The LC7 Light Chains of *Chlamydomonas* Flagellar Dyneins Interact with Components Required for Both Motor Assembly and Regulation

Linda M. DiBella,* Miho Sakato,* Ramila S. Patel-King,* Gregory J. Pazour,† and Stephen M. King*‡

*Departments of Biochemistry, and Molecular, Microbial, and Structural Biology, University of Connecticut Health Center, Farmington, CT 06030-3305; and †Program in Molecular Medicine, University of Massachusetts Medical School, Worcester, MA 01605

Members of the LC7/Roadblock family of light chains (LCs) have been found in both cytoplasmic and axonemal dyneins. LC7a was originally identified within *Chlamydomonas* outer arm dynein and associates with this motor's cargo-binding region. We describe here a novel member of this protein family, termed LC7b that is also present in the *Chlamydomonas* flagellum. Levels of LC7b are reduced ~20% in axonemes isolated from strains lacking inner arm I1 and are ~80% lower in the absence of the outer arms. When both dyneins are missing, LC7b levels are diminished to <10%. In *oda9* axonemal extracts that completely lack outer arms, LC7b copurifies with inner arm I1, whereas in *ida1* extracts that are devoid of I1 inner arms it associates with outer arm dynein. We also have observed that some LC7a is present in both isolated axonemes and purified 18S dynein from *oda1*, suggesting that it is also a component of both the outer arm and inner arm I1. Intriguingly, in axonemal extracts from the LC7a null mutant, *oda15*, which assembles ~30% of its outer arms, LC7b fails to copurify with either dynein, suggesting that it interacts with LC7a. Furthermore, both the outer arm γ heavy chain and DC2 from the outer arm docking complex completely dissociate after salt extraction from *oda15* axonemes. EDC cross-linking of purified dynein revealed that LC7b interacts with LC3, an outer dynein arm thioredoxin; DC2, an outer arm docking complex component; and also with the phosphoprotein IC138 from inner arm I1. These data suggest that LC7a stabilizes both the outer arms and inner arm I1 and that both LC7a and LC7b are involved in multiple intradynein interactions within both dyneins.

INTRODUCTION

Dyneins are large multisubunit complexes that function as microtubule-based molecular motors in eukaryotic cells (reviewed in (King, 2002; Vale, 2003). Ciliary and flagellar dyneins comprise the inner and outer arms and constitute a heterogeneous motor population that seems to have multiple force-generating roles. In these structures, the axonemal microtubules provide a scaffold for an extensive network of additional proteins that collectively regulate the waveform, beat frequency, and ultimately motility of these organelles. Therefore, defining the precise composition of these motor enzyme complexes and the mechanisms by which they are assembled and regulated is of major importance to understanding flagellar motility.

Overall, dynein molecular motors are built around two basic designs (for recent review, see Sakato and King, 2004). Cytoplasmic dynein and the outer arm and inner arm I1

axonemal dyneins contain two to three heavy chain motor units (~520 kDa each; HCs) that belong to the ancient AAA⁺ family of ATPases (Neuwald *et al.*, 1999; King, 2000). For example, *Chlamydomonas* outer arm dynein contains three distinct HCs (α, β, and γ) that exhibit different enzymatic and motor properties (for review, see DiBella and King, 2001). Dyneins also contain WD-repeat intermediate chains (ICs) that are associated with the N-terminal regions of the HCs and function in cargo and/or ATP-independent microtubule binding. Two general classes of light chains (LCs) are also part of the dynein complex. Several LC components so far found only in outer arm axonemal dynein possess putative regulatory regions (e.g., EF-hand and thioredoxin-like domains), associate directly with the HCs and likely influence motor function (Harrison *et al.*, 2002; Sakato and King, 2003). In addition, a leucine-rich repeat LC interacts directly with the γ outer arm dynein HC motor domain and also may play a regulatory role (Benashski *et al.*, 1999; Wu *et al.*, 2000).

Members of the second LC class associate with the ICs and are located within the cargo-binding region. LCs from this latter group belong to three distinct protein families and in cytoplasm, incorporation of discrete isoforms is thought to define specific motor subtypes that may recognize particular intracellular cargoes (DiBella *et al.*, 2001). Within flagella, the highly conserved LC8 protein associates with the outer dynein arms, inner arm I1, and the radial spokes, and also is involved in intraflagellar transport (King and Patel-King, 1995b; Harrison *et al.*, 1998; Pazour *et al.*, 1998; Yang *et al.*,

Abbreviations used: CBB, Coomassie Brilliant Blue; DC, docking complex; EDC, 1-ethyl-3-[3-dimethylaminopropyl]-carbodiimide; HC, heavy chain; IC, intermediate chain; LC, light chain; Robl, roadblock.

2001). LCs from the Tctex1/Tctex2 family were originally identified as putative distorters/sterility factors involved in the non-Mendelian transmission of murine *t*-haplotypes (Lader *et al.*, 1989; Huw *et al.*, 1995) and were subsequently found to be dynein LCs (King *et al.*, 1996; Patel-King *et al.*, 1997). In *Chlamydomonas*, LC2 (Tctex2a) is necessary for assembly of the outer arm motor unit onto the axoneme (Patel-King *et al.*, 1997; Pazour *et al.*, 1999), and Tctex2b is a component of inner arm I1 that contributes to the stability and motor function of that enzyme (DiBella *et al.*, 2004). Tctex1 is an integral component of inner arm I1 (Harrison *et al.*, 1998) and is also present in cytoplasmic dynein (King *et al.*, 1996) where it is involved in attachment of specific cargoes (Tai *et al.*, 1999).

The third LC family, LC7/Roadblock, was initially identified through molecular cloning of LC7 (henceforth termed LC7a) from the *Chlamydomonas* outer arm and isolation of *Drosophila* mutants defective in the *roadblock* gene (Bowman *et al.*, 1999). In flies, roadblock mutant larvae suffer from posterior, sluggish motility that eventually leads to complete paralysis. These mutants also exhibit mitotic defects, along with severe axonal loss and nerve degeneration as a result of cargo accumulation, which is consistent with defects in retrograde axonal cargo transport. Recent studies have identified two mammalian roadblock (Robl) isoforms (Robl1 and Robl2) and indicate that both LCs can bind to cytoplasmic dynein ICs (Susalka *et al.*, 2002) and form both homo- and heterodimers (Nikulina *et al.*, 2004). In addition, the levels of Robl LCs may be modulated in response to specific cellular states. For example, one member of this LC class expressed in rat visual cortex is down-regulated in response to light stimuli (Ye *et al.*, 2000). In *Chlamydomonas*, LC7a is an integral component of the outer arm and lack of this protein in the *oda15* null mutant results in arm assembly defects and aberrant flagellar motility (Pazour and Witman, 2000).

To further define the role of LC7/Roadblock LCs in dynein function, we have identified and analyzed an additional member of this family in *Chlamydomonas*. This LC, termed LC7b, shares 48% and 47% identity with the *Drosophila* Roadblock cytoplasmic dynein light chain and *Chlamydomonas* LC7a, respectively, and we demonstrate that LC7a and LC7b are *both* components of *Chlamydomonas* inner arm I1 and outer arm dynein. We find that association of LC7b is mediated, at least in part, by LC7a. Furthermore, zero-length cross-linking reveals that LC7b interacts directly with a component of the outer arm docking complex, the outer arm β heavy chain-associated thioredoxin, LC3, and with the IC138 IC that is required for phosphorylation-dependent control of inner arm I1 (Habermacher and Sale, 1996, 1997; Yang and Sale, 2000). These results provide the first evidence for how outer arm dynein is attached to the trimeric docking complex and suggest that members of this LC family play a role in both dynein-cargo attachment and motor regulation.

MATERIALS AND METHODS

Strains and Media

Wild-type (cc124) *Chlamydomonas reinhardtii* and the following mutant strains were used in this study: *oda1, oda9, ida1, ida4, pf14, pf18* (obtained from *Chlamydomonas* Genetics Center, Duke University), *oda15* (strain 3167.2; Pazour and Witman, 2000) and *pf28pf30* (Piperno *et al.*, 1990; WS4 isolate obtained from Dr. Winfield Sale, Emory University School of Medicine). All cells were grown in R (M medium plus 0.0075 M sodium acetate), or Tris-Acetate-Phosphate (TAP) media (Harris, 1989).

Purification of Axonemes and Dynein

Wild-type and mutant strains of *Chlamydomonas reinhardtii* were deflagellated with dibucaine by using standard methods (King *et al.*, 1986) and demembranated with 1% IGEPAL CA-630 (catalog no. I-3021; Sigma-Aldrich, St. Louis, MO; replaces Nonidet P-40). For dynein purification, isolated axonemes were first treated with 0.6 M potassium acetate and then extracted with 0.6 M NaCl (King *et al.*, 1986; Nakamura *et al.*, 1997). NaCl-extracted proteins were fractionated using either 5–20% sucrose density gradients or by anion exchange chromatography (see below). Samples were routinely electrophoresed in 5–15% polyacrylamide gradient gels and stained with Coomassie Brilliant Blue (CBB), or transferred to nitrocellulose for immunoblotting.

Anion Exchange Chromatography

To separate the different species of axonemal dynein, the 0.6 M NaCl axonemal extract was dialyzed against buffer A (20 mM Tris, pH 7.5, 60 mM KCl, 0.5 mM EDTA, 0.1 mM Tween 20, 1 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride), and applied to an anion exchange column (Mono Q HR5/5, Pfizer, New York, NY) using a Biologics chromatography work station (Bio-Rad, Hercules, CA). Proteins were eluted at a flow rate of 0.5 ml/min with a linear salt gradient of 0–50% buffer B (buffer A with 1 M KCl) and collected into 0.3-ml fractions. To initially identify fractions pertinent to this study, samples were electrophoresed in 8% polyacrylamide gels, before silver staining for protein detection (Merril *et al.*, 1981).

Molecular Analysis of LC7b

The entire coding region for *Chlamydomonas* LC7b was obtained by polymerase chain reaction (PCR) using wild-type *Chlamydomonas* first strand cDNA as the template. Both the forward primer 5′-GCGCTCTAGAATGTCGGATATC-GAGTC-3′ and reverse primer 3′-GCGCGAATTCTTATGTGGAGGC-CGCGTTGGG-5′ were designed based on the entire coding sequence derived from the *Chlamydomonas* expressed sequence tag AV387124 and incorporate an *Xba*I site at the 5′ end and an *Eco*RI site at the 3′ end, respectively. After restriction digestion, the gel-purified product was subcloned into pBluescript SK- (Stratagene, La Jolla, CA) across *Xba*I/*Eco*RI restriction sites. The sequenced clone was used to probe a Southern blot of *Chlamydomonas* genomic DNA, and a northern blot of *Chlamydomonas* RNA from nondeflagellated cells and from cells 30-min postdeflagellation that were actively regenerating flagella. The full-length cDNA also was used to isolate an ~20-kb genomic fragment containing the full-length *LC7B* gene from a λDashII (Stratagene) genomic DNA library previously generated from wild-type strain 1132D- (R. S.Patel-King and S. M.King, unpublished data).

Preparation of Recombinant Protein and Antibody

The full-length LC7b cDNA was subcloned into the pMAL-c2 vector (New England Biolabs, Beverly, MA) across the *Xmn*I and *Eco*RI restriction sites. This resulted in the LC7b protein fused to the C terminus of maltose binding protein (MBP) via a 19-residue linker containing a factor Xa cleavage site. The overexpressed fusion protein was purified by amylose affinity chromatography. The full-length fusion protein was used as the immunogen to generate rabbit antiserum CT116. Nitrocellulose membrane containing recombinant LC7b was used to affinity-purify the antibody (Olmsted, 1986). A MBP-LC7a fusion protein and the LC7a-specific antibody R7178 were described previously (Bowman *et al.*, 1999). Additional antibodies used in this study include 1869A (IC2) and 1878A (IC1) (King *et al.*, 1986), R4058 (LC8) (King and Patel-King, 1995b), R4930 (LC3) (Patel-King *et al.*, 1996), R5391 (LC2) (Patel-King *et al.*, 1997), R5932 (LC1) (Benashski *et al.*, 1999), αDC2 (DC2 kindly provided by Drs. Ritsu Kamiya, University of Tokyo, Tokyo, Japan; and Ken-ichi Wakabayashi, University of Connecticut Health Center, Farmington, CT) (Wakabayashi *et al.*, 2001), 12γB (γ HC) (King *et al.*, 1985), and αIC138 (Gri5-2; IC138) and αIC140 (IC140) (Yang and Sale, 1998) (both kindly provided by Dr. Winfield Sale, Emory University School of Medicine, Atlanta, GA).

