under light conditions that promote flowering or, especially for day neutral plants (whose transition to flowering is independent of the external environment), could be dependent on the age of the plant or on its size, as discussed by Khait (1986). An interesting possibility involves production of a growth promoter as one of the effects of flowering inhibitive hormone, so that an inhibition leads to its own reversion. Such a possibility could be associated with a limited amount of prereproductive growth that can be reduced (if inhibition is removed by external intervention) but not increased, which has been observed in some species (McDaniel 1984b).

As for identification of flowering inhibition with known hormones, the question is problematic. Auxin might be proposed as a strong candidate for this role. It is produced by young leaves and is known to inhibit flowering in numerous species (although there are cases when auxin actually promotes flowering; Zeevaart; 1978, Bernier, Kinet & Sachs 1981). While transported mostly basipetally (downwards), auxin has the capability of upward acropetal movement, at least for short distances (Thimann 1977; Schneider & Wightman 1978; Jacobs 1979).

It is known that auxin is most inhibitory when applied to short-day plants (which flower only if the duration of the light period is short enough). These plants do not bolt. Application of auxin to many bolting plants is ineffective. It was shown on the other hand that auxin acts mostly at the apex itself rather than at the leaves (Krekule & Privratsky 1974). One can speculate that the reason for this ineffectiveness lies in the capability of the apices of fast growing plants to escape from inhibition.

The possibility that auxin produced by the upper leaves can govern the behavior of the shoot apex was put forward in different contexts by Schwabe (1984) and Khait (1986).

The presentation above does not depend on particular implementations. We believe, however, that it is worth trying to identify specific hormones involved with known chemicals. We are aware of the fact that there is a confused state of affairs with respect to the general principles of action of specific phytohormones (Letham,
Goodwin & Higgins 1978). We therefore want to draw a clear line between the general theoretical considerations put forward in previous sections and the specific implementations suggested here.

One of the remarkable facts about phytohormones is their lack of specificity (Thimann 1977; Letham, Goodwin & Higgins 1978). Each of the known plant hormones influences a wide range of processes in plants. It is interesting to attempt to interpret such phenomena by assigning to each hormone only one aspect of plant behaviour to be governed directly, while other aspects that are dependent on the hormone in question are governed indirectly, because of interdependence between different processes. In the present work we demonstrated how a very specific action of one hormone, namely promotion of growth, can ultimately lead to a broad range of effects and therefore create an illusion that the hormone action is nonspecific.

Consideration of interactions between basic phenomena associated with specific hormones and examination of the possibility of periodic signals that have great information capacity can help to answer the question that arose recently, on the basis of the theory of system control as to whether in principle plants can exist whose communication system is based on hormonal signaling (Canny 1985). Future work on this topic should suggest a list of such basic phenomena together with a list of hormones associated with these phenomena and should show how observed plant behaviour could be obtained as a linear (or nonlinear) combination of these basic elements. In particular, promotion and inhibition of flowering may or may not be one of these basic phenomena. In this context one should take into account the possibility that promotion and inhibition of flowering or of some other mode of behaviour could be controlled by the same hormone when it arrives at the target at different frequencies. Alternatively, promotion, say, could be signalled by a certain frequency while inhibition occurs when there is an absence of any such regular signal.

Conclusions

In the present chapter we analyzed what types of hormonal signals could be employed by plants. We stressed the need for consideration of a potentially important class of periodic signals that is usually not discussed in the context of transition to flowering.

Transition to flowering is closely associated with the process of growth. Only growing plants can proceed from the vegetative to the reproductive mode of existence (Brink 1962). It is thus only natural that the growth rate of plants is an important factor during the evocation, as has actually been observed in many species. Various possible effects of the change in the plant growth rate on its mode of existence put forward above may help to elucidate the relation between these phenomena.

Along with McDaniel (1984a) we believe that the complexity observed in the regulation of flowering (as well as in other hormone-governed modes of plant
behaviour) has obscured some basic generalizations which would enable us to investigate the problem more fruitfully and would help to explain in a relatively simple way a large number of observations that previously appeared as a complex array of virtually inexplicable experimental data. We hope that the present work can help to make a small step toward better understanding of the basic processes behind the transition to flowering.

References


1. Introduction

In the organisms that serve as model systems in developmental biology, (such as the cellular slime mold Dictyostelium discoideum) there are four main patterning phenomena which should be explained:

(i) establishment of a differentiated pattern in natural development,
(ii) reestablishment of the pattern in cutting experiments,
(iii) behaviour in various transplantation experiments,
(iv) reestablishment of form by sorting out after cells are dispersed.

Establishment of the pattern may occur with or without cell proliferation. Reestablishment of the pattern in cutting experiments may come about either by regeneration of the lost tissue or by redifferentiation of part of the remaining cells, which leads to transformation of a fragment into a complete organism. There are two major possibilities for the establishment of the pattern without further proliferation; by sorting out of already differentiated cells, or by positional differentiation.

In the present work we shall deal with the establishment of the differentiated pattern and its reestablishment in cutting experiments in a one dimensional organism without proliferation or regeneration. We assume that initial polarity is established in the organism by some means and that all the following development results from positional differentiation.

