

Differential Light Chain Assembly Influences Outer Arm Dynein Motor Function

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Tctex1 and Tctex2 were originally described as potential distorters/sterility factors in the non-Mendelian transmission of *t*-haplotypes in mice. These proteins have since been identified as subunits of cytoplasmic and/or axonemal dyneins. Within the *Chlamydomonas* flagellum, Tctex1 is a subunit of inner arm I1. We have now identified a second Tctex1-related protein (here termed LC9) in *Chlamydomonas*. LC9 copurifies with outer arm dynein in sucrose density gradients and is missing only in those strains completely lacking this motor. Zero-length cross-linking of purified outer arm dynein indicates that LC9 interacts directly with both the IC1 and IC2 intermediate chains. Immunoblot analysis revealed that LC2, LC6, and LC9 are missing in an IC2 mutant strain (*oda6-r88*) that can assemble outer arms but exhibits significantly reduced flagellar beat frequency. This defect is unlikely to be due to lack of LC6, because an LC6 null mutant (*oda13*) exhibits only a minor swimming abnormality. Using an LC2 null mutant (*oda12-1*), we find that although some outer arm dynein components assemble in the absence of LC2, they are nonfunctional. In contrast, dyneins from *oda6-r88*, which also lack LC2, retain some activity. Furthermore, we observed a synthetic assembly defect in an *oda6-r88 oda12-1* double mutant. These data suggest that LC2, LC6, and LC9 have different roles in outer arm assembly and are required for wild-type motor function in the *Chlamydomonas* flagellum.

INTRODUCTION

Dyneins act as minus end-directed microtubule motors and are required for a variety of fundamental cellular activities. There are two general designs for these enzymes built around either a single or several heavy chain motor units (for recent review, see Sakato and King, 2004). The major cytoplasmic dynein isoform and both axonemal outer arms and inner arms of the I1 type¹ contain two, or, in some outer arms, three heavy chains. In all cases, these are associated with two or more intermediate chains (ICs) (usually WD-repeat proteins) and dimers of three different classes of light chains (LCs) (the LC8, Tctex1/Tctex2, and LC7/Roadblock families). These distinct dyneins also contain additional components specific to a particular enzyme class, such as the

light intermediate chains of cytoplasmic dynein and the regulatory light chains directly associated with the motor units of the *Chlamydomonas* outer arm.

The Tctex1 and Tctex2 proteins were first identified as candidates for distorter/sterility factors involved in the non-Mendelian transmission of mouse *t*-haplotypes (variant forms of a 30- to 40-Mb region of chromosome 17) (Lader *et al.*, 1989; Huw *et al.*, 1995). Subsequently, these proteins were found to be integral components of cytoplasmic (King *et al.*, 1996; King *et al.*, 1998) and/or axonemal (Patel-King *et al.*, 1997; Harrison *et al.*, 1998; Kagami *et al.*, 1998) dyneins and to associate directly with the intermediate chains (Mok *et al.*, 2001). In addition, phosphorylation of Tctex2-related proteins in salmonid fish and sea urchin dyneins has been correlated with the activation of sperm motility (Inaba *et al.*, 1999). These observations led to the hypothesis that defects in axonemal dynein function within sperm flagella provide the underlying molecular basis for this chromosome segregation abnormality (Patel-King *et al.*, 1997; Harrison *et al.*, 1998; Pazour *et al.*, 1999). The subsequent identification of an axonemal outer arm dynein heavy chain as an excellent candidate for the strongest distorter (Fossella *et al.*, 2000) and of a sperm motility kinase as the interacting responder (Herrmann *et al.*, 1999) has provided additional support for this model.

Sequence analysis (DiBella *et al.*, 2001) combined with NMR (Mok *et al.*, 2001; Wu *et al.*, 2001, 2005) and x-ray (Williams *et al.*, 2005) structural studies suggest that both Tctex1 and Tctex2 adopt a similar overall conformation. In cytoplasm, Tctex1 interacts with a variety of proteins in addition to dynein (e.g., Doc-2 [Nagano *et al.*, 1998], rhodop-

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Abbreviations used: DMP, dimethylpimelimidate; EDC, 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide; HC, heavy chain; IC, intermediate chain; LC, light chain; MBP, maltose-binding protein; UTR, untranslated region.

¹ This inner arm dynein is also known as subspecies f (Kagami and Kamiya, 1992).

Table 1. Strains used in this study

Strain	Description	Reference
cc124 g1	Wild type Used as parent for generation of null mutants; derived from a <i>nit1-305</i> × cc124 cross	Pazour <i>et al.</i> (1995)
<i>oda6-95</i>	IC2 frame-shift resulting in a premature stop; cannot assemble outer arms; slow swimming	Kamiya (1988); Mitchell and Kang (1993)
<i>oda6-r75</i>	<i>oda6-95</i> intragenic pseudorevertant; assembles outer arms and restores wild-type beat frequency	Mitchell and Kang (1993)
<i>oda6-r88</i>	<i>oda6-95</i> intragenic pseudorevertant; assembles outer arms, but exhibits reduced beat frequency	Mitchell and Kang (1993)
<i>ida1</i>	Defective for 1 α HC; lacks inner arm I1	Kamiya <i>et al.</i> (1991); Porter <i>et al.</i> (1992)
<i>ida4</i>	Defective for p28; lacks a subset of inner arms	Kamiya <i>et al.</i> (1991); LeDizet and Piperno (1995)
<i>oda3</i>	Defective for DC1; lacks outer arms and the outer arm docking complex	Kamiya (1988); Koutoulis <i>et al.</i> (1997)
<i>oda9</i>	Defective for IC1; lacks outer arms, but assembles the docking complex	Kamiya (1988); Wilkerson <i>et al.</i> (1995)
<i>oda12-1</i>	Null mutant for LC2; lacks functional outer arms	Pazour <i>et al.</i> (1999)
<i>oda12-2</i>	Lacks 3' end of LC2 gene; defective outer arm assembly	Pazour <i>et al.</i> (1999)
<i>oda12-1 + pBD14</i>	Null mutant for LC2, rescued with a 3.1-kb genomic region containing the wild-type LC2 gene	Pazour <i>et al.</i> (1999)
<i>oda13</i>	Null mutant for LC6; assembles outer arms and exhibits a minor swimming defect	Pazour and Witman (2000)
<i>oda15</i>	Null mutant for LC7a; assembles reduced levels of outer arms and inner arm I1	Pazour and Witman, (2000); DiBella <i>et al.</i> (2004a)
<i>oda6-r88 oda12-1</i>	Double mutant with altered IC2 and null for LC2	This study; Mitchell and Kang (1993); Pazour <i>et al.</i> (1999)
<i>pf14</i>	Defective for RSP3; lacks radial spokes; paralyzed flagella	Huang <i>et al.</i> (1981)
<i>pf18</i>	Lacks central pair microtubule complex; paralyzed flagella	Adams <i>et al.</i> (1981)
<i>pf28pf30</i>	W4 isolate; lacks outer arms and inner arm I1	Piperno <i>et al.</i> (1990)

sin [Tai *et al.*, 1999], polio virus receptor [Mueller *et al.*, 2002], mitochondrial voltage-dependent anion channel [Schwarzer *et al.*, 2002], neuronal voltage-gated Ca²⁺ channels [Lai *et al.*, 2005], Fyn kinase [Kai *et al.*, 1997; Mou *et al.*, 1998], and the Herpes capsid protein VP26 [Douglas *et al.*, 2004]) and has been proposed to act as an adaptor to attach specific cargoes to this motor (Tai *et al.*, 1999). In *Chlamydomonas*, one Tctex2 homologue (termed LC2) is required for assembly of functional outer arms (Patel-King *et al.*, 1997; Pazour *et al.*, 1999), whereas a related protein imparts stability to inner arm I1 and is necessary for wild-type motor activity (DiBella *et al.*, 2004b). Intriguingly, Tctex1 null alleles in *Drosophila* are viable, although these mutants are male sterile. Phenotypically, these strains exhibit defects in spermiogenesis where a “nuclear cap” of cytoplasmic dynein is incorrectly localized and a connection between the nucleus and basal body is not formed appropriately; they also have immotile sperm possibly due to the lack of Tctex1 in axonemal dyneins (Caggese *et al.*, 2001; Li *et al.*, 2004).

Although nine light chain components, including the essential Tctex2-related protein LC2, have so far been identified within the *Chlamydomonas* outer dynein arm (Piperno and Luck, 1979; Pfister *et al.*, 1982; Patel-King *et al.*, 1997; Pazour *et al.*, 1999), a protein clearly similar to Tctex1 has not previously been described in this motor enzyme. This had been somewhat surprising because obvious Tctex1 homologues are present in all other well-characterized dyneins containing multiple heavy chains, e.g., outer arms from sea urchin sperm flagella (Kagami *et al.*, 1998), mammalian cytoplasmic dynein (King *et al.*, 1996), and *Chlamydomonas* inner dynein arm I1 (Harrison *et al.*, 1998). Here, we identify a second Tctex1-like protein (termed LC9) encoded within the *Chlamydomonas* genome and demonstrate that it is an integral component of the outer dynein arm associated with

both intermediate chains. Thus, in this organism distinct Tctex1-related proteins are used within the flagellar inner and outer arm systems. Furthermore, we find that this LC9 protein together with LC2 (a Tctex2) and LC6 (related to the highly conserved LC8 protein; King and Patel-King, 1995) are missing in a mutant strain (*oda6-r88*) expressing a modified form of IC2 that allows for outer arm assembly but exhibits a reduced flagellar beat frequency close to that of cells lacking this motor (Mitchell and Kang, 1993). Our results indicate that in the presence of this IC2 mutation, these LCs are not required for assembly of the outer dynein arm but may play a role in the control of wild-type motor function. Analysis of additional mutants defective for LC2 (*oda12-1*) and LC6 (*oda13*) as well as an *oda6-r88 oda12-1* double mutant provides further support for this model and yields intriguing insight into the mechanisms of outer arm dynein assembly and the molecular architecture of the IC/LC complex.

