Isolation of a Sixth Dynein Subunit Adenosine Triphosphatase of *Chlamydomonas* Axonemes

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**Abstract.** This study of the axoneme led to the identification of a previously unknown adenosine triphosphatase (ATPase), which is likely a major component of inner dynein arms. The ATPase was isolated from a soluble fraction of axonemes obtained from pf28, a *Chlamydomonas* mutant lacking the outer dynein arms. The activity hydrolyzed up to 2.3 μmol of ATP·min⁻¹·mg⁻¹ of protein (at pH 7.2, in the presence of both Ca²⁺ and Mg²⁺), had a sedimentation coefficient of 11S in sucrose gradient, and cosedimented with four polypeptides of apparent molecular weight 325,000, 315,000, 140,000, and 42,000. Several arguments indicate that the new ATPase is a component of the inner dynein arms. Three or four polypeptides cosedimenting with the activity belong to a group of axonemal components that are deficient in the axonemes of pf23 and pf30, two mutants that display different levels of inner dynein arm deficiency. The 42,000 component is axonemal actin, a subunit of two other inner dynein ATPases. The two polypeptides of molecular weight >300,000 have electrophoretic mobility similar to that of high molecular weight components of outer and inner dynein arms. In spite of some similarities each ATPase isolated from inner or outer arms is composed of a different set of polypeptides. Different ATPases may be required for the modulation of localized sliding of adjacent outer double microtubules in the axoneme.

The outer and inner dynein arms generate the movement of cilia and flagella from their specific location on the microtubules of the axoneme. Studies of dyneins of sea urchin sperm, *Tetrahymena* cilia, and *Chlamydomonas* flagella have mainly focused on the morphology of dynein arms in situ and in vitro, on the identification of molecular components and ATPase activities of dynein arm substructures, on the analyses of formation and ATP-dependent dissociation of microtubule–dynein complexes. The literature on dyneins that describes these major subjects of investigation has been recently reviewed (2, 6, 21, 22).

Analyses of axonemal polypeptides of *Chlamydomonas* mutants, partially deficient or lacking outer or inner arms, allowed the identification of 13 outer and 10 inner dynein arm components (5). Many of these polypeptides are ATPase subunits. In fact, proteins extracted from the axonemes of wild-type cells with a high concentration of NaCl comprised five different ATPase activities carried out by dynein arm components (13, 14, 19, 20). Each ATPase is formed by at least one polypeptide of molecular weight >300,000 (HMW),¹ which may contain sites responsible for ATP binding (15) and be phosphorylated (20). Three outer dynein arm ATPases have completely different molecular composition and two inner dynein arm ATPases are assembled with the same polypeptides of low molecular weight, including an axonemal form of actin (18).

Additional ATPase activities located in the inner dynein arms may exist. In fact three HMW subunits of dynein arms have not been identified and analyzed after isolation. Moreover the morphology of inner dynein arms suggests the existence of two kinds of inner arms, each composed of two or three substructures bridging A and B microtubules (3). These substructures, like those of outer dynein arms, may be a site for ATPase activity occurring during the dissociation of microtubule–dynein linkages (6, 12).

Further studies of inner dynein arms could be developed with two *Chlamydomonas* mutants, oda38 and pf28 (4, 7, 10), each missing outer dynein arms but apparently normal in the remaining axonemal structures. The purification of a previously unknown ATPase activity from the axonemes of the mutant pf28 is described here. Four inner dynein arm components were copurified with the activity and two of them are of molecular weight >300,000.

