Genetic Dissection of the Central Pair Microtubules of the Flagella of *Chlamydomonas reinhardtii*

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**ABSTRACT**

Mutations at two loci, which cause an altered mobility of the flagella, affected the central pair microtubule complex of *Chlamydomonas reinhardtii* flagella. The mutations at both loci primarily affected the C1 microtubule of the complex. Three alleles at the PF16 locus affected the stability of the C1 microtubule in isolated axonemes. This phenotype has allowed us to determine that at least ten polypeptides of the central pair complex are unique to the C1 microtubule. The motility defect was correlated with the failure to assemble three of these ten polypeptides in vivo. The structural gene product of the PF16 locus was a polypeptide with molecular weight 57,000 as shown by analysis of five intragenic revertants and by analysis of axonemes from dikaryon rescue experiments. Three alleles at the PF6 locus affected the assembly of one of the two projections of the C1 microtubule and this projection was formed by at least three polypeptide components, which are a subset of polypeptides missing in isolated *pf16* axonemes. No structural gene product has been identified for the PF6 locus. The gene product is probably not one of the identified projection constituents as shown by analysis of dikaryon rescue experiments. Chemical extraction of isolated wild-type axonemes suggests that at least seven polypeptide components are unique to the C2 microtubule.

The central pair microtubules of flagella more closely resemble the cytoplasmic microtubules than the outer microtubules of the flagella by virtue of their structure and their lability. They are singlet microtubules with 13 protofilaments unlike the nearby outer doublet microtubules that are composed of a composite set of microtubules with 13 and 11 protofilaments (1). The outer doublet microtubules, in contrast to the central pair microtubules, arise as a continuation of the microtubules of the basal bodies. The central pair complex originates distal to the transition zone of the basal body and terminates in specialized structures associated with the flagellar membrane at its tip (2). In *Chlamydomonas reinhardtii* the central pair microtubules are sensitive to depolymerization by low ionic strength dialysis and by exposure to detergents (3, 4), whereas the outer doublet microtubules retain their integrity under these conditions.

The two microtubules of the central pair complex also differ from one another (5–7). In squid sperm, Linck and coworkers (8) have shown that the morphology of the C1 microtubule differs from that of the C2 microtubule. In *Chlamydomonas* the C1 microtubule has two projections that are 18 nm long as measured by thin-section electron microscopy. The C2 microtubule has two shorter projections of ~8 nm each. Furthermore, the C2 microtubule is more labile than the C1 microtubule (4).

We have analyzed mutations at two unlinked loci in *Chlamydomonas* that affect the C1 microtubule specifically with the aim of understanding the assembly of microtubules in vivo and the differentiation of different types of microtubules.

**MATERIALS AND METHODS**

**Mutant Strains:** Three independent mutations at the PF16 locus on linkage group IX have been identified, as well as six independent mutations at the PF6 locus on linkage group X. The first allele of each gene was isolated by R. Lewin (10) following ultraviolet irradiation. Two additional alleles of *pf16* (*pf16A* and *pf16B*) and five additional alleles of *pf6* (*pf6A–E*) were isolated following mutagenesis of strain 137c by nitrosoguanidine (11) and by ICR 191 (12), respectively. Each allele segregated two mutant and two wild-type zygospores in backcrosses to wild-type cells (137c) as would be expected for a single gene mutation. Each allele was shown to be recessive to its wild-type allele in temporary dikaryons. Allelism was then determined by the lack of complementation between new alleles and strains carrying known *pf* mutations in temporary dikaryons (13). For both new alleles of *pf16* (*pf16A* and *pf16B*) and two new alleles of *pf6* (*pf6A* and *pf6B*), identification was confirmed by linkage to *sr1* and to *gf19*, respectively (14).
Motility: In contrast to other mutations affecting the central pair complex (pf15, pf18, pf19, and pf20 [9, 6, 7]), the pf6 and pf16 mutants do not show paralyzed, rigid flagella. Rather, pf16 mutations lead to paralysis but the flagella show slight twitching at the flagellar tips and occasional wave forms. Flagella of pf6 cells show limited jiggling movements, which occasionally may propel the cells forward. These motility phenotypes were unchanged at 17°, 25°, or 32°C. Further studies were performed with cells grown at 25°C.

