Central-pair Microtubular Complex of *Chlamydomonas* Flagella: Polypeptide Composition as Revealed by Analysis of Mutants

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ABSTRACT Four mutants of *Chlamydomonas reinhardtii* representing independent gene loci have been shown to lack totally (*pf*-18, *pf*-19, and *pf*-15) or nearly totally (*pf*-20) the central microtubular pair complex in isolated axonemal preparations. Analysis of ^{35}S-labeled axonemal proteins, using two methods of electrophoresis, reveals that all four mutants lack or are markedly deficient in 18 polypeptides, ranging in molecular weight from 360,000 to 20,000, that are regularly present in wild-type axonemes. Analyses of axonemal proteins labeled by cellular growth on ^{32}P-labeled medium indicates that a subset of 8 of the 18 polypeptides are phosphorylated.

Mutant and wild-type axonemes and flagella have been analyzed for their content of tubulin subunits using a high resolution two-dimensional electrophoresis system combined with agarose gel overlays containing either anti-α or anti-β tubulin sera prepared from *Chlamydomonas* tubulins. The immunoprecipitates identify two major α tubulins, a major β tubulin, and a minor component which is also precipitated by the anti-β serum. None of these tubulins shows a specific defect in mutant axonemes, nor do the tubulin polypeptides show altered two-dimensional map positions in the mutant flagella.

The 18 polypeptides provide a useful signature for identifying other mutants affecting the central-pair microtubular complex. Such mutants could be useful in defining the structural or functional role of these polypeptides in the central microtubules. Efforts to obtain additional central-pair mutants based on the motility phenotype of the four mutants analyzed here have yielded mutants which are allelic to three of the four mutants.

The analysis of axonemal polypeptides in mutants of *Chlamydomonas reinhardtii* with defects in flagellar structures has proved to be a valuable approach to the study of assembly and function of eukaryotic flagella. Using electrophoretic methods which identify nearly two hundred axonemal polypeptides, wild-type axonemes have been compared with those from mutants lacking radial spokes (1, 2, 3) and inner or outer dynein arms (4). In each case, the comparison revealed that the mutant axonemes were deficient in a set of polypeptides, each set being unique for the missing structure. The missing polypeptides identify possible structural components of the radial spokes or dynein arms; they also serve as a signature by which additional mutants affecting these structures can be identified or by which polypeptides present in the course of enzyme purification can be identified with specific axonemal structures. The usefulness of such signatures is exemplified by the identification of several mutants affecting radial spokes (2, 3) and by progress in purification of dyneins (4, 5).

This paper is concerned with the flagellar and axonemal polypeptides of a series of mutants defective in the central microtubules and their appended structures. The four mutant strains which have been analyzed represent unlinked, independent loci. The mutants, *pf*-15, *pf*-18, *pf*-19, and *pf*-20, were isolated by R. A. Lewin (Scripps Oceanographic Institute),

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mapped by Ebersold et al. (6) and shown to be defective for central-pair structures by Randall and his colleagues (7, 8, 9). The results of our comparative analysis of axonemal proteins from mutant and wild-type strains indicate that all four mutants show total or partial deficiency of the same 18 polypeptides. The 18 polypeptides constitute a unique signature for the central-pair structure.

MATERIALS AND METHODS

Culture of cells (10), labeling with [^{35}S]sulfuric acid (4), labeling with [^{32}P]-phosphoric acid (2), preparation of flagella and axonemes (4), and electron microscopy (4) were performed as previously described.

Mutant Strains and Genetic Analysis

Cultures of *pf*-20 were provided by N. H. Chua (Rockefeller University), *pf*-15 and *pf*-18 by P. R. Levine (Harvard University), and *pf*-19 by G. B. Witman (Princeton University). The newly isolated central-pair defective mutants were obtained following chemical mutagenesis of wild-type strain 137c with nitrosoguanidine (NSG), methylmethanosulfate (MMS), or ICR-191 (Terochem Laboratories, Ltd., Edmonton, Canada) as previously described (3, 11). All genetic analysis was performed using standard techniques (12, 13).

One- or Two-dimensional Electrophoresis of Polypeptides

The methods used for two-dimensional electrophoresis of ^{35}S-labeled flagellar and axonemal polypeptides were as previously described (2). The separation of polypeptides with molecular weights >200,000 daltons was obtained on one-dimensional polyacrylamide gradient gels using the Neville gel system (14) as modified by Chua and Bennoun (15). β-mercaptoethanol (0.1%) was added to the cathodic buffer. The slab gels were composed of a 3.2% stacking gel and a running gel formed by a 4–8% acrylamide gradient containing SDS. The gels were electrophoresed 20 h at 20 mA/gel. Calibration of the gels was performed as previously described (5).

To obtain resolution of the flagellar and axonemal tubulin subunits, the methods used for two-dimensional electrophoresis were modified as follows: (*a*) the protein load was adjusted to optimize resolution of the α and β tubulins; 1.5 μg of ^{35}S-labeled proteins (specific activity, 170,000 cpm/μg) were loaded on the first dimension gels; (*b*) the proteins were applied to the cathodic side of the first dimension gel; (*c*) isoelectric focusing was prolonged to approach equilibrium (1); and (*d*) a continuous tris-glycine gel system (16) was used in the second dimension to optimize separation of the α and β tubulins.

