Monoclonal Antibodies Specific for an Acetylated Form of α-Tubulin Recognize the Antigen in Cilia and Flagella from a Variety of Organisms

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ABSTRACT Seven monoclonal antibodies raised against tubulin from the axonemes of sea urchin sperm flagella recognize an acetylated form of α-tubulin present in the axoneme of a variety of organisms. The antigen was not detected among soluble, cytoplasmic α-tubulin isoforms from a variety of cells. The specificity of the antibodies was determined by in vitro acetylation of sea urchin and *Chlamydomonas* cytoplasmic tubulins in crude extracts. Of all the acetylated polypeptides in the extracts, only α-tubulin became antigenic. Among *Chlamydomonas* tubulin isoforms, the antibodies recognize only the axonemal α-tubulin isoform acetylated in vivo on the ε-amino group of lysine(s) (L'Hernault, S. W., and J. L. Rosenbaum, 1985, *Biochemistry*, 24:473–478). The antibodies do not recognize unmodified axonemal α-tubulin, unassembled α-tubulin present in a flagellar matrix-plus-membrane fraction, or soluble, cytoplasmic α-tubulin from *Chlamydomonas* cell bodies. The antigen was found in protein fractions that contained axonemal microtubules from a variety of sources, including cilia from sea urchin blastulae and *Tetrahymena*, sperm and testis from *Drosophila*, and human sperm. In contrast, the antigen was not detected in preparations of soluble, cytoplasmic tubulin, which would not have contained tubulin from stable microtubule arrays such as centrioles, from unfertilized sea urchin eggs, *Drosophila* embryos, and HeLa cells. Although the acetylated α-tubulin recognized by the antibodies is present in axonemes from a variety of sources and may be necessary for axoneme formation, it is not found exclusively in any one subset of morphologically distinct axonemal microtubules. The antigen was found in similar proportions in fractions from sea urchin sperm axonemes enriched for central pair or outer doublet B or outer doublet A microtubules. Therefore the acetylation of α-tubulin does not provide the mechanism that specifies the structure of any one class of axonemal microtubules. Preliminary evidence indicates that acetylated α-tubulin is not restricted to the axoneme. The antibodies described in this report may allow us to deduce the role of tubulin acetylation in the structure and function of microtubules in vivo.

Microtubules are implicated in a variety of cellular functions including mitosis, cytokinesis, intracellular transport, the maintenance of cell shape, and the formation of motile systems such as eukaryotic cilia and flagella (5). In many cases, microtubules involved in different functions are organized into morphologically different arrays. Although the structure of a variety of microtubule frameworks has been described in great detail, the molecular mechanisms that specify the assembly of morphologically and functionally different microtubule arrays have yet to be determined. The diversity among microtubular structures may be generated by the association of microtubule components with accessory proteins localized in different parts of the cell (37), by the co-polymerization of different α- and β-tubulin subunits, or by a combination of these mechanisms. Structurally different tubulin subunits have been identified as the products of different genes (for review, see reference 4) and as the result of posttranslational modifications. At least three modifications of tubulin subunits

have been described: the phosphorylation of β-tubulin from brain (6), the removal of the carboxyterminal tyrosine from α-tubulin in vertebrate tissues (3), and the acetylation of the ε-amino group of lysine(s) in α-tubulin from *Chlamydomonas* flagella (18).

Posttranslational modification of tubulin subunits may be required for the assembly of particular kinds of microtubule arrays. The level of β-tubulin phosphorylation increases with the assembly of axonal microtubules during neurite outgrowth in mouse neuroblastoma cells (8). Immunofluorescence staining using polyclonal antibodies to the carboxyterminal sequence of α-tubulin with and without the terminal tyrosine demonstrated that the detyrosinated form of α-tubulin is involved in the formation of distinct subpopulations of microtubules (10). Acetylation of α-tubulin has been correlated with the assembly of the flagellar axoneme in *Chlamydomonas* (17). This modification is reversible; axonemal α-tubulin appears to be deacetylated during resorbtion of flagella (19). Although axonemal tubulin of other cells appears to be posttranslationally modified (9, 20, 30), the acetylation of α-tubulin has not yet been observed in other organisms or in other microtubule structures.

This report describes the identification and use of seven monoclonal antibodies that were raised against tubulin from the axonemes of sea urchin sperm flagella and that recognize an acetylated form of α-tubulin. Our results indicate that acetylation of α-tubulin is an important feature of axoneme assembly in a variety of organisms. Tubulin acetylation may play a prevalent role in the differentiation of microtubule structure and function.

MATERIALS AND METHODS

Preparation of Hybridomas and Monoclonal Antibodies: Seven independent cell lines producing IgGs against α-tubulin from sea urchin sperm axoneme were selected from among hybridomas derived from the fusion of a myeloma with mouse spleen cells in two separate experiments. The hybridomas are listed in Table I. The mice were immunized with a 15 S complex containing the 330,000-, 134,000-, and 126,000-mol-wt dynein subunits from the outer arms of sea urchin sperm axonemes. The immunogen also contained tubulin subunits, a 62,000-mol-wt polypeptide, and other minor components. The characterization of the immunogen and the procedures followed to obtain hybridomas and monoclonal antibodies against axonemal proteins have been described in a recent report (25). Several hybridomas secreting monoclonal antibodies to dynein subunits and to other axonemal components were isolated from the same hybridoma populations.

Two hybridomas, 3A5 and 4A2, secreting antibodies against tubulin from *Drosophila*, were prepared as follows. Embryonic tubulin was purified as described below by two rounds of assembly/disassembly from staged *Drosophila ORE-R* embryos 11–14 h old. Female BALB/c mice were hyperimmunized by several intraperitoneal and footpad injections of purified tubulin in Freund's adjuvant. Spleen cells were then fused to the parent myeloma cell line P3X63-Ag8.653 following a modification of the procedure of Galfre et al. (7) as described by Oi and Herzenberg (24). Positive hybridoma cell cultures were identified by plate assay using the immunogen as the test antigen. 3A5 was cloned three times and 4A2 was cloned twice by limiting dilution.

Gel Electrophoresis and Immunoblotting of Sea Urchin, Chlamydomonas, Tetrahymena, and Human Proteins: Electrophoresis was performed on slab gels of reduced size (6 cm long, 20 cm wide, and 0.075 cm thick). Volumes of the samples analyzed were 5–10 μl. Stacking buffer, cathodic and anodic buffers were prepared following the method of Neville (22). The stacking gel contained 3.2% acrylamide and 0.2% *N,N'*-methylene-bis-acrylamide. The running gel contained 5% acrylamide, 0.2% *N,N'*-methylene-bis-acrylamide, 25 mM Tris/glycine, pH 8.4, and 0.1% SDS (L-5750, lot 45C-0084, Sigma Chemical Co., St. Louis, MO). Electrophoresis was performed at 20 mA for 60 min.

Isoelectric focusing was performed on slab gels (14 cm long, 12 cm wide, and 0.075 cm thick. The focusing gel contained 1 ml each of pH 5–7 and 4–6 ampholines (LKB Instruments Inc., Bromma, Sweden). Gels and protein samples were prepared as described by Ferro-Luzzi Ames and Nikaido (6a) and Piperno et al. (26). 30–100-μg samples in a volume of 100 μl were applied at the acid end of the gel. Electrophoresis was performed at 1,000 V for 24 h.