**Materials and Methods**

*Chlamydomonas reinhardtii* mutant pf 28 was characterized by Mitchell and Rosenbaum (80). The mutant pf 30 was isolated and characterized by Bessie Huang, Zenta Ramanis, and David Luck (Rockefeller University, NY) and further analyzed by Brokaw and Kamiya (1). The axonemes of this mutant are partially deficient of inner dynein arms (1) and are missing two HMW components, III and IV. Mating type + gametes of strain 137c, pf 28, and pf 30 were cultured and labeled with [³⁵S]sulphuric acid as described by Luck et al. (9) except that the radioactivity used was 25 mCi/liter of medium. Axonemes were prepared following the procedure of Huang et al. (5). Extraction of polypeptide components from the axonemes in the pres-

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¹ Abbreviations used in this paper: HMW, molecular weight >300,000.
Table I. Solutions Used for Gel Electrophoresis of HMW Dynein Components

| Solution | Stacking gel | Resolving gel 3.2% | Resolving gel 5% | Cathodic buffer | Anodic buffer |
|----------|--------------|--------------------|-----------------|----------------|--------------|
| 1        | 1.87 ml      | 4.79 ml            | 7.52 ml         | 200 ml         |              |
| 2        | 9 ml         | 9 ml               | 9 ml            | 200 ml         |              |
| 3        | 5 ml         | 5 ml               | 5 ml            | 200 ml         |              |
| 4        | 3.8 ml       | 20 µl              | 20 µl           | 10 µl          | 1 ml         |
| 5        | 0.32 ml      | 0.14 ml            | 20 µl           | Urea           | 21.62 g      |
| 6        | 5 ml         | 5 ml               | 5 ml            | 200 ml         |              |
| 7        | 12.5 µl      | 12.5 µl            | 12.5 µl         | 12.5 µl        |              |
| H₂O      | 12.5 ml      | 26.71 ml           | 6 ml            | 944 ml         | 800 ml       |
| β-Met*   |              |                    |                 | 1 ml           |              |
| TEMED†   | 12.5%        |                    |                 |                |              |
| AP§       | 0.32 ml      | 0.14 ml            | 20 µl           | Urea           | 21.62 g      |
| Urea     |              |                    |                 |                |              |
| Total volume | 20 ml   | 45 ml              | 45 ml           | 1 liter        | 1 liter      |

Solutions 1–7 are described in Materials and Methods.  
* β-Mercaptoethanol.  
† N.N,N',N'-tetramethyl-ethylenediamine.  
§ 12.5% ammonium peroxydisulfate solution was prepared daily.

ence of NaCl was performed as in Piperno and Luck (19). Protein, ATPase, GTPase assays, chromatography on hydroxyapatite in the presence of 0.5 M NaCl, and two-dimensional gel electrophoresis were as described in Piperno and Luck (20). ATPase or GTPase assays were also performed in 0.15 M Tris.Cl, pH 7.2, 4 mM MgCl₂, plus 1 mM EGTA or 0.5 mM CaCl₂. The unit of ATPase activity was defined as the amount of enzyme which hydrolyzes 1 μmol of Pi per minute. Protein content of chromato-
graphic and sucrose gradient fractions was calculated from their ³⁵S radio-
activity and the specific radioactivity of the high salt axonemal extract by
making the assumption that specific radioactivity was equal for all polypep-
tides. Immunoblots were performed as in Piperno and Fuller (17). Transfer
to nitrocellulose of polypeptides of molecular weight >300,000 was per-
formed at 400 mA constant current for 24–26 h at room temperature. Detection of polypeptides required 1-4 d autoradiography of dried slab gels.

Electrophoresis in Polyacrylamide Gels of Dynein HMW Components

Electrophoresis and separation of dynein HMW components was performed as in previous studies (19) following a modified version of the method of Neville (II). This procedure allows the resolution of three HMW components of outer dynein arms I, II, and V, also referred to as α, β, and γ heavy chains (13) and of at least four HMW components of inner dynein arms III, IV, VI, and VII. Successive Roman numerals were assigned to reflect in-
creasing electrophoretic mobility. The original location of each component in the axoneme was deduced by comparing the molecular compositions of axonemes isolated from wild-type and arm defective mutants (5). Elec-

