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Patterning in the cellular slime moulds

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Abstract. The aim of the present article is to derive and illustrate in a simple form some of the important concepts in developmental biology. The development of the cellular slime mould *Dictyostelium discoideum* is an ideal model system for this purpose. I will outline the development of this organism at its multicellular stages and review some relevant studies focusing on the control of cell differentiation and pattern formation while deriving some key concepts in the current thinking about the control of development.

Keywords. Pattern formation; morphogenesis; *Dictyostelium.*

1. Introduction

How the shapes and patterns of living things are formed is a fascinating question. Unlike in artificial objects, shapes and patterns form by themselves in living things, and it is this autonomy, as well as the diversity of the shapes and patterns formed by different organisms, that have drawn the attention of many scientists from diverse disciplines to the problems of morphogenesis and pattern formation. The former term is preferred when the emphasis is placed on shape, whereas the latter is used in a wider sense whenever a regional difference arises within the body.

During a period of over a century, through the efforts of many biologists seeking general principles of morphogenesis and pattern formation, a body of theories and ideas has been developed. Recent progress in some areas of this field, which owes its success principally to the advancement of gene technology, has added much to our knowledge of these problems. The impact has been so large that classical work tends to be ignored. However, the importance of classical experiments, and the concepts derived therefrom, is increasing, rather than decreasing; one can easily get lost in the flood of new facts if one is not equipped with an appropriate view. The importance of theory, even though it may turn out to be wrong in the end, in interpreting the experimental results and designing further experiments cannot be overemphasized. The purpose of this article is to introduce some of the most basic ideas and concepts in the theoretical study of morphogenesis and pattern formation. We will do this by taking a simple organism called the cellular slime mould as an example.

There are reasons to choose as a representative of multicellular organisms, such an organism which, as we will see shortly, appears quite different from more familiar, "ordinary" organisms. As is the case in the field of physics, general principles in biology will be fully recognized by examining simple cases which can be more easily and more fully analysed. Indeed the very complex multicellular development can be looked at as consisting of elementary processes of which there are a relatively small number of different kinds. For instance, complicated structures of animals are formed, basically, by sequential transformations of a sheet of cells. In

fungi and plants, the branching pattern determines the basic morphology. In addition, cell movement and cell adhesion also play important roles in the formation of shape in many multicellular organisms. On the other hand, colour patterns on the body surface of animals are in many cases generated by the synthesis of different amounts of a pigment such as melanin, or synthesis of different pigments, by different epidermal cells, and this is most likely due to the differential expression of the genes among the cells encoding one or more enzymes (such as tyrosinase) involved in the biosynthetic pathways of the pigments.

In both instances expression of the genes causing the changes in question, be it shape change of the tissue or colouration of hair, need to be localized at specific regions of the organism and to occur at a specific time during the development. We can here conceive of a very fundamental question asking how, and when, a particular set of cells are selected to become distinct from surrounding cells. Among many model organisms for the study of development, the cellular slime moulds, in particular the asexual life cycle of one species *Dictyostelium discoideum,* provide us with an unrivalled opportunity to study such fundamental questions in biology. Cespite its very simple structure involving only a few cell types, it exhibits all the essential processes constituting multicellular development, such as organized cell movement, induction of cell differentiation, pattern formation, and morphogenetic movement. Unlike other multicellular organisms, cell multiplication, which is another important constituent of development, occurs only sparsely during the multicellular phase in the asexual life cycle of *Dictyostelium.* The virtual lack of cell multiplication is indeed one of its advantages because it makes the analysis of other facets of development easier.

It is quite appropriate therefore that the whole process leading to fruiting body formation is called development. In some slime mould species, including *D. discoideum,* yet another cycle called the macrocyst forming cycle is known to exist. This is quite different from the asexual cycle and involves sexual reproduction, and is also recognized as a very good model for studying the early events in sexual reproduction. In this article, we will specifically deal with the asexual life cycle of *D. discoideum.* We first briefly outline the development of *D. discoideuin* at its multicellular stages. Then focusing on several important aspects of the development, we will describe relevant studies in some detail, and examine the concepts derived from them. As we will see, these concepts apply to the development of many multicellular organisms.

