Test of Four Possible Mechanisms for the Temporal Control of Spindle and Cytoplasmic Microtubule Assembly in HeLa Cells*

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We have compared tubulin from exponentially growing and mitotic enriched populations of suspension-cultured HeLa cells. The proportion of the protein in the cell extract that was tubulin (4.6%) was the same in populations of both mitotic and log phase cells. The self assembly and copolymerization activity of extract tubulin were also compared for the two populations of cells. Approximately 60% of the tubulin in extracts of both mitotic enriched and log phase cells self assembled reversibly as determined by quantitative sedimentation assays. In copolymerization experiments, isotopically labeled tubulin from both mitotic enriched and log phase cell extracts was incorporated with equal efficiency into microtubules formed with microtubule protein purified from porcine brain. Greater than 80% of the tubulin in HeLa extracts participated in one cycle of reversible polymerization with carrier brain tubulin. In addition, the amount and type of microtubule-associated proteins (MAPs) were similar in the extracts of mitotic and log phase cells. The capacity of tubulin from the two populations to incorporate tyrosine into the COOH terminus of the α chain was also compared before and after partial digestion with carboxypeptidase A to release any endogenously bound COOH-terminal tyrosine. Once again, the results obtained for the material from the two sources showed no significant difference. In summary, no differences have yet been detected in the properties of microtubule protein in interphase and mitotic HeLa cells.

Since the assembly of the mitotic spindle in cultured cells can occur without concomitant protein synthesis (Taylor, 1963; Sisken and Iwaski, 1969), it seems likely that the principal control of microtubule formation operates at the level of polymerization from pre-existing subunits. Conceivably, the microtubules of the mitotic spindle might differ from the cytoplasmic microtubules present in interphase cultured cells, yet virtually no information on this question is currently available.

In this study we present a comparison of the microtubule protein from mitotic enriched and exponentially growing populations of suspension-cultured HeLa cells. Suspension cultures were chosen not only to obtain large numbers of cells, but to avoid the possible complication of change in shape displayed by most cells grown in monolayers as they round up to enter mitosis. HeLa cells in suspension culture are round throughout their cell cycle. Since the overwhelming majority of cells in exponentially growing cultures are in some stage of interphase, our study approximates a comparison of mitotic and interphase cells. This seemed to be a useful first step since if no differences were observed between these two populations, there would seem to be little point in carrying out a detailed study of the cell cycle.

Since there is only one report available on the amount of tubulin in interphase and mitotic cells (Forrest and Klevecz, 1972) and there are methodological problems with this paper, we first determined the concentration of tubulin in the two populations using methods recently shown to be reliable for the quantitation of tubulin in cell extracts. Since the control of spindle formation might be correlated with the polymerization competence of tubulin, we have also examined the activity of the tubulin in mitotic and log phase cells using a self assembly assay (Bulinski and Borisy, 1979) and a more sensitive technique involving copolymerization of HeLa extracts with brain microtubule protein. Since microtubule-associated proteins have been indicated to modulate the extent of tubule formation (Penningroth et al., 1976; Sloboda et al., 1976; Murphy et al., 1977), we have also used our in vitro polymerization assays of HeLa microtubules to compare the kind and amount of MAPs from the mitotic and log phase cells.

Another possible change in the tubulin of cells approaching mitosis involves changes in the amount of tyrosine covalently bound to the α subunit of tubulin. The level of activity of tubulin-tyrosine ligase, the enzyme which catalyzes this α tubulin modification, was found to change during the cell cycle of cultured Chinese hamster cells (Forrest and Klevecz, 1977). Although levels of tyrosination of tubulin seem to have no effect on tubulin polymerization in an in vitro system (Raybin and Flavin, 1977) it is not known whether or not changes in the covalently bound tyrosine could effect a mobilization of tubulin competent for polymerization into the mitotic spindle. Therefore, we have analyzed tubulin in mitotic and log phase cells with regard to its ability to be tyrosinated in the COOH-terminal end of the α chain and its content of tyrosine residues at that position in vivo.

