Paralyzed Flagella Mutants of *Chlamydomonas reinhardtii*

DEFFECTIVE FOR AXONEMAL DOUBLET MICROTUBULE ARMS

(Received for publication, October 5, 1978)

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In most motile eukaryotic cilia and flagella, two rows of arms (outer and inner) are found along subfiber-A of the peripheral doublet microtubules (1). Electron microscopic studies have shown that these microtubule arms are complex structures and that those found on the outer and inner rows differ in morphology (2–3). Previous studies have indicated that the doublet microtubule arms contain the major flagellar ATPases, dyneins (4–6), and that the arms are responsible for generating ATP-dependent sliding displacements between peripheral microtubules by forming transient cross-bridges with subfiber B on adjacent doublets (7–10).

For several years our laboratory has been exploring the use of genetics in the biflagellate alga, *Chlamydomonas* to study the structure, function, and assembly of flagella. Lewin was the first to demonstrate the feasibility of utilizing this approach in *Chlamydomonas*, when he isolated a large number of paralyzed flagella mutants (11). Several of these mutants, designated pf mutants of *Chlamydomonas reinhardtii*, were shown to represent independent loci distributed throughout the genetic map (12). Recent studies from our laboratory and others on pf mutants identified as specifically lacking central pair microtubules and associated structures, and mutants totally or partially defective for radial spoke structures, have provided new information as to the function, polypeptide composition, and assembly of these axonemal structures (13–17).

In this communication we report the isolation and identification of five paralyzed flagella mutants of *C. reinhardtii* with defects for the assembly of the axonemal doublet microtubule arms. In contrast to the recently reported cases of human sperm immotility in which electron microscopy of the flagella showed an absence of both the outer and inner arms (18, 19), the mutants described in this paper show specific ultrastructural defects for either the outer row or inner row of arms. The availability of these mutants in an organism which has several proven advantages for genetic and biochemical studies on flagella, provides a new and complementary means for studying the structure and function of the peripheral doublet arms.

In this study we have characterized the genetics and flagellar ultrastructure of the mutants. The mutant deficiencies for outer versus inner arms have been correlated with deficiencies for two different groups of wild type axonemal polypeptides resolved by polyacrylamide gel electrophoresis (11). Several of these mutants, designated pf mutants of *Chlamydomonas reinhardtii*, were shown to represent independent loci distributed throughout the genetic map (12). Recent studies from our laboratory and others on pf mutants identified as specifically lacking central pair microtubules and associated structures, and mutants totally or partially defective for radial spoke structures, have provided new information as to the function, polypeptide composition, and assembly of these axonemal structures (13–17).

**EXPERIMENTAL PROCEDURES**

The methods used for preparation of axonemal extracts, ATPase assays, and separation of polypeptides by one- and two-dimensional polyacrylamide gel electrophoresis are described in the accompanying paper (20).

**Mutant Strains and Genetic Analysis**—A culture of pf 13 was kindly provided by N. W. Gillham, Department of Zoology, Duke University. The new mutants, described in this communication, were isolated in our laboratory following chemical mutagenesis of wild type *Chlamydomonas* (20). N-nitrosoguanidine, Hepes, 4-(2-hydroxyethyl)-1-piperazinemethanesulfonic acid; SDS, sodium dodecyl sulfate.

The abbreviations used are: nitrosoguanidine, N-methyl-N-nitro-N-nitrosoguanidine. Heps, 4-(2-hydroxyethyl)-1-piperazinemethanesulfonic acid; SDS, sodium dodecyl sulfate.
multiple marker stocks to determine their map locations. The centromere distance for each mutant was calculated from the tetrateype frequency observed in crosses to ac-17 by the method of Gowans (24).