We suggest application of our model to the slime mold Dictyostelium discoideum, putting forward certain ideas which may help to merge the processes of sorting out and positional differentiation in this species. A comparison is made with other existing models.
2. Description of the Model and its Operation

(i) Formulation without memory

An important feature of developing organisms is their capability of regulating the relative proportions of their parts. For example in Dictyostelium discoideum more or less the same pattern is formed by mature organisms consisting of as few as 12 cells and as many as $10^5$ cells (Bonner 1967, Loomis 1975, MacWilliams & Bonner 1979). At certain intermediate stages of its development it is capable of reestablishing the same pattern in both parts of its cut body. (See Sec. 3.)

Our model is based on the following widely accepted principles.

1. Differentiation of a cell is a function of its chemical environment.
2. Chemicals which influence differentiation (morphogens) are produced by the differentiating cells themselves.
3. The chemical composition of a cell's secretion as well as its reaction to morphogens changes as the cell differentiates.
4. During the developmental process cells may switch their developmental path (up to a certain stage).

In many cases it appears that establishment of the stationary chemical pattern takes much less time than the cell differentiation. This leads us to an additional principle.

5. Establishment of a stationary chemical pattern is much faster than differentiation.

There are various ways to distinguish between different cell types. In our model the differentiation of cells is expressed explicitly by changes in the composition of their secretion (c.f. principle 3). It also becomes progressively more difficult for a more committed cell to change its parti-
cular path of development.

Because of the separation of time scales assumed in principle 5 the process of differentiation may be regarded as occurring by repetitions of double stages:
A. establishment of a stationary chemical distribution as a result of cell secretion,
B. taking of a small step along their differentiation paths by the cells in accord with the distribution of Stage A.

Thus, the processes that establish the chemical and differentiation patterns take place in parallel and the two patterns continuously influence each other. We take advantage of the fact that the stationary distribution of chemicals is established much faster than the differentiation of cells and neglect the establishment of this distribution on each intermediate stage.

Using the above developmental scheme several times one obtains a sequence of morphogen concentrations $C_i(k,x)$, where the index $i$ signifies the $i$-th chemical, $k$ stands for the $k$-th use of the scheme and $x$ is a space variable.

Suppose that the organism consists of two types of cells: t-cells and p-cells. It is widely assumed that there are organizing cells that govern the process of development by secreting morphogens. In our case these are t-cells. By contrast the p-cells react passively to various stimuli received from the governing cells.

Further presentation of our model will be given with reference to the following problem. There is a one dimensional array of cells in the interval $0 \leq x \leq L$. Initially there is a small tip of t-cells $(0 \leq x \leq R_0)$, while other cells are undifferentiated. (See Section 3 for further discussion.)
For morphogens produced by t-cells we choose two chemicals called \textit{inductor} and \textit{inhibitor}. The former induces t-type differentiation in cells while the latter inhibits this process and thus leads to p-differentiation. Inductor dominates in the immediate proximity of the t-cells while in more distant regions inhibitor is dominant. We assume that the inductor has a large breakdown rate while that of the inhibitor is small. Thus inductor concentrates only about the t-region where it is produced. Inhibitor is distributed almost homogeneously throughout the organism. With given production and breakdown rates of the inhibitor per cell its concentration depends on the size of the t region which produces it, and on the overall size of the organism throughout which it is eliminated. This provides a clue for the understanding of a regulation mechanism described below.

Quantitatively our requirements on the breakdown rates of the inductor and inhibitor are given by

\[ \sqrt{\frac{A^*}{D_1}} \gg 1, \quad \sqrt{\frac{B^*}{D_2}} \ll 1, \]  

(1a,b)

where \( A^* \) and \( D_1 \) are respectively the breakdown rate and the diffusion coefficient for inductor, while \( B^* \) and \( D_2 \) are the corresponding quantities for the inhibitor.

For a quantitative description of secretion by t-cells we introduce parameters \( d^*_1 \) and \( d^*_2 \) to characterize the source strength for the inductor and inhibitor. For the present we assume that the secretion of a cell depends only on its developmental stage and not on its external environment. Introduction of a dependence of cell secretion on environment only enriches the effects which the model provides. We show here that such effects are not necessary to simulate the regulation phenomenon both in natural development and in cutting experiments.
Suppose that a chemical pattern is established. How will cells interpret it for the purpose of differentiation? As assumed above inductor dominance over inhibitor causes t-type development, while a cell is forced into a p-path if inhibitor is dominant in its proximity. To express this in the simplest way we introduce the function

\[ C(k,x) = a(k,x) - h(k,x), \]  

where \( a \) and \( h \) are concentrations of inductor and inhibitor at point \( x \) after the \( k \)-th use of the differentiation scheme. (More generally \( C \) may be defined by \( C = m_1 a - m_2 h \), where \( m_1 \) and \( m_2 \) are constants expressing the relative influence of morphogens.) If at some part of the organism function \( C \), defined by (2), is positive then this part is induced to develop through the t-path; otherwise, if \( C \) is negative, p-development is induced.

