Cell cycle-related changes in the surface properties of amoebae of the cellular slime mould *Dictyostelium discoideum*

Paul T. Sharpe and D.J. Watts*

Department of Biochemistry, University of Sheffield, Sheffield S102TN, England

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Amoebae of the cellular slime mould *Dictyostelium discoideum* were harvested during exponential, axenic growth and were partitioned in a dextran–poly(ethylene glycol) two-phase system in a countercurrent distribution apparatus. Amoebae in G1-, S- and G2-phases of the cell cycle were located in different parts of the countercurrent distribution. Since partitioning separates cells with different surface properties, it is concluded that there are cell cycle-related changes in the surface properties, and thus plasma membrane structure, of the amoebae.

Dictyostelium discoideum *Partitioning* *Cell cycle* *Cell surface properties*

1. INTRODUCTION

Partitioning in aqueous, two-phase systems (e.g., dextran–poly(ethylene glycol) mixtures) is a well-established method for separating cells that differ in surface properties [1–3]. When a heterogeneous population of cells is subjected to partitioning on a countercurrent distribution apparatus, cells with identical surface properties should collect together and be left in a Poisson distribution [4]. Usually, however, each distribution is broader than the calculated, theoretical distribution (which is obtained if a homogeneous compound such as vitamin B-12 is partitioned) and must contain cells with slightly different surface properties. The behaviour of erythrocytes in two-phase systems has been particularly well-studied and a population of these cells seems to be heterogeneous and to give a somewhat broad distribution because it contains cells of different ages and because there are age-related changes in erythrocyte cell surface properties [5]. However, there has been no explanation for cell surface heterogeneity in a population of rapidly growing cells.

Amoebae of the cellular slime mould *Dictyostelium discoideum* seemed particularly suitable for further studies of the cell surface heterogeneity in a population of cells growing rapidly. These amoebae may be grown in axenic culture when the mean doubling time is 8–9 h [6] and amoebae harvested during exponential growth and partitioned in a dextran–poly(ethylene glycol) two-phase system have been found to give a rather broad distribution [7]. If the amoebae are washed free of nutrients and placed on a solid surface, a period of development is initiated during which the amoebae aggregate and differentiate to give, after about 24 h, the spore and stalk cells of fruiting bodies [8,9]. However, after only 9–11 h development, the cells are still amoeboid and partitioning then divides the cells into two populations [7]. One population has been shown to comprise amoebae largely in the G1-phase of the cell cycle whereas the other comprises amoebae largely in G2-phase [10]. Other studies [11] have also shown that there are few amoebae in S-phase or mitosis at 9–11 h development.

Because amoebae in different phases of the cell cycle during development have different surface properties, it seemed possible that the cell surface heterogeneity in growing amoebae might also be

* To whom correspondence should be addressed

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related to the cell cycle. To investigate this, the location of amoebae in the different phases of the cell cycle in the peak, produced by partitioning amoebae on a countercurrent distribution apparatus, has been determined.

2. EXPERIMENTAL

Amoebae of *D. discoideum* strain Ax-2 were grown at 22°C in HL5 glucose medium [6], harvested during exponential growth when at a density between 1 and 2×10^6 cells $\cdot$ ml^{-1} and washed once with distilled water at 4°C. Countercurrent distribution at 4°C was in the dextran T500–poly (ethylene glycol) 4000 two-phase system described in [7].

Amoebal DNA content was determined in assays where the fluorochrome Hoechst 33258, which binds specifically to DNA to become highly fluorescent, was used [10,12]. Nuclei were counted in amoebae of which the nuclei had been stained with the fluorescent dye 4′,6-diamidino-2-phenylindole [10].

Incorporation of [^{3}H]thymidine (1 mCi) was for 30 min in 70-ml cultures containing about 2×10^6 amoebae per ml HL5 glucose medium. After incorporation of radioactivity, amoebae were washed 5 times with HL5 glucose medium containing 5 mM thymidine at 5°C and once with distilled water at 4°C before partitioning in the two-phase system. After partitioning, amoebae were divided into 4 fractions (fig.1) which were washed and resuspended in distilled water at 5°C and the cell concentrations were determined. Samples were then treated as in [13]. KOH (0.2 M) was added to the fractions to give a final concentration of 0.1 M and the samples were incubated at 37°C for 20 min to hydrolyse RNA. Bovine serum albumin (0.1 ml at 200 μg $\cdot$ ml^{-1}) and calf thymus DNA (0.1 ml at 500 μg $\cdot$ ml^{-1}) were added after each sample had been cooled to 0°C. An equal volume of 20% (w/v) trichloroacetic acid at 0°C was then added to each sample and, after 15 min at 0°C, the samples were filtered through Whatman GF/C glass-fibre filters. Each filter was washed 4 times with 0.5 ml of 10% (w/v) trichloroacetic acid at 0°C and then with 1 ml methanol and 1 ml acetone. The filters were thoroughly dried and were immersed in scintillation fluid (0.2 g 1,4-di(2-phenyloxazolyl)-benzene and 5 g 2,5-diphenyloxazole per l toluene) for determination of radioactivity in a scintillation counter.

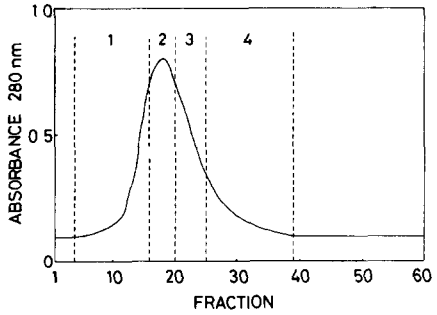

Fig.1. The distribution obtained by partitioning amoebae (harvested during exponential growth) on a countercurrent distribution apparatus was divided, as shown, into 4 fractions.

