HIGH MOLECULAR WEIGHT MAPs ARE PART OF THE MITOTIC SPINDLE

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ABSTRACT

We have found that the microtubule-associated proteins of high molecular weight are located in the mitotic spindle. Indirect immunofluorescence studies reveal that the pattern of distribution of these proteins is similar to that described for tubulin and corresponds to the known phases of mitosis.

KEY WORDS microtubule · microtubule-associated proteins · high molecular weight proteins · mitotic spindle · mitosis · indirect immunofluorescence

Although microtubule integrity has been known to be essential for mitosis for over 25 yr (9, 10) the manner by which microtubules affect chromosomal movement remains unclear (24). During prophase, soluble tubulin (perhaps derived from cytoplasmic microtubules) assembles into microtubules which, at metaphase, form the characteristic mitotic spindle. Colchicine, vinblastine, and other alkaloids, which bind to tubulin and thereby prevent microtubule assembly, abort mitosis at metaphase because the spindle cannot form or disintegrates soon after the addition of these drugs.

Although this information indicates that tubulin assembly into microtubules is necessary for the construction of the mitotic spindle, it does not tell us how the microtubules in the spindle actually bring about anaphase chromosome movement. It is generally accepted that the microtubule backbone, composed of tubulin, does not contract (18) and, thus, that the motive force must derive either from the assembly process itself (11, 12) or from another protein or proteins associated with microtubules in the spindle (6, 14, 20). Recently, a great deal of interest has focused on the microtubule-associated proteins (MAPs) which maintain a constant stoichiometric relationship with tubulin during repeated cycles of assembly-disassembly *in vitro* (2, 23). The high molecular weight MAPs stimulate tubulin assembly and form filaments (5) or periodic sidearms (1, 17) on the surface of

microtubules assembled *in vitro*. Such sidearms may link various intracellular organelles to microtubules in the living cells and participate in the movement of these structures during, for example, secretion or mitosis (26). Recently, we developed an antibody directed specifically against the high molecular weight MAPs and have shown by indirect immunofluorescence that these proteins are distributed along cytoplasmic microtubules *in vitro* (22). In the present study, we have found that the MAPs are located in the mitotic spindle and that the pattern of distribution of the protein corresponds to the known phases of mitosis.

MATERIALS AND METHODS

After SDS-polyacrylamide gel electrophoresis of rat brain microtubule protein, purified by two cycles of assembly-disassembly, gels were stained and the bands containing the high molecular weight MAPs were cut out, homogenized in complete Freund's adjuvant, and injected intradermally and intramuscularly into rabbits. 10 days to 2 wk after the third injection of approx. 90 μ g of MAP, the rabbits were bled, the serum was harvested, and monospecific antibodies to the MAPs were purified by affinity chromatography (7, 22). Radial immunodiffusion showed that the antibodies gave a single line against microtubule protein (tubulin and MAPs), a crude 100,000 g rat brain supernate, and the high molecular weight MAPs purified by SDS-column chromatography using Bio-Gel A 1.5 M (Bio-Rad Laboratories, Richmond, Calif.) (22). These lines fused with no visible spurs. No reaction was detectable against purified tubulin. Further, the precipitin lines were prevented by preincubating the antibody with purified MAP, whereas preincubation with purified tubulin had no effect. 3T3 fibroblasts were grown on glass cover slips in Dulbecco's Modified Eagle's medium supplemented

with 10% fetal calf serum and were studied during the logarithmic growth phase. Cover slips were placed in 3.5% formaldehyde (37°C) for 30 min, then in acetone (-5°C) for 7 min (21). After rinsing in phosphate-buffered saline pH 7.0 (PBS), a 0.11-mg/ml solution of anti-MAP antibody was applied for 30 min at 37°C. Cover slips were then rinsed in PBS, after which fluorescein-conjugated goat-anti-rabbit antibody (1:20 dilution in PBS) was applied for 30 min. After a final rinse in PBS, cover slips were mounted in glycerol-PBS (1:1)

and examined in a Zeiss photomicroscope equipped with epifluorescence optics.

RESULTS AND DISCUSSION

Fig. 1 shows examples of cells stained with the anti-MAP antibody during various stages of mitosis. Visualization of mitotic figures on control cover slips was blocked by preincubating the anti-MAP antibody with MAP but not with purified

FIGURE 1 Spindle fluorescence of 3T3 cells. (a) Early prophase, (b) late prophase, (c) metaphase, (d) early anaphase, (e) late anaphase, and (f) telophase.

tubulin. Non-immune rabbit IgG did not stain. The mitotic figures visualized in these cells with the anti-MAP antibody are indistinguishable from those seen with antitubulin antibody. Anti-MAP fails to stain the stem body (Fig. 1*f*), which also remains unstained with antitubulin antibody (3, 25) despite the fact that electron micrographs show numerous microtubules in this region (3). These observations suggest that the absence of stain in the stem body is due to exclusion of antibody from this region rather than to absence of tubulin or MAPs. In late anaphase, we commonly observe an intensely staining area at the poles of the spindle on the sides distal to the forming cleavage furrow (Fig. 1*e*), but the function of MAPs in this area is unknown.

The presence of the high molecular weight MAPs in the mitotic spindle suggests that these proteins have a role in chromosome movement. One possibility is that they form periodic sidearms along the microtubule surface which connect to chromosomes and also to other microtubules (15). Concerted sliding of adjacent microtubules powered by the MAPs would then move chromosomes connected to them. A dynein-like sliding filament mechanism for chromosome movement is supported by the findings of Sakai et al. (19) who showed that antiserum against the ATPase fragment A of sea urchin sperm flagellar dynein blocked ATP-dependent chromosome movement in glycerol-isolated mitotic apparatus of sea urchin and starfish eggs. Antiserum against starfish egg myosin was without effect. Further, the antidynein serum stained the mitotic apparatus of sea urchin eggs *in situ* (16).

Inoué and colleagues postulate that chromosomal movement is powered by microtubule assembly-disassembly *per se* (11-13). Other workers have suggested that a spindle-associated actin-myosin system pulls chromosomes to the poles during anaphase (6, 20). The work of Sakai et al. (19) and Mohri et al. (16) in invertebrates, together with our results in mammalian cells, indicates that the MAPs or related proteins are present in the mitotic spindle throughout the animal kingdom, and suggests that they power chromosome separation. Nevertheless, the other hypotheses are not ruled out by the present findings, particularly in view of the fact that the MAPs have not yet been demonstrated to have ATPase activity (4, 8). It is even possible that, during different stages of mitosis, different force-generating mechanisms prevail or that they work cooperatively

and simultaneously. Whatever the actual mechanism is, the evolutionary conservation of MAPs in the spindle is strong evidence that these proteins play an important role in normal mitotic division.

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