Analysis of Polypeptide Maps

Autoradiograms derived from paired or separate two-dimensional electrophoreses were superimposed and compared. The absence of spots or the appearance of new components were noted on each film. In this paper we have described only those differences that were reproducible in all maps analyzed. The data were derived from at least three independent axoneme preparations.

Antiserum-Agarose Overlay on Polyacrylamide Gels

Antiserum-agarose gels 0.1 × 7 × 7 cm were prepared in a glass mold. Rabbit sera in variable amounts (3–10% vol/vol, depending on the antibody titer) were added at 54°C to 1% molten agarose (Seakem; FMC Corp., Marine Colloids Div., Rockland, Maine) in 0.087 M barbital/HCl (pH 8.7). 0.1 × 7 × 7 cm polyacrylamide gel portions containing the polypeptides to be analyzed were incubated 1 h in 100 ml 2.5% Lubrol PX. Subsequently, polyacrylamide gels were rinsed in H_2O, sandwiched between antiserum-agarose gels and incubated in moist atmosphere for 24 h. Agarose gels were then washed in 100 ml 0.13 M NaCl, 0.01 M phosphate buffer (pH 7), four times over a period of 48 h and finally processed for autoradiography. All operations were performed at room temperature.

RESULTS

Description of the Mutant Strains

Mutant strains representing the original R. A. Lewin isolates were obtained from several sources as noted in Materials and Methods. Each was backcrossed to the 137c wild-type strain, and the map location for each mutant daughter strain was checked by recombination analysis with appropriate marker strains as shown in Table I.

In the course of characterizing *pf*-18 × 137c progeny, a second mutation resulting in defective motility was found to be present in the original stock. Because segregation of the defective motility phenotype from the *pf*-18 phenotype occurred at low frequency, the secondary mutation appears to be

TABLE I

Alleles of pf-18, pf-15, pf-19, and pf-20

Locus	Map location	Previously isolated alleles*	Newley isolated alleles	Mutagen	Phenotype
pf-18	II R	*pf*-18A-F	*pf*-18G	MMS	Paralyzed-rigid
			pf-18H	MMS	Paralyzed-rigid
			pf-18I	ICR-191	Paralyzed-rigid
			pf-18J	ICR-191	Paralyzed-rigid
			pf-18K	ICR-191	Paralyzed-rigid
			pf-18L	ICR-191	Paralyzed-rigid
			pf-18M	ICR-191	Paralyzed-rigid
			pf-18N	ICR-191	Paralyzed-rigid
pf-15	III L	*pf*-15, *pf*-15A-B	—	—	—
pf-19	X L	*pf*-19A-E	*pf*-19F	NSG	Paralyzed-rigid
			pf-19G	ICR-191	Paralyzed-rigid
pf-20	IV R	*pf*-20A-E	*pf*-20F	NSG	Paralyzed-rigid‡
			pf-20G	NSG	Paralyzed-rigid‡
			pf-20H	ICR-191	Paralyzed-rigid‡
			pf-20I	ICR-191	Paralyzed-rigid‡
			pf-20J	ICR-191	Paralyzed-rigid‡
			pf-20K	ICR-191	Paralyzed-rigid‡
			pf-20L	ICR-191	Paralyzed-rigid‡
			pf-20M	ICR-191	Paralyzed-rigid‡
			pf-20N	ICR-191	Paralyzed-rigid‡
			pf-20P	ICR-191	Paralyzed-rigid‡

* See reference 18.

‡ In addition to cells with paralyzed-rigid flagella the population included some cells with motile flagella.

closely linked to *pf*-18. A daughter (*pf*-18.8D$^+$) lacking the secondary mutation was selected and used for further analysis.

The motility phenotypes of daughter strains selected for biochemical analysis conform to those previously described (8): *pf*-18.8D$^+$ shows paralyzed rigid flagella; flagella of *pf*-15.3B$^+$ and *pf*-19.24B$^+$ show in addition some flexibility and jiggling, whereas *pf*-20.3C$^+$ shows a heterogeneous cell population which includes those with paralyzed rigid flagella, jiggly flagella, and some beating flagella which give some cells the appearance of wild-type swimming.

As seen in Table I, a number of alleles for the *pf*-18, *pf*-19, *pf*-20, and *pf*-15 loci have been previously identified (see reference 17). In our laboratory, 20 independently isolated mutant strains with the paralyzed rigid flagellar phenotype were recovered following chemical mutagenesis of wild-type 137c. Using complementation analysis in temporary dikaryons (18), we found each of these mutants to be a recessive allele for either *pf*-18, *pf*-19, or *pf*-20. Mutations for new loci were not identified. In the case of the eleven new alleles for *pf*-20, all showed a heterogeneous motility phenotype.