Evidence for a Second Mutation in Two pf16 Strains: In the course of biochemical analysis of isolated pf16 and pf16B axonemes, an alteration in the mobility in the pH gradient of two-dimensional gels was observed for a constellation of polypeptides (Fig. 3). The constellation consists of at least three polypeptides; all were shifted to a more basic location in these two strains. These polypeptides have an apparent molecular weight of 125,000. Because this electrophoretic change was observed in two of the three independently isolated allelics, we examined its linkage to the PF16 locus. In eight tetrams of a cross of pf16B × 137c and eight random spores from a cross of pf16 × 137c, the alteration segregated as expected of a single gene and there was no detectable linkage between the alteration and the PF16 locus (2:1:1:1 parental ditype:nonparental ditype:ternary type:nonparental ternary type; 1:1:5:3 parent:offspring). It was surprising that the same alteration was observed in two independently isolated alleles. The alteration when separated from the pf16 or pf16B lesion did not produce any noticeable change in the motility phenotype of the mutants nor did the new single mutant have a motility phenotype by itself. One possible explanation is that second mutation has some selective advantage during storage of mutants.

Reversion Analysis: Revertants of pf16B and pf6 were screened following mutagenesis by ultraviolet irradiation of gametic cells for 30 and 60 s as described (15). 47% and 87% killing was observed for pf16B cells, and 70% for pf6 cells. Each revertant was crossed to wild-type strain 137c and analyzed by standard techniques (16).

Extraction of the C2 Microtubule: Approximately 300 µg of axonemal protein was collected and resuspended in 50 mM NaCl, 4 mM MgCl₂, 0.25 mM HEPES, 0.005 mM SrCl₂, 0.025 M dithiothreitol. The suspension was made up to 0.001 M NaCl, 4 mM MgCl₂, 1 mM HEPES, 0.0001 M SrCl₂, 4 mM dithiothreitol, and 4 mM ATP (pH 7.2) in a total volume of 600 µl and placed at room temperature for 10 min. The suspension was dialyzed at 4°C for 4 h against 10 mM 2-(N-morpholino)ethanesulfonic acid (MES), 1 mM MgCl₂, 1 mM EGTA, 0.1 mM phenylmethylsulfonylfluoride (pH 6.4). It was centrifuged at 110,000 g for 30 min (17). Supernatant and the residual pellet was collected. Both fractions were examined by gel electrophoresis and the residual pellet was examined by thin-section electron microscopy.

Other Methods: Methods for culturing cells and formation of dikaryons (15), radiolabeling with [³⁵S]sulfate acid, and electron microscopy (11) have been previously described. The isolation protocol for flagella and axonemes was modified (11) in the final step by adding 0.1% NP-40 rather than 0.5% for preparation of axonemes and by collecting the flagella or axonemes by centrifugation at 35,000 g for 1 h. With these modifications we were able to obtain axonemes from wild-type 137c cells in which 87% of the axonemal cross sections showed both central pair microtubules as compared with 52% for the previous method. Two-dimensional gel electrophoresis was performed as described by Piperno et al. (18), with the modification that the pH gradient electrophoresis was run for 16 h at 1 mA. One-dimensional gel electrophoresis on 4-8% gradient gels was performed as described by Adams et al. (7).

RESULTS

Ultrastructural Analysis

Morphology of the central pair complex was studied in flagella attached to the cells, in isolated flagella, and in isolated axonemes. With intact flagella, there is poor penetration of tannic acid stain, and thin-section electron microscopy shows little detail of the central pair complex (7). In these flagellar cross sections, we simply were able to determine whether both central pair microtubules were present or absent. No differences in central pair morphology were detected between mutant and wild-type preparations in attached flagella. Both tubules were present in 97% of wild-type (137c mt⁺) cross-sectional images (n = 407), in 98% of pf16B images (n = 59), and in 97% of pf6 images (n = 73). Similar observations were made for isolated flagellar fractions from these strains (data not shown).

