The Inner Dynein Arms I2 Interact with a “Dynein Regulatory Complex” in Chlamydomonas Flagella

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Abstract. We provide indirect evidence that six axonomal proteins here referred to as “dynein regulatory complex” (drc) are located in close proximity with the inner dynein arms I2 and I3. Subsets of drc subunits are missing from five second-site suppressors, pf2, pf3, supf3, supf4, and supf5, that restore flagellar motility but not radial spoke structure of radial spoke mutants. The absence of drc components is correlated with a deficiency of all four heavy chains of inner arms I2 and I3 from axonemes of suppressors pf2, pf3, supf3, and supf5. Similarly, inner arm subunits actin, p28, and caltractin/centrin, or subsets of them, are deficient in pf2, pf3, and supf5. Recombinant strains carrying one of the mutations pf2, pf3, or supf5 and the inner arm mutation ida4 are more defective for I2 inner arm heavy chains than the parent strains. This evidence indicates that at least one subunit of the drc affects the assembly of and interacts with the inner arms I2.

The movement of Chlamydomonas flagella is generated by at least six types of dynein including the outer dynein arm, the inner dynein arm I1, and two types of inner dynein I2 and I3 (25). Outer dynein arm and inner dynein arm I1 are formed by three and two distinct heavy chains, respectively, and consist of the same subunits along the axoneme (14, 27). In contrast, the inner dynein I2 and I3 are each formed by two identical heavy chains and consist of different heavy chains depending on their location along the axoneme (25). Each dynein also comprises a distinct set of intermediate and light chains characterized by molecular weight ranging from 140,000 to 8000 (27, 35).

Two light chains, which are associated with inner arms I2 and I3, were referred to as actin and caltractin/centrin, respectively, on the basis of physical and chemical properties of the two proteins (13, 22, 29). The role of actin and caltractin/centrin in other cytoskeletal structures is to transmit tensile stress and provide Ca++-dependent regulatory mechanisms, respectively (28, 30). In contrast, the function of the same proteins within the inner arm structure has not been identified.

To investigate a possible function of actin and caltractin/centrin in the regulation of inner arm movement, we intended to determine whether the ATPase activity or stability of inner arms I2 and I3 is Ca++ sensitive in vitro. Moreover, we aimed to identify other axonomal proteins that interact with actin and caltractin/centrin in vivo. To reach these goals we analyzed complexes formed by actin, caltractin/centrin, and I2 and I3 inner arm heavy chains that were isolated from the outer dynein mutant pf28 (18). We also quantitatively analyzed I2 and I3 inner arm deficiency in inner arm defective mutants (9, 16) and suppressors of flagellar paralysis that generate bending of flagella similar to those of inner arm mutants (3, 11). These second-site suppressors are missing different subsets of six axonomal polypeptides and suppress flagellar motility without restoring the radial spoke structure (11).

We found that the suppressors are defective for I2 and I3 inner arm subunits to different extents. We also found that the polypeptides missing from the suppressors interact at least with inner arms I2 in wild-type strains. Therefore, we referred to these polypeptides as “dynein regulatory complex” (drc). The absence of drc components combined with the deficiency of inner arms I2 generate an extensive deficiency of I2 inner arms in recombinant strains carrying both suppressor and inner arm mutations. This and other evidence described here suggests that actin and caltractin/centrin are associated with I2 and I3 inner arms, probably forming a linkage between the drc and each of I2 and I3 inner arms.

Materials and Methods

Strains and Culture of Chlamydomonas Cells
Chlamydomonas strains were obtained from the Chlamydomonas Genetics Center (Duke University, Durham, NC) and the laboratory of David Luck (Rockefeller University, New York). Nomenclature and phenotype of the strains used for analysis of axonomal components are listed in Table I.

