Examination of the Chromosomes of *Polysphondylium pallidum* Following Metaphase Arrest by Benzimidazole Derivatives and Colchicine

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(Received 21 March 1979)

Techniques used previously to examine the mitotic chromosomes of *Dictyostelium discoideum* have been applied to the cellular slime mould *Polysphondylium pallidum*. Mitosis in *P. pallidum* is similar to that in *D. discoideum* except that a metaphase plate configuration is rare and that it has more chromosomes (n = 11 or 12) than *D. discoideum* (n = 7). Some metaphase arrest (mitotic index 14%) was achieved in *P. pallidum* when colchicine was used at a high concentration (10 mg ml⁻¹). Low concentrations of benzimidazole derivatives (thiabendazole, cambendazole, ben late and nocodazole) caused the arrest of cell division, mitotic indices greater than 50%, chromosome doubling and lowered plating efficiency. Growth of *P. pallidum* in axenic medium was investigated.

**INTRODUCTION**

*Polysphondylium pallidum* is a cellular slime mould with a branched, 'christmas tree'-shaped, asexual fruiting body. In this respect its morphogenesis is different from that of the cellular slime mould *Dictyostelium discoideum*, which has an unbranched fruiting body. Because the formation of the asexual fruiting body is neither essential for growth nor for genetic analysis, this morphogenetic process can, in principle, be examined genetically in the cellular slime moulds. For this reason attempts have recently been made to establish genetic systems in both *D. discoideum* (Newell, 1978) and *P. pallidum* (Francis, 1975). Mitosis in *D. discoideum* has been examined both ultrastructurally (Moens, 1976) and by light microscopy (Brody & Williams, 1974), and the chromosome number (n = 7) has been established (Robson & Williams, 1977; Zada-Hames, 1977). By contrast, no detailed reports of mitosis in *P. pallidum* have been published, although there has been an ultrastructural study of mitosis in a morphologically similar species, *Polysphondylium violaceum* (Roos, 1975). This paper reports a light microscopic examination of mitosis in *P. pallidum* and the determination of the chromosome number of two isolates. A mitotic index in excess of 50% was achieved using the benzimidazole derivatives thiabendazole, cambendazole, ben late and nocodazole.

**METHODS**

Strains and culture of amoebae of *P. pallidum*. Two isolates of *P. pallidum* were used in this study. PPHU8 is an axenic derivative of PN600 (formerly called Pp6; Francis, 1975) isolated in this laboratory. ANU122 was isolated from leaf litter in the New England National Park, N.S.W., Australia (Robson, 1978) using an adaptation of the method of Cavender & Raper (1965). The strains were stored desiccated on silica gel at 4 °C (Williams & Newell, 1976). When being used for experiments, *P. pallidum* was grown in the light on one-fifth strength SM agar (Sussman, 1966) or on the same medium containing 5 g charcoal l⁻¹ (Ajax activated decolourising powder). Fruiting body formation was enhanced on the charcoal-containing medium. Most studies were conducted on amoebae grown axenically in the medium of Watts & Ashworth (1970), containing

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Fig. 1. Axenic growth of *P. pallidum* amoebae. Flasks were inoculated with spores, each releasing a single amoeba on germination, of ANU122 (○), PN600 (●) and PPHU8 (●). PPHU8 is an axenic strain derived from strain PN600 as described in the Results.

(per litre): Oxoid bacteriological peptone, 14.3 g; Oxoid yeast extract, 7.15 g; glucose, 15.4 g; Na₂HPO₄, 12H₂O, 1.28 g; KH₂PO₄, 0.48 g; dihydrostreptomycin sulphate, 250 mg. The pH was adjusted to 7.0 with NaOH and the medium was autoclaved for 15 min at 121 °C, after which the pH fell to 6.5.

**Results**

**Axenic growth of *P. pallidum***

For convenience, the axenic medium used in this laboratory to grow axenic strains of *D. discoideum* was also used for *P. pallidum*. All four isolates of *P. pallidum* which have been tested (two of which are not reported here) grew in this medium, although there was a lag before growth commenced (Fig. 1, strains PN600 and ANU122). When axenically grown cells were grown on bacteria for 20 to 30 generations and then returned to axenic medium, this lag was not observed, as is shown for PPHU8, the axenic derivative of PN600, in Fig. 1. Axenic strains obtained from both isolates of *P. pallidum* grew with a doubling time of 7 to 12 h and had a stationary phase in excess of 10⁷ cells ml⁻¹ after several passages. Axenically grown amoebae of *P. pallidum* comprised a mixture of uninucleate cells and multinucleate cells, which contained variable numbers of nuclei; for example, the amoeba shown in Fig. 2(a) has 20 nuclei.

