ARMS AND BRIDGES ON MICROTUBULES IN THE MITOTIC APPARATUS

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INTRODUCTION

Except for reported differences in diameters, the findings on fine structure of microtubules (MT) involved in mitosis and meiosis are consistent throughout the literature. Whatever the sources of cells for study, plant (14, 10, 17, 6) or animal (14, 9, 13, 20), no deviations have turned up, other than in centrioles which have tubules arrayed in triplets.

Exceptions to the MT structure of the mitotic and meiotic apparatus are found in those tubules associated with cilia and flagella (2, 1). Certain of these, the peripheral tubules, commonly exist in pairs with additional structures—arms—attached to one tubule of the pair. The two central tubules

have no such arms but are enclosed by a central sheath.

Reports on fine structure of the mitotic apparatus (MA) of intranuclear divisions are not uncommon in the literature of both plant and animal cells. Recent among these are the Ichida and Fuller studies on the MA in fungus cells (6), and those of Jenkins (9) and Tucker (17) in protozoan cells. The fine structure of MT described by these authors is generally consistent with findings of others.

This report is concerned with the presence of arms and bridgelike structures associated with the MT in the interzone region of the intranuclear MA of the coenocytic green alga *Blastophysa rhizopus* Reinke. Such structures, to my knowledge, have not previously been discussed in the literature.

A complete study of the mitotic process at the fine structure level in *Blastophysa* is presently being carried out and a manuscript—including new information on kinetochore fine structure—will be submitted for publication at a later date.

MATERIALS AND METHODS

Algae (*Blastophysa rhizopus*) cultured according to a method described by Sears were placed on a 16:8-hr light-dark cycle (15). Specimens were collected at various times after the start of the dark period and were placed in 4.0% glutaraldehyde buffered at pH 7.2 with phosphate buffer for 1 hr, and were then washed for 5 min in buffer and were subsequently postfixed for 1 hr in 1.0% osmium tetroxide similarly buffered. The postfixed algae were dehydrated through a graded series of alcohols and embedded in Epon 812 which was polymerized overnight at 60°C. Sections were cut on an MT-2 Porter-Blum microtome, mounted on grids, and stained with uranyl acetate followed by lead citrate. Microscopy was done on an AEI (EM6B) electron microscope on loan to the Fertilization and Gamete Physiology Training Program at the Marine Biology Laboratory, Woods Hole, Mass.

RESULTS

Microtubules on the poleward side of the chromosome plates at anaphase always outnumber those seen in the interzone and consequently have an accompanying higher level of birefringence (7, 14). Only continuous MT are in the interzone at anaphase, while continuous as well as chromosomal MT are in the poleward regions. Since MT in the poleward region of *Blastophysa* at anaphase outnumber by a considerable degree—because of numerous and small chromosomes—those MT in the interzone region (H. J. Wilson, work in progress), the few profiles seen in Fig. 1 are indicative of the expected. The dark chromatin-like masses (*N*) in Fig. 1 are parts of the large nucleolus which fragments at late prophase. The lighter staining masses (*C*) are chromosomal, suggesting a section which is in close proximity to one of the separating chromosomal masses.

Three types of microtubules can be seen in transverse sections through the interzone region of the mitotic apparatus of *Blastophysa*. One type of MT shows the dense outer annular region with its less dense center which has been described frequently. Some of these MT are somewhat oval (see Discussion) in shape, with average dimensions of 200 A on the short axis and 250 A on the long axis. The annular portion is 40–50 A thick (*a* in Fig. 1).

The second type of MT is similar to the first in transverse section; however, this type possesses, in addition, an arm extending from the outer surface of the annular portion (*b* in Fig. 1; Fig. 3 *c*; Fig. 4, arrow). These arms are single, one arm per MT, and are thus not morphologically analogous to the 50-A-thick and 150-A-long paired arms described by Gibbons and Grimstone (2) in protozoan flagella, or to similar structures described earlier by Afzelius (1) in sperm tails. The single arms described here do not appear to be uniform in width or length, average measurements being 140 A in length (measured from the external annular surface to the tip of the arm) and 50 A in width. In some instances where this type of MT is adjacent to the nuclear envelope, the arm appears directly connected to the inner membrane of the envelope (Fig. 2, arrows). These MT are otherwise arranged in no particular pattern within the nucleus.