Native Gel Electrophoresis of MBP-LC Fusion Proteins

To examine the oligomeric state of LC7a and LC7b in solution, the MBP-LC fusion proteins were electrophoresed in gels of different acrylamide concentrations (at pH 8.8). The retardation coefficient (K_R) was determined from the negative slope of 100 [log ($R_f \times 100$)]. The standard proteins used were jack bean urease (545-kDa hexamer; 272-kDa trimer), bovine serum albumin (132-kDa dimer; 66-kDa monomer), ovalbumin (45 kDa), bovine carbonic anhydrase (29 kDa), and α-lactalbumin (14.2 kDa). A plot of log K_R vs. log M_r for these proteins was used as a standard curve from which the native molecular masses of the MBP fusion proteins could be determined.

Chemical Cross-linking of Purified Dynein with the Zero-Length Cross-Linker 1-Ethyl-3-[3-dimethylaminopropyl]-carbodiimide (EDC)

To investigate protein–protein interactions involving LC7b, purified dynein was treated with 0–20 mM EDC dissolved in 100 mM HEPES, pH 7.4. Samples were incubated at room temperature for 60 min, and the reactions were terminated by addition of β-mercaptoethanol in HMEK (30 mM HEPES,
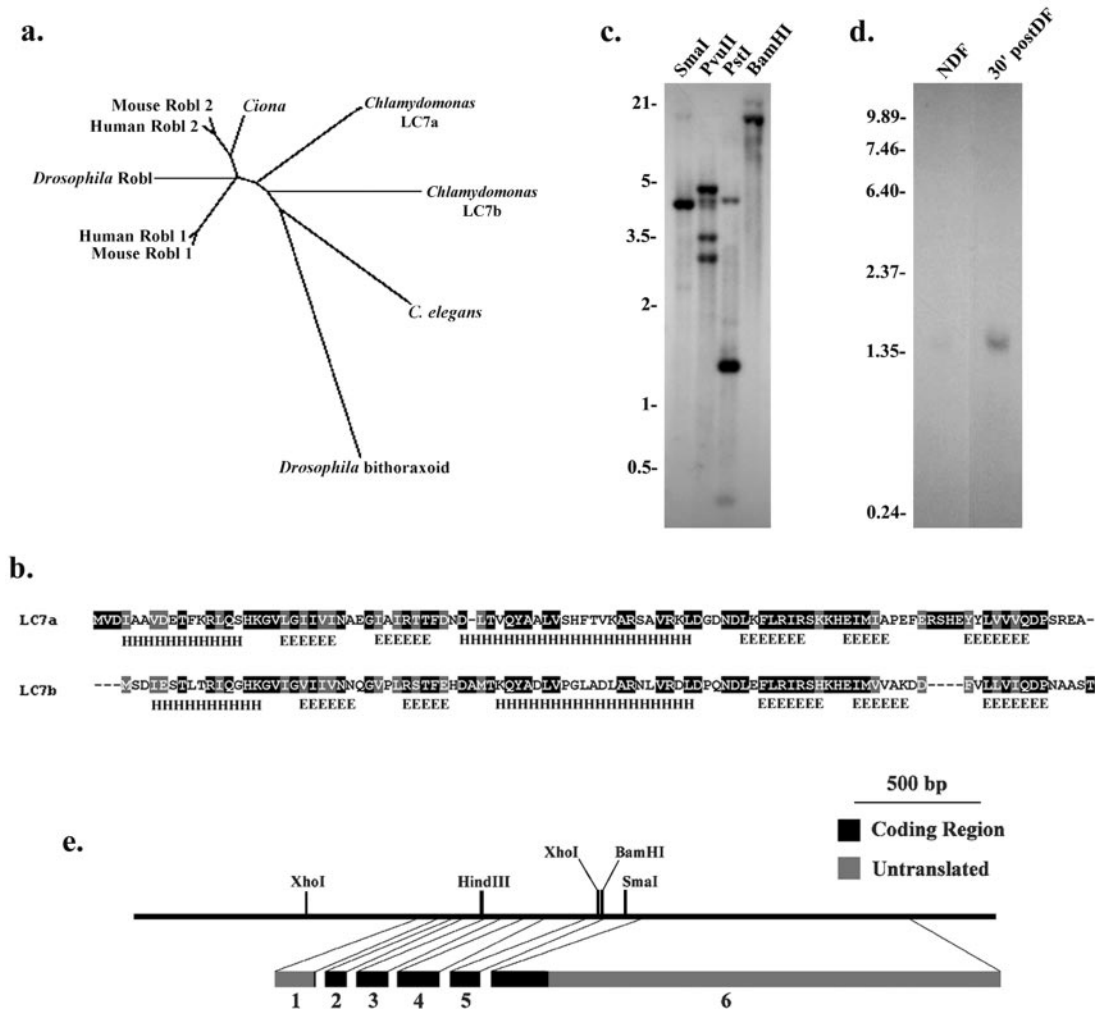
Figure 1. Molecular cloning and phylogenetic analysis of LC7b. (a) Phylogenetic analysis of the LC7/Roadlock family of LCs. Distances were calculated using ClustalW. *Chlamydomonas* LC7b (GenBank accession no. AV387124) shares 57% identity with the *Drosophila* cytoplasmic dynein LC roadblock (NP523771). The family members shown include two *Chlamydomonas* flagellar LCs, LC7a (AF140239), and LC7b (AV387124), *Drosophila* Robl (NP523771), human Robl1 (BC002481), and Robl2 (NM130897), mouse Robl1 (NM025947) and Robl2 (NM029297), *Ciona* Ci-rbDLC (AB074929), and *Caeronhabditis elegans* (NM063542). *Drosophila* bithoraxoid (NM169732) also has been identified as a distantly related member of this family. (b) The secondary structures of LC7a and LC7b were predicted using PredictProtein. E, extended sheet; H, helix. The alignment of LC7a and LC7b was generated with ClustalW by using default parameters and shaded using BOXSHADE. Identical residues are indicated in black; similar residues in gray. The two *Chlamydomonas* proteins share 47% sequence identity. (c) Southern blot of *Chlamydomonas* wild-type genomic DNA digested with either *Sma*I, *Pvu*II, *Pst*I, or *Bam*HI. A probe generated from the full-length *LC7B* coding region detected single bands in *Sma*I- and *Bam*HI-digested DNA, suggesting a single gene. (d) Northern blot of total RNA isolated from nondeflagellated cells (NDF) and cells actively regenerating their flagella for 30 min (30'postDF). A faint signal at ~1.4 kb was detected in NDF RNA and an up-regulated band of the same size was observed in 30'postDF RNA. (e) Map of the *LC7B* genomic region. RFLP analysis revealed that the *LC7B* gene maps to linkage group XXII/XXIII. The gene spans an ~1.4-kb region and includes six exons; 5' and 3' untranslated regions (UTRs, dark gray), coding regions (black).

pH 7.5, 5 mM MgSO$_4$, 0.5 mM EDTA, 25 mM KCl) to a final concentration of 0.1 M (Benashski and King, 2000).

Densitometric Analysis of Wild-Type and Mutant Axonemes

Various concentrations of wild-type and mutant axonemes were electrophoresed in 5–15% polyacrylamide gradient gels and either stained with Coomassie Blue or transferred to nitrocellulose. Blots were probed with CT116, 1878A (vs. IC1 of the outer arm; King *et al.*, 1986) or αIC140 (vs. IC140 of inner arm I1; Yang and Sale, 1998). Signals were developed using a chemiluminescent substrate. Densitometry was performed on several different axonemal amounts for each sample to ensure that signals were within the linear response range, by using an Alpha Innotech Imaging System and AlphaEase Software (Athens, GA). Mutant axoneme amounts were normalized to wild-type by using the Coomassie Blue-stained α/β tubulin dimer bands.

Electron Microscopy of oda15 Axonemes

Samples were prepared for electron microscopy by using the method of Schroeter *et al.* (1994). Axoneme samples were fixed with 2.5% glutaraldehyde, 1% tannic acid (#1764; Mallinckrodt, St. Louis, MO) in 100 mM sodium cacodylate, pH 7.4. Intact pellets were then transferred into a postfix solution (100 mM cacodylate, pH 7.4, 1% osmium tetroxide, 0.8% potassium ferricyanide) and subsequently washed in cacodylate buffer. Fixed samples were incubated in 1% tannic acid, 100 mM cacodylate, pH 7.4; stained en bloc with 0.5% aqueous uranyl acetate; and dehydrated in an ethanol series in propylene oxide. Samples were transitioned into plastic using increasing concentrations of Polybed (Polysciences, Warrington, PA) in propylene oxide. Blocks were polymerized at 60°C for 2–3 d, sectioned, and poststained with 2% aqueous uranyl acetate for 30 min and lead citrate (Sato, 1967). Sections were examined in a Philips CM10 transmission electron microscope operating at an accelerating voltage of 60 kV.
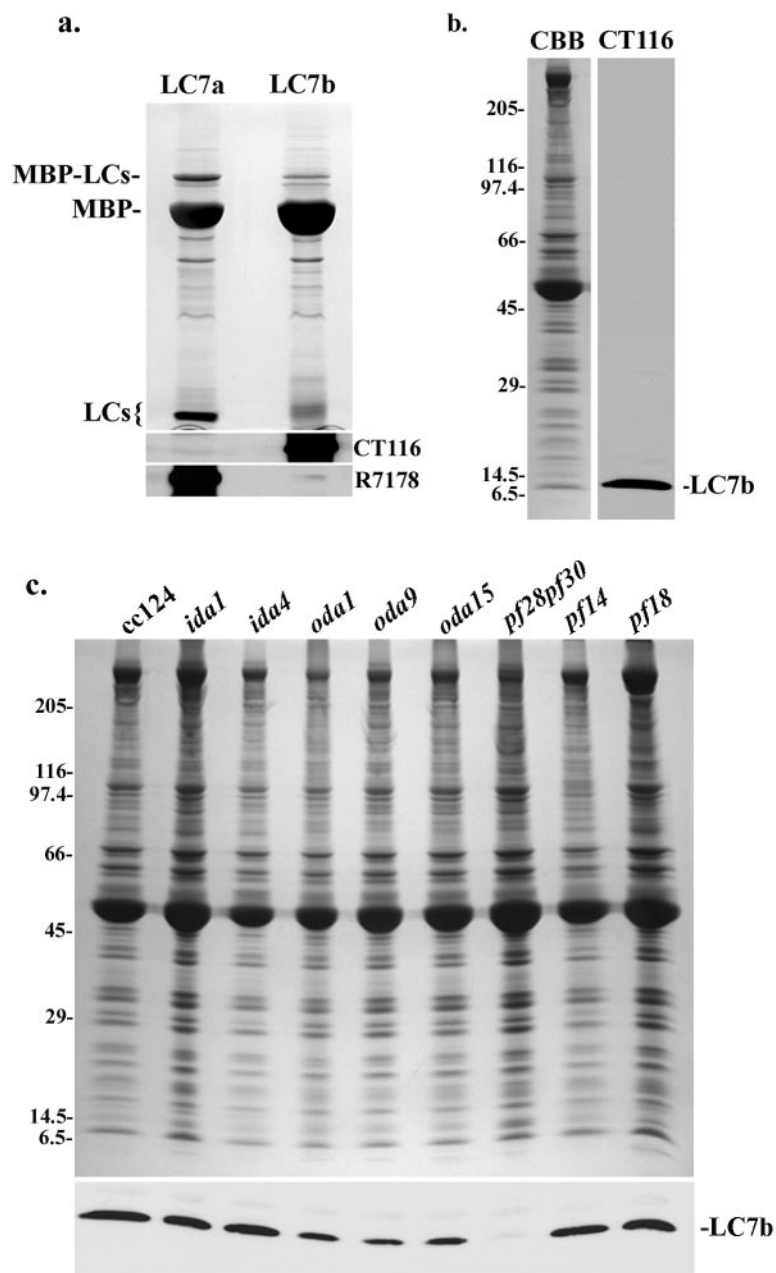
Figure 2. Antibody specificity and localization of LC7b in axonemes. (a) MBP fusions to LC7a and LC7b were digested with Factor Xa, and the LCs separated from MBP by SDS-PAGE, stained with Coomassie Blue (top), or transferred to nitrocellulose (bottom). Blots were probed with either affinity-purified rabbit polyclonal antibody CT116 (LC7b) or R7178 (LC7a) (Bowman *et al.*, 1999). Both antibodies are specific and only recognize their respective LCs. (b) Approximately 150 μg of wild-type (cc124) axonemes were electrophoresed in a 5–15% polyacrylamide gel. The Coomassie Blue-stained gel is shown at left. A blot of an identical sample was probed with affinity purified CT116, which recognized a single, discrete band of the appropriate molecular weight. (c) Approximately 150 μg of isolated axonemes from various strains were electrophoresed in 5–15% acrylamide gradient gels and stained with Coomassie Blue (top) or transferred to nitrocellulose and probed with CT116 (bottom). Strains used include wild-type (cc124), and mutants lacking various axonemal components: *ida1* (inner arm I1), *ida4* (inner arm subtypes a, c, and d from I2/3), *oda1* (outer arms and docking complex), *oda9* (outer arm), *oda15* (LC7a null mutant), *pf28pf30* (outer arm and inner arm I1), *pf14* (radial spokes), and *pf18* (central pair). Levels of LC7b are significantly reduced in axonemes that lack outer arms (*oda1*, *oda9*, *oda15*) and almost completely diminished in a mutant lacking both outer arms and inner arm I1 (*pf28pf30*). Molecular weight markers are indicated on the left.