MATERIALS AND METHODS

Chlamydomonas Strains

The *Chlamydomonas reinhardtii* strains used in this study are indicated in Table 1. The *oda6-r75* and *oda6-r88* pseudorevertants were generously provided by Dr. David Mitchell (SUNY Upstate Medical Center, Syracuse, NY). The *oda6-r88 oda12-1* double mutant was constructed using standard methods (Harris, 1989). The genotype of the tetrad progeny from this cross was determined using the PCR to obtain appropriate regions from both genes. The *oda6-r88* frame-shift mutation forms a novel *Bam*HI restriction site that was used to differentiate between wild-type and mutant versions; the *oda12-1* allele is a null and thus only the wild-type LC2 gene was detected by the PCR.

All strains were grown in R medium (medium I; Sager and Granick, 1953) containing 2.20 mM KH₂PO₄, 1.71 mM K₂HPO₄, and 7.5 mM sodium acetate). Flagella beat frequency was determined using the fast Fourier transform (FFT) method of Kamiya (2000).

Fractionation of Flagella and Dynein Purification

Flagella were detached using the dibucaine method and purified by standard procedures (Witman, 1986). After demembration with 1% IGEPAL CA-630 (replaces Nonidet P-40), dynein was extracted from the resulting axonemes by treatment with 0.6 M NaCl. The outer arm and associated docking complex were then purified by sucrose density gradient centrifugation in the presence of Mg²⁺ at low hydrostatic pressure (Takada *et al.*, 1992; DiBella *et al.*, 2004a). Samples were routinely electrophoresed in 5–15% acrylamide SDS gradient gels or 12.5% acrylamide slab gels and either stained with Coomassie blue or transferred to nitrocellulose for immunoblotting.

Preparation of Fusion Proteins and Antibodies

The LC9 coding sequence was obtained from first strand cDNA using the PCR, and residues 1–49 were subcloned into the pMAL-c2 vector across the *XmnI/XbaI* restriction sites. This resulted in the N-terminal region of LC9 attached to the C terminus of maltose-binding protein (MBP) via a short hydrophilic linker containing a factor Xa proteolytic cleavage site. Full-length LC9- and *Chlamydomonas* Tctex1-MBP fusion proteins were also prepared. The LC9(1–49) N-terminal fusion protein was used as the immunogen for antibody production in rabbit CT231, and the resulting serum was blot-purified against full-length recombinant LC9 after separation from MBP (Olmsted, 1986).

Other antibodies used in this study were blot-purified rabbit polyclonal antibodies R5932 (vs. LC1; Benashski *et al.*, 1999), R5391 (vs. LC2; Patel-King *et al.*, 1997), R4930 (vs. LC3; Patel-King *et al.*, 1996), CT61 (vs. LC4; M. Sakato and King, unpublished data), R4929 (vs. LC5; Patel-King *et al.*, 1996), R4928 (vs. LC6; King and Patel-King, 1995), R7178 (vs. LC7a; Bowman *et al.*, 1999), CT116 (vs. LC7b; DiBella *et al.*, 2004a), R4058 (vs. LC8; King and Patel-King, 1995), and anti-DC 2 (Wakabayashi *et al.*, 2001), and mouse monoclonal antibodies 12γB (vs. γHC; King *et al.*, 1985), 1878A (vs. IC1; King *et al.*, 1986), and 1869A (vs. IC2; King *et al.*, 1985). Antibody reactivity was detected using peroxidase-conjugated secondary antibodies and an enhanced chemiluminescent substrate (GE Healthcare, Little Chalfont, Buckinghamshire, United Kingdom).

Zero-Length Cross-linking with 1-Ethyl-3-(3-dimethylaminopropyl)-Carbodiimide (EDC)

Cross-linking with EDC was performed essentially as described previously (King *et al.*, 1991; DiBella *et al.*, 2004a). Isolated axonemes and purified dynein samples in 30 mM HEPES, pH 7.4, 5 mM MgSO₄, 0.5 mM EDTA, and 25 mM KCl (HMEK) buffer were treated with either 0 or 20 mM EDC in 100 mM HEPES, pH 7.4, for 60 min at room temperature. The cross-linking reaction was terminated by addition of β-mercaptoethanol in HMEK to a final concentration of 0.1 M before preparation for electrophoresis and immunoblotting.

Multi-Angle Laser Light Scattering

To assess the oligomeric state of LC9 and other related dynein components, MBP fusion proteins in 20 mM Tris-Cl, pH 7.4, 200 mM NaCl, and 1 mM EDTA were fractionated by gel filtration chromatography using a Superdex 200 column, and the peak fractions were analyzed by multi-angle laser light scattering using a Wyatt miniDAWN laser photometer (Wyatt Technology, Santa Barbara, CA). This allowed the native molecular mass of each protein peak to be determined directly and also provided a measure of the polydispersity of the protein sample.

Northern and Southern Blot Analysis

Northern and Southern blot analysis was performed as described previously (King and Patel-King, 1995). RNA was prepared both from nondeflagellated cells, and from cells that had been deflagellated and allowed to regenerate flagella for 30 min. For Southern analysis, wild-type genomic DNA was restricted with *Bam*HI, *Pst*I, *Pvu*II, and *Sma*I. The entire LC9 coding region was used to generate a ³²P-labeled probe.

Electron Microscopy

Wild-type and *oda6-r88 oda12-1* mutant axonemes were fixed with 2.5% glutaraldehyde, 1% tannic acid in 100 mM sodium cacodylate, pH 7.4, and postfixed with 1% osmium tetroxide, 0.8% potassium ferricyanide. Samples were stained en bloc with 1% tannic acid and 0.5% uranyl acetate, dehydrated in ethanol and propylene oxide, and embedded in Polybed (Polysciences, Warrington, PA). Sections were poststained with uranyl acetate and lead citrate and examined in a Philips CM-10 electron microscope at an accelerating voltage of 60 kV.

Computational Methods

Searches of the *Chlamydomonas* genome were performed using the BLAST interface at the Joint Genome Institute (Walnut Creek, CA) (<http://genome.jgi-psf.org/chlr2/chlr2.home.html>). The *Chlamydomonas* expressed sequence tag (EST) databases were searched using BLAST at the National Center for Biotechnology Information and at the Kazusa DNA Research

Center (Chiba, Japan). Sequence assembly was performed with the GCG suite of programs. Alignments were generated using ClustalW and processed with BOXSHADE. Residues within LC9 that are not conserved in *Chlamydomonas* inner arm Tctex1 were mapped onto the molecular surface of the Tctex1 NMR solution structure (PDB accession 1XDX; Wu *et al.*, 2005) and displayed using MOLMOL (Koradi *et al.*, 1996).

RESULTS

Identification of a Second Tctex1-like Protein (LC9) in *Chlamydomonas*

Previously, we reported the identification of a Tctex1-related LC within inner arm I1 from *Chlamydomonas* flagella (Harrison *et al.*, 1998). To assess whether additional members of this LC family exist in *Chlamydomonas*, we searched the genome sequence (version 1.0) for related molecules using inner arm Tctex1 (AAC18035) as the initial query sequence. We obtained a single high-scoring hit that was then used to examine the EST database. The entire coding sequence was initially assembled from a series of overlapping EST clones (BG849022, BG847628, BI721765, BI530285, BG847627, and BG849020). Subsequently, we used specific oligonucleotides to obtain this region from first strand cDNA using the PCR. This product was sequenced to confirm the assembly and used to generate fusion proteins (see below). The complete cDNA sequence is 996 base pairs in length and contains a single open reading frame that encodes a protein of 117 residues (here termed LC9) with a mass of 12,981 Da and a predicted pI of 4.37 (Figure 1a). The assembled sequence also contains two in-frame stop codons in the 5'-untranslated region (UTR) and a perfect copy of the *Chlamydomonas* polyadenylation signal in the 3'-UTR. Search of the genome sequence (version 2.0) and Southern blotting (our unpublished data) indicates that there is a single intron-less gene for LC9 in *Chlamydomonas* located on linkage group X between the KAT gene (encodes p60 katanin) and the GP441 marker. Northern analysis demonstrates that LC9 mRNA is of low abundance in nondeflagellated cells, but it is highly up-regulated after flagellar excision as is characteristic of integral flagellar components (Figure 1b).

Phylogenetic analysis and sequence comparisons with other members of this protein family are shown in Figure 1, c and d, respectively. The proteins used in the comparison include *Chlamydomonas* LC9, *Chlamydomonas* inner arm Tctex1, sea urchin outer arm LC3, murine Tctex1, human rp3, and three members of the Tctex2 LC subclass. *Chlamydomonas* LC9 clearly groups with other members of the Tctex1 subclass. Overall, LC9 shares 40% sequence identity (52% similarity) with *Chlamydomonas* inner arm Tctex1. The most highly conserved regions of LC9 include the C-terminal portion of helix α2 and the β2, β3, and β4 strands and their connecting loops (Figure 1d). Even so, mapping the amino acid differences between LC9 and *Chlamydomonas* Tctex1 onto the NMR solution structure of the latter (PDB accession 1XDX) revealed considerable variation over the entire molecular surface (our unpublished data).