The third type of MT seen in transverse section is characterized by two single MT joined together as a pair by a bridgelike connection (*c* in Fig. 1). No contact exists between MT of a pair except through the bridgelike structure. The dimensions of these MT vary from one pair to another, and in some instances individual tubules of a pair will vary, e.g. in Fig. 3 *b* the inside (less dense medulla) diameter of the left MT is 120 A while the inside diameter of the right MT is 150 A. The distance between paired MT (center to center) is variable while the bridge has a constant length. Thus, the variable distance between paired MT appears to be related to the arc formed by the bridge—the greater the arc, the shorter the distance between

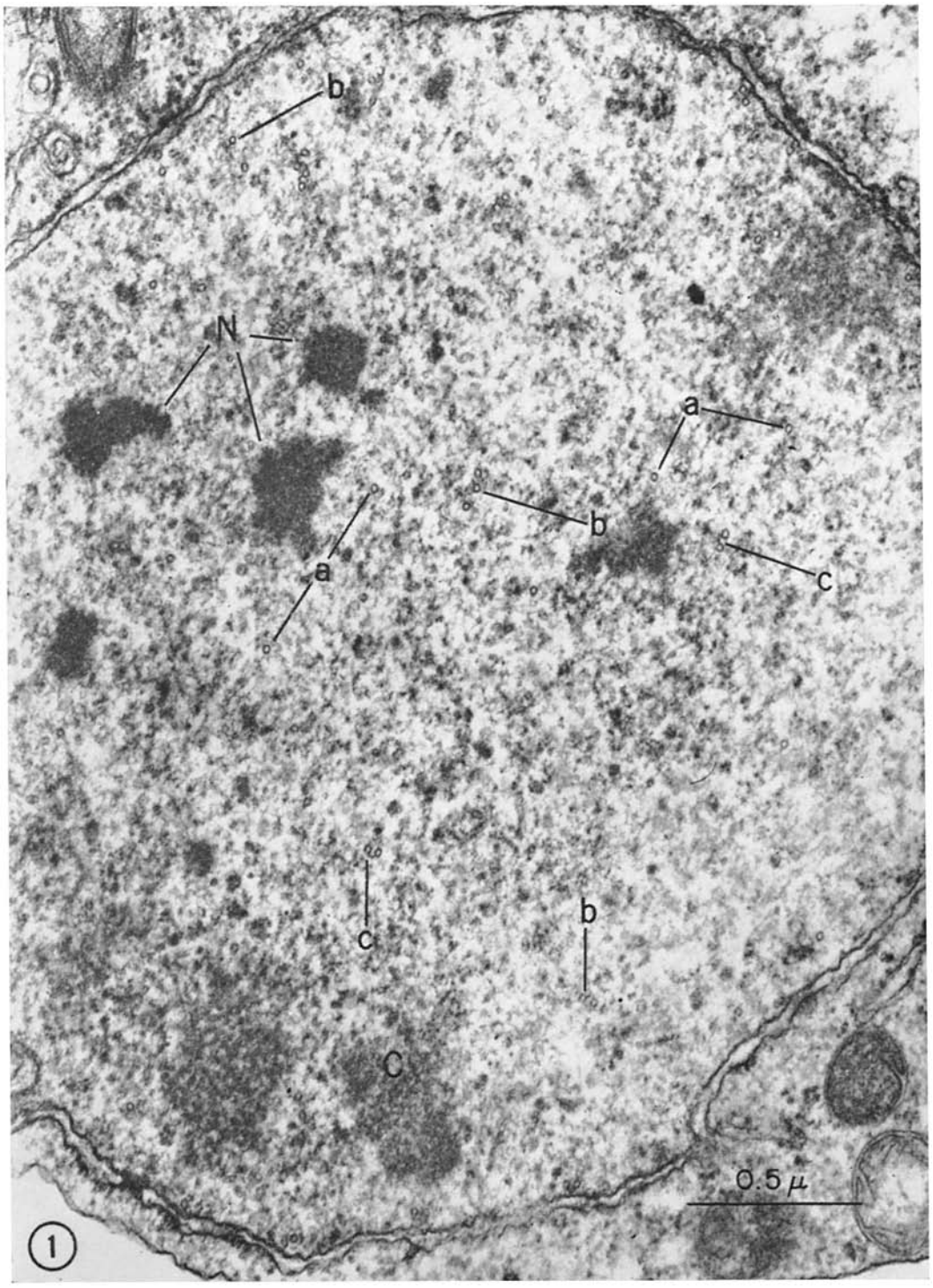

FIGURE 1 A transverse section through the interzone of a nucleus in anaphase showing the intact nuclear envelope and profiles of the three types of pole-to-pole MT: (*a*) the usual type of microtubule, (*b*) single MT with arms, and (*c*) pairs of MT joined by a bridgelike connection. Nucleolar fragments (*N*) and a small chromosomal mass (*C*) can also be seen. × 53,500.

