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The Outer Dynein Arm-Docking Complex: Composition and Characterization of a Subunit (Oda1) Necessary for Outer Arm Assembly

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To learn more about how dyneins are targeted to specific sites in the flagellum, we have investigated a factor necessary for binding of outer arm dynein to the axonemal microtubules of Chlamydomonas. This factor, termed the outer dynein arm-docking complex (ODA-DC), previously was shown to be missing from axonemes of the outer dynein armless mutants oda1 and oda3. We have now partially purified the ODA-DC, determined that it contains equimolar amounts of Mₐ ∼105,000 and ∼70,000 proteins plus a third protein of Mₐ ∼25,000, and found that it is associated with the isolated outer arm in a 1:1 molar ratio. We have cloned a full-length cDNA encoding the Mₐ ∼70,000 protein; the sequence predicts a 62.5-kDa protein with potential homologs in higher ciliated organisms, including humans. Sequencing of corresponding cDNA from strain oda1 revealed it has a mutation resulting in a stop codon just downstream of the initiator ATG; thus, it is unable to make the full-length Mₐ ∼70,000 protein. These results demonstrate that the ODA1 gene encodes the Mₐ ∼70,000 protein, and that the protein is essential for assembly of the ODA-DC and the outer dynein arm onto the doublet microtubule.

INTRODUCTION

Dyneins are large, multisubunit molecular motors that generate force against microtubules. Cilia and flagella contain three major classes of dyneins: cytoplasmic dynein, of which cytoplasmic dynein 1b/2 is the retrograde motor for intraflagellar transport (Pazour et al., 1998, 1999; Porter et al., 1999); axonemal inner arm dyneins, of which there may be as many as seven different forms (Porter and Sale, 2000); and axonemal outer arm dynein, of which only one form is currently known (Witman et al., 1994). Each of these different dyneins binds with high fidelity to sites that are specific for that particular dynein. Thus, these sites must be structurally or biochemically unique in a way that ensures that the correct dynein is targeted to them. The molecular basis for this specific binding is of great interest because it literally provides the foundation for the proper functioning of the flagellum. Moreover, knowledge of how these dyneins are targeted to their correct binding sites may provide a paradigm for how other axonemal components, e.g., the radial spokes and the projections of the central pair microtubules, are correctly positioned to form one of the most complex and highly ordered macromolecular structures in the cell.

To learn more about specific targeting of dyneins, we have been studying the Chlamydomonas reinhardtii outer arm dynein, which is the most well characterized of axonemal dyneins (Witman et al., 1994; Pazour and Witman, 2000). This dynein, which produces as much as four-fifths of the force for flagellar movement (Brokaw, 1994), is attached to specific sites on the A-tubules of the flagellar doublet microtubules and repeats at 24-nm intervals along the length of the doublet. It contains three dynein heavy chains (DHCs) (termed α, β, and γ), two intermediate chains (ICs) (termed IC78 and IC69), and several light chains (LCs) (Figure 6B).
The two ICs and most of the LCs are associated in an IC/LC complex located at the base of the dynein (King and Witman, 1990). One of the ICs, IC78, is in direct contact with tubulin in vivo (King et al., 1991) and is a microtubule-binding protein in vitro (King et al., 1995), so it is believed to be at least one of the dynein components that anchors the outer arm to the A-tubule. Studies of Chlamydomonas mutants lacking the outer dynein arm (oda mutants) indicate that an additional factor is necessary for efficient assembly of the outer arm onto flagellar doublet microtubules. When the Chlamydomonas outer arm dynein is removed from the wild-type axoneme by extraction with 0.6 M KCl in the absence of Mg$^{2+}$, it dissociates into a single-headed γ subunit (containing the γ DHC and two LCs) that sediments as a 12S particle, and a two-headed αβ subunit (containing the α and β DHCs, the two ICs, and all the remaining LCs) that sediments as a 21S particle (Piperno and Luck, 1979; Pfister et al., 1982; King and Witman, 1989). When these two particles were purified and added back to axonemes of the outer armless mutants oda2, oda4, oda5, or oda6, outer arms were reconstituted in their correct positions on the doublet microtubules (Takada and Kamiya, 1994). In contrast, if the subunits were added back to axonemes of the outer armless mutants oda1 or oda3, very few arms rebound. Efficient rebinding of arms to oda1 or oda3 axonemes required a factor that was present in the 0.6 M KCl extract of wild-type axonemes and sedimented at 7S. This factor apparently was missing in axonemes of oda1 and oda3, but present in axonemes of oda2, oda4, oda5, and oda6. The only discernible structural difference between axonemes of these two groups of mutants was that the latter had a small projection at the site where the outer arm normally would attach. These findings strongly suggested that the projection facilitates attachment of the dynein to the doublet microtubule, and that the 7S factor is the solubilized form of the projection. Partial purification of the factor and comparison of extracts from oda mutant axonemes showed that the factor was correlated with a polypeptide of $M_r \approx 70,000$ (Takada and Kamiya, 1994). Because the factor can assemble onto the doublet microtubules in the absence of the outer dynein arms in vivo, and is necessary for binding of the arms to the microtubules, it has been termed the outer dynein arm-docking complex (ODA-DC).

In this report, we show that the ODA-DC contains equimolar amounts of proteins of $M_r \approx 105,000$ and $\approx 70,000$ plus a third protein of $M_r \approx 25,000$, that it is present in a 1:1 stoichiometry with the outer dynein arm polypeptides, and that it remains associated with the outer dynein arm subunits when these subunits are isolated under conditions that keep them together as a three-headed αβγ complex (Takada et al., 1992). We have cloned and sequenced a full-length cDNA encoding the $M_r \approx 70,000$ protein; the sequence predicts a novel 62-kDa polypeptide with three long coiled-coil domains. Sequencing of the corresponding DNA from the outer armless mutant oda1 reveals that it has a mutation resulting in a stop codon just downstream from the translation initiation site; hence, it is unable to make the $M_r \approx 70,000$ ODA-DC protein. These results indicate that the $M_r \approx 70,000$ polypeptide is the ODA1 gene product and is essential for assembly of both the outer dynein arm and the ODA-DC onto the doublet microtubule. We previously showed that the ODA3 gene product corresponds to the $M_r \approx 105,000$ ODA-DC polypeptide and is predicted also to have three long coiled-coil regions (Koutoulis et al., 1997). Herein, we use a zero-length protein cross-linker to show that the ODA1 gene product is in direct contact with the ODA3 gene product in the soluble ODA-DC. Therefore, the ODA1 and ODA3 gene products interact with each other, possibly via their coiled-coil domains, and with an $M_r \approx 25,000$ subunit to form a structure that targets the outer dynein arm to its correct attachment site on the doublet microtubule. Potential homologs of the ODA1 gene are expressed in higher organisms, including humans, suggesting that the ODA-DC is widespread in ciliated animals.

