Cytoplasmic Microtubules Containing Acetylated α-Tubulin in *Chlamydomonas reinhardtii*: Spatial Arrangement and Properties

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**Abstract.** A monoclonal antibody, 6-11B-1, specific for acetylated α-tubulin (Piperno, G., and M. T. Fuller, 1985, *J. Cell Biol.*, 101:2085–2094) was used to study the distribution of this molecule in interphase cells of *Chlamydomonas reinhardtii*. Double-label immunofluorescence was performed using 6-11B-1, and 3A5, an antibody specific for all α-tubulin isoforms. It was found that acetylated α-tubulin is not restricted to the axonemes, but is also present in basal bodies and in a subset of cytoplasmic microtubules that radiate from the basal bodies just beneath the plasma membrane. Immunoblotting experiments of basal body polypeptide components using 6-11B-1 as a probe confirmed that basal bodies contain acetylated α-tubulin. In the cell body, 6-11B-1 stained an average of 2.2 microtubules/cell, while 3A5 stained an average of 6.5 microtubules. Although exposure to 0°C depolymerized both types of cytoplasmic microtubules, exposure to various concentrations of colchicine or nocodazole showed that the acetylated microtubules are much more resistant to drug-induced depolymerization than nonacetylated microtubules. Axonemes and basal bodies are already known to be colchicine-resistant. All acetylated microtubules appear, therefore, to be more drug-resistant than nonacetylated microtubules. The acetylation of α-tubulin may be part of a mechanism that stabilizes microtubules.

**Materials and Methods**

**Antibodies**

We used two monoclonal antibodies throughout this study. The first, produced by the 3A5 hybridoma (later referred to as 3A5), reacts with all species of α-tubulin tested thus far. The second, produced by the 6-11B-1 hybridoma (6-11B-1), is specific for acetylated α-tubulin (16). The cells were grown as described in reference 15 and the cell culture supernatants (harvest fluids) were used as the source of the antibodies. The 3A5 harvest fluid could be diluted 100 times and the 6-11B-1 harvest fluid 10 times without changing...
the staining observed. We duplicated some of the experiments using antibodies purified from serum-free harvest fluids (HB 102 medium, New England Nuclear, Boston, MA) by Protein A-Sepharose affinity chromatography. The 6-IIB-1-purified immunoglobulin could be used up to 100 μg/ml and still retain its specificity of staining.

**Immunofluorescence**

_Chlamydomonas_ gametic cells (strains 137+, 137-, or mutants) were grown for 4 d on plates low in sulphate and then resuspended in nitrogen-free medium at 20°C (II), at a density of ~3 x 10⁶ cells/ml. Under these conditions, wild-type cells regenerated full-length flagella in <1 h. All subsequent steps were performed at room temperature. After 1 h, the cells were collected by centrifugation and resuspended in autolysin at the same density as the autolysin solution was prepared by mixing 3 x 10⁶ mating-type plus gametes at room temperature with the same number of mating-type minus gametes in 200 ml of nitrogen-free medium. After 5 min the cells were transferred to ice and centrifuged three times for 10 min each at 1,000 g. The supernatant was frozen in aliquots at ~70°C until used. The digestion was allowed to proceed without shaking for 30 min, and the cells were then permeabilized and extracted by adding 6 vol of a solution containing 25% (wt/vol) glycerol, 1% (wt/vol) Nonidet-P-40, 100 mM 2 (N-morpholino) ethanesulfonic acid (Sigma Chemical Co., St. Louis, MO), 15 mM EGTA, 2.5 mM MgCl₂, 100 μM phenylmethylsulfonylfluoride, and 2 μM taxol (obtained from the Natural Product Branch of the National Cancer Institute, Bethesda MD) (pH 6.7). Glass coverslips were washed and immersed for 5 min in poly-L-lysine (1 mg/ml in water) and air-dried. After 5 min in the permeabilization solution, the cells were spotted on the coverslips and fixed for 45 min at 1% glutaraldehyde (Electron Microscopy Grade, Sigma Chemical Co.), 100 mM Tris-HCl (pH 7.5), 15 mM EGTA, and 2.5 mM MgCl₂. Remaining glutaraldehyde was reduced by incubation for 15 min in NaBH₄ (1 mg/ml in water). The coverslips were rinsed once in phosphate-buffered saline (PBS) (0.05 M sodium phosphate, 0.13 M NaCl [pH 6.5]) and then incubated four times: (a) 2 h in 6-IIB-1 harvest fluid; (b) 2 h or overnight in biotin-labeled sheep anti-mouse antisera (Amersham Corp., Arlington Heights, IL, diluted 1:100 in PBS); (c) 2 h or overnight in 3A5 harvest fluid; and (d) 1-2 h in a mixture of Texas Red-Streptavidin (Amersham Corp., diluted 1:100 in PBS) and fluorescein-labeled goat anti-mouse Fab fragment (Cappel Laboratories, Cochranville, PA, diluted 1:100 in PBS). The coverslips were rinsed three times for 5 min each in PBS after each incubation. The mounting medium consisted of 90% (vol/vol) glycerol, 10% (vol/vol) PBS, 1 mg/ml p-phenylene diamine (pH 7.15). Some of the results shown were obtained using similar single-labeling protocols.