Culture and Isolation of Flagella and Axonemes—Cells were grown on agar plates containing low sulfate media as previously described (17). For radioactive preparations H2SO4 was added to the growth media at 50 mM. Flagella were prepared as previously described (16) with the following modifications. Cells were deflagellated by pH shock in a solution containing 10 mM Hepes (pH 7.2), 0.2 mM SrCl2, and 1 mM dithiothreitol. Immediately following deflagellation MeCl2 was added to the suspension of flagella and cell bodies to give a final concentration of 4 mM. Although we have no evidence that proteases are present in our preparations, we routinely add Trypsin (Mobay Chemical Corp.) to a final concentration of 50 units/ml to the above suspension. Cell bodies were removed by differential centrifugation. Axonemal preparations were obtained by the addition of Nonidet P 40 to flagella suspensions to give a final concentration of 0.5%. At the time of detergent addition, NaCl was added to give a final concentration of 50 mM. Aliquots (-20 ml) of axoneme suspensions were pelleted through a 5-mL cushion of 5% sucrose. In comparison to the previously described protocol, this procedure gives better preservation of the central pair microtubule complex and higher, more consistent levels of Mg2+-dependent ATPase activity measured in isolated axonemes.

Electron Microscopy—Cells and isolated axonemes were fixed as pellets by the addition of 2% glutaraldehyde in 10 mM sodium cacodylate (pH 7.2) containing 1% tannic acid (Mallinkrodt). After fixation for 1 h at room temperature, the pellets were washed for 15 min in 10 mM sodium cacodylate (pH 7.2) containing 1% sodium sulfate. Following a brief wash in buffer alone, the pellets were fixed in 1% osmium tetroxide in 10 mM sodium cacodylate (pH 7.2) on ice for 30 min. After dehydration through a graded series of ethanol, the pellets were passed through propylene oxide and embedded in Epon 812. Sections cut on a Porter-Blum MT-2 ultramicrotome were stained with uranyl acetate and lead citrate (25), and examined in a Phillips 300 electron microscope.

RESULTS

Genetic Analysis of the Arm-defective Mutants—The five mutants investigated in the present studies are listed in Table I. As described in the following section, the mutants show specific ultrastructural defects for either the outer row or inner row of arms on the axonemal doublet microtubules. Four of the mutants are deficient for outer arms, while one has been identified as inner arm-defective. One of the outer arm mutants is pf-13, a mutant previously isolated by Lewin with uv irradiation and mapped by Ebersold et al. (12). The other four mutants were isolated in our laboratory following chemical mutagenesis with nitrosoguanidine or methylmethanosulfate.

Each of the strains has been back crossed to wild type strain 137c, and the mutant phenotype was found to segregate 2:2 as expected for single site mutations. The four outer arm mutants were tested for linkage by recombination analysis and for allelism by complementation testing in temporary heterokaryons as previously described (23). One of the newly isolated mutants was found to be closely linked and noncomplementing with pf-13; it has been designated pf-13A. Mapping data has confirmed the assignment of the pf-13 locus to linkage group IX, 30 map units to the right of the centromere (for a recent genetic map of C. reinhardtii, see Ref. 26). Genetic analysis has established that the other two outer arm mutants, which were independent isolates, are noncomplementing alleles for a locus distinct from pf-13. Mapping data have established that these two mutants represent mutations for a previously unmarked site on linkage group I, 10 map units to the left of the centromere. These mutants have been designated pf-22 and pf-22A. The inner arm-defective mutant has been mapped to a new site on linkage group XI, 29 map units to the left of the centromere; it has been designated pf-23.