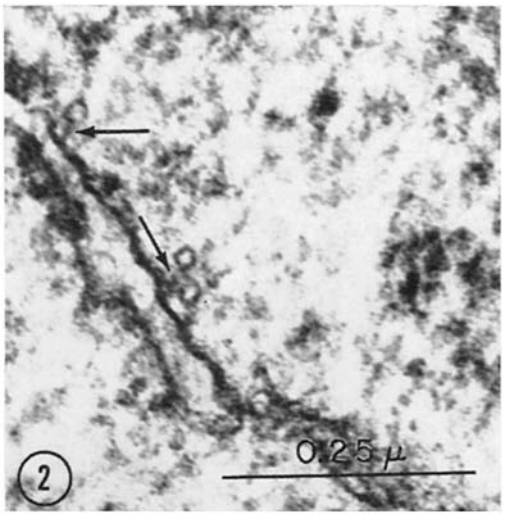

FIGURE 2 Profiles of a single MT (upper arrow) and a pair of MT (lower arrow) showing direct association with the inner membrane of the nuclear envelope. × 132,000.

MT. In Fig. 3 *a*, a distance of 300 A separates the MT, while in Fig. 3 *b* that distance is 400 A. The bridgelike connection is about 50 A thick. Fig. 3 *b* is an exception in that the bridge is thicker on one end and shows signs of being double.

Pairing does not seem to be the only way in which MT are associated. Fig. 4 shows four MT connected by three bridges. Such an arrangement could provide several different combinations of MT and arms or bridges if the MT and/or bridges are separated at selected points. There are indications of occasional connections between the bridgelike structures and the arms; however, these observations are not well established as yet (H. J. Wilson. Work in progress).

DISCUSSION

Recently, Stephens (16) stated that a class of proteins with an amino acid composition similar to that of actin is common to the major structural components of MT in outer fibers of cilia and flagella and in the mitotic apparatus. Other efforts to establish a structural and functional unity of the various MT have been put forth by Ledbetter and Porter (10), Inoué (7), Roth (13), and by the present author (14).

The most immediate implications of the findings in this paper relate to two recently reported observations: (*1*) The presence of the ATPase-protein (called dynein by Gibbons) in cilia of *Tetrahymena*, and (*2*) anaphase movement due to increases in length of pole-to-pole MT in the MA.

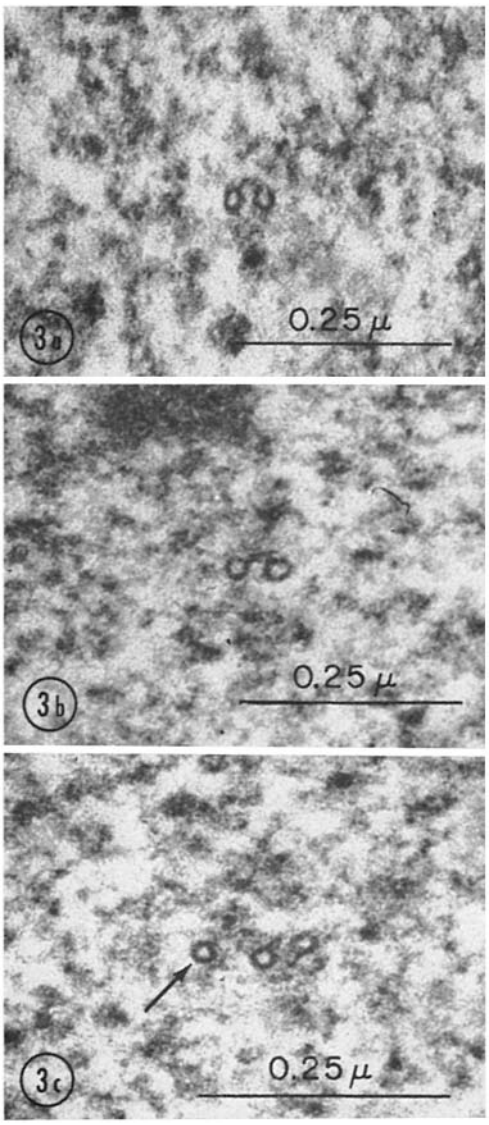

FIGURE 3 Higher magnifications from Fig. 1. Figs. 3 *a* and *b* show two pairs of MT with different measurements of center-to-center separation; and Fig. 3 *c* shows profiles of the usual MT (arrow) and of MT with arms. Figs. 3 *a* and *b*, × 119,000; Fig. 3 *c*, × 132,000.