RESULTS

Identification and Molecular Analysis of a Novel LC7/Roadblock Dynein Light Chain

To identify additional dynein components in *Chlamydomonas* flagella, we searched the *Chlamydomonas* expressed sequence tag (EST) and nonredundant databases by using LC7a from outer arm dynein (accession no. AF140239) as the initial query sequence. The search identified *Chlamydomonas* EST AV387124, which is 1362 base pairs in length and contains a single open reading frame encoding a 100-residue protein with a calculated molecular weight of 11,147 Da and a pI of 5.56. This protein (here termed LC7b) exhibits 47% sequence identity (63% similarity) with LC7a. A phylogenetic tree illustrating the relationship between LC7b and other members of this LC family is shown in Figure 1a. A secondary structure prediction made using Predictprotein (Rost and Sander, 1993) suggests that LC7b contains α-helices in the N-terminal and central sections of the polypeptide, with two short β strands between the helices and three additional strands in the C-terminal regions (Figure 1b). This predicted arrangement of secondary structural elements is consistent with similar analyses for other LC7/Roadblock family members (Koonin and Aravind, 2000; Nikulina *et al.*, 2004) and is clearly distinct from the LC8 (Liang *et al.*, 1999) and Tctex1/Tctex2 (Wu *et al.*, 2001) LC families.

A Southern blot of *Chlamydomonas* wild-type genomic DNA digested with *Sma*I and *Bam*HI produced single bands when probed with the full-length LC7b cDNA, suggesting that this protein is encoded by a single gene (Figure 1c). A single band of ~1.36 kb was readily detected in a northern blot of RNA isolated from cells that were actively regener-

ating flagella (Figure 1d); this band was barely detectable in RNA from nondeflagellated cells, suggesting that LC7b is a flagellar protein. The *LC7b* gene was cloned using the cDNA construct as the initial probe and found to contain six exons (Figure 1e). Restriction fragment length polymorphism (RFLP) analysis revealed that the *LC7B* gene is located on linkage group XII/XIII, between *TUB1* and *TUB2*.

LC7b Is Present in Flagellar Axonemes

The entire LC7b coding region was subcloned into the pMAL-c2 vector and expressed as an N-terminal fusion with MBP. The full-length fusion protein was used as the antigen to generate rabbit polyclonal antiserum CT116. After separation from MBP, immobilized LC7b protein was used to affinity purify the LC7b antibody. Western blotting against recombinant outer dynein arm LCs revealed that CT116 reacted only with its target antigen, and specifically did not recognize LC7a (Figure 2a) or any other axonemal dynein components (Figure 2b).

To determine whether LC7b is indeed an integral dynein LC, axoneme samples isolated from wild-type and mutant strains lacking various flagellar structures were probed with the CT116 antibody (Figure 2c). LC7b was detected in wild-type axonemes and similar levels were observed in mutants lacking a subset of inner arms I2/3 (*ida4*), radial spokes (*pf14*), and central pair components (*pf18*). The levels of LC7b were reduced by ~20% in axonemes from mutants lacking inner arm I1 (*ida1*), and by ~80% in the absence of the outer arms and the outer arm docking complex (*oda1*) or outer arms alone (*oda9*) (Table 1). The LC7a-null mutant, *oda15*, also showed a significant decrease. The most dramatic reduction (of >90%) was seen in a mutant (*pf28pf30*) lacking both the outer arms and inner arm I1. The decreased amounts of LC7b in mutants lacking individual dyneins and the severely diminished levels observed in the *pf28pf30* double mutant imply that this protein associates with both the outer arms and inner arm I1. This is consistent with the arrangement of four outer arms and one copy of inner arm I1 in the 96-nm axonemal repeat.

LC7a and LC7b are Both Components of the Outer Dynein Arm and Inner Arm I1

To confirm the location of LC7b, axonemes were treated with 0.6 M NaCl, which removes dynein components, and the soluble extracts were fractionated in 5–20% sucrose den-

Table 1.

Dynein subunit content

Strain	Subunit content, %[a]		
	LC7b	IC1	IC140
cc124	100	100	100
oda15	29	19	33
ida1	73	n.d.	[b]
oda1	13	[b]	n.d.
oda9	17	[b]	n.d.

n.d., not determined.

[a] Values were determined by quantitative densitometry of CBB-stained gels and normalized based on the α and β tubulin band signal.
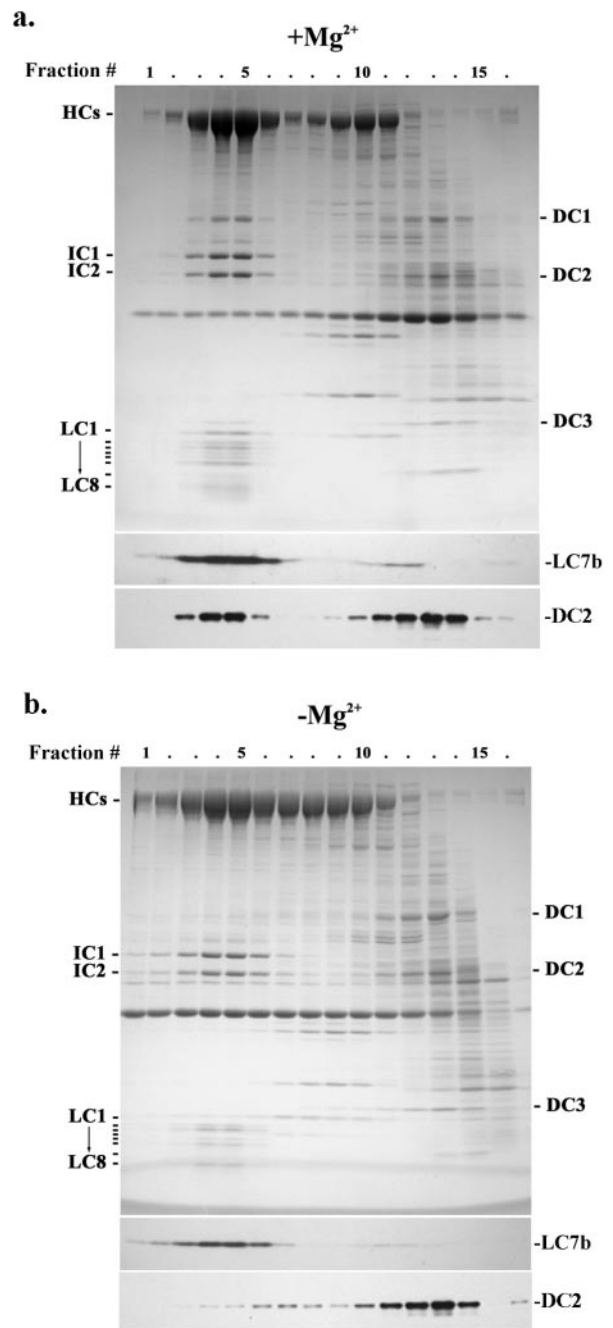
[b] not present.