Solution Properties of an MBP-LC9 Fusion Protein

Canonical Tctex1 is a dimeric protein as determined by native gel electrophoresis (DiBella *et al.*, 2001) and high-resolution structural studies (Williams *et al.*, 2005; Wu *et al.*, 2005). To assess whether LC9 can also form dimers, the coding region was subcloned into the pMAL-c2 expression vector, and the protein was expressed as a fusion with MBP. We then directly determined the molecular mass of the native MBP fusion protein using multi-angle laser light scattering (Figure 2a). An MBP fusion with Tctex1 from *Chlamydomonas* inner arm I1 is dimeric in this assay as expected

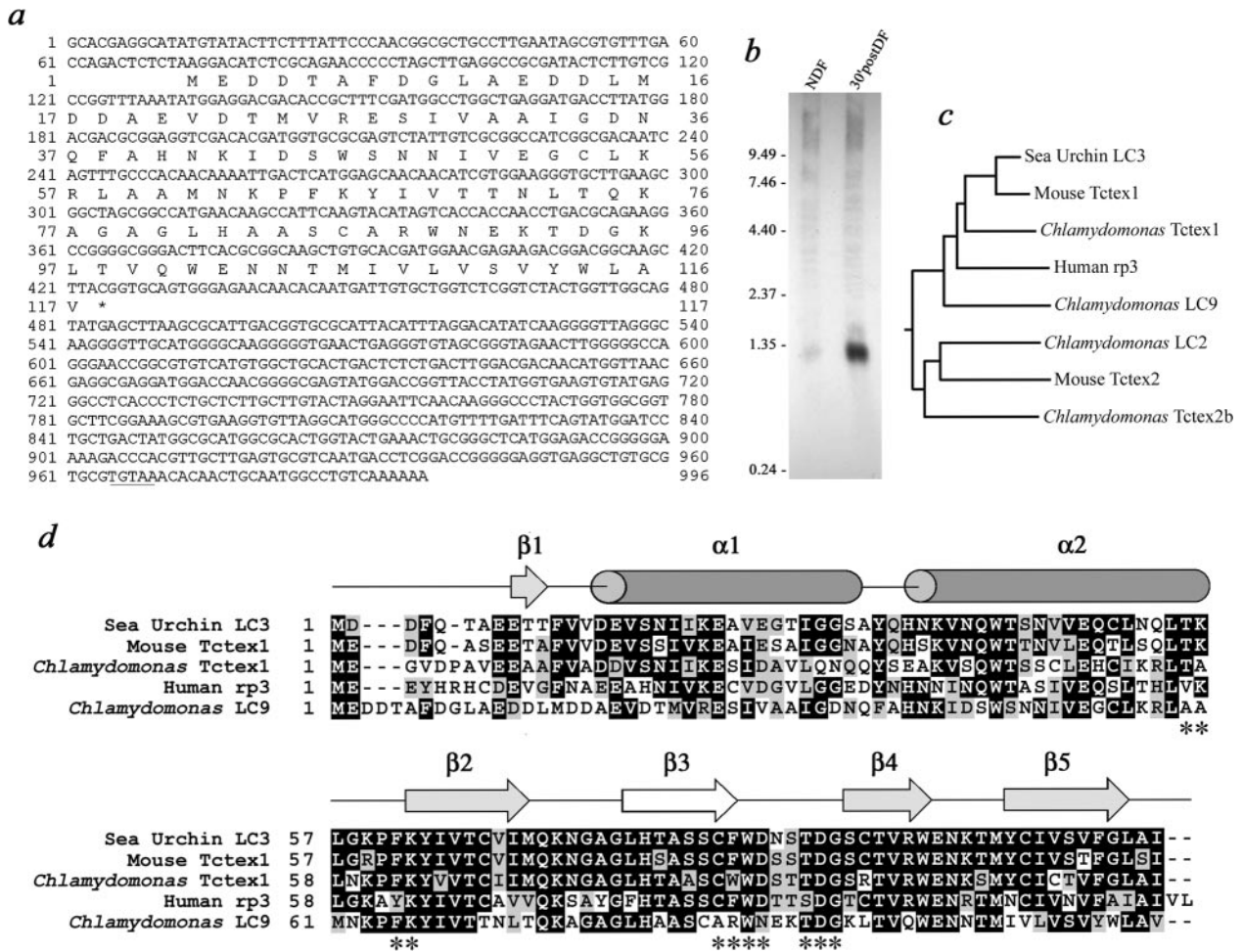


Figure 1. Molecular analysis of LC9. (a) Nucleotide and predicted protein sequence of LC9 originally derived from the overlapping ESTs BG849022, BG847628, BI721765, BI530285, BG847627, and BG849020 and confirmed by analysis of a full-length cDNA. The 5'-UTR contains two in-frame stop codons, and a perfect copy of the *Chlamydomonas* polyadenylation signal (underline) is present in the 3'-UTR. This sequence is available under GenBank accession no. DQ114947. (b) RNA samples from nondeflagellated cells (NDF) and from cells that had been deflagellated by pH shock and allowed to regenerate flagella for 30 min (30' postDF) were probed with the LC9 coding region. A single message of ~1.3 kb is greatly up-regulated after flagellar excision. (c) Rooted neighbor-joining phylogenetic tree illustrating the relationship between LC9, *Chlamydomonas* inner arm Tctex1 (AF039437), sea urchin outer arm LC3 (JC6573), murine Tctex1 (A32995), and human rp3 (U02556). Also included are three members of the Tctex1 light chain subclass; murine Tctex2 (U21673), *Chlamydomonas* outer arm LC2 (U89649) and *Chlamydomonas* inner arm Tctex2b (BK004867). (d) Sequence alignment of the Tctex1-related proteins shown in c was performed using ClustalW. The secondary structure of *Chlamydomonas* Tctex1 determined by NMR spectroscopy (Wu *et al.*, 2001, 2005) is shown above the alignment. The strand-switched dimer interface involves the β 3 strand (white), which hydrogen bonds to the β 2 strand from the other monomer. Residues in mammalian Tctex1 that interact with the cytoplasmic dynein IC (Mok *et al.*, 2001) are indicated by *.

from our structural studies (native molecular weight [mol. wt.] = 111,300 Da; calculated [calc.] monomer mol. wt. = 55,274 Da). The MBP-LC2 protein is monomeric as observed previously (native mol. wt. = 56,310 Da; calc. monomer mol. wt. = 58,351 Da) (DiBella *et al.*, 2001), whereas the MBP-LC9 fusion protein behaved as a mixture of both monomer and dimer under the same conditions (native mol. wt. = 72,150/126,200 Da; calc. monomer mol. wt. = 55,450 Da) (Figure 2a).

LC9 is Specifically Associated with the Outer Dynein Arm

To generate an antibody that specifically recognizes LC9, the MBP-LC9(1-49) protein was used as the immunogen and the resulting CT231 rabbit antiserum was blot-purified against full-length recombinant LC9 following digestion of the fu-

sion protein with factor Xa. To initially test the specificity of the CT231 antibody, *Chlamydomonas* Tctex1 and LC9 MBP fusion proteins were digested with factor Xa and electrophoresed to separate the LC moieties (Figure 2b). Immunoblotting revealed that LC9 was detected by CT231 but that no signal was obtained for the inner arm Tctex1 homologue with which it shares 40% sequence identity. The antibody was also tested against all recombinant outer arm dynein LCs and again was found to react solely with LC9 (Figure 2c). When used to probe axoneme samples, the CT231 antibody detected a single band of M_r ~13,000 corresponding to the predicted size of LC9 (Figure 2d).

To define the flagellar location of LC9, isolated flagella were first fractionated (Figure 3a) by detergent extraction to remove the membrane and matrix components. Nearly