Flagella of each of the five arm-defective mutants are paralyzed. In addition to this common motility phenotype, the mutants share another flagellar characteristic. In cultures growing exponentially the mutant cells assemble only short flagella and are slow to be released from the mother cell wall. Only at stationary phase of growth or as gametes do they form flagella of wild type lengths. pf-13 and pf-13A) or approximately two-thirds of the wild type lengths (pf-22, pf-22A, and pf-23). At this time, it is not clear how this characteristic may be related to the defective assembly of the doublet microtubule arms. It is known that this characteristic always segregates with the motility defect in crosses, and that in induced intragenic revertants restoration of motility is accompanied by restoration in the assembly of full length flagella during exponential growth.3

Ultrastructure of Wild Type and Mutant Axonemes—We have observed that the structural components of the axoneme in C. reinhardtii are not well resolved in thin section preparations fixed routinely with glutaraldehyde followed by osmium tetroxide fixation. Enhanced definition of axonemal structures has been obtained with tannic acid fixation. With this technique, new data on the fine structure of wild type flagellar axonemes has been obtained. Only details of the morphology and distribution of the doublet microtubule arms will be described here.

Fig. 1 contains electron micrographs of wild type flagella and isolated axonemes fixed in the presence of tannic acid. As previously described by Tinely et al. (27), we have observed that tannic acid does not readily penetrate flagellar membranes. The micrograph of a flagellum fixed in situ seen in Fig. 1A is a rare image in which tannic acid staining of the axoneme was detected. In this image and in high magnification cross-sections of isolated wild type axonemes (Fig. 1B) the "negative staining" effect of tannic acid on the subunits of the central pair and peripheral doublet microtubules is evident.

In transverse sections the outer and inner row of arms are seen to extend from subfiber-A of the doublet microtubules. In appropriate images (for example, see Fig. 1A) it is apparent that the outer arms project from the wall of subfiber-A at the junction between two tubulin protofilaments. A similar definition of the attachment site of the inner arms has not been obtained. In Chlamydomonas the arms on the outer and inner rows are morphologically dissimilar. The outer arms appear to be composed of two distinct segments. The proximal segment is curved and extends from subfiber-A for a distance of ~24 nm toward subfiber-B on the adjacent doublet. The distal segment of the outer arms, measuring ~14 nm in length, is positioned at an angle to the proximal segment and points in toward the center of the axoneme. In comparison, the inner arms appear less complex in structure. They extend from subfiber-A for a distance of ~18 nm, and are frequently seen

3B. Huang, Z. Ramants, and D. J. L. Luck, unpublished results.

### Table I

<table>
<thead>
<tr>
<th>Mutants</th>
<th>Mutagenic agent</th>
<th>Map location</th>
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<tr>
<td>Outer arm-deficient</td>
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</tr>
<tr>
<td>pf-13</td>
<td>UV irradiation</td>
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<tr>
<td>pf-23</td>
<td>Methylmethanosulfate</td>
<td>XI*</td>
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* Based on linkage to sr-1.
* Based on linkage to arg-7 and met-r.
* Based on linkage to pf-2 and eyc-10.
Arm-defective Mutants of Chlamydomonas reinhardtii

Fig. 1. Thin section electron micrographs of wild type flagella and isolated axonemes fixed in the presence of tannic acid. A, cross-section of a flagellum fixed in situ. The outer and inner rows of arms extend from subfiber-A of the peripheral doublets. In appropriate images (*) the outer arms are seen to extend from the wall of subfiber-A between two tubulin subunits. When optimally resolved, the outer arms are seen to consist of two defined segments (closed arrow). One of the doublets (open arrow) lacks outer arms. X 166,800. B, cross-section of an isolated axoneme. The appearance of the two rows of arms is similar to that observed in intact flagella. One of the doublets shows ill defined outer arm material (open arrow). The inner arms are readily distinguished from the nexin links (closed arrow). X 166,800. C, a section through a pellet of isolated axonemes. In most of the axonemes, one of the nine peripheral doublets lacks outer arms (*). X 67,500. D, median longitudinal section of a single axoneme. A frontal view of the outer (o) and inner (i) rows of arms along the single subfiber-A is seen at the right. The individual outer arms are distinct (lines). They appear uniformly tilted and repeat at ~24 nm along the length of the tubule. A repeating density, which is likely to correspond to individual inner arms, is seen along the inner aspect of the tubule (lines). These arms are also uniformly tilted, but repeat only at ~48 nm. X 128,000.