The mutant supf6 was characterized at the beginning of this study and was isolated as a motility mutant following a mutagenesis of the wild-type strain 137c with nitrosourea (8). It has slower motion than a wild-type strain and lacks four of the six axonomal polypeptides that compose the drc (Fig. 1). These four polypeptides are identical to those missing in the mutant pf3 (Table II). The motility of the mutant pf3, however, is slower than that

1. Abbreviation used in this paper: drc, dynein regulatory complex.
Table 1. Chlamydomonas Strains Used for Analysis of Axonemal Components

| Strain      | Motility of flagella | Flagellar length* | Inner arm heavy chain deficiency | Linkage group of defective gene | Defective system in the axoneme |
|-------------|----------------------|-------------------|----------------------------------|--------------------------------|---------------------------------|
| 137 (wild-type) | Slow motion          | 12.0 (1.5)        |                                  | XI                             | outer arms                      |
| pf28 (oda 2) | Slow motion          | 9.6 (1.1)         | 2, 3'                            |                               |                                 |
| pf22        | None                 | 4.0 (1.2)         | 2', 2                            | I                              | inner arm I2, I3                 |
| ida4        | Slow motion          | 10.5 (1.5)        | 1α, 1β, 2', 2, 3'                | XII                            | inner arm I2                     |
| pf23        | None                 | 4.0 (1.0)         |                                  | XI                             | inner arm I2, I3                 |
| pf2         | Slow motion          | 8.9 (1.3)         |                                  | XI                             | inner arm I2, I3                 |
| pf3         | Slow motion          | 9.1 (1.4)         |                                  | VIII                           | inner arm I2, I3                 |
| supp3       | Wild-type like motion| 12.6 (1.6)        |                                  | drc                            |                                 |
| supp4       | Wild-type like motion| 12.2 (1.5)        |                                  | drc                            |                                 |
| supp5       | Slow motion          | 11.0 (1.3)        |                                  | drc                            |                                 |
| ida4supp3   | Slow motion          | 7.6 (0.6)         | 2', 2, 3'                        | drc                            | inner arm I2, I3                 |
| ida4supp4   | Slow motion          | 10.0 (1.3)        | 2', 2, 3'                        | drc                            | inner arm I2, I3                 |
| ida4supp5   | Slow motion          | 11.1 (1.3)        | 2', 2                            | drc                            | inner arm I2                     |

* Flagellar length is expressed as an average of 15 determinations. Standard deviation is reported in parenthesis.

The mutations supp3, supp4, pf2, and pf3 suppress flagellar paralysis of the mutants pf1 and pf14 but not the paralysis of the mutants pf15 and pf18 (11). If the same criterion, namely observation of flagellar beating, was adopted in the analysis of suppression by the mutants supp3, supp4, pf2, pf3, and supp5, then the range of suppression of the mutant supp5 is wider than that of the mutants supp3, supp4, pf2, and pf3.

Each of the recombinant strains was isolated from nonparental ditype tetrads with the exception of pf2ida4 that was isolated from a tetraploidy.

Figure 1. Autoradiograms of 35S-labeled axonemal polypeptides resolved by two-dimensional electrophoresis. Only portions of the original maps resolving polypeptides in the 130,000-15,000 molecular weight range are shown. The gels are oriented with basic polypeptides on the left. (a) Wild-type polypeptides. Solid triangles and numbers indicate single or group of polypeptides. Subsets of them are missing in the mutants pf2, pf3, supp3, supp4, and supp5 (Table II). (b) supp5 polypeptides. Open triangles indicate the positions of wild-type polypeptides missing in supp5. Other differences between these two maps were not indicated because they were not reproduced in every experiment.
Tetrads were prepared by standard methods (5). Each recombinant was run for 17 h at 1.8 mA. Polypeptide maps were obtained through the cm gel containing ampholines. Nonequilibrium pH gradient electrophoresis blots (21) were performed as described previously.

* Huang et al. (1982).
† These components were resolved in two spots differing in apparent molecular weight and isoelectric point.

Electrophoresis in Polyacrylamide Gels

Electrophoresis of dynein heavy chains was performed as described (27). A discontinuous slab gel composed of 3.2% polyacrylamide stacking layer and a 3.6–5% polyacrylamide resolving layer was used. The slab did not contain urea.