Figure 3. LC7b is a component of the outer dynein arm. (a) A 0.6 M NaCl extract of *ida1* axonemes was loaded onto a 5–20% sucrose gradient either in the presence of Mg^{2+} (a) or without Mg^{2+} (b). After sedimentation under low (a) or high (b) hydrostatic pressure, fractions were electrophoresed in 5–15% acrylamide gels and either stained with Coomassie Blue (top) or blotted to detect LC7b and the docking complex protein DC2. In both a and b, positions of outer arm components are indicated to the left and outer arm docking complex components to the right. (a) In the presence of Mg^{2+}, the majority of LC7b comigrated with the trimeric outer arm in fractions 3–6. DC2 sedimented in two pools: one with the outer arm (fractions 3–6) and another at 7S (fractions 10–15). In the absence of Mg^{2+}, LC7b copurified with the αβ HC dimer complex (fractions 2–6), suggesting that it does not bind tightly to the γ HC which sedimented at ~12S (fractions 7–10). Under these conditions, the majority of DC2 was found at 7S (fractions 10–14), indicating that it completely dissociated from the outer arm in the absence of Mg^{2+}. In both a and b, a small amount of LC7b was observed at ~10S (fractions 10–12).
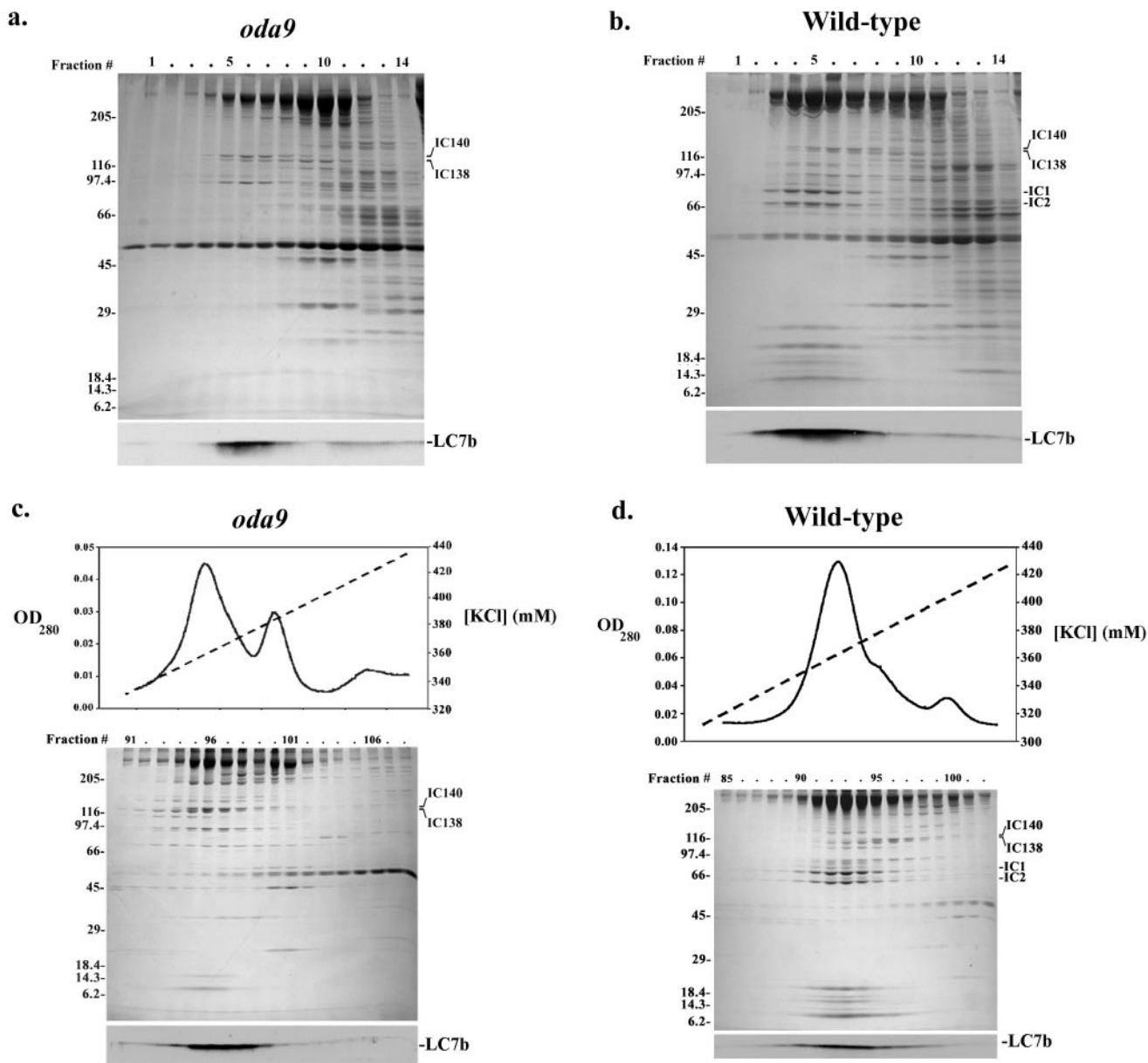
Figure 4. LC7b copurifies with both the outer arms and inner arm I1. In both a and b, 0.6 M NaCl axonemal extracts were loaded onto 5–20% sucrose gradients. After sedimentation, fractions were electrophoresed in 5–15% acrylamide gels and either stained with Coomassie Blue (top) or blotted to detect LC7b. Molecular weight markers are indicated at left. (a) In *oda9* extracts, the majority of LC7b comigrated with inner arm I1 (fractions 5–8). (b) In wild-type extracts, LC7b copurified with the outer arms and inner arm I1 (fractions 2–8), suggesting that it associates with both classes of arms. In both c and d, dialyzed 0.6 M NaCl axonemal extracts were subjected to anion exchange chromatography. A linear KCl gradient was used to elute the various dynein subspecies. After purification, fractions were electrophoresed in 5–15% acrylamide gels and either stained with Coomassie Blue (top) or blotted to detect LC7b. Molecular weight markers are indicated to the left and the positions of the outer arm and/or inner arm I1 ICs are indicated to the right. (c) In *oda9* extracts, inner arm I1 eluted at ~380–400 mM salt (fractions 94–99). LC7b also eluted within these same fractions. (d) In wild-type extracts, LC7b copurified with both sets of arms (fractions 90–99).

sity gradients. In *ida1* extracts, which lack inner arm subtype I1, the majority of LC7b copurified with the trimeric outer dynein arm (Figure 3a); minor pools of LC7b also were observed sedimenting higher in the gradient and likely represent dissociation products. In addition, when the same extract was fractionated under conditions that cause the outer arm to partially dissociate (i.e., the absence of Mg^{2+} and an increase in hydrostatic pressure; Nakamura *et al.*, 1997), LC7b comigrated with the $\alpha\beta$ HC dimer (Figure 3b).

The location of DC2 from the outer arm docking complex also is shown in Figure 3, a and b, and illustrates that significant levels of this protein copurify with LC7b only in the presence of Mg^{2+}. Furthermore, in *oda9* axonemal extracts, the majority of LC7b cofractionated with components of inner arm I1 (Figure 4a), whereas in wild-type, it copurified with both sets of arms (Figure 4b). To ensure that LC7b associated with each dynein, wild-type and *oda9* high salt extracts also were fractionated by anion exchange chroma-

tography. As predicted, LC7b copurified with inner arm I1 in *oda9* extracts (Figure 4c) and with both outer arm dynein and inner arm I1 in wild-type extracts (Figure 4d).

Previously, we demonstrated that LC7a was greatly reduced in axonemes prepared from mutants lacking outer dynein arms, although some protein could still be detected (Bowman *et al.*, 1999). Western blot analysis of wild-type and *oda1* axonemes using the LC7a-specific R7178 antibody (Figure 2a) confirmed that this protein is present in outer arm-less samples, although at lower levels (Figure 5a). Furthermore, we observed a significant LC7a signal in 18S dynein fractions from *oda1* extracts that had been fractionated in a sucrose density gradient (Figure 5b). These observations strongly suggest that the outer arm protein LC7a is also a component of inner arm I1.

The oligomeric state of the LC7 proteins was determined by native gel electrophoresis. Although the MBP-LacZ control (calculated molecular mass of 50.8 kDa) migrated as a monomer of M_r 56,200, the LC7a (54.4 kDa) and LC7b (50.8 kDa) fusion proteins migrated with M_r 104,800 and M_r 94,500, respectively. These results suggest that both proteins can form dimers in solution.

LC7b Interacts Directly with the Outer Arm Docking Complex and the Thioredoxin, LC3

To identify outer arm dynein components that interact with LC7b, dynein purified from the *ida1* mutant was treated with the zero-length cross-linker EDC and cross-linked products detected by immunoblotting (King *et al.*, 1991). A Coomassie Blue-stained gel of *ida1* outer arm dynein revealed that incubation with 20 mM EDC generated many cross-linked products as evidenced by a smear in the gel lane and the accumulation of material with very high M_r (Figure 6a). When an EDC-treated sample was probed with CT116, two major cross-linked products (M_r 94,700 and M_r 27,400) and a minor product (M_r 19,200) containing LC7b were detected (Figure 6b). The M_r of the largest product suggested that LC7b (11.1 kDa) had formed a complex with either an outer arm IC (IC1, 76.6 kDa; IC2, 63.5 kDa) or DC2 (62.2 kDa) from the docking complex. Immunoblotting using monoclonal antibodies specific for IC1 (1878A) and IC2 (1869A) revealed that LC7b had not cross-linked to either subunit (Figure 6, f and g). However, when the EDC-treated sample was probed for DC2, a product of identical M_r to that detected by the CT116 antibody was observed, strongly suggesting that DC2 and LC7b were part of the same cross-linked complex (Figure 6, c and e). Based on the size of the M_r 27,400 band, samples were probed with antibodies against all outer arm LCs and the docking complex DC3. Only the LC3 polyclonal antibody R4930 detected a band of similar size to that seen with the LC7b antibody, suggesting that these two LCs were in direct contact (Figure 6, d and h). In addition, the M_r 19,200 band was not detected by any antibody (including R7178 vs. LC7a) other than CT116, and because it also is present in cross-linked purified inner arm I1 dynein (see below), it likely represents an LC7b dimer. Immunoblot analysis of EDC-cross-linked samples using the R7178 antibody against LC7a did not reveal any major products; after prolonged exposure, several very minor bands (M_r of ~85,000–120,000) of unknown composition were detected.

LC7b Also Associates with IC138 from Inner Arm I1

In a similar manner to that described above, inner arm I1 purified from the *oda1* mutant was treated with EDC (Figure 7a) and probed with CT116. A major, reactive band of M_r 133,000 was detected, suggesting that LC7b had formed a

complex with an inner arm IC (Figure 7b). An immunoblot of EDC-treated *oda1* dynein using an antibody against IC140 revealed no cross-linked products of the appropriate M_r (our unpublished data). However, when a similar sample was probed for IC138, the same cross-linked product was detected (Figure 7, c and d). This product was not present in untreated dynein, indicating that it resulted from the zero-length cross-linking reaction and that LC7b and IC138 interact directly (Figure 7, b and c). An additional minor band of M_r 17,900 containing LC7b also was present in the cross-linked samples. Immunoblots using antibodies specific for Tctex1, Tctex2b, LC7a, and LC8 did not identify any similar sized cross-linked products, indicating that the M_r 17,900 band did not contain any of these LCs (our unpublished data). The simplest interpretation is that this band represents an LC7b dimer as also was observed in cross-linked outer arm dynein.

LC7a Is Required for the Stable Association of LC7b, the γ Heavy Chain, and DC2 with the Outer Arm

To further investigate the putative interaction between LC7b and DC2 detected by EDC cross-linking, we took advantage of the mutant *oda15*, which is defective for LC7a and exhibits a slow, jerky swimming (Pazour and Witman, 2000). Immunoblot analysis using the R7178 antibody confirmed that LC7a is completely missing in this strain, although considerable amounts of other outer arm dynein polypeptides (e.g., the γ HC-associated LC1 protein) are present (Figure 8a). This observation suggested that *oda15* still retains significant numbers of outer dynein arms. To assess this, we examined *oda15* axonemal cross-sections and found widely varying numbers of outer arms, ranging from a full comple-
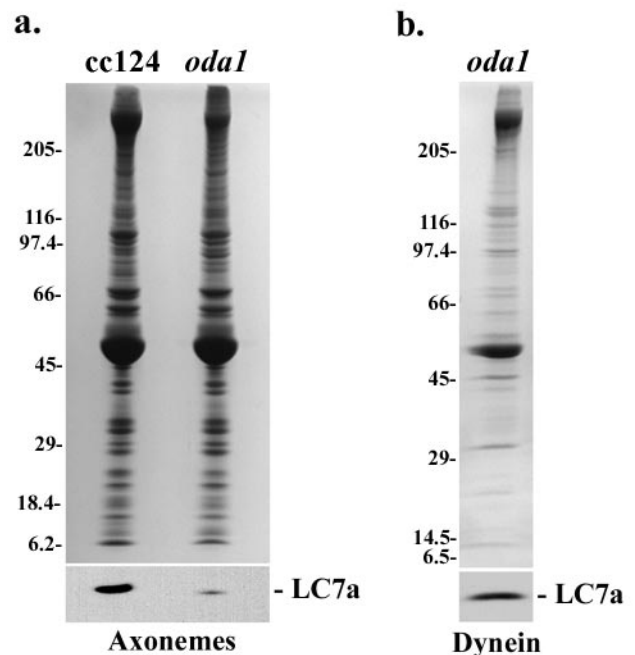