**MATERIALS AND METHODS**

**Strains**

*C. reinhardtii* strains used were wild type (137c), and outer armless mutants oda1-1, oda3-1, and oda6-1, all of which were derived from strain 137c (Kamiya, 1988). Cells were grown in liquid culture as described by Witman (1986).

**Characterization of the ODA-DC**

Flagellar axonemes from oda6 cells were isolated by the method of Witman (1986), washed with 0.5 M potassium acetate in 30 mM HEPES pH 7.5, 5 mM MgSO$_4$, 1 mM dithiothreitol, 0.5 mM EGTA, 0.5 mM EDTA (Nakamura et al., 1997), and then extracted with 0.6 M KCl in HMDEK (30 mM HEPES pH 7.5, 5 mM MgSO$_4$, 1 mM dithiothreitol, 0.5 mM EDTA, 25 mM potassium acetate) to solubilize the ODA-DC. Axonemes from oda3 cells, which lack the ODA-DC, were treated identically. The extracts were fractionated by 5–20% sucrose density gradient centrifugation under Mg$^{2+}$-free conditions (Piperno and Luck, 1979; Pfister et al., 1982). Fractions were collected from the bottom of the tube and their proteins analyzed by SDS-PAGE.

**Isolation of Three-headed Outer Arm Dynein**

Axonemes from wild-type cells were isolated, washed, and extracted as described above. The dynein extract was then fractionated by centrifugation (5-ml tube, Beckman SW50.1 rotor, 39,000 rpm for 7 h) through a 5–20% sucrose density gradient containing 5 mM Mg$^{2+}$ (Takada et al., 1992).

**SDS-PAGE and Band Quantitation**

Proteins from sucrose density gradient fractions were separated by electrophoresis in 5–20% polyacrylamide-SDS gels (King et al., 1986). The gels were stained with Coomassie blue and photographed on 35-mm Tech Pan film. Negatives were scanned with a Nikon Coolscan II film scanner. The resulting image files were analyzed and bands of interest integrated using one-dimensional gel analysis software (Quantity One; PDI, Huntington Station, NY).

**Immunoprecipitation of the ODA-DC**

In *Absence of Mg$^{2+}$*. Axonemes from wild-type cells were isolated, washed, and extracted as described above. The extract was dialyzed against TEDKS (30 mM Tris pH 7.5, 0.5 mM EDTA, 1 mM dithiothreitol, 25 mM KCl, 10% sucrose) containing 1 mM phenylmethylsulfonyl fluoride overnight at 4°C to dissociate outer arm dynein from the ODA-DC, and then dialyzed against phosphate-buffered saline.

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1 The three oda strains previously were referred to as oda1 strain 38, oda3 strain 73, and oda6 strain 95, respectively (Kamiya, 1988).
Table 1. Amino acid sequences of tryptic peptides from the M, ≈70,000 ODA-DC protein and nucleotide sequences of PCR primers used to clone the protein

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<thead>
<tr>
<th>Amino acid sequence of peptides</th>
<th>Nucleotide sequence of PCR primers</th>
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<tr>
<td>Peptide 1</td>
<td>Primer A (forward)</td>
</tr>
<tr>
<td>Peptide 2</td>
<td>Primer B (reverse)</td>
</tr>
<tr>
<td>Peptide 3</td>
<td>Primer C (forward)</td>
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<td></td>
<td>Primer D (reverse)</td>
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saline (PBS) (6.4 mM Na$_2$HPO$_4$, 0.15 mM KH$_2$PO$_4$, pH 7.5, 137 mM NaCl, 2 mM KCl) at 4°C for 5 h. To biotinylate the proteins in the extract, sulfo-N-hydroxysuccinimide-biotin (Pierce Chemical, Rockford, IL) was added to an ~50-fold molar excess over protein and the extract incubated at 24°C for 30 min. To remove unreacted biotin, the extract was dialyzed against PBS at 4°C overnight. The biotinylated extract was divided into two tubes (48 μg of protein/tube) and diluted to 200 μl with IP buffer 1 (3% bovine serum albumin [BSA], 1% Nonidet P-40, 10 mM Tris pH 8.0, 150 mM NaCl). Rabbit polyclonal IgG (4 μg) specific for the M, ~105,000 ODA-DC polypeptide (anti-DC105 antibody) (Wakabayashi et al., 2001) was added to one of the tubes and incubated on ice for 2 h. As a control, 4 μg of rabbit normal IgG was added to the other tube. A 0.5-ml aliquot of the 0.6 M KCl extract (2.8 mg/ml) was added to IP buffer 2 (HMDEK, 75 mM NaCl, 3% BSA, 0.1% Triton X-100, pH 7.4). This mixture was precleared by incubation with protein A-agarose (Roche Diagnostics, Tokyo, Japan) for 30 min at 4°C, followed by centrifugation. The supernatant was then incubated with the anti-DC105 antibody or normal rabbit IgG for 4 h followed by incubation with protein A-agarose for 1 h. The precipitated agarose beads were washed twice with IP washing buffer (IP buffer 2 with 0.05% Triton X-100) and twice with IP washing buffer without BSA. The immune complex was eluted by addition of an equal volume of 2× SDS-sample buffer and incubation at 100°C for 3 min. The eluted proteins were separated by electrophoresis in 10% SDS-polyacrylamide gels, transferred to a nitrocellulose membrane, probed with streptavidin-horseradish peroxidase (HRP) (Molecular Probes, Eugene, OR), and detected using an HRP-conjugated anti-rabbit IgG or anti-mouse IgG secondary antibodies and a chemiluminescent substrate.

**Isolation of M, ~70,000 ODA-DC Protein and Peptide Sequencing**

Proteins in 5–20% polyacrylamide gels of sucrose gradient fractions containing the ODA-DC from *oda-3* cells were transferred electrophoretically to polyvinylidene difluoride membrane (Immobilon-PSQ; Millipore) and stained with Ponceau S. A band at M, ~70,000, present in the 7S fraction from *oda-3* but absent in comparable fractions from *oda-3*, was excised and digested with trypsin. The resulting peptides were separated by reverse phase high-performance liquid chromatography, and the amino-terminal sequences of three of the peptides (peptides 1, 2, and 3; Table 1) were determined directly using an amino acid sequencer (model 477A; Applied Biosystems, Foster City, CA).