**Mitosis and chromosome numbers in *P. pallidum***

In general, interphase nuclei of *P. pallidum* were more intensely stained than those of similar preparations of *D. discoideum*, so that trypsin treatment to prevent cytoplasmic staining (Robson & Williams, 1977) was usually unnecessary. *Polysphondylium pallidum*
Fig. 2. (a) Multinucleate cell of axenically grown *P. pallidum* strain PPHU8 which contains 20 nuclei. Metaphase plate-like configuration in (b) *P. pallidum* strain PPHU8 (left, difficult to count the chromosomes) and strain ANU122 (right) and (c) *D. discoideum* strain AX3. (d) Condensed metaphase chromosomes and early metaphase chromosomes (11) of *P. pallidum* strain PPHU8. Early metaphase preparations of (e) PPHU8 and (f) ANU122 showing 11 and 12 chromosomes, respectively. (g) Two diploid metaphase in *P. pallidum* strain ANU122 treated for 23.5 h with thiabendazole at 10 μg ml⁻¹ in axenic medium; both nuclei contain 24 chromosomes. Bar markers represent 10 μm in (a) and 2 μm in (b to g).

Chromosomes became apparent during prophase and by metaphase they were very condensed. Only rarely were the chromosomes seen arranged in a flat ring at metaphase (Fig. 2b). This contrasts with metaphase in *D. discoideum* where chromosomes arranged in a ring on the metaphase plate are commonly observed (Fig. 2c). There was a tendency for *P. pallidum* chromosomes to disperse upon air drying, or alternatively for metaphase chromosomes to overlap (Fig. 2d), making accurate counts difficult. Careful analysis of well-spread late prophase–early metaphase nuclei indicated that PN600 (and PPHU8, the axenic derivative of PN600) have 11 chromosomes (Fig. 2e) and ANU122 has 12 chromosomes (Fig. 2f).

**Metaphase arrest in *P. pallidum***

The mitotic index of exponential phase axenic cultures of PPHU8 and ANU122 varied between 1.1 and 3.2% with a mean of 1.9% (four experiments) for ANU122 and 2.7% (four experiments) for PPHU8. To see whether colchicine was effective at inducing metaphase arrest, axenic cultures of strain PPHU8 were incubated for 16.5 h with colchicine at 10 mg ml⁻¹. At this concentration there was little or no increase in cell number. The mitotic indices of two such colchicine-treated cultures were 12.9% and 14.9%, respectively. The
mitotic index was not increased above control values when colchicine was used at 1 mg ml\textsuperscript{-1}. As a comparison, cells of strain PPHU8 treated with ben late at 100 \(\mu\)g ml\textsuperscript{-1} for 16.5 h gave mitotic indices of 57.6\% and 45.2\% in two experiments.

Since benzimidazole derivatives seemed to be much more potent antimitotic agents than colchicine, axenically grown cells of strain ANU122 were exposed to concentrations of thiabendazole (10 \(\mu\)g ml\textsuperscript{-1}), cambendazole (50 \(\mu\)g ml\textsuperscript{-1}), ben late (100 \(\mu\)g ml\textsuperscript{-1}) and nocodazole (7 \(\mu\)g ml\textsuperscript{-1}) which prevented an increase in cell number. The treated cells were sampled at intervals to determine the effect of these compounds (Fig. 3). All four benzimidazoles produced extremely high mitotic indices in ANU122, between 40 and 65\% at the time of maximum effect (Fig. 3). There was a short lag before ben late increased the mitotic index (Fig. 3\(d\)); thiabendazole, cambendazole and nocodazole were all effective within 5 h after the cells were exposed to them (Fig. 3\(a\), \(b\), \(c\)). In a preliminary examination of the plating efficiency and diploid formation in amoebae treated for 23.5 h, controls gave 100\% plating efficiency and all colonies were haploid. By contrast, treatment of cultures with thiabendazole, cambendazole, ben late and nocodazole resulted in low plating efficiencies (between 4 and 30\%) and at least 40\% of the colonies were diploid. Diploid and aneuploid metaphases were observed cytologically in cultures treated with a benzimidazole for 23.5 h (Fig. 2\(g\)).

**DISCUSSION**

Of all the cellular slime moulds, *P. pallidum* grows most readily in axenic culture. Whereas axenic media for some strains of *P. pallidum* were described in 1963 (Hohl & Raper, 1963; Sussman, 1963), a simple axenic medium, which supports the growth of certain mutants of *D. discoideum* (Williams et al., 1974; Williams, 1976), was not described until 1970 (Cocucci & Sussman, 1970; Watts & Ashworth, 1970; Loomis, 1971). There are no reports of sus-
tained growth of other cellular slime moulds on a simple axenic medium, although *P. violaceum* has recently been grown axenically (unpublished). The finding that all the isolates of *P. pallidum* tested grow after a lag in the axenic medium of Watts & Ashworth (1970) confirms that *P. pallidum* is easier to grow axenically than other cellular slime moulds. [Compare Fig. 1 with Fig. 1 of Williams (1976), where new axenic strains of *D. discoideum* were isolated.] However, it is likely that the axenic growth of *P. pallidum* requires at least one mutation, since strains which were obtained after axenic growth and then passaged on bacteria grew immediately upon re-inoculation into axenic medium; that is, the axenic strains appear to be genetically different from their parents. Recent studies on *D. discoideum* suggest at least three mutations are involved with rapid axenic growth in this species (North & Williams, 1978).

