**The Drosophila tctex-1 Light Chain Is Dispensable for Essential Cytoplasmic Dynein Functions but Is Required during Spermatid Differentiation**

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Variations in subunit composition and modification have been proposed to regulate the multiple functions of cytoplasmic dynein. Here, we examine the role of the Drosophila ortholog of tctex-1, the 14-kDa dynein light chain. We show that the 14-kDa light chain is a bona fide component of Drosophila cytoplasmic dynein and use P element excision to generate flies that completely lack this dynein subunit. Remarkably, the null mutant is viable and the only observed defect is complete male sterility. During spermatid differentiation, the 14-kDa light chain is required for the localization of a nuclear “cap” of cytoplasmic dynein and for proper attachment between the sperm nucleus and flagellar basal body. Our results provide evidence that the function of the 14-kDa light chain in Drosophila is distinct from other dynein subunits and is not required for any essential functions in early development or in the adult organism.

**INTRODUCTION**

The minus-end–directed microtubule motor cytoplasmic dynein has been implicated in a variety of cellular processes, including nuclear envelope breakdown, mitotic spindle assembly and orientation, chromosome movements, intracellular trafficking of organelles and mRNAs, and intraflagellar transport (reviewed in Karki and Holzbaur, 1999). The heavy chain subunit of dynein is known to provide ATPase and microtubule binding functions, and although more than one cytoplasmic dynein heavy chain has been identified, the major cytoplasmic dynein motor contains a homodimer of a single heavy chain. It remains unclear how this single cytoplasmic dynein motor is targeted to distinct organelles and cellular processes. One hypothesis is that the accessory intermediate, light intermediate, and light chain subunits of cytoplasmic dynein mediate its functional specialization. Consistent with this idea, in some eukaryotes these subunits are encoded by multiple genes that are differentially expressed and/or alternatively spliced as distinct transcripts in different tissues and cells (Gill et al., 1994; Pfister et al., 1996a; Bowman et al., 1999; Susalka et al., 2000; Tynan et al., 2000; Tai et al., 2001). In addition, the posttranslational modification of subunits may contribute to the heterogeneity of subunit composition in the dynein complex (Pfister et al., 1996b). Although the mutational analysis of the cytoplasmic dynein heavy chain has revealed a range of motor functions, the functional contribution of other individual subunits is not well understood.

The present study addresses the function of the 14-kDa dynein light chain. This light chain was first identified as a cytoplasmic dynein subunit in mammalian brain (King et al., 1996a) and as an axonemal dynein subunit within the specialized inner arm dynein of the Chlamydomonas flagella (Harrison et al., 1998). In Drosophila, a molecular study of the 14-kDa light chain gene reported defective male fertility for hypomorphic alleles, but the nature of the mutations left unresolved the significance of the 14-kDa light chain in cytoplasmic dynein (Caggese et al., 2001). Sequence analysis revealed that the 14-kDa dynein light chain is the product of a previously cloned mouse gene, tctex-1 (Lader et al., 1989; King et al., 1996b). The tctex-1 gene is part of the mouse t-complex, a chromosomal region that has been implicated in “transmission ratio distortion,” also referred to as “meiotic drive” (reviewed in Silver 1993; Olds-Clarke, 1997). One hypothesis is that distorted segregation results from mutations in dynein genes within the t-complex that contribute to the misregulation of axonemal assembly and motility (King et al., 1996b; Harrison et al., 1998).

Significantly, the physical interaction of tctex-1 homologs with a variety of proteins in mammalian cells, including Doc2 (Nagano et al., 1998), CDS (Bauch et al., 1998), Fyn kinase (Kai et al., 1997; Campbell et al., 1998), Trk kinase (Yano et al., 2001) rhodopsin (Tai et al., 2001), and poliovirus receptor CD155 (Mueller et al., 2002), suggests that tctex light chain also serves multiple cytoplasmic functions. However, the in vivo functional analysis of tctex-1 in vertebrates is made more difficult by multiple genes that encode related polypeptides. In mouse, there are four known copies of the tctex-1 gene on chromosomes 17 and in humans, a second gene, rp3, encodes a relatively divergent 14-kDa protein (55% identity) that can compete with the tctex-1 isoform for incorporation into the dynein complex (Tai et al., 2001).

