Analysis of Karyotype and Ploidy of *Dictyostelium discoideum* Using Colchicine-induced Metaphase Arrest

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SUMMARY

Conditions for obtaining colchicine-induced metaphase arrest of the chromosomes of the cellular slime mould *Dictyostelium discoideum* are described. Using this technique, which increases the mitotic index of exponentially growing amoebae from 1.5 to 11%, it has been demonstrated conclusively that *D. discoideum* has a haploid chromosome number of seven and that strain AX2 (ATCC24397) is not highly aneuploid as has recently been suggested. Each of the seven chromosomes can be identified by a characteristic banding pattern seen after staining with Giemsa.

INTRODUCTION

There have been several reports describing the chromosomes of *Dictyostelium discoideum* but confusion still exists as to the exact number of chromosomes in the haploid cell. Wilson (1952, 1953), Wilson & Ross (1957), Sussman (1961) and Brody & Williams (1974) have all suggested that the haploid chromosome number is seven. However, an early study by Bonner & Frascella (1952) proposed that there were only four chromosomes which were made up of seven arms joined by fine connexions, and recently Muroyama *et al.* (1975) have reported that the chromosomal elements seen at mitosis represent the seven arms of only five chromosomes. Muroyama *et al.* (1975) also presented evidence that strain AX2 of *D. discoideum* shows variable chromosome numbers between five and 12 with no more than 25% of the total cell population having the same chromosome number. Since strain AX2 is widely used for biochemical and genetic studies of development, it is important to determine whether it is indeed aberrant in karyotype.

One of the major problems in analysing the karyotype and ploidy of *D. discoideum* has been the low mitotic index of asynchronously dividing cells and this, coupled with the problem of obtaining discrete non-overlapping chromosomes, has made it difficult to examine significantly large numbers of mitotic nuclei. This paper reports a method of arresting the amoebae of *D. discoideum* in metaphase which enables many hundreds of well-separated chromosomes to be scored easily and precisely.

METHODS

Growth. Amoebae of strain AX2 (ATCC24397) were grown axenically in 60 to 70 ml of HL5 medium in 250 ml Erlenmeyer flasks at 22 °C as described by Watts & Ashworth (1970). The amoebae were grown either in medium containing 86 mM D-glucose (G-cells) or in medium with no added sugar (NS-cells).

Counting, sizing and plating efficiency of amoebae. Amoebae were diluted 1:100 with isotonic saline [0.4% (w/v) NaCl for NS-cells and 0.7% (w/v) NaCl for G-cells (Zada-Hames,

unpublished results)] and counted and sized using a model Z_B Coulter counter with a Coulter model P64 size distribution analyser attachment. Latex beads of known size were used for calibration. Plating efficiency was determined as described by Sussman (1966).

Metaphase arrest of amoebae. Immediately before use, colchicine (BDH), was dissolved in axenic medium at a concentration of 40 mg ml⁻¹ and filtered through a Millex disposable filter unit (pore size 0.45 µm). Cells in early-exponential growth (10⁵ to 10⁶ NS-cells ml⁻¹, 5 × 10⁵ to 2 × 10⁶ G-cells ml⁻¹), were diluted 1:1 with different proportions of fresh medium containing colchicine and fresh medium alone to give the desired final concentrations of colchicine.

Fixing and staining of amoebae. Amoebae were washed and fixed as described by Brody & Williams (1974). Half of the slides to be examined were treated with ribonuclease A (type 1-A from bovine pancreas; Sigma; 0.2 mg ml⁻¹ in 0.067 M Sorensen's phosphate buffer, pH 6.8) at 37 °C for 5 min before staining. This eliminated all visible cytoplasmic staining and enabled nuclei to be easily identified and scored. Slides were stained in 10% (v/v) Gurr's Giemsa stain (Improved R66) in 0.067 M Sorensen's phosphate buffer (pH 6.8) for 40 min. Care was taken not to draw the slides through the surface of the stain since on contact with the air a metallic sheen forms on the surface of the stain and this can lead to inferior results. Stain was always poured on to the slides, flushed off quickly at the end of the staining period with running tap water, and the slides were then rinsed briefly with distilled water.

Calculation of mitotic index. Slides were systematically scanned at ×1000 magnification and at least 2000 nuclei per sample were examined. Nuclei in all stages of mitosis from early prophase to late anaphase/telophase were scored as mitotic.

Microscopy and photography. Slides were examined with a Leitz Ortholux microscope using ×1000 magnification and green bright-field illumination. Photographs were taken with a Nikon F camera on Ilford Pan F film which was developed in Ilford Perceptol developer and printed on Ilford Ilfospeed photographic paper, number 5.