The axenically grown *P. pallidum* amoebae examined here contained multinucleate cells, thus exhibiting similar behaviour to *D. discoideum* amoebae grown in this medium (Robson & Williams, 1977; Zada-Hames & Ashworth, 1978; Sameshima et al., 1978). *Polysphondylium pallidum* strain WS320 had multinucleate cells when grown in a different axenic medium (Hohl & Raper, 1963), suggesting that multinucleate cells may be a feature of axenic cultures of cellular slime moulds.

By light microscopy, mitosis in *P. pallidum* resembles that of *D. discoideum*, with a few exceptions. The evidence for a metaphase plate in *P. pallidum* is not strong (Fig. 2b), whereas *D. discoideum* metaphase chromosomes are commonly observed in a tight ring (Fig. 2c). These findings are consistent with studies on the ultrastructure of mitosis in *P. violaceum* (Roos, 1975) and *D. discoideum* (Moens, 1976), where the absence of a metaphase plate in *P. violaceum* and the presence of this chromosomal arrangement in *D. discoideum* were reported. My finding that chromosomes of *P. pallidum* are more easily lost on spreading may reflect less secure binding of *Polysphondylium* chromosomes to the spindle than that observed for *D. discoideum*. Ultrastructural studies reveal that each kinetochore of *D. discoideum* has several microtubules attached to it, whereas only a single microtubule arises from each kinetochore of *P. violaceum* (Roos, 1975; Moens, 1976).

Since colchicine was effective only at high concentrations in *P. pallidum*, its action was similar to that found in *D. discoideum* (Zada-Hames, 1977). The antimitotic benzimidazole compounds tested here—thiabendazole, cambendazole, ben late and nocodazole—used at the same concentrations as with *D. discoideum* gave similar results (Welker & Williams, 1980). The only exception was that ben late was effective at metaphase arrest and chromosome doubling in *P. pallidum*, whereas it was ineffective at metaphase arrest and was only partially effective as a chromosome doubling agent in *D. discoideum* (Welker & Williams, 1980).

Our studies emphasize the need for caution when estimating chromosome numbers in the cellular slime moulds. Use of the benzimidazole derivatives produces large numbers of mitotic figures, but care must be taken to examine well-spread early metaphase chromosomes or the chromosome number may be underestimated. It is also important to use short incubation times with the benzimidazole derivatives, as large numbers of aneuploid and diploid metaphases are produced on prolonged incubation (Welker & Williams, 1980). In order to determine the chromosome number of *P. pallidum* with certainty, both karyotype analysis (Robson & Williams, 1977) of those *P. pallidum* isolates examined so far, and further studies on different isolates are needed. There are clearly more chromosomes than in *D. discoideum* (*n* = 7) and the number is likely to be *n* = 11 or 12; possibly the 11 chromosome isolates have two chromosomes fused.

While tentative chromosome numbers have been given for several species of cellular slime moulds (Wilson & Ross, 1957; Bonner, 1967; Muroyama et al., 1975) these must now be considered to be unreliable. *Dictyostelium discoideum* (*n* = 7) is the only cellular slime mould whose chromosome number is established (Wilson, 1953; Brody & Williams, 1974; Robson & Williams, 1977; Zada-Hames 1977). However, the techniques outlined in this and other
papers (Cappuccinelli et al., 1979; Welker & Williams, 1980) show that reliable estimates of chromosome numbers in cellular slime moulds are now possible.

There are several characteristics on which species of cellular slime moulds fall into two classes, Dictyostelium-like and Polysphondylium-like, for example, the chemotactic agent used (Wurster et al., 1976) and the distribution of spore granules (Traub & Höhl, 1976). Since P. violaceum appears to have a similar number of chromosomes to P. pallidum (unpublished) it will be interesting to see whether cellular slime moulds can also be divided into the same classes on the basis of chromosome number, Dictyostelium-like species having 7 chromosomes and Polysphondylium-like species having 11 or 12 chromosomes.

I thank Gill Robson and Peter Fokker, who did some of the preliminary experiments, and Drs H. Marchant, E. Smith and D. Welker for comments on the manuscript. I thank Dr D. Francis for the gift of strain PN600 and William Bliss for the gift of thiabendazole and cambendazole.

REFERENCES