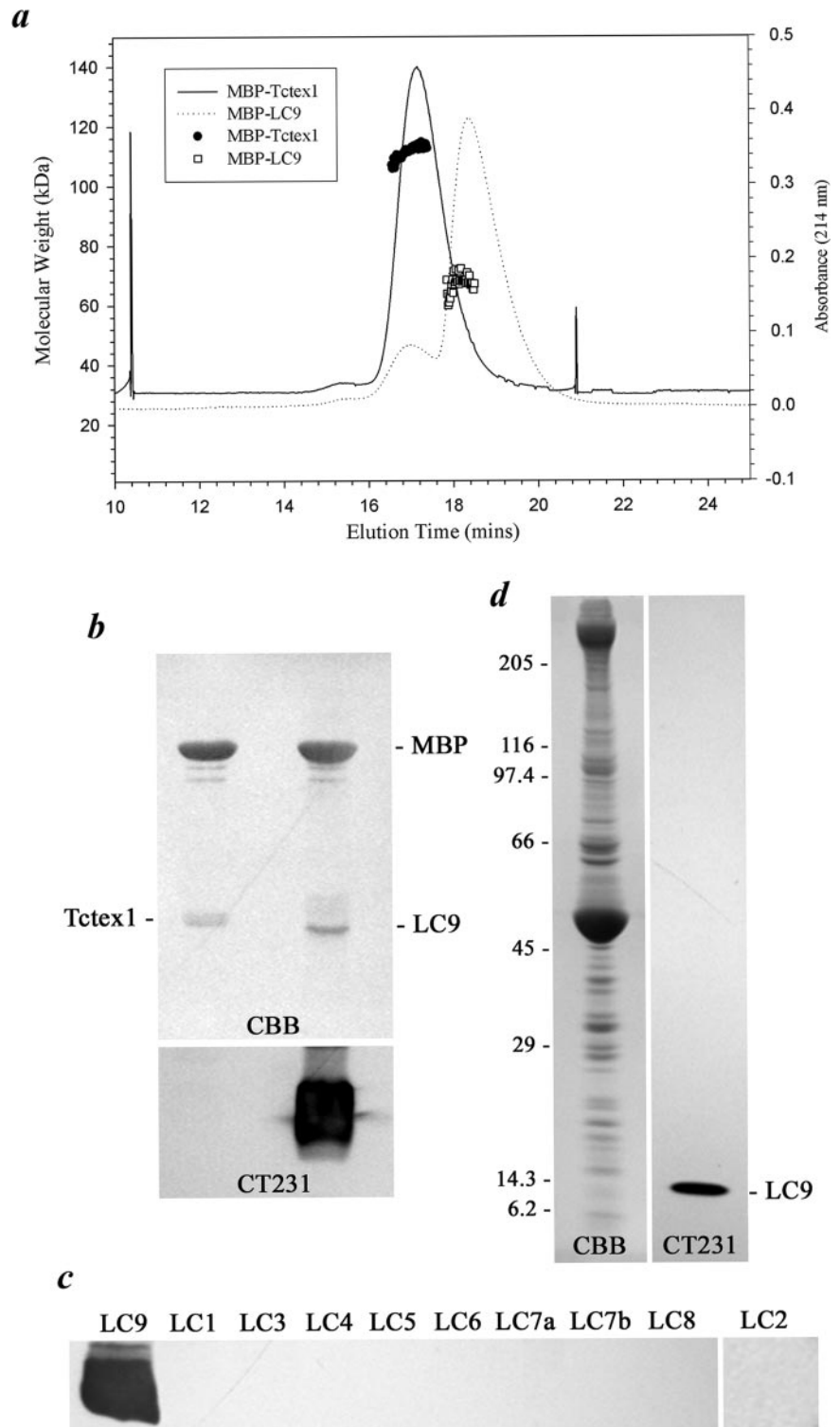


Figure 2. Oligomeric state of LC9 and specificity of the CT231 antibody. (a) The oligomeric state of fusion proteins containing LC9 and *Chlamydomonas* inner arm Tctex1 attached to MBP was determined by multi-angle laser light scattering. The absorbance at 214 nm of the gel filtration column elution profile for MBP-Tctex1 (solid line) and MBP-LC9 (dotted line) is shown. Individual molecular weight determinations are indicated by ● (MBP-Tctex1) and □ (MBP-LC9), respectively. MBP-Tctex1 is dimeric, whereas MBP-LC9 is mostly monomeric under the same solution conditions; a small peak of MBP-LC9 dimer is evident coeluting with MBP-Tctex1. (b) The Tctex1 and LC9 fusion proteins were digested with factor Xa, electrophoresed in a 15% acrylamide gel and either stained with Coomassie blue (top) or blotted to nitrocellulose and probed with CT231 (bottom). The antibody recognizes LC9 but not Tctex1 with which it shares 40% sequence identity. (c) Recombinant outer arm dynein LCs were probed with CT231 raised against LC9. No other outer arm LC component is recognized by this antibody. (d) Approximately 120 μ g of wild-type axonemes were electrophoresed in a 5–15% acrylamide gradient gel and stained with Coomassie blue (left) or probed with CT231 (right). Only a single axonemal protein of the appropriate M_r is recognized.

all LC9 protein was associated with the resulting demembrated axonemes. Treatment with 0.6 M NaCl solubilized ~60–70% of LC9. Subsequent fractionation of the high salt extract by sucrose density gradient centrifugation in the presence of Mg^{2+} and at low hydrostatic pressure revealed that LC9 copurified with the intact outer dynein arm (Figure 3b, fractions 5–7). To further assess this observation, axonemes were prepared from a

variety of mutants lacking specific axonemal structures including inner and outer arms, radial spokes and the central pair microtubule complex. LC9 was greatly reduced or missing only in those strains (*oda3*, *oda9*, and *pf28pf30*) that fail to assemble outer dynein arms (Figure 3c); the *oda15* strain assembles ~30% of outer arms (Pazour and Witman, 2000; DiBella *et al.*, 2004a) and therefore retains considerable amounts of LC9.

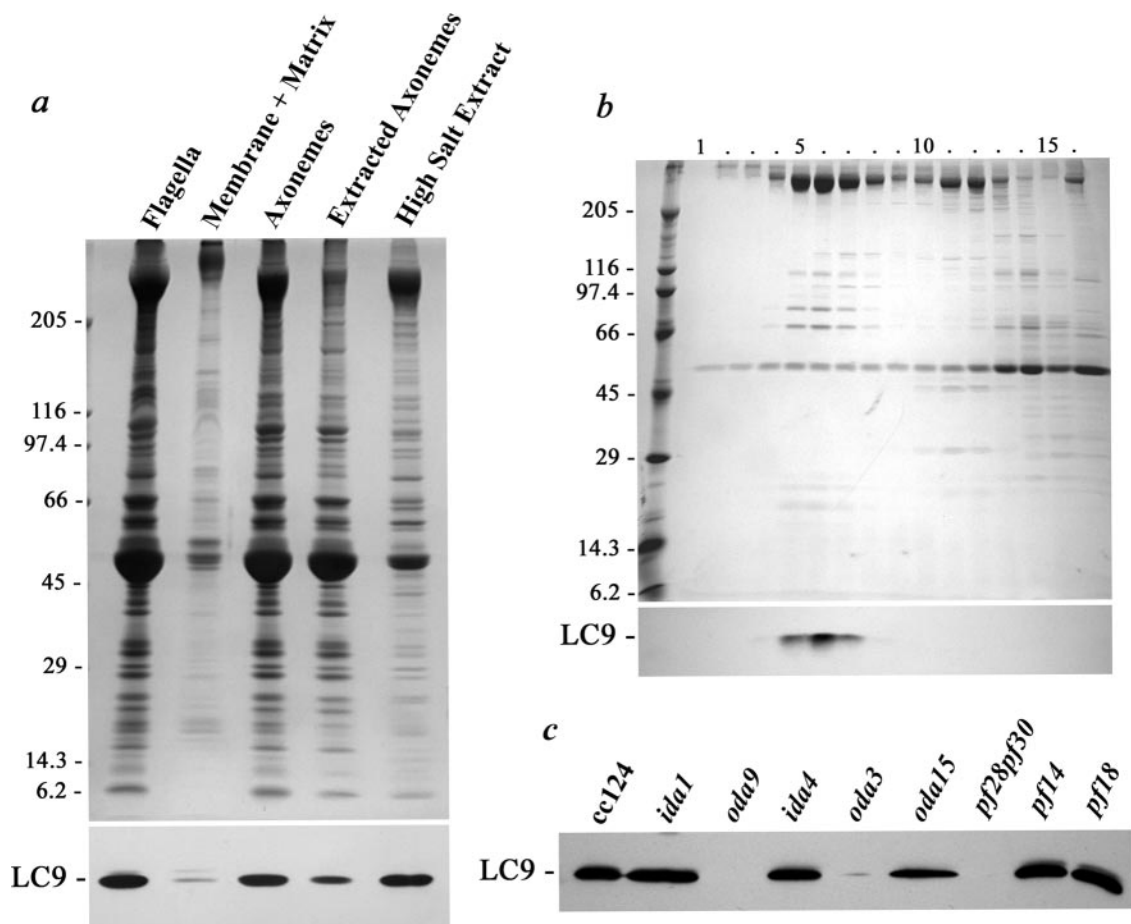


Figure 3. LC9 is a component of the outer dynein arm. (a) Approximately 150 μg of flagella were treated with detergent to yield the microtubular axoneme and solubilize the membrane and flagellar matrix components. Subsequently, the axonemes were extracted with 0.6 M NaCl to remove proteins associated via ionic interactions. Equivalent amounts of each sample were separated in a 5–15% acrylamide gradient gel and stained with Coomassie blue (top) or blotted and probed with CT231 (bottom). LC9 is specifically associated with the axonemes and most is solubilized by high salt treatment. The M_r markers are indicated at left. (b) The 0.6 M NaCl extract was sedimented through a 5–20% sucrose density gradient in the presence of Mg^{2+} , and equivalent volumes of each fraction were electrophoresed in a 5–15% acrylamide gradient gel (top, Coomassie blue; bottom, CT231 blot); the outer dynein arm sediments at ~ 23 S in fractions 5–7, as does LC9. The M_r markers are indicated at left. (c) Axonemes from wild-type *Chlamydomonas* (strain cc124) and from mutants lacking inner arm I1 (*ida1*), a subset of inner arms I2/3 (*ida4*), the outer arm (*oda9*), the outer arm and docking complex (*oda3*), both outer arms and inner arm I1 (*pf28pf30*), radial spokes (*pf14*), and central pair microtubule complex (*pf18*) were electrophoresed and probed with CT231. Also included is the strain *oda15*, which lacks LC7a and only assembles $\sim 30\%$ of outer arms (DiBella *et al.*, 2004a). The LC9 protein is specifically missing only in those strains that do not assemble outer dynein arms.

LC9 Interacts Directly with IC1 and IC2

To gain insight into the outer arm proteins with which LC9 associates, we used chemical cross-linking with the zero-length carbodiimide reagent EDC. Cross-links generated with EDC contain no cross-linker components in the final product because the carbodiimide is converted to a urea derivative, and thus two proteins must be in direct contact to be covalently joined. Treatment of purified outer arm dynein with 20 mM EDC generated two prominent bands (M_r $\sim 80,000$ and $90,000$) containing LC9 (Figure 4, left); these same bands were observed after EDC treatment of isolated axonemes (our unpublished data). Previously, we observed that both IC1 and IC2 yielded products of M_r 82,000 and 91,000, respectively, after EDC treatment (King *et al.*, 1991; DiBella *et al.*, 2004a), and the M_r of these LC9-containing products suggested that they may derive from attachment of LC9 to both IC1 (76,623 Da) and IC2 (63,519 Da). To test whether these bands indeed represent LC9-IC complexes,

blots of dynein samples treated with 20 mM EDC were cut lengthwise and one-half probed with CT231 and the other with either 1878A (vs. IC1; King *et al.*, 1986) or 1869A (vs. IC2; King *et al.*, 1985) (Figure 4, middle and right). After reassembly of the blots, we found that the lower and upper LC9-containing bands precisely comigrated with bands detected by 1869A and 1878A, respectively. These results indicate that LC9 is in direct contact with both ICs within the purified dynein particle and the axoneme.