Polypeptides were transferred from polyacrylamide gels to nitrocellulose sheets (HAWP 304 FO, Millipore Corp., Bedford, MA) following the method of Towbin et al. (35). Transfer of polypeptides in the range of 50,000 mol wt from SDS polyacrylamide gels was performed in 25% methanol, 192 mM glycine, 25 mM Tris, pH 8.3, at 400 mA for 90 min. Transfer of tubulin subunits from isoelectrofocusing gels was performed in 0.7% acetic acid at 400 mA for 3 h (35). After transfer, nitrocellulose sheets were incubated in 1× bovine serum albumin (BSA) solution overnight at room temperature and either used the next day or stored in BSA solution at 4°C until needed. BSA solution was made in a 100× stock solution consisting of 2% BSA fraction V, 2% Ficoll Type 40, 2% polyvinylpyrrolidone 40T (all from Sigma Chemical Co.), 2 mM EDTA, 10 mM Tris/Cl, pH 7.4. After being washed in 0.13 M NaCl, 0.01 M Na phosphate, pH 6.8, nitrocellulose sheets were incubated in hybridoma supernatant containing antibodies for at least 12 h at room temperature. Nitrocellulose sheets were then washed in 0.5% BSA fraction V, 0.25% Nonidet P-40, 2 mM EDTA, 0.14 M NaCl, 10 mM Tris/Cl, pH 7.4. ^{125}I-labeled secondary antibodies ($F(ab')_2$ fragment goat anti-mouse IgG, heavy and light chains, lot 15845, Cappel Laboratories, Cochranville, PA) were incubated with the nitrocellulose sheets. These were then washed and processed for autoradiography.

The quantitative dot-immunobinding assay was performed by the method of Jahn et al. (13) modified as follows. Nitrocellulose sheets upon which samples had been spotted were processed and incubated with antibodies as described above. The amount of radioactivity bound to axonemal and cytoplasmic tubulin from sea urchin was analyzed as a function of the amount of protein present in the samples. A linear relationship was obtained between 0.1 and 1 μg of sample protein. 0.5–0.7 μg sample protein was used for antibody dilution experiments.

Isolation of Axonemes and Cytoplasmic Soluble Tubulins: Sea urchin *Strongylocentrotus purpuratus* sperm axonemes were prepared as described by Piperno (25). Taxol-stabilized microtubule proteins were prepared from unfertilized sea urchin eggs by the modification of the method of Vallee (36) described by Scholey et al. (31). Taxol was obtained from Natural Products Branch of National Cancer Institute, Bethesda, MD. Cilia from sea urchin blastulae were prepared as described by Stephens (33). *Chlamydomonas* flagella and axonemes were prepared as described by Huang et al. (12). Membrane plus matrix proteins from *Chlamydomonas* flagella were prepared from 0.6 ml suspension of flagella that contained 8 mg/ml of protein, by the addition of 10% Nonidet P-40 to a final concentration of 1%. Soluble proteins were separated from the insoluble residue by centrifugation at 30,000 rpm in a SW65 Beckman rotor (Beckman Instruments Inc., Palo Alto, CA) for 30 min. Preparation of cytoplasmic tubulin from *Chlamydomonas* cell bodies was performed as described by Piperno and Luck (27). Human sperm was obtained from the Population Council, New York. Human sperm tails were prepared by differential centrifugation after sonication for 30 s at 50 W in 0.13 M NaCl, 0.01 M Na phosphate, pH 6.8, 0.1 mM phenylmethylsulfonyl fluoride at 0°C. Axonemes were obtained by the addition of 10% Nonidet P-40 to a final concentration of 0.5% and a final centrifugation at 20,000 rpm in a SS34 Sorvall rotor (E. I. DuPont de Nemours & Co., Inc., Sorvall Instruments Div., Newtown, CT) for 20 min. HeLa cells were given to us by Dr. N. Heintz of The Rockefeller University. Taxol-stabilized microtubules from HeLa cells were prepared by the method of Vallee (36). *Tetrahymena* cilia were a gift from Dr. Winfield Sale, Emory University, Atlanta, GA.

Acetylation of Proteins: Samples, containing 1–3 mg/ml of protein, of sea urchin egg extract, taxol-stabilized microtubules prepared from the egg extract, and cytoplasmic tubulin from *Chlamydomonas* cell bodies were each acetylated in 2 M Na acetate or 1 M Na phosphate, pH 8, by the addition of 1% acetic anhydride at 25°C. Under these conditions acetylation of ε-amino groups of lysine should be complete (21). However, some *O*-acetyltyrosine also may be formed. Tyrosyl residues may be deacylated by hydroxylamine at neutral pH (21). To ensure that the antigenicity of α-tubulin against antibody 6-11B-1 and all other antibodies of the set was due to acetylation of lysine and not of tyrosine, we treated the acetylated egg extract with 0.1% hydroxylamine, 5 mM Tris/Cl, pH 7. The α-tubulin remained antigenic after this treatment. Modified and unmodified proteins were dialyzed against 5 mM Tris/Cl, pH 8.3, 0.1 mM phenylmethylsulfonyl-fluoride before being processed for electrophoresis.

Sample Preparation, Electrophoresis, and Antibody Staining of Drosophila Proteins: *Drosophila* samples were prepared from the *ORE-R* wild-type strain. Sperm was isolated by dissection from seminal vesicles of male flies separated from females for 2 to 4 wk. Testes were dissected from

1- to 4-d-old males and either boiled in sample buffer immediately or labeled by incubation in the presence of [^{35}S]methionine as described by Kemphues et al. (14) before boiling. Embryonic tubulin was prepared from staged embryos in midembryonic development, 11.5–15.5 h after oviposition at 25°C. Tubulin was purified by two rounds of assembly/disassembly in 4 M glycerol and 1 mM GTP as described by Raff et al. (28). Two-dimensional gel electrophoresis was performed with nonequilibrium pH gradients in the first dimension as described by O'Farrell et al. (23) and as modified by Waring et al. (38) but using one part pH 3.5–10 (LKB Instruments Inc.) and four parts pH 5–6 (Serva Fine Biochemicals Inc., Garden City Park, NY) ampholines. One-dimensional SDS PAGE followed the procedure of Laemmli (16), except that the lower gel was 9% acrylamide and 0.52% *N,N'*-methylene-bis-acrylamide. Proteins separated by gel electrophoresis were transferred to nitrocellulose paper by the method of Towbin et al. (35). For antibody staining experiments, the nitrocellulose strips containing transferred proteins were preincubated for 1–2 h at room temperature in 10% fetal calf serum in phosphate-buffered saline (PBS) (3 mM KCl, 1.5 mM KH_2PO_4, 137 mM NaCl, 4 mM Na_2HPO_4, pH 7.2) with 0.05% polyoxyethylene sorbitan monolaurate (Tween 20). Strips were incubated in primary antibody in PBS/Tween, then incubated in peroxidase-conjugated IgG fraction of goat antiserum against mouse IgG, IgM, and IgA heavy and mixed light chains (Cappel 3211-0231). Bound antibody was localized by staining with 3,3' diaminobenzidine tetrahydrochloride. All antibody dilutions were made in fetal calf serum/PBS/Tween. Primary antibodies consisted of dilutions of supernatants from hybridoma cultures. Routine dilutions were 1:100 for 3A5 and 4A2 and 1:10 for 6-11B-1.

Differential Solubilization of Axonemal Microtubules and Electron Microscopy: For differential solubilization of axonemal components, a solution of 10% Sarkosyl (Na dodecyl sarcosinate, L-5125, lot 51F-0186, Sigma Chemical Co.) was added to a 1 mg/ml suspension of sea urchin sperm axonemes in 0.9 M sucrose, 0.1 M NaCl, 1 mM $MgCl_2$, 0.2 mM EDTA, 0.2 mM phenylmethylsulfonyl fluoride, 5 mM Tris/Cl, pH 7, 0.5% Nonidet P-40, to final concentrations of 0.2 and 0.4% Sarkosyl, and the suspensions were incubated at room temperature for 30 min. Soluble proteins were separated from insoluble residues by centrifugation in a Sorvall SS34 rotor at 17,000 rpm for 15 min. Outer doublet microtubules recovered from the treatment by 0.4% Sarkosyl were suspended at a protein concentration of 9 mg/ml in 0.1 mM EDTA, 0.5 mM dithiothreitol, 1 mM Tris/Cl, pH 8, and exposed to a temperature of 40°C for 4 min (34). The insoluble residue was separated by centrifugation as above. Axonemes and the 0.2 and 0.4% Sarkosyl-insoluble residues were processed for electron microscopy as described by Huang et al. (12). Quantitative analysis of axoneme cross sections was performed on several micrographs of thin sections taken in different parts of the pellets. More than 100 cross sections were examined in each case.