Figure 5. Outer dynein arm LC7a is also a component of inner arm I1. (a) Approximately 150 μg of wild-type (cc124) and *oda1* axonemes and (b) *oda1* purified 18S dynein were electrophoresed in 5–15% gradient gels then either stained with CBB (top) or transferred to nitrocellulose (bottom) and blotted to detect LC7a using the R7178 antibody. LC7a is present in *oda1* axonemes and is also a component of purified inner arm I1.
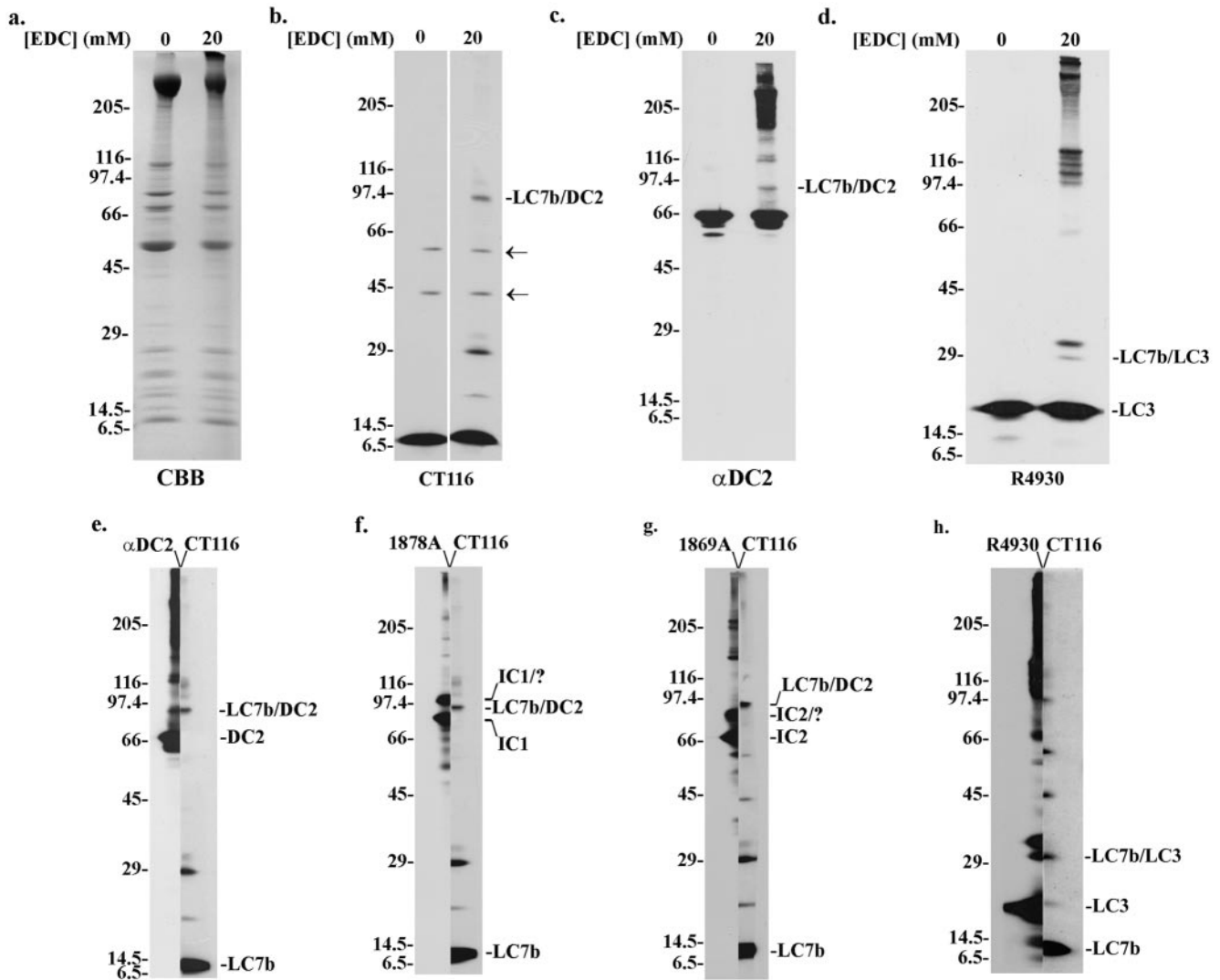
Figure 6. LC7b cross-links to DC2 from the outer arm docking complex and the LC3 thioredoxin. Purified outer arm dynein from *ida1* axonemes was cross-linked with 0 or 20 mM EDC. Samples were electrophoresed in 5–15% gradient gels and then either stained with CBB or transferred to nitrocellulose and blotted to detect various outer arm components. Molecular weight markers are indicated on the left. (a) CBB stained gel of 0 and 20 mM EDC-cross–linked dynein. (b) Blots of 0 and 20 mM EDC-cross–linked dynein probed with CT116 to detect LC7b-reactive products. Three cross-linked species of M_r ~19,200, 27,400, and 94,700 were identified. Two additional, nonspecific bands indicated by arrows were occasionally observed with this antibody preparation. (c) Identical samples to those shown in b were probed to detect DC2-containing species. (d) Samples similar to those shown in b and c were probed to detect LC3-containing species. (e) A single lane containing the 20 mM EDC-cross–linked sample was cut lengthwise and then probed with either αDC2 (left) or CT116 (right). Realignment of both halves revealed a common reactive band detected by both antibodies. (f and g) Similar procedure as in e, however the left sides of the blots were probed with monoclonal antibodies 1878A against IC1 (f), or 1869A against IC2 (g). The M_r 94,700 band contains both LC7b and DC2 but does not include IC1 or IC2. (h) A similar blot of EDC-treated dynein was probed with antibodies against LC7b and LC3. The M_r 27,400 band contains both proteins.

ment to none (mean 3.2), associated with the doublet microtubules (Figure 8b). This observation is consistent with the quantitative densitometry analysis (Table 1), which indicates that ~20% of outer arm IC1 is retained in *oda15* axonemes. Longitudinal sections revealed that, in regions containing outer arms, runs of motors were assembled with a 24-nm repeat as in wild-type. However, gaps occurred in the arrangement at apparently random intervals (Figure 8c), and many microtubule segments seemed completely devoid of attached dyneins. IC140 levels also were reduced to approximately one-third that seen in wild-type strains (Table 1),

suggesting that inner arm I1 assembly is also compromised in the *oda15* mutant.

Dyneins were extracted from *oda15* axonemes and sedimented in a sucrose density gradient under conditions that normally preserve association of the outer arm with the trimeric docking complex (Takada *et al.*, 1992; Figure 3). Electrophoretic and immunoblot analysis revealed that LC2, LC8, and both ICs from the outer arm cosedimented at ~18S in fractions 6–9 (Figure 9). However LC1 and the γ HC cosedimented at ~12S, as also occurs when Mg^{2+} is lacking in the gradient. As in wild-type samples, the peak of IC140
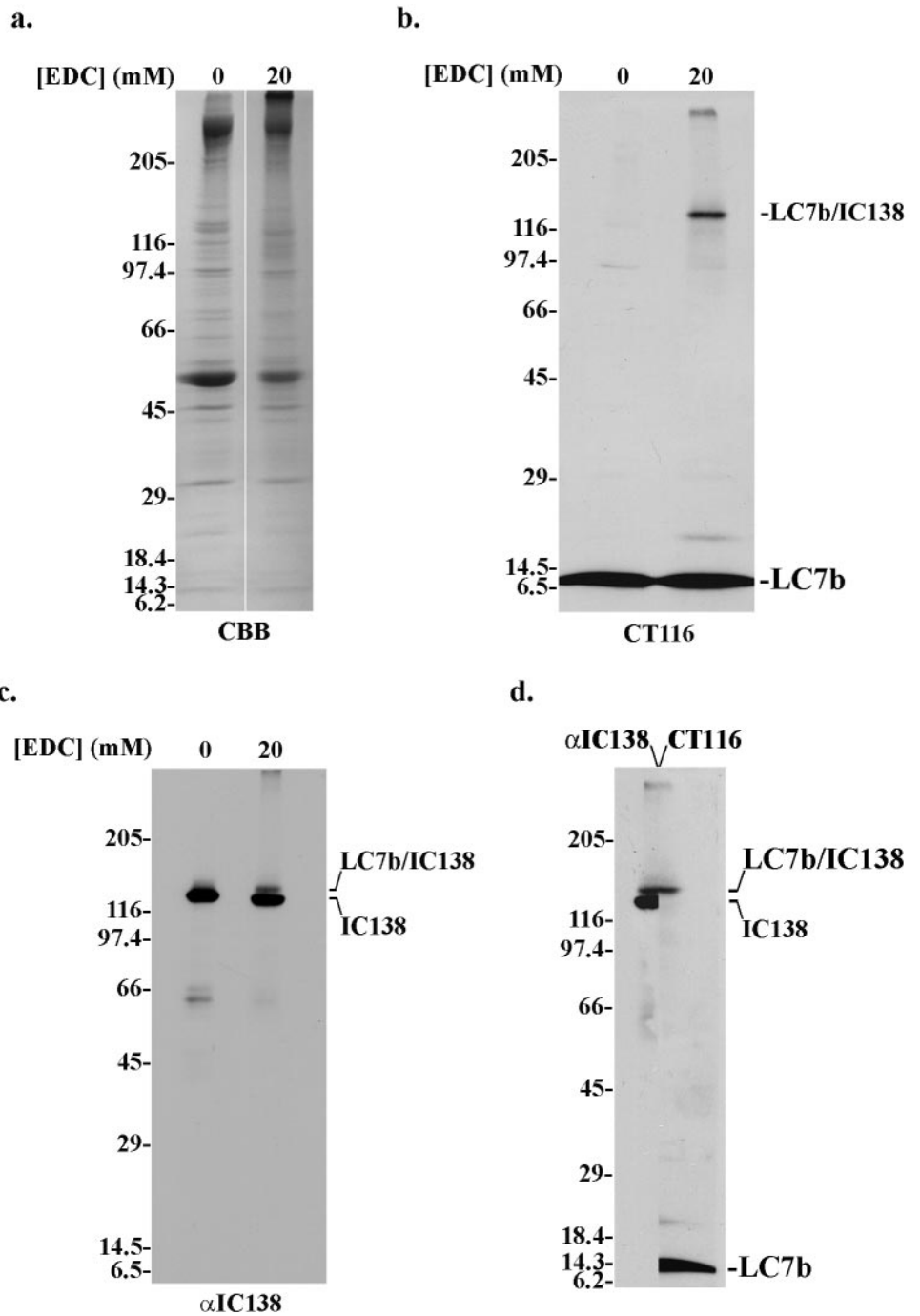
a.

[EDC] (mM)   0   20

205-

116-
97.4-

66-

45-

29-

18.4-
14.3-
6.2-

CBB

b.

[EDC] (mM)   0   20

205-

116-
97.4-

66-

45-

29-

14.5-
6.5-

-LC7b/IC138

-LC7b

CT116

c.

[EDC] (mM)   0   20

205-

116-
97.4-

66-

45-

29-

14.5-
6.5-

LC7b/IC138

IC138

αIC138

d.

αIC138 CT116

205-

116-
97.4-

66-

45-

29-

18.4-
14.3-
6.2-

LC7b/IC138

IC138

-LC7b

Figure 7. LC7b cross-links to IC138 from inner arm I1. Purified inner arm I1 dynein from *oda1* axonemes was chemically cross-linked with 0 or 20 mM EDC. Samples were electrophoresed in 5–15% gradient gels and then either stained with CBB or transferred to nitrocellulose and blotted to identify cross-linked products. Molecular weight markers are indicated on the left. (a) CBB-stained gel of 0 and 20 mM EDC-cross–linked dynein. (b) Blots of 0 and 20 mM EDC-cross–linked dynein probed with CT116 to detect LC7b-reactive products. One major cross-linked species at M_r 133,000, a minor product at M_r 17,900 and additional very high-molecular-weight products were obtained. (c) Similar sample as in b, probed with αIC138 to detect IC138-containing species. (d) Single lane of 20 mM EDC-cross–linked dynein divided lengthwise and probed with αIC138 (left) and CT116 (right). Alignment of the blots revealed that both LC7b and IC138 recognize the same band, indicating that they are in direct contact.

from inner arm I1 occurred slightly higher in the *oda15* sucrose gradient than the outer arm, suggesting that at least some of this dynein also assembled in the mutant. Interestingly however, LC7b did not comigrate with either the outer arms or inner arm I1, but rather completely dissociated and was found at the very top of the gradient. Furthermore, we observed that the docking complex protein DC2 also was released and sedimented at ~7S, rather than remaining associated with the outer arm (Figure 3). Consequently, we conclude that LC7a is required for the stable association of LC7b, the docking complex, and the γ HC subcomplex with the remainder of the outer arm dynein.