**Amplification and Cloning of Partial cDNA Encoding the M, ~70,000 ODA-DC Protein**

Wild-type cells were deflagellated by pH shock (Witman et al., 1972) in modified Sager and Granick Medium I (Witman, 1986), and allowed to regenerate new flagella. Total RNA was isolated from the cells ~30 min after deflagellation (Willerson et al., 1994). First strand cDNA was made from the RNA by using Superscript II reverse transcriptase (Invitrogen, Carlsbad, CA) and oligo-dT primer. cDNA fragments were amplified using specific primers (Table 1) designed from the peptide sequences of the M, ~70,000 protein. Polymerase chain reaction (PCR) products were subcloned between the EcoRI and BanHI sites of pBluescript II KS (−) (Stratagene, La Jolla, CA), and their ends were sequenced using Sequenase version 2.0 and the 7-deaza-dGTP sequencing kit (U.S. Biochemical, Cleveland, OH). A 750-base pair insert (pcST8-1) amplified by primers A and D was found to be correct, having additional sequences contained in peptides 1 and 2 but not used to design the primers. This cDNA was labeled with [*-32P]dCTP by using the Stratagene random primer labeling kit.

**Cloning of Full-Length cDNAs for the M, ~70,000 ODA-DC Protein**

A AZAP1 cDNA library of a *C. reinhardtii* wild-type strain (Willerson et al., 1995) was screened with the DNA probe pcST8-1. Positive phage plaques were subjected to a second round of screening. A phagemid pBluescript II SK (−) having a 2.3-kb insert (pcST773) was rescued by in vivo excision. pcST773 was digested with *Bam*HI, *Ban*HI, SacII, and *XhoI*, and four fragments were subcloned. The ends of the fragments were sequenced and the data used to design internal sequencing primers. Single-stranded DNA from both strands of clone pcST737 was isolated and completely sequenced.
Figure 1. ODA-DC composition and association with dynein. (A and B) SDS-PAGE analyses of fractions from sucrose density gradient centrifugations of high-salt extracts from axonemes of mutant strains \textit{oda6} (A) and \textit{oda3} (B). \textit{Oda6} axonemes have ODA-DCs but lack outer arms; \textit{oda3} axonemes lack both ODA-DCs and outer arms. Three proteins (Mr ~105,000, ~70,000, and ~25,000, black arrowheads in A) cosediment at 7S in the sucrose gradient fractionation of the \textit{oda6} extract, but are specifically missing in comparable fractions from the mutant \textit{oda3} (B, white arrowheads). The left lane in each gel was loaded with molecular weight standards (97.4, 66.2, 45.0, 31.0, 21.5, and 14.4 kDa). Gradients shown in A and B were centrifuged under standard conditions in the absence of Mg$^{2+}$ (Piperno and Luck, 1979; Pfister et al., 1982). (C) Immunoprecipitation of the ODA-DC in the absence of Mg$^{2+}$. The anti-DC105 antibody was used to immunoprecipitate the complex from...
Characterization of the Mutant Gene in oda1-1

oda1-1 cells were deflagellated by pH shock and allowed to regenerate flagella for 40 min under illumination, at which time total RNA was isolated from these cells and from oda1-1 and wild-type cells that had not been deflagellated. First-strand cDNA was made from the oda1-1 and wild-type RNA by using reverse transcriptase and oligo-dT primers. The PCR was then carried out with Elongase Enzyme Mix (Invitrogen), which contains Tag DNA polymerase and the proofreading Pyrococcus sp. GB-D polymerase for high fidelity, and by using two primers (Figure 2A, double underlines) designed to amplify the complete open reading frame encoding the M, ~70,000 ODA-DC protein. Products were cloned between the EcoRI and KpnI sites of pBluescript II KS (−) and subjected to in vitro transcription by using T7 RNA polymerase; the transcripts were then translated in vitro by using a reticulocyte lysate system (Promega, Madison, WI) containing [35S]methionine. The translation products were separated in 7.5 or 10% polyacrylamide gels and autoradiographed. Four clones obtained from oda1-1 were sequenced at their 5′ ends.

Figure 1 (cont.) betinylated 0.6 M KCl extracts of wild-type (wt) and oda1 (oda1) axonemes. The immunoprecipitated proteins were then separated by SDS-PAGE, transferred to nitrocellulose, and probed with streptavidin-HRP (lanes a–d). The anti-DC105 antibody immunoprecipitated three proteins (arrowheads, M, ~105,000, ~70,000, ~25,000) from wild-type axonemal extracts (lane b). None of these three proteins were immunoprecipitated from wild-type extracts by using rabbit normal IgG (lane a), or from oda1 axonemal extracts by using the anti-DC105 antibody (lane d) or rabbit normal IgG (lanes c). The wild-type betinylated 0.6 M KCl extract also was analyzed in Western blots probed with antibodies specific for the M, ~105,000 and ~70,000 proteins to confirm the relative mobilities of the betinylated proteins (lanes e and f). During the two overnight dialyses necessary to prepare the 0.6 M KCl extracts for immunoprecipitation, some proteolysis of the M, ~105,000 and ~70,000 proteins occurred, resulting in minor immunoreactive fragments (∗, lane b) running just below the intact proteins. Products used for the Western blots were stored at 4°C for several days longer to accentuate the proteolytic fragments (∗, lanes e and f). Numbers on left indicate molecular weight markers. (D) SDS-PAGE analyses of extract of wild-type axonemes centrifuged at 23S. Outer arm dynein intermediate chains (IC69 and IC78) and light chains are indicated by arrows and a bracket, respectively. A, B, and D chains also are shown. The complex also was shown to be present in 0.6 M KCl extracts of oda6 axonemes, and to sediment at 7S in Mg2+-free sucrose density gradients. Therefore, to partially purify the complex and positively identify its polypeptide components, axonemes of oda3 and oda6 were isolated, pre-extracted with 0.5 M potassium acetate to remove proteins that otherwise would contaminate the 0.6 M KCl fraction (Nakamura et al., 1997), and then extracted with 0.6 M KCl. The 0.6 M KCl extracts were fractionated by sucrose density gradient centrifugation, and the polypeptide composition of the fractions examined by SDS-PAGE. Polypeptides of M, ~105,000, ~70,000, and ~25,000 coeluted at 7S in the oda6 fractions but were uniquely missing from the oda3 fractions (cf. Figure 1, A and B). The M, ~70,000 polypeptide previously had been identified as a component of the ODA-DC (Takada and Kamiya, 1994). The present results provide evidence that the factor contains additional proteins of M, ~105,000 and ~25,000.

To confirm that the M, ~105,000, ~70,000, and ~25,000 polypeptides occur together as a complex, and to investigate whether the wild-type complex contains additional polypeptides not identified in the above-mentioned sucrose density gradient analyses, an antibody specific for the M, ~105,000 polypeptide (Wakabayashi et al., 2001) was used to immunoprecipitate the ODA-DC from an 0.6 M KCl extract of wild-type axonemes. Before the immunoprecipitation, the extract was dialyzed against a Mg2+-free buffer to dissociate the ODA-DC from the outer arm dynein, and then the

Computational Analysis

The GCG suite of programs (Devereux et al., 1984) was used for sequence assembly and protein structure predictions. The program COILS (Lupas et al., 1991; Lupas, 1996a) was used to predict regions of coiled-coil structure. The PSI-BLAST program (Altschul et al., 1997) was used to search databases for related sequences. The program CLUSTAL W (Thompson et al., 1994) was used to align homologs to the Chlamydomonas sequence. The PROSITE database was used to determine possible sites for post-translational modifications (Bairoch et al., 1995).

