A Comparison of the Macrocyst and Fruiting Body Developmental Pathways in *Dictyostelium discoideum*

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Several differences and similarities between the macrocyst and fruiting body pathways of development are described. First, as has been long assumed, starvation initiates the development of amoebae for both pathways. Second, amoebae make the decision to produce macrocysts or fruiting bodies before a critical time point approximately 12 h after the onset of starvation. After this time, amoebae are committed to fruiting body development. Substances that can push amoebae into one or the other pathway often do so by altering the timing of development. Finally, cell division may be required in order for amoebae to become competent to produce macrocysts; there is no such requirement for fruiting body development.

INTRODUCTION

It has been known since the work of Blaskovics & Raper (1957) that there are alternative developmental pathways in the life cycle of the cellular slime mould *Dictyostelium discoideum*. Depending upon a number of factors, amoebae that have been fed either aggregate into central collection points forming pseudoplasmodia which produce spores borne on a delicate stalk, or they can aggregate and produce macrocysts. As shown in the original observations of Blaskovics & Raper (1957) and in the subsequent work of Nickerson & Raper (1973a), Clark et al. (1973), Erdos et al. (1973a, b), Filosa & Dengler (1972) and O'Day & Lewis (1981), it is clear that shortly after opposite mating types are brought together, giant cells appear which accumulate a number of satellite or peripheral cells about them in a cluster. This cluster secretes a cellulose coat as the giant cell proceeds to engulf the smaller cells trapped within the cyst. As this process continues, two more cellulose coats are deposited and ultimately only the huge phagocytic cell remains occupying the resultant macrocyst. This giant cell then undergoes a series of divisions to produce numerous small amoebae. When this very resistant cyst can be germinated (Nickerson & Raper, 1973b), the new small amoebae emerge and start a new generation. It is presumed in some instances that the giant cell is a zygote and that the final cells emerging from the macrocyst are the haploid progeny. There is evidence in some species to support this idea (MacInnes & Francis, 1974; Francis, 1975; Wallace & Raper, 1979) but in other cases the possibility of asexual development or selfing remains strong (Bozzone & Bonner, 1982).

Cellular slime mould development is very sensitive to environmental cues. Cultural conditions favouring macrocyst or fruiting body formation have been extensively investigated by many workers (Blaskovics & Raper, 1957; Weinkauff & Filosa, 1965; Nickerson & Raper, 1973a; Wallace, 1974, 1977). Darkness, temperatures greater than 20 °C, wet culture conditions and the absence of phosphate lead to macrocyst development while the converse favours sorocarp formation in all of the cellular slime mould species tested thus far.

Exposure of amoebae to light or darkness during the early part of vegetative growth is crucial in determining the developmental fate of these cells. In *D. discoideum*, amoebae grown in the dark for 24 h develop macrocysts even if amoebae are switched to the light for additional vegetative growth (Erdos et al., 1976). Similar results have been obtained for *D. rosarium* (Chang & Raper, 1981) and *D. purpureum* (Hirschy & Raper, 1964).
Control of macrocyst or fruiting body development by volatile factors has been reported in *D. mucoroides* and *D. discoideum*. Weinkauff & Filosa (1965) demonstrated that macrocyst production in *D. mucoroides* is dependent on some volatile factor that is not CO₂. Evidence in support of a volatile hormone which can induce macrocyst formation in the V12 strain of *D. discoideum* has been reported (O’Day & Lewis, 1975; Lewis & O’Day, 1977). This induction takes place in V12 even in the absence of cell contact with its mate NC4; NC4 is thought to produce this species-specific pheromone.

Aside from the obvious morphological differences between the macrocyst and fruiting body pathways of development, there are other major differences of exceptional interest which are the subject of this study. As will be shown, environmental cues which guide the amoebae into one pathway or the other often do so by altering the timing of development. The macrocyst pathway is available to amoebae during a sharply delimited time period. Once a certain time in development has passed, cells are committed to the asexual fruiting body mode of development. Finally, cell division following spore germination may be necessary for macrocyst formation while it is unnecessary for fruiting body development.

In addition to these differences between macrocyst and sorocarp development, there are some very interesting similarities in these two pathways. As will be demonstrated, starvation is a crucial environmental cue that signals the onset of development in both.