RESULTS

Mitotic index of amoebae growing exponentially in the absence of colchicine

When amoebae of *D. discoideum* strain AX2 were fixed and stained as described above, mitotic nuclei could easily be distinguished (Fig. 1*a*). In slides not treated with ribonuclease, interphase nuclei were stained pale pink, cytoplasm pale blue and mitotic chromosomes deep mauve; in ribonuclease-treated cells, the cytoplasmic stain was completely eliminated thus facilitating scoring of nuclei. Both treatments gave the same estimate of mitotic index.

Over 20000 nuclei in cultures of exponentially growing amoebae were examined and the mitotic index was always between 1.5 and 2%.

Effect of colchicine on amoebae

Colchicine at concentrations of 5 to 10 mg ml⁻¹ inhibited cell growth (Fig. 2), and resulted in increased cell volumes (Fig. 2) and size distributions that were frequently bimodal (Fig. 3), as might be expected if cells were being blocked at mitosis. Similar concentrations of colchicine increased the mitotic index from 1.5 to 11% (Fig. 4) without affecting cell viability as measured by plating efficiency. The extent of growth inhibition and metaphase arrest varied slightly depending on the source and batch of colchicine but for routine work a concentration of 20 mg ml⁻¹ added to the cells for 14 to 16 h consistently gave mitotic indices of

Fig. 1. Nuclei and chromosomes of *D. discoideum* AX2 amoebae. (a) Mitotic and interphase nuclei in G-cells. Bar marker represents 10 μm . (b), (c) Typical appearance of mitotic figures in G-cell cultures arrested in metaphase by exposure to 20 mg colchicine ml^{-1} for 15 h: the chromosomes are compact and well-separated. Bar markers represent 2 μm .

Fig. 2

Fig. 2. Effect of colchicine on the growth and mean cell volume of *D. discoideum* AX2 amoebae. NS-cells in the early-exponential phase of growth were exposed to various concentrations of colchicine and after 16 h the percentage increase in cell number (○) and in mean cell volume (●) was determined for each culture.

Fig. 3

Fig. 3. Effect of colchicine on the size distribution of *D. discoideum* AX2 amoebae. NS-cells in the early-exponential phase of growth were exposed to 10 mg colchicine ml^{-1} for 14.5 h: ---, size distribution before addition of colchicine; —, size distribution after exposure to colchicine for 14.5 h.

around 10%. Cells exposed for this length of time showed condensed and well-separated metaphase chromosomes (Fig. 1 b, c) which could easily be counted; longer exposure times did not increase the mitotic index and often resulted in slight deterioration of chromosome structure.

NS-cells were more sensitive to colchicine than G-cells (Table 1) and thus were used for most of this study.

Fig. 4. Effect of colchicine on the mitotic index of *D. discoideum* AX2 amoebae. NS-cells in the early-exponential phase of growth were exposed to various concentrations of colchicine for 16 h and then the mitotic index was determined.

Table 1. *Effect of colchicine on NS-cells and G-cells*

Cells were exposed to 10 mg colchicine ml⁻¹ for 14.5 h and then the mitotic index and percentage increase in cell number and mean cell volume were determined. Control cultures were treated in the same way as those exposed to colchicine (see Methods), but were diluted with fresh medium.

	NS-cells	G-cells
Percentage increase in cell number of control cultures	177	253
Percentage increase in cell number of colchicine-treated cultures	45	115
Percentage increase in mean cell volume of colchicine-treated cultures	53	7
Mitotic index of colchicine-treated cultures	9.1	5.1

Chromosome number of D. discoideum AX2

Chromosome counts were made on cultures grown either in the absence or in the presence of colchicine to ensure that colchicine was not altering the cell chromosome complement. The results were the same, although it was far more difficult to count chromosomes in cells not exposed to colchicine due both to the scarcity of mitotic nuclei and to the much higher incidence of chromosome overlap. The haploid chromosome number of *D. discoideum* AX2 was found to be seven (Figs 1*b*, *c* and 6*c*) and more than 70% of amoebae contained this number (Fig. 5). Up to 25% of the cells contained six or fewer chromosomes. Aneuploid amoebae with all possible chromosome numbers between seven and 14 were observed but these represented only 2 to 7% of the total. Diploids were seen at a frequency of up to 2%.

At metaphase the chromosomes were arranged radially in a ring, suggesting that they were telocentric, and fine connexions which joined either all (Fig. 6*a*) or some (Fig. 6*b*, *d*, *e*, *f*,) of the chromosomes could sometimes be seen. However, most mitotic nuclei showed no such interchromosomal connexions and all seven chromosomes could be seen as discrete units (Figs 1*b*, *c* and 6*c*).