We neglect memory for the present and assume that the differentiation of a cell depends only on its current chemical environment, that is on \( C(k,x) \).

To summarize, if t-cells occupy the interval \((0,R)\) then the equations for the subsequent distribution of the inductor \((a)\) and inhibitor \((h)\) are:

\[
\begin{align*}
D_2 \frac{d^2 h}{dx^2} - B^* h + d_2 &= 0, \quad x < R; \\
D_2 \frac{d^2 h}{dx^2} - B^* h &= 0, \quad x > R; \\
D_1 \frac{d^2 a}{dx^2} - A^* a + d_1^* &= 0, \quad x < R; \\
D_1 \frac{d^2 a}{dx^2} - A^* a &= 0, \quad x > R.
\end{align*}
\]
The boundary conditions correspond to the assumption that there is no leakage of morphogen at the ends of the organism:

\[
\frac{dh}{dx}_0 = \frac{dh}{dx}_L = \frac{da}{dx}_0 = \frac{da}{dx}_L = 0.
\]  

(4)

The solution of (3) and (4) is

\[
h = \frac{d_2}{B}\left\{1 - \frac{\cosh\sqrt{B}x}{\sinh\sqrt{B}L} \sinh[\sqrt{B}(L - R)]\right\}, \quad x < R; \quad (5a)
\]

\[
h = \frac{d_2}{B}\frac{\sinh\sqrt{B}R}{\sinh\sqrt{B}L} \cosh[\sqrt{B}(L - x)], \quad x > R; \quad (5b)
\]

\[
a = \frac{d_1}{A}\left\{1 - \frac{\cosh\sqrt{A}x}{\sinh\sqrt{A}L} \sinh[\sqrt{A}(L - R)]\right\}, \quad x < R; \quad (5c)
\]

\[
a = \frac{d_1}{A}\frac{\sinh\sqrt{A}R}{\sinh\sqrt{A}L} \cosh[\sqrt{A}(L - x)], \quad x > R. \quad (5d)
\]

Here

\[
A = \frac{A^*}{D_1}, \quad B = \frac{B^*}{D_2}, \quad d_1 = \frac{d_1^*}{D_1}, \quad d_2 = \frac{d_2^*}{D_2}.
\]

Using (1), we see that the solution of (5c,d) may be approximated by

\[
a = \frac{d_1}{A} - \frac{d_1}{2A} e^{-\sqrt{A}(R - x)}, \quad x < R; \quad (6a)
\]

\[
a = \frac{d_1}{2A} e^{-\sqrt{A}(x - R)}, \quad x > R.
\]

Hence except for the transition zone between the two regions \((x \neq R)\) the concentration of the inductor is given by

\[
a \approx \frac{d_1}{A}, \quad x < R; \quad a \approx 0, \quad x > R. \quad (6b)
\]

To a first approximation, the concentration of inhibitor is equal to a constant:

\[
h \approx d_2 R/BL \quad (6c)
\]
Thus \( C_R(x) = a(x) - h(x) \) is given by

\[
C_R = \frac{d_1}{A} - \frac{d_2}{B} \frac{R}{L}, \quad x < R, x \neq R; \tag{7}
\]

\[
C_R = -\frac{d_2}{B} \frac{R}{L}, \quad x > R, x \neq R.
\]

[Here the subscript \( R \) on \( C \) explicitly indicates that t-cells are located in the interval \((0, R)\).] A "boundary layer" connects these two constants in the vicinity of \( R \) [see (6a)] with

\[
C_R(R) \approx \frac{1}{2} \frac{d_1}{A} - \frac{d_2}{B} \frac{R}{L}. \tag{8}
\]

Assume that the parameters are such that \( C(R_0) > 0 \), while \( C(L) < 0 \). Then there exists some \( R_1 \) \((R_0 < R_1 < L)\) such that \( C(R_1) = 0 \). We thereby obtain a new differentiated pattern with t-cells in \((0, R_1)\) and p-cells in \((R_1, L)\).

We return now to the first stage of our differentiation procedure. Solving system \((3, 4)\) with \( R = R_1 \) we obtain a new chemical pattern, which is then translated into a new differentiation pattern with some different value of \( R \). From (8) one sees that as \( R \) grows \( C(R) \) diminishes and thus with suitable values of parameters \( A, B, d_1, d_2 \) there is a value \( R = R_* \) such that

\[
C_{R_*}(R_*) = 0. \tag{9}
\]

The pattern in which t-cells are located in \((0, R_*)\) while p-cells are located in \((R_*, L)\) is stable to perturbations. That is, if for some reason \( R \) becomes greater or smaller than \( R_* \) it will decrease or grow until eventually it is again equal to \( R_* \). Fig. 1 illustrates the regulation process.
Fig. 1

(a)

(b)

(c)
In the beginning of the differentiation process there are t-cells located in a small segment \([0,R_0]\) while all other cells are undifferentiated. T-cells secrete morphogens. Fig. la shows the distribution of the inductor \((a)\) and inhibitor \((h)\) over the body of the organism when \(R_0 = 1\). A graph of the function \(C = a - h\) may be seen in Fig. 1b.