**METHODS**

*Growth and maintenance of stock cultures.* Stock cultures of *Dictyostelium discoideum* strains NC4 and V12 were maintained on *Escherichia coli* B/r as a food source on nutrient agar plates (per 1 distilled H₂O: 10 g peptone, 10 g dextrose, 0.381 g Na₂PO₄, 0.45 g KH₂PO₄, 20 g Difco agar) at 22 °C. Stocks were recultured weekly. For long term storage, spores were lyophilized and stored at 4 °C.

*Formation of macrocysts.* Macrocysts were produced by mixing equal numbers of amoebae or spores of NC4 and V12 (~5 × 10⁶ of each strain for spores and ~1 × 10⁵ of each strain for amoebae) and plating them on 0.1% lactose-peptone agar (per 1 distilled H₂O: 1 g peptone, 1 g lactose, 0.284 g Na₂PO₄, 0.272 g KH₂PO₄, 15 g Difco agar) or non-nutrient agar (per 1 distilled H₂O: 20 g Difco agar). Salt solution (2 ml) (Bonner, 1947) was added to each dish to immerse the amoebae. In some experiments, spores were washed and heat shocked according to the method of Cotter & Raper (1968). In brief, NC4 and V12 spores were harvested separately with salt solution and centrifuged at 1100 g for 12 min. The supernatant was poured off and spores were resuspended in salt solution. The resulting spore suspensions were heat shocked at 45 °C for 30 min and then plated separately on nutrient agar with *E. coli* B/r. Growth cultures were incubated in the dark and synchronously developing amoebae capable of forming macrocysts could be obtained by harvesting such growth plates 12–18 h after inoculation and separating amoebae from *E. coli* B/r by differential centrifugation (Bonner, 1947).

Experiments were designed so that the capacity of amoebae to produce macrocysts was measured rather than the preference of amoebae to develop macrocysts instead of fruiting bodies (Filosa & Chan, 1972; Robson & Williams, 1981). For optimal macrocyst production cultures were wet and incubated in the dark at 24 ± 1 °C. Cells that did not form macrocysts generally became amorphous cell clumps under these conditions. Since the cultures were wet, fruiting body development did not or only rarely occurred on plates and hence fruiting body counts cannot be included in these experiments. In experiments where fruiting body formation was investigated, amoebae were incubated on dry plates in the light. Cultures were scored for the number of macrocysts per field 1 week after amoebae of opposite mating type were mixed together.

*Scoring cultures.* Macrocyst production was measured by counting the number of macrocysts per field (7 mm²) of at least 10 random fields of a Petri plate (86 mm in diameter) viewed at a magnification of 54 × with a dissecting scope. At least four replicates of each treatment were done and each experiment was performed two to four times; each data point represents at least 40 samples.

**RESULTS**

*Pheromone and inhibitor studies.*

In a first attempt to determine whether exogenous chemical cues were involved in macrocyst development, NC4 and V12 spores and *E. coli* B/r were plated together on 0.1% lactose-peptone agar. Under these conditions, signals produced by amoebae of either mating type or by *E. coli* would be detected and further experiments could perhaps separate these factors. Activated charcoal was included in the cultures either on the surface of the agar (charcoal-down) or on a small platform above the agar (charcoal-up). Plates were incubated under macrocysts forming...
Macrocyst development in D. discoideum

Fig. 1. Relationship between macrocyst production and the presence and position of activated charcoal in culture. Activated charcoal was present on the agar (charcoal-down) or above the agar on a small platform (charcoal-up). The area of one field was 7 mm². Each column represents the mean and each bar represents the standard error of the mean of at least 40 samples.

Table 1. Sterile bacterial filtrate reduces macrocyst production

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of macrocysts per field*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard salt solution</td>
<td>135.3 ± 12.7</td>
</tr>
<tr>
<td>Bacterial filtrate</td>
<td>54.5 ± 5.7</td>
</tr>
</tbody>
</table>

* Mean of at least 40 samples ± S.E.

conditions as described above. This experimental design is essentially similar to that used by Weinkauff & Filosa (1965).

Macrocyst production increased markedly in charcoal-down treatments and decreased in charcoal-up treatments as compared with the no charcoal control (Fig. 1). The inhibition of macrocyst production observed in the charcoal-up treatment is consistent with the existence of a volatile activator of mating. It is possible that this volatile activator is the same substance as the volatile hormone reported previously (O'Day & Lewis, 1975; Lewis & O'Day, 1977).