MATERIALS AND METHODS

Cell Growth and Synchrony—HeLa cells, strain H, were grown as described previously (Bulinski and Borisy, 1979). Log phase cells were harvested at densities of 4 × 10^6/ml. Mitotic enriched populations

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The abbreviation used is: MAPs, microtubule-associated proteins.
were prepared by the nitrous oxide synchrony method of Rao (1968) with the following modifications: cell populations were subjected to the thymidine block for 18 to 20 h, released for 4 h, and blocked with nitrous oxide at 85 ppm for 9 h in a 400-ml capacity pressure vessel (Artisan Industries, Waltham, Mass.). In this way up to 2 x 10^6 cells with mitotic indices of 72 to 87% were obtained. Mitotic indices were determined by light microscopy, as follows: drops of cell suspensions were made on glass slides, fixed with formaldehyde and 3:1 ethanol-acetic acid and applied to microscope slides. The air-dried slides were stained with a solution containing 0.4% crystal violet, 10% ethanol, 0.2% sodium oxalate, and destained with water.

Cell and Tissue Extracts and Microtubule Protein Preparation—HeLa cell extracts and microtubule protein were prepared as described previously (Bulinski and Borisy, 1979). Porcine brain extracts and microtubule protein were prepared according to Borisy et al. (1975).

Tubulin and Protein Determinations—Gel electrophoretic, colchicine-binding, DEAE-retention, and radioimmune assays are described in detail elsewhere. Protein determinations were performed according to Lowry et al. (1951).

Self Assembly Assay—Aliquots (1.0 ml) of extracts, prepared from mitotic enriched or log phase populations, were warmed to 37°C for 30 min. Microtubules were quantitated by a temperature-reversible, sedimentation method. Polymer was first sedimented by centrifugation at 37°C at 48,000 x g for 30 min. The pellets were resuspended in a volume of 0.25 ml and depolymerized for 30 min at 0°C. Irreversible aggregates were removed by centrifugation at 0°C at 48,000 x g for 30 min. The protein concentrations of the resultant Gs (see terminology of Borisy et al., 1975) and the original extracts were determined, and the tubulin content was measured by densitometry of stained polyacrylamide gels which separated α and β tubulin.

Copolymerization Assay—HeLa cells were grown with [3H]leucine (Amersham/Searle, catalog No. TRK-170) present in the usual growth medium for 48 h. For one- or two-cycle copolymerizations, 0.5 μCi/ml of [3H]leucine was used, while for 7-cycle preparations, 25 μCi/ml was used. Extracts were prepared from 'H-labeled mitotic or log phase HeLa cells and mixed with twice-cycled brain microtubule protein (4 to 5 mg/ml) in a volume:volume ratio of 1 part HeLa to 10 parts brain. A Cs fraction of the mixture was prepared as above, and total radioactivity was determined for 0.1-ml aliquots in 10 ml of tritrisol fluor (Benson, 1966) in a Packard scintillation counter. The proportion of extract and Gs protein which was tubulin was determined by slicing unstained gels with a Gilson gel slicer (Gilson Biomedical, Middleton, Wis.) and counting the radioactivity in each slice as above. Tubulin was localized on duplicate stained gels.

Incorporation of Tyrosine into Tubulin from the Cell Extracts—Cell lysates from log phase and mitotic populations of cells were prepared as described above and centrifuged at 105,000 x g for 60 min. The cell extracts were dialyzed against 0.1 M piperazine N,N'-bis(2-ethanesulfonic acid) (pH 6.94) containing 0.1 mM MgCl₂ and 0.1 mM GTP. A portion of each extract was incubated in the presence of casein phosphatase A (5 to 10 μg/ml of extract protein) for 20 min at 23°C. Under these conditions total release of aromatic residues from the COOH terminus of brain α-tubulin has been observed (Rodriguez and Borisy, 1978). After release of the aromatic residues the reaction virtually stops at glutamate, which is the next residue, yielding tubulin with an increased number of sites for in vitro incorporation of tyrosine. The capacity for incorporation of tyrosine under the conditions described by Barra et al. (1973) was measured in cell extracts of both populations in the presence of partially purified porcine brain ligase prepared according to Raybin and Flavin (1977). d-Phenylalanine (30 mM) was added to the reaction mixture to block the activity of casein phosphatase A after the digestion step.

Typically an aliquot of the extract (2 to 3 mg of total protein) was incubated for 30 min at 37°C in the presence of ligase (specific activity 0.82 nmol/min/mg of protein), 30 mM ATP, 12.5 mM MgCl₂, 50 mM KCl, and 0.4 μCi of [14C]Tyrosine (Amersham/Searle, catalog No. CF-74). Acid-insoluble radioactivity was measured as described (Barra et al., 1973).