In isolated axonemal preparations, staining was effective and additional features of the central pair complex were visible. When we determined the frequency of cross-sectional axonemal images that had two central microtubules, pf16B axonemes were clearly different from both pf6 and 137c mt⁺ preparations (Table I). For 137c mt⁺, 87% of axonemal cross sections (n = 303) showed two central microtubules and for pf6 the value was 89% (n = 460). In the case of pf16B preparations, only in 8% of the images (n = 430) were both microtubules present. In the remainder of the pf16B cross-sectional images, 60% showed only one tubule, and 32% showed no central tubules.

In addition, we examined wild-type axonemes that had been subjected to chemical extraction. Axonemes were treated with high salt concentrations (17) and then dialyzed against low ionic strength buffer for 4 h. The suspension was subjected to centrifugation and the residual axonemal pellet and the extracted material in the supernatant were collected. By thin-section electron microscopy, 90% of the images of the residual axonemes in the pellet fraction (n = 278) had a single microtubule, 3% had two microtubules, and 7% had no central microtubules.

In axonemal images the C1 central microtubule can be seen to have projections of ~18 nm, which are longer than the projections of the C2 microtubule (5). We used this criterion to identify the persistent tubule among 110 pf16B axonemal cross sections showing a single central microtubule. In 106 cases (95%), the C2 microtubule persisted. Similar observations were made for pf16 axonemes (96%; n = 71). In direct contrast, it had been observed previously that in wild-type axonemal preparations the C2 microtubule is more likely to be labile (4, 7). In our preparations most cross sections with only a single microtubule from pf6 axonemes (96%; n = 43), from 137c axonemes (93% n = 34), or from extracted 137c axonemes (97%; n = 250) contained the C1 microtubule. Thus, it appeared that pf16 lesions specifically altered the stability of the C1 microtubule in the majority of isolated axonemes. Since in pf16B axonemes, 32% of the cross sections (compared with 1.3% in 137c and 1.7% in pf6 axonemes) showed the absence of both microtubules, it is likely that the mutation secondarily affects the stability of the C2 microtubule.

As already noted isolated axonemes from pf6 cells showed no significant instability of the central microtubules. A mutant defect became apparent when samples of C1 and C2 microtubules from axonemal cross sections of pf6 and 137c were analyzed for the number of projections that each carried. As shown in Table II, there was in pf6 preparations a specific paucity of C1 images that showed two projections and a marked excess of C1 images that showed only a single projection. No such difference was found in the analysis of C2 images from pf6 and 137c. These observations suggest that lesions at the PF6 locus specifically affect formation of one
TASTE II

Number of Cross-sectional Images from 137c and pf6 Gametic Cells

| No. of projections | C1 microtubule | C2 microtubule |
|--------------------|----------------|----------------|
|                    | 137            | 137            |
| 0                  | 3 (0.04)*      | 8 (0.13)       |
| 1                  | 25 (0.33)      | 52 (0.84)      |
| 2                  | 47 (0.63)      | 2 (0.03)       |
| Total              | 75             | 62             |

|                    | pf6            | pf6            |
|--------------------|----------------|----------------|
| 0                  | 8 (0.13)       | 4 (0.06)       |
| 1                  | 52 (0.84)      | 24 (0.39)      |
| 2                  | 34 (0.45)      | 34 (0.35)      |
| Total              | 62             | 62             |

*Numbers in parentheses indicate the frequency of each class.

**FIGURE 1** Thin-section electron micrograph illustrating the morphology of isolated axonemes from wild-type (A) and pf6 (B) cells. (A, top) a transverse section of a wild-type axoneme in which the central tubules are clearly seen to be differentiated by the presence of two long projections (C1 microtubule on the right) and two short projections (C2 microtubule on the left); (bottom) a longitudinal section that passes through a wild-type axoneme in the plane indicated by slashes at top. The long projections on the C1 microtubule extend from the microtubule in two rows with a longitudinal periodicity of ~17 nm. (B, top) a transverse section of a pf6 axoneme in which one of the two long projections on the C1 microtubule is missing; (bottom) a longitudinal section that demonstrates that pf6 cells are defective for the assembly of one specific row of long projections normally associated with the C1 microtubule. x 128,000.