Two-dimensional electrophoresis of axonemal proteins was performed as described (26). Samples were applied at the anode of a 12 × 14 × 0.075 cm gel containing ampholines. Nonequilibrium pH gradient electrophoresis was run for 17 h at 1.8 mA. Polyptide maps were obtained through the application of a 0.6 × 14 cm strip of the ampholine-gel on a slab gel containing 4–11% polyacrylamide (20).

Quantitative Analysis of Axonemal Components

Quantitation of axonemal components was performed directly with image data obtained from the PhosphorImager (Molecular Dynamics, Sunnyvale, CA). The PhosphorImager is 10 times more sensitive than an X-ray film and gives a linear response in a range of 10:100,000 d.p.m.

Other Procedures

Determination of flagellar length (25), preparation of flagella through the exposure of cells to pH 4 (25), preparation of dynein fractions by exposure of the axoneme to high ionic strength (24), and sedimentation on a sucrose gradient. Inner arm heavy chains 2', 2, 3, and 3' do not contain II heavy chains 1a and 1b (Fig. 2). Although the ratio of each heavy chain to the others in the 1S fraction is different than in the axoneme, the 1S fraction is suitable for an analysis of all four heavy chains together.

We identified actin and caltractin/centrin by immunoblots of all the polypeptides contained in the 1S protein fraction (Fig. 3 a). A mAb against chicken gizzard actin (17) (Fig. 3 b) and a polyclonal antibody to Chlamydomonas caltractin (13) (Fig. 3 c) bind actin and caltractin/centrin, respectively. A polyclonal antibody against chicken back muscle bound specifically to actin in a similar experiment (not shown). This evidence confirmed that both actin and caltractin/centrin are bound to at least one of the I2 and I3 inner arm heavy chains. Axonemal actin may not form long filaments because rhodamine-labeled phalloidin (4) does not bind to the axoneme in detectable amounts (result not shown).

Extraction and isolation of the 1S protein fraction used for the immunoblots was performed at high ionic strength, in 0.5 M NaCl, 0.1 mM EGTA, and 10 mM Tris Cl (pH 7.4) for actin or caltractin. (Lane a) Coomassie blue-stained polypeptides contained in an 1S protein fraction. The position of molecular weight standards is indicated on the left side. Two aliquots of the same 1S fraction were electrophoresed in parallel and then transferred to nitrocellulose. (Lane b and c) Autoradiograms of the corresponding immunoblots obtained using specific antibodies and 125I-labeled secondary antibodies. (Lane b) Immunoblot incubated with anti-actin antibodies. (Lane c) Immunoblot incubated with anti-caltractin antibodies.

**Table II. Deficiency of Axonemal Polypeptides in the Suppressors**

| Component   | Mr x 10^-3 | p<sub>f2</sub> | p<sub>f3</sub> | sup<sub>o</sub>sup<sub>2</sub> | sup<sub>o</sub>sup<sub>4</sub> |
|-------------|------------|---------------|---------------|----------------|----------------|
| 1           | 108        | +             | +             | +              | +              |
| 2           | 83         | +             | +             | +              | +              |
| 3           | 65         | +             | +             | +              | +              |
| 4           | 60         | +             | +             | +              | +              |
| 1           | 5          | 40            | +             | +              | +              |
| 6           | 29         | +             | +             | +              | +              |

* This study.
† These components were resolved in two spots differing in apparent molecular weight and isoelectric point.

**Figure 3.** Electrophoretogram of axonemal polypeptides of the mutant pf28. A portion of the original autoradiogram resolving polypeptides in the 500,000–400,000 molecular weight range is shown. Equal amounts of radioactivity were analyzed. (Left lane) Unfractionated axonemal polypeptides (Right lane) 1S fraction obtained by extraction of axonemal polypeptides, followed by sucrose gradient sedimentation both in the presence of 0.5 M NaCl and 0.1 mM EGTA. Bands referred to as 1a, 1b, 2', 2, 3, and 3' are inner dynein arm heavy chains.