As a result of inductor and inhibitor action on the cells a differentiation step occurs. The boundary between the two regions moves to the right (see arrow). Now one obtains t-cells in a segment \([0,R_1]\), where \(R_1 = 1.38\). Newly differentiated cells begin production of morphogens along with the "veteran" t-cells and a new chemical pattern is established (Fig. 1c). As the process continues the boundary between t- and p-regions approaches a stable limit at point \(R^*_k\). If for some reason the differentiation process passes this point, that is for some \(k, R^*_k > R^*_1\), in succeeding steps the boundary will be moved back toward \(R^*_1\). (See Fig. 1d.) As the process continues the differences in the boundary position become progressively smaller. This is shown in Fig. 1e, a plot of successive values \(R^*_k\) for two different initial values \(R_0\).

Here and in Fig. 2 we use \(A = 10, B = 10^{-4}, d_1 = 20, d_2 = 3 \cdot 10^{-4}\) in eqns (6a) and (6b).
If the system has parameters which satisfy

\[ \frac{1}{2} \frac{d_1 B}{d_2 A} = \varepsilon < 1 \]  

(10a)

then

\[ R_* = \varepsilon L. \]

In other words the fraction of t-cells

\[ \frac{R_*}{L} = \varepsilon \]  

(10b)

does not depend on L. Eq. (10a) is obtained straightforwardly from (8) and (9).

(ii) Effects of memory; cutting experiments

Mathematically the regulation phenomenon was obtained without assuming that there is memory in the system: the differentiation state of each cell depended only on the current distribution of morphogens. Biologically this is an oversimplification. The present differentiation state of cells depends on their history. As will be seen from the discussion below, even mathematically the model without memory will fail if one considers cutting experiments.

Suppose that the organism is cut into two parts. We first note that the problem of reestablishment of the differentiation pattern in the posterior part can be dealt with relatively easily. There are two possibilities.

a) The organism is cut at a point \( y < R_* \). Then there is a t-segment in the posterior section, which regulates the ratio exactly as in the natural process described above.

b) Suppose \( y > R_* \). We now assume that a small t-tip is established by the polarity of the system as in the beginning of the natural regulation
process so that once again the problem reduces to one previously solved. (In Section 3 support for this assumption is presented.)

Let us turn our attention to the anterior part. It consists mostly or entirely of t-cells. To be specific let us assume the latter. These cells continue secretion of the inhibitor at the previous rate. But now the inhibitor is distributed over a smaller region than before and thus its concentration grows. At the same time the concentration of the inductor does not grow appreciably because even in the parent organism it was located mostly in the t-region and its immediate proximity. From (7) one thus obtains

\[ C(x) = \frac{d_1}{A} - \frac{d_2}{B} \]

for the entire organism. From (10a)

\[ \frac{d_1}{A} = 2\varepsilon \frac{d_2}{B} , \text{ hence } C(x) = (2\varepsilon - 1) \frac{d_2}{B} . \tag{11} \]

If \( \varepsilon > \frac{1}{2} \), \( C \) is positive throughout the anterior part. Therefore t-cell differentiation will be maintained. Accordingly, to be capable of reestablishing pattern in cutting experiments the organizer cell region may not include more than a half of the organism. A system with too many leaders is vulnerable!

If \( \varepsilon < \frac{1}{2} \), \( C(x) \) becomes negative [eq.(11)], that is inhibitor concentration dominates over that of inductor and cells are driven towards redifferentiation.

At this point the memory of the cells becomes crucial. If there were no memory the whole anterior section would redifferentiate into p-cells in one stage of the differentiation scheme. Observations show that redifferentiation is a lengthy process, which is also one of the foundations of
our model (principle 5). Introduction of memory prevents such a drastic and unrealistic change in the organism. Moreover, it also helps to produce gradations in redifferentiation because the more anterior cells are more t-differentiated and would take longer to change their type. After some time (that is several applications of our differentiation procedure) the less t-differentiated cells in the posterior end become p-cells. When the correct amount of such cells [defined by (10)] has appeared, the regulation process stops and there is a new organism with the same ratio of t- and p-cells in it as in the parent organism.

To express the effect of memory we introduce a function \( g \) which characterizes the level of differentiation of each individual cell. In general, \( g \) is an accumulative function, that is its present value depends on the cell's environmental history. For our problem with two competing morphogens

\[
g(k, x) = g[C(1, x), \ldots, C(k - 1, x)], \quad (12)
\]

where \( C \) is defined by (2).

The absolute value of \( g \) shows the level of commitment of the cell to its particular path of development, or in other words how much it has differentiated through one of the paths. Positive values of \( g \) are associated with the t-path while negative values of \( g \) characterize the level of cell's p-differentiation. For undifferentiated cells \( g = 0 \).