Previously, a Tctex1-binding motif (K/R)(K/R)XX(K/R) has been identified in the mammalian cytoplasmic dynein IC (Mok *et al.*, 2001). In addition, a second binding region VS(K/H)(T/S)X(V/T)(T/S)(N/Q)V has been proposed to exist in certain Tctex1-associated proteins (Sugai *et al.*, 2003), although this motif is either truncated or does not occur in some known binding partners. Examination of the *Chlamydomonas* IC1 and IC2 sequences identified perfect copies of the Lys/Arg-rich motif in the N-terminal regions of both

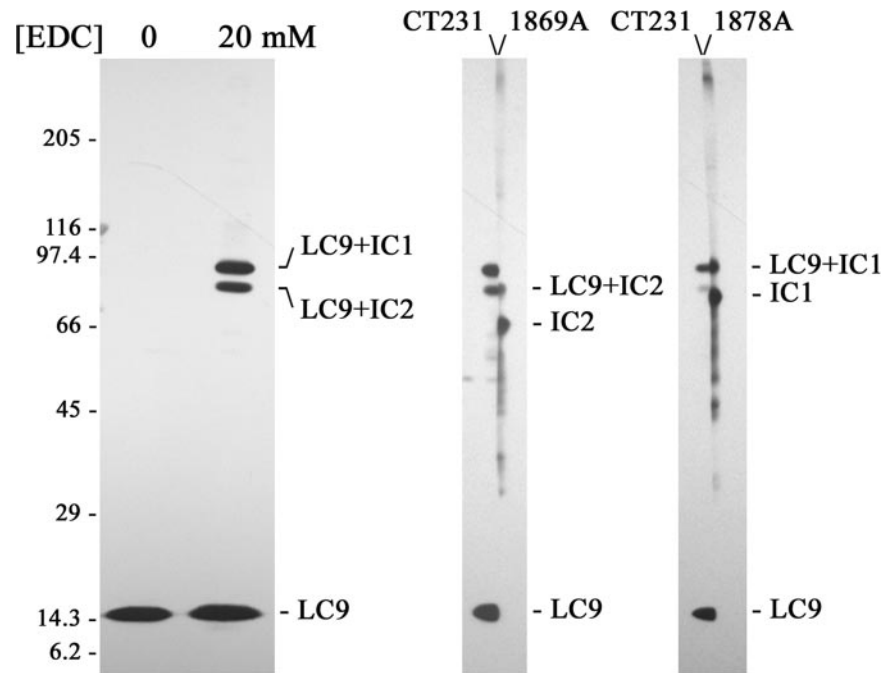


Figure 4. LC9 interacts with both outer arm dynein intermediate chains. Purified outer arm dynein from the *ida1* strain that lacks inner arm II was treated with 0 or 20 mM EDC, electrophoresed, and probed with the CT231 antibody (left). In the absence of EDC, only the LC9 band was observed. However, after EDC treatment two additional prominent bands of $M_r \sim 80,000$ – $90,000$ were obtained. In the center and right panels, single lanes of 20 mM EDC-treated dynein were cut lengthwise, and half were probed for LC9 and the other half for either IC2 (monoclonal antibody [mAb] 1869A) or IC1 (mAb 1878A). Reassembly of the blots revealed that the two LC9 cross-linked products also contain IC2 ($M_r \sim 80,000$ band) and IC1 ($M_r \sim 90,000$ band).

proteins ($_{18}\text{KKTRK}_{22}$ in IC1 and $_{98}\text{KKVEK}_{102}$ in IC2) that may represent LC9 interaction sites. No complete correlate of the second motif is discernible in either IC, although the IC2 region (residues 31–54) altered in *oda6-r88* (see below) does exhibit some weak similarity to the C-terminal part of this motif.

Incomplete Assembly of the IC/LC Complex in an *oda6* Pseudorevertant

Strains defective at the *oda6* locus that encodes IC2 are unable to assemble outer dynein arms (Kamiya, 1988; Mitchell and Kang, 1991). The original *oda6-95* mutation removes a single base pair and results in a frame-shift at residue 54 that inserts 13 novel residues before a premature stop codon (Figure 5a). Because the *oda6-95* strain does not assemble outer arms, it consequently exhibits a reduced flagellar beat frequency of ~ 20 – 25 Hz rather than 50 – 60 Hz observed for wild-type *Chlamydomonas*. Mitchell and Kang (1993) isolated two classes of *oda6* intragenic pseudorevertants exemplified by strains *oda6-r75* and *oda6-r88*. Both strains contain additional frame-shifts that, when combined with the original *oda6-95* alteration, restore the IC2 reading frame (Figure 5a). However, although both pseudorevertant strains assemble outer dynein arms, only the *oda6-r75* mutation also results in restoration of wild-type flagellar beat frequency; in *oda6-r88* beat frequency increases to only 30 – 35 Hz (Figure 5a) (Mitchell and Kang, 1993). Because the putative LC9 binding site in IC2 is located near the region disrupted by the *oda6-95* mutation, we reasoned that the different phenotypes of the pseudorevertants might reflect differential LC assembly. To test this possibility, axonemes were prepared from wild-type, *oda6*, *oda6-r75*, and *oda6-r88* strains (Figure 5b) and probed with antibodies against all outer arm dynein LCs. Axonemes from *oda6* lack most outer arm LCs with the exception of LC7a, LC7b, and LC8, which are present because they are also components of other axonemal structures (King, 2002; DiBella *et al.*, 2004a). Both pseudorevertant classes contain LC1, LC3, LC4, LC5, LC7a, LC7b, and LC8 (the data for LC1 and LC3 are shown in Figure 5c). How-

ever, although LC2, LC6, and LC9 are present in *oda6-r75*, they are completely absent from both intact flagella (our unpublished data) and demembrated axonemes (Figure 5c) prepared from *oda6-r88*.

LC6 Is Not Required for Assembly of LC2 and LC9

No mutants defective for LC9 have been identified so far. However, the *oda13* strain is an LC6 insertional null allele that assembles outer dynein arms and exhibits a minor swimming defect (Pazour and Witman, 2000). Therefore, to test whether the failure of LC9 assembly in *oda6-r88* is a consequence of the lack of LC6 or whether the *oda6-r88* alteration affects LC9 association more directly, we prepared axonemes from *oda13* and examined the LC content (Figure 6, a and b). We observed that the *oda13* mutant completely lacks LC6 as reported previously (Pazour and Witman, 2000), but it contains wild-type amounts of LC2, LC3, and LC9. Thus, LC9 assembly onto the ICs is not contingent on LC6. Although *oda13* exhibits a clear alteration in flagellar beat frequency compared with the parental strain (the peak in the FFT spectrum becomes much broader although the maximal value remains close to the wild-type level; Figure 6c, bottom), the *oda6-r88* defect is much more severe (Figure 5a). This suggests that lack of LC6 alone is not responsible for the *oda6-r88* phenotype.

The *oda6-r88* Mutation Is Not an Extragenic Suppressor of *oda12-1*

The absence of LC2 in outer arms of the *oda6-r88* strain is intriguing, because we observed previously that an LC2-null mutant (*oda12-1*) does not assemble functional outer arms and that this phenotype may be rescued by transformation with a genomic fragment containing only the wild-type LC2 gene (Pazour *et al.*, 1999). To test whether the *oda6-r88* mutation might act as an extragenic suppressor of *oda12-1* and bypass the requirement for LC2 in outer arm assembly, we constructed an *oda6-r88 oda12-1* double mutant. The genotype of the tetrad progeny from the cross was determined using the PCR to assay for the presence of the LC2 gene and