The increase in macrocyst formation caused by the charcoal-down treatment suggests that there is a diffusible inhibitor of macrocyst development, assuming that this factor is adsorbed by activated charcoal. One possible mechanism for this observed reduction of macrocyst production is that the inhibitor might be a product of the E. coli B/r. To test this, cultures of amoebae of opposite mating types were exposed to sterile bacterial filtrates which were prepared from E. coli B/r grown for 48 h by centrifuging out the bacteria and filtering the supernatant through a 0.45 µm Millipore filter. To make certain that this filtrate was sterile, portions of it were placed on nutrient agar. No growth occurred. Exposure of mating amoebae to this bacterial filtrate clearly reduced macrocyst production; macrocyst formation was 40% that of the control level (Table 1). There is, however, no certainty that the E. coli inhibitor is the only one. There could be an amoeba-generated inhibitor as well (see O'Day & Lewis, 1981). The interesting and
most salient point here is that macrocyst formation is probably inhibited by a block of the starvation cue caused by the presence of bacterial filtrate. Experiments relating directly to the starvation cue and to information regarding the mechanism of the mating inhibitor from sterile bacterial filtrate will be discussed later.

**Differences between amoebae of the macrocyst and fruiting body pathways of development**

**Cell determination.** In heterothallic strains of *D. discoideum*, Erdos et al. (1976) showed, by shifting vegetative amoebae from dark to light or vice-versa, that the decision to follow a macrocyst pathway occurred prior to aggregation. In the next series of experiments reported here, these results have been confirmed and extended.

Synchronously developing populations of amoebae were prepared by washing and heat shocking NC4 and V12 spores and culturing them separately on nutrient agar with *E. coli* B/r. After 10 h incubation in the dark, cells were harvested and separated from the bacteria by differential centrifugation; NC4 and V12 amoebae were then plated separately on non-nutrient agar. At various times these synchronously developing NC4 and V12 amoebae were reharvested, mixed in equal proportions and placed on non-nutrient agar under a layer of salt solution in order to test the ability of amoebae that had been developing under fruiting body conditions to produce macrocysts. After 1 week incubation under macrocyst-forming conditions, the number of macrocysts was counted.

The most striking result of this experiment was that macrocyst production was highest when cells of opposite mating type were mixed just prior to aggregation (~9 h after starvation). The capacity of amoebae to produce macrocysts dropped precipitously at 11-12 h after starvation (Fig. 2). Suspensions of single amoebae of NC4 and V12 from cultures that had been starved for longer than 12 h could not form macrocysts when mixed together and immersed in salt solution in the dark. Evidently, there is a relatively narrow timespan prior to aggregation during which cells become determined to develop macrocysts or fruiting bodies.

In order to test the possibility that the *E. coli* B/r inhibitor described previously might be causing a delay in slime mould development and in this way influencing macrocyst production, the following experiment was done. Dark-grown amoebae of NC4 and V12 were harvested, washed and plated on non-nutrient agar or on bacterial suspension agar. Bacterial suspension agar was made by mixing 2% agar with a suspension of *E. coli* B/r. After this agar had hardened non-nutrient agar was poured on top making a 0.15 mm agar barrier to prevent physical contact between amoebae and the bacteria. Bacterial suspension agar was used rather than *E. coli* sterile filtrate because large enough quantities of sterile filtrate of sufficient concentration could not be obtained.
Table 2. Non-dividing cells and macrocyst development

<table>
<thead>
<tr>
<th>Time after heat shock (h)</th>
<th>No. of macrocysts per field*</th>
<th>Developmental stage†</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>3.3</td>
<td>Cells, clumps, aggregation starting</td>
</tr>
<tr>
<td>14</td>
<td>2.2</td>
<td>Cells, clumps, aggregation starting</td>
</tr>
<tr>
<td>16.5</td>
<td>0.1</td>
<td>Clumps of cells, aggregation</td>
</tr>
<tr>
<td>18.5</td>
<td>1.3</td>
<td>Clumps of cells, aggregation</td>
</tr>
<tr>
<td>20.5</td>
<td>0.3</td>
<td>Clumps of cells, aggregation</td>
</tr>
<tr>
<td>23.5</td>
<td>3.7</td>
<td>Aggregation, fingers</td>
</tr>
<tr>
<td>27.5</td>
<td>2.1</td>
<td>Aggregation, slugs</td>
</tr>
<tr>
<td>29.5</td>
<td>1.8</td>
<td>Aggregation, slugs, fruiting bodies</td>
</tr>
<tr>
<td>31.5</td>
<td>1.3</td>
<td>Aggregation, slugs, fruiting bodies</td>
</tr>
<tr>
<td>33.5-37</td>
<td>2.2</td>
<td>Aggregation, slugs, fruiting bodies</td>
</tr>
</tbody>
</table>

* Mean of at least 40 samples.
† Developmental stage of NC4 and V12 amoebae when they were mechanically dissociated and combined to assay for macrocyst production.

