Proc. Natl. Acad. Sci. USA
Vol. 75, No. 10, pp. 4962–4966, October 1978
Cell Biology

Identification of tubulin from the yeast *Saccharomyces cerevisiae*

(radioimmunoassay/copolymerization/isolated nuclei/drug binding/gel electrophoresis)

PETER BAUM*, JEREMY THORNER*, AND LAWRENCE HONIG†

* Department of Bacteriology and Immunology, and † Department of Molecular Biology, University of California, Berkeley, California 94720

Communicated by Robley C. Williams, July 10, 1978

ABSTRACT **A tubulin-like protein was identified in the lower eukaryote *Saccharomyces cerevisiae*. The following criteria were used: (*i*) copolymerization of the ^{35}S-labeled yeast protein with porcine brain tubulin; (*ii*) immunoprecipitation of the ^{35}S-labeled yeast protein with antiflagellar tubulin antibody; (*iii*) the presence of the yeast protein as a constituent of isolated yeast nuclei; and (*iv*) splitting of the yeast protein in a gel electrophoretic system containing sodium dodecyl sulfate that resolved the α- and β-tubulin chains from other sources. This protein did not appear to have significant affinity for the plant alkaloid, Colcemid.**

The role of microtubule assembly and disassembly in the mechanism of chromosome segregation and other mitotic events is not well understood (1). The unicellular eukaryotic microorganism *Saccharomyces cerevisiae* has advantages for investigating the genetic control of microtubule synthesis and assembly and the involvement of these organelles in chromosome segregation and cell division. First, the cytology of mitosis in baker's yeast appears analogous in most respects to that found in higher eukaryotes (2–4). Second, large amounts of homogeneous, and if need be synchronous, populations of cells can be prepared (5). Third, techniques for mutagenesis, chromosome mapping, complementation tests, and other genetic manipulations are well developed (6, 7). Last, unlike *Chlamydomonas* and *Tetrahymena*, which are also amenable to genetic analysis, the yeast system is simpler in that the cells are nonmotile and hence contain only cytoplasmic and spindle microtubules. Here we present immunological and biochemical evidence that cells of *S. cerevisiae* contain a protein species that is similar to tubulin, the major component of the microtubules of higher eukaryotic cells (8).

MATERIALS AND METHODS

Chemicals. [*ring C-methoxy*-^{3}H]Colcemid, Na^{125}I, and $H_2{}^{35}SO_4$ were purchased from New England Nuclear. Methyl 2-benzimidazole carbamate (MeBzaC) was the gift of H. J. Thome (Dupont Chemical Co.). [*G*-^{3}H]MeBzaC was prepared by catalytic tritium exchange (Amersham/Searle). All other chemicals were reagent grade.

Organisms and Growth Conditions. *S. cerevisiae* P2180A and its propagation in complex and minimal media have been described (9). *Escherichia coli* C-1a (from R. Calendar) was grown in 1% Bacto-tryptone/0.8% NaCl.

Preparation of Antitubulin Antibodies. Porcine brain microtubule protein was prepared as described (10). Tubulin was purified from the microtubule protein either by preparative polyacrylamide slab gel electrophoresis (11) or by ion-exchange chromatography on DEAE-cellulose (12). Adult female New Zealand white rabbits were immunized with (A) the crude microtubule protein, or with (B) the electrophoretically purified tubulin, or with the chromatographically purified tubulin that (C) had been denatured with sodium dodecyl sulfate (NaDodSO$_4$) or that (D) had been crosslinked with glutaraldehyde (13) and then denatured with NaDodSO$_4$. Primary injections (1 mg of protein) were in complete Freund's adjuvant, followed by boosters (1 mg of protein) in incomplete Freund's adjuvant. For some sera, the IgG fraction was purified by ammonium sulfate fractionation and DEAE-cellulose chromatography. A similar regimen was used to prepare antiserum directed against purified yeast phosphoglycerate kinase. Microtubule protein from sea urchin sperm tail was the gift of W. Z. Cande. Antiserum against *Chlamydomonas reinhardtii* flagellar tubulin (14) was the gift of G. Piperno and D. Luck.