Flagellar and Axonemal Morphology

As illustrated in Fig. 1*A*, flagella of *C. reinhardtii* show a classical 9+2 organization of microtubules. The central-pair microtubules extend the entire length of the flagellum and have been shown to terminate in a specialized flagellar membrane-associated "cap structure" (19, 20) (not illustrated). As previously described (21, 22, 23), the central tubules each bear periodic projections along their length and are linked to one another by dense material.

Fig. 2*A* illustrates the morphology of wild-type axonemes obtained by detergent treatment of isolated flagella. In cross sections, the central tubules are clearly seen to be differentiated by the presence of two long or two short projections. In appropriate longitudinal images, pairs of central-tubular projections appear to interact with individual radial spokes which project from subfiber A of the outer doublets. With the methods used in this study for preparing axonemes, we have observed, as previously reported (21), that one of the central-pair microtubules shows instability. In a typical wild-type preparation from which we obtained electrophoretic characterization of axonemal polypeptides, out of 432 axonemal cross sections which were examined, 32% showed the loss of one of the central tubules. Although not apparent in all cases, the loss of central-pair structure appeared to be specific for the tubule bearing short projections. We have also observed by thin-section and negative-stain electron microscopy that the central microtubular cap structure is lost following treatment of isolated wild-type flagella with 0.5% nonidet P-40 (NP-40).

The flagellar morphology of the four central-pair defective strains analyzed in this study correspond to those previously described for the mutants (7, 8, 23). In each of the mutants the central-pair microtubule complex has been replaced by a core of dense material running longitudinally down the center of the axoneme. This dense material is illustrated in the longitudinal and cross-sectional images of *pf*-18 flagella shown in Fig. 1*B*. As previously reported by Warr et al. (8), we have observed that the "leaky" motility phenotype of *pf*-20 is correlated at the ultrastructural level with the presence of a proportion of flagellar transverse and longitudinal sections showing one or two wild-typelike central tubules. Examination of 69 flagellar cross sections of *pf*-20.3C$^+$ revealed twenty-four 9+2, three 9+1, and forty-two 9+0 axonemal profiles.

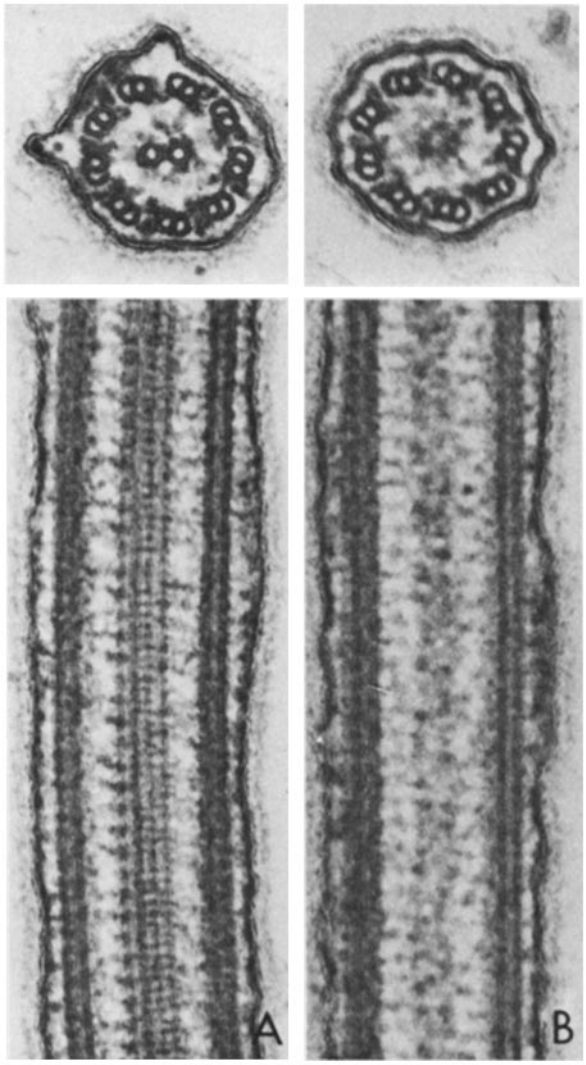

FIGURE 1 Transverse and longitudinal thin-section electron micrographs illustrating the morphology of wild-type (*A*) and *pf*-18 (*B*) flagella fixed *in situ*. *A*, flagella of wild-type *Chlamydomonas* show the classical organization of nine outer doublet and two central singlet microtubules. The central-pair microtubules each bear periodic projections along their length and are linked to one another by dense material. *B*, in *pf*-18 flagella, the central pair microtubule complex has been replaced by a core of dense material running longitudinally down the center of the axoneme. × 110,000.

In each of the four mutants we have observed that during the course of flagellar isolation and conversion to axonemes by treatment with 0.5% NP-40 the dense core material is lost. As illustrated in Fig. 2*B*, isolated axonemes from *pf*-18, *pf*-19, and *pf*-15 show completely empty centers. In the case of *pf*-20.3C$^+$, we have noted that in 448 cross sections, 429 showed no central pair structures, while 21 (<5% of the total) showed the presence of one of the central-pair tubules. The morphological studies just described gave indication that for our proposed study of polypeptides associated with the central pair and its appended structures, axonemal preparations from wild-type and the four mutant strains provided useful starting material.