RESULTS

Monoclonal Antibodies to the Major α-Tubulin of Sea Urchin Sperm Axoneme Do Not Bind to the α-Tubulin of Unfertilized Sea Urchin Egg

Seven monospecific monoclonal antibodies against the major form of α-tubulin of sea urchin sperm axoneme have the following similar characteristics. They bind to the major form of α-tubulin in the axoneme but do not recognize α-tubulin isolated from the cytoplasm of unfertilized sea urchin egg by taxol-induced polymerization. The binding of the antibodies to the α-tubulins of sperm axonemes and unfertilized eggs was analyzed by immunoblots of gel electrophoretograms and by a quantitative immunobinding assay of proteins fixed on nitrocellulose as described in Materials and Methods. A monoclonal antibody raised against embryonic α-tubulin of *Drosophila*, 3A5, and a polyclonal antibody to axonemal α-tubulin of *Chlamydomonas* (27), both of which cross-react with α-tubulins from a wide variety of organisms, were used as positive controls.

Fig. 1*A* shows electrophoretograms of approximately equal amounts of protein from sperm axonemes and taxol-stabilized microtubules from sea urchin egg. The two patterns are very similar. Two major bands, the α- and β-tubulins, are widely separated in both electrophoretograms. A small number of

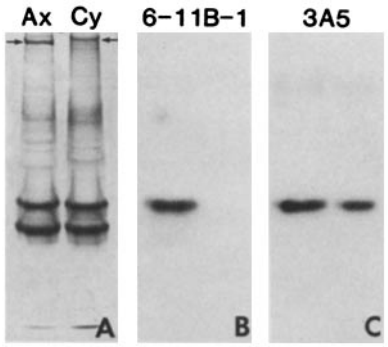

FIGURE 1 Antibody binding to α-tubulin of sea urchin sperm axoneme and taxol-stabilized microtubules from sea urchin egg. (*A*) Silver-stained polypeptides separated by PAGE. Arrows indicate dynein subunit of the axoneme (*Ax*, left lane) and the high molecular weight components of the taxol-induced microtubules (*Cy*, right lane). 1 μg of axoneme and microtubule protein were applied to the gel. Two sets of the same samples were electrophoresed in parallel and then transferred to nitrocellulose. (*B* and *C*) Autoradiograms of nitrocellulose incubated with antibody 6-11B-1 (*B*) and 3A5 (*C*).

minor components including the dynein subunit of the axoneme and the high molecular weight components of the taxol-induced microtubules (see arrows) are also visible. Fig. 1, *B* and *C* show immunoblots of the same samples incubated with, respectively, 6-11B-1, one of the antibodies to sea urchin axonemal α-tubulin, and 3A5, the antibody to embryonic tubulin of *Drosophila*. Antibody 6-11B-1 binds only to the α-tubulin of the axoneme and does not recognize any component of the sample of egg microtubules. In contrast, the control antibody, 3A5, binds to α-tubulins that are present in both samples. The polyclonal antibody to α-tubulin of *Chlamydomonas* formed the same binding pattern as the antibody 3A5 (not shown). Antibodies 6-9H-1, 3-4B-8, 4-11F-2, 4-4H-12, C-202-13, and B-37-6 were applied at the same dilution as antibody 6-11B-1 to parallel blots and showed similar binding properties. Although the staining varied in intensity with the application of different culture supernatants, each antibody recognized the α-tubulin from sperm axoneme but did not bind to any component of the egg microtubule sample.

A quantitative analysis of the binding properties of each antibody was performed by the dot assay described in Materials and Methods. Fig. 2 shows that similar amounts of the antibody 3A5 bound to aliquots of axonemes and of cytoplasmic tubulin over a wide range of antibody dilution. Antibody 6-11B-1 also bound to axonemal proteins in the same range of dilution but did not appreciably recognize cytoplasmic tubulin at any antibody dilution. The binding activities of the other six antibodies are described in Table I. All components of the set showed the same bias. They either did not bind or had very low affinity for α-tubulin from taxol-induced cytoplasmic microtubules.

Sea urchin sperm axonemes are formed by at least two α-tubulin isoforms, which occur in the axoneme in different amounts and can be resolved by gel isoelectric focusing (the major form of α-tubulin is occasionally resolved into three components). Both the major and the minor α-tubulin isoforms are detected by the antibody 3A5 in immunoblots of isoelectric focusing gels (Fig. 3*A*). The minor α-tubulin isoform, which migrates in a more basic position (Fig. 3*A*, arrow), is not recognized by antibody 6-11B-1 (Fig. 3*B*) or by

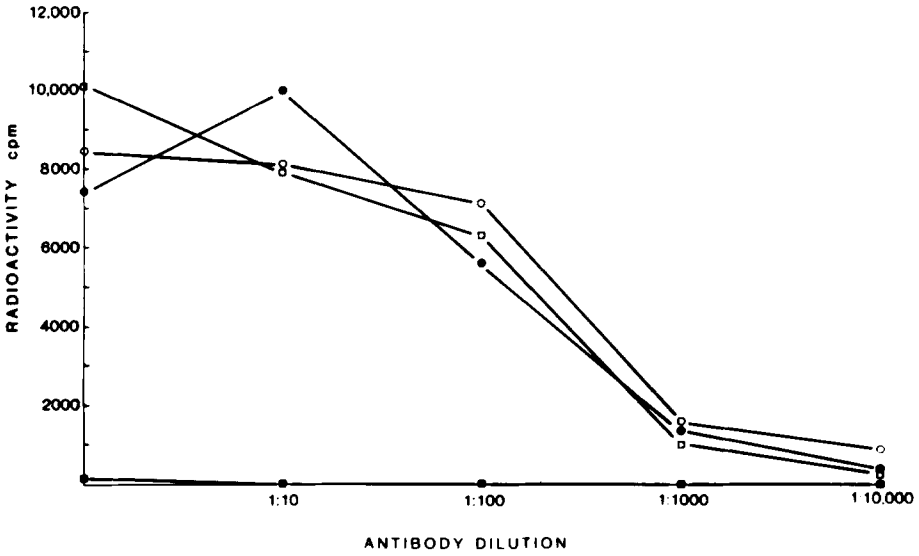

FIGURE 2 Antibody binding to protein of sea urchin sperm axoneme and taxol-stabilized microtubules from sea urchin egg, as measured by a quantitative dot assay. 0.7 μg protein was applied to nitrocellulose and incubated with different dilutions of antibody as described in Materials and Methods. ○, binding curve of antibody 3A5 to axoneme tubulin; ●, binding curve of antibody 3A5 to cytoplasmic tubulin; □, binding curve of antibody 6-11B-1 to axoneme tubulin; ■, binding curve of antibody 6-11B-1 to cytoplasmic tubulin.

any of the six other antibodies of the set. The major α-tubulin isoforms, which migrate in the more acidic position, are recognized by the general antibody 3A5 and all seven specific antibodies. The presence of the epitope in the major axonemal α-tubulin coincides with the migration of the protein to a more acidic position in isoelectric focusing gels.