The sizes of the individual chromosomes varied from metaphase to metaphase and so could not be used as a means of identification. However, in some instances distinctive banding patterns were visible along the chromosomes enabling each to be positively identified (Fig. 6*c*). Although not all banded metaphases showed the same degree of banding it was usually possible to recognize most chromosomes, chromosomes 1, 2, 5 and 7 being the most constant in appearance. Figure 6 shows haploid chromosome sets in which the

Fig. 5. Distribution of chromosome numbers in *D. discoideum* AX2 amoebae. NS-cells in the early-exponential phase of growth were exposed either to 20 mg colchicine ml⁻¹ for 16 h and 558 mitotic nuclei were scored (open bars), or to 10 mg colchicine ml⁻¹ for 16 h and 212 mitotic nuclei were scored (closed bars).

chromosomes have been identified and numbered. Since no two chromosomes are constantly connected or adjacent to one another, they cannot represent the arms of a single chromosome.

DISCUSSION

All previous studies on the chromosomes of *D. discoideum* have used asynchronously growing cultures in which the low mitotic index has made it difficult to examine large numbers of mitotic nuclei. This paper reports a method of arresting amoebae at metaphase which produces readily-countable, well-separated, condensed chromosomes and therefore allows many hundreds of mitotic nuclei to be scored on a single slide. The values presented here for mitotic indices may be slightly exaggerated, since multinucleate cells are present at a level of 20 to 30% in axenic cultures (Zada-Hames, unpublished results) and the method of scoring mitoses counts each nucleus and not each cell. However, axenically growing cultures contain the same proportions of multinucleate cells whether grown in the absence or presence of colchicine and thus the comparison of mitotic index between the two is valid. When slides are prepared to demonstrate intact cells it is difficult to score mitotic nuclei unambiguously owing to lack of chromosome spreading.

From the evidence presented in this paper it is concluded that the haploid chromosome number of *D. discoideum* is seven and that each chromosome can be identified by its distinctive banding pattern. A recent study by Moens (1976) has shown that there are seven pairs of kinetochores in haploid cells of *D. discoideum* which lends support to this conclusion. It has also been shown here that strain AX2 is not highly aneuploid since more than 70% of the amoebae in any population have seven chromosomes. The recent report of Muroyama *et al.* (1975) that the chromosome number of *D. discoideum* is five and that strain AX2 shows extremely variable chromosome numbers was presumably due to difficulty in obtaining well-separated unconnected chromosomes for counting and the rather small size of their

Fig. 6. Haploid chromosome sets of *D. discoideum* AX2 amoebae. (The cell type, colchicine concentration and exposure time are noted in parentheses.) (a) A late metaphase figure showing all the chromosomes joined in a ring (G-cells, 20 mg ml⁻¹, 15 h). (b) A late metaphase figure showing a fine connexion between only two of the chromosomes (G-cells, 20 mg ml⁻¹, 15 h). (c) An early metaphase figure showing distinctive banding along each chromosome; each chromosome has been assigned a number. No interchromosomal connexions are evident (G-cells, 5 mg ml⁻¹, 15 h). (d), (e), (f), (g), Early metaphase figures showing connexions between some of the chromosomes, banding and random arrangement of chromosomes. The chromosomes have been numbered according to identification based on (c) (all from G-cells, 20 mg ml⁻¹, 15 h). All bar markers represent 2 µm.

sample (62 cells). Although up to 25% of the mitotic nuclei have six or fewer chromosomes, these are probably the result of chromosome overlap (especially in the case of those with six chromosomes) or chromosome loss during slide preparation. Indeed, these cultures give plating efficiencies of greater than 95% which suggests that the proportion of cells with less than the haploid chromosome number is rather small. Aneuploid amoebae with chromosome numbers between seven and 14 presumably represent transient intermediates in the process of haploidization of diploid nuclei (Sinha & Ashworth, 1969; Brody & Williams, 1974).

The data presented here indicate that diploids are found at a frequency of no more than 2%. This agrees well with the studies of Sussman & Sussman (1962) and Brody & Williams (1974) who concluded that the frequency of diploids in *D. discoideum* was no greater than 1%. Sackin & Ashworth (1969) have reported that diploids constitute 8% of the total population in strain NC4 but they based their conclusion on an analysis of spore size distribution and thus their estimate may not be as accurate as those based on direct examination of mitotic figures. The diploids seen in cultures of *D. discoideum* probably arise spontaneously but whether they occur as a result of cell fusion followed by karyogamy (Wilson & Ross, 1957; Huffman & Olive, 1964; Fukui, 1974; Fukui & Miyake, 1975) or by failure of cytokinesis following nuclear division is currently being investigated.

Note added in proof. Robson & Williams [*Journal of General Microbiology* (1977) **99**, 191–200], using release from starvation conditions to increase mitotic index, have also shown the haploid chromosome number of *D. discoideum* to be seven and presented a karyotype based on Giemsa banding in which all seven chromosomes can be identified. Although there are minor differences in the banding reported in this paper and that of Robson & Williams, we have agreed upon a standardized scheme of chromosome numbering and thus the chromosomes described in one paper can be directly correlated to those in the other.

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