RESULTS

Logarithmic phase and synchronized mitotic populations of suspension-grown HeLa cells were used to compare the tubulin of interphase and mitotic cells. The mitotic cells were collected by the nitrous oxide technique of Rao (1968) which has previously been demonstrated to be reversible and to block cells without preventing the assembly of spindle microtubules (Brinkley and Rao, 1973; Cox et al., 1979). Light micrographs of examples of the cell populations used are shown in Fig. 1. In the mitotic enriched populations used, an average of 76% of the cells were in mitosis. In the log phase cell populations used to represent interphase cells, only 2.5% of the cells were undergoing mitosis. Therefore, the proportion of mitotic cells was enriched more than 30-fold by the mitotic synchrony method we used, allowing us to compare extracts in which most of the tubulin was derived from mitotic or interphase cells, respectively.

We first compared the proportion of cell protein which was tubulin in the mitotic and log phase populations. Extracts were prepared from cells by methods which were described previously (Bulinski and Borisy, 1979). The tubulin in the extracts was quantitated by three different assays, a DEAE-retention assay, a colchicine-binding assay, and a radioimmunoassay. These assays have been described in detail in another report.

The results of the comparison are shown in Table I. The DEAE-retention assay, which measures the proportion of
Table I

<table>
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<th>Assay method</th>
<th>Mitotic enriched populations</th>
<th>Log phase populations</th>
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<tr>
<td></td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td>DEAE-retention</td>
<td>5.5</td>
<td>5.4</td>
</tr>
<tr>
<td>Colchicine binding</td>
<td>4.0</td>
<td>4.3</td>
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<tr>
<td>Radioimmunoassay</td>
<td>4.2</td>
<td>4.2</td>
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<tr>
<td>Average of all assays</td>
<td>4.6</td>
<td>4.6</td>
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*The three assay methods used to determine tubulin content are described in detail in another report.*

*Average mitotic index 76%.

*Average mitotic index 2.5%.

The extract protein of 55,000 molecular weight which binds to DEAE-Sephadex at moderate salt concentration, gave values of 5.5 and 5.4% for mitotic and log phase extracts, respectively. The decay-corrected colchicine-binding assays also yielded similar values for the mitotic and log phase extracts. In the radioimmunoassay, purified radioactive HeLa tubulin was bound to tubulin antibody and titrated with nonradioactive HeLa tubulin in order to generate a standard curve for the assay of HeLa extracts. When this standard curve was used to quantitate tubulin in mitotic and log phase extracts, the tubulin percentage obtained was 4.2 in both kinds of extracts. Table I demonstrates that an average of the three tubulin assays yields identical values for the proportion of tubulin in mitotic and log phase HeLa extracts.

Since we have shown that tubulin content is identical in mitotic cells and log phase cells, we examined the biological activity of tubulin in the two populations of cells. Biologically active tubulin spontaneously polymerizes into microtubules under optimal *in vitro* assembly conditions. We have previously shown that good yields of spontaneously polymerized microtubules could be obtained from extracts prepared from log phase HeLa cells (Bulinski and Borisy, 1979). We used two quantitative measures of polymerization to determine the proportion of tubulin in cell extracts which was biologically active (Table II). We measured the amount of protein which was present in the C,S, the crude microtubule protein obtained from a single cycle of polymerization and depolymerization. Gel electrophoresis was used to determine the proportion of the C,S material which was tubulin. By dividing the amount of tubulin present in the C,S by the amount of tubulin in the extract, the percentage of activity of extract tubulin was obtained. In this self assembly assay, 59.0% of the mitotic and 57.8% of the log phase tubulin was designated active.

Although close to 60% of the tubulin in the extracts was considered active as determined by our self assembly assay, it would be incorrect to conclude that the remaining 40% of the tubulin was inactive. We previously found that the equilibrium monomer concentration of HeLa tubulin in extracts is approximately 0.5 mg/ml. Since tubulin comprises only 4.6% of the extract protein, this 0.5 mg/ml of monomeric tubulin would represent 30 to 40% of the total tubulin in the extracts used for the assay (30 to 40 mg/ml of total protein). Thus, if we were to correct the self assembly data for the amount of active monomer in the supernatant, we would calculate that 90 to 100% of the tubulin was competent to assemble. To test this possibility more directly, we measured the proportion of HeLa tubulin that would copolymerize with brain microtubule protein.

