A Mutant of *Dictyostelium discoideum* with Alternative Pathways of Differentiation Depending on Culture Conditions

By SHUJI ISHIDA

Department of Botany, Faculty of Science, Kyoto University, Kyoto 606, Japan

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A mutant (*dev*-1515), which forms either spores or stalk cells depending on the cultural conditions, was isolated from *dev*-1510, a mutant of *Dictyostelium discoideum* NC-4. When grown with *Escherichia coli* on a nutrient-rich medium, *dev*-1515 formed a hemispherical cell mass in which most cells differentiated into spores. Culture with *E. coli* on a nutrient-intermediate medium inhibited amoebae from aggregating normally and they differentiated into stalk cells only. Development of washed vegetative cells of the mutant proceeded further without bacteria on either medium. Cells cultured in a roller tube were profoundly affected by the ionic strength of the medium. Almost all the cells in an agglutinate differentiated into stalk cells in a medium of low ionic strength, while most cells differentiated into spores in a medium of high ionic strength.

INTRODUCTION

After ceasing vegetative growth, amoebae of the cellular slime mould *Dictyostelium discoideum* aggregate to form slug-shaped cell masses. The slug consists of two types of cells, the anterior prestalk and the posterior prespore cells, which eventually form the stalk cells and the spores of the fruiting body.

To understand the mechanisms underlying this development, a number of biochemical studies have been made on the whole developing cell mass. However, it is difficult in such cases to determine whether the changes in question are due to differentiation of spores or stalk cells since both occur simultaneously. Three methods have been used to study changes specific for differentiation of each cell type: (1) isolation of anterior and posterior segments by cutting slugs (e.g. Newell *et al.*, 1969; Brenner, 1977); (2) separation of prespore from prestalk cells by density gradient centrifugation (e.g. Maeda *et al.*, 1973; Oohata & Takeuchi, 1977); (3) use of a mutant which forms only stalk cells or spores depending on the cultural conditions. The use of the first two methods is restricted to that period in development when prestalk and prespore cells are separable in the cell mass, and hence cannot be used throughout development. The third method was used by Hamilton & Chia (1975) who found that a mutant (P-4), isolated by Hohl & Raper (1964), formed only stalk cells in the presence of cyclic AMP (Chia, 1975). Recently, I have reported the isolation of a mutant (*dev*-1510) which forms either only spores or only stalk cells depending on the cultural conditions (Ishida, 1980*a*). On a nutrient-rich medium, this mutant stopped development after forming hemispherical cell masses in which the majority of cells differentiated into spores. Cells of the mutant at the early aggregation stage on nutrient-poor medium, and shifted from 21 °C to 27 °C, stopped development before papilla formation and most of them differentiated into stalk cells. Without temperature shift-up, however, they developed further and the ratio of spores to stalk cells was almost normal in the terminal structure. As such an abrupt temperature shift-up would bring about many changes in the cells, the aim of the present work was to isolate from *dev*-1510 mutants which stop development before papilla formation in the

hope of selecting a mutant which gives rise to a homogeneous population of either stalk cells or spores, depending on cultural conditions, without any further manipulation.

METHODS

Strains. The mutant *dev*-1510 (Ishida, 1980*a*) from *Dictyostelium discoideum* KYH (NC-4) and a mutant (*dev*-1515) isolated from it were used in the present study.

Media and growth. To examine the effects of culture media on the development of *dev*-1515, amoebae were grown on a nutrient-rich medium (R-medium) or a nutrient-intermediate medium (I-medium), at 21 °C, in association with *Escherichia coli* B/r. The R-medium consisted of 2% (w/v) agar containing 1% (w/v) glucose, 1% (w/v) peptone and 13 mM-Na/K phosphate buffer (pH 6.2). The I-medium consisted of 2% (w/v) agar containing 0.5% (w/v) lactose and 0.5% (w/v) peptone. To obtain vegetative cells both strains were grown with *E. coli* in liquid shake culture in medium containing 0.5% (w/v) lactose, 0.5% (w/v) peptone and 100 mM-mannitol at 21 °C (Ishida, 1980*b*). The cells were harvested at a density of about 10^6 ml⁻¹ and washed three times in 1% (w/v) Bonner's salt solution (BSS) (Bonner, 1947).

Roller tube culture. This was carried out according to the method of Takeuchi *et al.* (1977). A suspension of 5×10^6 washed vegetative amoebae in 3 ml solution of the required ionic strength was placed in a test tube which was sealed by a Teflon-silicone stopper and rotated around the long axis at 21 °C at 28 rev. min⁻¹.

RESULTS

Isolation of a mutant with alternative pathways of differentiation

The ratio of stalk cells to spores of *dev*-1510 is dependent on the terminal structures formed: the ratio was either unusually high or low in a structure which stopped development before papilla formation depending on the cultural conditions, while the ratio was normal in a structure formed after that stage regardless of cultural conditions (Ishida, 1980*a*). Therefore, a mutant was sought which stopped development before papilla formation in order to give a more homogeneous population of either spores or stalk cells than *dev*-1510. Amoebae of *dev*-1510 were treated with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (0.1 mg ml⁻¹) for 30 min, and then washed and cultured at 21 °C with *E. coli*. After incubation for 4–5 d, clones which stopped development before papilla formation were isolated. Of 10 such mutants obtained, *dev*-1515 produced the most homogeneous population of stalk cells and spores on the I- and R-media.