As has been said above, differentiation manifests itself in our model by a change in the composition of the cells secretion. For our problem, in terms of \( g \) this is expressed by

\[
d_i(g, x) = \begin{cases} 
  d_i & \text{if } g(x) > 0, \text{ that is if } x \text{ belongs to t-region.} \\
  0 & \text{if } g(x) < 0, \text{ that is if } x \text{ belongs to p-region.}
\end{cases} \quad (13)
\]
We assume that differentiation of a cell consists of two parts:
1. A short term change which may appear through internal production of some chemical or its consumption from outside. It is short term in the sense that it fades quickly if not backed up or restored continuously.
2. A long term change in cell structure as a result of a continual influence of the short term change. This change does not fade quickly (and after some stage the process may be not reversible). The final level of differentiation is determined by this change.

For a description of the short term change we use the function \( C \).

To describe a change in the structure of cells (long term change) we assume that the differentiation function \( g \) satisfies
\[
g(k, x) = b_1 g(k-1, x) + f(k, x), \quad 0 < b_1 \leq 1. \tag{14a}
\]
Here \( i \) is equal to 1 or 2 depending on which path of development is taken by the cell located at point \( x \). t-cells are associated with \( i = 1 \), p-cells with \( i = 2 \). The coefficients \( b_i \) characterize the memory of a cell. The term \( f(k, x) \) describes a change that has occurred in a cell during the \( k \)-th application of the differentiation procedure. We assume that
\[
f(k, x) = C(k, x). \tag{14b}
\]
If \( C(k, x) \) is positive, a cell located at point \( x \) differentiates further through the t-path if it is already a t-cell or diminishes its p-differentiation if it is a p-cell. In terms of \( g \) one can say that earlier we simulated regulation with memory coefficients equal to zero.

To stress the passive nature of p-differentiation we define \( b_2 = 0.5 \), while \( b_1 = 1 \). P-cells are ready to receive new influences from t-cells and thus they have a shorter memory than t-cells.
Let us return to the differentiation scheme. We use (3) and (4) as before to find concentrations of morphogens. In the previous case only the latest concentrations influenced the differentiation pattern. Now they contribute their part along with the morphogen distributions obtained in previous stages, with weights given by the memory parameters \( b_i \). Memory is seen to lead to a slow down in the pattern formation process because at each stage there is a region (usually small) which is forced to change its commitment from \( p \) to \( t \). At the beginning of our calculations we assume a weak differentiation to \( t \)-cells in \((0, R_0)\); i.e. cells in this interval secrete but \( g(x) \approx 0 \) for all \( x \). The first step in the differentiation procedure may look rather drastic because of the large difference between \( R_0 \) and \( R_1 \). The cause of this difference, is that most of the cells in the organism were undifferentiated until this step and thus even a small commitment to this or that path produces an illusion of drastic change. As the process continues the differences in the boundary (between \( R_n \) and \( R_{n+1} \)) become progressively smaller.

The greater \( b_i \) is, the more difficult is the process of change. However, because the criterion for the end of the process is the same as in the previous case \([eq.(9)]\) the final pattern is also the same. In the cutting experiments one obtains a wave like process of redifferentiation in accord with the ideas presented above. See Fig. 2.

(iii) General discussion of the model

The reason why it was possible to produce a workable model which describes some of the important phenomena of developmental biology using simple linear equations lies in the fact that we used an internal regulation mechanism. Each cell regulates the production of morphogens by its developmental level, which in turn depends on the environment that is
Fig. 2. In contrast to the case without memory (see Fig. 1) the boundary obtained after the k-th step of differentiation does not coincide with \( R_c \), the root of function \( C(k,x) \). This is because cells located in \((R_{k-1}, R_c)\) have previously been committed to the p-path in Fig. 2(a), which deals with the same equation as Fig. 1, with the addition of memory defined by (13) and (14) with \( b_1 = 1, b_2 = 0.5 \). The differentiation process becomes slower in comparison to the comparable process without memory (Fig. 2b; compare with Fig. 1c). As the process continues a gradation in the level (g) of cell differentiation is established (Fig. 2c). Suppose now that the organism is cut into two parts. Fig. 2d shows a graph of function \( C \) in the anterior part just after the cutting (at \( k = 40 \)). Now the process of dedifferentiation begins. After several steps in the differentiation procedure the posterior cells become p-cells (Fig. 2e) and gradually the pattern is reestablished (Fig. 2f). [Note different scales for positive and negative vertical axes in (c) and (f).]
created by the cells themselves. We did not employ explicit feedback, which is usually used in regulation models and which implies interactions between the two chemicals and leads to nonlinear equations. Here is a nonlinearity hidden in the rules for cell behaviour, such as eq.(13), but the calculations required in our model are linear).

We defined the differentiation function $g$ as a scalar. This was possible because there were only two cell types in our problem. If the number of cell types is greater than two, then $g$ should contain several components. Moreover, in the process of redifferentiation described above, cells proceed from one type to the other via the undifferentiated state ($g = 0$) — yet it seems plausible that in nature a cell does not return to its earlier undifferentiated state. This suggests adding another component to the differentiation function even in the case of two cell types.