**Results**

**Actin and Caltractin/Centrin Form a Complex with at Least One Inner Dynein Arm Heavy Chain**

We intended to confirm that actin and caltractin/centrin are associated with I2 and/or I3 inner dynein arm heavy chains and determine whether the stability or the activity of the complexes is Ca<sup>2+</sup>-sensitive.

To confirm that actin, caltractin/centrin, and I2 and I3 inner arm heavy chains are part of the same complexes we analyzed a protein fraction derived primarily from I2 and I3 inner arms. The protein fraction was extracted from axonemes of the outer dynein arm mutant pf28 and purified by sedimentation on a sucrose gradient. Inner arm heavy chains I2 and I3 sediment as 1S particles and remain associated with proteins of lower molecular weight (27). An electrophoretogram resolving axonemal polypeptides of molecular weight close to 500,000 shows that the protein fraction containing 1S particles is enriched in I2 and I3 heavy chains 2', 2, 3, and 3' and does not contain II heavy chains 1a and 1b (Fig. 2). Although the ratio of each heavy chain to the others in the 1S fraction is different than in the axoneme, the 1S fraction is suitable for an analysis of all four heavy chains together.

We identified actin and caltractin/centrin by immunoblots of all the polypeptides contained in the 1S protein fraction (Fig. 3 a). A mAb against chicken gizzard actin (17) (Fig. 3 b) and a polyclonal antibody to Chlamydomonas caltractin (13) (Fig. 3 c) bind actin and caltractin/centrin, respectively. A polyclonal antibody against chicken back muscle bound specifically to actin in a similar experiment (not shown). This evidence confirmed that both actin and caltractin/centrin are bound to at least one of the I2 and I3 inner arm heavy chains. Axonemal actin may not form long filaments because rhodamine-labeled phalloidin (4) does not bind to the axoneme in detectable amounts (result not shown).

Extraction and isolation of the 1S protein fraction used for the immunoblots was performed at high ionic strength, in 0.5 M NaCl, 0.1 mM EGTA, and 10 mM Tris Cl (pH 7.4) for actin or caltractin. (Lane a) Coomassie blue-stained polypeptides contained in an 1S protein fraction. The position of molecular weight standards is indicated on the left side. Two aliquots of the same 1S fraction were electrophoresed in parallel and then transferred to nitrocellulose. (Lane b and c) Autoradiograms of the corresponding immunoblots obtained using specific antibodies and 125I-labeled secondary antibodies. (Lane b) Immunoblot incubated with anti-actin antibodies. (Lane c) Immunoblot incubated with anti-caltractin antibodies.

**Figure 2.** Electrophoretograms of 35S-labeled axonemal polypeptides of the mutant pf28. A portion of the original autoradiogram resolving polypeptides in the 500,000–400,000 molecular weight range is shown. Equal amounts of radioactivity were analyzed. (Left lane) Unfractionated axonemal polypeptides (Right lane) 1S fraction obtained by extraction of axonemal polypeptides, followed by sucrose gradient sedimentation both in the presence of 0.5 M NaCl and 0.1 mM EGTA. Bands referred to as 1a, 1b, 2', 2, 3, and 3' are inner dynein arm heavy chains.
Figure 4. Sedimentation profile and electrophoretograms of 35S-labeled polypeptides from pf28 axonemes. (a) Sedimentation in a 5–20% sucrose gradient of a protein fraction previously chromatographed on a hydroxyapatite column and then dialyzed against a solution of low ionic strength in the presence of 0.1 mM EGTA. The direction of sedimentation was from right to left. (A) Proteins; (A) Mg-activated ATPase activity. (+) Mg-activated ATPase in the presence of 0.2 mM Ca++. (b) Electrophoretograms of all polypeptides contained in odd fractions 1–31. Triangles indicate actin, p28, and caltractin/centrin cosedimenting with inner arm heavy chains. The position of molecular weight standards is indicated on the left side.