**FIGURE 2** Portion of autoradiograph from one-dimensional gradient gel electrophoretogram resolving polypeptides from 360,000 to 220,000. Axonemes from pf16, pf6, and 137c radiolabeled cells are shown. Approximately 0.5 μg of protein (specific activity = 100,000 cpm/μg) were loaded per lane. The band above CP3 is variable in its presence and probably represents contamination by flagellar matrix proteins. Values at right represent molecular weight x 10^3.

**Biochemical Analysis**

Previous work had shown that the central pair complex in axonemes isolated from pf18 mutant cells (6, 7) was unstable and was lost during the preparation of axonemes. In comparing radiolabeled polypeptides from isolated pf18 and 137c axonemes, a set of 18 polypeptides was missing or reduced consistently in this mutant (7). However, in these preparations only 52% of the axonemes from wild-type cells had a complete central pair complex by electron microscopic analysis. Using minor modifications of the isolation protocol (see Materials and Methods) we were able to obtain wild-type preparations in which 87% of the axonemal cross sections had a complete central pair complex. In comparing pf18 and 137c axonemes using this protocol, 23 polypeptides were missing from the pf18 preparations compared with wild-type preparations. Two different electrophoretic gel systems were used for this analysis. The first method resolves polypeptides with molecular weights from 360,000 to 160,000 by one-dimensional SDS acrylamide gradient gels. Four polypeptides in this molecular weight range (CP1–CP4) are missing (6, 7) and are indicated in Fig. 2. The second method resolves polypeptides from 160,000 to 15,000 by two-dimensional gel electrophoresis. 19 polypeptides in this molecular weight range (CP5–CP23) are missing and are indicated in Fig. 3. It should be noted that CP19–CP23 had not been identified as consistently deficient in the earlier analyses of pf18 axonemes (6, 7).

Axonemal polypeptides from pf16 and pf6 cells were ana-
FIGURE 3 Autoradiograph of $[^{35}S]$sulfuric acid radiolabeled polypeptides from isolated 137c axonemes resolved by two-dimensional electrophoresis. The first dimension is nonequilibrium isoelectric focusing in a pH gradient and the second dimension is 4–11% acrylamide gradient gel electrophoresis in the presence of SDS. Nineteen polypeptides missing or diminished in the mutant pf18 are indicated. This mutant completely lacks a central pair microtubule complex in isolated axonemal preparations (6, 7). The constellation of polypeptides indicated by the asterisk is the polymorphism described in Materials and Methods.

lyzed and compared with wild-type axonemes. In both cases, the sets of polypeptides consistently found to be deficient in these mutants comprised a subset of the group of polypeptides deficient in the isolated axonemes from pf18 cells. The subset of 10 polypeptides missing from pf16B axonemes is listed in Table III. In three independent alleles (pf16, pf16A, pf16B) the same complement of polypeptides was defective. However in pf16A axonemes, CP12 and CP18 were reduced but were not missing. The presence of these two prominent polypeptides may indicate the severity of the defect associated with this allele.

Polypeptides from isolated pf16B and pf16 flagella were also analyzed. They were missing only three of the ten polypeptides absent in axonemes. The missing components were CP14, CP17, and CP19 (Table III). In summary, there are 10 unique polypeptides associated with the C1 microtubule.
absence of three of these polypeptides results in paralyzed flagella (as shown by the analysis of the isolated flagella) and their absence destabilizes the normally stable C1 microtubule in isolated axonemes.

Polypeptides from isolated pf6 axonemes were examined and it was found that the isolated axonemes were missing a subset of the pf16 axonemal signature. They were lacking CP9, CP12, and CP18 (Table III). Two additional alleles of pf6 (pf6A and pf6B) showed the same molecular phenotype in axonemes. Isolated flagella from pf6 were also missing CP9, CP12, and CP18. The lesions of PF6 appeared to affect one of the two projections of the C1 microtubule. By electron microscopic analysis, a single lateral array of projections was deficient and by biochemical analysis, it appeared to comprise a unique set of three polypeptides. Furthermore, this projection did not appear to play a role in stabilizing the C1 microtubule.