DISCUSSION

Chlamydomonas Contains Two Members of the LC7/Roadblock Family

Previously, we identified LC7 (here termed LC7a) within the outer dynein arm as a founding member of the LC7/Roadblock class of dynein light chains (Bowman *et al.*, 1999). Here, we describe a second member of this LC family, termed LC7b, found in *Chlamydomonas* that is ~50% identical to both LC7a and the *Drosophila* cytoplasmic dynein LC, roadblock. The LC7/Roadblock dynein LCs belong to an ancient superfamily involved in the regulation of NTPases
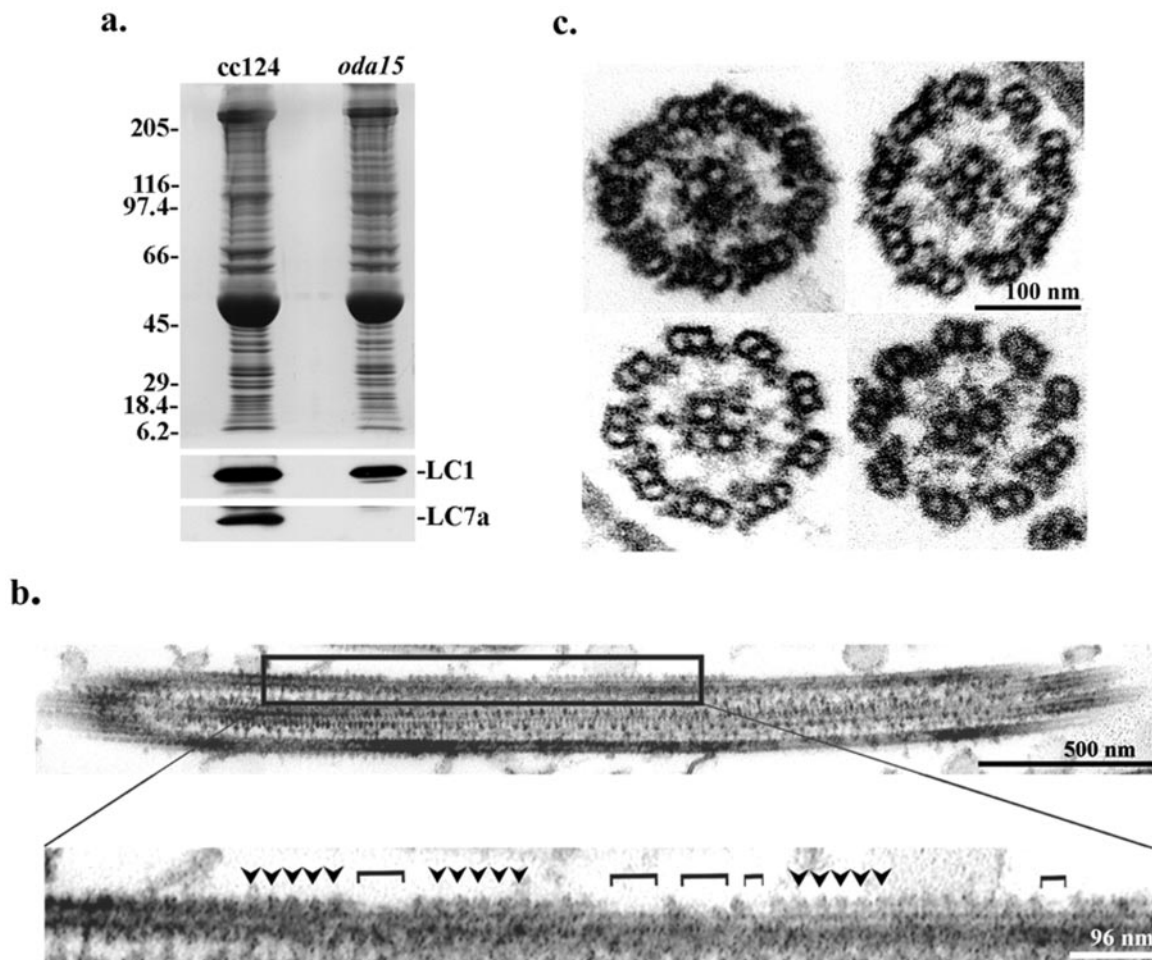
Figure 8. Axonemes from *oda15* contain randomly placed outer dynein arms. (a) Approximately 50 μg of wild-type (cc124) and *oda15* (LC7a null mutant) axonemes were electrophoresed in 5–15% gradient mini gels then either stained with Coomassie Blue or transferred to nitrocellulose and blotted to detect the outer arm proteins, LC1 (using antibody R5932; Benashski *et al.*, 1999) and LC7a (R7178). Reduced levels of LC1 were found in *oda15*, signifying the presence of outer arms; however, the LC7a protein was completely absent. (b) A longitudinal section of an *oda*15 axoneme prepared by thin section electron microscopy. The section outlined is shown at higher magnification, below, and shows stretches of outer arms (arrowheads) and randomly spaced gaps (brackets) where arms are missing. (c) Random cross sections of *oda15* axonemes reveal a widely varying complement of outer arms.

(Koonin and Aravind, 2000). One member of this family, MglB from *Myxococcus xanthus*, seems to modulate the GTPase MglA, which is involved in the regulation of gliding motility (Hartzell and Kaiser, 1991a; 1991b). Mammals also contain two members of this family that are differentially expressed (e.g., Robl1 but not Robl2 has been detected in brain). Furthermore, levels of Robl are dramatically altered in response to light stimuli in the rat visual cortex (Ye *et al.*, 2000), and the balance between Robl1 and Robl2 in human hepatocellular carcinoma tissue is shifted compared with healthy liver tissue (Jiang *et al.*, 2001).

LC7a and LC7b Associate with Both the Outer Dynein Arm and Inner Arm I1

Four outer dynein arms and one inner arm I1 normally assemble within a 96-nm repeat along the axonemal microtubules. Consistent with this arrangement, we found that LC7b levels are ~80% below wild type in mutants completely lacking the outer arms or outer arms and docking complex (*oda1*, *oda9*) and ~20% reduced in the absence of inner arm I1 (*ida1*) (Table 1). LC7b is almost completely absent from the axoneme when both classes of motor are missing (*pf28pf30*), and furthermore, this protein copurifies with both dyneins in sucrose density gradients and following anion exchange chromatography. These data indicate that this LC associates with both classes of dynein arm and further implies that it cannot assemble in the absence of other dynein components. This latter property is a common feature of many dynein proteins and is consistent with their preassembly into subcomplexes within the *Chlamydomonas* cytoplasm (Fowkes and Mitchell, 1998).

Previously, we identified LC7a within *Chlamydomonas* outer arm dynein, and found that this protein was greatly reduced, but not absent, in mutant axonemes prepared from strains lacking that dynein (Bowman *et al.*, 1999). In this report, we demonstrate that LC7a is also an integral component of inner arm I1. Consequently, these two dyneins contain the same two members of this LC class. In cytoplasmic dynein, Robl1 and Robl2 are capable of forming both homo- and heterodimers (Susalka *et al.*, 2002; Nikulina *et al.*, 2004), suggesting that they assemble in pairs within the dynein motor unit. We observed that both LC7a and LC7b fusion
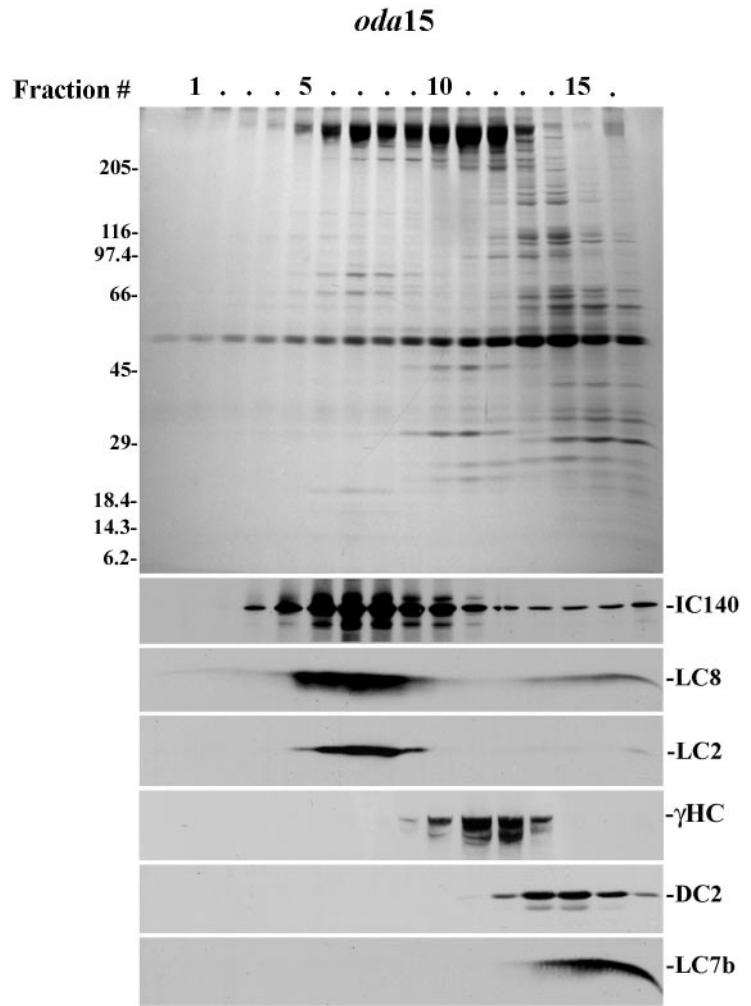
*oda*15

Fraction # 1 . . . 5 . . . . 10 . . . . 15 .

205-
116-
97.4-
66-
45-
29-
18.4-
14.3-
6.2-

-IC140
-LC8
-LC2
-γHC
-DC2
-LC7b

Figure 9. LC7a provides stability to the outer dynein arm. Sucrose gradient (containing Mg^{2+}) fractionation of an *oda15* high salt extract performed under conditions identical to those used for Figure 3a. Samples were electrophoresed in 5–15% gradient gels and either stained with Coomassie Blue (top) or blotted to detect IC140 from inner arm I1; LC8 from the outer arms, inner arm I1, and radial spokes; the outer arm LC, LC2; the outer arm γ HC; DC2 from the outer arm docking complex, and LC7b. IC140, LC8 and LC2 comigrated at 18S (fractions 6–10); however, both the γ HC and DC2 dissociated and migrated at ~12S and ~7S, respectively. In addition, LC7b did not cosediment with components of either the outer arms or inner arm I1, suggesting that LC7a stabilizes the association of this component with dynein.

proteins also form dimers in solution, and our cross-linking data further suggests that at least LC7b forms homodimers within both axonemal dyneins. Thus, the LC7/Roadblock LCs follow the general characteristic that LC components within the dynein IC/LC complex exist as dimers, e.*g.*, the inner arm proteins Tctex1 (Harrison *et al.*, 1998; Wu *et al.*, 2001) and Tctex2b (DiBella *et al.*, 2004); outer arm LC6 (Pfister *et al.*, 1982; King and Patel-King, 1995b), and LC8, which is present in both dyneins (Pfister *et al.*, 1982; King and Patel-King, 1995b; Liang *et al.*, 1999) (Figure 10). The one apparent exception to this rule is the LC2 (Tctex2a) component of the outer arm, which is present at a stoichiometry of one per dynein (Pfister *et al.*, 1982; Patel-King *et al.*, 1997). However, we have recently identified an additional outer arm component related to Tctex1 that may act as its partner (L.M.DiBella and S. M.King, unpublished data).

LC7a Is Not Absolutely Required for Outer Dynein Arm Assembly

Many components (including some LCs) of both the outer dynein arm and inner arm I1 are essential for assembly of the motor unit onto the axoneme, e.g., the 1α and 1β HCs from inner arm I1 (Kamiya *et al.*, 1991; Porter *et al.*, 1992; Myster *et al.*, 1997; Perrone *et al.*, 2000); IC140 from the I1 dynein (Perrone *et al.*, 1998; Yang and Sale, 1998); the outer arm β and γ HCs (Mitchell and Rosenbaum, 1985; Kamiya,

1988; Sakakibara *et al.*, 1993); IC1 and IC2 from the outer arm (Kamiya, 1988; King *et al.*, 1995; Wilkerson *et al.*, 1995); LC8, a LC required for IFT that also is found in the outer arm, inner arm I1, and radial spokes (King and Patel-King, 1995b; Pazour *et al.*, 1998); and the outer arm LC, LC2 (Pazour *et al.*, 1999). However, LC7a is apparently not absolutely required for this process, because the LC7a-null mutant *oda15* can assemble ~20% of its outer arms and ~35% of the I1 class of inner arm. Analysis of purified dyneins from this strain indicated that the outer arms were unstable, because LC7b, the γ HC, and DC2 dissociated from the motor unit after extraction even when purified in the presence of Mg^{2+} under conditions that maintains their integrity within wild-type dynein. Furthermore, LC7b also completely dissociated, suggesting that it requires LC7a to form stable interactions with both dyneins.

