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.

The assembly and differentiation of structures composed of microtubules are analyzed with some advantages in simple organisms that are suitable for genetic analysis and contain well characterized microtubule frameworks. The unicellular, biflagellated alga Chlamydomonas reinhardtii is suited to this purpose. The flagellar apparatus is located anteriorly in interphase Chlamydomonas cells and includes paired basal bodies from which two flagella extend outward. Basal bodies and flagellar axonemes are composed of single, double, and triple microtubules. Twelve or more microtubules form a bipolar basket encircling the cell body underneath the plasma membrane (17). These microtubules form four bundles of continuous filaments at one pole in the basal body region, while they are single and may be discontinuous in the middle part and posterior end of the cell. During mitosis, a mitotic spindle assembles inside the nuclear envelope (2, 7).

These different microtubular systems contain at least three α-tubulin isoforms (14). Two isoforms are coded by two α-tubulin genes which are both transcribed and code for extremely similar proteins (19). The third isoform is generated by posttranslational modification. L'Hernault and Rosebaum have proven by chemical analysis that Chlamydomonas axonemal microtubules contain acetylated α-tubulin and have proposed that reversible tubulin acetylation might control the assembly and disassembly of axonemal microtubules (8–10). However, the acetylation of α-tubulin had not yet been observed in other microtubule structures.

A series of monoclonal antibodies specific for acetylated α-tubulin was recently characterized and used to demonstrate that acetylated α-tubulin is present in flagellar axonemes from a variety of organisms, including Chlamydomonas (16). We have used one of these antibodies to study Chlamydomonas microtubules by immunofluorescence microscopy and immunoblotting. All microtubules stained by this antibody will be referred to in the text as "acetylated microtubules." In this paper we show that acetylated microtubules are not restricted to the axoneme but are also present in Chlamydomonas cell bodies. These cytoplasmic acetylated microtubules are notably more resistant to antimitotic drugs than nonacetylated 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 6-11B-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 × 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 above and allowed to stand on ice for various lengths of time in the autolysis solution. We first attempted to permeabilize the cells in ice-cold permeabilization solution, but this resulted in a very poor extraction. Therefore, we subsequently performed this step at room temperature, since no microtubule repolymerization occurred during this step.

**Basal Body Preparation**

Basal bodies relatively free of contaminants were prepared as described by Snell et al. (21). Cell lysis by Nonidet P-40 was performed at 0°C and the centrifugations were at 4°C. Sucrose fractions containing basal bodies were collected, negatively stained, and analyzed by electron microscopy. The fraction collected at the 55–60% sucrose interphase after the last centrifugation appeared to contain structurally intact basal bodies that were not contaminated by axonemes or cytoplasmic microtubules. These basal bodies were further analyzed by SDS polyacrylamide gel electrophoresis, electrophoretic transfer of proteins, and antibody binding.

**Electrophoresis and Immunoblotting**

The samples were analyzed by electrophoresis on a Neville 4–11% polyacrylamide gradient gel, transferred on nitrocellulose, and detected by immunostaining as described by Piperno and Fuller (16).

**Results**

**The Whole Axoneme and a Subset of Cytoplasmic Microtubules Contain Acetylated α-Tubulin**

We performed single- and double-labeling immunofluorescence experiments using the 3A5 antibody, which recognizes all types of α-tubulin tested, and the 6-11B-1 antibody, which is specific for acetylated α-tubulin. We devised the double-labeling procedure described in Materials and Methods to be able to evaluate quantitatively the prevalence of acetylated vs. nonacetylated microtubules in a given cell. The specificity of either antibody was the same in single-labeling experiments. This evidence eliminated the possibility that artifacts due to the double-labeling protocol, such as competition between the antibodies, were occurring. These experiments show that acetylated α-tubulin is present throughout the length of the axonemes and in the proximal portion (towards the basal bodies) of a few of the cytoplasmic microtubules.

The pattern of staining observed when gametic cells of the strain 137+ (mating type plus) are incubated with the 3A5 antibody is shown in Fig. 1, A', B', and C'. Fig. 1, A' and B', show two cells after double staining. Fig. 1 C shows one cell after single-labeling. The axonemes are brightly and uniformly stained, as is the region of the basal bodies. In some instances, the axonemes were split into bright stain filaments or filament bundles that are composed presumably of outer doublet and central pair microtubules (for example, see Fig. 1 C). In the cytoplasm, up to 12 microtubules or microtubule bundles can be discerned, apparently extending from one pole of the cell to the other under the plasma membrane. Their average number is 6.5 (see below) and their length is 6–10 μm.

The pattern of microtubules to which 6-11B-1 binds is clearly different (Fig. 1, A–C). Fig. 1, A and B, show the same cells as Fig. 1, A' and B'; Fig. 1 C shows a different cell than Fig. 1 C'. Although the staining of the axonemes by 6-11B-1 is indistinguishable from the staining obtained...
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, ActI(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 (acyetylated 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-
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 3

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.

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Figure 4

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 (□).

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 acetylated 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...
Figure 5. Stability of acetylated and nonacetylated microtubules exposed to different treatments. Wild-type cells were treated and double-stained as described in Materials and Methods. The three panels show the percentages of acetylated (—□—) and nonacetylated (—●—) microtubules remaining after (A) treatment with various concentrations of colchicine for 100 min, (B) treatment with various concentrations of nocodazole for 45 min, and (C) exposure to 0°C for various lengths of time.

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

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 6-11B-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 \( \alpha \)-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 \( \alpha \)-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 \( \alpha \)-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 \( \alpha \)-tubulins that are present in both samples. In contrast, 235-2 binds only to the dynein present in the axoneme. Therefore, acetylated \( \alpha \)-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 \( \alpha \)-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 some other "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 regrowth 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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