The assembly of *Chlamydomonas* axonemes is accompanied by the acetylation of a major form of α-tubulin, which causes the modified subunit to migrate at a more acidic position in the isoelectric focusing dimension of two-dimensional gels (18, 20). To determine whether the epitope recognized by 6-11B-1 and by the other antibodies of the set could be formed by acetylation of α-tubulin in sea urchin axonemes, we investigated whether in vitro acetylation of cytoplasmic α-tubulin from sea urchin eggs would allow it to be recognized by the specific antibodies.

Chemical Acetylation of Cytoplasmic α-Tubulin Forms the Epitope Recognized by the Antibody 6-11B-1

Chemical acetylation of crude extracts and purified tubulin from unfertilized sea urchin eggs was performed by acetic anhydride under conditions that allow complete and specific modification of the ε-amino group of lysines. Although many polypeptide constituents of the samples appeared to be modified by the acetic anhydride, only the α-tubulin subunit was recognized by 6-11B-1 after modification.

Fig. 4, *A* and *D* show the electrophoretograms of polypeptides present in samples of crude extracts and purified tubulin from unfertilized egg before and after chemical acetylation. Many unresolved bands are visible in the patterns formed by the extracts (Fig. 4*A*). The migration pattern of many of the polypeptides in the two electrophoretograms is different. Therefore, many polypeptides in the crude extract have been modified. As shown in Fig. 4*D*, in vitro acetylation causes both α- and β-tubulin to migrate more slowly in SDS gels.

Immunoblots of electrophoretograms identical to those presented in Fig. 4, *A* and *D* are shown in Fig. 4, *B* and *C*, and *E* and *F*. Modified and unmodified samples were blotted in duplicate, and the nitrocellulose strips were incubated with either the general antibody 3A5 or with the specific antibody 6-11B-1. Fig. 4, *B* and *C* show the autoradiograms of the blots

TABLE I. *Monoclonal Antibodies to α-Tubulin of Sea Urchin Sperm Axonemes**

Hybridomas	Binding to axonemal α-tubulin‡	Binding to cytoplasmic α-tubulin‡	Ratio
	cpm	*cpm*	
6-11B-1	6,348	71	89
6-9H-1	5,880	153	38
3-4B-8	4,396	150	29
4-11F-2	4,298	54	80
4-4H-12	454	25	18
C-202-13§	2,668	26	102
B-37-6§	1,415	86	16
3A5‖	7,169	5,647	1.3

* Each antibody was tested at a dilution of 1:100 in culture medium.
‡ Binding activity was detected by ^{125}I-labeled secondary antibody. Amounts of the secondary antibody were measured in counts per minute. 350 background counts were subtracted. Numbers of counts per minute are means of two determinations.
§ These hybridomas were isolated from a population obtained by an independent fusion. They were also selected by a different screening method (25).
‖ This hybridoma secrets an antibody to embryonic α-tubulin of *Drosophila*. The antibody recognizes a variety of α-tubulins from different organisms and was used to test for the presence of α-tubulin in the samples analyzed (see Fig. 1*C*).

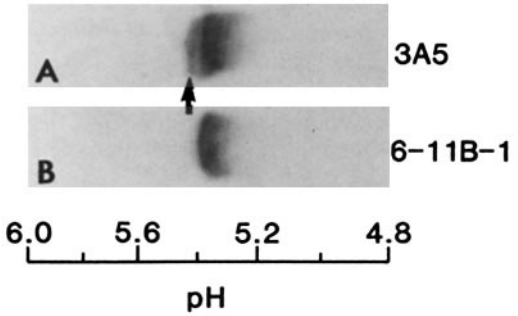

FIGURE 3 Antibody binding to α-tubulin isoforms of sea urchin sperm axoneme resolved by gel isoelectric focusing. After electrophoresis, samples were transferred to nitrocellulose. (*A* and *B*) Autoradiograms of nitrocellulose incubated with antibody 3A5 (*A*) and 6-11B-1 (*B*). The minor α-tubulin isoform is indicated by an arrow. The values of the pH gradient are indicated at the bottom.

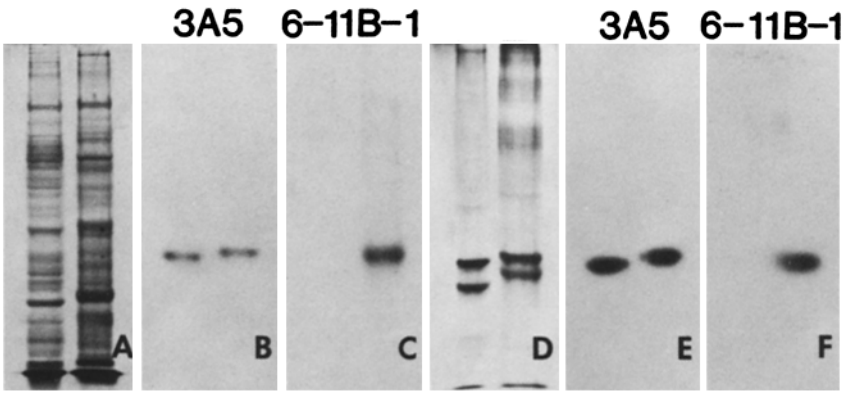

FIGURE 4 Antibody binding to chemically acetylated cytoplasmic α-tubulin from unfertilized sea urchin eggs. (*A* and *D*) Polyacrylamide gel electrophoretograms of polypeptides stained by silver. 8 μg crude extract (*A*) and 1 μg protein from taxol-stabilized microtubules (*D*) were applied to the gel. Acetylated samples are in the right lane of each panel. Two sets of the same samples were electrophoresed in parallel, transferred to nitrocellulose, and incubated with antibody. (*B*, *C*, *E*, and *F*) Autoradiograms of nitrocellulose. (*B*) Crude extracts incubated with 3A5. (*C*) Crude extracts incubated with 6-11B-1. (*E*) Cytoplasmic tubulin incubated with 3A5. (*F*) Cytoplasmic tubulin incubated with 6-11B-1.

of the extracts. The antibody 3A5 binds only to one band, corresponding to α-tubulin in both nonacetylated and acetylated extracts (Fig. 4 *B*). As in the purified tubulin sample, the modified α-tubulin from extracts migrates more slowly than the unmodified α-tubulin. Although antibody 6-11B-1 does not recognize α-tubulin in the unmodified egg extract it does recognize a single component in the acetylated extract, and this polypeptide has the same mobility as the acetylated α-tubulin (Fig. 4*C*). An identical result was obtained with the immunoblots of taxol-stabilized microtubule protein (Fig. 4, *E* and *F*).

Immunoblots of unmodified and modified extracts identical to those shown in Fig. 4*C* were obtained with all six of the other specific antibodies listed in Table I. All of them bind to cytoplasmic α-tubulin only after it has been acetylated (not shown). Although the binding of all seven specific antibodies to the α-tubulin is indeed correlated with the modification of the tubulin subunit, we do not yet know whether each antibody recognizes the same antigenic determinant.

The Antibody 6-11B-1 Binds to Axonemal α-Tubulin of Different Organisms

Complete amino acid sequences of α-tubulin from a variety of organisms are known and show a great degree of homology. Since the existence of ε-*N*-acetyllysine in the structure of the major α-tubulin from *Chlamydomonas* flagella has been proven by chemical analysis of the modified amino acid (18), we have assayed for the presence of the epitope recognized by the antibody 6-11B-1 in α-tubulins prepared from axonemes of *Chlamydomonas* and other organisms. Posttranslational acetylation of α-tubulin is correlated with axoneme assembly in *Chlamydomonas* (17). It is possible that acetylation of α-tubulin during axoneme assembly is a general mechanism and may be essential for the formation of the axoneme. In this case we would expect that the epitope recognized by 6-11B-1 might be present in axonemal tubulin from many organisms but not in cytoplasmic soluble tubulin from homologous systems. We found that all axonemal tubulins tested to date, including the tubulin from human sperm, contain at least one α-subunit that is recognized by the 6-11B-1 antibody. In contrast, soluble cytoplasmic tubulins from *Chlamydomonas* cell bodies and flagella, *Drosophila* embryos, or HeLa cells are not recognized.