trophoresis of axonemal proteins in amounts >1 µg does not resolve the same

Results

Dynein Arm Components of Dynein Arm–defective Mutants

At the outset, the mutant pf 28, which lacks the outer arms (IO), and the mutant pf 30, which is partially defective for inner dynein arms (I), were analyzed as were other existing outer and inner arm–defective mutants (5). HMW polypep-
tides of axonemes were resolved and named following the procedures originally used for dynein arm components of Chlamydomonas (19). Fig. 1 shows a portion of the electrophoretograms of wild-type and pf 30 axonemal proteins where three major bands, previously identified as outer arm components, are indicated with Roman numerals I, II, and V.
These components are absent in the electrophoretogram of pf28 axonemal proteins. In contrast, inner arm components III and IV are present in the electrophoretograms of wild-type and pf28 proteins but absent in that of pf30. Arm components VI, VII, and VIII are present in every electrophoretogram and each is partially resolved as two bands. The apparent molecular weight of each of these components was calculated in a previous study and is >300,000 (19). The estimated molecular weight of components III and IV was 325,000 and 315,000, respectively. Two polypeptides, components VI, VII, and VIII are present in every electrophoretogram and each is partially resolved as two bands.

Table II. Salt Extraction of Axonemal Mg++--activated ATPases

|                | Total protein | Protein extracted | Total ATPase | ATPase extracted |
|----------------|---------------|-------------------|--------------|-----------------|
| mg            | mg            | %     | U      | mg           | %     |
| Wild type     | 1.87          | 0.49  | 26     | 0.80         | 0.61  | 76   |
| pf28          | 10.00         | 2.70  | 27     | 0.32         | 0.14  | 44   |
| pf30          | 2.11          | 0.58  | 28     | 0.99         | 0.69  | 70   |

The unit of ATPase activity was defined as the amount of enzyme that hydrolyzes 1 μmol of Pi per minute. Chromatograms of these preparations of extracted proteins are shown in Fig. 3.

Chromatography of Polypeptides from Dynein Arms

Electrophoretic and biochemical analyses of chromatographic fractions of pf28 axonemal proteins suggested that a new ATPase activity copurifies with components III and IV. This activity was extracted from the axonemes in the presence of 0.5 M NaCl, 1 mM ATP, 4 mM MgCl2, 1 mM dithiothreitol, and 10 mM Hepes (pH 7.2), and was separated by chromatography on hydroxypatite from the inner dynein arm ATPases composed of polypeptides VI and VII as previously described (20).

Percentages of proteins and Mg++--activated ATPase activities extracted from the axonemes of wild-type pf28 and pf30 are reported in Table II. 39–68% (average was 49 ± 11%) of ATPase activity was extracted from four different preparations of pf28 axonemes and 18–25% (average was 21 ± 3%) remained in the residue. Evidence showing that all HMW components of pf28 axonemes are extracted to the same extent will be described in the second part of this section.

Chromatograms of proteins and Mg++--dependent ATPase activities, present in salt extracts of wild-type pf28 and pf30 axonemes, are shown in Fig. 3. Although the salt extract of pf28 axonemes contained approximately five times the amount of protein of the other two extracts, protein and activity elution profiles of each chromatogram are similar in the 0.05–0.17 M sodium phosphate range used for elution. However, the profiles obtained with the pf28 extract (Fig. 3 b) differ from the others in the range 0.15–0.25 M of sodium phosphate. A major peak of protein and ATPase activity eluted at 0.18 M Na phosphate is present in the chromatogram of wild-type and pf30 proteins (Fig. 3, a and c) but absent in the chromatogram of pf28 proteins (Fig. 3 b). These chromatographic separations were interpreted on the basis of previous analyses (20). In fact, the two inner dynein arm ATPases associated with subunits VII and VI are eluted between 0.05–0.12 M sodium phosphate and outer dynein arm ATPases and their subunits I, II, and V form the peak eluted at 0.18 M sodium phosphate. Electrophoretic analysis of HMW polypeptides of wild-type axonemes showed that components I, II, III, IV, and V are not resolved by chromatography in these conditions (20).
Pools designated A–F from the chromatogram of pf28 axonemal proteins were made with fractions eluted in the 0.07–0.23 M sodium phosphate range. Polypeptides contained in pooled chromatographic fractions were analyzed by gel electrophoresis, enzymatic assays, and sedimentation on sucrose gradient after a dialysis against 5 mM TrisCl, pH 8.3, 0.1% NP-40, and 0.1 mM phenylmethylsulfonyl fluoride in the presence or absence of 0.2 mM EDTA, 0.2 mM EGTA. Fig. 4 shows the electrophoretograms of HMW components from a chromatogram similar to that shown in Fig. 3 b. Components III and IV are present in pools C, D, E, and F and are partially purified from components VI, VII, and VIII. These last are enriched in pools A, B, and C and each is partially or completely resolved in two bands, as seen in Fig. 1. Electrophoresis of polypeptide fractions from chromatograms of wild-type salt extracts confirmed that components III and IV are eluted between 0.15–0.25 M sodium phosphate, as are the outer dynein arm components I, II, and V (not shown).