Radioimmunoassay of Tubulin. To determine the titer of a given antiserum or antibody, we added approximately 0.8 ng of ^{125}I-labeled tubulin (^{125}I-tubulin) (~30,000 cpm), labeled by the method of Hunter (15), to a series of tubes containing 0.23 ml of 100 mM NaCl/1 mg of bovine serum albumin per ml/1% Triton X-100/50 mM Tris buffer, pH 7.5. Various amounts of antitubulin antiserum or purified IgG were added and incubated in a final volume of 0.25 ml for 16 hr at 4°. To facilitate subsequent precipitation, we supplemented each tube with normal rabbit IgG, to yield a total of 15 μg. Goat anti-rabbit IgG (Antibodies Inc., 500 μg) was added to give a final volume of 0.3 ml, and incubation continued for another 6 hr at 4°. The precipitates were collected by centrifugation at 4500 × *g* for 20 min at 4°. The pellets were washed twice by successive resuspensions and centrifugations in the same buffer, and the radioactivity present in the final pellets was measured in a gamma counter (model 1195, Searle). Controls consisted of either sera or purified IgG fractions from the same rabbits before immunization or from otherwise normal rabbits. To measure crossreacting material, the procedure was essentially the same except that the competing antigen was incubated with the antitubulin serum or antibody for 4 hr at 4° before the ^{125}I-tubulin was added.

Preparation of Extracts. Extracts for copolymerization and immunoprecipitation experiments were made from yeast cells grown at 30° to a cell density of 1–2 × 10^7/ml in 200 ml of minimal medium, which was 2% glucose, 0.2 mM Na_2SO_4, 2 mM $MgCl_2$, 20 mM NH_4Cl, 30 mM Na,K-PO_4 (pH 6.8), and vitamins (in ng/ml) [*p*-aminobenzoate (200), biotin (16), inositol (2000), pantothenate (400), pyridoxine (400), riboflavin (200), and thiamine (400)], containing 10–100 μC_i of $^{35}SO_4{}^{2-}$. The cells were harvested by centrifugation, resuspended in 2 ml of ice-cold lysis buffer [1 mM phenylmethylsulfonyl fluoride/0.5 mM $MgCl_2$/1 mM ethylene glycol bis(β-aminoethyl ether)-*N*,*N*′-tetraacetic acid/0.1 mM EDTA/2 mM 2-mercaptoethanol/10% glycerol/100 mM 2-(*N*-morpholino)ethanesulfonate (Mes) (pH 6.4), and ruptured by three passages through a French pressure cell at 20,000 lbs./inch^2 (1 lb./inch^2 = 6.895 kilopascals). The extracts were clarified at 4° by centrifugation at 20,000 × *g* for 20 min, followed by centrifugation at 100,000

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

Abbreviations: MeBzaC, methyl 2-benzimidazole carbamate; NaDodSO$_4$, sodium dodecyl sulfate.

× *g* for 1 hr. Extracts for radioimmunoassays of crossreacting material and for drug-binding studies were made in a similar manner from cells grown in broth. Protein concentration was determined by the method of Lowry *et al.* (17), with bovine serum albumin as a standard.

Isolation of Nuclei. Nuclei were prepared by minor modifications of the procedure of Wintersberger *et al.* (18).

Gel Electrophoresis. Electrophoresis of proteins (19) in slabs (0.1 × 30 × 15 cm) of 7.5% polyacrylamide gel containing $NaDodSO_4$ was performed at pH 9.2. Under these conditions, α- and β-tubulin subunits were well resolved.

RESULTS

Yeast Extracts Contain Material Immunologically Related to Tubulin. Microtubule protein preparation D yielded serum of the highest titer against porcine brain tubulin, as judged by radioimmunoassay. However, such antiserum was relatively specific for brain tubulin, in that binding of brain ^{125}I-tubulin was only weakly competed for by heterologous sea urchin flagellar microtubule protein (Fig. 1*A*). In contrast, antiserum prepared against *Chlamydomonas* flagellar tubulin was also able to bind brain ^{125}I-tubulin, and this binding was competed for by the sea urchin protein (Fig. 1*B*). Despite these differences in specificity, it was possible to demonstrate that extracts of yeast cells, but not extracts of *E. coli* cells, contained material that competed for binding of ^{125}I-tubulin by both types of antitubulin serum (Fig. 2). That the observed inhibition represented competition for binding rather than degradation of the