Protein Cross-Linking

Preparation of 0.6 M KCl extracts of wild-type axonemes was as described under “Characterization of the ODA-DC” but without the 0.5 M potassium acetate wash. The zero-length cross-linker 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) (Sigma Chemical, St. Louis, MO) was added to aliquots of the extract to various final concentrations and the mixtures incubated at 24°C for 1 h. The cross-linking reactions were stopped by addition of equal volumes of 2× SDS-PAGE sample buffer. The cross-linked products were separated by electrophoresis on 6% SDS-polyacrylamide gels, transferred to nitrocellulose membranes, probed with a rabbit polyclonal antibody specific for the M, ~70,000 ODA-DC polypeptide (anti-DC70 antibody) (Wakabayashi et al., 2001), and immunoreactive bands were detected with HRP-conjugated anti-rabbit IgG and a chemiluminescent substrate. The membrane was then stripped and reprobed with the anti-DC105 antibody.

RESULTS

ODA-DC Contains Three Polypeptides

The ODA-DC previously was shown to be absent from axonemes of the outer dynein armless mutants oda1 and oda3, but to be present and functional in axonemes of mutants oda2, oda4, oda5, and oda6 (Takada and Kamiya, 1994). The complex also was shown to be present in 0.6 M KCl extracts of oda6 axonemes, and to sediment at 7S in Mg2+-free sucrose density gradients. Therefore, to partially purify the complex and positively identify its polypeptide components, axonemes of oda3 and oda6 were isolated, pre-extracted with 0.5 M potassium acetate to remove proteins that otherwise would contaminate the 0.6 M KCl fraction (Nakamura et al., 1997), and then extracted with 0.6 M KCl. The 0.6 M KCl extracts were fractionated by sucrose density gradient centrifugation, and the polypeptide composition of the fractions examined by SDS-PAGE. Polypeptides of M, ~105,000, ~70,000, and ~25,000 coeluted at 7S in the oda6 fractions but were uniquely missing from the oda3 fractions (cf. Figure 1, A and B). The M, ~70,000 polypeptide previously had been identified as a component of the ODA-DC (Takada and Kamiya, 1994). The present results provide evidence that the factor contains additional proteins of M, ~105,000 and ~25,000.

To confirm that the M, ~105,000, ~70,000, and ~25,000 polypeptides occur together as a complex, and to investigate whether the wild-type complex contains additional polypeptides not identified in the above-mentioned sucrose density gradient analyses, an antibody specific for the M, ~105,000 polypeptide (Wakabayashi et al., 2001) was used to immunoprecipitate the ODA-DC from an 0.6 M KCl extract of wild-type axonemes. Before the immunoprecipitation, the extract was dialyzed against a Mg2+-free buffer to dissociate the ODA-DC from the outer arm dynein, and then the
proteins in the extract were biotinylated. The immunoprecipitated proteins were separated by SDS-PAGE, transferred to a nitrocellulose membrane, and detected using streptavidin-HRP. Three major bands corresponding to the Mr \(90,000\), \(70,000\), and \(45,000\) polypeptides were detected in the wild-type immunoprecipitate (Figure 1C, lane b). Except for two proteolytic fragments and bands present in the normal IgG control (Figure 1C, lane a), no additional bands were observed. These three polypeptides were specifically missing in immunoprecipitates prepared identically from 0.6 M KCl extracts of \(oda1\) axonemes (Figure 1C, lane d), which lack the ODA-DC. Western blot analyses of the wild-type 0.6 M KCl extract verified that the biotinylated Mr \(90,000\), \(70,000\), and \(45,000\) ODA-DC polypeptides comigrated with the two major high-molecular-weight proteins in the immunoprecipitate (Figure 1C, lanes e and f). These results confirm that the three putative ODA-DC polypeptides occur together in a complex in wild-type axonemes. Moreover, because a functional ODA-DC can be isolated from the 0.6 M KCl extract in HMDEK, it is likely that these three polypeptides are sufficient for ODA-DC function and constitute the entire ODA-DC.

### The ODA-DC Is Associated with Soluble Three-headed Outer Arm Dynein

When the outer dynein arm is removed from the wild-type axoneme by extraction with 0.6 M KCl and then purified by sucrose density gradient centrifugation in the presence of Mg\(^{2+}\), the \(\alpha\), \(\beta\), and \(\gamma\) subunits remain associated as a “three-headed” complex that sediments at 23S (Takada et al., 1992). In contrast to the “two-headed” \(\alpha \beta\) subunit and “single-headed” \(\gamma\) subunit isolated in the absence of Mg\(^{2+}\), this three-headed dynein is competent to bind to the correct sites on \(oda1\) axonemes without addition of exogenous ODA-DC (Takada et al., 1992). This raised the question of whether the ODA-DC might remain associated with the soluble three-headed dynein under these conditions. To investigate this, the three-headed dynein was prepared by the method of Takada et al. (1992), with the sucrose gradient centrifugation carried out at relatively low hydrostatic pressure to prevent dissociation of the outer arm dynein subunits (Nakamura et al., 1997). SDS-PAGE analysis of the resulting sucrose density gradient fractions revealed that the Mr \(90,000\), \(70,000\), and \(45,000\) polypeptides now cosedimented with each other and with the outer arm polypeptides at 23S (Figure 1D). Therefore, in the presence of Mg\(^{2+}\) and low hydrostatic pressure, the ODA-DC remains associated with the \(\alpha\), \(\beta\), and \(\gamma\) outer arm dynein subunits. This is the first demonstration that the ODA-DC and the outer dynein arm directly interact with each other. That all three ODA-DC polypeptides now sediment together at 23S provides additional evidence that they are all part of the same complex.

To obtain further evidence for the direct interaction of the ODA-DC and outer arm dynein in the presence of Mg\(^{2+}\), a 0.6 M KCl extract in HMDEK was prepared from wild-type axonemes. The ODA-DC was immunoprecipitated from the extract by using the antibody specific for the Mr \(90,000\) ODA-DC polypeptide. Western blot analysis indicated that, as expected, the immunoprecipitate contained both the Mr \(90,000\) and \(70,000\) ODA-DC polypeptides (Figure 1E, lanes c and d). To determine whether dynein was immunoprecipitated with the ODA-DC, the blots were probed with mouse monoclonal antibodies 1869A and 1878A specific for the outer arm dynein intermediate chains IC69 and IC78, respectively (King et al., 1991). Both intermediate chains were detected (Figure 1E, lanes a and b). These results confirm that the ODA-DC is associated with outer arm dynein in the presence of Mg\(^{2+}\).