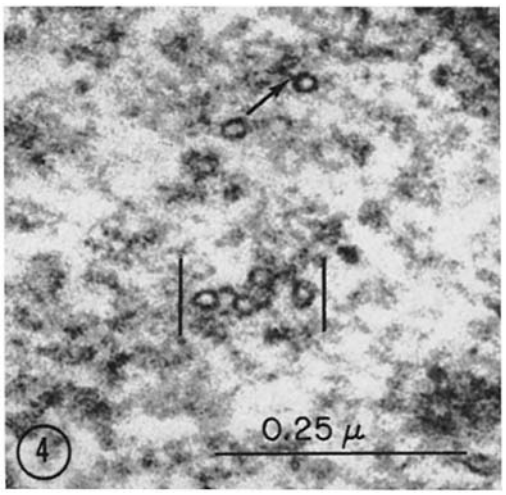

FIGURE 4 A micrograph showing profiles of four MT joined by three bridgelike connections (inside brackets) and a single MT with an arm (arrow). × 132,000.

Arms, Bridges, and Dynein

The ATPase-protein named dynein by Gibbons (3) and later isolated by Gibbons and Rowe (4) was found in the arm portions of outer ciliary MT. The failure of other investigators to observe secondary structures such as these has prevented establishment of a structural basis for an analogous protein on cytoplasmic and mitotic apparatus MT; however, the presence of ATPase has been reported by Mazia et al. (11) and subsequently by others. The absence of an ATPase-protein from MA preparations has been a barrier to efforts by those attempting to establish unity between the MT of MA, cilia, and flagella, on the one hand, and similar structures in striated muscle cells (5), on the other. To this general regard, the recent work by Young and Nelson (19) on reversible interactions of actin and myosin-like compounds from bull sperm tails with muscle actin and myosin is of considerable interest. An analysis of the intranuclear MA of *Blastophysa*—with its arms and bridgelike structures might—prove fruitful in this regard.

Arms, Bridges, and Expansion of Pole-to-Pole MT

The second implication of the arms and bridgelike structures relates to the increase in length of continuous (pole-to-pole) MT during anaphase. Increases in length of continuous MT as a means of separating chromosomes at anaphase were postulated by Ris (12). Suggestions for a mechanism of action for such an hypothesis have been presented by Roth (13) and Inoué (7) in proposing the addition of MT subunits at a point(s) along the continuous MT. This proposal has also been advanced by the present author (14) in collaboration with others. On the basis of birefringence studies, Inoué (7, 8) stated that MT subunits can actually pass from chromosomal MT to continuous MT.

It was proposed earlier that MT may be attached at points along their length (14). Recently, Jenkins (9) suggested that such attachments between continuous and chromosomal MT might occur on the poleward side of the anaphase chromosomes. The present report shows that continuous MT are attached by bridgelike structures in the interzone region. This finding does not exclude the possibility of similar connections between continuous and chromosomal MT in the polar region as suggested by Jenkins. What appear to be cross-bridges in the poleward region at metaphase can be seen in a micrograph by Tucker (17, Fig. 6); unfortunately, no discussion of that particular micrograph was included. At the present time, connections have not been observed on the poleward side in *Blastophysa;* however, a search for these connections is of primary concern in the continuing work.

Thus, a morphological basis for the addition of MT subunits and subsequent increase in MT (continuous MT) length is presented here. That there are indications of MT attached by the arm to the nuclear envelope is not unexpected, since Inoué (7) has shown that membranes as well as kinetochores and centrioles may act as centers of organization for MT subunits. With regard to kinetochores, it should be pointed out that the kinetochore-microtubule relationship proposed by Inoué may be morphologically demonstrable (18; H. J. Wilson, work in progress). Since the interphase nucleus of *Blastophysa* possesses a nucleolus, a morphological basis for intranuclear synthesis of MT proteins exists. The absence of a nucleolus or definite ribosomes in *Blepharisma* prompted Jenkins (9) to speculate on a mechanism of transporting necessary MT proteins across the nuclear envelope in the micronucleus.

The two implications discussed above are not the only possibilities for the observations presented

in this paper, although this author does favor these two, particularly the anaphase movement possibility. Other possibilities, e.g. cross-bonds for stability of the gel-like nature of the MA, and muscle-like cross-links involved in movement, will be discussed in the already mentioned work which is presently in preparation.

I would like to express my thanks to Drs. Charles R. Austin, Wayne R. Ferris, Shinya Inoué, Leonard Nelson, and Raymond E. Stephens for their criticisms and suggestions during the writing of this manuscript, and to Miss Pamela Nelson for help with its structure. Thanks are also due Mr. James Sears for providing *Blastophysa* in culture, and Miss Victoria Goon for her much needed understanding throughout this study.

This work was supported in part by the training grants DE00184 (Comparative and Developmental Biology) and 5-TI-HD26-07 (Fertilization and Gamete Physiology) from the National Institutes of Health.

Received for publication 26 August 1968, and in revised form 12 November 1968.

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