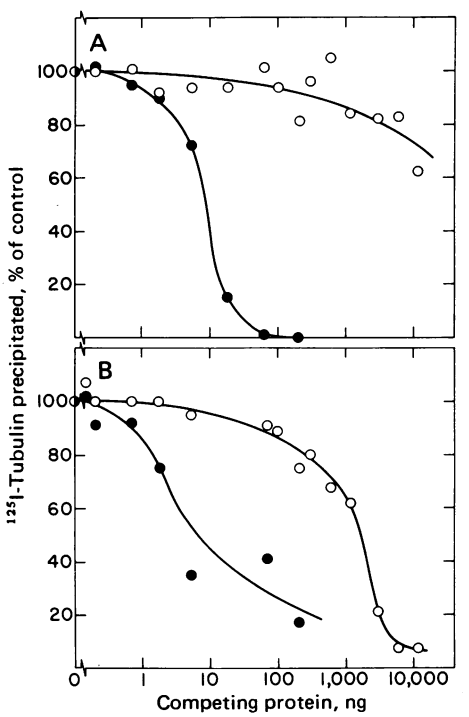

FIG. 1. Radioimmunoassay of tubulin. Inhibition of the binding of brain ^{125}I-tubulin to purified anti-brain tubulin IgG (1 μg) (*A*) or to anti-*Chlamydomonas* tubulin serum (10 μg) (*B*) by increasing amounts of either brain tubulin purified by DEAE-cellulose chromatography (●) or sea urchin flagellar microtubule protein (○) was measured. Maximum specific binding of the ^{125}I-tubulin that could be obtained with these antibody preparations was greater than 70% of the input radioactivity. The amount of IgG or antiserum used was such that the binding observed in the absence of competing antigen was 50% of maximum. The brain tubulin was essentially homogeneous and the sea urchin microtubule protein was at least 60% tubulin, as judged by densitometer tracings of profiles from polyacrylamide gel electrophoresis.

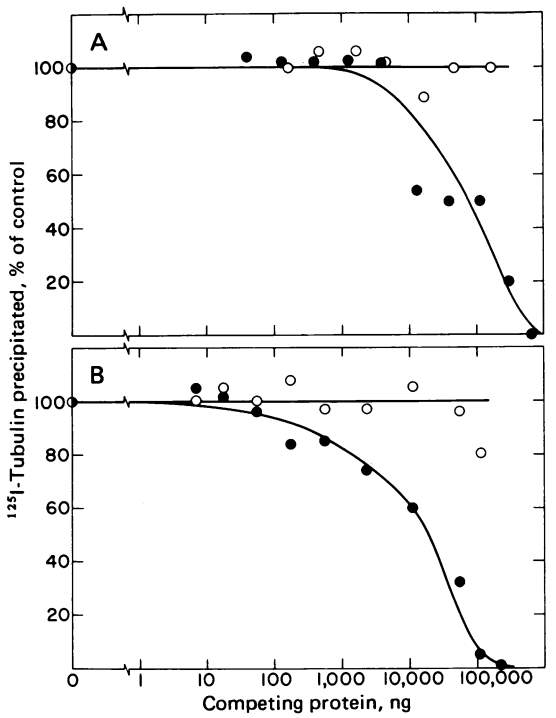

FIG. 2. Quantitation of crossreacting material in extracts. Extracts of *E. coli* (○) and *S. cerevisiae* (●) were tested for their ability to inhibit the binding of brain ^{125}I-tubulin either to purified anti-brain tubulin IgG (*A*) or to anti-*Chlamydomonas* tubulin serum (*B*) by the radioimmunoassay presented in the legend to Fig. 1.

labeled tubulin was supported by the fact that, even at the highest concentrations of yeast and bacterial extract used, >90% of the radioactivity was still acid-precipitable at the end of the incubation period in the radioimmunoassay. As expected, yeast extract competed against the binding of labeled tubulin by the anti-*Chlamydomonas* tubulin serum better than against the anti-brain tubulin serum, by about an order of magnitude.

Yeast Protein Can Be Copolymerized with Brain Microtubules. The immunological relatedness suggested that putative yeast tubulin might be incorporated into microtubules if yeast extract were included in the *in vitro* assembly systems that have been devised for brain (10). When brain microtubules were formed by several cycles of assembly and disassembly in the presence of ^{35}S-labeled yeast extracts, autoradiography revealed that the final pellets were greatly enriched in a species that migrated just slightly faster than brain tubulin marker upon electrophoresis in polyacrylamide gels containing $NaDodSO_4$. Under conditions that resolved the α- and β-chains of brain tubulin well (Fig. 3), autoradiography showed that the major species in the copolymerized yeast proteins also split into two bands (α' and β') which were distinct from phosphoglycerate kinase and enolase, two abundant soluble proteins. That these two bands were recovered because they became integral components of microtubules rather than nonspecifically associated with the brain proteins was supported by the following additional observations. First, the α' and β' bands were retained and greatly enriched over their presence in whole extract (Fig. 3), after as many as three cycles of polymerization and depolymerization. Second, addition of high concentrations of commercial preparations of phosphoglycerate kinase (Boehringer, 100 μg/ml), enolase (Sigma, 400 μg/ml), or bovine serum albumin (400 μg/ml) to the copolymerization mixtures reduced neither the total radioactivity recovered in the final pellets nor the intensity of the α' and β' bands in autoradiograms of the electrophoretically separated proteins (results not shown).