Mixtures of radioactively labeled HeLa extract and a 25- to 30-fold excess of brain microtubule protein were polymerized and depolymerized for seven complete cycles in a polymerization buffer optimal for HeLa microtubule polymerization.

In these copolymerization experiments, protein from both mitotic and log phase HeLa cells was analyzed. In order to quantitate the degree of equivalence in the behavior of the HeLa and brain tubulin, we calculated a copolymerization index. This index measures the efficiency with which HeLa tubulin polymerizes and depolymerizes relative to the carrier brain tubulin. The copolymerization index is computed by first measuring the proportion of tubulin which participates in each polymerization or depolymerization step. These proportions are computed as the ratios of tubulin: (H,P)/(C,S) for a polymerization step or (C,S)/(H,P) for a depolymerization step. For HeLa, which was isotopically labeled, the amount of tubulin was assayed by radioactivity; brain tubulin was determined by the Lowry assay for protein (Lowry et al., 1951). The proportion of tubulin polymerizing or depolymerizing for both HeLa and brain ranged between 0.60 and 0.95 over the seven cycles examined. The important quantity, however, is the ratio of proportions, HeLa/brain, for each step. This is the copolymerization index. If the HeLa and brain tubulin copolymerized with equal efficiency, we would expect a copolymerization index of 1.0 for each cycle. Fig. 2 is a plot of the copolymerization index of HeLa and brain tubulin during the seven reversible assembly cycles. Data for both mitotic and log phase protein approximate the behavior expected for an equal efficiency of copolymerization of HeLa and brain tubulin.

We used copolymerization experiments to obtain an independent estimate of the activity of tubulin from log phase and mitotic cells. The activity was calculated as the percentage of extract tubulin which remained after one complete cycle of reversible assembly, that is, after the first polymerization and depolymerization steps. The resultant values were similar for tubulin from mitotic and log phase cells; more than 80% of the tubulin was biologically active under these conditions, confirming the calculation based on the results for the self assembly assay. Table II compares the values obtained in the

### Table II

<table>
<thead>
<tr>
<th>Assay used</th>
<th>Mitotic enriched populations</th>
<th>Log phase population</th>
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<tbody>
<tr>
<td>Self assembly*</td>
<td>59.0</td>
<td>57.8</td>
</tr>
<tr>
<td>Copolymerization*</td>
<td>81.8</td>
<td>80.7</td>
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*See text for details.*

![Fig. 2: Copolymerization index of HeLa and porcine brain tubulin. The index measures the degree to which isotopically labeled HeLa tubulin polymerizes or depolymerizes relative to the carrier brain tubulin. See text for details.](http://www.jbc.org/)

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copolymerization and self assembly assays. We could detect no difference in the activity of HeLa tubulin derived from the mitotic and interphase stages of the cell cycle.

We also compared the purified microtubule protein obtained from mitotic and log phase cells. Because the number of mitotic cells we could obtain was limited, we used the copolymerization technique to examine microtubule protein from mitotic cells. Fig. 3, a comparison of the microtubule protein from log phase and mitotic cells, shows a densitometric trace of a stained gel of self assembled material from log phase cells and a radioactivity profile of copolymer from mitotic cells. In both log phase and mitotic cells, tubulin was the major component of the purified microtubule protein, accounting for 94% of the stained material on the gel of log phase protein and 95% of the radioactivity in the gel of mitotic protein. In addition, two minor peaks were detected in gels of both kinds of material. These species had molecular weights of approximately 125,000 and 210,000 as denoted by arrows in Fig. 3. In microtubule protein from both log phase and mitotic cells the 125,000 MAP comprised approximately 2% of the protein and the 210,000 comprised about 2.5%. Therefore, microtubules purified from mitotic cells contained the same kind and amount of MAPs as microtubules purified from log phase cells.

We also compared the ability of tubulin in the mitotic and log phase HeLa extracts to incorporate tyrosine at the COOH-terminal end of the α chain. Nath et al. (1978) reported that HeLa tubulin had no capacity to serve as a substrate for in vitro tyrosination. It is not clear why they failed to detect this activity. However, by using high sensitivity assay conditions we have found that native cytoplasmic tubulin had a measurable acceptor capacity for incorporation of tyrosine with the characteristics described for brain cytoplasmic tubulin (Arce et al., 1975). In these experiments extracts of log phase and mitotic HeLa cell populations at protein concentrations of 30 to 40 mg/ml were used.