Analysis of Axonemal Proteins

Comparison of axonemal proteins from mutant and wild-type preparations gave indication that two types of electropho-

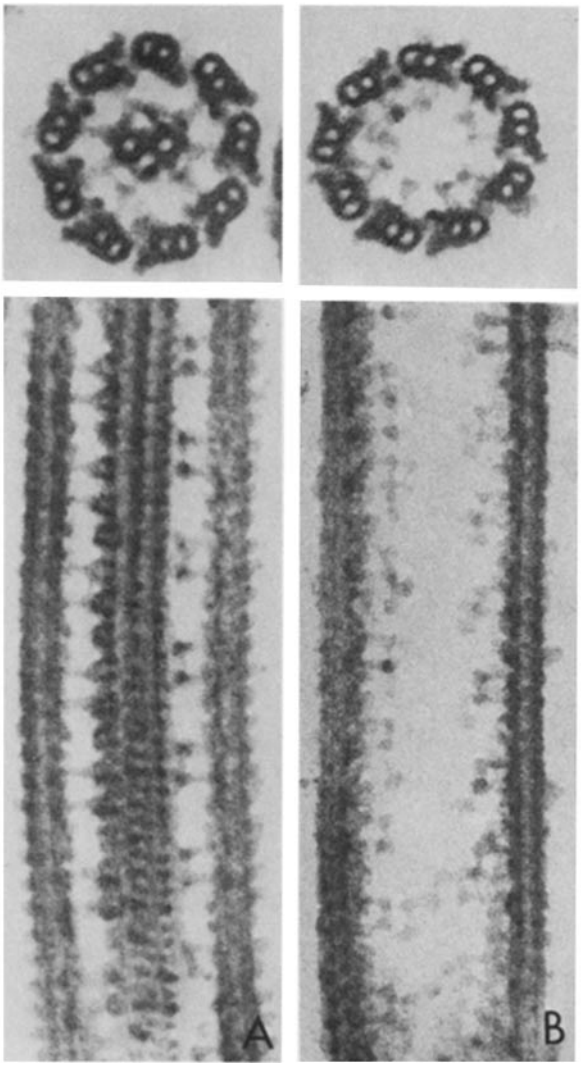

FIGURE 2 Thin-section electron micrographs illustrating the morphology of isolated axonemes from wild type (*A*) and *pf*-18 (*B*). *A*, top panel: a transverse section of an isolated wild-type axoneme in which the central tubules are clearly seen to be differentiated by the presence of two long or two short projections. Bottom panel: a longitudinal section of a wild-type axoneme in which pairs of central tubule projections appear to interact with individual radial spokes projecting from the outer doublet on the left. *B*, isolated axonemes from *pf*-18 showing the absence of any central-pair associated structures in the central region of the axonemes. × 150,000.

retic procedures were required to reveal the mutant deficiencies: a two-dimensional method, i.e., nonequilibrium electrophoresis in an ampholine gradient followed by gradient gel electrophoresis in SDS, to characterize polypeptides in the molecular weight range 200,000–15,000; and a one-dimensional electrophoretic procedure to characterize polypeptides of molecular weights >200,000.

Fig. 3 shows a comparison of wild-type and mutant preparations analyzed to reveal polypeptides in the molecular weight range 360,000–220,000. As indicated, four polypeptides regularly found in wild type were missing in the central-pair defective mutants. Only in the case of *pf*-20 were small and variable quantities of components 1–4 detected. In Fig. 3, a band of slightly lower mobility than component 2 is visible in the mutant preparations. Its occurrence in mutant and wild-type preparations is variable and independent of component 2. The components labeled I, II, V, and X are high-molecular-weight subunits of axonemal dyneins (5) and do not show significant variation among wild-type and mutant preparations. They serve as a control for equal axonemal mass loading in the five electrophoretic lanes.

Additional deficiencies for mutant axonemes were found in the molecular weight range 200,000–15,000. Two-dimensional electrophoretograms for wild-type and *pf*-18 axonemal proteins are shown in Figs. 4*A* and *B*. Comparisons of a number of preparations of this kind reveal consistent absence or deficiency in *pf*-18 of 14 polypeptides regularly present in wild type. The same 14 polypeptides were found to be missing completely or present in very reduced amounts in *pf*-15, *pf*-19, and *pf*-20. The data for all four mutants are summarized in Table II. It should be noted that data summarized in this table are based on analysis of at least five different preparations for each mutant. Where a component has never been detected it is noted (−); in the categories designated R at least some preparations showed traces of the polypeptide.