Three different protein samples of *Chlamydomonas* were assayed by immunoblotting: the axoneme, a crude fraction containing soluble tubulin from the cell body, and the membrane plus matrix from flagella. Fig. 5*A* shows the electrophoretograms of the polypeptides contained in the three samples. The tubulin subunits are not resolved and migrate as a major band of apparent molecular weight 50,000. Parallel samples were transferred to nitrocellulose by blotting. The blots were incubated separately with antibody 3A5 (Fig. 5*B*) and the antibody 6-11B-1 (Fig. 5*C*). Whereas the general antibody, 3A5, binds to α-tubulin in each sample, the specific antibody, 6-11B-1, binds only to axonemal tubulin. The tubulin present in the sample of flagellar membrane and matrix proteins is not recognized by the antibody 6-11B-1, although it is derived from the same cellular compartment that contains the axoneme. Therefore, in *Chlamydomonas* flagella, the antigenic determinant is associated specifically with the form of α-tubulin assembled in the axoneme.

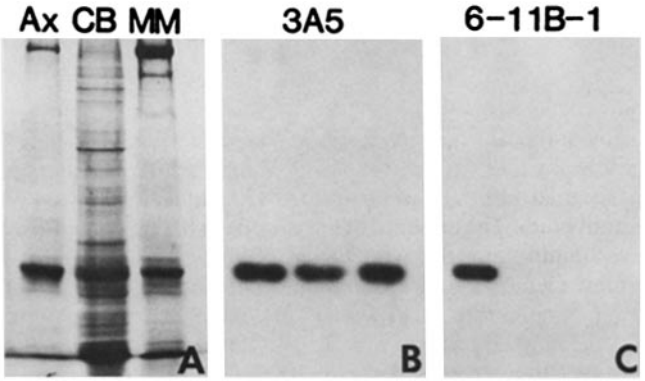

FIGURE 5 Antibody binding to *Chlamydomonas* α-tubulin from axonemes, cell body, and membrane plus matrix of flagella. (*A*) Polyacrylamide gel electrophoretograms of 1 μg polypeptide from each sample after staining by silver. From left to right, tubulin samples are from axoneme (*Ax*), cell body (*CB*), and membrane plus matrix (*MM*). Two sets of the same samples were electrophoresed in parallel, transferred to nitrocellulose, and incubated with antibody. (*B* and *C*) Autoradiograms of nitrocellulose incubated with antibody 3A5 (*B*) and 6-11B-1 (*C*).

Antibody 6-11B-1 binds specifically to the acetylated form of α-tubulin in *Chlamydomonas*. Blots of *Chlamydomonas* axoneme α-tubulins resolved in isoelectric focusing gels proved that antibody 6-11B-1 bound to the major, acetylated form of α-tubulin but not to the minor, unmodified α-tubulin (not shown). Although antibody 6-11B-1 did not recognize soluble forms of α-tubulin from *Chlamydomonas* cell body, 6-11B-1 did recognize cytoplasmic α-tubulin of *Chlamydomonas* after the protein sample was acetylated in vitro, as

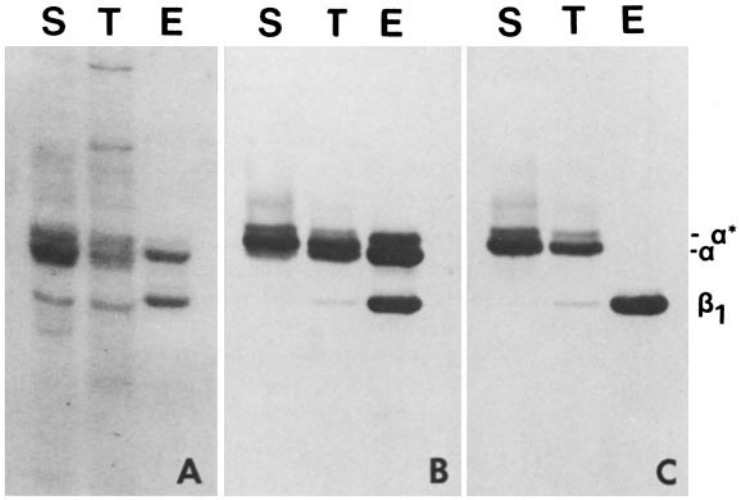

FIGURE 6 Antibody binding to α-tubulins from *Drosophila* sperm, testis, and embryos. *Drosophila* proteins were separated by PAGE, transferred to nitrocellulose, and stained with antibodies as described in Materials and Methods. Identical samples were loaded in parallel in *A*, *B*, and *C*. Lane *S*, sperm isolated from 1.5 seminal vesicles; lane *T*, 1.5 testes from males 1–5 d old; lane *E*, assembly-purified embryonic tubulin. (*A*) Electrophoretogram stained with Coomassie Blue. (*B*) Immunoblot stained with antibody 3A5. (*C*) Immunoblot stained with antibody 6-11B-1. Blots *B* and *C* were also stained with 4A2, a monoclonal antibody that recognizes the somatic β-tubulin, β_1, but does not cross react with the major testis β tubulin, β_2. The major polypeptide component in the Coomassie Blue–stained sperm sample in *A*, lane *S* is not α-tubulin. Although it migrates in the α-tubulin position in the SDS dimension, in two-dimensional gels it migrates at a more neutral pH than tubulin (15). Each major form of α-tubulin (α) has a corresponding minor α-tubulin isoform (α*) that migrates above it.

shown for the cytoplasmic sea urchin tubulin.

The acetylation of α-tubulin does not differentiate flagellar axonemes from ciliary axonemes. The antigen that is recognized by the antibody 6-11B-1 is present also in axonemes of cilia from sea urchin blastulae and *Tetrahymena*. Only the α-tubulin was recognized in immunoblots of polypeptides from both preparations (not shown).

The antibody 6-11B-1 binds to α-tubulin from *Drosophila* sperm, but not to soluble α-tubulin from *Drosophila* embryos. Fig. 6 shows the pattern of antibody binding to proteins from *Drosophila* sperm (*S*), testis (*T*) and assembly purified tubulin from *Drosophila* embryos (*E*). Fig. 6*A* shows the electrophoresed proteins stained with Coomassie Blue. The α- and β-tubulins are widely separated. Parallel gel lanes containing identical samples were transferred to nitrocellulose and stained with antibody 3A5 (Fig. 6*B*) and antibody 6-11B-1 (Fig. 6*C*). The blots shown in Fig. 6, *B* and *C* were also stained with 4A2, a monoclonal antibody that recognizes the somatic form of β-tubulin (β_1) but not the testis-specific β-tubulin from *Drosophila*. The anti-β_1 antibody was included to demonstrate that the amount of embryonic tubulin applied to the gel was similar in both cases. Antibody 3A5 recognizes all known *Drosophila* tubulin isoforms resolved in the gel system used for these experiments (Fig. 6*B*). Antibody 6-11B-1 stains α-tubulins from sperm and testis but fails to recognize α-tubulins from embryos (Fig. 6*C*). All of the other specific monoclonal antibodies in the set listed in Table I were tested against testis and purified embryonic tubulin and showed the same specificity as 6-11B-1. To rule out the possibility that acetylated α-tubulin isoforms may have been selectively lost from the embryo tubulin preparations during two rounds of microtubule assembly-disassembly, the first five antibodies of the set were also tested against a high speed supernatant from a crude embryo extract. Again the antibodies failed to recognize embryonic tubulin (data not shown).

