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 en-
velope breakdown (2). In fungal mitoses of cen-
triole-lacking styles, the microtubules composing 
the metaphase spindle arise, in most instances, in 
the vicinity of variously shaped microtubule or-
ganizing 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 myxo-
mycetes but recently one has been found, sur-
prisingly, 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 
plasmodia 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 syn-
chrony and the course of mitosis can be reconstructed 
from samples taken at closely spaced intervals. Nu-
clear behavior in the growing plasmodium was moni-
tored by examining small fragments of plasmadium 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. Speci-
mens 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 enlarge-
ment, 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 nu-
cleus in early prophase. The nucleolus is still com-
 pact but has already taken up an off-center posi-
tion. In the center of the nucleus, occupying a de-
pression in the nucleolus, an amorphous osmi-
ophilic zone, 0.3-0.5 μm 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. $\times 37,500$.

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 opportunely prophase confirm the recent findings of Sakai and Shi-
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 microtubules 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.
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.

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