The procedure used yielded three kinds of specimens. Some were not permeabilized and showed an intense chlorophyll red fluorescence. These "nonextracted" cells were never considered. Some were "disrupted" cells, where the flagella and the cytoplasmic microtubules were preserved and all other structures were lost as observed by phase-contrast and immunofluorescence microscopy (see, for example, Fig. 1, A' and B'). In others, the cell shape was well preserved and the chloroplast and the nucleus could be seen in phase-contrast microscopy. However, the disappearance of the chloroplast red background fluorescence in the chloroplast attached to the successful permeabilization and extraction of these "intact" cells (see, for example, Fig. 1 C'). The frequency of the different classes of specimens varied from one coverslip to the next. Photography required that all microtubules be in the same focal plane and sufficiently spread apart to be distinguished from one another. These conditions were met much more frequently in disrupted cells, which are therefore overrepresented in the illustrations.

Although all results were also obtained in the absence of taxol, it was included in the permeabilization solution because it allows the preservation of a greater number of nonacetylated microtubules. When taxol was omitted in control experiments, we observed a decrease of up to 30% in the number of nonacetylated microtubules and only a slight (~10%) decrease in the number of acetylated microtubules. Therefore, it is clear that taxol does not cause the acetylation of the microtubules but simply helps preserve the nonacetylated microtubules.

**Colchicine, Nocodazole, and Cold Treatments**

For the colchicine treatment, the cells were resuspended in nitrogen-free liquid medium for 1 hr, collected by centrifugation, and resuspended for 1 hr in the dark in the same medium containing various concentrations of colchicine (Aldrich Chemical Co., Milwaukee, WI). The cells were then processed as usual, except that all solutions until the fixation step contained colchicine and all incubations were performed in the dark. Nocodazole (Al-

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with 3A5, only a few cytoplasmic structures are visible. It is clear from the double-labeling experiments that the structures stained by 6-11B-1 represent the proximal part or the entire length of a few of the microtubules detected by 3A5 (compare Fig. 1, A and B with Fig. 1, A' and B'). The average number of cytoplasmic microtubules totally or partially acetylated is 2.2 (see below).

In similar experiments, gametic cells of the strain 137- (mating type minus) were stained by both antibodies. In all respects, acetylated microtubules found in these cells were identical to those of mating type plus cells (not shown). This indicates that spatial arrangement and properties of the cytoplasmic acetylated microtubules are not related to sexual differentiation.

Two monoclonal antibodies were used as negative controls of the specificity of the antibody binding. One, produced by the C-241-2 hybridoma, is specific for a high molecular weight dynein polypeptide of sea urchin sperm axonemes (15), and the other, Actl(20), is specific for Dictyostelium actin. Neither antibody cross-reacts with Chlamydomonas proteins, as judged by immunoblotting (Piperno, G., unpublished observations). These antibodies stained the axonemes extremely weakly and failed to detect any cytoplasmic structure (result not shown).

**Mutants Lacking Flagella Contain Normal Cytoplasmic Acetylated Microtubules**

The analysis of bald 1 mutant cells, whose flagellar apparatus consist only of transition zone material contained within the cell wall (Goodenough, U., personal communication), shows that the acetylation of cytoplasmic microtubules occurs even in the absence of flagella. Moreover, cytoplasmic acetylated microtubules can be distinguished from single outer doublet and central pair microtubules derived from split axonemes.