Four α-tubulin isoforms from *Drosophila* testis are resolved in our two-dimensional gel system (Fig. 7, *A* and *B*). Two major α-tubulin isoforms are separated in the isoelectric focusing dimension. Each major α-tubulin has a minor α-tubulin (α*), which migrates just above it in the SDS dimension. The more acidic of the two major α-tubulins is the major α-tubulin in mature, motile sperm (29). The two major α-tubulins in testis are barely resolved in the one-dimensional gel shown in Fig. 6*B*, and both bind the antibody 3A5.

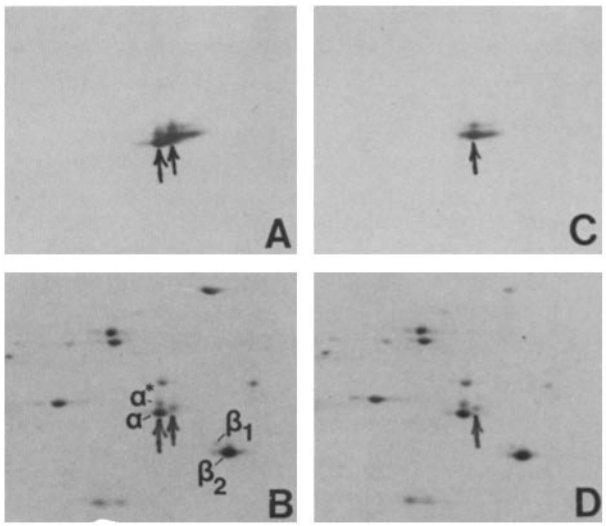

FIGURE 7 Identification of α-tubulin isoforms that are recognized by antibody 6-11B-1 in *Drosophila* testis. Testis from males 1–4 d old were cultured in vitro for 1 h in [^{35}S]methionine to label newly synthesized proteins. The proteins were separated by electrophoresis then transferred to nitrocellulose. After antibody staining, the position of the antigen was marked on the blot with radioactive ink (arrows). Autoradiograms of the blots show the position of the antigen with respect to other testis proteins. The acid end of the gels is to the right. (*A*) Testis α-tubulins stained with antibody 3A5; (*B*) autoradiogram of *A*. (*C*) Testis α-tubulins stained with antibody 6-11B-1; (*D*) autoradiogram of *C*. The somatic (β_1) and the testis-specific (β_2) β-tubulin are indicated as β_1 and β_2, respectively; minor and major α-tubulins are indicated as α* and α.

Antibody 6-11B-1 stains only one of the two major α-tubulin isoforms in testis (Fig. 7*C*). To determine which of the testis tubulins were recognized by antibody 6-11B-1 we stained testis proteins separated by two-dimensional PAGE. Testis were cultured for 1 h in [^{35}S]methionine and the testis proteins were separated on two-dimensional polyacrylamide gels then transferred to nitrocellulose. After peroxidase staining with antibody, the position of the antigen was marked with radioactive ink (Fig. 7, *A* and *C*, arrows), and the nitrocellulose was placed on film. The resulting autoradio-

grams show the position of the antigen with respect to the radioactively labeled testis proteins (Fig. 7, *B* and *D*). Although the two major α-tubulin isoforms are present in adult testis in roughly equal amounts, radioactive label is preferentially incorporated into the more basic form when testis are cultured in [^{35}S]methionine for a short period. (Fig. 7, *B* and *D*; see also reference 29). The general antibody, 3A5, recognizes all four of the testis α-tubulin isoforms resolved in our gel system (Fig. 7*A*). The specific antibody, 6-11B-1, recognizes only one of the two major α-tubulin isoforms plus the minor tubulin that migrates directly above it. As shown by the position of the single arrow in Fig. 7*D*, 6-11B-1 recognizes the more acidic of the two major α-tubulins, the form found in sperm.

To assay for the presence of acetylated α-tubulin in tissues of vertebrates we chose two cells of human origin, human sperm and HeLa cells. Proteins from human sperm and tubulin polymerized by addition of taxol to an extract of HeLa cells were analyzed by one-dimensional gel electrophoresis and immunoblots. The antibody 6-11B-1 recognized axonemal α-tubulin from human sperm but not cytoplasmic tubulin from HeLa cells.

Fig. 8*A* shows the electrophoretograms of the two samples. Blots from parallel gel lanes were incubated, respectively, with antibody 3A5 (Fig. 8*B*), and antibody 6-11B-1 (Fig. 8*C*). Several major bands are resolved in the electrophoretogram of human sperm; however, only two major components, presumably the α- and β-tubulin subunits, are resolved in the electrophoretogram of taxol-induced microtubules from HeLa cells (Fig. 8*A*). The general antibody, 3A5, recognizes α-tubulin in both samples, whereas antibody 6-11B-1 binds α-tubulin only in the sample of human sperm (Fig. 8, *B* and *C*). A preparation of axonemes from human sperm was tested in similar conditions to confirm that the antigen was associated with flagellar microtubules. The antibody 6-11B-1 recognized α-tubulin in that sample but bound also to two minor components of lower molecular weight (not shown). These minor antigens were presumably produced by proteolysis of α-tubulin after the flagellum was detached from the cell body by sonication.

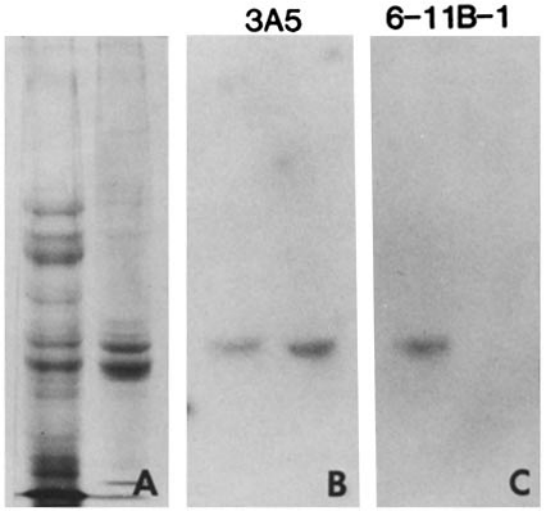

FIGURE 8 Antibody binding to human α-tubulin from sperm and HeLa cells. (*A*) Polyacrylamide gel electrophoretograms of polypeptides from human sperm (left lane) and taxol-stabilized microtubules from HeLa cells (right lane) after staining with Coomassie Blue. Two sets of the same samples were electrophoresed in parallel and then transferred to nitrocellulose. (*B* and *C*) Autoradiogram of the nitrocellulose gel blot incubated with antibody 3A5 (*B*) and 6-11B-1 (*C*).

Localization of the Antigen in the Axoneme of Sea Urchin Sperm

The framework of the axoneme is formed by three different kinds of microtubules: the single microtubules that are located in the center of the axoneme, and the A and B tubules of the peripheral doublets. These three kinds of microtubules have different stability and are associated with different appendages (11, 39). However, none of these differences appears to be specified by the presence of acetylated α-tubulin. This modified subunit was found to be distributed in the same proportion in all three kinds of axonemal microtubule.

Sea urchin sperm axonemes were subjected to a series of extraction steps. First, both of the central pair microtubules were solubilized in the presence of 0.4% Sarkosyl, then the B tubule of the outer doublet microtubules was solubilized by treatment at 40°C, as described in Materials and Methods. The central pair, the outer doublet, and the B and A microtubules all contained the antigens recognized by the antibodies 6-11B-1 and 3A5 in approximately the same ratio. There was no evidence of enrichment of the acetylated form of α-tubulin in any of the fractions.

