INTRANUCLEAR MICROTUBULE ORGANIZING CENTER IN EARLY PROPHASE NUCLEI OF THE PLASMODIUM OF THE SLIME MOLD, *PHYSARUM POLYCEPHALUM*

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INTRODUCTION

The nuclei of the plasmodial phase of the true slime mold (myxomycetes) perform intranuclear mitosis which is *not* accompanied by centrioles, while those of the myxamoebae exhibit an astral type of mitosis with centrioles and nuclear envelope breakdown (2). In fungal mitoses of centriole-lacking styles, the microtubules composing the metaphase spindle arise, in most instances, in the vicinity of variously shaped microtubule organizing centers (MTOC) (13) or spindle pole bodies (1). These may take the form of circular or rectangular, electron-opaque, layered plaques as in yeasts and mycelial ascomycetes (14, 17), or vaguely textured spherical structures repeatedly described in basidiomycetes (5, 10, 11).

No such structure has been described in myxomycetes but recently one has been found, surprisingly, deep inside prophase nuclei of *Physarum* (15). The present study provides independently made observations confirming the occurrence of this novel type of MTOC in *Physarum*.

MATERIALS AND METHODS

Cultures of *Physarum polycephalum*, kindly supplied by Professor J. Ohta, Ochanomizu University, Tokyo, were maintained plasmodia on sterile, semidefined medium according to the method of Daniel and Rusch (3), slightly modified by Okada and Ohta (12). Surface plasmodia were obtained by the fusion of microplasmodia growing on filter paper (TOYO No. 4, Toyo Roshi Co. Ltd., Tokyo) supported by a layer of glass beads in a Petri dish according to Guttes and Guttes (6). The nuclei of plasmodia divide in synchrony and the course of mitosis can be reconstructed from samples taken at closely spaced intervals. Nuclear behavior in the growing plasmodium was moni-

tored by examining small fragments of plasmodium by phase-contrast microscopy. As soon as plasmodial nuclei were seen to be entering division, pieces of plasmodium were removed at intervals of 2–4 min and fixed in 3% glutaraldehyde in 0.1 M cacodylate buffer at pH 7.2 containing 0.005 M calcium chloride. After overnight washing, samples were postfixed with 1% OsO₄ in the same buffer, and were soaked in 0.5% aqueous uranyl acetate. Dehydration was carried out in a graded acetone series. It was followed by soaking in methyl methacrylate and embedding in Vestopal W (Madame Martin Jaeger, Geneva, Switzerland) (9). Sections were cut with glass knives on a Porter-Blum MT-2 ultramicrotome and stained with uranyl acetate followed by Reynolds' lead citrate. Specimens were viewed in a JEM 7A electron microscope at 80 kV.

RESULTS

During interphase the nucleolus lies in the center of the nucleus, and the chromosomes are near the periphery. Under the phase-contrast microscope the onset of mitosis can be recognized by enlargement, loss of density, and fragmentation of the nucleolus and its removal to an eccentric position inside the nucleus. Fig. 1 shows a section of a nucleus in early prophase. The nucleolus is still compact but has already taken up an off-center position. In the center of the nucleus, occupying a depression in the nucleolus, an amorphous osmophilic zone, 0.3–0.5 μ m in diameter, can be seen from which bundles of microtubules are diverging. This region will, tentatively, be referred to as MTOC. Fig. 1 provides a median section of it and shows it set off from the matter of the nucleolus by a band of low density some 500 Å wide. Figs. 2–4 show two sections chosen from a long series of closely spaced sections of another prophase nu-

FIGURE 1 Section of a prophase nucleus. Microtubules (*MT*) diverge from the organizing center (*OC*) which rests in a depression of the nucleolus (*NC*). Chromosomes (*CH*) are randomly scattered throughout the nucleus. $\times 37,500$.

cleus. The nucleolus of this nucleus has begun to disintegrate and appears as a loose aggregate of clumps of dense material. The chromosomes appear as dense patches of fibrous elements scattered through the rest of the nucleoplasm (Figs. 1 and 2). Highly enlarged views of the MTOC, sectioned in two different planes, are provided by Figs. 3 and 4. Broad bundles of microtubules diverge from the region of the MTOC. They traverse the plane of the section at slightly different angles to their long axes. There is a suggestion that the microtubules are held together by dense, amorphous cementing material. Bundles diverge in many directions from their geometrical origin at the MTOC but nowhere penetrate deeply into the nucleolus.