Fig. 4 (last two lanes) also shows the electrophoretograms obtained with protein samples of equal mass of wild-type axonemes and pf28 salt extract. Bands of components III, IV, VI, VII, and VIII of pf28 salt extract are as intense as the bands formed by outer arm components I, II, and V. Therefore, there was enrichment of each HMW component in pf28 salt extract (see Fig. 1 for comparison) and each HMW component appears to be extracted to the same extent. Salt-insoluble residues of pf28 axonemes contained similar amounts of each HMW component at the trace level (not shown).
Figure 5. Electrophoretograms of all 35S-labeled polypeptides contained in pools A–F, as they are resolved in a 4–11% polyacrylamide gradient gel of reduced size (see legend to Fig. 2). The load of radiolabeled proteins was 3,000 cpm for each sample. HMW components, tubulin subunits, and actin shown in the autoradiogram of polypeptides are indicated by arrows. The identification of these components was based on their electrophoretic mobility. The position of molecular weight standards is indicated on the left.

Fig. 5 shows the electrophoretograms of all polypeptides present in pools A–F. Each pool contains HMW dynein components, tubulin subunits, a 42,000-mol-wt polypeptide (Fig. 5, arrows), as well as a discrete number of other polypeptides.

ATPase activities of pools A–F were assayed in different conditions and the corresponding specific activities are reported in Table III. Specific ATPase activities of each pool are similar in different conditions. However, specific ATPase activities of pools A and B, which contain the highest concentrations of components VII and VI, are approximately two times higher than the specific activities of pools E and F, which contain only trace amounts of these components. Therefore, one or more previously unknown ATPase activities may be present in pools E and F. Components III and IV are good candidates for subunits of the ATPase since they are present in pools E and F at high concentration and in a relatively pure state. ATPase activity of pools D and F, measured at pH 7.2 in the presence of Mg** and EGTA, respectively, was 2.3 and 3.9% of the ATPase activity. Therefore, the activity present in pools D and F appears to be specific for ATP, similar to all other dynein arm ATPases (19, 20).

Table III. Specific ATPase Activities of Chromatographic Fractions of pf28 Axonemal Proteins

| Pool | pH 8.8, Mg** | pH 7.2 Mg**, EGTA | pH 7.2, Mg**, Ca** |
|------|-------------|------------------|-------------------|
| A    | 1.11        | 1.03             | 1.16              |
| B    | 0.89        | 0.82             | 0.86              |
| C    | 0.67        | 0.54             | 0.66              |
| D    | 0.68        | 0.58             | 0.75              |
| E    | 0.50        | 0.52             | 0.58              |
| F    | 0.51        | 0.42             | 0.58              |

Specific ATPase activity is expressed in µmol-min⁻¹·mg⁻¹. Average values obtained in duplicate assays are reported.

