SHORT COMMUNICATION

Immunofluorescence of Microtubular Structures During the Cell Cycle of *Dictyostelium discoideum*

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Microtubules and microtubule-organizing centres of *Dictyostelium discoideum* were investigated using indirect immunofluorescence with antibodies against a microtubule-associated protein from a rabbit preimmune serum. The microtubule system showed a cell cycle dependent variation. The cytoplasmic microtubule network present in interphase disappeared in mitosis, in contrast to the astral fibres and central spindle fibres which remained evident. In both cell cycle stages microtubules radiated from microtubule-organizing centres which corresponded to the nucleus-associated organelles in interphase and the spindle pole bodies in mitosis.

INTRODUCTION

Microtubular structures can be visualized by immunofluorescence using specific antibodies from animals injected with purified antigens (Fuller *et al.*, 1975; Sherline & Schiavone, 1977; Connolly *et al.*, 1977) or using preimmune sera from untreated animals (Karsenti *et al.*, 1977; Connolly & Kalnins, 1978; Maunoury, 1979). In *Dictyostelium discoideum*, antibodies against purified mammalian brain tubulin cross-react with tubulin of growing amoebae (Cappuccinelli *et al.*, 1978). Such antibodies can be used to stain the tubulin system in interphase cells of *D. discoideum* and related organisms (Unger *et al.*, 1979). Similar results can be obtained using a rabbit preimmune serum thought to contain antibodies against a component of *D. discoideum* microtubules.

In this paper we report the use of a preimmune serum to demonstrate the microtubular pattern of mitotic cells. In addition to staining the microtubules it is possible to visualize some of the microtubule-organizing centres such as nucleus-associated organelles in interphase and spindle pole bodies in division stages. Kinetochores, however, could not be seen.

METHODS

Organism and culture conditions. Myxamoebae of *D. discoideum* (strain Ax2, haploid) were grown in axenic culture at 22 °C in HL-5 medium containing 86 mM-glucose (Watts & Ashworth, 1970). Cells were collected during the exponential phase of growth, attached to polylysine-coated slides (Clarke *et al.*, 1975) and allowed to spread for at least 30 min.

Indirect immunofluorescence. Preimmune serum containing anti-microtubule antibodies was obtained from an untreated female New Zealand rabbit (age 18 months). The γ -globulin fraction was purified by ammonium sulphate precipitation (Harboe & Ingild, 1973) and used at a final concentration of 1 to 2 mg ml⁻¹.

Immunofluorescence staining was performed as previously described (Unger *et al.*, 1979). Briefly, the attached cells were fixed with formaldehyde, treated with methanol and acetone, incubated with the antibody solution and stained with fluorescein isothiocyanate-conjugated anti-rabbit- γ -globulin antibodies (FITC-AB, Behring-Werke, Hamburg, F.R.G.). In order to reduce non-specific binding, FITC-AB were incubated overnight with an acetone powder of *D. discoideum*, before use for staining. Photographs were taken on Kodak Tri-X Pan film, developed in Microdol-X and printed on Orwo extrahard paper.

The following controls were performed: (a) incubation with FITC-AB without pretreatment with anti-microtubule antibodies; (b) incubation with FITC-AB after treatment with a rabbit serum that did not contain anti-microtubule antibodies; (c) cold treatment (0 °C for 30 min) before complete staining; (d) microtubule poisoning with nocodazole (7 $\mu\text{g ml}^{-1}$ for 12 h at 22 °C) or thiabendazole (10 $\mu\text{g ml}^{-1}$ for 12 h at 22 °C) before complete staining.

RESULTS

In interphase and during division a typical pattern of fluorescent fibres was visualized using preimmune serum antibodies (Fig. 1). Interphase microtubules radiated from cytocentres that corresponded to nucleus-associated organelles and extended into the cytoplasmic space (Fig. 1*a*). Frequently the fluorescent nucleus-associated organelles had the appearance of rings in both normal interphase cells and in cells incubated at low temperatures or treated with microtubule inhibitors (Fig. 1*a, b, c*, arrows). Usually only a few microtubules were present in pseudopods. After cold treatment or microtubule poisoning with thiabendazole or nocodazole most of the fibres disappeared and only the nucleus-associated organelles with short microtubules radiating from them could be found (Fig. 1*b, c, d*). Low temperatures and the inhibitors used are known to destroy microtubules in mammalian cells, *D. discoideum* and some other micro-organisms (De Brabander *et al.*, 1976; Cappuccinelli & Ashworth, 1976; Girbardt, 1979; see also Dustin, 1978, for additional references). It may be concluded that the stained fibres were microtubules. Using immunochemical methods it has been shown that the microtubular component bound by the antibodies is not tubulin itself but another microtubular protein which can be considered a microtubule-associated protein (unpublished results).