Fig. 9, *A–C* show the electron micrographs of intact axonemes and of axonemes extracted by 0.2% and 0.4% Sarkosyl. The axonemes appear well preserved, and several substructures, including dynein arms, the central pair projections, and the radial spokes, are clearly distinguished (Fig. 9*A*). After exposure to 0.2% Sarkosyl the axonemes appear extracted, all of the projections are no longer visible, and the axonemal framework is disrupted in some cases. However, both central pair and the outer doublet microtubules appear intact in most cross-sections (Fig. 9*B*). After extraction with 0.4% Sarkosyl, 67% of the central pair microtubules were solubilized, but the outer doublet microtubules appear well preserved, as shown in six selected cross-sections in Fig. 9*C*. Therefore, the extract obtained with 0.4% Sarkosyl was enriched in tubulin depolymerized from central pair microtubules. The preferential solubilization of the B tubule from the residual doublet microtubules was performed by thermal treatment at 40°C for 4 min. The insoluble fraction was analyzed by electron microscopy in negative staining (not shown). This fraction was composed mainly of microtubule A from outer doublet tubules.

Binding of antibodies 6-11B-1 and 3A5 to the axoneme fractions described above was tested by quantitative dot immunobinding assay. At each extraction step, both the solubilized protein and the remaining unsolubilized axoneme structures were tested for antibody binding. The results of one representative experiment are listed in Table II. Although the supernatants from axonemes exposed to 0.4% Sarkosyl and axonemes exposed to 0.4% Sarkosyl and then treated at 40°C are enriched in, respectively, tubulin from the central pair and from the B tubule, they appear to contain the same amount of acetylated tubulin found in the axonemes, outer doublet, and A tubule. No significant differences were observed in the ratios of antibodies 6-11B-1 and 3A5 bound to the antigens. Therefore the α-tubulin subunits that are present in each fraction are modified by acetylation to the same extent.

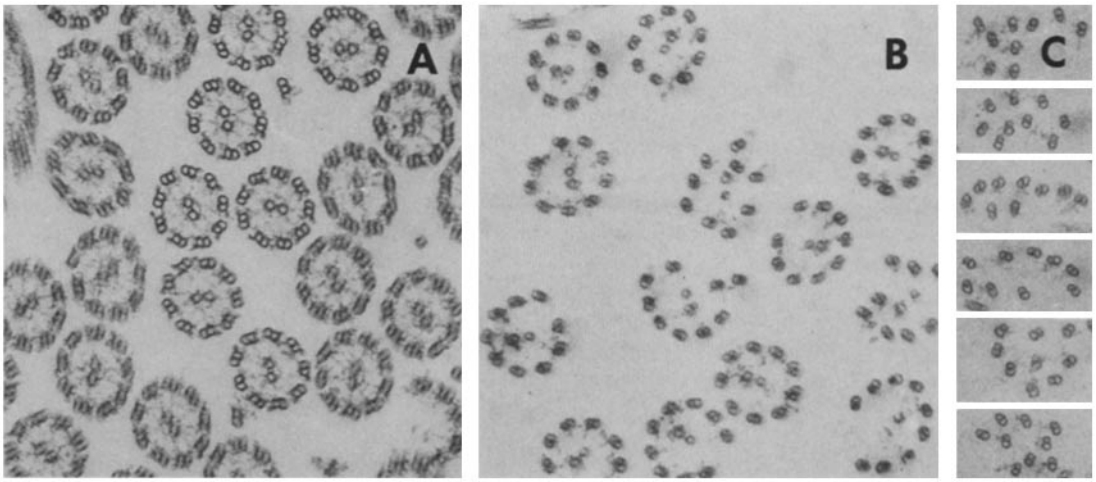

FIGURE 9 Thin-section electron micrographs of axonemes isolated from *Strongylocentrotus purpuratus* sperm flagella. Sections through pellets of intact axonemes (*A*), axonemes extracted by 0.2% Sarkosyl (*B*), and axonemes extracted by 0.4% Sarkosyl (*C*). × 55,000.

TABLE II. *Localization of the Epitope Recognized by Antibody 6-11B-1 in Different Fractions of the Axoneme*

	% axonemes lacking central pair microtubules	% protein solubilized*	Antibody 6-11B-1 bound	
			Antibody 3A5 bound	
Untreated axonemes	1			0.56
Axonemes exposed to 0.2% Sarkosyl	4	16	Soluble fraction	0.48
			Insoluble fraction	0.55
Axonemes exposed to 0.4% Sarkosyl	67	39	Soluble fraction	0.46
			Insoluble fraction	0.51
Axonemes exposed to 0.4% Sarkosyl and at 40°C for 4 min		59	Soluble fraction	0.53
			Insoluble fraction	0.46

* Percentage of protein solubilized in 0.2 or 0.4% Sarkosyl refers to total axonemal protein. However, percentage of protein solubilized by thermal treatment refers to protein content of axonemes extracted by 0.4% Sarkosyl.

Protein fractions enriched in tubulin from central pair microtubules were also prepared by dialysis of a suspension of axonemes against a solution of low ionic strength (34). The soluble fraction containing primarily central pair tubulin and the insoluble fraction containing outer doublet tubulin were analyzed with antibodies 6-11B-1 and 3A5. Again, the antigen recognized by 6-11B-1 was found in both fractions in the same relative amount (not shown).

DISCUSSION

The Seven Monoclonal Antibodies Are Specific for an Acetylated Form of α-Tubulin

We have isolated and characterized a set of seven monoclonal antibodies that appear to be specific for an acetylated form of α-tubulin. Two lines of evidence support this conclusion. First, α-tubulin from crude extracts of sea urchin unfertilized eggs or from *Chlamydomonas* cell bodies, which was not originally recognized by the antibodies, became antigenic after treatment with acetic anhydride. This treatment results primarily in the in vitro acetylation of the ε-amino group of lysine residues (21). Chemically acetylated β-tubulin subunits and other polypeptides in the same cytoplasmic extracts did not become antigenic. Therefore, the antibodies do not recognize acetylated lysine alone but require the context of the α-tubulin molecule. The antigenic determinant may consist of one or more acetylated lysines set in a particular α-tubulin sequence. Alternatively, the determinant may be formed when the conformation of a specific α-tubulin amino acid sequence is altered by acetylation of lysine residues in the molecule.

The second line of evidence is that the acetylation of α-tubulin in vivo during axoneme assembly in *Chlamydomonas* is correlated with the appearance of an α-tubulin isoform that is recognized by the seven antibodies. Antibody binding assays against α-tubulin isoforms from *Chlamydomonas* revealed that the antibodies recognize the acidic isoform of α-tubulin which is a product of posttranslational acetylation on the ε-amino group of lysine (18). Only the modified α-tubulin is antigenic. Unmodified α-tubulin isoforms present in *Chlamydomonas* flagella or cell bodies do not bind the antibodies. Since the antigen was detected in assembled axonemal tubulin from *Chlamydomonas* flagella, but not in tubulin from the flagellar membrane-plus-matrix fraction, the α-tubulin may become acetylated only as it is incorporated into axonemal microtubules (see also reference 17).

In both the in vitro acetylation experiments and the acetylation of *Chlamydomonas* α-tubulins in vivo, α-tubulins, which did not originally cross-react with the antibodies, become antigenic after modification. This result indicates that the antigenic determinant is formed in a polypeptide domain of α-tubulin that may be the same or very similar in soluble, cytoplasmic α-tubulin isoforms from *Chlamydomonas* cell bodies (32) and sea urchin unfertilized eggs and in the flagellar microtubules from these organisms. The antibody used as a control in these experiments, 3A5, clearly recognizes a different determinant. 3A5 binds to α-tubulin isoforms in sea urchin eggs, *Chlamydomonas* cell bodies, *Drosophila* embryos, and HeLa cells. Its binding does not seem to be altered by acetylation of α-tubulin either in vivo or in vitro.