In Drosophila, the ortholog of the tctex-1 gene was first reported as the dynein light chain 90F (Dlc90F; Li et al., 1998). Drosophila tctex (dltcex) protein is encoded by a single gene (Li et al., 1998; Caggese et al., 2001; this study), but its role in cytoplasmic dynein function is not well characterized. To test whether this light chain might direct a specific...
cytoplasmic dynein function in Drosophila, we have generated a null mutation in the gene and characterized the null phenotype. Our results demonstrate that although this light chain is a bona fide component of dynein motors in multiple tissues, it is absolutely required only in spermatogenesis and is therefore the first dynein subunit found to be nonessential in Drosophila.

MATERIALS AND METHODS

Fly Stocks
The fly stocks of P element insertion lines i(3)05089/TM3, ry Sb Ser, and i(3)05089/TM3, and deficiency Dj(3)1JD2/TM3 (89E1-F4; 91B1-2) were obtained from the Bloomington Drosophila Stock Center (Bloomington, IN). The ry h b p e' stock was obtained from the Bowling Green Stock Center (Bowling Green State University, Bowling Green, OH) and was isogenized in this laboratory for a lethal-free third chromosome. The 12-3 source of transposase was provided by a stock Dj(3)/TM3 Sb 12-3 obtained from our colleague Dr. Michael Simmons (University of Minnesota, St. Paul, MN). The p50-green fluorescent protein (GFP) transgenic line was described previously (Wojcik et al., 2001). Oregon R flies were used as wild-type controls. Flies were raised on standard yeast-cornmeal-agar medium at 25°C.

Polymerase Chain Reaction (PCR) Reactions
The coding region of the Dlcf090 gene was obtained by amplification of cDNAs derived from a 2- to 14-h embryonic library, by using sense primer 5'-GATGGGACTACCGCCAGG-3' (base pairs 161-180) and the antisense primer 5'-GACAGCTCTCTACTCTCCTC-3' (base pairs 590-610 of the cDNA sequence; GenBank no. 708968). PCR products were subcloned into the pGEM-T vector (Promega, Madison, WI). In screening for deletions of Dlcf090, template DNA was isolated from single male flies according to the method of Engel et al. (1990) with minor modifications. A single fly was homogenized with a pipette tip in 50 μl of buffer containing 10 mM Tris-Cl, pH 8.2, 1 mM EDTA, 25 mM NaCl, and 200 μM/ml proteinase K. After incubation at 37°C for 20 min, the solution was heated at 95°C for 2 min. Two microliters of DNA were used as a template, with sense primer 5'-GAGA TGA ACG TGT CTT GCA G-3' located 390 bp upstream of the cDNA, and antisense primer 5'-CAA TGT CTG CCA TGG AAA CG-3', which is 310 base pairs 3' to the polyadenylation site of the Dlcf090 transcript. A predicted PCR fragment from wild-type genomic DNA is 1249 bp. In the excision experiment, we screened for a PCR fragment either bigger or smaller than the wild type.

DNA and RNA Hybridization
Genomic DNA for Southern blot experiments was prepared from adult flies as described previously (Rasmussen et al., 1994). Southern and Northern blots were prepared by standard methods. Hybridizations were carried out in a solution of 40% formamide, 5% dextran sulfate, 5 × SSC, pH 6.8, 50 mM Tris-HCl, pH 7.5, 1 × Denhardt's solution, 1% SDS, and 0.1 mg/ml single-strand salmon sperm DNA at 42°C for overnight. Final washes were 2× SSC, 0.2% SDS at 42°C for low-stringency and 0.1× SSC, 0.1% SDS at 65°C for high-stringency conditions. The cosmid genomic DNA clone was isolated by screening a cosmid library made from fly stocks of P element insertion lines (89E1-F4; 91B1-2) were obtained from the Bowling Green Stock Center (Bowling Green, OH)) and was isogenized in this laboratory for a lethal-free third chromosome (Bowling Green Stock Center, Bowling Green, OH). The Dlcf090 genomic region and the nearest genomic region and the nearest loci of buffer containing 10 mM Tris-Cl, pH 8.2, 1 mM EDTA, 25 mM NaCl, and 200 μM/ml proteinase K. After incubation at 37°C for 2 min. Two microliters of DNA were used as a template, with sense primer 5'-GAGA TGA ACG TGT CTT GCA G-3' located 390 bp upstream of the cDNA, and antisense primer 5'-CAA TGT CTG CCA TGG AAA CG-3', which is 310 base pairs 3' to the polyadenylation site of the Dlcf090 transcript. A predicted PCR fragment from wild-type genomic DNA is 1249 bp. In the excision experiment, we screened for a PCR fragment either bigger or smaller than the wild type.