2. Brief overview of development

The most complex structure formed in the development of this organism is a fruiting body. Figure 1 schematically shows the development of *D. discoideum.* The fruiting body (figure 1j) consists of a spherical mass of spores and a supporting stalk, the latter of which is composed of cells (stalk cells) with cell wall and a large vacuole. At the base of the stalk is a basal disc, which is composed of cells very much similar to stalk cells. Thus there are basically only two cell types constituting the fruiting body.

It should be noted that the spore-to-stalk ratio is roughly constant over a wide range of fruiting body size–about 8:2 by cell number (Bonner and Slifkin 1949; Stenhouse and Williams 1977). Such a well-proportioned fruiting body structure is

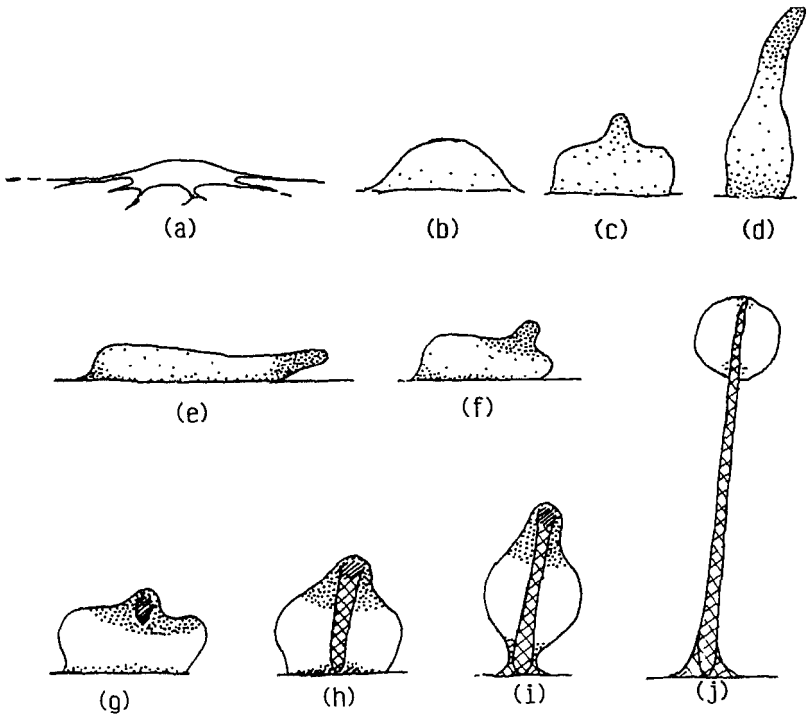

Figure 1. Morphogenesis of *D. discoideum.* (▦), prestalk, anterior-like, and rear-guard cells; (▨), prestalk cells (and rear-guard cells) in the process of maturation;(▦), mature stalk cells;(□), undifferentiated cells (a,b), prespore cells (c–i), or mature spores (j). Some prespore cells are already differentiating in (b).

thought to be adaptive to the protection and dispersal of spores. For fruiting bodies to be formed without error in the varying natural environment, this organism has attained in the course of evolution remarkable abilities of regulating cell differentiation and cell movement, which will be summarized in the following.

2.1 *Aggregation and slug formation*

Under favourable conditions, starved cells of *D. discoideum* aggregate to form multicellular bodies (figure 1 a,b). The aggregation of dictyostelid slime moulds is mediated by species-specific chemoattractants collectively called acrasins. In *D. discoideum,* cAMP (3',5'-cyclic adenosine monophosphate) is the natural chemoattractant (Konijn *et al* 1968). Cyclic AMP not only mediates the aggregation but also plays a central role in the control of cell differentiation in the later development (see below). As the process of aggregation completes, a tip appears at the top of each cell mound (figure 1 c) and elongates to form what is called a

standing slug or a first finger (figure 1d). Here, depending on the environmental conditions, either of the two developmental courses is chosen: (i) to proceed to the formation of a fruiting body, or (ii) to fall over and crawl on the substratum, like a slug (hence the cell mass at this stage of development is called migrating slug, also called pseudoplasmodium or grex, figure le).