### Molar Ratios of ODA-DC Proteins

To determine the stoichiometries of the ODA-DC polypeptides relative to themselves and to the outer dynein arm, quantitative densitometry was carried out on gels such as that shown in Figure 1D. The amount of Coomassie blue dye bound to a protein is approximately proportional to the number of positive charges on the protein (Tal et al., 1980). The combined mole percentages of arginine, lysine, and histidine in the Mr \(90,000\) protein, the Mr \(70,000\) protein, IC78, and IC69 are 18, 16, 15, and 13, respectively. Therefore, one would expect the values obtained by quantitative densitometry of a Coomassie blue-stained gel to accurately reflect the relative amounts of these four proteins. From the gel shown in Figure 1D, the molar ratios of the Mr \(90,000\) protein, IC69, and the Mr \(70,000\) protein relative to IC78 were determined to be 0.92, 1.05, and 1.05, respectively (Table 2). Similar results were obtained with other gels. These results indicate that the Mr \(70,000\) ODA-DC protein is present in an equimolar amount with the Mr \(90,000\) ODA-DC protein. Moreover, because the outer dynein arm

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<th>Polypeptide</th>
<th>Relative amount*</th>
<th>Mass (kDa)</th>
<th>Molar ratio</th>
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<tbody>
<tr>
<td>Mr (90,000)</td>
<td>1.00</td>
<td>83.4</td>
<td>0.92 (1)</td>
</tr>
<tr>
<td>IC78</td>
<td>1</td>
<td>76.5</td>
<td>1.05 (1)</td>
</tr>
<tr>
<td>IC69</td>
<td>0.87</td>
<td>63.4</td>
<td>1.05 (1)</td>
</tr>
<tr>
<td>Mr (70,000)</td>
<td>0.85</td>
<td>62.2</td>
<td>1.05 (1)</td>
</tr>
</tbody>
</table>

* Average of two lanes.
Figure 2. Sequence and predicted coiled-coil structure of the Mr, 70,000 ODA-DC protein. (A) Nucleotide sequence of a cDNA clone encoding the protein, and its deduced amino acid sequence. An in-frame stop codon just upstream of the predicted translation initiator ATG is indicated by an underline and an asterisk; the stop codon at the end of the long open reading frame is marked by an asterisk. Lines under the deduced amino acids indicate sequence that exactly matches that obtained by direct microsequencing of three tryptic peptides from the Mr, 70,000 protein. Two nucleotide sequences that were used for PCR primers to amplify the complete protein coding region from oda1-1 and wild-type first-strand cDNA are indicated by double underlines in the 5'- and 3'-untranslated regions. A complete Chlamydomonas polyadenylation signal sequence (TGTAA) is marked by a row of asterisks. The C at nucleotide position 283, which is changed to a T in oda1-1, is shown in bold. These sequence data are available from GenBank/European Molecular Biology Laboratory/DNA Data Bank of Japan under accession no. AY039618. (B) The deduced amino acid sequence of the Mr, 70,000 protein was analyzed using the program COILS (MTIDK matrix, with a 2.5 weighting of hydrophobic positions a and d), which estimates the probability that a region of polypeptide will form a coiled-coil structure (Lupas, 1996a). Regions A, B, and D (amino acids 27–60, 120–215, and 323–370, respectively) have a high probability (≥99%) of forming coiled-coils. Region C (amino acids 234–262) is predicted to be α-helical, but when the NEWCOILS program is run with weighting of hydrophobic positions a and d, this region is not predicted to form a coiled coil.
contains one copy of each IC (King and Witman, 1989), there appears to be one ODA-DC for each outer arm dynein.

Visual examination of gels indicated that the \( M_r \approx 25,000 \) ODA-DC protein is present in about the same amount as the \( M_r \approx 20,000 \) outer dynein arm LC, but in only about one-half the amount of the \( M_r \approx 22,000 \) outer dynein arm LC. The outer dynein arm is estimated to contain one copy of the \( M_r \approx 20,000 \) LC and two copies of the \( M_r \approx 22,000 \) LC (King and Witman, 1989). Therefore, it is likely that the three ODA-DC proteins are present in the complex in a 1:1:1 molar ratio.

**Sequence and Structure of the \( M_r \approx 70,000 \) ODA-DC Polypeptide**

The \( M_r \approx 70,000 \) ODA-DC polypeptide from gels such as that shown in Figure 1A was transferred to polyvinylidene difluoride membrane, stained with Ponceau S, excised, and digested with trypsin. The resulting fragments were isolated by high-performance liquid chromatography and their amino-terminal sequences determined directly (Table 1). From these sequences, PCR primers were designed (Table 1) for reverse transcription-PCR; total RNA from wild-type cells was used as template. Primer A designed from the sequence PGDPFFAQ in peptide 1 and primer D designed from the sequence MAQNVAA in peptide 2 yielded a 750-base pair product (pcST8-1). Sequencing the ends of this product revealed that it encoded that the clone \( ZAPII \) that was regenerating their coiled-coil structures (Figure 2B). In addition, there is a region between amino acids 234 and 262 that is predicted to form an \( \alpha \)-helix but does not contain a heptad repeat; 57% of the amino acids in this region are charged. This region is similar in structure and sequence to a portion of the \( M_r \approx 105,000 \) ODA-DC protein (see DISCUSSION). The amino-terminal 26 amino acids, of which eight are glycine, are predicted to form a random coil. The carboxyl-terminal 59 amino acids, of which 42% are charged, are also predicted to form a random coil; this region includes a glutamic acid-rich cluster (residues E_{207} to E_{227}).

A PSI-BLAST search of the databases by using the entire predicted amino acid sequence of the \( M_r \approx 70,000 \) ODA-DC protein revealed one potential homolog in *Leishmania major* (accession no. CA555364). Two potential homologs in *Drosophila melanogaster* (A AF55345 and A AF56123), two potential human homologs (AK057357 and AK057488) that appear to be variants expressed from the same gene, and one mouse homolog (BC013491) that is very similar to the human homolog AK057488. An alignment of the *Leishmania* homolog, one of the *Drosophila* homologs, and the two human homologs to the *Chlamydomonas* protein is shown in Table 3; BLAST E values and predicted masses and isoelectric points for the homologs are given in Table 3. The proteins are similar in predicted size, and the identities extend throughout the proteins’ predicted sequences, suggesting that they are true homologs. The human homologs are known from full-length (AK057357) or partial (AK057488) testis cDNAs; comparison of their predicted carboxyl-terminal amino acid sequences reveals that the last 11 residues of the former are replaced by 249 different residues in the latter, suggesting that the two transcripts are produced by alternative splicing. The gene encoding these proteins maps to chromosome position 19q13 (Human Genome Project Working Draft, University of California, Santa Cruz, CA). No close matches were found in yeast, *Caenorhabditis elegans*, or *Arabidopsis*, all of which lack motile cilia.