The seven monoclonal antibodies have behaved similarly in all assays performed to date. It is possible that they recognize the same antigenic determinant. We did not have any

reason to predict the isolation of seven hybridomas secreting antibodies with such similar properties from two different fusions. The immunogen was a 15 S dynein fraction from sea urchin sperm axoneme that contained nine polypeptides, including high molecular weight dynein subunits and axonemal α- and β-tubulin isoforms (25). The initial screen may have biased the selection of positive hybridomas toward those secreting antibody against tubulin, since α- and β-tubulin are the most prevalent polypeptides in the axoneme preparations used as antigen in the screen. However, of the 17 cell lines isolated from the two fusions, none secreted antibodies against β-tubulin, and all of the anti-α-tubulins selected were specific for the acetylated isoform.

The Antigen Is Found in Axonemal Microtubules from a Variety of Sources

The α-tubulin isoform recognized by the seven monoclonal antibodies has been observed in every cell or tissue containing axonemal structures tested to date. The antigen has been detected in axonemes from organisms as phylogenetically disparate as single cell microorganisms, marine invertebrates, insects, and mammals. The antigen is present in both flagellar and ciliary axonemes, which generate different kinds of movement.

The axoneme is a complex structure made of several distinct kinds of microtubules. The central pair microtubules and the A and B tubules of outer doublets have different structures and are associated with different appendages (11). Our extraction experiments have shown that acetylated α-tubulin is present in similar proportions in the central pair microtubules and in the A and B tubules of the outer doublets. Therefore, the acetylation of α-tubulin does not appear to provide the mechanism that specifies the structure or the associated appendages of any one class of microtubules in the axoneme. Even the differential stability under extraction conditions that distinguishes each of the central pair microtubules (39) does not seem to be due to tubulin acetylation. However, the extraction experiment(s) would not have distinguished between the possibility that both central pair microtubules contain acetylated α-tubulin and the possibility that one of the central pair microtubules is made up completely of unmodified subunits and the other is acetylated twice as much as the outer doublet microtubules. Analysis of *Chlamydomonas* mutants that lack central pair microtubules indicates that the outer doublet microtubules of the axoneme contain both acetylated and nonacetylated α-tubulins. Two forms of α-tubulin, corresponding to the modified and unmodified isoforms, are present in the axonemes from both wild-type and mutant cells (1).

The location of the unmodified α-tubulin in the outer doublet microtubules is not known. Unmodified α-tubulin could be localized in specific parts of the axonemes, for instance in tips of the A microtubule, which extend beyond the B microtubule at the distal end of cilia and flagella. Alternatively, unmodified α-tubulin may form a co-polymer with the acetylated isoform and be found throughout the microtubules. It is possible that the modified and unmodified α-tubulin isoforms in the axoneme are in an equilibrium mediated by acetyltransferase(s) and deacetylase(s) and that the activity of these enzymes in vivo regulates the state of assembly of the axonemes. L'Hernault and Rosenbaum (17–19) have shown that acetylation occurs in *Chlamydomonas* flagella during flagellar assembly and is reversed during flagellar resorption. The acetyltransferase(s) and the deacetylase(s) probably are present in the same cellular compartment as the axoneme. If these enzymes are located in the flagellum they may remain active during the isolation of flagella and axonemes in our preparations.

Sea urchin, *Chlamydomonas*, and *Drosophila* axonemes all have an α-tubulin isoform that is antigenic for the seven monoclonal antibodies described in this paper and that migrates in an acidic position in isoelectric focusing gels. *Polytomella*, *Crithidia*, and *Physarum* also have an axonemal α-tubulin isoform with an isoelectric point more acidic than that of cytoplasmic α-tubulins (9, 20, 30). The appearance of this acidic isoform has been correlated with the posttranslational modification of α-tubulin in these organisms. This modification may also be due to acetylation. This hypothesis may be easily confirmed using the antibodies described in this report.

The Antigen Is Not Restricted to Axonemal Microtubules

The seven monoclonal antibodies did not recognize soluble, cytoplasmic α-tubulin isoforms from unfertilized sea urchin eggs, *Chlamydomonas* cell bodies, *Drosophila* embryos, and HeLa cells. However, each of these protein fractions was isolated in a way that would enrich for tubulin precursors or tubulin depolymerized from labile microtubules that disassembled after cell breakage. The first step after homogenization in each preparation was centrifugation at high speed to remove cellular debris. Stable microtubules such as those that form axonemes, centrioles, and basal bodies probably remained intact during the fractionation and therefore did not contribute any tubulin to the soluble protein fraction. Therefore, the evidence indicates that not all α-tubulin isoforms are acetylated but does not rule out the existence of acetylated α-tubulin isoforms in microtubule structures other than the axoneme.

Preliminary experiments indicate that the antigen is probably not restricted to axonemal tubulins. Antibody binding to proteins from isolated basal bodies of *Chlamydomonas* showed that acetylated α-tubulin is also present in that structure. The absence of axonemes from cellular fractions that contained basal bodies was tested by monoclonal antibodies against high molecular weight polypeptide V from dynein outer arm (12) (Piperno, G., unpublished results).

Acetylated α-tubulin isoforms may also be found in nonaxonemal microtubular structures in *Crithidia*. The cortex of *Crithidia* and other kinetoplastids contains a framework of subpellicular microtubules that can be prepared in fairly pure form and are stable under isolation conditions. The microtubules of this pellicular fraction are formed in part by an α-tubulin identical to the axonemal α-tubulin of that organism (30). It is possible that posttranslational acetylation of α-tubulin is important also for the maintenance of this stable microtubule array.

The antigen recognized by the seven monoclonal antibodies is present in substantial amounts in a variety of *Drosophila* tissues that are thought not to contain any significant axoneme-like structures. The general antibody, 3A5, recognized two major α-tubulins present in roughly equal amounts in blots of one-dimensional gels of protein samples prepared from larval discs, brain, and salivary glands. In these experi-

ments intact tissues were solubilized in boiling sample buffer, and there was no centrifugation step. The specific antibody, 6-11B-1, recognized only one major protein band from each tissue, and the antigen migrated in the α-tubulin position. 6-11B-1 also recognized a major protein in the α-tubulin position on one-dimensional gels from adult heads, thoraces, and whole bodies from which germline tissue had been removed, either by dissection or genetically, using a grandchildless mutation (Fuller, M., unpublished results).

The unequal distribution of α-tubulins with and without the 6-11B-1 determinant in tubulin preparations from certain cell types and organelles leads us to speculate that the modification of α-tubulin by acetylation may play an important role in the specification of tubulin structure and function. It is possible that posttranslational acetylation ofα-tubulin may distinguish stable microtubules organized in complex arrays from labile microtubule populations. Analysis of the distribution of the 6-11B-1 antigen in cells that contain both kinds of α-tubulin isoforms should help determine if the acetylated isoform of α-tubulin participates in structurally or functionally distinct microtubule arrays. Ultimately the antibodies described in this report may help elucidate the possible role of acetylation of α-tubulin in the regulation of tubulin function in vivo.

Gianni Piperno thanks Dr. Giorgio Vidali for his advice about in vitro acetylation of proteins, Eleana Sphicas for the electron microscopy of axonemes, and Jerrienne Barrett and Anne Gibbons for technical assistance. Margaret Fuller thanks Drs. Michiko Watanabe and Karen Palter for helpful advice during the isolation of monoclonal antibodies, and Joan Caulton for faithful technical assistance. She also thanks Dr. Anthony Mahowald for use of his population cages for staged embryo collections and Dr. Elizabeth C. Raff for support and useful discussions.

G. Piperno was supported by grant GM28702 from the National Institutes of Health. M. Fuller was supported by a Postdoctoral Fellowship from the Jane Coffin Childs Fund for Medical Research, National Institute of Child Health and Human Development (NICHHD) grant 1-R01-HD-16739 to E. C. Raff, a Junior Faculty Research Award from the American Cancer Society, and by NICHHD grant 1-R01-HD-18127 to M. Fuller.

Received for publication 20 May 1985, and in revised form 25 July 1985.

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