In this mutant, apart from the absence of the axonemes, the patterns of staining with both antibodies are similar to

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**Figure 1.** Antitubulin immunofluorescence staining of wild-type cells. Cells in A, A', B, and B' were double-stained and cells in C and C' were single-labeled. A-C show the microtubules stained by 6-11B-1 (specific for acetylated α-tubulin) and A', B', and C' show the microtubules stained by 3A5 (specific for all α-tubulin isoforms). Bars, 10 μm.
those obtained with wild-type cells. Fig. 2 A shows a cell single-labeled with 6-11B-1 and Fig. 2 A' shows another cell labeled with 3A5. Fig. 2, B and B', shows two cells after double-labeling. Fig. 2 B shows the fluorescence detecting 6-11B-1 and Fig. 2 B' shows the fluorescence detecting 3A5. 3A5 stains a large number of cell-length cytoplasmic microtubules, while only one to three of these are stained by 6-11B-1. The region of the basal bodies is brightly stained by both antibodies.

Cells from the strain uni 1, which have only one flagellum located in trans to the eyespot (5), also showed a wild-type-like pattern of staining of their cytoplasmic microtubules (results not shown). It seems therefore that the number of cytoplasmic microtubules containing acetylated α-tubulin bears no direct relationship to the number and position of the flagella.

Properties of Cytoplasmic Microtubules Containing Acetylated α-Tubulin: Resistance to Antimitotic Drugs and Cold-lability

To determine whether the presence of acetylated α-tubulin in the microtubule lattice confers different properties on the whole microtubule, we attempted to distinguish qualitatively the microtubules that contain acetylated α-tubulin from the bulk of cytoplasmic microtubules. We tested their stability in the presence of colchicine, nocodazole, and, at 0°C, conditions known to depolymerize microtubules (3).

For these experiments, it was important to compare the behavior of both classes of microtubules in the same cells. The cells were double-labeled and examined under the microscope. In every cell, where the cytoplasmic microtubules could be distinguished from one another and from the flagella, the cytoplasmic microtubules stained by either antibody were counted. In every case, 60–90 cells were considered.

Fig. 3 shows double-labeled cells after exposure to 2 mg/ml colchicine for 100 min. Fig. 3, A and B, shows the fluorescence detecting 6-11B-1 and Fig. 3, A' and B', shows the fluorescence associated with 3A5. The flagella are stained by both antibodies. In the cytoplasmic domain, one to three microtubules remain, stained equally well by both antibodies. The cytoplasmic acetylated microtubules are often but not always shorter than those in untreated cells. A typical situation is shown on Fig. 3, B and B', where one of the microtubules is long, and the other is a short stub. These results indicate that after a 100-min exposure to concentrations of colchicine ≥1 mg/ml, all remaining microtubules are stained equally well by both antibodies and are therefore acetylated.

To assess quantitatively the effect of exposure to colchicine in Chlamydomonas cells, we counted the cytoplasmic microtubules stained by 6-11B-1 or 3A5 in double-labeled cells. Fig. 4 shows the percentage of cells containing different numbers (N) of microtubules at varying concentrations of colchicine. The plain bars indicate microtubules detected by 3A5 (i.e., the total population of microtubules) and the hatched bars indicate the microtubules stained by 6-11B-1 (acetylated microtubules). In untreated cells, 3A5 stains an average of 6.5 microtubules/cell while 6-11B-1 stains an average of 2.2 microtubules/cell.

In each cell, the number of acetylated microtubules (stained by both antibodies) and nonacetylated microtubules (stained by 3A5 but not by 6-11B-1) was determined. Fig. 5 A shows the percentage of acetylated and nonacetylated microtubules remaining after exposure to different concentrations of colchicine, relative to the numbers obtained in un-

Figure 2. Antitubulin immunofluorescence staining of bald 1 cells. Cells A and A' were single-stained and cells in B and B' were double-labeled. A and B, microtubules stained by 6-11B-1 (specific for acetylated α-tubulin). A' and B', microtubules stained by 3A5 (specific for all α-tubulin isoforms). Bars, 10 μm.
treated cells. As Fig. 5A shows, the number of nonacetylated microtubules decreases rapidly after exposure to colchicine, while under the same conditions, the population of acetylated microtubules is much less affected. After exposure to 0.6 mg/ml colchicine for 100 min, the average number of nonacetylated microtubules decreases by 20%, while 86% of the nonacetylated microtubules are depolymerized. The colchicine resistance of acetylated microtubules is not absolute, since about one half of them are depolymerized after exposure to high concentrations of colchicine.