Antibodies and Protein Analysis
An polyclonal antiserum "1246" was raised in rabbits against the synthetized peptide "DDSREESQFIVDVEVSC," and affinity purified over a column of the same peptide bound to agarose gel (procedures carried out by QCB). Extracts from whole flies were made in PMEG buffer (100 mM PIPES, pH 6.5, 5 mM MgOAc, 5 mM EGTA, 0.1 mM EDTA, 0.5 mM dithiothreitol, and 0.9 M glycerol) plus protease inhibitors (10 μg/ml aprotinin, 1 μg/ml leupeptin and pepstatin, 0.1 μg/ml each of soybean trypsin inhibitor, n-tosyl-l-arginine methyl ester, and benzamidine). Preparation of crude dynein by ATP-sensitive affinity to taxol-stabilized microtubules was as described previously (Hays et al., 1994). Sedimentation of crude dynein or soluble extracts of tissues through 5-20% sucrose gradients was as described previously (McGrail et al., 1995). Immunoprecipitation experiment procedures were as described previously (Boylan et al., 2000). SDS-PAGE of 5-17% acrylamide gradient gels. Western blots were probed with anti-light chain (LC) 1246 diluted 1:3000; anti-dynein heavy chain (HC) monoclonal antibody PI14 (McGrail and Hays, 1994) diluted 1:10,000; anti-dynein intermediate chain (Chemicon Interna-
tional, Temecula, CA) diluted 1:1500 and processed with the Tropix chemiluminescent system (Applied Biosystems, Foster City, CA).

Meiotic Recombination and P Excision
Lethal mutations on the chromosomes i(3)05622 and i(3)05689 were removed by meiotic recombination of the chromosomes with a lethal-free third chromo-
mosome containing multiple genetic markers, ru h b p e' e'. The lethal-free P insertion chromosome derived from i(3)05689 was renamed F5. For P excision experiments, flies of genotype F5/TM3 Sb was mass mated to Df(3R)TM3 Sb, 12-3. Individual F5/TM3 Sb, 12-3 males were then crossed to i(3)TM3 Sb, ry females. From each vial, one progeny male with rosy eyes (ΔP/TM3 Sb, ry) was selected and crossed to i(3)TM3 Sb, ry females. Three days later, DNA templates for PCR were prepared from single male flies, and the resulting products were screened for altered migration of DNA fragments on gels. Once a candidate was identified, the balanced progeny of AP, ry/TM3 Sb, ry from the crosses with the male were used to establish a stock.

Cytology
Testes were dissected from 1- to 2-old males in TB1 (15 mM potassium phosphate, pH 6.7, 80 mM KCl, 16 mM NaCl, 5 mM MgCl₂, and 1% polyethy-
lene glycol 8000) and placed on Superfrost Plus slides (Fisher Scientific, Pitts.
burg, PA). Testes on slides were fixed transversely to expose spermatozoa.

Electroretinogram Recordings and Examination of Rhabdomere Morphology
Morphylogy of rhabdomeres was examined using the optical neural technique described by Franceschi and Kirschfeld, 1977. Electrotetrograms (ERGs) were recorded from nonanesthetized flies imobilized with polyethyl-
ylene glycol 8000 and placed on Superfrost Plus slides (Fisher Scientific, Pittsburgh, PA). Testes on slides were ruptured to expose spermatozoa and de
gated by an electromagnetic shutter. Responses were recorded and analyzed using a Labview data acquisition interface and custom software.

RESULTS
The Tctex-1 Homolog Is a Component of Cytoplasmic Dynin in Drosophila
We initiated our study of the function of the 14-kDa dynein LC in Drosophila development by isolating a cosmid genomic DNA clone, by using a cDNA probe that encodes the fly homolog of the mouse Tctex-1 gene. A restriction map of the Dlcf090 genomic region and the nearest flanking genes on either side are shown in Figure 1A. Our Southern blot anal
To examine whether the Dlc90F gene product is assembled into the cytoplasmic dynein motor complex in Drosophila, we generated a polyclonal antibody against a peptide derived from the first 20 residues of Dlc90F (Figure 1B). On Western blots, the antibody recognizes a band of mobility ~14 kDa in extracts isolated from whole flies and individual tissues. This polypeptide is enriched in dynein prepared by microtubule affinity from both ovaries and embryos (Figure 2A). After further purification of the ATP-eluted dynein fraction by sucrose density gradient sedimentation, the 14-kDa polypeptide cosegregates with the ~19S dynein complex, indicating that this light chain is present as a subunit within the dynein complex (Figure 2B). Immunoprecipitation experiments by using antibodies against the cytoplasmic dynein HC, IC, and 14-kDa LC subunits confirm they exist as a complex in embryos and ovaries (our unpublished data).