(27). To determine whether the actin and caltractin/centrin, both Ca++ binding proteins, bind to inner arm heavy chains or regulate the ATPase activity of the 11S complexes in a Ca++-dependent manner, we isolated the 11S particles by a different procedure.

The I2 and I3 inner arm heavy chains were isolated by chromatography on hydroxyapatite column and then sedimented under conditions of low ionic strength (20) in the presence of 0.1 mM EGTA or 0.1 mM CaCl2. These conditions led to the isolation of 11S fractions that are suitable for ATPase activity assays and similar in purity to those obtained by sedimentation at high ionic strength. An 11S protein fraction containing Mg++-activated ATPases was isolated in a sucrose gradient containing 0.1 mM EGTA, 10 mM Tris Cl (pH 7.4) (Fig. 4 a). Electrophoretograms of polypeptides contained in odd fractions of the sucrose gradient show that the 11S peak is formed by dynein heavy chains, actin, and caltractin/centrin (Fig. 4 b). A 28,000 molecular weight polypeptide, referred to as p28, is also a component of the 11S complexes. Tubulin subunits and other minor polypeptides are contaminants because they do not form a sedimentation peak coincident to that of the inner arm heavy and light chains. Inner arm heavy chains 2', 2, 3, and 3' were identified as components of the 11S peak through gel electrophoresis resolving the inner arm heavy chains (not shown).

A similar 11S protein fraction was obtained when the sedimentation on a sucrose gradient was performed at low ionic strength in the presence of 0.1 mM CaCl2 (not shown). Therefore, the association between I2 and I3 heavy chains and the set of proteins including actin, p28 and caltractin/centrin is Ca++ insensitive and stable at high and low ionic conditions.

Mg++-activated ATPase activities of 11S particles are insensitive to Ca++ ions. Mg++-activated ATPase activities of I2 and I3 inner arm heavy chains are similar in the absence or presence of 0.2 mM CaCl2 (Fig. 4 a).

**Actin, Caltractin/Centrin, and p28 Bind to Inner Arm Heavy Chains and Other Axonemal Subunits**

To determine whether actin, p28, and caltractin/centrin associate with all I2 and I3 inner arm heavy chains or a subset of them, we analyzed qualitatively and quantitatively axonemal polypeptides of three inner arm mutants, pf22, ida4, and pf23 that are defective for subsets of heavy chains 2', 2 and 3' (16, 25).

The mutants pf22, ida4, and pf23 were compared to a wild type strain. An electrophoretogram resolving dynein heavy chains (Fig. 5) shows that the mutant pf22 lacks heavy chains 2 and 3'. The mutant ida4 lacks heavy chains 2' and part of heavy chain 2 and finally the mutant pf23 lacks heavy chains 2', 2 and 3'.

Axonemal polypeptides of molecular weight lower than 200,000 from the mutants pf22, ida4, and pf23 were resolved by two-dimensional gel electrophoresis. The positions of actin, p28, and caltractin/centrin in the two-dimensional maps were identified by comparison with a map resolving the low molecular weight components of an 11S protein fraction from the mutant pf28 (Fig. 6 a; see Fig. 4 for the characterization of the 11S fraction). Actin, p28, and caltractin/centrin are present in axonemes of the mutants pf22 and pf23 (Fig. 6, b and d). In contrast, p28 is virtually absent from axonemes of the mutant ida4 (Fig. 6 c).

Ratios between the radioactivity values of actin, p28, and caltractin/centrin and the radioactivity values of an insoluble axonemal component (26), referred to as s in the maps (Fig. 6, b–d) are reported in Table III. Multiple gels containing...
Figure 6. Autoradiograms of $^{35}$S-labeled axonemal polypeptides resolved by two-dimensional electrophoresis. Only portions of the original maps resolving polypeptides in the 55,000-15,000 molecular weight range are shown. The gels are oriented with basic polypeptides on the left. (a) Map resolving actin, p28, and caltractin/centrin that are present in an 11S protein fraction (see Fig. 4, a and b for the characterization of the 11S fraction). The protein p28 is resolved into two components that differ in their isoelectric point. (b) Polypeptides from the mutant pf22. (c) Polypeptides from the mutants ida4. (d) Polypeptides from the mutant pf23. Solid triangles indicate the position of actin, p28, and caltractin/centrin. Open triangles indicate the positions of wild-type p28 components missing in the mutant ida4. The polypeptide labeled s was adopted as internal standard in each sample.