In Fig. 4*A* it will be noted that components 6, 8, 9, and 12 of the central-pair signatures are labeled as multiple components consisting of two or more polypeptides in the same molecular-weight class, but with unit differences in isoelectric behavior or as a cluster with differences in isoelectric behavior and mobility in the second electrophoretic dimension (component 9). Since in the four mutants, deficiency or absence of all components in each group was coordinate, we considered them to be a single class, and based on our experience with heterogeneous polypeptides associated with radial spokes (2), we questioned whether the basis of heterogeneity of these components was protein modification by phosphorylation.

To study this question, we carried out experiments in which cultures of wild-type and *pf*-18 strains were pulse-labeled in vivo with $^{32}PO_4$ and their axonemal proteins separated by the two electrophoretic methods. Comparative analyses of *pf*-18 allowed us to identify without ambiguity eight polypeptides in wild-type axonemal ^{32}P-labeled electrophoretograms. As indicated in Table II, components 1, 3, 5, 6, 8, 9, 12, and 16 were labeled by ^{32}P. Heterogeneity of components 6, 8, 9, and 12

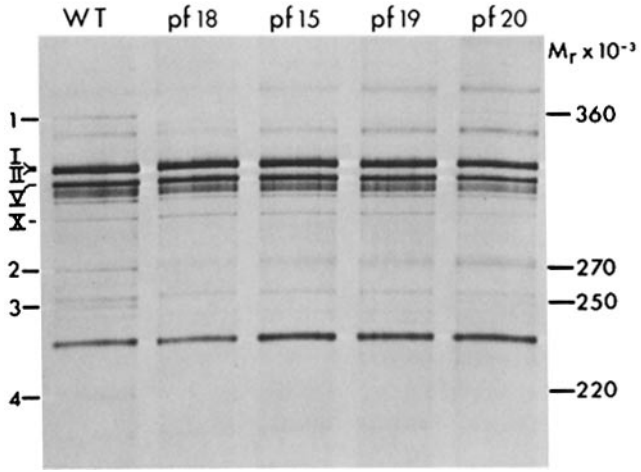

FIGURE 3 Autoradiogram of the one-dimensional separation of high-molecular-weight polypeptides from wild-type (*wt*) and mutant axonemes. Only a portion of the slab gel is shown. Four wild-type bands (numbered 1–4) are absent on *pf*-18, *pf*-15, and *pf*-19, and present at diminished intensities in *pf*-20. The positions of the high molecular weight dynein subunits I, II, V, and X (5) are indicated for reference and serve as a control for equal mass loading in the five electrophoretic lanes.