Does LC7b Mediate Attachment of the Outer Arm to the Docking Complex?

The trimeric docking complex (DC1-DC3) is essential for correct placement of the outer dynein arm onto the axoneme (Koutoulis *et al.*, 1997; Wakabayashi *et al.*, 2001; Takada *et al.*, 2002), although a fraction of dyneins can assemble in the absence of DC3 (Casey *et al.*, 2003). Our cross-linking data reveal that DC2 and LC7b are in direct contact in purified outer arm dynein. This finding, together with the observa-
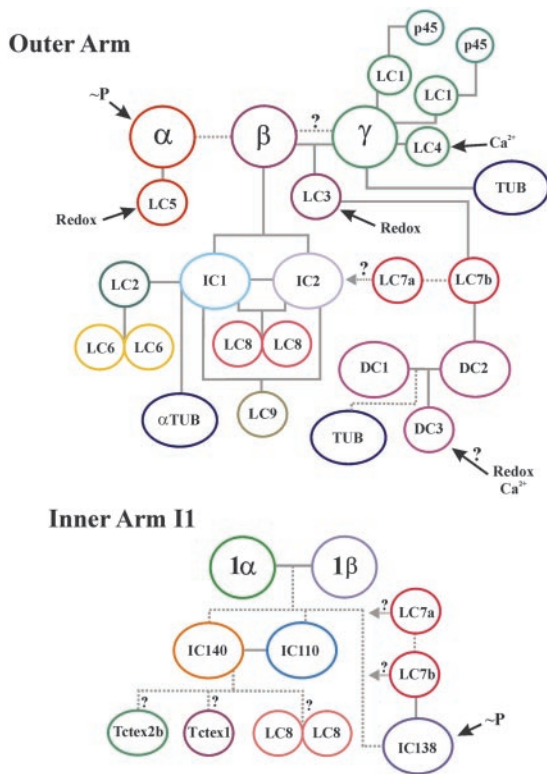
Figure 10. Protein interaction maps of the outer dynein arm and inner arm I1. Diagrams illustrating intradynein associations within the outer arm and inner arm I1 are shown. Experimentally determined interactions are indicated by solid connections and dashed lines represent predicted associations. Phosphorylated subunits are identified by "~P." Ca^{2+} binding and potential redox sites are also indicated. Within the outer arm, the three HCs (α, β, and γ) each have single LCs that are tightly associated with the stem regions of these polypeptides (LC5, LC3, and LC4, respectively; King and Patel-King, 1995a; Patel-King *et al.*, 1996; Harrison *et al.*, 2002). The γ HC associates with two additional copies of LC1 that bind directly to the HC motor domain and also interact with an unidentified 45-kDa axonemal protein (Benashski *et al.*, 1999). It remains unclear whether the heavy chains interact directly with each other. The cargo-binding region of the outer arm consists of two ICs (IC1 and IC2; Pfister *et al.*, 1982) and several LCs [LC2 (Patel-King *et al.*, 1997; Pazour *et al.*, 1999); LC6 and LC8 (King and Patel-King, 1995b); LC7a (Bowman *et al.*, 1999); and LC7b] that participate in an elaborate series of interactions. This complex interacts with the N-terminal stem regions of the HCs through the ICs; however, it is possible that additional associations with this region of the motor unit exist. The outer arm seems to be linked to the docking complex (DC1-DC3; Koutoulis *et al.*, 1997; Takada *et al.*, 2002; Casey *et al.*, 2003) via LC7a and LC7b. LC7b is in direct contact with DC2 and together with the γ HC, they require LC7a to form a stable association with the rest of the outer arm. Additional IC1-tubulin (indicated as *TUB* on the diagram) (King *et al.*, 1991) and γ HC-tubulin (Sakakibara and Nakayama, 1998) interactions also have been identified. A novel Tctex1-related protein (labeled as LC9) also associates with the outer arm through the ICs (L. M.DiBella and S. M.King, unpublished observations). Inner arm I1 dynein contains two distinct HCs (1α and 1β), and a cargo-binding region composed of three ICs (IC110, IC138, and IC140) and several LCs (Tctex1, Tctex2b, LC8, LC7a, and LC7b). Interactions exist between 1α and 1β and cross-linking experiments have identified associations between IC110 and IC140, and IC138 and LC7b. IC138 is a key regulatory subunit of this dynein. LC7a also seems to mediate a stable interaction between LC7b and this inner arm species.

tion that both proteins dissociate from dyneins extracted from *oda15* axonemes suggest that these proteins interact in the flagellum. This is the first evidence that links a component of the docking complex to the outer arm. Although the interaction of LC7b and DC2 represents the only identified connection between these two structures (Figure 10), this does not rule out the possibility of additional interactions between other dynein and docking complex components. The instability of the γ HC in this mutant raises the possibility that it also interacts with LC7b. However, in *ida1* sucrose gradients purified under conditions that cause the γ HC and DC2 to dissociate from the remaining components of the outer arm, LC7b copurifies with the $\alpha\beta$ HC dimer complex. EDC cross-linking revealed that LC7b is also in direct contact with the β HC-associated thioredoxin LC3. This observation raises the intriguing possibility that LC3 and the DC3 docking complex protein that binds Ca^{2+} in a redox-sensitive manner (Casey *et al.*, 2003), are in close proximity.

A protein interaction map for the outer dynein arm is shown in Figure 10 (top) and illustrates the complexity of associations that exist within this dynein motor. In addition to direct associations with LC3 and DC2, additional potential linkages involving LC7a and other subunits within the cargo-binding region are indicated. For example, as the Robl1 and Robl2 cytoplasmic dynein LCs both bind to the IC74 IC in a region distinct from the LC8 and Tctex1 binding sites (Susalka *et al.*, 2002), IC1 and IC2 from the axonemal outer arm are also potential LC7a and LC7b interacting partners.

LC7b Interacts with a Regulatory Subunit of Inner Arm I1

Within inner arm I1, we found that LC7b cross-links to IC138; however, because LC7b also detaches from inner arm I1 in the absence of LC7a, it must again form additional associations within this dynein (Figure 10). Both cAMP-dependent protein kinase (Hasegawa *et al.*, 1987; Howard *et al.*, 1994) and casein kinase 1 (Yang and Sale, 2000) have been implicated in the regulation of microtubule sliding through inner arm I1 (Smith and Sale, 1992). Moreover, IC138 is the only known phosphorylated subunit within this dynein; implicating it as a major regulatory component involved in this process (Habermacher and Sale, 1997). Interaction of LC7b with IC138 places it in an intriguing position within this dynein that may transduce regulatory signals.

In conclusion, we have identified a novel member of the LC7/Roadblock family in *Chlamydomonas*. We show that both LC7a and LC7b associate with outer arms and I1 inner arms and that LC7a mediates a stable association between several dynein components, including LC7b within both dyneins. These LCs participate in multiple interactions within the outer arm and their relationship with the docking complex seems to play a major role in the assembly and stability of this dynein motor. In addition, within inner arm I1, LC7b associates with a major regulatory subunit in a manner that is stabilized by LC7a.

REFERENCES

Benashski, S.E., and King, S.M. (2000). Investigation of protein-protein interactions within flagellar dynein using homobifunctional and zero-length crosslinking reagents. Methods 22, 365–371.

Benashski, S.E., Patel-King, R.S., and King, S.M. (1999). Light chain 1 from the Chlamydomonas outer dynein arm is a leucine-rich repeat protein associated with the motor domain of the γ heavy chain. Biochemistry 38, 7253–7264.

Bowman, A.B., Patel-King, R.S., Benashski, S.E., McCaffery, J.M., Goldstein, L.S., and King, S.M. (1999). Drosophila roadblock and Chlamydomonas LC7, a conserved family of dynein-associated proteins involved in axonal transport, flagellar motility, and mitosis. J. Cell Biol. 146, 165–180.

Casey, D.M., Inaba, K., Pazour, G.J., Takada, S., Wakabayashi, K., Wilkerson, C.G., Kamiya, R., and Witman, G.B. (2003). DC3, the 21-kDa subunit of the outer dynein arm-docking complex (ODA-DC), is a novel EF-hand protein important for assembly of both the outer arm and the ODA-DC. Mol. Biol. Cell 14, 3650–3663.

DiBella, L.M., Benashski, S.E., Tedford, H.W., Harrison, A., Patel-King, R.S., and King, S.M. (2001). The Tctex1/Tctex2 class of dynein light chains: dimerization, differential expression, and interaction with the LC8 protein family. J. Biol. Chem. 276, 14366–14373.

DiBella, L.M., and King, S.M. (2001). Dynein motors of the Chlamydomonas flagellum. Int. Rev. Cytol. 210, 227–268.

DiBella, L.M., Smith, E.F., Patel-King, R.S., Wakabayashi, K.I., and King, S.M. (2004). A novel Tctex2-related light chain is required for stability of inner dynein arm I1 and motor function in the Chlamydomonas flagellum. J. Biol. Chem. 279, 21666–21676.

Fowkes, M.E. and Mitchell, D.R. (1998). The role of preassembled cytoplasmic complexes in assembly of flagellar dynein subunits. Mol. Biol. Cell 9, 2337–2347.

Habermacher, G., and Sale, W.S. (1996). Regulation of flagellar dynein by an axonemal type-1 phosphatase in Chlamydomonas. J. Cell Sci. 109, 1899–1907.

Habermacher, G., and Sale, W.S. (1997). Regulation of flagellar dynein by phosphorylation of a 138-kD inner arm dynein intermediate chain. J. Cell Biol. 136, 167–176.

Harris, E.H. (1989). The Chlamydomonas Sourcebook: A Comprehensive Guide to Biology and Laboratory Use. Academic Press, Inc.

Harrison, A., Olds-Clarke, P., and King, S.M. (1998). Identification of the t complex-encoded cytoplasmic dynein light chain Tctex1 in inner arm I1 supports the involvement of flagellar dyneins in meiotic drive. J. Cell Biol. 140, 1137–1147.

Harrison, A., Sakato, M., Tedford, H.W., Benashski, S.E., Patel-King, R.S., and King, S.M. (2002). Redox-based control of the γ heavy chain ATPase from Chlamydomonas outer arm dynein. Cell Motil. Cytoskeleton 52, 131–143.

Hartzell, P., and Kaiser, D. (1991a). Function of MglA, a 22-kilodalton protein essential for gliding in Myxococcus xanthus. J. Bacteriol. 173, 7615–7624.

Hartzell, P., and Kaiser, D. (1991b). Upstream gene of the mgl operon controls the level of MglA protein in Myxococcus xanthus. J. Bacteriol. 173, 7625–7635.

Hasegawa, E., Hayashi, H., Asakura, S., and Kamiya, R. (1987). Stimulation of in vitro motility of Chlamydomonas axonemes by inhibition of cAMP-dependent phosphorylation. Cell Motil. Cytoskeleton 8, 302–311.

Howard, D.R., Habermacher, G., Glass, D.B., Smith, E.F., and Sale, W.S. (1994). Regulation of Chlamydomonas flagellar dynein by an axonemal protein kinase. J. Cell Biol. 127, 1683–1692.

Huw, L.Y., Goldsborough, A.S., Willison, K., and Artzt, K. (1995). Tctex 2, a sperm tail surface protein mapping to the t-complex. Dev. Biol. 170, 183–194.

Jiang, J., Yu, L., Huang, X., Chen, X., Li, D., Zhang, Y., Tang, L., Zhao, S. (2001). Identification of two novel human dynein light chain genes, DNLC2A and DNLC2B, and their expression changes in hepatocellular carcinoma tissues from 68 Chinese patients. Gene 281, 103–113.