Two types of control experiments indicated that the tyrosine was covalently incorporated into the COOH terminus of extract tubulin. Radioactive tyrosine incorporated into the native protein of log phase cell extracts was found to be rapidly removed by carboxypeptidase digestion. Furthermore, only one radioactive band, with the mobility of tubulin, was detected in a gel of the sample. These observations demonstrate that [3H]tyrosine was incorporated into tubulin in the cell extracts in the same way as previously described for brain tubulin.

Consequently, extract tubulin from the two cell populations was further examined in a series of experiments in which the incorporation of [3H]tyrosine was measured in preparations from log phase and mitotic enriched populations of cells processed simultaneously. Table III shows that the HeLa tubulin did have the capacity to serve as a substrate for in vitro tyrosination and that the capacity was increased after treatment with carboxypeptidase A to release the endogenous COOH-terminal residues. The important point, however, is that no significant difference in the level of tyrosination was detected before or after treatment with carboxypeptidase A for the two cell populations. Thus, under the experimental conditions used in this study, we may conclude that HeLa tubulin from mitotic and interphase cells has no alteration as far as this peculiar post-translational modification is concerned.

In addition, we have analyzed the tubulin purified from log phase populations of HeLa cells by three cycles of assembly and disassembly. The acceptor capacity for tyrosine was measured before and after treatment with carboxypeptidase A. From the increase in acceptor capacity after carboxypeptidase treatment, we calculate that 40% of the tubulin molecules contained endogenous COOH-terminal tyrosine.

**DISCUSSION**

In our comparison of mitotic and interphase tubulin from HeLa cells, we sought to test possible mechanisms by which the timing of assembly of mitotic spindle tubulin could be controlled in vivo. In the first mechanism, the intracellular tubulin concentration would vary during the cell cycle. Increases in the cytoplasmic tubulin concentration at the onset of mitosis would shift the microtubule assembly equilibrium in favor of polymerization of spindle tubulin. Our data showing the constancy of the proportion of tubulin in mitotic and interphase cells argues against this mechanism, in keeping with the conclusions drawn from protein synthesis inhibitor studies (Taylor, 1963; Sisken and Iwasaki, 1969). In the second mechanism for temporal control of spindle microtubule assembly, changes in the polymerization activity of cellular tubulin would precede the assembly of tubulin in the mitotic spindle. This model predicts the existence of a substantial pool of inactive tubulin which can be mobilized when required. Altering the proportion of cytoplasmic tubulin which was competent for polymerization would have the same effect as altering

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<th><strong>Table III</strong></th>
<th>Incorporation of [3H]tyrosine into extract tubulin*</th>
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<tr>
<td>Cell population</td>
<td>cpm/mg of protein</td>
</tr>
<tr>
<td>Log phase</td>
<td>1974 ± 213</td>
</tr>
<tr>
<td>Mitotic enriched</td>
<td>2677 ± 314</td>
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*Preparation of extracts and conditions for incorporation of [3H]tyrosine, before and after treatment with pancreatic carboxypeptidase A, were as described under "Materials and Methods." Values represent mean ± S.E.M. for three separate experiments.
Mobilization of tubulin from a pool which is incompetent to polymerize molecular weight between 200 and 220,000 and two proteins groups of MAPs in HeLa microtubule protein preparations tend to large proportion loss of activity during the preparative procedures, and/or by assay. Both mitotic and log phase extracts contained similar mitotic and interphase cells. Preparations and in similar proportions in the copolymer obtained with carboxypeptidase during interphase. Relative to tubulin in the log phase microtubule protein preparation, the tubulin in a cultured cell line is competent to polymerize about the endogenous content of aromatic residues at the COOH-terminus of a tubulin. The determinations were made before and after treatment with carboxypeptidase A in order to obtain information about the endogenous content of aromatic residues at the COOH-terminal position which are removed by the protease.