As expected, NC4 and V12 amoebae, alone or mixed together, did not develop at all on bacterial suspension agar. Even after 10 d only cells and small clumps of cells were present, while on non-nutrient agar development was normal. At 12, 24 and 36 h after inoculation on bacterial suspension agar, some of the amoebae were harvested, washed and replated on non-nutrient agar. Although no development of unharvested amoebae took place on the remaining bacterial suspension agar, the recultured cells developed normally. When mixed cultures of NC4 and V12 amoebae were replated after 12 h incubation on bacterial suspension agar, they were still able to produce macrocysts. However, such mixed cultures replated after 24 and 36 h exposure to bacterial suspension agar were not able to do so even though environmental conditions were appropriate for macrocyst formation; only sorocarps developed. It would appear that if the delay in development caused by the E. coli-produced inhibitor was sufficiently long, amoebae were prevented from forming macrocysts, perhaps because the time for some critical developmental switch had passed. This alteration in timing caused amoebae to become determined to develop sorocarps.

Cell division requirement. In many developing systems, a round of cell division is required before changes in developmental programmes can be enacted (Holtzer et al., 1972). In microbial mating systems, cell division often precedes cell fusions that accompany zygote formation. Although starvation is necessary for the initiation of macrocyst development, it is not sufficient. In contrast, starvation is sufficient for initiation of sorocarp development. Evidence given below strongly suggests that cell division prior to starvation is required for macrocyst development.

NC4 and V12 spores were washed, heat shocked and plated separately on non-nutrient agar and incubated in the dark. Germination was allowed for 12 h and then the resulting unfed, and therefore non-dividing, cells were harvested every 2 h, mixed and replated on non-nutrient agar. These cultures were incubated in the dark for 1 week and then the number of macrocysts was scored.

Although the cell density was appropriate for macrocyst development, very few were formed at any time point while fruiting body formation proceeded normally (Table 2). If one compares these data with those in Fig. 2, the requirement for the feeding of amoebae prior to starvation is quite evident.

In a second experiment, a dilution series of washed NC4 and V12 amoebae that had been grown on nutrient agar in the dark in association with E. coli B/r was prepared. NC4 and V12 suspensions of various densities were then mixed and plated on non-nutrient agar. After 1 week incubation in the dark, the number of macrocysts was counted.

In a similar fashion, NC4 and V12 spores were washed, heat shocked and a dilution series of each strain was prepared. NC4 and V12 spore suspensions of various densities were mixed and the resulting cultures were assayed for macrocyst production. As expected, macrocyst formation decreased as cell or spore density decreased (Fig. 3). However, macrocyst production in spore cultures was much lower than in amoebal cultures of similar density, once again demonstrating
Fig. 3. Relationship between macrocyst production and the density of NC4 and V12 spores (■) or amoebae (▼). Each point represents the mean of at least 40 samples.

Table 3. Requirement of cell division for macrocyst development

The growth conditions shown are those under which NC4 and V12 amoebae were grown prior to harvest. NC4 and V12 amoebae were combined on non-nutrient agar and cultures were assayed for macrocyst production.