**bald 1** cells were analyzed using the same procedure. Fig. 6 shows a **bald 1** cell double-labeled after exposure to 2 mg/ml colchicine for 100 min. Fig. 6A shows the fluorescence associated with 6-11B-1, and Fig. 6A' shows the fluorescence detecting 3A5. One cytoplasmic microtubule is resistant to colchicine and is acetylated. A brightly stained dot is present in the region of the basal bodies. This, along with the bright staining of this region observed in wild-type cells suggested to us that the microtubules forming the basal bodies also contain acetylated α-tubulin. We later found this to be the case (see following section). In other cells, the number of colchicine-resistant, acetylated microtubules was one to three (result not shown).

We tested the action of nocodazole on acetylated and nonacetylated microtubules using the experimental design used to test the effect of colchicine. Fig. 5B shows the percentage of acetylated and nonacetylated microtubules remaining after treatment with different concentrations of nocodazole. The percentage of microtubules remaining are calculated relative to the control cells treated with dimethyl sulfoxide alone. Acetylated microtubules are totally resistant to a concentration of nocodazole of 8 µg/ml, while 43% of

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**Figure 3.** Antitubulin immunofluorescence staining of wild-type cells after exposure to colchicine. The cells were exposed to 2 mg/ml colchicine for 100 min and double-stained as described in Materials and Methods. A and B, microtubules stained by 6-11B-1 (specific for acetylated α-tubulin). A' and B', microtubules stained by 3A5 (specific for all α-tubulin isoforms). Bars, 10 µm.

**Figure 4.** Number of microtubules stained in the cytoplasm of wild-type cells. The cells were exposed to various concentrations of colchicine for 100 min and double-stained as described in Materials and Methods. The histograms show the percentage of cells containing N microtubules stained by 6-11B-1 (□) and by 3A5 (■).
the nonacetylated microtubules are depolymerized by this concentration. Higher drug concentrations (12 and 15 μg/ml) lead to depolymerization of microtubules of both types. Due to the limited solubility of nocodazole, we could not use drug concentrations above 15 μg/ml. This experiment indicates that acetylated microtubules are markedly more resistant than nonacetylated microtubules to depolymerization induced by intermediate concentrations of nocodazole.

Exposure of the cells to 0°C affects both acetylated and nonacetylated microtubules equally. Fig. 7, A, A', B, and B' shows cells double-labeled after exposure to cold for 15 min (see Materials and Methods for details). Fig. 7, A and B, shows the fluorescence detecting 6-IIB-1, while Fig. 7, A' and B', shows the fluorescence detecting 3A5 in the same cells. After 15 min on ice, most cells do not contain any cytoplasmic microtubules. This experiment also shows that no repolymerization occurs at room temperature during the permeabilization of the cells. The kinetics of depolymerization of microtubules by exposure to cold was studied by the method used for the drug studies. Fig. 5 C shows the percentage of acetylated and nonacetylated microtubules remaining after exposure to cold for different lengths of time. No
significant difference was found in the behavior of acetylated microtubules as compared with nonacetylated microtubules.

**Isolated Basal Bodies Contain Acetylated α-Tubulin**

The cellular region where the basal bodies are located appeared brightly stained in Figs. 1–3, 6, and 7 by both 3A5 and 6-IIB-1. In particular Fig. 6 shows that in the mutant bald 1, in the absence of flagella, the cytoplasmic microtubules seem to radiate from an intensely stained spot. These observations raised the question of whether the microtubules forming the basal bodies contain acetylated α-tubulin as do the axonemes and a subset of cytoplasmic microtubules. To answer this question we prepared basal bodies free of axonemes and cytoplasmic microtubules and analyzed the polypeptide components by SDS polyacrylamide gel electrophoresis and immunoblots. The evidence indicates that basal body microtubules do contain acetylated α-tubulin.

Fig. 8 A shows electrophoretograms of approximately equal amounts of protein from axonemes and basal bodies. The two patterns obtained by silver staining are different. Dyneins and tubulin polypeptides form two major bands in the axonemal sample. In contrast, polypeptide components from the basal body sample form several prominent bands. Although a major band, presumably tubulin, is present in the basal body sample, other high molecular weight components with the electrophoretic mobility of dyneins are absent. Fig. 8, B and C, shows immunoblots of the same samples incubated respectively with 6-IIB-1 and 235-2, an antibody against component V of *Chlamydomonas* dynein outer arm (reference 4, and Piperno, G., unpublished results). 6-IIB-1 binds to α-tubulins that are present in both samples. In contrast, 235-2 binds only to the dynein present in the axoneme. Therefore, acetylated α-tubulin is present in the sample of basal bodies whereas band V, the major component of the dynein family, is absent.