3. RESULTS

3.1. *Amoebal DNA content*

Table 1 shows the DNA content of amoebae in the 4 pooled fractions from the partitioning profile (fig.1). Amoebae from the leading and trailing edges of the peak had a similar DNA content but the DNA content of amoebae in the centre of the peak was higher. Since amoebae grown in axenic culture tend to be multinucleate [10,14,15], the differences in DNA content could have arisen had

Table 1

DNA content of amoebae and incorporation of [^{3}H]thymidine into amoebal DNA

	Fraction			
	1	2	3	4
DNA content (pg/cell)	0.28	0.38	0.36	0.28
[^{3}H]Thymidine incorporation (cpm per 10^7 amoebae)	2040	1140	1050	750

Amoebae were subjected to partitioning immediately after being harvested from axenic culture (for measurement of DNA content) or after incubation with [^{3}H]thymidine. Each peak produced by partitioning was divided into 4 fractions (see fig.1). Results are given as means for 3 experiments

amoebae with most nuclei been distributed by partitioning preferentially into the centre of the peak but it was found that amoebae in each of the 4 fractions had, on average, a similar number of nuclei (about 1.5 nuclei per amoeba). Hence, amoebae in the centre of the peak must have had a high DNA content because they were in the G2-phase of the cell cycle. Amoebae with lower DNA contents in the leading and trailing edges of the peak must have been in the G1- and S-phases of the cell cycle.

3.2. *Incorporation of [^{3}H]thymidine*

Only amoebae in S-phase of the cell cycle should be active in nuclear DNA synthesis and it should therefore be possible to identify these amoebae by their rate of incorporation of [^{3}H]thymidine into DNA. Amoebae were incubated with [^{3}H]thymidine for a time short in comparison with the duration of S-phase (2.1 h [15]) and were then subjected to partitioning. In agreement with [13,16,17], it was found that only small amounts of [^{3}H]thymidine were incorporated into amoebal DNA but, nevertheless, it was apparent that [^{3}H]thymidine had been incorporated preferentially into the DNA of amoebae in the trailing edge (fraction 1) of the peak produced by partitioning (table 1). Thus, these amoebae were in the S-phase of the cell cycle and the amoebae with a similar DNA content, but in the leading edge of the distribution, must have been in G1-phase.

There was also some incorporation of [^{3}H]thymidine into the DNA of amoebae identified as being in G1- and G2-phases. This was also found in [13] and was attributed to incorporation into mitochondrial DNA which is independent of the cell cycle [18]. When *D. discoideum* amoebae are incubated in [^{3}H]thymidine, the specific activity of mitochondrial DNA is 8–10-times greater than that of nuclear DNA [19] with the result that incorporation of [^{3}H]thymidine into mitochondrial DNA is unusually high in comparison with incorporation into nuclear DNA.

The viabilities of amoebae in the leading and trailing edges of the distribution were found to be similar (about 93% viable cells). There would therefore seem to be no possibility that amoebae in S-phase were evenly distributed throughout the peak but that incorporation of [^{3}H]thymidine by amoebae in the leading edge was poor because this part of the distribution contained fewer viable amoebae than the trailing edge.

4. DISCUSSION

Amoebae of *D. discoideum* in the G1-, S- and G2-phases of the cell cycle were located in different parts of the countercurrent distribution. Amoebae in G1- and S-phases were in the leading and trailing edges, respectively, whereas amoebae in G2-phase were in the centre of the distribution. The location of the small proportion of amoebae in mitosis [11] was not determined. Because partitioning separates cells only on the basis of differences in cell surface properties [1], amoebae in the various phases of the cell cycle must have had different surface properties and thus different plasma membrane structures. The overall distribution of the amoebae must have been made up from at least 3 individual distributions (i.e., one containing amoebae in G1-phase, another containing amoebae in G2-phase and the third containing amoebae in S-phase) and this would account for the distribution being broader than that expected for a homogeneous population of cells. Because the individual distributions overlap, division of the countercurrent distribution into the 4 fractions indicated in fig.1 could not have been expected to give samples each containing amoebae in only one phase of the cell cycle. Fraction 1, containing mainly amoebae in S-phase, would have been contaminated with amoebae in G2-phase as would fraction 4 which was enriched with amoebae in G1-phase. Similarly, although fractions 2 and 3 were enriched with amoebae in G2-phase, there would have been considerable contamination with amoebae in G1- and S-phases. In consequence, the average DNA content per amoeba in fractions 2 and 3 could not be expected to be double that in fraction 4 (table 1), even though amoebae in G2-phase were in fractions 2 and 3 and amoebae in G1-phase were in fraction 4.

Although the surface properties of *D. discoideum* amoebae change as the amoebae progress through the phases of the cell cycle, this is not a process of gradual change. Thus amoebae in G1- and S-phases were at opposite sides of the countercurrent distribution which indicated that amoebae in these two successive phases of the cell cycle had markedly different surface properties. Previous

studies with a few other cell types [20] have also indicated that there may be cell cycle-related changes in cell surface properties and structure, but these studies have all had to make use of cells that could be grown in synchronous culture. The method used to study cell cycle-related changes in the surface properties of *D. discoideum* amoebae has the advantage that it can be used with cells that cannot be grown in synchronous culture and that partitioning is an extremely sensitive method for detecting changes in cell surface properties and structure. The method could therefore be applied to a wide range of cell types and it would then be possible to determine whether the tendency for cell surface structure to change as cells pass through the phases of the cell cycle is a general phenomenon.

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