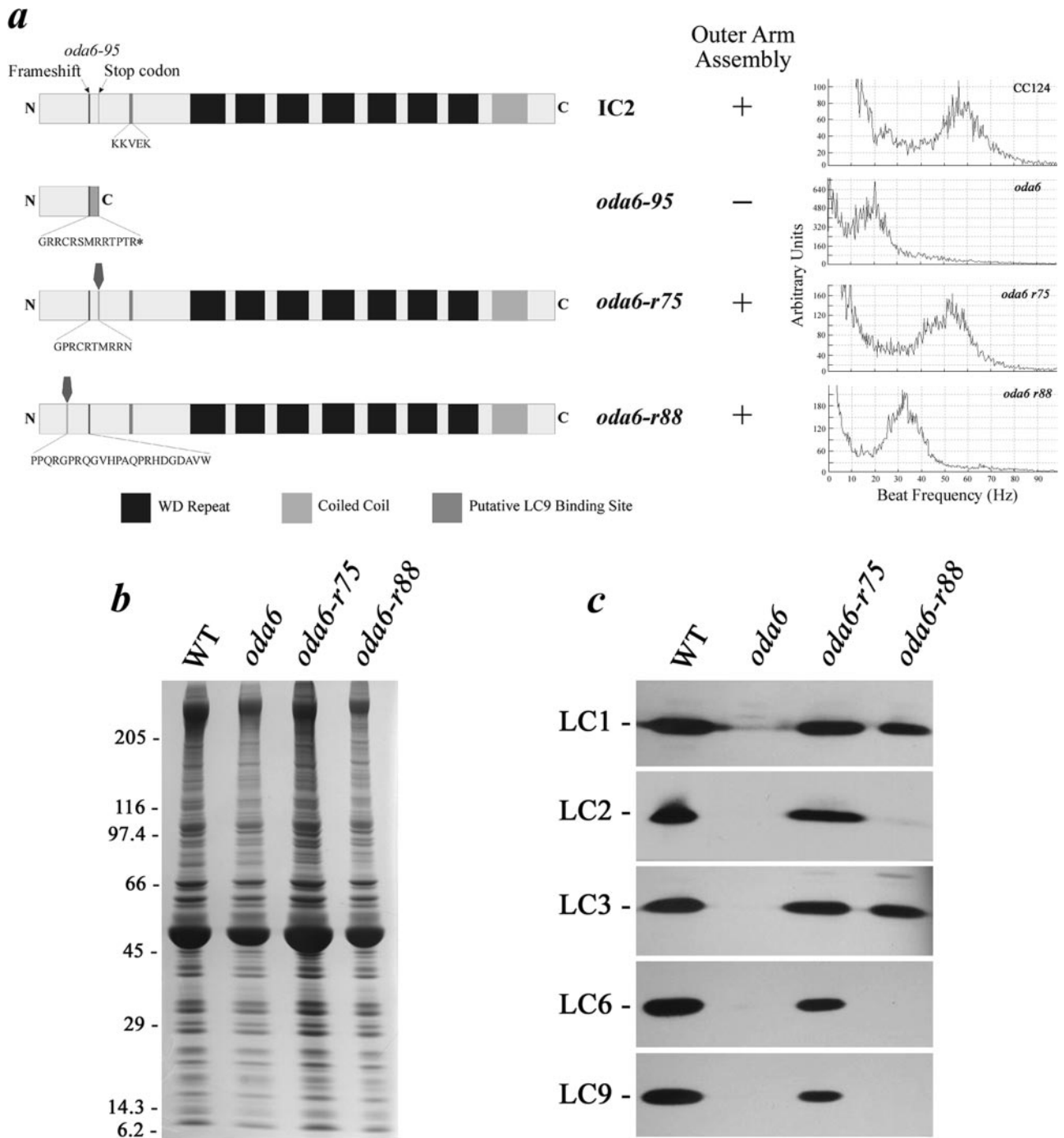


Figure 5. An *oda6* pseudorevertant lacks LC2, LC6, and LC9. (a) Diagram of the IC2 protein indicating the location of WD-repeats, the C-terminal coiled coil domain, a putative LC9 interaction site, and the original *oda6-95* frame-shift that results in a premature stop codon is shown at top. Maps of IC2 from *oda6-95* and the two pseudorevertants (*oda6-r75* and *oda6-r88*) are shown below the wild type (data are from Mitchell and Kang, 1993). In these diagrams, residues that are altered from the original and their location are indicated. Whether the strain is capable of outer arm dynein assembly and flagellar beat frequency analysis is indicated at right. (b) Axonemes from wild-type, *oda6*, *oda6-r75*, and *oda6-r88* strains were electrophoresed in a 5–15% acrylamide gradient gel and stained with Coomassie blue. The M_r markers are at left. (c) Identical samples to those shown in b were blotted and probed with antibodies R5932, R5391, R4930, R4928, and CT231 to detect LC1, LC2, LC3, LC6, and LC9, respectively. All these outer arm proteins are missing in *oda6* but are restored in *oda6-r75*. In contrast, LC2, LC6, and LC9 are not present in axonemes from the *oda6-r88* pseudorevertant, which assembles outer arms but does not rescue the beat frequency defect.

to assess whether the IC2 gene contained the novel *Bam*HI restriction site introduced by the *oda6-r88* frame-shift mutation (Figure 7a). FFT analysis of the tetrad progeny revealed

that the *oda6-r88 oda12-1* strain had the characteristic beat frequency of *oda* mutants (~20 Hz) and was clearly distinguishable from the *oda6-r88* revertant (~30–35 Hz) (Figure

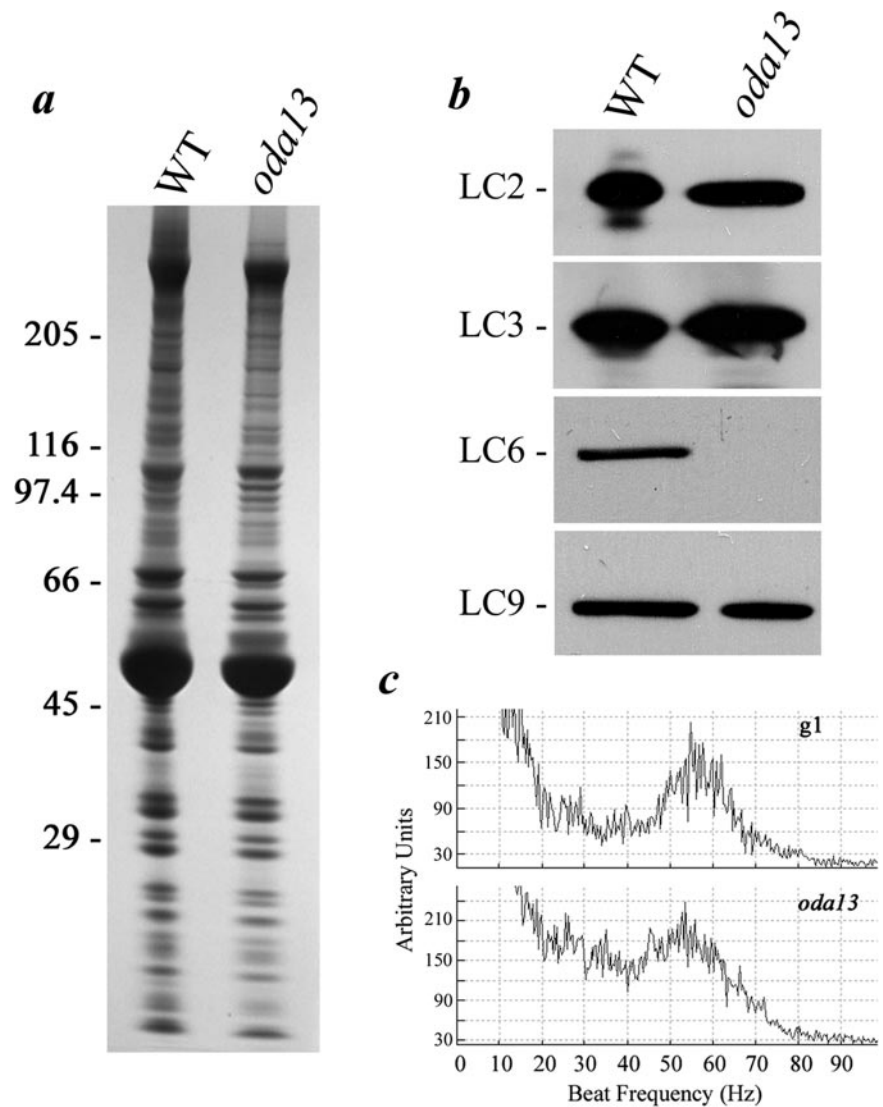


Figure 6. LC9 assembly is not dependent on LC6. (a) Flagellar axonemes were isolated from wild-type and the *oda13* strain, and ~100 μ g of each was electrophoresed in a 5–15% acrylamide gradient gel and stained with Coomassie blue. The M_r markers are at left. (b) Similar samples were blotted to nitrocellulose and probed with antibodies R5391, R4930, R4928, and CT231 to detect LC2, LC3, LC6, and LC9, respectively (right). Only LC6 is missing in the *oda13* mutant axonemes, indicating that this protein is not required for the assembly of either LC2 or LC9. (c) FFT analysis of the *oda13* mutant and the parental wild-type strain (g1) reveals only a subtle reduction in the peak flagellar beat frequency for the mutant.

7b). Thus, *oda6-r88* does not functionally suppress the *oda12-1* motility phenotype.

The oda6-r88 oda12-1 Double Mutant Exhibits a Synthetic Assembly Defect

Previously, we demonstrated that no morphologically distinguishable outer arms were identified in *oda12-1* axonemes by thin-section electron microscopy (Pazour *et al.*, 1999). In an attempt to further define the differences between *oda6-r88* and *oda12-1*, which both completely lack LC2, we tested *oda12-1* axonemes for the presence of several other outer arm components (Figure 7, c and d). Immunoblot analysis revealed that this mutant retains considerable amounts of several outer arm-specific proteins including the γ HC, IC1, IC2, and LC1; a reduced amount of LC6 and very minor amounts of LC9 were also detectable. We also examined axonemes from the *oda12-2* allele, which lacks only the 3' end of the LC2 gene and found that they incorporate similar levels of LC9 to those observed in *oda12-1*. Rescue of the *oda12-1* strain with a 3.1-kb *HindIII* fragment (pBD14) containing the LC2 gene (Pazour *et al.*, 1999) restored LC9 assembly to wild-type levels (our unpublished data).

Assembly of the outer arm docking complex was unaffected by either the *oda6-r88* or *oda12-1* mutations. However, we did observe a synthetic interaction between these alleles as the double mutant contained essentially undetectable amounts of the γ HC and greatly reduced quantities of both IC1 and IC2 (Figure 7d); as expected, essentially no outer arms were detected by EM (Figure 7e). Intriguingly however, LC1 was assembled in this strain even in the absence of its target heavy chain (HC), suggesting that this LC can be incorporated independently of the remainder of the arm.

DISCUSSION

In this report, we have identified an additional member (LC9) of the Tctex1 family as an integral component of *Chlamydomonas* outer arm dynein. Mutant analysis has revealed that LC2, LC6, and LC9 play an important and interconnected role in generating a functional dynein particle. Furthermore, we have made several intriguing observations concerning the interplay of light chain incorporation and outer arm dynein assembly that yield insight into the mechanisms by which these motor enzymes are constructed.