We also analyzed supernatant and pellet fractions of chemically extracted 137c axonemes. Based on the electron microscopic phenotype (Table I) of the residual axonemal pellet, we expected that central pair polypeptides representing components of the C2 microtubule would be enriched in the supernatant fraction. The supernatant fraction may also include labile components of the C1 microtubule. We found that 14 of the 23 central pair signature polypeptides were extracted into the supernatant by the chemical treatment. Comparing supernatant and pellet, seven polypeptides were found predominantly in the supernatant fraction (Table III; CP3, 5, 6, 7, 10, 11, and 15). These polypeptides are likely to represent unique components of the C2 microtubule. The other seven polypeptides were extracted partially; they are CP1, 8, 13, 16, 21, 22, and 23. Except for CP1 in the second set, the central pair polypeptides that were solubilized by the chemical treatment did not include the 10 polypeptides found missing from pf16 axonemes. The polypeptides that are solubilized partially might be localized in both the C1 and C2 microtubules.

### Gene Product Analysis of pf16B

To ask about the identity of the gene product of the PF16 locus and to determine its role in the flagellum, we attempted to isolate intragenic revertant mutations of the motility defect following ultraviolet-irradiation of gametic cells. Seven potential intragenic revertants of pf16B were isolated. Each was an independent isolate and was found to be linked to the original pf16B mutation as assessed by the absence of recombination between each of the new mutations and the pf16B mutation in 12 tetrads in backcrosses to 137c (<4.0 centiMorgans [cM]). One isolate (R3) was examined further and no recombination was observed between the pf16B mutation and the second mutation in 138 tetrads from crosses to wild-type cells (137cmt). Although these secondary mutations may define a closely linked locus (ranging from <4 cM to <0.34 cM), we will assume that these secondary mutations are intragenic revertants.

Two of the revertants (R6 and R10) restored wild-type motility to the cells, whereas five of the revertants (R1, R3, R5, R7, and R8) showed some departure from wild-type motility at 25°C. Biochemical analyses of isolated axonemes from R6 and R10 cells showed the return of the 10 polypeptides missing in axonemes of the parent pf16B cells to their wild-type position. The second group of revertants (R1, R3, R5,

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**Table III**

Summary of Axonemal and Flagellar Polypeptide Deficiencies

| CP   | Mol wt $\times 10^{-3}$ | pf16B axonemes | pf16B flagella | pf6 axonemes | pf6 flagella | 137c extracted* axonemes | extracted | residual |
|------|------------------------|----------------|----------------|-------------|-------------|--------------------------|----------|---------|
| 1    | 360*                   | -/+            | +              | +           | +           | -/+                      | +        | -       |
| 2    | 270*                   | -              | +              | +           | +           | -                        | -        | +       |
| 3    | 250*                   | +              | +              | +           | +           | -                        | +        | -       |
| 4    | 220*                   | -              | +              | +           | +           | -                        | -        | +       |
| 5    | 142*                   | +              | +              | +           | +           | -                        | -        | +       |
| 6    | 128*                   | +              | +              | +           | +           | -                        | +        | -       |
| 7    | 106*                   | +              | +              | +           | +           | -                        | +        | -       |
| 8    | 97*                    | -              | +              | +           | +           | -                        | +        | -       |
| 9    | 97*                    | -              | +              | +           | +           | -                        | +        | -       |
| 10   | 84*                    | +              | +              | +           | +           | -                        | +        | -       |
| 11   | 84*                    | +              | +              | +           | +           | -                        | +        | -       |
| 12   | 66*                    | -              | +              | +           | +           | -                        | +        | -       |
| 13   | 62*                    | +              | +              | +           | +           | -                        | +        | -       |
| 14   | 57*                    | -              | -              | +           | +           | -                        | -        | +       |
| 15   | 56*                    | +              | +              | +           | +           | -                        | -        | +       |
| 16   | 45*                    | +              | +              | +           | +           | -                        | -        | +       |
| 17   | 32*                    | -              | +              | +           | +           | -                        | -        | +       |
| 18   | 20*                    | -              | -              | -           | -           | -                        | -        | +       |
| 19   | 128                    | -              | -              | -           | -           | -                        | -        | +       |
| 20   | 110                    | -              | +              | +           | +           | -                        | -        | +       |
| 21   | 56                     | +              | +              | +           | +           | -                        | +        | -       |
| 22   | 25                     | +              | +              | +           | +           | -                        | +        | -       |
| 23   | 14                     | +              | +              | +           | +           | -                        | +        | -       |

* Polypeptides identified by Adams et al. (7).
* Column entitled extracted refers to polypeptides removed from axonemes by salt treatment and dialysis and recovered in the supernatant after centrifugation at 110,000 g for 30 min. Column entitled residual refers to polypeptides remaining bound to the axonemes and recovered in the pellets.