A new differentiation function can be defined by

$$G = (g, \tau),$$

where $g$ is the differentiation function defined above while $\tau$ characterizes the maturity of a cell, that is how different it is from an undifferentiated cell. In our case (and also in most other in vivo and in vitro observations) maturity may be associated with time, but it cannot be identified with it because there are experiments in which cells were forced to revert to the initial undifferentiated state. ([Concerning Dictyostelium discoideum such experiments are described by Finney, Varnum & Soll (1979).] We leave for future work exploration of the role of maturity in differentiation.

As has been mentioned above, one can disregard the process during which the chemical pattern is established and consider only its final dis-
tribution at each step of the developmental scheme, provided that establishment of this pattern takes considerably less time than differentiation. In quantitative terms this requirement takes the form

\[ \left[ D \left( \frac{n}{L} \right)^2 + A^* \right] t \gg 1, \]  

(17)

where \( t \) is the time from the beginning of the process.

Criterion (17) may be obtained by solving the time dependent version of (3c,d) with the right side replaced by \( \partial a/\partial t \). From (17) one can see that morphogens establish a stationary pattern suitably fast either if they diffuse rapidly or break down quickly. One may expect that the inhibitor exemplifies the former possibility, while the inductor exemplifies the latter.

Suppose that the characteristic time for differentiation is one hour and the length of an organism is 1 mm. Then for a morphogen that has diffusion as a decisive factor for the establishment of the stationary distribution we require

\[ D \gg \frac{1}{10} \frac{L^2}{t} \approx 3 \cdot 10^{-7} \text{ cm}^2/\text{sec}. \]  

(18)

The diffusion coefficient of a molecule with molecular weight of several hundred would be large enough to satisfy requirement (18) (Crick 1970). Moreover criterion (17) was obtained with zero initial conditions, but in fact the chemical distributions do not change markedly from one use of the scheme to the other (at least after the initial redistribution occurred in the first application of our differentiation scheme). Thus one can conclude that the range of chemicals which may play the role of inhibitor is wider than that limited by requirement (18).
The model may be generalized to 3-dimensional problems. Now one must take into account that there are two different ways for elimination of morphogens — by internal mechanisms (breakdown, neutralization, absorption) or by diffusion or excretion to the exterior. For the one-dimensional case of long, thin organisms both ways may be mathematically described by the same terms used here \((A\cdot C,B\cdot C)\). When one deals with a many-dimensional problem one should distinguish between these two possibilities. While the same method of description remains correct for the internal mechanism of elimination, the external mechanism must be described by boundary conditions. Qualitatively it is obvious that for large organisms (when their volume is large relative to surface) external elimination becomes less effective.

3. Applications

The model has been constructed with the cellular slime mold Dictyostelium discoideum (D.d.) in mind (Bonner 1967, Loomis 1975). The life cycle of this species consists of several stages having very different natures. If there is a food supply, the cells of D.d. live separately from each other and proliferate by dividing. However, when the local environment is depleted of food, the cells collect in large streaming patterns to form groups of up to \(10^5\) cells. These aggregates become integrated by secretion of a surface sheath covering the whole organism. After several changes of form the aggregate produces a fingerlike structure called a slug which may fall onto the surface and migrate horizontally, still surrounded by the sheath. At this stage cells virtually do not divide and it has been shown that the few existing divisions are not essential for the development of the mature organism (Cappuccinelli et al 1979).
Later the slug transforms into a fruiting body. This consists of stalk cells formed from the anterior part of the slug, and spores formed by posterior cells. Thus, cells which are located in the anterior region of the slug, occupying 20-30% of the whole body (the fraction is strain dependent), are called prestalk cells. The remaining posterior cells are referred to as prespore cells. Thus, during the process of development pre-aggregation cells of one undifferentiated type transform into two different types of cells. It has been shown that prestalk cells, which are identified with t-cells in our model, play an organizing role in D.d. (Rubin and Robertson 1975).

Differentiation in D.d. manifests itself most clearly by the difference in the external appearances of stalk cells and spores. Late prespore cells are also easily distinguishable from their prestalk counterparts. In intermediate stages the differences are more subtle (Loomis 1982). It is very difficult to establish the moment when cells begin differentiation into prestalk and prespore cells.

One reason why we did not explicitly discuss D.d. in the previous part of the paper is that there is a difference of opinion concerning the way of establishment of the differentiated pattern in this particular species (MacWilliams & Bonner 1979, Gross et al 1981). Some workers believe that there is a positional differentiation in D.d. (e.g. Sussman and Schindler 1978). Others suggest that each cell differentiates independently of its position and only later is the pattern established as prestalk cells sort out in the anterior part of the slug (i.e. Sternfeld & David 1981). We believe that our model contains principles that may be applicable in a general developmental context, so that we do not want to connect it too strongly with one specific organism.
The idea of sorting out may be reconciled with our model by suggesting that sorting out is preliminary to differentiation in a sense that there are cells which for some reason are inclined to locate themselves in the anterior part of the slug more than other cells in a given population. (The idea is analogous to that suggested by Leach et al (1973) for scaling cells of different populations.) Early stage anterior cells seem essentially undifferentiated because if separated from other cells or transplanted into other populations they differentiate into prespore cells and stay in the posterior part of the slug. The formation of the normal organism in this case consumes a similar time period to that in the first case. That is, the fate of the cell population depends upon which other cells it is mixed with (Leach et al 1973).