The doublet so affected does not appear to have a constant relationship with the central pair microtubules and the orientation of doublets around them.

Fig. 1D shows a longitudinal section of a wild type axoneme in which a frontal view of both rows of arms is seen along a single subfiber-A. The individual arms on the outer row are distinct; they are observed to be uniformly tilted, repeating at ~24 nm center-to-center along the length of the tubule. The arms on the inner row are less distinct. In Fig. 1D a repeating density which is likely to correspond to individual inner arms is seen. The inner arms, like those on the outer row, appear to be uniformly tilted. In contrast to the outer arms, the inner arms repeat at a period of only ~48 nm.

Fig. 2 shows representative cross-sectional images of isolated axonemes from the five paralyzed flagella mutants which we have identified as defective for the doublet microtubule...
arms. In these micrographs it is immediately apparent that \( pf-13 \), \( pf-13A \), \( pf-22 \), and \( pf-22A \) are morphologically similar; outer arms are missing on most of the doublet microtubules while inner arms are present. In contrast, the mutant \( pf-23 \) shows a distinct absence of inner arms with the apparent normal assembly of outer arms. This deficiency for either the outer or inner row of arms is also apparent in micrographs of mutant flagella fixed in situ. Except for the arm defects, each of the five mutants has been observed to be wild type for the other major axonemal components resolved by thin section electron microscopy; these include the central pair microtubules and associated projections, radial spokes, and nexin links.

As illustrated in Fig. 2, in \( pf-13 \), \( pf-13A \), \( pf-22 \), and \( pf-22A \) not all of the doublet microtubules in every mutant axonemal cross-section are devoid of outer arm material. What appear to be intact outer arms and incomplete or abnormal outer arms can be seen on individual doublets. Similar observations have been made for \( pf-23 \) with respect to inner arm structures.

Table II gives the frequency with which outer and inner arm material was detected in an examination of 50 cross-sectional images of isolated wild type and mutant axonemes. These data confirm that although the \( pf-13 \) and \( pf-22 \) mutants are quantitatively deficient for outer arms, each of the mutants shows some assembly of outer arm structures. Of the four mutants, \( pf-13A \) shows the greatest loss of outer arms. From

\[ \begin{array}{|c|c|c|}
\hline
& Outer arms & Inner arms \\
\hline
Wild type & 91 & 100 \\
\hline
pf-13 & 16 & 95 \\
pf-13A & 5 & 99 \\
pf-22 & 22 & 90 \\
pf-22A & 19 & 95 \\
pf-23 & 85 & 24 \\
\hline
\end{array} \]
this analysis, it is also evident that pf-23 is somewhat “leaky” for the assembly of inner arms.

As seen in Table II, the defects in the mutants appear to be specific for either the outer or inner arms. pf-13 and pf-22 mutants showed wild type frequencies for inner arms, and pf-23 showed wild type frequencies for outer arms. To confirm these observations at the level of the fine structure of the arms, selected electron micrographs of wild type and mutant axoneme cross-sections were subjected to rotational image reinforcement (2, 28).

Fig. 3 shows the results of rotational reinforcement on images of wild type, pf-13A, and pf-23 axonemes. In the rotated image of pf-13A no evidence of outer arm material is seen, and the inner arms do not appear to differ in form or dimension from those observed in wild type. Essentially identical results were obtained with the other three outer arm-defective mutants. In the rotated image of pf-23, inner arms are distinctly absent, although the reinforcement of the nexin links which normally originate from subfiber-A in the vicinity of the inner arms is apparent. The reinforced image of the outer arms in pf-23 is similar to that observed for wild type. These results have confirmed our initial identification of pf-13 and pf-22 mutants as defective specifically for the outer arms, and pf-23 for the inner arms.