Kamiya, R. (1988). Mutations at twelve independent loci result in absence of outer dynein arms in Chlamydomonas reinhardtii. J. Cell Biol. 107, 2253–2258.

Kamiya, R., Kurimoto, E., and Muto, E. (1991). Two types of Chlamydomonas flagellar mutants missing different components of inner-arm dynein. J. Cell Biol. 112, 441–447.

King, S. (2000). AAA domains and organization of the dynein motor unit. J. Cell Sci. 113, 2521–2526.

King, S.M. (2002). Dynein motors: structure, mechanochemistry, and regulation. In: Molecular Motors, ed. M. Schliwa, Munich, Germany: Wiley-VCH GmbH, 45–78.

King, S.M., Dillman, J.F., III, Benashski, S.E., Lye, R.J., Patel-King, R.S., and Pfister, K.K. (1996). The mouse t-complex-encoded protein Tctex-1 is a light chain of brain cytoplasmic dynein. J. Biol. Chem. 271, 32281–32287.

King, S.M., Otter, T., and Witman, G.B. (1985). Characterization of monoclonal antibodies against Chlamydomonas flagellar dyneins by high-resolution protein blotting. Proc. Natl. Acad. Sci. USA 82, 4717–4721.

King, S.M., Otter, T., and Witman, G.B. (1986). Purification and characterization of Chlamydomonas flagellar dyneins. Methods Enzymol. 134, 291–306.

King, S.M., and Patel-King, R.S. (1995a). Identification of a Ca$^{(2+)}$-binding light chain within Chlamydomonas outer arm dynein. J. Cell Sci. 108, 3757–3764.

King, S.M., and Patel-King, R.S. (1995b). The M_r = 8,000 and 11,000 outer arm dynein light chains from Chlamydomonas flagella have cytoplasmic homologues. J. Biol. Chem. 270, 11445–11452.

King, S.M., Patel-King, R.S., Wilkerson, C.G., and Witman, G.B. (1995). The 78,000-$M_{(r)}$ intermediate chain of Chlamydomonas outer arm dynein is a microtubule-binding protein. J. Cell Biol. 131, 399–409.

King, S.M., Wilkerson, C.G., and Witman, G.B. (1991). The M_r78,000 intermediate chain of Chlamydomonas outer arm dynein interacts with alpha-tubulin in situ. J. Biol. Chem. 266, 8401–8407.

Koonin, E.V., and Aravind, L. (2000). Dynein light chains of the roadblock/LC7 group belong to an ancient protein superfamily implicated in NTPase regulation. Curr. Biol. 10, R774–R776.

Koutoulis, A., Pazour, G.J., Wilkerson, C.G., Inaba, K., Sheng, H., Takada, S., and Witman, G.B. (1997). The Chlamydomonas reinhardtii ODA3 gene encodes a protein of the outer dynein arm docking complex. J. Cell Biol. 137, 1069–1080.

Lader, E., Ha, H.S., O'Neill, M., Artzt, K., and Bennett, D. (1989). tctex-1, a candidate gene family for a mouse t complex sterility locus. Cell 58, 969–979.

Liang, J., Jaffrey, S.R., Guo, W., Snyder, S.H., and Clardy, J. (1999). Structure of the PIN/LC8 dimer with a bound peptide. Nat. Struct. Biol. 6, 735–740.

Merril, C.R., Goldman, D., Sedman, S.A., and Ebert, M.H. (1981). Ultrasensitive stain for proteins in polyacrylamide gels shows regional variation in cerebrospinal fluid proteins. Science 211, 1437–1438.

Mitchell, D.R., and Rosenbaum, J.L. (1985). A motile Chlamydomonas flagellar mutant that lacks outer dynein arms. J. Cell Biol. 100, 1228–1234.

Myster, S.H., Knott, J.A., O'Toole, E., and Porter, M.E. (1997). The Chlamydomonas Dhc1 gene encodes a dynein heavy chain subunit required for assembly of the I1 inner arm complex. Mol. Biol. Cell 8, 607–620.

Nakamura, K., Wilkerson, C.G., and Witman, G.B. (1997). Functional interaction between Chlamydomonas outer arm dynein subunits: the γ subunit suppresses the ATPase activity of the αβ dimer. Cell Motil. Cytoskeleton 37, 338–345.

Neuwald, A.F., Aravind, L., Spouge, J.L., and Koonin, E.V. (1999). AAA$^+$: a class of chaperone-like ATPases associated with the assembly, operation, and disassembly of protein complexes. Genome Res. 9, 27–43.

Nikulina, K., Patel-King, R.S., Takebe, S., Pfister, K.K., and King, S.M. (2004). The roadblock light chains are ubiquitous components of cytoplasmic dynein that form homo- and heterodimers. Cell Motil. Cytoskeleton 57, 233–245.

Olmsted, J.B. (1986). Analysis of cytoskeletal structures using blot-purified monospecific antibodies. Methods Enzymol. 134, 467–472.

Patel-King, R.S., Benashki, S.E., Harrison, A., and King, S.M. (1996). Two functional thioredoxins containing redox-sensitive vicinal dithiols from the Chlamydomonas outer dynein arm. J. Biol. Chem. 271, 6283–6291.

Patel-King, R.S., Benashski, S.E., Harrison, A., and King, S.M. (1997). A Chlamydomonas homologue of the putative murine t complex distorter Tctex-2 is an outer arm dynein light chain. J. Cell Biol. 137, 1081–1090.

Pazour, G., and Witman, G.B. (2000). Forward and reverse genetic analysis of microtubule motors in Chlamydomonas. Methods 22, 285–298.

Pazour, G.J., Koutoulis, A., Benashski, S.E., Dickert, B.L., Sheng, H., Patel-King, R.S., King, S.M., and Witman, G.B. (1999). LC2, the Chlamydomonas homologue of the t complex-encoded protein Tctex2, is essential for outer dynein arm assembly. Mol. Biol. Cell 10, 3507–3520.

Pazour, G.J., Wilkerson, C.G., and Witman, G.B. (1998). A dynein light chain is essential for the retrograde particle movement of intraflagellar transport (IFT). J. Cell Biol. 141, 979–992.

Perrone, C.A., Myster, S.H., Bower, R., O'Toole, E.T., and Porter, M.E. (2000). Insights into the structural organization of the I1 inner arm dynein from a domain analysis of the 1β dynein heavy chain. Mol. Biol. Cell *11*, 2297–2313.

Perrone, C.A., Yang, P., O'Toole, E., Sale, W.S., and Porter, M.E. (1998). The *Chlamydomonas IDA7* locus encodes a 140-kDa dynein intermediate chain required to assemble the I1 inner arm complex. Mol. Biol. Cell *9*, 3351–3365.

Piperno, G., Ramanis, Z., Smith, E.F., and Sale, W.S. (1990). Three distinct inner dynein arms in *Chlamydomonas* flagella: molecular composition and location in the axoneme. J. Cell Biol. *110*, 379–389.

Pfister, K.K., Fay, R.B., and Witman, G.B. (1982). Purification and polypeptide composition of dynein ATPases from *Chlamydomonas* flagella. Cell Motil. *2*, 525–547.

Porter, M.E., Power, J., and Dutcher, S.K. (1992). Extragenic suppressors of paralyzed flagellar mutations in *Chlamydomonas reinhardtii* identify loci that alter the inner dynein arms. J. Cell Biol. *118*, 1163–1176.

Rost, B., and Sander, C. (1993). Prediction of protein secondary structure at better than 70% accuracy. J. Mol. Biol. *232*, 584–599.

Sakakibara, H., and Nakayama, H. (1998). Translocation of microtubules caused by the αβ, β and γ outer arm dynein subparticles of *Chlamydomonas*. J. Cell Sci. *111*, 1155–1164.

Sakakibara, H., Takada, S., King, S.M., Witman, G.B., and Kamiya, R. (1993). A *Chlamydomonas* outer arm dynein mutant with a truncated β heavy chain. J. Cell Biol. *122*, 653–661.

Sakato, M., and King, S.M. (2003). Calcium regulates ATP-sensitive microtubule binding by *Chlamydomonas* outer arm dynein. J. Biol. Chem. *278*, 43571–43579.

Sakato, M., and King, S.M. (2004). Design and regulation of the AAA^+ microtubule motor dynein. J. Struct. Biol. *146*, 58–71.

Sato, J. (1967). A modified method for lead staining of thin sections. J. Electron Microsc. *16*, 133

Schroeter, D., Speiss, E., Paweletz, N., and Benke, R. (1994). A procedure for rupture-free preparation of confluently grown monolayer cells for scanning electron microscopy. J. Electron Microsc. Tech. *1*, 219–225.

Smith, E.F., and Sale, W.S. (1992). Regulation of dynein-driven microtubule sliding by the radial spokes in flagella. Science *257*, 1557–1559.

Susalka, S.J., Nikulina, K., Salata, M.W., Vaughan, P.S., King, S.M., Vaughan, K.T., and Pfister, K.K. (2002). The roadblock light chain binds a novel region of the cytoplasmic dynein intermediate chain. J. Biol. Chem. *277*, 32939–32946.

Tai, A.W., Chuang, J.Z., Bode, C., Wolfrum, U., and Sung, C.H. (1999). Rhodopsin's carboxy-terminal cytoplasmic tail acts as a membrane receptor for cytoplasmic dynein by binding to the dynein light chain Tctex-1. Cell *97*, 877–887.

Takada, S., Sakakibara, H., and Kamiya, R. (1992). Three-headed outer arm dynein from *Chlamydomonas* that can functionally combine with outer-arm-missing axonemes. J. Biochem. *111*, 758–762.

Takada, S., Wilkerson, C.G., Wakabayashi, K., Kamiya, R., and Witman, G.B. (2002). The outer dynein arm-docking complex: composition and characterization of a subunit (oda1) necessary for outer arm assembly. Mol. Biol. Cell *13*, 1015–1029.

Vale, R.D. (2003). The molecular motor toolbox for intracellular transport. Cell *112*, 467–480.

Wakabayashi, K., Takada, S., Witman, G.B., and Kamiya, R. (2001). Transport and arrangement of the outer-dynein-arm docking complex in the flagella of *Chlamydomonas* mutants that lack outer dynein arms. Cell Motil. Cytoskeleton *48*, 277–286.

Wilkerson, C.G., King, S.M., Koutoulis, A., Pazour, G.J., and Witman, G.B. (1995). The 78,000 $M_{(r)}$ intermediate chain of *Chlamydomonas* outer arm dynein is a WD-repeat protein required for arm assembly. J. Cell Biol. *129*, 169–178.

Wu, H., Maciejewski, M.W., Benashski, S.E., Mullen, G.P., and King, S.M. (2001). ^1H, ^{15}N and ^{13}C resonance assignments for the Tctex1 dynein light chain from *Chlamydomonas* flagella. J. Biomol. NMR *20*, 89–90.

Wu, H., Maciejewski, M.W., Marintchev, A., Benashski, S.E., Mullen, G.P., and King, S.M. (2000). Solution structure of a dynein motor domain associated light chain. Nat. Struct. Biol. *7*, 575–579.

Yang, P., Diener, D.R., Rosenbaum, J.L., and Sale, W.S. (2001). Localization of calmodulin and dynein light chain LC8 in flagellar radial spokes. J. Cell Biol. *153*, 1315–1326.

Yang, P., and Sale, W.S. (1998). The M_r140,000 intermediate chain of *Chlamydomonas* flagellar inner arm dynein is a WD-repeat protein implicated in dynein arm anchoring. Mol. Biol. Cell *9*, 3335–3349.

Yang, P., and Sale, W.S. (2000). Casein kinase I is anchored on axonemal doublet microtubules and regulates flagellar dynein phosphorylation and activity. J. Biol. Chem. *275*, 18905–18912.

Ye, F., Zangenehpour, S., and Chaudhuri, A. (2000). Light-induced down-regulation of the rat class 1 dynein-associated protein robl/LC7-like gene in visual cortex. J. Biol. Chem. *275*, 27172–27176.