2.2 *Cell differentiation in the slug*

In the slug, cells differentiate into either of the two cell types: prestalk cells or prespore cells. In figure 1, prestalk cells (and their relatives, see below) are indicated by dots. As the names indicate, these are precursor cell types of stalk cells and spores, respectively, and as long as there is no significant disturbance in subsequent development, cells of each precursor cell type turn into their respective mature form in the fruiting body. It is therefore reasonable that we find a fairly constant proportion between these cell types (prespore : prestalk ≈ 7 : 3 to 8 : 2) (e.g. Hayashi and Takeuchi 1976). At an early stage of slug formation (figure lc), a pattern of spatial distributions of the two cell types emerges. Normally the anterior region of the slug (about 1/6 to 1/3 by length) consists of prestalk cells (prestalk region) while prespore cells constitute the rest of the slug (prespore region) (Raper 1940). Non-prespore cells present in the posterior region of the slug are called anterior-like cells (Sternfeld and David 1981). In particular, those located at the rear-most part of the slug are called rear-guard cells, the majority of which eventually constitute the basal disc of the fruiting body (Bonner 1957). It has been shown that there is a sparse but constant interconversion among anterior prestalk cells, anterior-like cells, and rear-guard cells (Bonner 1957; Kakutani and Takeuchi 1986), and despite the difference in their localizations, these cells seem to be basically of the same cell type with very minor differences (Devine and Loomis 1985).

Several lines of evidence indicate that anterior prestalk cells consist of subclasses which can be distinguished by their responses to the factors that influence stalk cell differentiation (Sobolewski and Weeks 1988) and by the expression of stage- and cell type-specific genes (Jermyn *et al* 1989; Jermyn and Williams 1991). The spatial distribution of these sub-cell types suggests that they each represent one of the steps that lead to the process of stalk cell differentiation.

2.3 *Regulation*

In normal development, prestalk cells and prespore cells are destined to become stalk cells and spores, respectively. However, removal of either cell type causes many of the remaining cells to redifferentiate so that the normal cell type ratio is restored. For instance, if a slug is cut into prestalk region and prespore region and if the environment favours slug migration, a significant fraction of the prestalk cells in the isolated prestalk region are induced to differentiate into prespore cells, and eventually a normally proportioned fruiting body results (Raper 1940; Bonner *et al* 1955; Sakai 1973). If, however, the environmental condition is such that it induces immediate fruiting (see below), a very stalky fruiting body forms (Raper 1940; Sampson 1976). On the other hand, the posterior fragment of the slug invariably rounds up, and it takes a few hours before a new tip regenerates to resume

migration or start fruiting body formation depending on the environmental condition. Within the round cell mass, it is reported, some anterior-like cells sort out to the region where a new tip will form. Meanwhile, some of the prespore cells turn into prestalk cells or new anterior-like cells, and the overall cell-type proportion is restored (Sternfeld and David 1982).

2.4 *Induction of fruiting*

Several factors have been reported to influence the choice between migration and fruiting. For instance, when the ambient humidity is low, newly formed slugs form fruiting bodies without migration, and slugs already in migration are efficiently induced to fruit by desiccation (Bonner and Shaw 1957). Also slugs tend to be induced to fruit on substrata of high osmolarity or high ionic strength (Slifkin and Bonner 1953). Apparently loss of water by whatever means induces fruiting, or in other words, keeping a sufficient amount of water is essential for slugs to migrate (Bonner *et al* 1982). Ammonia is another important factor which encourages migration (Schindler and Sussman 1977). Since slug cells generate a large amount of ammonia themselves, high ambient pH alone is normally sufficient to keep slugs migrating, and by the same token, low pH induces fruiting body formation.