Axonemal polypeptides from wild-type or inner arm mutants pf22, ida4, and pf23 were analyzed.

Both actin and p28 are deficient in all three mutants. Caltractin/centrin is deficient in the mutant ida4. No evidence was found suggesting that actin or p28 or caltractin/centrin are associated only to a subset of heavy chains.

In summary, the mutant pf23 lacks heavy chains 2'; 2, and 3' and is deficient in actin and p28, whereas the mutant ida4 lacks heavy chain 2'; part of 2 and is more deficient for actin, p28, and caltractin/centrin than the mutant pf23. Therefore, a greater loss of heavy chains as in the mutant pf23 does not correlate with a greater deficiency of light chains. Conversely, the lesser loss of heavy chains in the mutant ida4 does not correlate with a less pronounced deficiency of light chains. This evidence and the evidence obtained by the isolation of 11S particles indicate that all three light chains, or a subset of them, bind to the axoneme through the heavy chains as well as other molecules.
Some Suppressors of Flagellar Paralysis Are Inner Arm Mutants

Second binding sites for actin or p28 or caltractin/centrin could be on the tubulin subunits or other axonemal proteins that are in close proximity with the inner arm heavy chains. To test the latter hypothesis, we turned to the analysis of a group of mutants each lacking a distinct subset of axonemal proteins referred to as drc (see Introduction and Materials and Methods).

The position of the drc within the axoneme is unknown but an interaction between drc and inner dynein arm subunits is suggested by the observation that the suppressors pf2 and pf3 generate bending patterns of flagella similar to those of inner arm mutants (3).

Quantitative analysis of axonemal polypeptides resolved by two-dimensional gel electrophoresis revealed that the mutants pf2, pf3, and supf5 are defective for actin to approximately the same extent. In addition, the mutant pf3 is defective for p28 and caltractin/centrin (Table III). Therefore, the lack of drc components 1 and 2 or 3 and 4 was correlated with a deficiency of one or three inner arm light chains. These deficiencies could not be predicted from qualitative analyses of inner arm components. All four inner arm heavy chains 2', 2, 3, and 3' are present in the axonemes of the mutants pf2, pf3, supf3, supf4 and supf5 (Fig. 7). In contrast, quantitative analyses of electrophoreograms revealed that heavy chains 2', 2, 3, and 3' are present in reduced amounts in the axonemes and flagella of the mutants pf2, pf3, supf3, and supf5. The ratios between radioactivity values of combined heavy chains 2', 2, 3, and 3' and radioactivity values of combined γ and 1β chains of outer arm and inner arm II (27) are reported in Table IV.

There is a parallel between these results and the results obtained with the quantitative analysis of actin, p28, and caltractin/centrin. The mutants pf3 and supf5 are the most defective for inner arm heavy chains and actin, whereas the mutant supf4 is similar to wild-type. For each strain the deficiency of inner arm heavy chains in samples of axonemes is similar to the deficiency of the same components in samples of flagella. Therefore, inner arm heavy chains were not extracted preferentially when flagellar membrane and matrix proteins were separated from the axoneme.

Although we did not identify the axonemal subunits binding actin or p28 or caltractin/centrin, we found that the loss of components 1 and 2 or 3 and 4 of the drc is correlated with the defect of assembly of all four heavy chains of inner arms I2 and I3. Components 1, 2, 3, and 4 do not appear to be components of inner arms I2 and I3 because they are absent when inner arm heavy chains are only deficient. Instead they may form a binding site for these arms or modify their subunits to make them assembly-competent. In both cases they should interact directly with one or more subunits of the inner arms I2 and I3. To test this hypothesis, we determined whether the loss of a subset of drc components 1, 2, 3, and 4 affects the assembly of a specific type of inner arm in the presence of a preexisting inner arm defect.