<table>
<thead>
<tr>
<th>Growth condition</th>
<th>No. of macrocysts per field*</th>
<th>No. of divisions†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nutrient agar with bacteria</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Harvested after 10 h growth</td>
<td>49.1</td>
<td>1–2</td>
</tr>
<tr>
<td>Harvested after 14 h growth</td>
<td>19.5</td>
<td>2</td>
</tr>
<tr>
<td>Non-nutrient agar with bacteria</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Harvested after 10 h growth</td>
<td>3.6</td>
<td>0</td>
</tr>
<tr>
<td>Harvested after 14 h growth</td>
<td>0.2</td>
<td>0</td>
</tr>
</tbody>
</table>

* Mean of at least 40 samples.
† The number of times NC4 and V12 amoebae divided after germination and before they were combined on non-nutrient agar for the macrocyst assay.

the possible requirement of cell division for the development of the ability to initiate macrocysts. In all of the experiments discussed thus far, the possibility remains that it was not actually cell division that was required for macrocyst initiation but simply that cells had to be well-fed in order to have sufficient energy to undergo macrocyst development. One last experiment was done in an attempt to separate these two variables, that is, feeding and cell division. In this experiment, a known number of amoebae were cultured under different nutritional conditions and the number of cell divisions prior to starvation was measured as was macrocyst production. Clearly, there is a strong relationship between cell divisions that precede development and the number of macrocysts formed (Table 3). Even in the situation where abundant food was available, no macrocysts were produced unless at least some amoebae had undergone division. However, it was not possible to arrange culture conditions so that cell division occurred in the absence of feeding. Perhaps food is required in order for amoebae to have sufficient energy to undergo cell division and thus become capable of macrocyst initiation.

**Similarity between the pathways: starvation cue**

It is well known that starvation of amoebae is required for fruiting body development (Raper, 1940; Marin, 1976) and it has been long assumed although not directly demonstrated that it is also necessary for macrocyst development. Of course it is not surprising to find that starvation is required for the initiation of macrocyst development thus establishing an important similarity between these two modes of development. The experiments described here are the result of an effort to see whether amoebae became determined to develop fruiting bodies a certain number of hours after germination or starvation. In the first type of experiment, two groups of NC4 and V12 spores were washed and heat shocked at the same time but the resulting amoebae were
starved for different time periods. Approximately 12 h after inoculation on to nutrient agar with *E. coli* B/r, one group of NC4 and V12 cultures (A) were harvested, washed and plated separately on non-nutrient agar to initiate synchronous development. Every 3 h, these developing NC4 and V12 cells were harvested a second time, dissociated into a suspension of single cells and pairs of cells, combined in single culture on non-nutrient agar, immersed in salt solution and incubated in the dark.

Approximately 18 h after inoculation on to nutrient agar with *E. coli* B/r, the second group of NC4 and V12 amoebae (B) were harvested from nutrient agar, washed and plated separately on non-nutrient agar as described above. Similarly, these cells were harvested from non-nutrient agar every 3 h, mixed and recultured on non-nutrient agar. In summary, spores of group A and those of group B germinated simultaneously but were fed for different time periods and thus amoebae of each group began starvation after different time spans of vegetative growth.

As shown in the time course experiments discussed previously, there is a peak in macrocyst initiation in both populations that occurs 9–10 h after the onset of starvation regardless of the number of hours after germination (Fig. 4). This peak in the capacity of amoebae to produce macrocysts precedes the onset of aggregation. In addition, these results clearly show that the cue for macrocyst development is starvation as it is for sorocarp formation. Therefore, one of the earliest decisions a population of starving amoebae makes is whether to produce macrocysts or fruiting bodies; at this point these two developmental pathways diverge.

It was shown earlier that sterile bacterial filtrate can reduce macrocyst production. The mechanism of this inhibition by bacterial filtrate may be due to a blockage of the starvation cue. If this is true, one would expect that media rich in nutrients should be able to block a starvation cue and thus inhibit macrocyst development. This proved to be the case; when dark-grown NC4 and V12 amoebae were washed free of bacteria and plated together under a layer of salt solution on media containing glucose (10 mg ml⁻¹) or amino acids (0·5 mg ml⁻¹) (Table 4), macrocyst production was drastically reduced.

### Table 4. Macro cyst development on media containing glucose or amino acids

<table>
<thead>
<tr>
<th>Medium</th>
<th>No. of macrocysts per field*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-nutrient agar</td>
<td>49·1 ± 4·5</td>
</tr>
<tr>
<td>+ Glucose (10 mg ml⁻¹)</td>
<td>5·2 ± 1·0</td>
</tr>
<tr>
<td>+ Amino acids (0·5 mg ml⁻¹)†</td>
<td>5·4 ± 1·1</td>
</tr>
</tbody>
</table>

* Mean of at least 40 samples ± S.E.
† Complete mixture of amino acids, 0·5 mg of each ml⁻¹.