In our further analysis of the mutants described in the following sections, pf-13A and pf-22 were examined as representative mutants for the two unlinked cistrons affecting outer arm assembly.

**Separation of Axonemal Polypeptides in Polyacrylamide Gels**—To correlate the structural defects observed in the mutants with deletions or alterations in axonemal polypeptides, isolated axonemal and flagellar preparations from wild type and mutant cells labeled with [35S]sulfate to high specific activity were examined by polyacrylamide gel electrophoresis. As described in the accompanying paper (20), two complementary procedures were used to analyze SDS-solubilized axonemal polypeptides. Polypeptides with molecular weights from 240,000 to 15,000 were resolved by a two-dimensional separation: first, electrophoresis in a polyacrylamide slab gel containing Ampholines, and second, electrophoresis in a polyacrylamide gradient slab gel containing SDS. With this technique ~160 axonemal components are resolved (20). Since polypeptides with molecular weights greater than 240,000 are not well resolved in this system, high molecular weight components were separated in low percentage polyacrylamide gradient slab gels containing SDS and a gradient of urea.

Fig. 4 shows an autoradiogram of the one-dimensional separation of high molecular weight polypeptides from wild type and mutant axonemes. Only a portion of the slab gel comprising a molecular weight range of ~400,000 to 290,000 is shown. It is in this region that the mutants have been observed to show distinct alterations when compared to wild type. As previously described (20), in wild type flagellar and axonemal...
preparations 10 polypeptides are resolved in the molecular weight range of 330,000 to 300,000. These components are numbered 1 to X in order of decreasing molecular weight. Polypeptides I, II, and V are the most intensely labeled. Coomassie blue staining of these polypeptides indicates that the higher amount of label parallels the higher amount of mass. In the wild type preparation the intense labeling of Polypeptide V obscures Polypeptides IV and VI, which are more minor components in terms of labeling. Polypeptide IX sometimes appears split into two bands as seen in this wild type preparation.

The high molecular weight polypeptide patterns obtained from axonemes of the mutants differ from wild type both qualitatively and quantitatively. In the slot containing pf-13A axonemes it is apparent that the three major polypeptides I, II, and V are either absent or greatly reduced in amount when compared to wild type. In addition, the less intensely labeled Polypeptide X is missing. Like pf-13A, pf-22 also shows a deficiency for Polypeptides I, II, V, and X, but each of these components is more represented in this mutant. pf-22 differs from pf-13A in that an additional band, Polypeptide VIII, is missing.

The profile obtained for the inner arm mutant, pf-23, is strikingly different from that of the outer arm mutants. In pf-23, like wild type, Polypeptides I, II, and V represent the major bands present in this high molecular weight range. pf-23 also differs from the outer arm mutants in that Polypeptides III, IV, VI, and VII are reduced or missing. pf-23 shows one common deficiency with pf-22; Polypeptide VIII is absent.

The pattern and level of deficiencies for high molecular weight polypeptides described for isolated mutant axonemes has also been observed in intact flagellar preparations from the mutants.

Fig. 5 shows autoradiograms of the two-dimensional separation of axonemal polypeptides. Only a portion of the original maps is shown comprising the molecular weight range 130,000 to 15,000. A, pf-23, B, pf-13A. The maps of the two mutants are complementary in that those polypeptides observed to be deficient in one are present in the other at wild type levels. Polypeptides which are present in reduced amounts. These polypeptides (indicated by arrows with open heads) range in molecular weight from 110,000 to 28,000 and are numbered 1' to 5' in order of decreasing molecular weight. The polypeptides observed to be deficient in pf-23 are present in the map of pf-13A and are indicated by arrows with closed heads.