Our results show that with both the self assembly and copolymerization assays, mitotic and log phase HeLa extracts contained equal proportions of active tubulin. Furthermore, the assays indicate that for both cell populations, between 80 and 100% of the extract tubulin was competent to polymerize under our in vitro conditions. Therefore, the HeLa tubulin which is inactive represents a small proportion of the cellular tubulin, perhaps arising during preparation of the extracts, and the proportion which is inactive does not change significantly as cells progress from interphase to mitosis. These results support the suggestion of Spiegelman et al. (1977) that a class of assembly-incompetent tubulin may not exist. However, their conclusions had to be qualified because of the tendency for a large proportion (65%) of the tubulin to aggregate in an amorphous, colchicine-insensitive manner. Hence, they could not exclude the possibility that the activity of tubulin was regulated but that the regulation was obscured under their in vitro conditions. Other reports (Wiche and Cole, 1979; Duenges et al., 1979) of low yields of tubulin self-assembled from cultured cells might have been taken to provide circumstantial evidence for the existence of an inactive tubulin pool. However, these results can be accounted for by overestimates of the tubulin content of the cell extracts, by loss of activity during the preparative procedures, and/or by limiting concentrations of assembly-promoting MAPs. Thus, our results provide the first demonstration that the bulk of tubulin in a cultured cell line is competent to polymerize reversibly. This finding has permitted us to conclude that the tubulin which assembles into the mitotic spindle is apparently not mobilized from a pool which is incompetent to polymerize during interphase.

We have previously demonstrated the existence of two groups of MAPs in HeLa microtubule protein preparations (Bulinski and Borisy, 1979). The two groups, three proteins of molecular weight between 200 and 220,000 and two proteins of molecular weights 120 and 125,000 were also detected as peaks of radioactivity in preparations of copolymer formed from isotopically labeled mitotic HeLa and carrier brain microtubule protein. These MAPs are present at the level of 5% relative to tubulin in the log phase microtubule protein preparations and in similar proportions in the copolymer obtained from mitotic cells. Additional evidence for a constant proportion of MAPs to tubulin is obtained from the self assembly assay. Both mitotic and log phase extracts contained similar tubulin concentrations. Since the tubulins have the same activity in the copolymerization assay, the fact that the self assembly assay values are nearly identical means that the two tubulins have the same equilibrium monomer concentrations. Changing the proportion of MAPs to tubulin at a fixed tubulin concentration changes the equilibrium monomer concentration of brain tubulin (Murphy et al., 1977) and HeLa tubulin. From the determinations at the equilibrium monomer concentrations of tubulin in extracts of mitotic and log phase cells, we infer that the proportion of MAPs to tubulin is comparable in mitotic and interphase cells.

The possibility that a modification of the tubulin molecule is involved in the mechanism for temporal control of assembly of the spindle microtubules was also explored. We have analyzed bulk cytoplasmic tubulin in our HeLa extracts for its capacity to bind tyrosine at the COOH-terminal end of the α chain. The determinations were made before and after treatment with carboxypeptidase A in order to obtain information about the endogenous content of aromatic residues at the COOH-terminal position which are removed by the protease, leaving tubulin in condition to serve as substrate for in vitro incorporation. The results indicate that tubulin from the extracts of this line of human cells has a measurable acceptor capacity to incorporate tyrosine covalently into the COOH terminus in vitro and that tyrosine is present in native tubulin at the same position. However, no significant difference has been found between the extracts from mitotic or log phase populations of cells. This observation is in agreement with the notion that modification of the COOH terminus of a tubulin by addition or removal of aromatic amino acid residues has minimal effects on tubulin polymerization (Raybin and Flavin, 1977).

Because of these results and others presented in this report, certain mechanisms for temporal control of assembly of spindle microtubules are disfavored. Our data are inconsistent with control mechanisms based on alteration of amount, activity, or tyrosination state of tubulin or amount or kind of MAPs present in cells. However, our failure to detect any differences in the microtubule protein of mitotic and interphase cells should not be interpreted as indicating that none exist. Negative evidence of the type we have collected cannot exclude the possibility of some as yet undetected modification of tubulin or MAPs important in regulating formation of the mitotic spindle.

It will be important to continue to search for differences between interphase and mitotic microtubules. However, should negative evidence for differences continue to mount, then one would be driven to consider more seriously the possibility that control of assembly resides in factors extrinsic to the microtubules. Control might be exerted through the cellular milieu such as by altering free calcium levels, or through the activation of microtubule- nucleating sites at the centrosomes and kinetochores.

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J C Bulinski, J A Rodríguez and G G Borisy


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