We have used electron microscopy of negatively stained specimens, electrophoresis, and immunoblot analyses to ensure that axonemes do not contaminate the basal body fraction. Cytoplasmic acetylated microtubules were not present in the same fraction since they are cold-labile and would not have survived during the basal body preparation. Therefore we conclude that the basal bodies themselves contain acetylated α-tubulin.
Discussion

Cytoplasmic Acetylated Microtubules

In this work, we took advantage of the specificity for acetylated α-tubulin of the monoclonal antibody produced by the hybridoma 6-11B-1 to show that, in interphase *Chlamydomonas reinhardtii* cells, acetylated α-tubulin is not restricted to the axonemes but is also found in the basal bodies and in a few cytoplasmic microtubules. We show further that these cytoplasmic acetylated microtubules are more resistant than nonacetylated microtubules to the depolymerizing effects of colchicine and of nocodazole.

The 6-11B-1 antibody is specific for acetylated α-tubulin in immunoblotting experiments. We believe that this antibody has the same specificity when used for immunofluorescence, since in these and other experiments involving various organisms such as tissue culture cells and sea urchin eggs, immunofluorescence and immunoblotting give concordant results regarding the presence or absence of material reacting with 6-11B-1 (Piperno, G., unpublished observations).

Until the acetylation of cytoplasmic and basal body microtubules is chemically proven, of course, the possibility remains that 6-11B-1 might recognize a conformation brought about by acetylation in the axonemes and by other similar "modification" in the cell body. It is more likely, however, that 6-11B-1 recognizes true acetylated α-tubulin. In fact, this explains why L'Hernault and Rosenbaum (8) detected a molecule with properties similar to those of α3-(acetylated, axonemal) tubulin in deflagellated cell bodies.

Several pieces of evidence indicate that cytoplasmic acetylated microtubules constitute a true, distinct subpopulation of microtubules in *Chlamydomonas* cells before fixation: (a) They all originate from the basal body region. (b) As discussed in Materials and Methods, taxol is not needed to observe them, indicating that this agent does not cause their polymerization. Moreover, the acetylated microtubules are less affected than nonacetylated microtubules by the presence of taxol in the permeabilization solution. This indicates a greater in vitro stability of the acetylated microtubules, which is likely to be related to their greater in vivo stability. (c) The lack of staining of the whole complement of microtubules by 6-11B-1 is not caused by the antibody's lack of affinity or its insufficient concentration in the harvest fluid. 6-11B-1 can stain extremely thin filaments, possibly single microtubules, in split axonemes. In addition, the concentrations of the harvest fluids or of the purified immunoglobulin used in the experiments did not affect the specificity of the staining. (d) The resistance of the microtubules stained by 6-11B-1 to colchicine and nocodazole is much higher than that of the total population of cytoplasmic microtubules. This point will be detailed further.

Cytoplasmic Drug-resistant Microtubules

We found that very high concentrations of colchicine (≥0.6 mg/ml) were needed to affect acetylated microtubules within 100 min, probably because of the low affinity of *Chlamydomonas* tubulin for the drug (1, 18). Moreover, we were unable to dissolve β- and γ-lumicolchicine (Sigma Chemical Co.) at the same concentrations, and therefore we could not confirm that these analogues of colchicine, which do not bind to tubulin (3), would be ineffective in our system. Although we could not prove that the action of colchicine is truly mediated by its binding to tubulin monomers, this direct interaction is likely to occur. The total number of cytoplasmic microtubules, as detected by 3A5, decreases markedly upon exposure to colchicine. The dose–response curve for this phenomenon is very similar to that obtained (18) when the inhibition by colchicine of the regeneration of the flagella was under study. The effect of colchicine on flagellar assembly was negligible up to a concentration of 0.1 mg/ml, and was maximal at a concentration of 1 mg/ml and above.

The concentration of colchicine needed to observe 50% of the maximal effect was 0.45 mg/ml. We found that the total number of cytoplasmic microtubules is affected by colchicine in a very similar fashion. This resemblance might reflect a similarity in molecular events concerning the action of colchicine on assembly of axoneme microtubules and disassembly of cytoplasmic microtubules.