The map of pf-13A shows an absence of nine different polypeptides which are normally present in wild type. Arrows with open heads indicate the positions where these polypeptides are normally found. These nine polypeptides are identified in the map of pf-23 by arrows with closed heads. Two of the polypeptides missing in pf-13A, numbered 1 and 2, have molecular weights of 86,000 and 73,000, respectively. All of the other missing polypeptides have lower molecular weights in the range of 19,000 to 15,000. The map obtained from the outer arm mutant, pf-22 (not shown) is similar to that of pf-13A. The nine polypeptides observed to be missing in pf-13A are present in small amounts in pf-22.

Table III shows the values of molecular weight characterizing those polypeptides resolved by two-dimensional electrophoresis observed to be deficient in the arm-defective mutants.

![Fig. 5](image_url)
ties. The mutants have been compared to wild type with respect to Mg$^{2+}$-dependent ATPase activity found in suspensions of intact axonemes and in high salt axonemal extracts containing nearly all measured axonemal ATPase activity (20). The results of such an analysis are seen in Table IV. When compared to wild type, the specific activities found in the outer arm mutants, pf-13A and pf-22, are extremely low, in the order of 10 to 20% of that measured for wild type axonemes and high salt extracts. The inner arm mutant, pf-23, also shows a deficiency for axonemal ATPase activity, but not to the extent observed in the outer arm mutants. The specific activity of pf-23 axonemes and high salt extracts is 60 to 80% of that measured for wild type.

ATPase activities present in high salt extracts from wild type and mutant axonemes were fractionated by sucrose density gradient centrifugation after dialysis against a low ionic strength solution. Fig. 6A shows the sedimentation profiles obtained from a wild type extract. Proteins were labeled with $^{35}$S, and their sedimentation profile was obtained by radioactivity measurements. Three peaks of radioactivity are resolved in the wild type gradient. Coincident with the second and third peaks are two peaks of Mg$^{2+}$-dependent ATPase activity, a peak characterized by high specific activity, sedimenting at $\sim 18$ S, and a peak of lower specific activity, sedimenting at $\sim 12$ S.

Fig. 6B shows the results of sucrose density gradient centrifugation of a 0.5 M NaCl extract from the outer arm mutant pf-13A. In contrast to wild type the 18 S peak of radioactivity and ATPase activity are completely missing from the mutant gradient. In addition, the 12 S peak of radioactivity appears sharper and only a small peak of ATPase activity sedimenting at $\sim 13$ S is seen. This small peak of activity observed in pf-13A may represent residual 12 S activity or a distinct activity which is also present in wild type but is obscured by the larger amount of 12 S activity.

Profiles of radioactivity and ATPase activity obtained with extracts from pf-22 axonemes are similar to those observed for pf-13A (results not shown). In this mutant a small peak of activity which sediments at $\sim 13$ S is also apparent. The only significant difference in the profiles of the two mutants is the presence of a small amount of activity in pf-22 sedimenting at 18 S.

In contrast to the outer arm mutants, the profiles obtained from the inner arm mutant, pf-23, resemble those of wild type (results not shown). The 12 S and 18 S peaks of radioactivity and ATPase activity are present in the mutant.

**DISCUSSION**

In this communication we have reported the isolation and identification of five paralyzed flagella mutants of *C. reinhardtii* which show specific ultrastructural defects for either the outer row or inner row of arms on the axonemal doublet microtubules. Four of the mutants were identified as selectively deficient for outer arms, and were found to represent mutations for one of two unlinked genetic loci (pf-13, pf-13A, pf-22, and pf-22A). One mutant was identified as inner arm-defective and has been shown to contain a lesion for a third locus (pf-23).