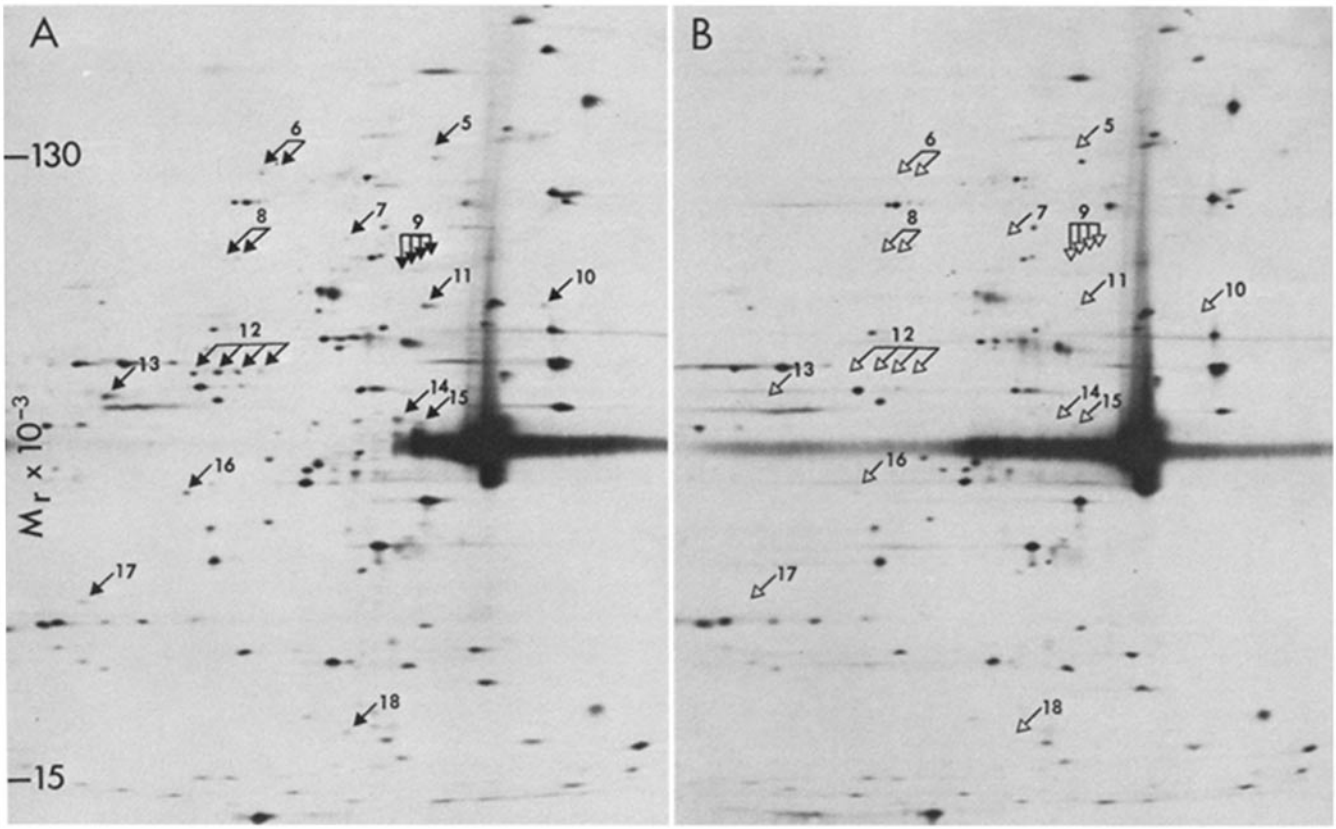

FIGURE 4 Autoradiograms of the polyacrylamide gel slabs used for the two-dimensional separation of ^{35}S-labeled axonemal polypeptides. The relevant portions of the original maps are shown. Proteins were applied at the anode. Basic polypeptides are on the left side. Tubulin subunits are not resolved and form a streak as a consequence of overloading. *A*, wild-type axonemes. Arrows point to 14 polypeptides which are absent in the map shown in *B*. Polypeptides are numbered from 5 to 18 in order of decreasing molecular weight. *B*, *pf*-18 axonemes. Arrows with open arrowheads indicate the positions where polypeptides are missing. Only those reproducible differences between the mutant and wild-type axonemes are described.

could be explained as representing a single polypeptide with multiple states of phosphorylation.

Analysis of axonemal polypeptides gave a consistent signature for the central pair, composed of 18 polypeptides ranging in molecular weight from 360,000 to 20,000. A subset of 8 of the 18 appear to be phosphorylated.

Analysis of Flagellar Proteins

Having established a central-pair polypeptide signature for axonemes, we analyzed wild-type and mutant flagellar preparations to determine whether any of these components were present in flagella either as soluble components or assembled into the dense core material. The results are shown in Table II. In all four mutants, seven components, 1, 3, 4, 5, 11, 12, and 16, were found to be present in the flagella at their wild-type positions. It should be noted that in each mutant, the flagellar polypeptides were totally deficient in six or more components. There is the possibility that one of the missing components represents the gene product.

Microtubular Proteins

To determine whether unique tubulins were associated with central-pair structures, we analyzed tubulin subunits in axonemal preparations of wild-type and mutant strains. To explore whether the central-pair defect resulted from a mutation in microtubular proteins resulting in an electrophoretic tubulin variant, we carried out the same analysis in flagellar preparations of *pf*-15, *pf*-18, *pf*-19, and *pf*-20.

These analyses were made by modifying the two-dimensional electrophoresis procedures of Fig. 4 to obtain better resolution of tubulin subunits. Details of the modifications are given in Materials and Methods. A typical two-dimensional resolution of the tubulin region for a wild-type flagellar preparation is shown in Fig. 5 *B*. As indicated, α tubulin is resolved as two separate components, while β tubulin appears to be a single component. This pattern showed no qualititative variation in flagellar preparations or in axonemal preparations of *pf*-15, *pf*-18, *pf*-19, or *pf*-20. Components 14 and 15 of the central-pair signature and a flagellar actinlike protein previously described (24) are present in the region of the map shown in Fig. 4 *B* and are indicated by arrows.

As previously noted, components 14 and 15 were absent in axonemes of all four mutant strains. Since these components are in the molecular-weight range of tubulin subunits, we tested their relatedness to tubulin using an immunochemical approach. A two-dimensional gel of the type shown in Fig. 5 *B* was sandwiched between agarose gels containing antisera to α and β tubulin subunits from *Chlamydomonas* axonemes (25). After 24 h, the gels were separated, washed, and subjected to autoradiography. As shown in Fig. 5 *A*, the anti-α serum immunoprecipitated both α tubulin components but not components 14 or 15. Component 15 has a molecular weight higher than that of tubulin subunits. In Fig. 5 *A* it would be detected above the streak which is caused by traces of α components. Fig. 5 *C* illustrates the immunoreplica obtained with the anti-β serum. In addition to the precipitate corresponding to the β

TABLE II

Summary of Axonemal and Flagellar Polypeptide Deficiencies of Central-Pair Mutants