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Fig. 4. Relationship between macrocyst production and the time at which starved amoebae of opposite mating type were mixed. Spores of groups A and B were germinated together but amoebae of group B (■) fed for 6 h longer than those of group A (▲). Each point represents the mean of at least 40 samples.
From this study, several differences and similarities have emerged between macrocyst and fruiting body development. First, starvation initiates the development of amoebae for both pathways. Depletion of nutrients seems to activate a developmental timer in cells. Second, amoebae make the decision to produce macrocysts or fruiting bodies before a critical time point which occurs approximately 12 h after the onset of starvation. After this time, amoebae are committed to fruiting body development and cannot produce macrocysts when cultured under a layer of salt solution and incubated in the dark. Substances that can push amoebae into one or the other pathway often do so by altering the timing of development. Finally, cell division may be required in order for amoebae to become competent to produce macrocysts; there is no such requirement for fruiting body development.

Cell determination in heterothallic and homothallic cellular slime moulds

The light regime to which heterothallic amoebae are exposed during the early part of vegetative growth is crucial in determining the developmental fate of these cells (Erdős et al., 1976). In such heterothallic cultures, the production of giant cells is a key event in determining the developmental fate of a population of amoebae: cytophagic cells appear prior to aggregation in these cultures (Wallace, 1977; O'Day, 1979). The developmental fate of heterothallic cultures is not easily switched. As demonstrated in the experiments described here, the capacity to form macrocysts disappears before cells become aggregation competent. Dissociated aggregates and slugs cannot be redirected to form macrocysts when immersed in salt solution. When cultured on dry plates, amoebae from these later developmental stages reaggregate and produce fruiting bodies only (Fig. 2).

In contrast, homothallic species are much more developmentally labile. When aggregates and slugs of D. mucoroides were immersed in saline, 78% of the aggregates and 21% of the slugs could be redirected to form macrocysts (Filosa et al., 1975). Similarly, AC4, a homothallic strain of D. discoideum could be pushed toward macrocyst formation even at very late stages of development (Wallace, 1977). Again in contrast to heterothallic macrocyst formation, giant cells are never seen in homothallic cultures until after aggregation (Filosa & Dengler, 1972). Free giant cells have only rarely been seen in AC4 and when they are formed, they are not present in proportion to the final yield of macrocysts (Wallace, 1977). All of these observations point to the inescapable conclusion that although cell determination has been clearly demonstrated for heterothallic macrocyst development, the situation for homothallic development is quite different.

Macrocyst initiation peaks at approximately 10–12 h after starvation and then drops sharply (Fig. 2). This time period is also very important for other developmental events for cellular slime moulds. As measured by an erythrocyte agglutination assay, discoidin activity increases 400-fold from the beginning of differentiation to 12 h of development (Rosen et al., 1973). Contact sites A are expressed and cells begin to exhibit end-to-end contacts that are typical of aggregating cells (Müller & Gerisch, 1978). At this stage, expression of a large number of new genes is initiated; specific cell–cell contact may be essential for this induction (Blumberg et al., 1982). Of importance to macrocyst development, Szabo et al. (1982) have shown that giant cell formation begins at approximately 10 h of development. All of these observations suggest that a population of starving amoebae faces a series of decisions that must be made prior to aggregation at around 10 h after starvation. One of the earliest choices to be made is whether to produce macrocysts or fruiting bodies. Altering the timing of development by addition of sterile bacterial filtrates, amino acids, glucose or certain lectins (unpublished data) results in the inhibition of macrocyst production and the amoebae become committed to fruiting body development.

Cell division requirement

Recent studies have revealed a relationship between the number of cell divisions and the timing of cilium formation in sea urchin embryos (Masuda, 1979) and of rRNA synthesis in amphibian embryos (Misumi et al., 1980). Such observations suggest that developmental timing is controlled by events in the cell division cycle involving cytokinesis, nuclear division or DNA
replication. Results discussed in this paper support the idea that adequate feeding enables cellular slime mould amoebae to undergo cell division thus causing the amoebae to become capable of producing macrocysts. The fact that amoebae from germinated spores can develop fruiting bodies suggests that this mode of development represents a baseline and that alternative pathways such as macrocyst formation utilize different developmental programmes which can operate only after some event during cell division permits them to be expressed. The mechanism that makes available in amoebae that have divided, information that was not available in newly germinated amoebae is a most intriguing question for future research.

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