2.5 *Fruiting body formation* (*or "culmination"*)

At the beginning of culmination, the tip of the slug stops forward movement, and the entire slug rounds up. At this stage, prestalk cells turn into true stalk cells in the small region (indicated by the hatched area in figure 1 g-i) inside the apical tip, and the stalk elongates by continuously adding new stalk cells to its tip (Raper and Fennell 1952). This process continues until most of the prestalk cells enter the stalk tube to become mature stalk cells. Meanwhile prespore cells become mature spores. As will be discussed later, there seems to be a complicated network of cell interactions to achieve the right fruiting body structure.

3. Some important aspects of development

Several phenomena can be identified as important elements in the development of this organism: i.e., cell differentiation, control of cell differentiation by diffusible molecules, diversification or "division of labour" within the cell population, proportioning, spatial pattern formation, and regulation. These are indeed the elementary processes of multicellular development in general.

3.1 *Cell differentiation*

For each starved *Dictyostelium* cell, development is the process of differentiating into either a spore or a stalk cell. The term "cell differentiation" has been defined in a number of different ways, but one of the simplest ways would be to define it as a process in which a cell comes to express a set of specific genes and consequently attains a specific character. As a cell goes through the process of development, a

number of genes are expressed in succession. Some of them are likely under the control of a common regulatory gene, so the timing and duration of their expression are about the same and their functions closely related to each other. Those genes that are expressed only during a certain period of development are called specific to that developmental stage. A number of stage-specific genes are known to exist. During the early stages of development all the cells sequentially express the same set of stage-specific genes. Later in development, however, some cells start to express the genes that are normally not expressed by other cells. These genes define the corresponding cell type, and are called cell-type-specific genes. Thus we have prespore-specific genes and prestalk-specific genes. There are also genes that start to be expressed even later in development such as those expressed during spore maturation.

3.2 *Morphogens*

Several endogenous factors are known to influence cell differentiation, and it is believed that these, and possibly some other yet unknown factors, work together to control cell differentiation during later development. Such substances that control cell differentiation during developmental processes are generally called morphogens (after Turing 1952). In *Dictyostelium,* cAMP, ammonia. differentiation inducing factor (DIF), and adenosine are thought to be morphogens. These are all stable, small molecules. This is a common feature of the candidate morphogens mediating long-range cell interactions in multicellular development, such as retinoic acid in vertebrates. This is in contrast with the case in which the basic pattern is formed within continuous cytoplasm, in which morphogens could be proteins such as the *bicoid* gene product in the early development of *Drosophila.*

3.2a *cAMP*: The rate of cAMP production peaks at the aggregation stage, but it continues to be produced throughout development. This nucleotide is required for both prestalk and prespore differentiation, although there is evidence suggesting that prespore differentiation requires more cAMP, or more prolonged exposure to cAMP,than prestalk differentiation (Ishida 1980). For instance, depletion of cAMP in the slug leads to dedifferentiation of prespore cells (Okamoto 1986; Wang *et al* 1988). On the other hand, cAMP given externally inhibits some step later in stalk cell differentiation (Berks and Kay 1988, 1990).

3.2b *Ammonia:* Ammonia is continuously produced in a quantity by cells throughout development. It inhibits fruiting body formation, and this is most likely due to its inhibitory effect on stalk cell differentiation. Indeed, in monolayer culture of mutants capable of differentiating into stalk cells and spores without morphogenesis, ammonia suppresses stalk cell differentiation and, at the same time, promotes spore differentiation (Gross *et al* 1983). It has been shown that ammonia influences the choice of individual cells between the stalk and spore pathways, not solely inhibiting stalk cell maturation, although there are some contradictory reports in this regard (Neave *et al* 1983; Dominov and Town 1987; Bradbury and Gross 1989; Berks and Kay 1990). The effect of ammonia is strongly pH-dependent, being more pronounced at higher pH, indicating that the effective form is NH_3. The ammonia effect is therefore most likely mediated by elevation of intracellular pH (Inouye 1988b).