In addition, we observe a pool of 14-kDa LC that is not complexed with dynein. When cytosolic extracts, rather than crude dynein preparations, are fractionated through sucrose gradients, the majority of the 14-kDa subunit sediments outside the complex, with a lower S value (Figure 2B).

The 14-kDa LC Is Required for Male Fertility

To analyze the function of the 14-kDa LC during Drosophila development, we carried out a mutational analysis of the Dlc90F gene. Using restriction fragment length polymorphism analysis, we confirmed that a deficiency in the region Df(3R)DG2 contains a deletion removing the Dlc90F gene (supplemental Figure 2). A P element insertion line,
A P element insertion results in partial loss-of-function of Dlc90F, and imprecise excision of the P element abolishes 14-kDa LC expression. (A) A Western blot probed with the 14-kDa LC antibody. Ovary extracts were centrifuged to separate insoluble material, and equal total protein was loaded in each lane. LC expression in flies of genotype Df(3R)DG2/F5 is reduced but not eliminated. (B) Ovary extracts were prepared as in A from the P insertion line e155/e155; balanced siblings with one copy of Dlc90F (e155/+), and wild-type (+/+). (C) No protein is detected in extracts from whole male or female adult flies homozygous for e155. WT, wild-type; S, supernatant; P, pellet.

I(3)05089, was obtained and shown by Southern blot experiments to contain a single insertion. Plasmid rescue and sequence analysis indicated that the P element is located within the 5’ untranslated region (UTR) of the Dlc90F transcript. Although the P-insertion chromosome is homozygous lethal, complementation tests with the de script. Although the P-insertion chromosome is homozygous lethal, the P element is located in the 5’ UTR of the Dlc90F transcript, indicating that the lethal mutation in the stock is not a result of the P insertion. We removed the background lethal mutation by meiotic recombination. Because the original name of the stock, I(3)05089, was based on the lethal phenotype, we redesignated the lethal-free P chromosome F5. Flies homozygous for F5 or hemizygous for F5/Df(3R)DG2 are viable and male flies are sterile.

To demonstrate that the observed male sterility is associated with the P insertion, the P element was excised from its position by using a P element transposase. Of 11 excisions examined, three lines were homozygous viable and fertile, indicating that the P insertion disrupts the function of the Dlc90F gene and that the 14-kDa LC is required for male fertility. Because the P element is inserted in the 5’ UTR of Dlc90F transcript, we tested whether the insertion abolished expression of Dlc90F. As shown in Figure 3A, by using the antibody specific for the 14-kDa LC in Western blot experiments, the amount of the 14-kDa LC protein is substantially reduced in the hemizygous mutant (Df/F5) flies compared

with the heterozygous siblings or wild-type flies; however, some protein of the expected size is still produced. This result demonstrates that the P element insertion leads to a partial loss-of-function of Dlc90F.

Previously, we have shown that cytoplasmic dynein function is essential for cell viability and Drosophila development (Gepner et al., 1996). To determine whether the function of the 14-kDa LC is also essential, we isolated a null mutation of Dlc90F by imprecise excision of the P element from the F5 chromosome. Analysis of the Drosophila genome reveals that the Dlc90F gene is closely flanked by genes on either side (Figure 1A; BDGP database). Expressed sequence tag clones representing both genes are identified in the Drosophila genome project, indicating they are bona fide genes. To generate a mutation that removes only the Dlc90F gene, we first conducted P element excision screens by selecting loss of an eye marker carried by the P element and then identified by PCR a deletion that removes the Dlc90F gene. Of 75 excision events scored, one, designated e155, produced a PCR fragment shorter than wild type (see supplemental Figure 2). Sequence analysis demonstrated that the chromosome in e155 contains a 381-bp deletion in the Dlc90F gene. The 5’ break point is at the position where the P element is originally inserted (bp 46 in the 5’ UTR of the cDNA) and the 3’ break point is at bp 426 of the cDNA. This deletion removes the translation initiation codon and most of the coding sequence of Dlc90F (Figure 1B). The P element excision also leaves a 16-bp footprint in the insertion site. Thus, the predicted transcript of the deleted Dlc90F gene in e155 flies is 200 nt and, if translated from an internal in-frame methionine codon, would produce a peptide of 12 residues derived from the C terminus of the 14-kDa LC. However, due to the insertion of the 16-bp P element footprint, three out-of-frame ATG codons have been created, which are likely to prevent the use of the internal methionine codon of Dlc90F. Furthermore, structural analysis of the 14-kDa LC has predicted that the last 12 aa correspond to a β-sheet that has no predicted function (Mok et al., 2001; Makokha et al., 2002). Consistent with these molecular data, the epitope recognized by the 14-kDa LC antibody is not detected in e155 homozygotes (Figure 3). We conclude that e155 represents a null mutation of Dlc90F.