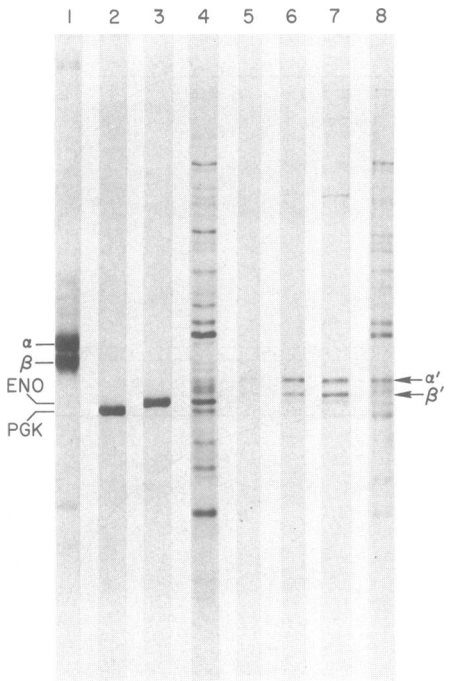

FIG. 3. Copolymerization of ^{35}S-labeled yeast proteins with brain microtubules. Brain microtubule protein (2.5 mg/ml) was subjected to one cycle of assembly and disassembly (10) just before use and portions (400 μl) of the cold depolymerized material were mixed with 25-, 100-, and 300-μl samples of a ^{35}S-labeled yeast extract (3.4 mg/ml, 3.2×10^4 cpm/μg). The mixtures were carried through two cycles of assembly and disassembly, and then repolymerized. The final polymerized pellets were rinsed gently with buffer and dissolved in 200 μl of electrophoresis sample buffer by boiling for 3 min, and portions (5 μl) were subjected to gel electrophoresis. The radioactive species were revealed by autoradiography of the dried gel on Kodak XS-5 x-ray film for 3 days. Nonradioactive species were stained with Coomassie brilliant blue. Lane 1: porcine brain tubulin (α and β) (2.5 μg); lane 2: yeast phosphoglycerate kinase (PGK) (2 μg); lane 3: yeast enolase (ENO) (2 μg); lane 4: ^{35}S-labeled yeast extract (34,000 cpm); lanes 5, 6, and 7: copolymerized pellets from mixtures of ^{35}S-labeled yeast extract and brain microtubule protein, wt/wt, of ~1:10 (500 cpm), ~1:3 (1800 cpm), and ~1:1 (3500 cpm), respectively; lane 8: ^{35}S-labeled yeast nuclei (10,500 cpm).