Polypeptide #	Molecular weight	Axoneme*				Flagella				^{32}P-label	Multiple spots
		pf-18	*pf*-15	*pf*-19	*pf*-20	*pf*-18	*pf*-15	*pf*-19	*pf*-20		
	× 10^3										
1	360	−	−	−	R	+	+	+	+	+	ND
2	270	−	−	−	R	−	−	−	+	−	ND
3	250	−	−	−	R	+	+	+	+	+	ND
4	220	−	−	−	R	+	+	+	+	−	ND
5	142	−	−	−	−	−	−	−	−	+	−
6	128	−	R	R	−	+	+	+	+	+	+
7	106	−	−	R	−	+	−	+	−	−	−
8	97	−	−	−	−	−	+	−	+	+	+
9	97	−	−	−	−	−	−	−	−	+	+
10	84	−	−	−	−	−	−	−	−	−	−
11	84	R	−	−	−	+	+	+	+	−	−
12	66	−	−	−	−	+	+	+	+	+	+
13	62	−	−	−	−	−	+	−	−	−	−
14	57	−	−	−	−	+	−	+	+	−	−
15	56	−	−	−	−	+	−	+	+	−	−
16	45	R	R	R	R	+	+	+	+	+	−
17	32	−	−	−	−	−	−	−	−	−	−
18	20	−	−	−	−	+	−	+	+	−	−

* − component not present; +, component detected; R, component absent or detected at low levels in some preparations; and ND, not determined.

subunit, a minor component characterized by more basic isoelectric point is detected (see arrow). However, components 14 or 15 were not precipitated.

Immunoprecipitation obtained by antiserum-agarose overlay appears to have high sensitivity. As it is shown in Fig. 5*A* and *C* all tubulin subunits were detected in trace amounts in the streaks. Nonetheless, additional experiments were performed with higher (2–4 times) amounts of proteins to exclude the possibility that components 14 or 15 did not precipitate because of their presence in the gels below critical concentrations. Again it was found that those components did not react with antitubulin antisera.

The study of microtubular proteins in flagellar and axonemal preparations from wild-type and the four central-pair deficient mutants, provide no evidence for a unique tubulin species (electrophoretic mobility or isoelectric point) associated with the central pair, nor is there positive evidence for a mutationally altered tubulin in any of the four mutants.

DISCUSSION

Analysis of axonemal polypeptides in four central-pair defective mutants of *C. reinhardtii* reveals that all show absence or marked deficiency of the same set of 18 polypeptides. The polypeptides, which are regularly found in wild-type axonemes range in molecular weight from 20,000 to 360,000. Although the four mutants, *pf*-15, *pf*-18, *pf*-19, and *pf*-20, each represent a mutation at a different locus, all show in their isolated axonemes absence of the central-pair complex. The set of 18 polypeptides serve as a signature which is unique. None of the 18 polypeptides have been found to be missing or reduced in amount in mutant axonemes lacking radial spokes (2, 3), the outer dynein arm (4), or the inner dynein arm (4). Three of the 18 polypeptides described here are likely to correspond to the three polypeptides, molecular weights >220,000 found to be missing in *pf*-19 and *pf*-15 axonemes in an earlier report (23).

It is likely that part or all of the signature components represent structural proteins of the central-pair microtubules and their appended structures, but not one of the eighteen missing polypeptides appears to be a microtubular protein subunit. Components 14 and 15, which are close in electrophoretic migration in a pH gradient and in apparent molecular weight to α and β tubulin subunits, do not cross react with the specific antisera for these polypeptides. The significance of the immunoprecipitation of one minor component which is more basic than the β tubulin subunit cannot be assessed yet. Since the antiserum was directed against the β subunit purified by preparative electrophoresis (25), it is possible that minor axonemal components were not resolved and were injected in rabbits as contaminants. In that case precipitation of the minor spot was caused by antibodies which are not specific for β tubulin. Alternatively, the minor component is a β tubulin subunit and it should be identified by additional work.

In wild-type flagella and axonemes examined by highly resolving two-dimensional methods, microtubular proteins are observed to be present as two α components and one β component. This pattern corresponds to the resolution of *Chlamydomonas* flagellar tubulin observed by Lefebvre et al. (26). In axonemes isolated from each of the mutants, in which the central-pair tubules are missing, the same microtubular-protein-subunit pattern was observed, giving indication that neither of the two α tubulin components are specifically associated with the central-pair microtubules.

Central-pair α and β tubulins from sea urchin sperm have been reported to differ from their outer doublet counterparts in amino acid composition and in their tryptic peptide maps (27). If this were true in *Chlamydomonas* flagella, the expectation would be that the central-pair specific tubulin subunits would appear as variants in the two-dimensional gel electrophoretograms. No specific central-pair tubulin has been identified in our experiments. With our present methods it is not possible to carry out quantitative analyses to correlate absence of the central-pair microtubules of mutant axonemes with an expected 12% reduction in the major α and β tubulin regions. The absence of these data makes somewhat tentative our conclusion that in *Chlamydomonas* axonemes, central-pair tubulin does not constitute a unique species.