One can say that the potential capability of some cells to become prestalk cells is greater than in others. This capability, which may be associated with sorting out towards the anterior part of the slug and even with some differences in external appearance, should be distinguished from actual differentiation.

The difficulty in determining the essential features that truly characterize the cell type may be illustrated by the finding of eight different cell types in the slug (Francis & O'Day 1971). Scrive et al (1981) report about five different cell types in the "undifferentiated" aggregation stage of the D.d. With further improvements of experimental technique one can expect that further individual features of cells may be found. These differences should not be referred to as differentiation. The purpose of the latter notation is to help to classify cells into preferably small numbers of classes which play essentially different roles in the organism. Thus it is generally agreed that the mature D.d. organism consists of two cell types (or three if basal disc cells are distinguished).
Let us give a picturesque analogy to illustrate what we mean by potential capability, in contrast to differentiation. Given two illiterates, there is a good possibility that after instruction one of them will be able to read much better than the other. But before they begin to study they are both equally illiterate, that is they are at the same level of "differentiation".

It may be that there are a few cells which differentiate into pre-stalk cells independently of their position, sort out in the anterior part the slug and induce differentiation in other cells. A similar process was observed in cutting experiments where so-called anterior-like cells appeared to sort out into the anterior part of the posterior slug to initiate the reestablishment of the pattern (Sternfeld & David, 1981). The process of reestablishment of the pattern proceeds in a wavelike fashion in both parts of the parent slug (MacWilliams & Bonner, 1979). Thus both processes, sorting out and positional differentiation, might contribute to the establishment of the pattern.

The question of the practical definition and measurement of the differentiation function \( g \) is closely connected to the problem of determination of the essential features of differentiation. We believe that for both ways of pattern formation it is very important to scale quantitatively the level of cell differentiation through the available paths. One of the leading methods for determining the developmental path of a cell which provides a possibility of quantitative measurement is a fluorescent technique (Hayashi & Takeuchi, 1976). According to this method cells of the D.d. aggregate are disaggregated at different stages of their development and specially stained. The fluorescent intensities of individual cells are measured. The prespore cells which are associated with cytoplasmic granules are of high fluorescent intensity while prestalk
cells display low intensity. The question is whether one can scale prespore
development by intensity, that is whether one can say that the higher is
the fluorescent intensity the more committed is the cell to the prespore
path.

Three chemicals have been proposed as candidates for the role of
morphogens: cyclic-AMP, ammonia and an unknown chemical called differen-
A recent study (Gross et al 1981) has shown that cyclic-AMP is needed for
both differentiation paths. Ammonia - a candidate for inhibitor - is
highly diffusible, while DIF has not been found in the prespore region
and therefore it is plausible that it breaks down before it has diffused
more than a few cell diameters. Thus it appears that both DIF and ammonia
can satisfy our requirements on morphogens for the application of our
principle 5.

Until now there is no evidence that DIF and ammonia react with each
other (Gross et al 1981). Thus the absence of reaction terms in (3) is
supported. This is a very important point because other models crucially
depend upon reaction terms. (See below.)

In D.d. a polarity in external conditions is present from the very
beginning of the post aggregation stage because of the vertical form of
the aggregate. These conditions in particular provide more intense con-
tact with the atmosphere by the anterior (upmost) region as compared to
other parts of the aggregate. This fact has already been used for a qualita-
tive explanation of the establishment of pattern and of the chances for
the standing slug to topple (Sussman & Schindler 1978).

There is much data on different D.d. mutants with various ratios of
the two types of cells. From formula (10a) one can see that the difference
between these mutants may be in the comparative strength of their prestalk
cells as sources of inductor and inhibitor and/or in their capability to break down the morphogens.

4. Comparison With Other Models

Several models have been proposed for the description of pattern formation in general and for D.d. in particular. MacWilliams and Bonner (1979) provide a critical review of the most important models concerning D.d. The model considered most promising by these authors is the activator-inhibitor model (see e.g. Meinhardt 1978, Gierer 1981). Recently two versions of the Turing model were proposed (Lacalli & Harrison 1978, Pate & Othmer 1983).

While there are some differences between these models they are rather similar to each other. One can term them all "direct chemical pattern formation models". Here we point out aspects of these models which we believe are their weak points.

1. The differentiated pattern in these models is established by cells reading out a stable chemical pattern. The process of differentiation is thus divided into two successive stages, that of the establishment of the chemical pattern by a nonlinear feedback system with strong reaction between morphogens (described by a system of diffusion equations with nonlinear kinetics) and a differentiation by cells which accomplish this according to the chemical pattern. Thus differentiation occurs after establishment of the chemical pattern and hence has no influence on the latter process. This may be regarded as a one time use of our differentiation scheme with the emphasis put on the establishment of chemical pattern. By contrast our model is based on the notion that the establishment of the chemical pattern and cell differ-
entiation are not separated in time. Cells themselves are the main source of the morphogens and they change the concentrations of morphogens while differentiating. These ideas are stressed by our principles 2 and 3.