Figure 6. Sucrose gradient sedimentation and electrophoretograms of polypeptides. (a) Sedimentation profile of ~4.5 µg of protein (133,000 cpm/µg) contained in pool F. Centrifugation of a 5–20% sucrose gradient in 0.1% NP-40, 0.2 mM EDTA, 0.2 mM EGTA, 5 mM Tris-Cl (pH 8.3) was performed in a rotor (model SW55; Beckman Instruments, Inc., Palo Alto, CA) at 44,000 rpm for 10 h at 5°C. 125I-Catalase labeled by the chloramine T method was sedimented as standard in a parallel gradient. Recovery of protein was 126% and recovery of ATPase activity in the peak of activity was 32%. Direction of sedimentation was from right to left. (●) Proteins; (○) ATPase activity. (b) Electrophoretograms of polypeptides of molecular weight >300,000 contained in sucrose gradient fractions 11–17, in pf28, and in wild-type axonemes. Slots were loaded with 40-µl aliquots of the fractions or with 40 µl of 15% sucrose (from the 20% stock solution used for the sucrose gradient) containing 50,000 cpm of axonemal proteins. (c) Electrophoretograms of all polypeptides contained in even fractions 4–24 of the sucrose gradient. Electrophoretic conditions are as in the experiment shown in Fig. 5. Slots were loaded with 10 µl from each fraction. Superimposed on fractions 10–16 are reported values of ATPase activity expressed in units × 10³ as in a. Triangles indicate polypeptides of molecular weight 140,000 and 42,000 that cosediment with the activity. The position of molecular weight standards is indicated on the left.
Partial Purification of a New ATPase

The identification of a new ATPase was achieved after those proteins contained in chromatographic fractions of pf28 salt extract were sedimented on a sucrose gradient. Fig. 6a shows the sedimentation profiles of proteins and ATPase activity of a pool prepared as pool F was. The peak of activity has a sedimentation coefficient of 11S. Specific ATPase activity at the peak is 1.4 µmol·min⁻¹·mg⁻¹ at pH 7.2 in the presence of both Mg²⁺ and Ca²⁺. Additional experiments performed with pool F proteins indicated that no peak of ATPase activity other than the 11S peak is present in the gradient (not shown). Fig. 6b shows the electrophoretograms of HMW polypeptides contained in fractions II-17 of the sucrose gradient. Components III and IV are prevalent in these fractions and are isolated from other HMW components in fraction I4. Their distribution approximately coincides with that of protein and ATPase activity, although they are present in different concentration in fractions I2-I6. Component VI in fraction I5 and component VII in fraction I2 are present in trace amounts. Other sedimentations were performed with pool E and pool D proteins, which contain higher concentrations of components VI and VII. These experiments confirmed that components III and IV sediment with a coefficient of 11S and are partially separated from components VI and VII, which sediment with coefficients of 10-11 and 12.5S, respectively (not shown). Isolated HMW components III and IV were also obtained from pool E proteins, and specific ATPase activity up to 2.3 µmol·min⁻¹·mg⁻¹ was found at the 11S peak of sedimentation.

The polypeptides were applied at the anode of the gel used for isoelectric focusing and detected by autoradiography. A portion of the original map is shown. The two major components are α and β tubulin subunits.

One Component of the 11S Complex Is Actin

The 42,000-mol-wt polypeptide cosedimenting with the 140,000-mol-wt polypeptide and components III and IV was identified as actin on the basis of its electrophoretic properties. Fig. 7 shows a portion of a two-dimensional map resolving α, β tubulin subunits and the 42,000-mol-wt polypeptide contained in the 11S sedimentation peak. The lowest component migrates as actin. An extensive characterization of this polypeptide was reported elsewhere (18). No other axonemal component has the same electrophoretic behavior in two-dimensional gel electrophoresis.