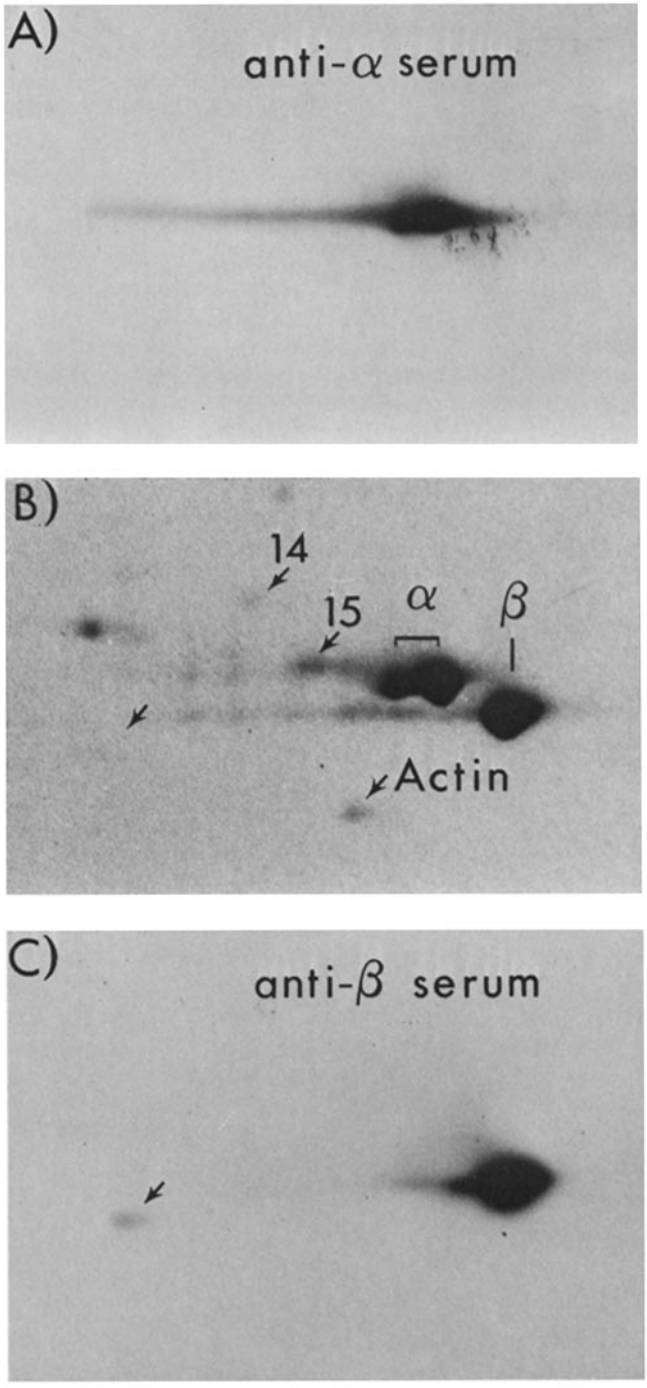

FIGURE 5 Immunochemical analysis of wild-type flagellar tubulins. *B*: autoradiogram of the two-dimensional resolution of ^{35}S-labeled flagellar polypeptides obtained with the procedural modifications indicated in the Materials and Methods. Only a portion of the map containing the tubulin subunits is shown. A similar gel portion from a paired two-dimensional electrophoresis was sandwiched between agarose gels containing anti-α or anti-β sera. *A* and *C*: autoradiograms of agarose gels containing immunoprecipitates. *A*, polypeptides precipitated by an antiserum directed against α tubulin. *C*, polypeptides precipitated by anti-β serum.

Included in the 18 signature polypeptides may be structural components of the short and long projections of the central microtubules (23) or the dense material linking the microtubules. Since the central-pair "cap" structures at the flagellar tips (20) are lost in our preparations of wild-type axonemes, it is unlikely that their component polypeptides are part of the signature. Clearly, understanding of the function and structural site of the 18 polypeptides requires additional experiments. A most powerful use of the central-pair signature will be the screening and identification of new mutants affecting the central-pair complex. This type of screening seems all the more important because, based on the experience of Randall and his colleagues (8) and our own, identification of central-pair defective mutants by the paralyzed rigid flagellar phenotype only results in the isolation of more alleles for the *pf*-15, *pf*-18, *pf*-19, and *pf*-20 gene loci.

Previous studies have shown that the central-pair microtubules of cilia and flagella have several properties similar to those of cytoplasmic microtubules. Structurally, they are singlet microtubules composed of 13 protofilaments (28) which, in contrast to the other doublet microtubules, are assembled with no differentiated association with structures of the basal bodies (29). Chemically, the central-pair microtubules show cold-lability (30) and can be readily solubilized into native tubulin subunits with colchicine-binding activity (31). In light of these similarities with cytoplasmic microtubules, it is of interest to note that several of the signature polypeptides of the central pair show similarities in molecular weight and modification by phosphorylation to microtubule-associated proteins from brain and other sources (32, 33, 34, 35). Specifically, one of the high molecular weight components 1–4 may correspond to microtubule-associated proteins (MAPs) 1 or 2, and components 12–15 may be related to tau proteins. If any of the central-pair signature polypeptides are analogous to MAPs or tau, the polypeptides of flagellar origin may affect the polymerization of purified tubulin from a heterologous cytoplasmic source. There is already evidence that flagellar extracts from *Chlamydomonas* and sea urchin sperm stimulate the in vitro polymerization of brain tubulin (36).

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