Flies homozygous for e155/e155 and hemizygous for e155/Df(3R)DG2 are viable and males are sterile. When homozygous e155/e155 females are mated with heterozygous e155/+ males, equal numbers of homozygous and heterozygous progeny are produced, suggesting that neither maternal nor zygotic expression of the 14-kDa LC is needed for normal development in flies. We found no distorted segregation of tctex-1 in the mutant. Both mutant and wild-type sperm are able to fertilize eggs, and no abnormal ratio of genotypes in the progeny is observed. Our data demonstrate that although the 14-kDa light chain is a bona fide component of Drosophila cytoplasmic dynein, its function in the cytoplasmic motor is not essential and is dispensable for most developmental processes in Drosophila.

No Effect on Rhabdomere Formation and Function

It has been shown that the mammalian tctex-1 dynein light chain can directly bind the cytoplasmic tail of the transmembrane protein rhodopsin (Tai et al., 1998). Rhodopsin is a visual light transduction protein; mutations affecting rhodopsin and its localization can promote degeneration of photoreceptors and cause retinitis pigmentosa. The polarized transport of rhodopsin-containing vesicles in vertebrate cell extracts was reported to depend on cytoplasmic dynein (Tai et al., 1999). In Drosophila, the analogous trafficking of
rhodopsin from the endoplasmic reticulum to the rhabdome is important for the development and function of invertebrate photoreceptors (Kumar and Ready, 1995). However, we found no evidence that the 14-kDa LC is important for polarized transport in rhabdomere development. The \textit{e155/e155} \textit{flies} do not exhibit a rough-eye phenotype as observed by scanning electron microscopy, and no obvious retinal degeneration was observed by imaging rhabdomeres by using an optic neutralization technique (our unpublished data). In addition, we assayed whether the null mutant exhibits a defective light response by recording the ERG, which measures the light-evoked electrical response of the retina. The ERG recorded from \textit{e155/e155} \textit{flies} was normal in appearance and in amplitude (see MATERIALS AND METHODS; supplemental Figure 1).

Spermatogenesis of \textit{Dlc90F} Mutant Males

Spermatogenesis in \textit{Drosophila} is well characterized (reviewed in Fuller, 1993). It begins with the stem cell divisions that give rise to founder spermatogonial cells. At the apical end of the testis, primary spermatogonia go through four synchronous mitotic divisions to give rise to a cyst of 16 spermatocytes. Subsequently, the 16 spermatocytes undergo two meiotic divisions to generate 64 spermatids. As a consequence of incomplete cytokinesis during both mitotic and meiotic cell divisions, the 64 postmeiotic spermatids are interconnected by cytoplasmic bridges in a common cyst.

Next, the flagellar axonemes are assembled from basal bodies embedded in each spermatid nucleus. As the axonemes elongate, nuclear shape is streamlined and the spermatid nuclei are driven toward the basal end of the testis. After elongation, the syncytial spermatids are separated into individual sperm by an individualization complex (IC), a cone-shaped complex of cytoskeletal and membrane proteins that translocates the length of the cyst from the nuclei to the ends of the tails, eliminating excess cytoplasm and investing each sperm in its own membrane. Finally, the sperm bundles coil and motile spermatozoa are moved into the seminal vesicles.

