CONTROL OF CELLULAR DIFFERENTIATION BY TEMPERATURE IN THE CELLULAR SLIME MOULD DICTYOSTELIUM DISCOIDEUM

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SUMMARY
The effects of low temperature on morphogenesis and cellular differentiation of Dictyostelium discoideum were examined. During incubation at 5 °C, the vegetative and preaggregation cells never developed, but cell masses at the aggregation or slug stage developed to form hemispherical, or dumbbell-shaped multicellular structures. By staining with FITC-antispore IgG, the structures formed after 10 days of incubation of tipped aggregates at 5 °C were found to be composed of 90% spores, 5% prespore cells and 5% non-stained cells. Since only 20% of the total cells constituting the tipped aggregate had been prespore cells at the beginning of incubation, this showed that spore differentiation proceeded even at low temperature, while stalk differentiation was completely inhibited. Similar results were obtained when the cells were incubated at 3 °C. However, at 0 °C, morphogenesis and cellular differentiation did not occur, although most of the prespore cells at the late culmination stage differentiated incompletely into spores. Possible reasons for the high proportion of spores being induced by low temperature are discussed.

INTRODUCTION
After cessation of vegetative growth, Dictyostelium discoideum cells aggregate to form a hemispherical mound with a tip that acts as an organizing centre during further development. The cell mass stands on the substratum and then lies down to migrate as a slug under suitable conditions. After certain periods of migration, the slug constructs a fruiting body consisting of a mass of spores (sorus), a stalk and basal disc. During this process, the tip region of the cell mass secretes the stalk sheath in a cylindrical form and enters the sheath to differentiate into stalk cells with cellulosic walls. With the formation of the stalk sheath and stalk cells, the posterior cells rise into the air and finally differentiate into spores.

The ratio of spores to stalk cells in a fruiting body is known to be kept almost constant, irrespective of its size (Bonner & Slifkin, 1949). This ratio is the same as the prespore: prestalk ratio in a slug (Hayashi & Takeuchi, 1976, 1981), and thus the final cell proportioning in a fruiting body is predetermined at the slug stage (MacWilliams & Bonner, 1979). A close relationship has been found between cell proportioning and morphogenesis (Ishida, personal communication), especially the formation of the tip at the slug stage.

The present work was originally undertaken to investigate whether or not the cell motility is involved in cellular differentiation as well as morphogenesis. During examination of the factors affecting cell motility, a low temperature around 5 °C was
found preferentially to induce spore differentiation and inhibit stalk formation, leading to a higher proportion of spores. The possible function of low temperature is discussed with special reference to cell proportioning.

MATERIALS AND METHODS

Culture

*Dictyostelium discoideum* NC4 cells were incubated with *Escherichia coli* B/r for various periods on standard nutrient agar media (Bonner, 1947) at 21°C, to obtain cells or cell masses at certain developmental stages. These were then transferred to agar plates kept at various temperatures. In another experiment, cells at the growth phase were harvested and washed four times in a chilled Bonner's salt solution (BSS) (Bonner, 1947). To obtain developmentally synchronized cell masses, the washed cells were resuspended in the salt solution at a density of approx. 2x10^6 cells/ml, and two or three drops of the suspension were deposited on a Millipore filter (pore size, 0.45 μm) supported by a filter pad soaked in BSS and then incubated at 21°C. After various periods of incubation, the filters were transferred to a nutrient agar medium and incubated at 5°C.

Immunohistochemistry

Fluorescein isothiocyanate (FITC)-conjugated immunoglobulin G (IgG) produced against *Dictyostelium mucoroides* spores was used for the identification of prespore cells and spores, since this antispore IgG is known to react specifically with acid mucopolysaccharide, the major component of prespore vesicles and spore coats (Maeda, 1971; Ikeda & Takeuchi, 1971). The antibody was a generous gift from Dr I. Takeuchi of Kyoto University. Cells were stained with the FITC-antispore IgG according to the method of Takeuchi (1963).

To prepare histological sections, cell masses on agar blocks were fixed in absolute methanol, embedded in Paraplast (Sherwood), and sectioned 5 μm thick.

To estimate the ratio of prespore cells and spores to total cells, cell masses on a Millipore filter were filtrated through a nylon mesh (pore size, 40 μm; NBC Industries) to remove single cells and small cell clumps. The filtrated cell masses were mechanically dissociated in 10mM-Tricine (pH 7.0) containing 20mM-EDTA by being passed several times through a syringe needle. The dissociated cells were collected and resuspended in 60% (v/v) methanol, and then a drop of the suspension was air-dried on a coverslip.

RESULTS

Effects of low temperature on morphogenesis and cellular differentiation

When preaggregation and postaggregation cells were transferred to 7–21°C, they developed into normal fruiting bodies. Below 5°C, however, the progress of morphogenesis was greatly affected and vegetative cells did not develop even after 1 month of incubation at 5°C. This was also the case for early aggregates shifted to the low temperature. In a tipped aggregate, cells within it moved toward the tip at 5°C, eventually forming a hemispherical or spherical mass with or without a supporting neck (Fig. 1A,B). The majority of cells in a hemispherical mass were found to be spores, according to their shape and stainability with FITC-antispore IgG (Fig. 1C), though a small number of cells adhering to the substratum had remained undifferentiated. The spherical mass with the neck was composed exclusively of spores, while cells in the neck were highly vacuolated. Similar results were obtained in standing or migrating slugs shifted to 5°C. Serial longitudinal sections of a dumbbell-shaped structure derived from a standing slug showed that this structure was composed of a
Fig. 1. Side-views of a hemispherical mass (A) and a spherical mass with a neck (B), both formed after 1 month of incubation of aggregates at 5°C. C. Fluorescent micrograph of a section (10 μm thick) of a hemispherical mass stained with FITC-antispore IgG. Most cells show a spore-like appearance with surfaces stained with IgG. A,B, ×25; C, ×450.

major portion of spore-like stained cells and a minor portion of non-stained vacuolated cells (Fig. 2A). The stained cells were distributed evenly in the cell mass, and their envelopes were strongly stained with IgG (Fig. 2C) as in the case of the spores. This contrasts with the case of the prespore cells in a normal migrating slug (Fig. 2A). The non-stained and vacuolated cells in the dumbbell-shaped structure seemed to differentiate incompletely into stalk cells, because they were lysed by treatment with 0.1% Triton X-100. In all of the cases examined, neither the stalk sheath nor the stalk cell wall formed.