**Mutant oda1-1 Has a Defect in the Gene Encoding the \( M_r \approx 70,000 \) ODA-DC Protein**

Axonemes of the mutants oda1 and oda3 lack the ODA-DC as well as the outer dynein arm (Takada and Kamiya, 1994), suggesting that the primary defect in these mutants involves the ODA-DC polypeptides. Indeed, the ODA3 gene previously was found to encode the \( M_r \approx 105,000 \) polypeptide (Koutoulis et al., 1997), which we herein show is a component of the ODA-DC (see above). Therefore, ODA1 was a strong candidate gene for the \( M_r \approx 70,000 \) protein.

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**Table 3. Potential homologs of the *Chlamydomonas* \( M_r \approx 70,000 \) ODA-DC protein**

<table>
<thead>
<tr>
<th>Organism</th>
<th>Name</th>
<th>Accession no.</th>
<th>BLAST E value</th>
<th>BLAST identities/positives (%)</th>
<th>Predicted masses</th>
<th>Predicted pI</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Leishmania</em></td>
<td>L1648.02</td>
<td>CAB55364</td>
<td>5e-27</td>
<td>25/46</td>
<td>70,226</td>
<td>5.12</td>
</tr>
<tr>
<td><em>Drosophila</em></td>
<td>CG14905</td>
<td>AAF55345</td>
<td>3e-20</td>
<td>25/47</td>
<td>56,181</td>
<td>6.34</td>
</tr>
<tr>
<td>Human</td>
<td>Unnamed (cDNA)</td>
<td>AK057357</td>
<td>3e-19</td>
<td>23/44</td>
<td>55,346</td>
<td>6.11</td>
</tr>
<tr>
<td>Human</td>
<td>Unnamed (cDNA)</td>
<td>AK057488</td>
<td>3e-14</td>
<td>27/50</td>
<td>N.A.</td>
<td>N.A.</td>
</tr>
</tbody>
</table>

N.A., not applicable; complete sequence not available.
As a rapid initial test to assess if the oda1-1 mutant had a defect in the gene for the Mr, ~70,000 protein, we determined whether that protein could be synthesized in vitro beginning with oda1-1 RNA. From the wild-type nucleotide sequence, we designed a pair of PCR primers to amplify the complete coding region for the Mr, ~70,000 protein. One primer used sequence 100 base pairs upstream from the initiator ATG in the 5'-untranslated region; the other used sequence next to the poly(A) tail in the 3'-untranslated region (Figure 2A, double underlines). RNA was isolated from oda1-1 cells that were actively regenerating flagella, as well as from nonregenerating wild-type and oda1-1 cells. First-strand cDNA was made using reverse transcriptase, and the PCR carried out with the above-mentioned primers. A large amount of a single product of 2.3 kb was obtained when the cDNA template was prepared from oda1-1 cells that were regenerating flagella (Figure 4A); a smaller amount of the 2.3-kb product was obtained with cDNA prepared from either wild-type cells or oda1-1 cells that were not regenerating flagella. The PCR products from wild-type and regenerating oda1-1 cells were ligated into pBluescript II KS (--) and cloned into Escherichia coli strain XL1Blue. Two wild-type clones and four oda1-1 clones were transcribed using T7 RNA polymerase, and the transcripts translated in a rabbit reticulocyte lysate containing [35S]methionine. Although all clones produced transcripts of the expected size (Figure 4B), only the wild-type transcripts yielded protein of Mr, ~70,000 (Figure 4C). With oda1-1 transcripts, smaller protein bands were detected but none had an intensity comparable to that of the Mr, ~70,000 band produced by wild-type transcripts. These results strongly suggested that strain oda1-1 has a mutation in the gene encoding the Mr, ~70,000 protein and that the complete protein is not expressed in vitro.

This was confirmed by sequence analysis of the 5' ends of the oda1-1 cDNA clones. In all four clones, the C at nucleotide position 283, 135-base pairs downstream from the initiator ATG, was changed to T (Figure 2A). This single-base replacement converts a CAG codon specifying glutamate to the stop codon TAG. This would result in the translation of only 45 of the 552 amino acids encoded by the wild-type ODA1 gene. It is likely that the minor bands Mr, ~60,000 produced by the oda1-1 transcripts (Figure 4C) are the result of translation initiation at internal sites downstream from this stop codon.

The Mr, ~70,000 and ~105,000 ODA-DC Polypeptides Are Cross-linked by EDC

Both the Mr, ~70,000 and ~105,000 ODA-DC polypeptides are predicted to have extended coiled-coil regions that are likely to function in dimerization or heterodimer formation (see DISCUSSION). This raised the question of whether the
two proteins interact with each other to form a heterodimer that then binds the \( M_r \sim 25,000 \) protein to form the ODA-DC, or whether each polypeptide associates with another copy of itself to form a homodimer, with the two types of homodimers possibly coming together during assembly of the complete ODA-DC. To investigate this, we used the zero-length cross-linker EDC to examine interactions between the ODA-DC proteins in 0.6 M KCl extracts of wild-type axonemes. KCl extracts were chosen for these experiments because interactions between the ODA-DC and tubulin should be minimized in the high-salt environment, thus simplifying the number of cross-linked products obtained. As shown in Figure 5, EDC generated a series of cross-linked products that migrated between \( M_r \sim 175,000 \) and \( M_r \sim 210,000 \) and contained both the \( M_r \sim 70,000 \) and \( M_r \sim 105,000 \) ODA-DC polypeptides. The apparent size of the smallest of these conjugates is of the size expected for a simple heterodimer of the \( M_r \sim 70,000 \) and \( M_r \sim 105,000 \) polypeptides, but it is not clear why multiple, more slowly migrating products appeared simultaneously. One possibility is that intermolecular cross-linking at different sites produced products with different relative mobilities, a phenomenon postulated to account for a band-spreading observed for cross-linked products of \( \alpha \) and \( \beta \) spectrin (Huang and Richards, 1977). In any case, all of the products appear to contain both ODA-DC polypeptides, the amounts of the monomeric proteins decrease si-

Figure 5. Cross-linking of the ODA-DC proteins by EDC. A 0.6 M KCl extract of wild-type axonemes was incubated with increasing concentrations of EDC (0, 0.2, 0.5, 1, 2, 4, 6, 10, and 20 mM). The proteins were then separated on a 6% SDS-polyacrylamide gel and transferred to a nitrocellulose membrane. The right panel shows the blot probed with the anti-DC70 antibody; the left panel shows the same blot stripped and reprobed with the anti-DC105 antibody. Multiple cross-linked products migrating between \( M_r \sim 175,000 \) and \( M_r \sim 210,000 \) appear simultaneously, are recognized by both antibodies, and become progressively more prominent as the EDC concentration is increased. Numbers on left indicate molecular weight markers.