Gross examination showed no morphological abnormality in the testes of the \textit{Dlc90F} mutant males. However, the seminal vesicles of \textit{Dlc90F} mutants did not swell after they were held away from females for several days. Under these conditions, we find no motile sperm in the mutant testes, whereas wild-type seminal vesicles accumulate motile sperm and look swollen (Figure 4). In \textit{e155/e155} mutant testes, cysts at the 16-spermatocyte stage seem to be normal. Both mitotic and meiotic figures are readily seen using phase-contrast microscopy, suggesting that any defect is postmeiotic. Early “onion stage” spermatids present a typical, geometrically regular pattern within the cyst, as the 64 nuclei are each paired with a similarly sized spherical mitochondrial derivative. Flagella elongate, although sperm tails are bundled less tightly than those of wild type, and the coiling of tails in the mutant is compromised and less com-

Figure 4. Morphology of mutant testis and spermatid nuclei. Testis morphology was examined using a phase-contrast microscope. Testes were dissected from wild-type (A) and \textit{e155/e155} adult males (B) held away from females for 6 d. The base of a testis is located in the center of the coil. Arrow points to seminal vesicle. (C–F) Confocal sections of testes from 1- to 2-d-old adult males stained with fluorescent Oligreen dye to visualize nuclei. (C) Elongated and bundled spermatid nuclei in each cyst are visible near the base of wild-type testis (arrow). (D) In the \textit{e155} mutant, nuclei are not properly bundled and seem scattered throughout the length of the testis. (E) Nuclei of \textit{e155} mutant spermatids are scattered along sperm tails of several cysts seen in high magnification. Some nuclei have undergone proper elongation (arrow), whereas others have not (arrowhead). Region shown is near base of testis. (F) Scattered nuclei in a single cyst from \textit{e155} mutant testis. Nuclei have not successfully elongated and are randomly oriented. Bar (C–F), 10 \textmu m.
Examination of mutant testes using electron microscopy showed that axonemal structure is not grossly affected by loss of the 14-kDa LC (our unpublished data).

To determine the defect in spermiogenesis, mutant testes were first stained with a fluorescent DNA dye to visualize nuclear shape and positioning. In wild type, the 64 spermatid nuclei in a cyst elongate together in a synchronized manner, aligned in parallel and positioned toward the basal end of each cyst. The elongated nuclear bundles are located within the basal third of a wild-type testis (Figure 4C). In testes from the 14-kDa null mutant, spermatid nuclei are not aligned or bundled at the end of the cyst (Figure 4D). Instead, spermatid nuclei within each cyst are scattered and positioned in varied orientations along the entire length of the cyst. In addition, mispositioned nuclei frequently fail to elongate or only partially elongate (Figures 4D, 5, and 7). Mutant nuclei that do maintain a normal position at the basal end of the cyst seem to elongate normally.

The aberrant organization of sperm tails seen by phase-contrast microscopy could result from defects in sperm individualization (Fabrizio et al., 1998). To examine this possibility more closely, we double-stained mutant testes to visualize DNA and F-actin filaments. The IC comprises 64 cones of actin that form around the spermatid nuclei after elongation and DNA condensation (Fabrizio et al., 1998; Rogat and Miller, 2002; Noguchi and Miller, 2003). Despite the mispositioning of most spermatid nuclei within the mutant cyst, the individualization process in the mutant seemed remarkably normal. Cones of actin were assembled and translocated along the axonemes (Figure 5). Typical groups of actin cones were observed moving away from the nuclear heads. In some cases, individual cones were observed in the middle of a sperm tail bundle, but nonetheless seemed to have assembled normally (Figure 5). These results argue that the individualization machinery in mutant testes is functional and that the observed defects in the individualization of spermatids are secondary, possibly due to the aberrant positioning of nuclei throughout the cyst of elongating spermatids.

The 14-kDa LC Acts to Mediate Cytoplasmic Dynein Localization at Nuclear Membranes of Early Spermatids

We examined the distribution of cytoplasmic dynein in the wild-type and 14-kDa LC null mutant. We visualized the cytoplasmic dynein motor by using a monoclonal antibody specific for the Dhc64C dynein heavy chain. Throughout spermatogenesis, a pool of cytoplasmic dynein seems to be homogeneously distributed. In wild-type early spermatids (i.e., onion stage), a striking concentration of cytoplasmic dynein in a hemispherical “cap” over one side of the nucleus is observed (Figure 6). No obvious enrichment of dynein in other stages of spermatogenesis was obtained under our experimental conditions. To verify the observed pattern of
dynein localization, we used a transgene encoding a GFP-tagged p50 dynamitin subunit of dynactin. The p50-GFP transgene is driven by the endogenous promoter and can fully rescue a lethal mutation in the p50 dynactin subunit (Wojcik et al., 2001). The p50-GFP gene product shows an identical pattern of localization to a hemispherical cap on early spermatid nuclei (Figure 6C). Previous studies have shown that in onion stage spermatids, a second layer of nuclear membrane comes to form a hemispherical cap on one side of the nucleus (Tates, 1971). In Dic90F mutants, the hemispherical pattern of dynein localization is lost or greatly diminished, suggesting that the 14-kDa LC acts to mediate the association of cytoplasmic dynein with the nuclear membrane (Figure 6).