3.2c *DIF*: This substance was first described as an endogenous diffusible factor which induces isolated cells to form mature stalk cells (Town *et al* 1976). It induces expression of some of the stalk specific genes at the transcriptional level (Williams *et al* 1987). DIF consists of five related molecular species with more than 95% of its activity associated with a single species termed DIF-1. The chemical structures of three forms out of five have been determined (Kay *et al* 1989). CIF is also produced throughout development, but its level peaks at the stage of slug formation, corresponding to the time when prestalk cells begin to appear (Brookman *et al* 1982). A putative DIF-receptor has been found and examined in detail (Insall and Kay 1990). DIF also inhibits prespore differentiation and, furthermore, causes transdifferentiation of prespore cells to prestalk cells (Kay and Jermyn 1983), indicating that it is a pathway-specific differentiation inducer.

3.2d *Adenosine*: This is one of the degradation products of cAMP. Adenosine was first shown to interfere with aggregation by reducing the affinity of cAMP receptor proteins to its ligand (Newell and Ross 1982). It also prevents prespore differentiation (Weijer and Durston 1985), and enzymatic reduction of its level in the slug causes transdifferentiation of a significant fraction of anterior prestalk cells into the prespore cell type (Schaap and Wang 1986).

3.3 *Division of labour*

For fruiting body formation, both spores and stalk cells must arise. While a spore is a viable and resistant cell, a stalk cell is a dead cell which cannot transmit its genes to posterity. Then why do not all the cells become spores? This is a question related to evolution but if one asks "how" instead of "why", it is an example of a general question in developmental biology asking how a group of cells descended from a single cell give rise to different cell types. In *Dictyosteliuin,* one can conceive that part of the cells receive a signal that induces, or interferes with, either spore or stalk cell differentiation, most probably through the action of one or more of the above morphogens.

As mentioned earlier, cAMP is itself not a pathway-specific inducer, but there are possibilities that it can still have pathway-specific effects. For instance, it could have different effects at different concentrations, as mentioned above. If, as has been repeatedly suggested to be the case (Schaap and Wang 1984; Weijer *et al* 1984b), cAMP continues to be a signalling substance organizing the multicellular structure with its effect being transmitted as periodical pulses, just as in the aggregation process, a modification of its frequency, such as gating of the pulses, could give rise to a pathway-specific effect.

On the other hand, ammonia and DIF are pathway-specific signalling substances, acting in favour of prespore and prestalk differentiation, respectively, although the mode of action seems to be quite different between the two. There is a possibility that other important factors still elude our search.

Whatever the regulators of differentiation are, there needs to be a negative feedback system so that any one cell type will not overwhelm the others. If such a system exists and works accurately enough, it would also serve as a mechanism for tissue proportioning and proportion regulation (see below).

3.4 *Spatial pattern*

As shown in figure 1, prestalk cells and prespore cells show a distinct spatial distribution within the slug. Indeed the different cell types (including sub-cell types of prestalk cells) are arranged so that the slug is always ready to set out for culmination. Culmination provides a different kind of spatial pattern. As shown earlier, prestalk cells become mature stalk cells within a very limited region, suggesting the presence of precise spatio-temporal control over the maturation of prestalk cells (Inouye 1988a).