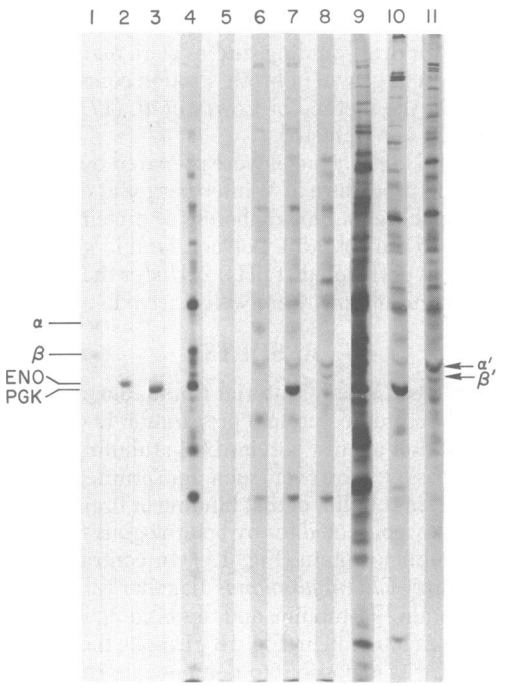

FIG. 4. Immunoprecipitation of ^{35}S-labeled yeast proteins by antitubulin antibody. Samples (~1.0 mg) of ^{35}S-labeled yeast extracts (~5 mg/ml, $\sim 2 \times 10^5$ cpm/μg) were mixed with 5–10 mg of normal rabbit serum, antiserum against yeast phosphoglycerate kinase, or antiserum against *Chlamydomonas* tubulin, in a final volume of 0.3–0.5 ml, and incubated for 16 hr at 4°. Protein A-Sepharose beads (Pharmacia, 250–400 μg of protein A) were added and incubated with gentle agitation for an additional 4 hr at 4°. The beads were sedimented by centrifugation at $1000 \times g$ for 2 min at 4°, washed twice by successive resuspensions and recentrifugations in buffer, and finally resuspended in electrophoresis sample buffer. The bound proteins were solubilized and subjected to electrophoresis and autoradiography, as described in the legend to Fig. 3. Lane 1: porcine brain tubulin (α and β) (~1 μg); lane 2: yeast enolase (ENO) (~1 μg); lane 3: yeast phosphoglycerate kinase (PGK) (~1 μg); Exp. 1—lane 4: yeast extract ($\sim 7 \times 10^5$ cpm); lane 5: no rabbit serum added ($\sim 5 \times 10^3$ cpm); lane 6: normal rabbit serum ($\sim 4 \times 10^4$ cpm); lane 7: antiserum against yeast PGK ($\sim 7 \times 10^4$ cpm); lane 8: antiserum against *Chlamydomonas* tubulin ($\sim 6 \times 10^4$ cpm). Exp. 2—lane 9: yeast extract ($\sim 4 \times 10^5$ cpm); lane 10: antiserum against yeast PGK ($\sim 4 \times 10^4$ cpm); lane 11: antiserum against *Chlamydomonas* tubulin ($\sim 4 \times 10^4$ cpm).

Last, with weight ratios of yeast extract protein to brain microtubule protein as high as 5:1, microtubules were still observed in the electron microscope and, although somewhat fewer in total number, these tubules were often much longer than those seen in control samples lacking yeast extract. Microtubules were not seen in samples with yeast extract alone.

Yeast Nuclei Contain Tubulin-Like Proteins. Studies with the electron microscope have shown that microtubules in yeast cells only are found attached to the spindle pole body of the nuclear membrane (2, 3). Because both extra- and intranuclear microtubules radiate from this structure, microtubule components should be among the proteins recovered in isolated yeast nuclei. The α′ and β′ bands were found to be among the major components in preparations of nuclei from growing cultures of yeast cells (Fig. 3).

Tubulin-Like Proteins Can Be Immunoprecipitated from Yeast Extracts by Antitubulin Antibody. The radioimmunoassays suggested that another way to confirm the relatedness of the α′ and β′ bands to tubulin subunits was to use antitubulin antibody to recover these proteins from yeast extracts. Indeed, these same bands were among the proteins found in immunoprecipitates obtained with antiflagellar tubulin serum, but not with either normal rabbit serum or antiserum against phosphoglycerate kinase (Fig. 4). The antitubulin antibody seemed to retain the α′ band somewhat preferentially. The apparent differential recovery of the α′ band may be accounted for, at least in part, by the presence of a contaminating protein of molecular weight similar to that observed in controls, or may reflect a difference in the content of sulfur-containing amino acids between α′ and β′, or may be due to selective degradation of the β′ protein during the extended time used in the immunoprecipitation regimen.

Yeast Extracts Do Not Contain a Colcemid-Binding Protein. We felt that the drug-binding properties of α′ and β′ might provide an additional means for demonstrating their relatedness to other eukaryotic tubulins. Haber *et al.* had reported (20) that an activity that bound the antimitotic drug Colcemid could be detected in crude extracts of yeast cells. They suggested that this component might be tubulin dimer because it eluted at the excluded volume of a Sephadex G-100 column and was acidic, as judged by its retention on DEAE-cellulose columns. We were unable to reproduce the results of DEAE-cellulose chromatography found by Haber *et al.*, but were able to detect an apparent Colcemid-binding activity on gel filtration columns. However, this activity also was excluded from Sephadex G-200 (Fig. 5*A*), and even from Sepharose CL-6B (Fig. 6). Digestion of the sample with 500 μg of Pronase per ml prior to chroma-