Molecular Interactions between drc and Inner Arm Subunits

Assuming that drc subunits modify or are contiguous to subunits of inner arms I2 and I3 we expect to find that a deficiency of a specific inner arm is enhanced in recombinant strains carrying both suppressor and inner arm mutations.

For our analysis we isolated five recombinants between the inner arm mutant ida4 and each of the five suppressors pf2, pf3, supf3, supf4, and supf5. The mutant ida4 was chosen instead of the mutant pf2 or pf3 because it is the least defective in inner arm heavy chains, lacking only heavy chain 2' and being defective for heavy chain 2 (Figs. 5 and 8).

Recombinant strains pf2ida4, pf3ida4, and supf5ida4 all show an enhanced loss of inner arm heavy chain 2 compared to the parent strains. Recombinants pf2ida4 and
Table IV. Quantitative Analysis of Inner Arm Heavy Chains*  

|          | Wild-type | pf2 | pf3 | supp3 | supp4 | supp5 |
|----------|-----------|-----|-----|-------|-------|-------|
| Axonemes | 0.95      | 0.81| 0.50| 0.77  | 1.11  | 0.55  |
| Flagella | 0.95      | 0.78| 0.54| 0.78  | 0.93  | 0.60  |

* Numbers are ratios between radioactivity values of combined heavy chains 2', 2, 3, and 3' and radioactivity values of combined heavy chains 1 and 1'. Five determinations of radioactivity were performed on the same electrophoretogram. Standard deviations were lower than 7% in each case.

Figure 8. Electrophoretograms of 35S-labeled axonemal polypeptides of a wild-type strain, mutants supp3 and ida4 and recombinant strains pf2ida4, pf3ida4, supp3ida4, supp4ida4 and supp5ida4. Portions of the original autoradiograms resolving polypeptides in the 500,000-400,000 molecular weight range are shown. Equal amounts of radioactivity were analyzed in each lane. Bands referred to as 2', 2, 3, and 3' are I2 and I3 inner arm heavy chains.

Discussion

The Presence of Caltractin/Centrin and Actin within the Axoneme

The transition between ciliary and flagellar types of motion of *Chlamydomonas* axonemes can be observed in vivo (31), with demembranated cell models (15) and with isolated axonemes (2). In each case the transition depends on the Ca++ concentration of the medium where the axoneme moves. At Ca++ concentrations above $10^{-6}$ M axonemes assume a flagellar type of waveform instead of the usual ciliary type, containing the heavy chains 2' and 3' and radioactivity values of combined heavy chains 2' and 3'. Five determinations of radioactivity were performed on the same electrophoretogram. Standard deviations were lower than 7% in each case.

In summary, ida4, an inner arm mutation that causes a partial defect in inner arms I2, combined independently with each of the suppressor mutations pf2, pf3, and supp5, resulted in a total loss of inner arms I2. The suppressors pf2, pf3, and supp5, differ from the suppressor supp3 and supp4 because they are deficient for actin and drc components 1, 2, or 3. Therefore, the loss of heavy chain 2 in ida4 recombinants is caused by a synergistic effect of defects affecting different parts of the inner arm structure. This evidence supports a model where part of the drc interacts with inner arms I2.

The Location of the “Dynein Regulatory Complex”

The test for the existence of an interaction between drc and dynein arms through the analysis of recombinant strains car-
raining both drc and inner arm defects has a precedent in the analysis of a recombinant carrying supp, and pf22 mutations (11). The mutant supp, has outer dynein arms but carries an outer arm β heavy chain that is defective. The mutant pf22 is deficient for outer dynein arms and lacks inner arms components 2 and 3' (25). In contrast, the recombinant supp, pf22, lost completely the outer dynein arms. Therefore, more than one structural defect is needed to cause the loss of outer dynein arms, which likely are bound to the axoneme through more than one site. Similarly a loss of drc subunits in combination with a deficiency of inner arms I2 resulted in the loss of inner arms I2 in each recombinant pf22, pf3, and supp. This evidence supports the conclusion that the drc and inner arms I2 interact directly.