There has been a controversy for over a decade concerning the mechanism whereby the prestalk-prespore pattern arises. Some think that the spatial distributions of prestalk cells and prespore cells are consequences of the non-uniform distribution within the cell mass of the morphogens that control cell differentiation. Indeed, cAMP, DIF and adenosine are all reported to show non-uniform distributions within the slug (Otte *et al* 1986; Brookman *et al* 1987; Wang *et al* 1988). There may also be regional differences within the cell aggregates in the more general sort of variables, such as the rate of oxygen supply, which would in themselves not induce any cell differentiation but may have significant influences on the action of the morphogens (cf., Sternfeld 1988). The regional difference of the combined effects of these variables might serve as the basis of the prestalk-prespore pattern. In developmental biology, such properties varying in space are called gradients since they usually vary in graded manners. The direction of the gradient (in which the parameters consisting the gradient vary in space most) normally coincides with the polarity of the body, or part of the body under consideration (collectively called a field), and these are generally thought to be the basis of the organization of body structure. In the case of a slug, the field is the entire slug and the polarity will be its longitudinal axis starting from the tip. According to this view, it may be argued that cells know their positions within the cell mass by sensing the gradient and that they differentiate accordingly. This hypothetical mechanism that teaches cells their positions within the field has been termed positional information (Wolpert 1969).

The other hypothesis finds the cause of the diversity within the cells themselves: the levels of the inducers may be the same throughout the tissue but it seems reasonable to assume that there is a difference between the cells in the responsiveness to the inducers. There is evidence that intrinsic components influence the fate of individual cells. To give an example, it is known that the cell cycle phase at the beginning of development strongly affects the fate of the cells (Weijer *et al* 1984a; Gomer and Firtel 1987; Maeda *et al* 1989), and this might be due to a difference which might exist between the cells at different cell cycle phases in sensitivity to the morphogens. However, the distribution of the cells over the substratum before aggregation is random without relationship to the cell-cycle phase of each cell, and there is no noticeable cell sorting during the aggregation process. This means that for the prestalk-prespore pattern in the slug to arise within the cell aggregate on the basis of cell cycle phase difference, there must be cell-sorting after the formation of cell aggregates. Indeed there is ample evidence that cell-sorting does occur at this stage in the way required for the formation of the prestalk-prespore pattern within cell aggregates (Takeuchi 1969; Maeda and Maeda 1974).

It should be noted, however, that the two possibilities are not mutually exclusive

but differ in the relative contribution of the external signals in the decision by individual cells of their fate; any developmental system will have both external and internal components that affect cell differentiation, and if the external signals override the intrinsic predisposition of the cells, the system will appear to be governed by positional information, whereas if the latter predominates, the fate of individual cells will be determined in an autonomous way.

The above question may appear only to have resulted from the idiosyncrasy of this organism in which cells can relatively, easily change their positions within the tissue. Indeed in most multicellular organisms cells usually adhere to each other tightly to form rigid tissues in which changing position seems difficult. Nevertheless the distinction should be borne in mind because it exists. In ordinary multicellular organisms, predisposition and position usually go together due to the fact that most cells remain attached to each other after cell division. However, even in such organisms, there are often cases in which one should discriminate between the internal and external components that control the cells' fate, and the intrinsic parameters in such cases, which usually vary gradually over the field, are sometimes referred to as the positional values.

3.5 *Proportioning*

As shown earlier, prestalk-to-prespore ratio is more-or-less constant. As in the case of *Dictyostelium* development, a constant proportion of different cell types is a general property required for the formation of a constant, well-proportioned body in multicellular organisms. In most cases, however, little is known about the molecular mechanism of this constancy. In fact, few attempts have been made to elucidate the mechanism of tissue proportioning, and *D. discoideum* is one of the few exceptions in which the problem of proportioning has been the primary concern. In most studies concerning cell-type proportioning, this problem has been considered to be part of the problem of pattern formation. However proportion and pattern can be distinguished conceptually, and it is only following such a distinction that the major advancement was made.

Experimentally-based models for the maintenance of constant proportion proposed so far, all involve negative feedback interactions between the two cell types. It is quite natural that we find those factors described above, such as cAMP and CIF, to be the main actors in this play. Although our knowledge of the mechanism for the maintenance of constancy is still far removed from satisfaction, the constant prestalk-to-prespore proportion is most likely achieved by a network of cell interactions of which a negative feedback loop mediated by one or other morphogens plays the central part (Weijer and Durston 1985; Blaschke *et al* 1986; Inouye 1989).