We have correlated the ultrastructural defects in the mutants with deficiencies for a number of wild type axonemal polypeptides resolved by two different polyacrylamide gel electrophoretic procedures (20). In our analysis each of the mutants was observed to be pleiotropic as might be expected of macromolecular assembly mutants. In the case of the outer arm mutants, pf-13A and pf-22, the common ultrastructural defect was correlated with deficiencies for a common set of 13 axonemal polypeptides, four high molecular weight polypeptides (range 330,000 to 300,000), and nine lower molecular weight polypeptides (range 86,000 to 15,000). Not only did the mutants for two unlinked cistrons show a defect for the same group of polypeptides, but the level to which they were found to be deficient for these polypeptides could be correlated with the extent of loss of outer arms characterizing the mutants. pf-13A, the mutant identified as the most defective for outer arms, showed the greatest deficiency for the polypeptides in question. In the mutant characterized as more leaky for outer arms, pf-22, these polypeptides were found to be present in higher amounts, although reduced in intensity when compared to wild type.

In contrast to the outer arm mutants, the inner arm mutant pf-23 showed a deficiency for a different group of nine axonemal polypeptides, four in the molecular weight range of 330,000 to 300,000, and five in the molecular weight range of 110,000 to 28,000. The one exception to this division of outer arm versus inner arm mutant-deficient polypeptides was high molecular weight Polypeptide VIII. This polypeptide was found to be markedly reduced in pf-23 and also absent in the
outer arm mutant pf-22. At the present time we do not know the significance of this shared deficiency. In pf-13A, the mutant showing the greatest degree of loss of the outer arms, Polypeptide VIII has been observed to be present at wild type levels.

The possibility exists that some of the mutant-deficient polypeptides may be derived from axonemal structures other than the doublet microtubule arms. At the present time, except possibly for high molecular weight Polypeptide VIII, we have no evidence for this. We can exclude that the polypeptides in question are derived from the central pair microtubules and associated structures or the radial spokes since they have all been found to be present in mutants lacking these structures. In addition, it should be noted that our results from analysis of the mutants are consistent with those obtained by chemical dissociation of wild type axonemes from *Chlamydomonas*. As reported in the accompanying paper (20), brief exposure of wild type axonemes to a high salt solution results in the solubilization of the majority of outer and inner arms. Under these conditions, all of the polypeptides observed to be deficient in the arm-defective mutants are among those axonemal components which are completely or partially extracted.

Assuming that the mutant-deficient polypeptides are true components of the outer and inner arms, the data indicate that the doublet microtubule arms are complex structures and that the arms found on the outer and inner rows differ in polypeptide composition.

Our results from analysis of the mutants give indication that in wild type axonemes, the mass contribution of the outer arms must be greater than that of the inner arms. This is based on the observation that three of the polypeptides (I, II, and V) observed to be deficient in the outer arm mutants, are the most prominent high molecular weight components (in terms of mass and labeling) found in wild type axonemes. In comparison, the four high molecular weight polypeptides (III, IV, VI, and VII) affected uniquely in the inner arm mutant, pf-23, are present in lower quantities. These data are consistent with our morphological observations that the outer arms in *Chlamydomonas* repeat along the length of subfiber-A with twice the frequency observed for the inner arms (24 nm versus 48 nm).

Previous studies have indicated that the doublet microtubule arms contain the major axonemal ATPases, dynesins (4–6). We have observed that the outer arm mutants, pf-13A and pf-22, show a common marked deficiency for Mg$^{2+}$ dependent ATPase activity in both axonemes and high salt axonemal extracts containing nearly all axonemal ATPase activity. The levels of activity fell in the range of 10 to 20% of that measured for wild type. The inner arm mutant, pf-23, also shows a reduction in ATPase activity, but the levels of activity found in the mutant were much higher, on the order of 60 to 80% of that measured for wild type.

Sucrose density gradient fractionation of ATPase activities contained in high salt extracts from wild type axonemes resolves two activities sedimenting at ~12 S and 18 S. When such an analysis was performed on extracts from the inner arm mutant, pf-23, both the 12 S and 18 S peaks of activity were observed to be present. In contrast, the marked deficiency in axonemal ATPase activity found in the outer arm mutants, pf-13A and pf-22, was correlated with the complete absence or a marked deficiency for both the 12 S and 18 S activities.