**Abbreviations used in this paper:** cM, centiMorgans; MAP, microtubule-associated protein.
R5, R7, and R8) showed the return of nine of the ten polypeptides to their wild-type positions and the tenth polypeptide, CP14, was replaced by a polypeptide with a more basic isoelectric point (CP14') (Fig. 4). From in vitro mixing experiments of isolated axonemes from R1 or R3 cells with wild-type axonemes, both polypeptides (CP14 and CP14') were observed (Fig. 4). When R1 and R3 axonemes were mixed together in vitro, a single polypeptide (CP14') was observed (data not shown).

The observed change in the mobility of CP14 in some of the revertants suggests that CP14' may be an altered structural product of the PF16 locus; however, it is not proof. Further evidence that CP14 is the structural gene product was provided by dikaryon analysis (15). Temporary dikaryons were constructed between pf16B and 137c cells, in which the pf16B parent was radiolabeled with $^{35}$S]sulfuric acid but the wild-type parent (137c) was not radiolabeled. When in situ rescue of the motility defect of the pf16B flagella occurs in the dikaryon in the presence of a protein synthesis inhibitor, anisomycin, any polypeptide that must come exclusively from the pool of wild-type flagellar polypeptides rather than the combined mutant and wild-type pools will return unlabeled. It was observed that all the polypeptides except CP14 contained radiolabel when the axonemes from pf16B x 137c dikaryons were isolated and gel electrophoretograms were analyzed by autoradiography. Thus, CP14 could only be supplied by the pool of polypeptides from the unlabeled wild-type parent. Both methods suggest that CP14 is the gene product of the PF16 locus.

The revertants were also analyzed in dikaryons to assess dominance relationships. In dikaryons of pf16B x pf16B-R3, in which both parents were radiolabeled, the paralyzed flagella regained motility. The altered CP14' polypeptide and not the normal CP14 was observed in axonemes isolated from these dikaryons (data not shown). This datum shows that the revertant is dominant to the pf16B allele. Additionally, the altered gene product of two revertants, pf16B-R1 and pf16B-R3, were each found to be co-dominant with the wild-type
PF16 gene product as shown by the presence of both CP14 and CP14' in axonemes isolated from wild-type by revertant dikaryons in which the flagella had been amputated and regenerated in the mixed cytoplasm (data not shown). These results show that the pf16 flagella in the presence of wild-type or revertant cytoplasm regained motility and that the two microtubules were again stable in isolated axonemes, which again suggests that CP14 is the PF16 gene product.

**Gene Product Analysis of pf6**

We attempted to isolate intragenic revertants of the motility defect of pf6 cells. Only a single potential intragenic revertant (pf6-R4) was isolated. No recombination was observed between pf6 and the second mutation in 43 tetrads (<1.1 cM). These cells showed wild-type motility patterns. Biochemical analysis of isolated axonemes from pf6-R4 cells showed the return of the three polypeptides missing in axonemes of pf6 cells to their wild-type position. No evidence for a structural gene product of the PF6 gene was obtained using dikaryon rescue analysis. When radiolabeled pf6 cells were mated to unlabeled 137c cells, all three polypeptides that were missing returned with label in the isolated axonemes. Two alternative explanations exist. One is that the gene is a structural component of the axoneme but that we have not resolved it in either of our gel systems. Alternatively, the gene product may be extrinsic to the axoneme.