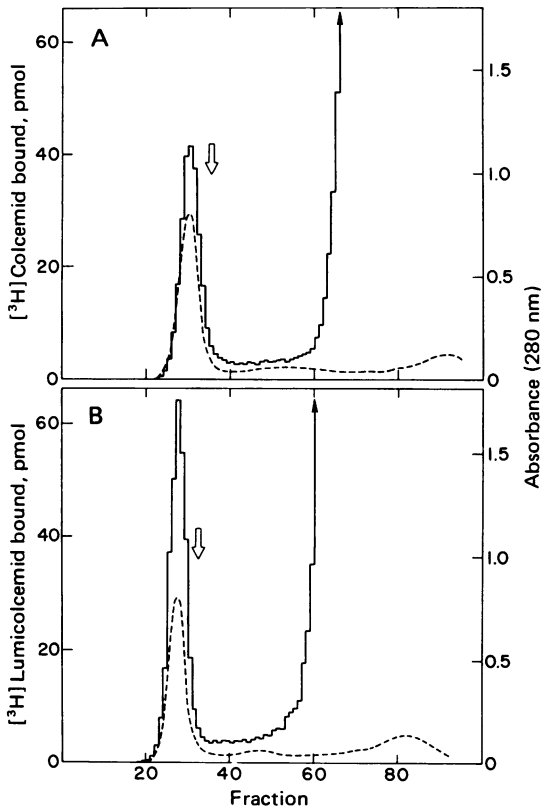

FIG. 5. Sephadex G-200 chromatography of drug-binding activity. (*A*) To 1 ml of a yeast extract (15 mg/ml) in 50 mM potassium phosphate (pH 6.8) was added 10 μl of 10 mM GTP and 50 μl of 10 mM [^{3}H]Colcemid (6.8×10^8 cpm/ml) in 2 mM acetic acid, and the mixture was incubated at 30° for 1 hr. The solution was chilled rapidly in ice and, after addition of β-galactosidase (Worthington, 4 μg), was subjected to filtration through a bed (1.9 × 31.5 cm) of Sephadex G-200 that had been equilibrated at 4° with 0.1 M KCl 50 mM potassium phosphate (pH 6.8). Fractions of 1 ml were collected. (*B*) Essentially the same procedure was followed except that 50 μl of [^{3}H]lumicolcemid was added. The [^{3}H]lumicolcemid was prepared just before use by irradiating glass capillaries containing portions of the [^{3}H]Colcemid stock solution for 50–70 hr at a distance of 2.5 cm from a short-wavelength UV lamp (model V41, Ultra-Violet Products). Completeness of the conversion was monitored by the change in absorbance at 355 nm (16). Arrows mark the elution position of the peak of β-galactosidase activity, as measured by *o*-nitrophenyl-β-D-galactopyranoside hydrolysis. (—) Radioactivity; (- - -) $A_{280\ nm}$.

tography on Sepharose 6B reduced the absorbance of the excluded peak by 90% but only lowered the radioactivity in these fractions by 16% (results not shown). In contrast, predigestion with a mixture of 500 μg each of phospholipases A and B per ml reduced both the radioactivity and the absorbance in the excluded fractions by 50% (results not shown). Moreover, as much or more binding to this fraction was obtained when Colcemid that had been converted to its inactive derivative, lumicolcemid, was used (Fig. 5*B*). It has been amply demonstrated that lumicolcemid does not bind to brain tubulin (16), but does bind nonspecifically to membrane fractions from various tissues (21).

MeBzaC is a drug whose herbicidal and fungicidal properties have been attributed to an antimitotic action, perhaps involving direct binding to tubulin (22, 23). Even though this drug, like Colcemid, is a rather hydrophobic molecule, in preliminary experiments [^{3}H]MeBzaC-binding fractions of yeast extracts were well included in elution profiles of Sepharose 6B columns (results not shown).