2. There is no memory included in the direct chemical pattern formation models while all biological systems have memory. We believe that to simulate any differentiation process, one needs a function which describes in some way the level of the differentiation of cells through the time course of the process.

3. To obtain a pattern, the earlier models require reactions between the morphogens. As far as D.d. is concerned there is no evidence of reactions between major candidates for the roles of morphogens.

A major initial goal of activator-inhibitor and Turing models was to show that inhomogeneous pattern may be established in initially homogeneous or nearly homogeneous tissue by unstable perturbations. (We on the other hand assume the existence of polarity at the outset.)

Of the four phenomena indicated in the introduction that should be embraced by an overall model of the type we deal with, only two were subjects of discussion in the present work. And even for these two types only one kind of regulation - by positional differentiation - was considered. The main restriction of the present model is an exclusion of relative cellular movement. An extension of the model to include movement phenomena is now under way.

Another restriction of the model is its omission of proliferation and growth. (For D.d. development these factors seem to be unimportant.) Growth can easily be included for specific applications by suggesting that the length of the organisms changes by some law (which may or may not use our functions C and g) for each successive use of the differentiation
scheme.

The phenomena of cell movement and proliferation are also beyond the scope of the direct-chemical pattern formation models discussed above.

To mention one possible sphere for application of our model, in a vast field of biology, botany, there is certainly no sorting out in natural development. Thus we believe that our model with some modifications may be successfully used for describing development in plants.

5. Conclusions

On the basis of the five principles presented above we were able to simulate the phenomenon of regulation in initial development, and in cutting experiments, for an organism consisting of two cell types. The organizing t-cells produce both inductor a and inhibitor h. Inductor has a very short range, so that both its production and its decay occur almost entirely in the t-region (whose length at a given stage is R). Net production at rate $d_1 R$ balances elimination at rate $aAR$. Thus $a$ has approximately the constant value $d_1/A$ in the t-region, falling rapidly to an effectively zero concentration throughout almost all of the p-region.

Inhibitor has a long range so that its concentration is approximately constant throughout the organism (length L). Production by t-cells at rate $d_2 R$ balances elimination at rate $hBL$, yielding $h \approx d_2 R/BL$. Thus the key fact emerges that the level of inductor is independent of the extent $R$ of t-cells, while the level of inhibitor depends on $R$ only through the ratio $R/L$ of t-cells to the total number of cells.

At this stage, cells at point $x$ tend to retain their t-character
if and only if \( C_R(x) = a(x) - h(x) > 0 \). The pattern will remain unchanged provided that the boundary point \( x \) where \( C_R(x) = 0 \) coincides with the boundary \( x = R \) of the previous stage. Thus t-cells are stably contained in the interval \( 0 < x < R^* \) if \( C_R^*(R^*) = 0 \).

Since the formulas for \( a \) and \( h \) contain \( R \) in the combination \( R/L \), the just cited equation for \( R^* \) yields \( R^*/L = \varepsilon \), where \( \varepsilon \) is a combination of parameters that does not include \( L \). Thus the fraction of t-cells is the same whatever the length of the organism: regulation has occurred.

Reestablishment of the pattern in cutting experiments stems from the different levels of cell differentiation prior to cutting. In our model, this level is described by a (history-dependent) memory function \( g \) [formulae (12) and (14)]. The resistance of cells to redifferentiation is directly proportional to their commitment to one of the two available developmental paths. The correct pattern is established by the mechanism used in the initial regulation.

References

של מלדלת הגלף היגה תופעה שכיחה באררגמיזטים. התけばו התבגרו דבריך לביצוע מיצובים אלה בשידור ההופעות. לט臌ennessee שלוש שיטות שונות שלمدرسة המביעהiminary הרבחה אפואר大切ית spannonefrad בבלבלמשלס התが増えו. מאמציה מאמץ לת%A הסדרה בבלבלמשלם התotence החרירה כבש השינה השגרה.

כדייה המקדיש של התערובות מספרים בקביעות ב חברים המבכרית.

כדיברנו אספנטים שרגים של השפעה של המראה הבינדול של צמחים על הקוצרי הררך. מתכונת שתפי בה שרגים של הסיבורים מהזוריים. מאמצי שטח וחבל הסיבורים בתיאוריך ממעביר לבריכת פעילות התוכנית ספגטרית לשפעה.

אותה מסירות של עלים.localizedים על המעבד התוך.

מודל מعزي מדלי חסיל הלוערצות יצירה (פואר) באורגניזם מע糗. מנעל מסגרת לתמך. שירוקו גלול בראגרביזים מוקשים. קדם של המדל הזה מ notwithstanding דהפרמציאלה לתינאירורה שין רכילות במקהלה הד-מקלד. מודע שירוקו לדיקטואטולוגים בדקדוקייאדואים.