Whatever the actual mediator is, we are faced with a problem of reconciling the maintenance of constant proportion with the formation of a stable coherent pattern: in the former, due to the negative feedback loop, cells tend to suppress the differentiation of other cells into the same cell type as themselves, whereas in the latter, cells of the same type need to cluster. This apparent discrepancy may be resolved in different ways. Most simply, differentiated cells may become insensitive to negative feedback inhibition by the same cell type once they differentiate. This possibility applies only to the case where spatial pattern is formed by cell-sorting.

Another possibility is that cells that have differentiated into one cell type exert positive as well as negative effects on surrounding cells. If the inhibition extends a long distance while the activation is local, a coherent spatial pattern could arise while keeping the cell-type proportion constant. This type of mechanism, called an activator-inhibitor model, is a special case of what is called a reaction-diffusion system, which has been a subject of extensive mathematical studies. The pioneering work by Turing (1952, known as the Turing model) and the one developed by Gierer and Meinhardt (1972) are among the most important mathematical models based on this idea. The activator-inhibitor model, and its variants, have been successfully applied in studies of various developmental system including *Dictyostelium* (*e.g.,* Meinhardt 1983).

3.6 *Regulation*

The ability of the cellular slime moulds to regulate their cell-type ratio is one of the most spectacular examples of this kind. Although such flexibility in the fate-map of an entire organism may be an extreme case, regulation is a universal phenomenon probably present in any multicellular development. Even in the nematode, which shows a typical mosaic development, experimentally induced regulation has been observed. In *Dictyostelium,* regulation has been studied *in vitro,* using culture systems designed to eliminate positional factors, as well as *in vivo,* using migrating slugs (Bonner *et al* 1955; Sakai 1973; Oyama *et al* 1983; Akiyama and Inouye, 1987; Inouye 1989). For example, using the monolayer culture of dissociated slug cells, one can examine the effects of various substances on isolated prestalk or prespore cells. From studies of this kind, it has been shown that prespore cells release a substance, or substances, that inhibit(s) conversion of prestalk cells into prespore cells, and it is suggested that prestalk-to-prespore conversion induced in isolated prestalk fragments is due to the decrease in the level of that inhibitor (Inouye, in preparation). It should be noted that the initial establishment of the constant proportion can also be explained by this same hypothesis. It may well be possible that pattern regulation is an extra benefit of the machinery for building a proportionate body structure in normal development.

3.7 *Timing*

The temporal relationship between different events is also very important in building up correct structures. Culmination in the development of *Dictyostelium* provides an excellent example of how timing can affect the morphology of the terminal structure. As shown earlier, prestalk cells continuously differentiate into mature stalk cells within a very limited region, suggesting the presence of precise spatio-temporal control over the maturation of prestalk cells. Also there is evidence that the transformation of prespore cells to mature spores during culmination occurs in an orderly manner. Unlike stalk cell maturation, however, spore maturation occurs within a short time, suggesting the presence of an extracellular signal that synchronizes the event (Bonner 1967). In addition, spore maturation must occur at the right time during culmination, because otherwise the final structure of the fruiting body would be aberrant. Thus there seems to be a rather

complicated network of interactions among cells of different cell types as well as interactions within the same cell type to achieve the right fruiting body structure. It is interesting to note that the difference in the fruiting body morphology among different species of dictyostelid slime moulds may be explained, at least in part, by the different timing of stalk and spore maturation (Bonner 1982).

4. Concluding remark

As we have seen, the development of the cellular slime moulds is an ideal model system in which to study biological pattern formation and morphogenesis. Because of their simple life cycle, fundamental processes of development take place in a relatively pure form. This makes it feasible to design and perform analytical studies, which in other more complex organisms are often almost impossible. This advantage is particularly significant in relation to the theoretical study of development because theory can be more easily tested by experiments with a simple system showing minimal complications due to other processes which might occur simultaneously.

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