When a cell mass at the early culmination stage was transferred to 5°C, a very short stalk sheath filled with a few stalk and prestalk cells was formed in addition to a predominant number of spores (data not shown). On the other hand, a cell mass at the late culmination stage was found to develop into a fruiting body, though the ratio of spores seemed to be considerably higher than that in a normal fruiting body. At 3°C cells developed as at 5°C but more slowly.

A lower temperature like 0°C exerted drastic effects on morphogenesis and cellular differentiation, such as aborting the development of postaggregation cells. If the cells were prespore ones at the late culmination stage when transferred to 0°C, they usually differentiated into spores. However, these spores were round (Fig. 3), not ellipsoidal like normally developed spores.

Temporal changes of prespore and spore differentiation during incubation at 5°C

Development on a Millipore filter proceeded as described in the preceding section, but more synchronously than on agar, even at 5°C. Thus, cells or cell masses on the
Fig. 2
Temperature and Dictyostelium development

Fig. 3. Fluorescent micrograph of round spores obtained at 0°C from prespore cells at the late culmination stage. ×600.

Fig. 4. Temporal change in the proportion of prespore cells and spores stainable with FITC-antispore IgG in tipped aggregates during 10 days of incubation at 5°C. Total number of stained cells (●—●), prespore cells (○—○) and spores (▲—▲). Values were obtained from three separate experiments. Bars indicate standard deviations.

Fig. 2. Fluorescent micrographs of a longitudinal section of a normal migrating slug (a) and serial sections of a dumbbell-shaped structure (b) formed after 1 month of incubation of a standing slug. Each section (5 μm thick) was stained with FITC-antispore IgG. c. Higher magnification of the section marked with * in b. Most cells are stained well with IgG. a, ×250; b, ×180; c, ×400.
filter were used to examine temporal changes in the proportion of spores and prespore cells in those incubated at 5 °C at various developmental stages. When cells were incubated at 5 °C before the aggregation stage most of them could not be stained by FITC-antispore IgG even after 1 month of incubation. In the case of a tipped aggregate incubated at 5 °C, the ratio of non-stained cells decreased from about 80% to 5% of the total cells (Fig. 4). At the beginning of incubation at 5 °C, all of the stained cells (20% of the total cells) were prespore cells containing prespore vesicles. The ratio of spores in the stained cells began to increase rapidly after 4 days of incubation and finally reached 94% of the total stained cells (corresponding to 90% of the total cells) after 10 days of incubation (Fig. 4). Before differentiation, prespore cells gradually increased during the first 4 days, followed by a rapid decrease in the ratio to about 5% of the total cells during another 4 days of incubation (Fig. 4).

**DISCUSSION**

The most important aspects of the present findings can be summarized as follows: (1) even at low temperatures such as 3–5 °C cells can differentiate into spores, but not into stalk cells; (2) the ability of cells to differentiate into spores is acquired after the late aggregation stage; (3) spores formed at low temperature have a tendency to become round.

The effect of low temperature on the cell proportioning in *Dictyostelium* was first reported by Bonner & Slifkin (1949). They demonstrated that fruiting bodies with a higher proportion of spores are formed when cell masses grown at room temperature are exposed to 2°C for 2 days at the migration or culmination stage. This is basically consistent with the present findings.

Although the reason for the high proportion of spores in cell masses formed under low temperature is unknown, one explanation may be that the prestalk cells are converted into prespore cells by the mechanism that regulates the prespore:prestalk ratio, because of the inhibition of stalk differentiation by low temperature. An alternative reason may be that the cells directed to differentiate into stalk cells are selectively lost from the cell mass at low temperature. For clarification, the actual behavior of prestalk cells during incubation at low temperature needs to be examined. A preliminary observation that cell-type conversion from prestalk to prespore cells occurs in a prestalk isolate at low temperature (unpublished data) suggests that the latter explanation should be excluded. The above fact also suggests that the prestalk cells at the slug stage are distinguishable from cells with some prestalk-specific proteins (Borth & Ratner, 1983) and antigens (Tasaka, Noce & Takeuchi, 1983) at the aggregation stage, because the latter could not differentiate into prespore cells at low temperature. Therefore, the inhibitory effect of low temperature on cells before the late aggregation stage is considered to block the acquisition of prestalk competence. It is clear from the present work that the acquisition of prespore competence is also temperature-dependent. The cells with prespore competence, however, are able to differentiate into spores even at low temperature. On the other hand, the prestalk cells differentiate...
into spores by cell-type conversion, but not into stalk cells. These facts indicate that prespore and spore-specific genes are expressed selectively at low temperature.

Finally, the fact that spores formed at 0°C were round suggests that the establishment of spore shape is attributable to microtubules. In this connection, it is noteworthy that *Dictyostelium* microtubules are disassembled at low temperature (White, Tolbert & Katz, 1983).

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REFERENCES


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