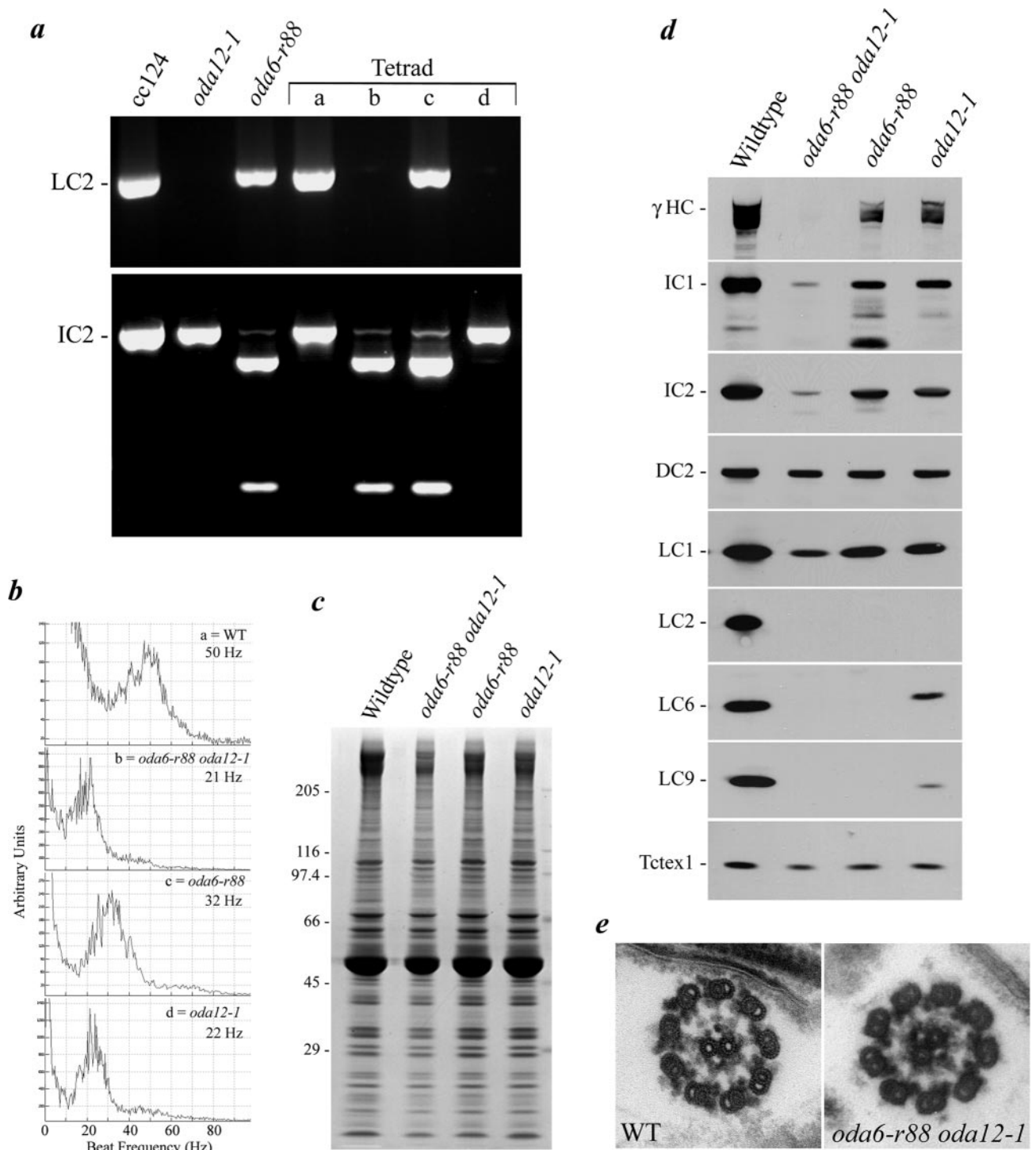


Figure 7. The *oda6-r88* allele is not an extragenic suppressor of *oda12-1*. (a) PCR analysis of genomic DNA from the progeny of an *oda6-r88* × *oda12-1* cross and both wild-type and parental mutant strains. Top, a 585-base pair segment of the LC2 gene that is completely missing in the *oda12-1* null allele. Bottom, a *Bam*HI digest of a 495-base pair region of the IC2 gene that includes the novel restriction site introduced by the *oda6-r88* reversion. In this tetrad type tetrad, progeny "b" represents the double mutant. (b) Flagellar beat frequency analysis of the four tetrad products identified in a. The double mutant exhibits a beat frequency similar to *oda12-1* and is clearly reduced from that observed for *oda6-r88*. (c) Axoneme samples from each tetrad product were electrophoresed in a 5–15% acrylamide gradient gel and stained with Coomassie blue. The M_r markers are indicated at left. (d) Samples identical to those shown in c were blotted to nitrocellulose and probed to detect various components of the outer dynein arm. The Tctex1 protein from inner arm I1 was used as a loading control. (e) Cross-section electron micrographs of wild-type and *oda6-r88 oda12-1* mutant axonemes. No outer arms are detectable in the double mutant.

Distinct Members of the Tctex1 Family are Present in Inner and Outer Arm Dyneins

Genomic analysis revealed that *Chlamydomonas* contains two members of the Tctex1 family of dynein LCs. Previously, we found that *Chlamydomonas* Tctex1 is a component of inner arm I1 (Harrison *et al.*, 1998) and demonstrate here that the second member of this family (LC9) is also an integral axonemal component. Sequence analysis indicates that the LC9 protein is a rather divergent member of this LC class as is the alternate mammalian cytoplasmic dynein LC (currently termed rp3) (King *et al.*, 1998). LC9 copurifies with outer arm dynein in sucrose density gradients and is missing only in those mutant strains that are devoid of outer arms. Thus, the presence of a Tctex1-like protein seems to be a general feature of the design of dyneins containing two or more HCs [see Sakato and King (2004) for further discussion].

Lack of LC2, LC6, and LC9 Correlates with Defective Outer Arm Motor Function

Zero-length cross-linking with EDC revealed that LC9 interacts directly with both IC1 and IC2. The *oda6-95* frame-shift mutant cannot synthesize full-length IC2 and consequently fails to assemble outer arms (Kamiya, 1988; Mitchell and Kang, 1993). Two classes of intragenic pseudorevertants have been identified that insert additional frame-shifts and allow for dynein assembly (Mitchell and Kang, 1993). Importantly, the beat frequency of *oda6-r75* reverts almost to wild-type (>50 Hz), whereas that of *oda6-r88* remains relatively low (~30–35 Hz), although it is still greater than mutants lacking all outer arms (e.g., *oda1* ~20–25 Hz). Based on these data, Mitchell and Kang (1993) suggested that the region defective in IC2 from *oda6-r88* may in some way regulate motor activity or be required for assembly of a LC. We have now found that *oda6-r88* outer arm dynein completely lacks three LC components, which likely all interact independently with this region (discussed below). Tctex1 is a structural homologue of the highly conserved LC8 protein (Mok *et al.*, 2001; Wu *et al.*, 2001, 2005; Williams *et al.*, 2005), and structural studies of LC8 interactions with its target peptides (Liang *et al.*, 1999) indicate that each LC-IC interface involves at least seven residues. Because the IC segment defective in *oda6-r88* is only 23 residues in length, the independent binding of these three LCs would require this entire region. Thus, it becomes highly probable that it is the LC deficiency in this strain, rather than the IC alteration per se, that results in nonfunctional dynein.

Analysis of the *oda13* null allele indicates that lack of LC6 has only a minor effect on flagellar beat frequency and does not result in a severe swimming defect as seen in *oda6-r88*. This suggests that it is defective assembly of LC2 and/or LC9 in *oda6-r88* that causes outer arm dysfunction. Previously, we demonstrated that strains lacking LC2 did not assemble outer arms that could be identified by electron microscopy nor was any IC1 observed (Pazour *et al.*, 1999). We have now found that *oda12-1* does contain some outer arm proteins, including IC1. The reason for this apparent discrepancy remains uncertain, although we have observed that both ICs are much more sensitive to proteolysis in strains lacking LC2 (*oda12-1* and *oda6-r88*), presumably because the N-terminal region is more exposed. Even so, the outer arm dyneins assembled in *oda12-1* are completely nonfunctional as flagellar beat frequency of this strain is indistinguishable from those mutants (e.g., *oda1*) that completely lack this motor. These observations present an intriguing conundrum as they indicate that outer arms assembled in an LC2-null background are not functionally equivalent to assembled arms that lack this component.

A number of possibilities might account for these observations. First, these LCs may actually assemble in *oda6-r88* flagella but be so weakly bound to the dynein arm that they dissociate after demembration. This scenario does not seem to be correct because none of these LCs could be detected in intact flagella from this strain. Second, the *oda12* insertional alleles may be missing a second gene close to LC2 that is actually responsible for the assembly/functional defects. Our examination of the *Chlamydomonas* genome revealed that another apparent dynein LC related to LC8 is encoded close to the *ODA12* locus. This gene is missing in the *oda12-1* null allele but is present in *oda12-2*, which lacks only the 3' end of the LC2 gene. Furthermore, this LC8-related gene is not restored in *oda12-1* strains transformed with a wild-type LC2 gene construct (pBD14), which rescues both outer arm assembly and the motility defect (Patel-King, Pazour, and King, unpublished observations) (Pazour *et al.*, 1999). Thus, although an additional potential dynein gene is missing in *oda12-1*, it is not responsible for the differential outer arm function observed in the *oda6-r88* and *oda12-1* strains.