At the onset of mitosis the cytoplasmic microtubule network disappeared. The nucleus-associated organelle split and two fluorescent spindle pole bodies were seen connected by a short bar (Fig. 1*e, f, g*). During the nuclear division the spindle pole bodies gradually separated and the connecting microtubular bundle was observed as a sharp small line (Fig. 1*g-l*). In multinucleate cells these processes occurred synchronously for all the nuclei. The fluorescence was more evident in the middle region of the central spindle (Fig. 1*h, k*) at spindle lengths between 3 and 11 μm , representing early anaphase to telophase states (Moenes, 1976). In addition to the fluorescent line connecting the spindle poles, only a few short microtubules radiating from the pole into the nucleus and cytoplasm were seen. At the end of mitosis, when cell separation takes place, the fluorescent line progressively disappeared and cytoplasmic microtubules radiating from nucleus-associated organelles became increasingly evident (Fig. 1*l*). At this point of the cell cycle the fluorescent mid-body characteristic of this stage for higher cells could not be seen.

DISCUSSION

In interphase cells the microtubule network revealed by preimmune serum antibodies against a microtubule-associated protein(s) of *D. discoideum* was identical to that obtained after staining with anti-tubulin antibodies (Unger *et al.*, 1979). However, rabbit preimmune γ -globulins were preferred in this study because they gave a lower background and a higher resolution of the microtubule complex.

Microtubule patterns showed marked differences during the cell cycle. The extensive network of cytoplasmic microtubules disappeared at the onset of mitosis during which only

Fig. 1. (a–d). Immunofluorescence staining of the microtubule network in an interphase multinucleate amoeba of *Dictyostelium discoideum* (a), and the disappearance of the cytoplasmic microtubules after cold treatment (b) or treatment with nocodazole (c) or thiabendazole (d). Arrows indicate nucleus-associated organelles with a ring-like appearance.

(e–l). Immunofluorescence of the mitotic apparatus of *D. discoideum* amoebae at different stages of mitosis: metaphase (e, f); anaphase (g, h); telophase (i, j); late telophase stage (k); nearly separated cells (l). The mitotic stages were estimated from the distance between the spindle pole bodies (Moens, 1976). All the bar markers represent 5 μ m.

the central spindle and some short pieces of astral microtubules were evident. The disappearance of cytoplasmic microtubules may have been due to their disassembly at the beginning of mitosis, as occurs in mammalian cells (Weber *et al.*, 1975), or to a redistribution of the protein(s) that bind the preimmune serum. The electron microscopic results of Moens (1976) indicate the persistence of cytoplasmic microtubules during *D. discoideum* mitosis. However, these results must be interpreted with caution because they were not obtained by a quantitative technique. Microtubules radiated from centres, termed nucleus-associated organelles, in interphase and spindle pole bodies in mitosis. These organelles represent different functional states of a microtubule-organizing centre in progressive stages of the cell cycle. As shown in *D. discoideum* (Moens, 1976) and in the related slime mould *Polysphondylium violaceum* (Roos, 1975), ultrastructural changes occur in these microtubule-organizing centres during the cell cycle.

The fluorescence of nucleus-associated organelles was not homogeneous. The rings that could be seen in normal interphase cells and in interphase cells after microtubule poisoning or cold treatment probably represent fluorescent material arranged at the surface of a sphere or a cylinder. Interphase nucleus-associated organelles of *D. discoideum* are cylindrical with dense granules at the surface as seen by electron microscopy (Moens, 1976). It is probable that these granules contain material that binds the preimmune antibodies. Such ring-like fluorescent structures were not seen in the spindle pole bodies of division stages. This may suggest that the arrangement of the structural components containing the binding sites for preimmune serum changes during spindle pole body formation.

Electron microscope observations indicate that the spindle of *D. discoideum* contains a small bundle of pole-to-pole microtubules connecting spindle pole bodies with kinetochores (Moens, 1976). In our experiments, however, kinetochores could not clearly be identified. This may be due to the absence of the protein target for the antibodies, thus suggesting a difference in their chemical composition from that of other similar fibres. Pole-to-pole directed microtubules that were evident after separation of the spindle pole bodies had the appearance of a continuous line with increased fluorescence in the middle. The area of increased fluorescence disappeared at cell separation and can be interpreted as the region in which pole-to-pole directed microtubules overlap, in accordance with the ultrastructural results of Moens (1976). The decreased fluorescence of the central spindle near to the spindle pole bodies may also be due to a shadowing effect by the chromosomes condensed in later division stages in the vicinity of the spindle pole.

In the electron microscope, the kinetochores appear as distinct structures with a diameter of about half a spindle pole body. However, they could not be clearly identified by immunofluorescence. This may depend on experimental conditions, a difference in the molecular composition, or on the concentration of identical proteins in these two kinds of microtubule-organizing centres. The molecular structure of these microtubule-organizing centres has not yet been analysed and at present it is not possible to draw any conclusion.

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