Development of the mutant on the two culture media with bacteria

When cultured with *E. coli* on the I-medium, the *dev*-1515 cells could not normally aggregate. The use of a time-lapse video-tape recorder (Inoue & Takeuchi, 1979) revealed that cells aggregated to a collection point for a while, then stopped movement, but soon resumed movement towards another point. Consequently, small aggregates consisting of only two or three cell layers were scattered among a large number of unaggregated cells. All the cells differentiated into stalk cells which were vacuolated and enclosed by walls stainable by Calcofluor (Harrington & Raper, 1965).

Mutants cells cultured with *E. coli* on the R-medium underwent aggregation, but no further development. 'Squashes' of the hemispherical cell masses formed revealed that all the cells except for a layer adjacent to the agar differentiated into spores. However, unaggregated cells as well as those adjacent to the agar in a cell mass differentiated into stalk cells. Spores formed were elliptical and capable of germinating in the presence of 1% (w/v) cellulase (Sigma) after heat-shock treatment for 30 min at 45 °C, although untreated spores hardly germinated. When the cell masses formed were separated from free cells by filtering through a nylon mesh (30 µm pore size) and then dissociated by sonication, over 90% of cells were found to be spores.

Development of the mutant without bacteria

After growth in liquid shake culture, amoebae were washed and plated at a density of 5×10^6 cells cm⁻² on the I- and R-media containing streptomycin sulphate (0.1 mg ml⁻¹). The

Table 1. *Dependence on medium ionic strength of differentiation of dev-1515 in agglutinates made by roller tube culture*

Washed vegetative cells of *dev-1515* were cultured in roller tubes with various concentrations of Bonner's salt solution (BSS: 10 mM-KCl, 10 mM-NaCl, 3 mM-CaCl₂). After 2 d, the agglutinates made were dissociated by sonication and spores and stalk cells were scored microscopically.

Concentration of BSS	Stalk cells (%)	Spores (%)
0.1×	<10	<10
0.5×	>80	<10
1×	>80	10–20
2×	60–80	10–30
4×	30–60	30–60
6×	10–30	60–80
8×	<10	>80
10×	<10	>80

development of the cells on both media proceeded further than in the presence of bacteria. Cells on the I-medium formed aggregation centres consisting of several cell layers in which those in the upper two or three layers differentiated into spores while the rest differentiated into stalk cells. Cells on the R-medium formed aberrant fruiting bodies, standing slugs and hemispherical cell masses. In the aberrant fruiting bodies and standing slugs the cells differentiated into both types with a normal ratio, as was observed with the parent strain, *dev-1510* (Ishida, 1980*a*). In the hemispherical cell masses, however, almost all the cells formed spores except for a layer adjacent to the agar. Similar development of *dev-1515* was observed when the R-medium was replaced by 2% (w/v) glucose, 2% (w/v) peptone or 3 × BSS, indicating that the changes produced by incubation on I- or R-media are caused by osmotic changes rather than changes of nutrients.

Differentiation of the mutant in roller tube culture

Since the mutant cells underwent homogeneous differentiation in an aggregate devoid of papilla, roller tube cultures of *dev-1515* cells were made. It was found that cells formed agglutinates in which spores and stalk cells differentiated and that this differentiation was profoundly affected by the ionic strength of the medium. Low ionic strength favoured differentiation into stalk cells, while high ionic strength favoured differentiation into spores (Table 1). It was found that medium containing 2.5 mM-KCl, 2.5 mM-NaCl and 5 mM-CaCl₂ was optimum for stalk formation and medium containing 80 mM-KCl, 60 mM-NaCl and 10 mM-CaCl₂ was optimum for spore formation. In the former medium, all the cells differentiated into stalk cells, which were vacuolated and enclosed by a cell wall. In the latter medium, all the cells differentiated into spores, except those in the outermost layers which formed stalk cells. Spores formed could germinate in the presence of 1% (w/v) cellulase after heat activation at 45 °C for 30 min.

DISCUSSION

The mutant *dev-1515* was characterized not only by the inability to form papilla, but also by the ability to produce the most homogeneous population of either stalk cells or spores among mutants so far isolated, especially in roller tube culture. The parental strain, *dev-1510*, gave rise to considerably less homogeneous populations in roller tube culture under the same conditions as employed for *dev-1515* (unpublished data).

Previously, the only mutant known to produce a homogeneous population of either cell type was P-4 which differentiates into stalk cells only in a culture containing cyclic AMP (Chia, 1975). Hamilton & Chia (1975) have examined biochemical changes accompanying stalk formation by assaying the activities of several enzymes. However, the results must be interpreted with caution, since Kay *et al.* (1978) have shown that wild-type cells plated on

agar containing high concentrations of cyclic AMP become prespore cells first and then transform into stalk cells. For this kind of study, the mutant *dev-1515* has an advantage over P-4 in that it forms either spores or stalk cells depending on cultural conditions, and further that the pathway of differentiation can be changed without exogenous cyclic AMP.

The *dev-1515* cells formed stalk cells in a medium of low ionic strength in roller tube culture and formed spores at high ionic strength. Although the reason for this phenomenon is presently unknown, it seems to be related to the fact that when *dev-1510* and *dev-1515* cells form spores and stalk cells in a cell mass, cells adjacent to the medium differentiate into stalk cells while the rest form spores (Ishida, 1980*a*). A similar phenomenon was observed with wild-type cells by Takeuchi *et al.* (1977) who showed that prespore cells in a slug adjacent to agar lose the prespore antigen during slug migration, and that this loss is prevented by the addition of cyclic AMP, KCl and imidazole buffer to the agar (M. Tasaka & I. Takeuchi, personal communication). These facts suggest that when a substance(s) involved in spore formation leaks out into the agar, cells tend to differentiate into stalk cells. It is possible that a medium of high ionic strength somehow prevents the leakage of the substance.

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