The enhancement of inner arm I2 defect in recombinants pf22, pf3, and supp is correlated with the deficiency of actin in suppressors pf2, pf3, and supp (Tables III and IV). Therefore, suppressor defects that lead to loss of I2 inner arms in the recombinants may derive from defective interactions between inner arm light chains and drc components. These polypeptides may include the defective gene products of suppressors pf2, pf3, and supp.

The location of the drc within the axoneme has never been determined. We propose that the drc is located in close proximity with inner arms I2 and I3 since it affects the assembly of inner arms I2 and I3 when it is defective. In a position close to inner arms I2 and I3, the drc could be located between the radial spokes S1 and S2 and the inner arms that are a target of the drc activity.

A complex double-rowed organization of inner arms was described by Muto et al. (19) as the result of tilt-series thin section EM of Chlamydomonas flagella. Within a repeating unit of 96 nm the inner arms appeared to be formed by a row of structures consisting of four electron dense structures and three pairs of densities located centripetally and centripetally, respectively. Muto et al. (19) postulated that both rows of densities are formed by inner arm heavy chains. In contrast, we propose that one row of densities contains the drc and is located in a centripetal position closer to the radial spoke stalks. This hypothesis can be tested through EM of axonemes from mutants lacking drc components.

From its putative location between the stem of inner arm I2 and radial spoke stalks the drc may regulate the activity of radial spokes. The spokes in bend regions of the axoneme form an angle with central pair and doublet microtubules instead of being perpendicular as they are in straight regions of the axoneme (34). The drc also may regulate the motion of inner arms I2 and I3 toward the proximal region of the axoneme (6). Lastly, it may form a specific binding site of inner arms I2 and I3 on the surface of doublet microtubules (33).

It was proposed that the mechanisms regulating the formation of bending waves may operate primarily through variations of mechanical properties of the axonemal substructures (7). A position of the drc close to radial spokes and inner arms would allow the formation of a physical linkage between these substructures. This linkage could generate a conformational and not a chemical change of protein subunits of the three systems in response to axonemal bending.

Within 96-nm sections of each outer doublet actin, p28 and caltractin/centrin may regulate the function of both inner arms I2 and I3 and the drc. The possible integration with other axonemal substructures appears to be a characteristic of every intermediate and light chain that was identified as an inner arm component (9). A polypeptide referred to as I' (molecular weight 110,000) was found to be missing in the radial spoke mutant pf5 in association with the loss of two subunits of the radial spoke stalk (10). Moreover, a second inner arm component referred to as I" (molecular weight 83,000) is missing in all mbo mutants, mutants that are both defective for proximal beak-like projections and able to generate only flagellar type of bending of the axonemes (32). Therefore, several kinds of regulation may be mediated through inner arm intermediate and light chains and have inner arm heavy chains as a main terminal.

The assembly and/or function of complexes formed by I2 and I3 inner arm heavy chains, p28, and caltractin/centrin may be regulated also through posttranslational modifications because each of these subunits is phosphorylated in vivo (23). In addition, the presence of caltractin/centrin as part of these complexes provides a site for functional regulation through changes of Ca++ concentration. Both kinds of regulations have the inner dynein arms as a main terminal since the outer dynein arms do not have a strong influence toward changing the waveforms of flagella (3).

In summary, we have shown that the drc interacts with inner arms I2 in vivo. To integrate this observation in a more general model of the mechanism generating specific bending patterns of flagella, we intend to determine whether the radial spokes form a continuous structure with the drc and whether Ca++ ions above 10-6 M level change the signaling occurring between radial spokes and inner arms.

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