The nuclear cap enriched for cytoplasmic dynein and dynactin in wild-type spermatids lies juxtaposed to the newly formed basal body, as marked by an antibody against centrosomin (Figure 6). We reasoned that dynein localization at the nuclear membrane might help to maintain attachment of spermatid nuclei to the spermatid basal bodies. To explore this possibility, nuclei and basal bodies were visualized by staining for DNA and γ-tubulin, respectively, in elongating cysts. In wild-type, each of 64 nuclei is closely linked with a basal body throughout elongation and individualization (Figure 7A). In contrast, in Dic90F mutant testes, few nuclei were seen to tightly associate with the basal body. As predicted, nuclei found in the middle of sperm tail bundles were not associated with a basal body (Figure 7, B and C). The few nuclei that maintained proper position at the basal end of the testis also seemed to maintain close linkage to the basal body.

**DISCUSSION**

Here, we demonstrate that the dynein 14-kDa light chain is not essential in *Drosophila*. Our data indicate that this *Drosophila* dynein subunit is only necessary for proper postmeiotic spermatid development and male fertility. This restricted phenotype distinguishes the 14-kDa light chain from other dynein subunits and supports the hypothesis that cytoplasmic dynein subunits can mediate specific dynein functions.

**Diversity of Dynein Function: Roles of Subunits**

If the subunits of cytoplasmic dynein provide its functional diversity, then mutations in different subunits might be expected to generate different phenotypes. Yet to date, strong loss-of-function mutations in different cytoplasmic dynein subunits show extensive overlap in the resulting mutant phenotypes. Beyond the heavy chain motor subunit, the intermediate, light intermediate, and light chains seem to be important in multiple dynein functions, but exactly how individual subunits contribute to the diversification of dynein function is not clear.

To date, some of the strongest evidence that dynein subunits can be functionally specialized comes from the analysis of the light chains in mammalian cells. Studies of rhodopsin transport in mammals reveal unique functions for specific LC family members. The tctex-1-dependent transport of rhodopsin can be inhibited by the overexpression of rp3 light chain, which does not bind rhodopsin, but competes for binding to the IC (Tai et al., 1999, 2001). Thus, tctex-1 and rp3 are not functionally redundant light chain isoforms. The *Drosophila* visual system shares many structural and functional features with the vertebrate visual system. Similar to the vertebrate system, rhodopsin photopigments move through the endoplasmic reticulum and Golgi and then are transported to the apical surface of the photoreceptor cells for incorporation into the specialized membranes of the rhabdomere. *Drosophila* rhabdomeres are the functional equivalent of the vertebrate membrane discs in the photoreceptor outer segment. In *Drosophila*, rhodopsin accounts for ~65% of membrane proteins in rhabdomeres and mutations in the rhodopsin gene *ninaE* cause rhabdomere degeneration (Kurada and O’Tousa, 1985). In vertebrates, *Drosophila* cytoplasmic dynein is implicated in rhabdomere assembly by its participation in apical vesicle transport during rhabdomere morphogenesis (Fan and Ready, 1997). However, the null Dic90F mutant displays normal rhabdomere development, and we found no alteration in the electroretinogram recorded from the 14-kDa LC null mutant compared with the control sibling flies. Given the essential requirement for rhodopsin in phototransduction, we conclude that the transport of rhodopsin is not defective in the null mutant and so does not depend on the 14-kDa LC in *Drosophila*.

**Functional Redundancy and Nonessential Regulatory Functions for the 14-kDa LC**

We have considered the possibility that another functionally redundant protein compensates for the loss of Dic90F. Is another related light chain encoded by the *Drosophila* genome? Our results and others have shown there is only a single tctex-1 family member in *Drosophila* (Cagnese et al., 2001). We used low-stringency Southern blots and extensive database searches to rule out the existence of a second related gene. Thus, any essential function of the 14-kDa LC protein in rhodopsin transport or any other process is not being masked by the presence of multiple related genes. However, we cannot exclude the possibility that an unre-
The 14-kDa LC mutant demonstrates that the dynein motor complex can assemble and function in the absence of the 14-kDa light chain. On the other hand, the robust expression of this light chain and its assembly into the 19S complex suggest that an additional, nonessential dynein regulatory function is yet to be understood.