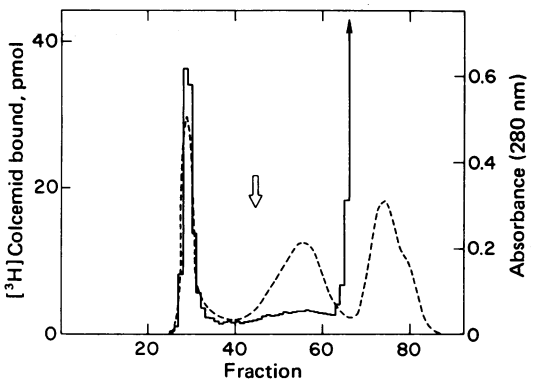

FIG. 6. Sepharose CL-6B chromatography of drug-binding activity. A portion (1 ml) of a yeast extract (16 mg/ml), prepared and incubated with [^{3}H]Colcemid as described in the legend to Fig. 5, was subjected to filtration through a bed (1.9 × 32 cm) of Sepharose CL-6B that had been equilibrated at 4° with 0.1 M KCl/50 mM potassium phosphate (pH 6.8). Fractions of 1 ml were collected. Arrow marks the elution position of the peak of β-galactosidase activity. (—) Radioactivity; (- - -) $A_{280\ nm}$.

DISCUSSION

The major protein component of the microtubular organelles in higher eukaryotic cells is an acidic protein, tubulin (1, 8). Each native tubulin molecule seems to be a heterodimer of two distinct polypeptide chains (α and β), each of about 50,000 molecular weight (24). Depending on the tissue or organism, tubulin may represent 3–20% of the total soluble protein of higher cells. The high affinity of tubulin for certain plant alkaloids with antimitotic activity (especially colchicine and Colcemid) has provided a probe for identifying and purifying tubulins from higher systems (25). Despite these biochemical advantages, study of the regulation of tubulin synthesis and of the control of other aspects of microtubule assembly and function in higher cells has been hampered by the difficulty of obtaining mutants defective in these processes in higher systems. Because of their genetic tractability, lower eukaryotic organisms offer the possibility of such an approach to these problems. In particular, the synthesis and function of spindle and cytoplasmic microtubules should be amenable to attack in the yeast *S. cerevisiae*, where the role of these organelles in cellular functions is not complicated by the presence of cilial or flagellar structures. As the first step in such an analysis, we have shown here that yeast cells contain a protein analogous to higher cell tubulins, on the basis of several immunological and biochemical criteria.

By a radioimmunoassay (Fig. 1), yeast extracts appeared to contain material which competed for the binding of brain tubulin by antibodies directed against both homologous and heterologous tubulins (Fig. 2). To determine if the immunological relatedness reflected functional characteristics common to all tubulins, we attempted to polymerize yeast proteins along with porcine brain microtubules assembled *in vitro*. We found that certain yeast proteins did interact selectively with such microtubules. The major yeast species incorporated, which we have termed α′ and β′ (Fig. 3), seemed to be of somewhat lower molecular weight (46,000 and 45,000, respectively) than their higher cell counterparts (50,000 and 48,000, respectively), as judged by their mobilities upon gel electrophoresis with respect to yeast protein standards of known molecular weight. This copolymerization method had been used to demonstrate the presence of α and β tubulin-like proteins in extracts of the filamentous fungus *Aspergillus niger* (26). In fact, during the course of our work, two preliminary reports appeared that described copolymerization studies with yeast extracts. In

agreement with our results, one of these other investigations (27) found that two major microtubule-associated yeast bands had mobilities on gel electrophoresis similar to, but greater than, those for the α and β chains of chicken brain tubulin. In contrast, in a study using a different gel electrophoretic system (28), the major copolymerized yeast components comigrated with the α and β subunits of rat brain tubulin.

Since the microtubules of yeast cells remain associated with the nucleus throughout the cell cycle (2, 3), the presence of the α' and β' bands as constituents of isolated yeast nuclei supported their assignment as yeast tubulin subunits (Fig. 3). Further confirmation of the specification of the α' and β' bands as tubulin-like molecules was their selective immunoprecipitation by antitubulin antibody (Fig. 4).

We examined the possibility that the α' and β' components could be detected by their affinity for antimitotic drugs known to have a mode of action through direct interaction with tubulins. Our results indicated that the Colcemid-binding activity of yeast extracts reported previously (20) was not due to a tubulin-like protein, but probably to nonspecific association of the drug with membrane fragments or lipid micelles (Figs. 5 and 6). In this regard, yeast extracts resemble those of other lower eukaryotes in which the affinity for this plant alkaloid is very low or nonexistent (29, 30). More promising were our preliminary results with MeBzaC, which interacts with a tubulin-like protein in extracts of *A. niger* (23). A MeBzaC-binding fraction of a molecular size appropriate for tubulin dimer could be detected in yeast extracts by column chromatography.

Taken together, the findings presented here strongly suggest that the α' and β' bands are the subunits of yeast tubulin. Although these proteins appear to constitute only 1% or less of the total soluble protein in high-speed supernatant solutions of yeast extracts, large-scale purification of these species would appear to be the best way to further characterize these proteins and to confirm their identity as yeast tubulin subunits.