Discussion

A previously unknown ATPase has been identified as a component of a protein fraction extracted from the axoneme of the mutant pf28. Partial purification of the molecular complex carrying out the ATPase activity was achieved with a three-step procedure consisting of preferential extraction of dynein arms from the axonemes, chromatography on hydroxyapatite in the presence of 0.5 M NaCl, and sedimentation on a sucrose gradient. In the past this approach led to the purification of two inner dynein arm ATPases from axonemes of wild-type cells. The application of the same procedure to axonemes of pf28, a mutant lacking outer dynein arms, has allowed the isolation of a third ATPase, which is likely located in the inner dynein arms.

The new ATPase differs from previously identified inner dynein arm ATPases in its chromatographic and sedimentation properties, molecular composition, and specific activity. In fact, the activity is carried out by a complex comprising component III and/or IV, the polypeptide of 140,000 mol wt, and actin. Fractions containing the ATPase in its highest specific activities did not include any subunit of known inner dynein arm ATPases except actin. Other molecules present in the active fractions do not form a peak of sedimentation coincident with the ATPase activity. Whether the HMW components could hydrolyze ATP in the absence of the 140,000-mol-wt polypeptide and actin was not determined.

The sedimentation coefficient of the complex carrying the activity is 11S. This is a reasonable value for a protein of 400,000 mol wt and is close to those of the inner dynein arm ATPases, which are comprised between 10 and 12.5S. However, the 11S complex appears to contain two HMW components instead of one, as the 10-11 and 12.5S complex. Therefore three complexes have similar sedimentation behavior and apparently very different molecular weight. This paradox is explained if HMW component III and IV are different electrophoretic forms of the same polypeptide. Alternatively, two complexes containing HMW component III or IV have similar sedimentation coefficient. Hints in support of this last explanation are found in Fig. 6, a, b, and c. The peak formed by the ATPase activity is skewed toward the denser
part of the gradient. The concentration of component IV in
the peak of activity is higher than that of component III, al-
though these components apparently were equimolar before
sedimentation. Finally, the actin component of the complex
appears to be enriched in fractions sedimenting with coeffi-
cient above 11S. All these facts suggest that two complexes
appear to be enriched in fractions sedimenting with coeffi-
cients above 17S. The 12.7S sedimentation peak contained
two HMW components and at least four intermediate and
low molecular weight polypeptides. The 17.7S complex
was formed by two HMW components, termed III and IV, and
a polypeptide of molecular weight 140,000. Actin was not
found in the 17.7S complex. Assuming that the HMW com-
ponents III and IV identified here are identical to those indi-
cated by Goodenough et al. (4), then the 17.7S particle is an
intermediate product of dissociation of an inner dynein arm
subunit. This product probably is further dissociated into
the two different 11S complexes that were mentioned above.
A similar pattern of dissociation from an 18 into a 13.1 and a
14.5S particle was found to occur with one subunit of
Chlamydomonas outer arm dynein (13). A control of the
identity of the 17.7S complex could be performed with the
axonemes of the mutant pf 30 which are missing the HMW
components III and IV.

Several lines of evidence indicate that the subunits of the
new ATPase are inner dynein arm components. First, the
same polypeptides or a subset of them appear to be deficient
in axonemes of two mutants, pf 23 and pf 30. Electron micro-
scopic analysis revealed that both mutants are deficient but
not completely depleted of inner dynein arms, pf 23 being
more defective than pf 30 (1, 5). The deficiency of ~75% of
the inner arms from the axonemes of pf 23 was correlated with
the deficiency of 11 polypeptides, including components
III and IV, the polypeptide of molecular weight 140,000 and
actin. The lesser degree of deficiency of pf 30 has been cor-
related with the absence of III and IV, and the 140,000 pol-
ypeptide, components that copurify with the new ATPase.
Therefore, this ATPase may derive from a substructure or a
differentiated form of inner dynein arms (1, 3). Second, the
four polypeptides analyzed in this study are extracted from
the axonemes, as the subunits of all other dynein arm ATP-
ases, in a process that is selective for dynein arms. In fact,
20 putative outer and inner dynein arm components, toget-
er with ~30 other polypeptides, are extracted from wild-type
axonemes in conditions causing the disassembly of nearly
the totality of outer and inner dynein arms. In contrast, >200
other axonemal polypeptides and substructures like radi-
al spokes, nexin links, and one of the central microtubule
pair are remaining preferentially in the insoluble residue
(19). Third, the new ATPase activity consists of a complex
containing at least one polypeptide of molecular weight
>300,000 (HMW), which has a distinctive electrophoretic
behavior. These HMW polypeptides, as all the HMW
subunits of ATPases located in the dynein arms, migrate in
a polyacrylamide gel with similar and exceptionally low mo-