**DISCUSSION**

Mutations at the pf16 locus result in the singular loss from axonemal preparations of the C1 microtubule of the central pair complex. When compared with axonemes from wild-type cells, the mutants showed loss or striking diminution of ten axonemal polypeptides regularly present in the wild-type preparations. The missing polypeptides, CP1, 2, 4, 9, 12, 14, 17, 18, 19, and 20, range in relative molecular weight from 360,000 to 20,000 and constitute a subset of 23 polypeptides missing from isolated axonemes of mutants at the pf18 locus. Axonemes from pf18 cells lack both the C1 and C2 central microtubules (5).

When wild-type axonemes were treated with high salt concentrations and dialyzed against low ionic strength buffer, the C2 central pair microtubule is selectively solubilized. Examination of the distribution between supernatant and pellet of central pair axonemal polypeptides, as defined by the pf18 axonemal deficiencies, showed that a subset of seven polypeptides CP3, 5, 6, 7, 10, 11, and 15 are exclusively present in the supernatant fraction. These data together with the pf16 results, suggest that each subset of central pair components is unique respectively to the C1 or the C2 microtubule.

At present there is no evidence to indicate that tubulin subunits, the major components of the central pair, show any structural variation between the C1 and C2 tubules. An analysis of α and β tubulin in two-dimensional gels showed no differences between wild-type and central pair defective (pf18) axonemes (7). Since only two α tubulin and two β tubulin genomic sequences have been identified in C. reinhardtii (20, 21), any explanation of the specific properties of the variety of flagellar and cellular microtubules based exclusively on tubulin subunits would require that the diversity be based on transcriptional processing or post-translational modification. An explanation based on the association of other polypeptides with the tubulin subunit lattice seems equally plausible.

A variety of proteins have been found associated with preparations of cytoplasmic microtubules, including microtubule-associated protein (MAP 1 (M, = 350,000) and MAP 2 (M, = 270,000) (22, 23), the tau (r) MAPs (M, = 55,000–62,000) (24), and lower molecular weight MAPs (M, = ~30,000) (25). It has been shown that MAP 1 or MAP 2 proteins reassembled onto microtubules appear as filamentous projections or arms at regularly spaced intervals (26–28). The high molecular weight MAPs and the tau MAPs (29) have been found to influence the stability of microtubules reassembled from brain tubulins.

It is likely too, in the case of Chlamydomonas flagellar central microtubules, that the specific and different properties of the C1 and C2 microtubules are based on the unique subset of nontubulin polypeptides associated with each microtubule. The case seems clear for one class of projections of the C1 microtubule. The absence from pf6 flagella and axonemes of polypeptides CP9, 12, and 18, a subset of the unique C1 polypeptides, is correlated with the absence of one class of projections. In this case the mutant defect appeared not to alter the stability of the C1 microtubule in axonemal preparations.

In the case of pf16 mutants, three polypeptides were missing from flagella (CP14, 17, and 19) but it has not been possible to determine whether loss of the polypeptides is associated with a structural alteration. When mutant flagella are converted to axonemes, there was very extensive loss of the C1 microtubule, the more stable of the central pair microtubules in wild-type axonemes, and partial loss of the C2 microtubule. The altered stability of the C1 central microtubule suggests that one of the three polypeptides missing from pf16 flagella may interact with the tubulin lattice. The likely candidate for such direct or indirect interaction is CP14, the polypeptide (M, = 57,000) that appears to be the PF16 gene product.

It is interesting that pf16B-R3, a strain selected from reversal experiments and likely to represent an intragenic revertant (<0.34 cM) showed a heat-sensitive phenotype. At temperatures below 21°C, motility was wild-type and the complement of axonemal polypeptides was complete (see Fig. 4). The cells become progressively more paralyzed with increasing temperature and the C1-specific polypeptides were absent from axonemes maintained and isolated at 32°C. Preliminary results suggest that it will be possible to use this mutant to isolate additional suppressors at the restrictive temperature with the aim of identifying other genes involved in the same morphogenetic process (30, 31).

Analysis of mutants at the PF6 and PF16 loci provides additional evidence that specialized properties of cellular microtubules may depend on nontubulin polypeptides associated with the tubulin lattice. The pf16 mutants appeared to affect stability of the tubulin lattice, and genetic analysis using a temperature-sensitive variant may permit extensive dissection of the interaction of α and β tubulins with associated proteins.

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