Figure 4. Amplification, in vitro transcription, and in vitro translation of cDNAs encoding the \( M_r \sim 70,000 \) ODA-DC protein. (A) Amplified cDNA made from first strand cDNA by using a pair of primers (Figure 2A) designed to amplify the full-length open reading frame encoding the \( M_r \sim 70,000 \) protein. Template RNAs were from wild-type cells (lane 1), \( oda1-1 \) mutant cells that had been deflagellated and were in the process of regenerating new flagella (lane 2), and \( oda1-1 \) cells that were not deflagellated (lane 3). The left lane was loaded with a 1-kb DNA ladder (Invitrogen). (B) Formaldehyde-agarose gel (1.2%) of RNA transcribed from cDNA clones derived from the PCR products shown in A. The products were cloned in pBluescript II KS (−) and transcribed in vitro by using T7 RNA polymerase. Two wild-type (wt) and four \( oda1-1 \) (\( oda1 \)) clones were examined; all produced RNA of the expected size. The left lane shows an RNA ladder (0.24–9.5 kb) (Invitrogen). (C) SDS-PAGE analysis of products obtained by in vitro translation of RNAs shown in B. The wild-type RNAs (wt) produced a protein of \( M_r \sim 70,000 \) (arrow), whereas the \( oda1-1 \) RNAs (\( oda1 \)) failed to produce a protein of this size. The right lane (L) is a product from luciferase mRNA (61 kDa). Bars at the left indicate molecular weight markers (76,000 and 52,000).
multaneously and concomitantly with the appearance of the cross-linked products, and there is no evidence for cross-linking of homodimers of either protein. Because EDC generates covalent linkages solely between groups that interact directly, these results indicate that the M₄, ~70,000 ODA-DC polypeptides are intimately associated with each other, and support the hypothesis that the two proteins interact with one another to form a heterodimer.

DISCUSSION

The ODA-DC Has Three Subunits

To understand the mechanism by which the outer dynein arm is targeted to its correct binding site on flagellar microtubules, we have been characterizing the C. reinhardtii ODA-DC, which originally was described as a “7S” factor when released from axonemes by high-salt extraction (Takada and Kamiya, 1994). This factor assembles onto flagellar doublet microtubules in the absence of outer arms in vivo, but is necessary for the outer dynein arm to attach to the doublet microtubules (Takada and Kamiya, 1994). Hence, the ODA-DC must be an important intermediary in the binding of outer arm dynein to its unique attachment site within the flagellar axoneme.

Herein, we present three independent lines of evidence that the ODA-DC contains polypeptides of M₁, ~105,000, ~75,000, and ~25,000: 1) Sucrose density gradient centrifugation was used to partially purify the ODA-DC after its release from the axoneme by a modified high-salt extraction procedure (Nakamura et al., 1997) that reduces the number of “background” polypeptides in the high-salt extract and in subsequent sucrose gradient fractions. Under these conditions, the 7S fraction from axonemes of the outer armless mutant oda1, which has the ODA-DC (Takada and Kamiya, 1994), contained polypeptides of M₁, ~105,000, ~70,000, and ~25,000 that were missing in equivalent fractions from the mutant oda3, which lacks the ODA-DC. These results strongly suggested that the ODA-DC contains these three polypeptides. The M₁, ~70,000 polypeptide previously was reported to be a component of the ODA-DC (Takada and Kamiya, 1994). That we were able to detect the presence or absence of the M₁, ~105,000 and ~25,000 polypeptides in sucrose gradient fractions probably was due to the reduced number of background bands resulting from our modified extraction conditions. 2) An antibody specific for the M₁, ~105,000 protein coimmunoprecipitated the M₁, ~70,000 and ~25,000 proteins but no other proteins from a 0.6 M KCl extract of wild-type axonemes. These results confirmed that all three proteins were part of the ODA-DC and indicated that the three proteins are likely to constitute the complete ODA-DC in wild-type cells. 3) The three polypeptides shifted simultaneously from the 7S to the 23S fraction in sucrose density gradients centrifuged under conditions where the ODA-DC remained associated with the more rapidly sedimenting outer dynein arm (see below).

The ODA-DC Interacts Directly with the Outer Dynein Arm

If the ODA-DC is directly responsible for binding the outer arm to the doublet microtubule, then it should interact with the outer arm. When the outer dynein arm was removed from the wild-type axoneme by extraction with high salt and subjected to sucrose density gradient centrifugation in the presence of Mg²⁺, the α, β, and γ DHCs remained associated with each other as a three-headed particle that sediments at 23S (Takada et al., 1992). We found that under these conditions the three polypeptides of the ODA-DC remained associated with the outer arm polypeptides and cosedimented with them at 23S. Therefore, the ODA-DC interacts directly with the outer arm. This interaction was confirmed by experiments in which the outer dynein arm intermediate chains were found to coimmunoprecipitate with the ODA-DC from high-salt extracts prepared in the presence of Mg²⁺. The association of the ODA-DC with the three-headed dynein undoubtedly explains the ability of this dynein to bind efficiently to oda1 and oda3 axonemes, which lack the ODA-DC (Takada et al., 1992). Because the entire outer arm dynein appears to be able to assemble in the cytoplasm of oda1 and oda3 mutants (Fowkes and Mitchell, 1998), the ODA-DC is not promoting outer arm assembly simply by binding the αβγ subunits together. More likely, it forms a direct link between the outer dynein arm and the outer doublet microtubule.

ODA-DC Polypeptide Stoichiometry

Quantitative densitometry indicated that the M₁, ~70,000 and ~105,000 ODA-DC proteins are present in an equimolar ratio in a 23S particle consisting of the ODA-DC and the outer dynein arm; visual comparison of band intensities suggested that the M₁, ~25,000 ODA-DC protein also is present in equimolar amounts with the two larger ODA-DC subunits. Therefore, it is likely that the three ODA-DC subunits occur in a 1:1:1 molar ratio. These data alone do not distinguish whether the ODA-DC is a heterotrimer or a higher order polymer, such as a hexamer. However, we also found that there was one copy of each ODA-DC polypeptide per soluble three-headed outer arm dynein. Therefore, the simplest model is that the ODA-DC is a heterotrimer, and that each outer arm is associated with a single ODA-DC.