### Table IV. Match of ATPase Subunits and Polypeptides

| Polypeptides deficient in pf23 | Apparent molecular weight | 11S ATPase | 10-11S ATPase | 12-5S ATPase |
|-------------------------------|--------------------------|------------|--------------|--------------|
| III                           | 325,000                  | +          | -            | -            |
| IV                            | 315,000                  | +          | -            | -            |
| VI                            | 315,000                  | -          | +            | -            |
| VII                           | 310,000                  | -          | -            | +            |
| VIII                          | 310,000                  | -          | -            | +            |
| 1'                             | 110,000                  | -          | -            | +            |
| 2'                             | 83,000                   | -          | -            | +            |
| 3'                             | 42,000                   | +          | +            | -            |
| 4'                             | 28,000                   | -          | +            | +            |
| 5'                             | 28,000                   | -          | +            | +            |

* Criteria used for the denomination of polypeptides and the determination of their apparent molecular weight were as described (5). The identification of subunits of each ATPase with components deficient in the axoneme of the mutant pf 23 was based on electrophoretic properties of each polypeptide in one- and two-dimensional gel electrophoresis.

† The sedimentation peak of the 11S ATPase also contains a 140,000 subunit that is not reported in this table. The deficiency of this polypeptide in the axo-

### Notes

- Actin has been found in each complex that has ATPase ac-

### Additional Information

Additional inner dynein arm ATPases may exist. Table IV shows that not all inner dynein arm components, as they are defined for their deficiency in the axonemes of the mutant pf 23, were isolated in the form of an ATPase. HMW compo-

- Actin has been found in each complex that has ATPase ac-

### References

- Goodenough et al. (4) have also used the mutant pf 28 for the characterization of inner arm dynein subunits. They ana-

### Conclusion

- Fourth, the ATPase copurifies with actin as the two

### Further Reading

- The multiplicity of inner dynein arm ATPases indicates that inner dynein arms are differentiated at the level of their
substructures. Assuming that each substructure contributes to the sliding of adjacent outer double microtubules, differences among these substructures create possible sites for the modulation of localized movements of the axoneme. The modulation itself is probably changed by mechanisms depending on phosphorylation and/or the presence of Ca++ ions, as in other moving structures. Therefore the substructures of inner dynein arms could be envisioned as slightly different axonemal “motors” endowed with very complex systems of regulation. Comprehensive studies of inner and outer dynein arms eventually will explain the molecular mechanisms generating and controlling the movements of the axonemes. In short, they provide models for the analysis of molecules that generate movement in other systems of microtubules.