We are grateful to Denyse Goff and Steven Prescop for their enthusiastic technical assistance and to Dr. Leslie Wilson for useful comments and suggestions throughout this work. This investigation was supported by U.S. Public Health Service Research Grant GM21841 from the National Institute of General Medical Sciences and Biomedical Research Support Grant 1S07RR07006 from the Department of Health, Education and Welfare (to J.T.), and by U.S. Public Health Service Predoctoral Traineeships, GM07232 (to P.B.) and GM01389 (to L.H.).

1. Snyder, J. & McIntosh, J. R. (1976) *Annu. Rev. Biochem.* **45**, 699–720.
2. Matile, P. H., Moor, H. & Robinow, C. F. (1969) in *The Yeasts*, eds. Rose, A. H. & Harrison, J. S. (Academic, New York), Vol. 1, pp. 219–302.
3. Byers, B. & Goetsch, L. (1975) *J. Bacteriol.* **124**, 511–523.
4. Peterson, J. B. & Ris, H. (1976) *J. Cell Sci.* **22**, 219–242.
5. Mitchison, J. M. & Carter, B. L. A. (1975) *Methods Cell Biol.* **11**, 201–219.
6. Mortimer, R. K. & Hawthorne, D. C. (1975) *Methods Cell Biol.* **11**, 221–233.
7. Sherman, F., Fink, G. R. & Lawrence, C. R. (1974) *Methods in Yeast Genetics: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).
8. Olmsted, J. B. & Borisy, G. G. (1973) *Annu. Rev. Biochem.* **42**, 507–540.
9. Bisson, L. & Thorner, J. (1977) *J. Bacteriol.* **132**, 44–50.
10. Weingarten, M. D., Suter, M. M., Littman, D. R. & Kirschner, M. W. (1974) *Biochemistry*, **13**, 5529–5540.
11. Wiche, G. & Cole, R. D. (1976) *Exp. Cell Res.* **99**, 15–22.
12. Eipper, B. A. (1972) *Proc. Natl. Acad. Sci. USA*, **69**, 2283–2287.
13. Weber, K., Wehland, J. & Herzog, W. (1976) *J. Molec. Biol.* **102**, 817–829.
14. Piperno, G. & Luck, D. J. L. (1977) *J. Biol. Chem.* **252**, 383–391.
15. Hunter, W. M. (1967) in *Handbook of Experimental Immunology*, ed. Weir, D. M. (Blackwell's, London), 2nd Ed., pp. 17.1–17.36.
16. Wilson, L., Bamburg, J. R., Mizel, S. B., Grisham, L. M. & Creswell, K. M. (1974) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **33**, 158–166.
17. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275.
18. Wintersberger, U., Smith, P. & Letnansky, K. (1973) *Eur. J. Biochem.* **33**, 123–130.
19. Laemmli, U. K. (1970) *Nature (London)* **227**, 680–685.
20. Haber, J. E., Peloquin, J. G., Halvorson, H. O. & Borisy, G. G. (1972) *J. Cell Biol.* **55**, 355–367.
21. Stadler, J. & Franke, W. W. (1974) *J. Cell Biol.* **60**, 297–303.
22. Hammerschlag, R. & Sisler, H. D. (1973) *Pestic. Biochem. Physiol.* **3**, 42–54.
23. Davidse, L. C. & Flach, W. (1977) *J. Cell Biol.* **72**, 174–193.
24. Luduena, R. F., Shooter, E. M. & Wilson, L. (1977) *J. Biol. Chem.* **252**, 7006–7014.
25. Wilson, L. & Bryan, J. (1974) *Adv. Cell Mol. Biol.* **3**, 21–72.
26. Sheir-Neiss, G., Nardi, R. V., Gealt, M. A. & Morris, N. R. (1976) *Biochem. Biophys. Res. Commun.* **96**, 285–290.
27. Shriver, K. & Byers, B. (1977) *J. Cell Biol.* **75**, 297 (abstr.).
28. Water, R. D. & Kleinsmith, L. J. (1976) *Biochem. Biophys. Res. Commun.* **70**, 704–708.
29. Burns, R. G. (1973) *Exp. Cell Res.* **81**, 285–292.
30. Flanagan, D. & Warr, J. R. (1977) *FEBS Lett.* **80**, 14–18.