Structure of the M₁, ~70,000 ODA-DC Polypeptide

Beginning with peptide sequence obtained directly from microsequencing of tryptic fragments from the M₁, ~70,000 ODA-DC polypeptide, we cloned and sequenced a full-length cDNA encoding the protein. The sequence predicts a 99% likely to form a coiled-coil structure. The M₁, ~70,000 protein is associated. The M₁, ~70,000 ODA-DC protein has three regions (~34, ~96, and ~48 amino acids in length) totaling 178 amino acids that are >99% likely to form a coiled-coil structure. The M₁, ~105,000 ODA-DC protein likewise has three regions (~120, ~70, and ~36 amino acids) totaling ~236 amino acids that are >99% likely to form a coiled-coil. Coiled-coils commonly function in dimerization and heterodimer formation (Lupas, 1996b). As discussed above, each ODA-DC is likely to contain just one copy of each of its component polypeptides. Moreover, experiments using the zero-length protein cross-linker EDC.
In the $M_r \sim 105,000$ protein this sequence consists of an imperfect 11-amino acid tandem repeat (Koutoulis et al., 1997) that previously was found to be closely related to repeats in mammalian trichohyalin, a protein that interacts with intermediate filaments (Fietz et al., 1993; Lee et al., 1993). In trichohyalin these repeats are proposed to form a single-stranded $\alpha$-helical rod that is stabilized by ionic interactions between successive turns of the helix (Lee et al., 1993); it has been proposed that these repeats interact with charged residues on intermediate filaments. Weak repeats with similar structure and composition also occur after a long coiled-coil region in troponin T and caldesmon (e.g., Ks$_{12}$-Q27 in chicken caldesmon; Bryan et al., 1989); these regions are candidates for the tropomyosin-binding sites of these proteins (Bryan et al., 1989). Therefore, this part of the $M_r \sim 70,000$ protein may be involved in protein–protein interactions.

The carboxyl-terminal 59 amino acids of the $M_r \sim 70,000$ protein contain a short glutamic acid-rich region followed by a region with a high percentage of positively and negatively charged residues. Both tubulin and IC78 of outer arm dynein have highly charged basic and acidic domains, so it is possible that this portion of the $M_r \sim 70,000$ protein interacts with those proteins. The $M_r \sim 105,000$ protein likewise has a glutamic acid cluster near its carboxy terminus.

The size of the $M_r \sim 70,000$ protein estimated by SDS-PAGE is greater than the 62.5-kDa mass predicted from nucleotide sequence. Disparities in this direction and of this magnitude or more are commonly observed in proteins with similar structure (e.g., chicken caldesmon, $M_r 120,000–150,000$ estimated vs. 87.0 kDa predicted [Bryan et al., 1989]; C. reinhardtii $M_r \sim 105,000$ ODA-DC protein, $M_r \sim 105,000$ estimated vs. 83.4 kDa predicted [Koutoulis et al., 1997]).

The $M_r \sim 70,000$ ODA-DC Protein Is Encoded by the ODA1 Gene

Outer dynein armless mutants having defects at 15 different ODA loci (ODA1-ODA15) have been reported, of which 12 (oda1-oda10, oda12, and oda15) lack the complete outer arm and have a slow swimming phenotype (Kamiya, 1988; Koutoulis et al., 1997; King, 2000; Pazour and Witman, 2000). The genes ODA2, ODA4, ODA6, ODA9, ODA11, ODA12, ODA13, and ODA15 encode the $\gamma$ and $\beta$ DHCs, IC69, IC78, the $\alpha$ DHC, LC2, LC6, and LC7, respectively, of the outer arm dynein (Mitchell and Kang, 1991; Sakakibara et al., 1991, 1993; Wilkerson et al., 1994, 1995; Pazour and Witman, 2000). A sixth ODA gene (ODA3) has been cloned and found to encode the $M_r \sim 105,000$ ODA-DC protein (Koutoulis et al., 1997). We now report that the ODA1 gene encodes the $M_r \sim 70,000$ ODA-DC protein. Sequencing of mutant oda1-1 cDNA encoding the $M_r \sim 70,000$ protein revealed that the mutant DNA has a point mutation that converts codon 46, which in wild-type DNA specifies a glutamine, to a stop
Functions of the \( M_r \sim 70,000 \) Protein

The fact that a defect in the \( M_r \sim 70,000 \) protein leads to loss of the ODA-DC in \( oda1-1 \) indicates that the \( M_r \sim 70,000 \) protein is essential for assembly of the ODA-DC, and confirms that loss of the ODA-DC leads to an inability to target outer dynein arms to doublet microtubules. In contrast, loss of a major outer arm dynein structural protein in \( oda2, oda4, oda6, \) and \( oda9 \) results in failure of the outer arm to assemble, but does not lead to loss of the ODA-DC (Takada and Kamiya, 1994; Koutoulis et al., 1997). Therefore, the ODA-DC can assemble and bind to the correct position on flagellar doublet microtubules independently of the outer arm dynein.

How the ODA-DC itself assembles onto the correct site on the doublet microtubules is not known. One possibility is that the ODA-DC is a rod-like structure that links together with adjacent ODA-DCs to form a filament running longitudinally along the doublet (Figure 6B); assembly of this filament might be initiated on the correct microtubule protofilament by some structure at the base of the axoneme, or by some discontinuity in the tubulin lattice. This model is consistent with the coiled-coil nature of the ODA-DC and the outer dynein arm, but would be inconsistent with the ODA-DC and the outer dynein arm but would be defective in phosphorylation of the targeted site.

Homologs of the \( M_r \sim 70,000 \) ODA-DC Protein Are Present in Higher Organisms

A BLAST search of gene and protein databases revealed potential homologs of the \( Chlamydomonas \) \( M_r \sim 70,000 \) ODA-DC polypeptide in the protozoan \( Leishmania \), in \( D.\) \( spheroides \), and in mice and humans, but not in organisms such as yeast, \( C.\) \( elegans \), and \( A.\) \( thaliana \) which lack motile cilia. This is the first evidence that the ODA-DC occurs in higher organisms. In humans, primary ciliary dyskinesia (PCD), an inherited disorder in which ciliary and flagellar movement is impaired, seems most frequently to be caused by loss of the outer dynein arms (Afzelius and Mossberg, 1995). As a result of this defect, PCD patients develop bronchiectasis and chronic sinusitis; male patients are infertile. Inasmuch as the \( M_r \sim 70,000 \) ODA-DC gene is necessary for outer arm assembly in \( Chlamydomonas \), its potential human homolog \( AK057357 \) is now a candidate gene for those cases of PCD in which the outer dynein arms are missing. \( AK057357 \) maps to chromosome 19q13. Interestingly, in some PCD patients lacking the outer dynein arm, the defective locus has been mapped to this same region (Meeks et al., 2000). Further studies are warranted to determine whether a defect in \( AK057357 \) causes PCD.

ACKNOWLEDGMENTS

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