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References

1. Brokaw, C. J., and R. Kamiya. 1987. Bending patterns of Chlamydomonas flagella. IV. Mutants with defects in inner and outer dynein arms indicate differences in dynein arm function. Cell Motil. Cytoskeleton. 8:68–75.
2. Goodenough, U. W., and J. E. Heuser. 1985. Outer and inner dynein arms of cilia and flagella. Cell. 41:341–342.
3. Goodenough, U. W., and J. E. Heuser. 1985. Substructure of inner dynein arms, radial spokes, and the central pair/projection complex of cilia and flagella. J. Cell Biol. 100:2008–2018.
4. Goodenough, U. W., B. Gebhart, V. Mermall, D. R. Mitchell, and J. E. Heuser. 1987. High-pressure liquid chromatography fractionation of Chlamydomonas dynein extracts and characterization of inner-arm dynein subunits. J. Mol. Biol. 194:481–494.
5. Huang, B., G. Piperno, and D. J. L. Luck. 1979. Paralyzed flagella mutants of Chlamydomonas reinhardtii defective for axonemal doublet microtubule arms. J. Biol. Chem. 254:3001–3009.
6. Johnson, K. A. 1985. Pathway of the microtubule-dynein ATPase and the structure of dynein: a comparison with actomyosin. Annu. Rev. Biophys. Biophys. Chem. 14:161–188.
7. Kamiya, R., and M. Okamoto. 1985. A mutant of Chlamydomonas reinhardtii that lacks the flagellar outer dynein arm but can swim. J. Cell Sci. 74:181–191.
8. King, S. M., T. Otter, and G. B. Witman. 1985. Characterization of monoclonal antibodies against Chlamydomonas flagellar dyneins by high-resolution protein blotting. Proc. Natl. Acad. Sci. USA. 82:4717–4721.
9. Luck, D. J. L., G. Piperno, Z. Ramanis, and B. Huang. 1977. Flagellar mutants of Chlamydomonas: studies of radial spoke-defective strains by dikaryon and revertant analysis. Proc. Natl. Acad. Sci. USA. 74:3456–3460.
10. Mitchell, D. R., and J. L. Rosenbaum. 1985. A motile Chlamydomonas flagellar mutant that lacks outer dynein arms. J. Cell Biol. 100:1228–1234.
11. Neville, D. M. 1971. Molecular weight determination of protein-dodecyl sulfate complexes by gel electrophoreses in a discontinuous buffer system. J. Biol. Chem. 246:6328–6334.
12. Okagaki, T., and R. Kamiya. 1986. Microtubule sliding in mutant Chlamydomonas axonemes devoid of outer or inner dynein arms. J. Cell Biol. 103:1895–1902.
13. Pfister, K. K., and G. B. Witman. 1984. Subfractionation of Chlamydomonas 18 S dynein into two unique subunits containing ATPase activity. J. Biol. Chem. 259:12072–12080.
14. Pfister, K. K., R. B. Fay, and G. B. Witman. 1982. Purification and polypeptide composition of dynein ATPases from Chlamydomonas flagella. Cell Motil. 2:525–547.
15. Pfister, K. K., B. E. Haley, and G. B. Witman. 1984. The photoaffinity probe 8-azidoadenosine 5’ triphosphate selectively labels the heavy chain of Chlamydomonas 12 S dynein. J. Biol. Chem. 259:8499–8504.
16. Piperno, G. 1984. Monoclonal antibodies to dynein subunits reveal the existence of cytoplasmic antigens in sea urchin egg. J. Cell Biol. 98:1842–1850.
17. Piperno, G., and M. T. Fuller. 1985. Monoclonal antibodies specific for an acetylated form of α-tubulin recognize the antigen in cilia and flagella from a variety of organisms. J. Cell Biol. 101:2085–2094.
18. Piperno, G., and D. J. L. Luck. 1979. An actin-like protein is a component of axonemes from Chlamydomonas flagella. J. Biol. Chem. 254:2187–2190.
19. Piperno, G., and D. J. L. Luck. 1979. Axonemal adenosine triphosphatases from flagella of Chlamydomonas reinhardtii: purification of two dyneins. J. Biol. Chem. 254:3084–3090.
20. Piperno, G., and D. J. L. Luck. 1981. Inner arm dyneins from flagella of Chlamydomonas reinhardtii. Cell. 27:331–340.
21. Piperno, G., and D. J. L. Luck. 1982. Outer and inner arm dyneins from flagella of Chlamydomonas reinhardtii. Cell Motil. (Suppl.) 1:95–99.
22. Tang, W.-J. Y., C. W. Bell, W. S. Sale, and R. G. Gibbons. 1982. Structure of the dynein-1 outer arm in sea urchin sperm flagella. I. Analysis by separation of subunits. J. Biol. Chem. 257:508–515.

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