Within minutes of the early prophase stage described above, the greatly enlarged nucleolus

begins to break up into numerous fragments which are distinguishable from the chromosomes only by the presence inside them of characteristic electron-opaque granules (8). Microtubules which earlier on were seen diverging from a single dense, central focus are now arranged in the double cone of the familiar metaphase spindle, with tubules diverging from two diametrically opposed points close to the inner leaf of the nuclear envelope (Fig. 5). No plaques or other forms of MTOC were observed at the poles of the spindle, in accord with the observations of earlier students of plasmodial mitosis (2, 7, 15).

DISCUSSION

The present independent observations on the intranuclear MTOC in the early opportunistically prophase confirm the recent findings of Sakai and Shi-

FIGURE 5 Late prophase nucleus. The chromosomes (*CH*) are about to arrange themselves on the metaphase plate between two diametrically opposed cones of diverging microtubules (*MT*). $\times 35,000$.

genaga (15), who referred to this structure as the primordium of spindle microtubules and discussed it with regard to the intranuclear mitosis in their review of the earlier studies on plasmodial mitosis.

The pattern of bundles of microtubules diverging from a dense granular focus in *Physarum* bears some resemblance to the vast arrays of microtu-

bules diverging from an intracytoplasmic "centroplast" in the heliozoan *Raphidiophrys*, recently described and strikingly illustrated by Tilney (16). It is, in fact, this similarity which makes identification of the intranuclear focus in *Physarum* as an MTOC more plausible than the opposite view of its being a center of convergence of microtubules

FIGURE 2 No. 5 of a series of 19 consecutive sections of another prophase nucleus. The nucleolus (*NC*) is less dense than that of the nucleus of Fig. 1. In the center of the nucleus and partly surrounded by nucleolar material lies the MTOC. $\times 8,000$.

FIGURE 3 A more highly enlarged view of the MTOC of Fig. 2. Bundles of microtubules (*MT*) are seen diverging from the MTOC. *OC*, organizing center. $\times 90,000$.

FIGURE 4 Section no. 8 of the nucleus of Figs. 2 and 3. It is a glancing section of the MTOC which seems to be composed of randomly oriented short tubules, probably representing oblique sections of tubules which at this level are not yet aligned in diverging bundles. *MT*, microtubules; *OC*, organizing center. $\times 90,000$.

arising randomly at the periphery of the nucleus, which would be equally reasonable on the grounds of geometry alone. On the evidence presented as well as the observations suggesting the division of MTOC (15), it is probable, though it has not been proven, that the diverging microtubules of early prophase are later used in the assembly of the metaphase spindle. How this is done (if indeed, it is done) and whether all of the numerous prophase tubules are used for this purpose remains to be elucidated. In the course of his stimulating comprehensive review of past and present ideas on the origin, nature, and function of centrioles, Fulton (4) rightly concludes that it is unlikely that any of the claims of the existence of intranuclear centrioles in the older literature will be confirmed by modern investigators. The recent discovery of a strange, near crystalline spindle precursor inside micronuclei by Hauser (8) and of an intranuclear MTOC in *Physarum* by Sakai and Shigenaga (15) and the confirmation of these latter findings by the present writer suggest, however, that the occurrence of *noncentriolar* intranuclear spindle organizers need no longer be discounted.

The author extends thanks to Dr. C. F. Robinow at the University of Western Ontario, Canada, for help with the manuscript. He is also grateful to Dr. J. Ohta, Ochanomizu University, Tokyo, for supplying the slime mold, and to Mrs. Kyoungsun Park for her technical assistance throughout this work.

Received for publication 20 July 1972, and in revised form 5 December 1972.

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