Although quite well understood in vitro (13), the mode of action of colchicine in vivo and at the molecular level is not known (see reference 3 for a review). Moreover, exposure to concentrations higher than 4 μg/ml colchicine causes side effects in tissue culture cells (3). Side effects might in fact be extensive in *Chlamydomonas* because of the high drug concentration used. Therefore, to verify the existence of a correlation between microtubule resistance to the action of tubulin-specific drugs and α-tubulin acetylation, it was important to test another antimitotic drug. Nocodazole, another microtubule-depolymerizing drug that binds to tubulin by a mechanism distinct from that of colchicine, was used. As for colchicine, both acetylated and nonacetylated microtubules are affected by the drug, but the acetylated microtubules are more resistant than the nonacetylated microtubules. In con-
clusion, acetylated microtubules appear to be more stable than nonacetylated microtubules, inasmuch as they are more resistant to two independently acting microtubule-depolymerizing drugs.

It may seem surprising that acetylated microtubules are no more cold-resistant than nonacetylated microtubules. After exposure to 0°C for 15 min, all acetylated and nonacetylated microtubules are depolymerized. When shorter exposure times (3, 5, and 10 min) were used, more cytoplasmic microtubules were preserved but again acetylated microtubules showed no greater resistance. However, the action of cold in vivo can be expected to be less specific than exposure to drugs because exposure to 0°C affects most enzymatic processes, protein–protein and protein–water interactions, and other cellular processes such as diffusion in the cytoplasm and energy metabolism. Moreover, the depolymerization process itself may be different. In vitro experiments suggest that, while both colchicine-induced and cold-induced depolymerization can proceed by endwise shortening (13), cold also induces internal breaks along the whole length of the microtubule (12).

Microtubule-associated proteins may also contribute to the resistance to depolymerization of the microtubules. Cold-stability of rat brain microtubules has been shown in vitro to be linked to the presence of substoichiometric amounts of specific microtubule-associated proteins (6), therefore, it is conceivable that acetylation of α-tubulin increases the drug-stability of a given microtubule in Chlamydomonas without affecting its cold-lability. Other instances of cold-labile, drug-resistant microtubules have been described previously (3). It remains to be seen whether α-tubulin acetylation is also involved in those cases. Experiments performed with 3T3 cells and HeLa cells have shown that tissue culture cells also contain subsets of cytoplasmic microtubules that are acetylated. These acetylated microtubules are, as in Chlamydomonas, cold-labile and drug-resistant. (Piperno, G., unpublished results).

Function of Acetylated Microtubules

The function of the acetylated microtubules is not known. Although all axonemes tested so far are acetylated (16), the control of axoneme assembly and disassembly is probably not the only function of tubulin acetylation, since it also occurs in the cell bodies. Acetylated microtubules were found in gametic cells of both mating types. Vegetative cells, which were not used in this study because of their lower susceptibility to autolysin, also contain similar structures (LeDizet, M., unpublished observations). Therefore, the presence of acetylated microtubules in the cytoplasm is not strictly correlated with gametic or sexual differentiation. Since all acetylated microtubules (in the cytoplasm, axonemes, and basal bodies) exhibit a higher stability, they might remain in the cytoplasm as seeds upon which regrowth would occur. The fact that basal bodies are acetylated is important in that respect, because the axonemes grow from them. Moreover, some cytoplasmic microtubules are only acetylated proximal to the basal bodies. These acetylated microtubule segments could be involved in specialized functions. For instance, they could constitute a spatial link between the basal bodies and the nucleus and are perhaps part of the nucleo-flagellar apparatus studied by Wright et al. (22).

Microtubules in axonemes and basal bodies are already stabilized by cross-linking structures, and the cold-sensitive, drug-resistant cytoplasmic microtubules may correspond to the bundles of closely associated microtubules seen by electron microscopy in the anterior part of Chlamydomonas cells (17). Therefore, the acetylation may be a consequence rather than a cause of the stability of a microtubule. Microtubules stabilized by other mechanisms would undergo significant acetylation of α-tubulin, thereby locking the tubulin dimer in a conformation that is more energetically favorable for the maintenance of microtubule assembly.

Acetylated α-tubulin was found in axonemes from a variety of organisms (16). We therefore expect cytoplasmic acetylated microtubules not to be restricted to Chlamydomonas but to be a feature of many cell types. In that case the role of α-tubulin acetylation in microtubule dynamics and microtubule interactions with other cellular elements could be studied using tissue culture cells as a model system. Drugs that stabilize microtubules, such as taxol (3), may induce the formation of acetylated microtubules and create favorable conditions to analyze the role of acetylation of α-tubulin in the reversible differentiation of microtubule frameworks.

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