These results suggested that some of the polypeptides observed to be deficient in the outer arm mutants are associated with the 12 S and 18 S ATPase activities. As reported in the accompanying paper (20), the major ATPases contained within these two peaks have been further purified and found to be two different dyneins. In their most purified form both the 12 S and 18 S dyneins have been observed to be multicomponent. Table V lists the outer arm mutant-deficient polypeptides and those polypeptides which have been observed to co-purify with the 12 S and 18 S dyneins. As seen in the table, all of the polypeptides observed to be deficient in the outer arm mutants were found to be associated with either the 12 S or 18 S dyneins: Polypeptides V and III with the 12 S dynein, and Polypeptides I, II, and X, and 1, 2, 4, 5, 6, 7, 8, and 9 with the 18 S dynein. Two additional polypeptides (10 and 11) have been found to co-purify with the 18 S dynein, but are present in both pf-13 and pf-22 mutants. If Polypeptides 10 and 11 are true components of the 18 S dynein, they appear to be selectively retained in the mutants.

These findings suggest that both the 12 S and 18 S dyneins are localized in the outer arms. Several alternatives exist for how these two multicomponent complexes might be associated with the outer arms. The observation that single site mutations for two unlinked cisatrons result in a deficiency for both activities and associated molecules suggests that the two dyneins are likely to be found in a single arm. It is of interest to note that ultrastructurally the outer arms appear to be composed of two well defined segments. The possibility is not excluded, however, that the outer arms within a given row or on different doublet of the axoneme may be heterogeneous, bearing one or the other ATPase complex. In this case, based on the morphology of the mutants, both the pf-13 and pf-22 mutant gene products must be extrinsic to the dyneins and constituents of all outer arms.

The nature of the lesser deficiency for axonemal ATPase activity which we observed for the inner arm mutant, pf-23, was not resolved by fractionation of ATPases on sucrose gradients. Both the 12 S and 18 S peaks of ATPase activity were found to be present in the mutant, an observation that correlates well with the evidence that the polypeptides associated with the major ATPases contained within these peaks are present in pf-23. In the accompanying paper (20) it is noted that a third ATPase which appears to be molecularly

<table>
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<th>Table V</th>
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<td>Polypeptides deficient in outer arm mutants (pf-13A and pf-22)</td>
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<td>---------</td>
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<tr>
<td>High molecular weight polypeptides (M&lt;sub&gt;e&lt;/sub&gt; = 330,000–300,000)</td>
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<tr>
<td>Lower molecular weight polypeptides (M&lt;sub&gt;e&lt;/sub&gt; = 86,000–15,000)</td>
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*From the accompanying paper (20).

As indicated in the accompanying paper (20), Polypeptides I and X are present in all fractions containing the 18 S enzyme activity, but the peak distribution of these polypeptides is displaced from the enzyme activity.
distinct from the 12 S and 18 S dyneins may be present in wild type axonemes. It is of interest to note that four polypeptides (2', 3', 4', and 5') which are characteristically deficient in pf-23 have been found to be associated with this activity. The possibility that inner arms contain a unique ATPase activity is being explored by additional genetical and biochemical studies.

In summary, our analysis of outer and inner arm-defective mutants has led to the identification of a number of polypeptides which are likely to be components of these structures. This analysis has provided evidence that the arms are more complex in polypeptide composition than previous studies have indicated, and suggests further that the arms on the outer and inner rows are composed of different molecules. In the case of the outer arm mutants, a marked reduction in axonemal ATPase activity has been correlated with a specific deficiency for the ATPase activity and polypeptides associated with 12 S and 18 S dyneins recently purified from wild type Chlamydomonas (20).

Acknowledgments—We are grateful to Virginia Kozler, Zenta Ramanis, and Leslie Hanrahan for their excellent assistance in the genetic and electron microscopic aspects of this study, and to Diann Lewis and Diann Petrino for their expert assistance in the biochemical phases of the work.

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