The Differentiation Responses of *Dictyostelium discoideum* Amoebae at Various Times During Synchronous Growth

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Synchronous cultures of the cellular slime mould, *Dictyostelium discoideum*, were prepared by a selection method which involved low-speed centrifugation of exponential cultures. The method gave a relatively high degree of synchronous growth and cell doubling and a discrete phase of DNA synthesis was discernible. Amoebae which were induced to sporulation at different times during synchronous growth differed in their differentiation responses. Those which were about to divide produced more fruiting bodies and had higher levels of α -mannosidase and *N*-acetylglucosaminidase than cells removed to sporulation conditions at other times during synchronous growth.

INTRODUCTION

The physiological and biochemical changes which accompany sporulation of the cellular slime mould *Dictyostelium discoideum* have been extensively studied and reviewed (Loomis, 1975). Although it is also well known that many similar qualitative and quantitative changes occur during the growth–division cycle of other eukaryotic microbes (Mitchison, 1971), the growth of *D. discoideum* has received little attention. The study of growth has been facilitated by the isolation of axenic strains (Sussman & Sussman, 1967; Watts & Ashworth, 1970; Ashworth & Watts, 1970; Loomis, 1971) which also exhibit normal sporulation. Exponential growth of the axenic strain Ax-2 has been described (Watts & Ashworth, 1970; Ashworth & Watts, 1970) but studies of the cell cycle have been based either on induced synchrony of temperature-sensitive mutants (Katz & Bourguignon, 1974) or of ‘stationary phase’ cells, which when transferred to fresh growth medium, divide synchronously (Zada-Hames & Ashworth, 1978). In this paper we describe a method for obtaining synchronous cultures of *D. discoideum* which is based on a selection principle, and investigate the differentiation competence of the cells at various times during synchronous growth.

METHODS

Organism and growth conditions. *Dictyostelium discoideum* Ax-2 strain, was kindly provided by S. Minter, Essex University, U.K. Amoebae were grown on the medium described by Watts & Ashworth (1970) except that Difco yeast extract was substituted for the Oxoid product. Cultures were grown in 50 ml medium in a 250 ml conical flask, or in 100 ml medium in a 500 ml flask. All cultures, whether exponential or synchronous, were incubated at 22 °C on a reciprocal shaker at 80 rev. min⁻¹.

Preparation of synchronous cultures. Synchronous cultures were prepared by a modification of the low-speed centrifugation method described by Chagla & Griffiths (1978). Exponential cultures were first centrifuged in sterile, stoppered centrifuge tubes (50 ml) at 570 rev. min⁻¹ (35 g, r_{av} = 10 cm) for 2 min in an MSE bench centrifuge. The supernatant appeared to contain a high proportion of non-viable cells and was discarded. The cell pellet was resuspended in sufficient conditioned growth medium (prepared by centrifuging another exponential culture of the same age so that it was completely free of cells) to restore the original culture volume. The reconstituted culture was again centrifuged at 500 rev. min⁻¹ and the resulting supernatant, which contained about 10% of the cells which were present in the original exponential culture, was decanted carefully into a clean, sterile conical flask and constituted the synchronous culture. The degree of synchrony was estimated by the synchrony index (*F*) of Blumenthal & Zahler (1962) which has a value of 1.00 for a perfectly synchronous culture.

Cell counts. Amoebae were counted without prior fixation in a Fuchs-Rosenthal haemocytometer (Weber & Sons, Lancing, Sussex, U.K.). A minimum of 200 cells were counted for each sample.

Measurement of DNA. Samples (2–5 ml) were washed twice with distilled water and sonicated for 10 s at 40 W. Trichloroacetic acid (5%, w/v final concentration) was then added to the samples and DNA was extracted using the procedures described by Herbert *et al.* (1971). DNA was measured by a fluorimetric method (Kissane & Robins, 1955), with Sigma calf thymus DNA (type IIs, sodium salt) as the standard.

Induction of differentiation. Amoebae were collected by centrifugation and washed with water. Development was initiated by transferring washed cell suspensions to (i) Millipore filters (AABP047) supported on absorbent pads which were soaked either with a solution containing 50 mM-phosphate buffer (pH 6.4), KCl (1.5 mg ml⁻¹) and MgCl₂ (0.5 mg ml⁻¹) or with water alone, and (ii) 2% (w/v) non-nutrient agar plates. For both methods incubation was at 22 °C.

Cell suspensions were spread over a fixed area of agar or Millipore filter so that the number of cells per unit area was the same. The number of fruiting bodies formed after 48 h incubation on agar was estimated by examining known areas (75 mm²) using a template. Eight to twelve areas were examined for each sample and at least 400 fruiting bodies were counted. All the fruiting bodies (450–1500) which had formed on Millipore filters were counted. Each sample was duplicated and the averages of the two estimates were expressed as a percentage of the highest values obtained.