A third possibility is that the *oda6-r88* mutation acts as an extragenic suppressor of *oda12-1* and removes the requirement for LC2 in outer arm assembly. If correct, the *oda6-r88* *oda12-1* double mutant should have had a flagellar beat frequency similar to *oda6-r88* rather than *oda12-1*; however, we observed the converse. Moreover, we found that the double mutant had a synthetic assembly defect in that several outer arm-specific proteins including the γ HC and both ICs were dramatically reduced compared with either parent. One clear compositional difference between *oda6-r88* and *oda12-1* is the presence of LC6 and a small amount of LC9 in axonemes of the latter strain. Thus, it is feasible that assembly of LC6 in the absence of LC2 results in defective motility, but that the absence of both proteins is less detrimental.

Finally, it is also possible that LC2 is not essential for dynein assembly/function per se, but rather that it interacts in the cytoplasm with another protein that is itself required for assembly or activation of a functional motor. If this second protein were unstable in the absence of LC2, then the *oda12* mutant would contain nonfunctional dyneins as we have observed. In contrast, although *oda6-r88* does not incorporate LC2 into the flagellum, that protein is likely still present in the cell body and thus could interact with and stabilize the hypothetical assembly/activating factor. This could allow for the production of at least some active dynein motors.

Implications for Intermediate Chain/Light Chain Complex Architecture

The IC/LC complex of the *Chlamydomonas* outer arm consists of two ICs and LC2, LC6, LC7a, LC7b, LC8, and LC9. We demonstrated previously using dimethylpimelimidate (DMP) cross-linking that LC2 and LC6 are within 9.2 Å of each other in situ and potentially interact directly (DiBella *et al.*, 2001). Analysis of the LC6-null mutant *oda13* revealed that both LC2 and LC9 assemble at wild-type levels in the absence of LC6. We also found in the *oda12-1* mutant that reduced amounts of LC6 and low levels of LC9 could assemble in the total absence of LC2, whereas in *oda6-r88* all three LCs were completely missing. We have obtained no evidence to suggest that either LC2 or LC6 associates directly with LC9, although lack of LC2 dramatically reduces the amount of LC9 that is incorporated. Thus, it seems most likely that each of these LCs interacts directly with one or both ICs and that disruption of the interface with IC2 is

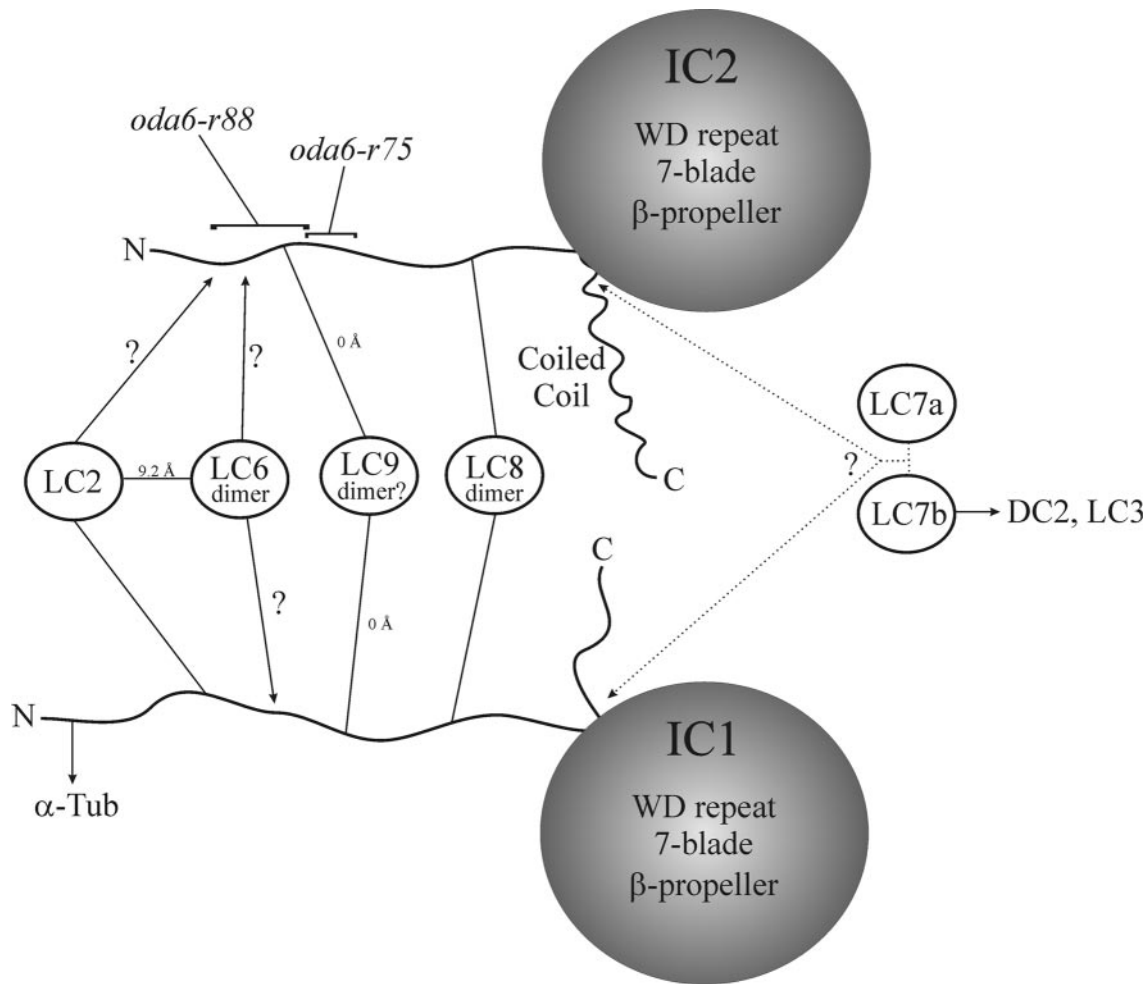


Figure 8. Model for IC/LC interactions within the outer dynein arm. A model for protein-protein associations within the IC/LC complex of outer arm dynein is shown. Both ICs consist of an N-terminal region (in IC1 this interacts directly with α -tubulin), seven WD-repeats that form a β -propeller, and a C-terminal segment (in IC2 this is predicted to form a coiled-coil). LC2, LC6, and LC9 likely interact with IC2 via the region defective in *oda6-r88*; the region altered in *oda6-r75* is not necessary for their assembly. Both LC2 (Mitchell and Rosenbaum, 1986) and LC9 (this study) have been shown to interact directly with IC1. Putative LC8 binding sites within both ICs are located relatively close to the WD-repeat region. The precise order of association sites on IC1 and within the *oda6-r88*-defective region of IC2 is speculative. Analysis of other dyneins has suggested that members of the LC7/Roadblock family interact with IC regions either N-terminal to the first WD-repeat (Susalka *et al.*, 2002) or C-terminal of the last repeat (Hendrickson *et al.*, 2004); due to the interlocking design of the WD-repeat β -propeller, these regions are spatially close together. Maximal distances (in angstroms) between components are based on DMP and EDC cross-linking (King *et al.*, 1991; DiBella *et al.*, 2001, 2004a).

sufficient to disallow LC assembly. A model depicting IC/LC interactions is shown in Figure 8.

Although the regions of IC1 and IC2 N-terminal of the WD-repeats are almost completely distinct, there are at least four short motifs that are apparently conserved between the two proteins. One of these occurs within the 23-residue region of IC2 that is altered in *oda6-r88* and contains both basic and acidic residues. Because we found that LC9 could be directly cross-linked to both ICs using EDC, this motif [(K/Q)(D/E)YIPxxP] represents a potential interaction site. Both axonemal ICs also contain a KKxxK motif suggested to be involved in Tctex1 binding to the cytoplasmic dynein IC (Mok *et al.*, 2001). In IC2, this motif is located C-terminal of the regions defective in *oda6-r88* and *oda6-r75*, whereas in IC1 it is immediately N-terminal to the (K/Q)(D/E)YIPxxP motif.

The IC location at which the LC7a and LC7b components (axonemal homologues of the roadblock cytoplasmic dynein

LCs) bind remains uncertain. Analysis of cytoplasmic dynein has suggested that an IC region immediately N-terminal of the first WD-repeat (or possibly including the repeat) is necessary (Susalka *et al.*, 2002), whereas study of the *bop5* mutant version of IC138 within *Chlamydomonas* inner arm I1 has implicated the seventh WD-repeat and/or the C-terminal region in the interaction of LC7b with that dynein (Hendrickson *et al.*, 2004). Due to the interlocking assembly of the WD-repeat β -propeller (the first β strand of repeat #1 hydrogen bonds to the last strand of repeat #7; see, e.g., Sondak *et al.*, 1996), these regions are likely in proximity at the tertiary structure level and may both contribute to LC7/roadblock LC binding (Hendrickson *et al.*, 2004).

Heavy Chain-independent Assembly of the Motor Domain-associated Light Chain

Previously, we demonstrated that the leucine-rich repeat protein LC1 associates directly with the motor domain of the

γ HC via a strong hydrophobic association (Benashski *et al.*, 1999; Wu *et al.*, 2000). Furthermore, we determined by EDC cross-linking that LC1 interacts directly with an additional as yet unknown axonemal protein (p45) that does not copurify with the outer dynein arm. The interaction between LC1 and p45 is ionic and is disrupted by the high salt treatment required to extract the outer arm from the axoneme (Benashski *et al.*, 1999; Wu *et al.*, 2000). Analysis of axonemes from the *oda6-r88 oda12-1* double mutant revealed that considerable amounts of LC1 assemble even in the complete absence of the γ HC. This implies an alternative, dynein HC-independent, assembly pathway for LC1, and further suggests that this protein mediates a direct linkage between the γ HC motor domain and other axonemal components.

In conclusion, identification of a Tctex1-like protein within *Chlamydomonas* outer arm dynein and analysis of mutants defective for components of the IC/LC complex, has revealed a functional contribution of LC binding to dynein motor activity and provided insight into the molecular architecture of this portion of the dynein particle.

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