Measurement of enzyme activities during development. After 4 h development on membrane filters, samples were taken by scraping off the cells into 3 ml distilled water. These samples were then frozen until required when they were thawed and sonicated for 10 s at 20 kHz, 500 W in an MSE sonicator. The levels of α -mannosidase were measured by the method described by Loomis (1970), with 5 mM-*p*-nitrophenyl- α -mannoside as the substrate. *N*-Acetylglucosaminidase was measured with 8 mM-*p*-nitrophenyl-*N*-acetylglucosamine as the substrate (Loomis, 1969). In both cases, one unit of enzyme is that amount which liberates 1 nmol nitrophenol min⁻¹.

RESULTS AND DISCUSSION

The change in numbers of *D. discoideum* amoebae after the low-speed centrifugation procedure is shown in Fig. 1. Two cycles of cell division were followed and these gave synchrony indices (*F*) of 0.72 for the first cycle and 0.46 for the second cycle. In the first cycle, division occupied 1.9 h and the ratio of cell numbers after and before division (N/N_0) was 1.98; in the second cycle these values were 1.0 h and 1.65, respectively. In 14 experiments synchrony indices for the first cycle ranged from 0.6 to 0.78 (mean 0.70, s.d. 0.06) and N/N_0 ratios ranged from 1.83 to 1.98 (mean 1.90, s.d. 0.05). Division times varied from 1.25 to 2.25 h (mean 1.81, s.d. 0.34). There was some variation in the lag before the cells divided [1.25–2.4 h (mean 1.91, s.d. 0.30)] and the cell cycle times, measured as the time between the start of successive divisions, varied from 5.5 to 6.0 h. There was a sharp increase in the DNA content of the cells during the growth of the synchronous cultures (Fig. 2).

Cells taken at different times from the synchronous cultures, and induced to differentiate by transfer to agar plates and Millipore filters, differed in their sporulation responses and in their levels of α -mannosidase and *N*-acetylglucosaminidase. Amoebae which were induced to undergo development just prior to cell division gave the highest level of fruiting body formation. The lowest number of fruiting bodies was formed by amoebae removed from the synchronous cultures towards the end of the division phase.

The two enzymes which were measured in our experiments have been shown to increase rapidly during the initial stages of differentiation of *D. discoideum* (Loomis, 1969, 1970) and are therefore sensitive indicators of the differentiation responses of amoebae. There appears to be complete agreement in the rank order of the samples in relation to fruiting body formation and enzyme levels (Table 1).

Some important features of the selection method described here are that the degree of synchrony and the ratios of cell numbers before and after cell division were comparable with the values obtained with synchronous cultures of other eukaryotic microbes [which were also established by centrifugation (Lloyd *et al.*, 1975; Chagla & Griffiths, 1978)]. The procedure would seem, therefore, to be a suitable method for further studies of the growth of *D. discoideum*.

The cell cycle time of 5.5–6.0 h was shorter than that reported for exponential cultures of the Ax-2 strain (Zada-Hames & Ashworth, 1978) and for temperature-induced synchronous cultures of the Ax-3 strain (Katz & Bourguignon, 1974). The lengths of the DNA synthesis (S) phase and

Fig. 1. Changes in cell number during synchronous growth of *D. discoideum*. The synchronous culture contained 13% of the original exponential culture. Cell numbers were estimated every 0.25 h in a haemocytometer. Synchrony indices (F) for the first two cycles of cell division are shown.

Fig. 2. Changes in cell number (a) and DNA content (b) of *D. discoideum* during synchronous growth. Cell suspensions (2–5 ml) were washed and sonicated. DNA was extracted and measured fluorimetrically. (F , synchrony index).

Table 1. *Fruiting body formation and enzyme levels of developing cells taken at various times from synchronous cultures*

Cells were taken at the times indicated during synchronous growth (shown in Fig. 2) and development initiated as described in Methods. After 48 h, the number of fruiting bodies was estimated, and expressed as a percentage of the maximum value. The enzymes α -mannosidase and *N*-acetylglucosaminidase were assayed after 4 h development; enzyme units are nmol nitrophenol released min^{-1} .

Time at which cells were removed from synchronous cultures (h)	Fruiting bodies formed (%)	Enzyme activity [units (mg protein) ⁻¹]	
		α -Mannosidase	<i>N</i> -Acetylglucosaminidase
0.25–0.5	85	4.61	18.40
1.5–1.6	100	5.55	25.80
2.9–3.25	55	1.42	9.20
4.5–4.65	58	3.52	17.20

the G_2 phase appeared to be shorter in our cultures than in previous estimates, although the G_1 phase was of about the same duration. The discrepancies between our results and those of previous workers concerning the length of the DNA synthesis phase could be due to the different methods which were used to measure DNA levels. Synchronous cultures do not, however, allow

the various phases of the cell cycle to be delineated with absolute precision and the times reported here would need to be confirmed by observations of single cells. It follows therefore, that as the method we have described for obtaining synchronous cultures does not give complete synchrony, it is not possible to be more precise about the relationship between differentiation competence and the different phases of the cell cycle. Our experiments do suggest however, that there are large differences at different points in the cell cycle in the extent to which cells are able to initiate and complete differentiation, and that further consideration should be given to the nature of the transition from growth to differentiation in this organism.

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