The *Drosophila* 14-kDa LC subunit may not always associate with the dynein motor complex. Our immunoblot analyses of fractionated whole ovary and embryo extracts reveal that the majority of the 14-kDa LC subunit does not cosediment with the 19S complex and is present in a “free” pool of light chain. Previously, Tai et al. (1998) reached a similar conclusion based on immunocytology and immunoprecipitation experiments in mammalian cells. The free pool of the 14-kDa LC may represent the dynamic assembly of the dynein complex. Alternatively, the 14-kDa light chain may function in other cellular processes outside the dynein motor pathway and in doing so interact with and regulate other proteins. This possibility was previously demonstrated for the LC8 dynein light chain in its role as a component of both the myosin V and nitrous-oxide synthase complexes. Nonetheless, the limited phenotype of the 14-kDa LC null argues against this polypeptide acting in many places outside the dynein motor in *Drosophila*.

The Roles of the 14-kDa LC in Spermatid Differentiation

Our data provide new findings that show the 14-kDa LC is dispensable for most cytoplasmic dynein functions, but it is required for differentiation of spermatozoa in *Drosophila*. The *Dlc90F* null mutant is completely male sterile. A previous study of P element-induced mutations in this gene reported reduced fertility and sterility depending on the allele (Caggese et al., 2001). However, the mutations analyzed affected only the 5′ noncoding sequences and were inconclusive regarding the null phenotype. Moreover, no data on the cytological basis of the phenotype were presented. Our results establish the male sterile phenotype for a null 14-kDa LC mutant and shed light on the defects in spermatid development that underlie the observed sterility.

A striking defect observed in the *Dlc90F* null mutant testis was the mispositioning of spermatid nuclei during the elongation of flagellar axonemes. In a wild-type testis, all nuclei are maintained in a cluster as flagellar elongation takes place. By comparison, the mutant spermatid nuclei are not clustered but scattered along the entire length of the cyst. This defective nuclear position correlates with a disrupted linkage between the nucleus and the flagellar basal body. Immunolocalization of γ-tubulin, a component of the basal body, revealed that the basal body was no longer closely associated with the nucleus (Figure 7). During elongation, flagellar axonemes assemble from basal bodies embedded in each spermatid nucleus. As the axonemes elongate, the spermatid nuclei are moved toward the base of the testis. In the mutant, loss of the connection between the axoneme and nucleus may prevent maintenance of proper nuclear position within the elongating cyst. How is the connection between the spermatid nucleus and flagellar basal body maintained? Our immunocytological observations reveal a novel nuclear cap of cytoplasmic dynein that lies juxtaposed to the associated flagellar basal body. Moreover, live imaging of a GFP-tagged dynactin component, p50-GFP, provides further evidence of the concentrated hemispherical cap of dynein. This distribution of dynein closely resembles the distribution of a second nuclear membrane that are stable and becomes limited to the nuclear side adjacent to the centriolar body (Tates, 1971). At this stage of spermatid differentiation, the centriole that will

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**Figure 7.** Detachment of spermatid nuclei from basal bodies. Confocal images were collected from testes stained to show DNA (green) and γ-tubulin (red). (A) In wild-type cysts, all 64 spermatid nuclei are elongated, tightly bundled, and attached to basal bodies, which are visualized here by γ-tubulin. (B) At the basal end of the mutant testis, few of the 64 nuclei have elongated properly. Elongated nuclei are less tightly bundled, with fewer nuclei in each bundle. Some are in the vicinity of the basal body, but few are actually attached. (C) Nuclei that are severely mispositioned and scattered in the middle of a mutant sperm tail bundle do not associate with basal bodies. Bar, 5 μm.

The 14-kDa LC null demonstrates that the dynein motor complex can functionally compensate for the loss of *Dlc90F*.

Although the phenotype of the 14-kDa LC null is restricted to spermiogenesis, this does not reflect an absence of this subunit from dynein motors involved in other processes. *Dlc90F* gene expression is not restricted to testis and is abundant in ovaries and early embryos (Caggese et al., 2001; our unpublished data). We show that the 14-kDa LC cosediments on sucrose gradients with the 19S motor complex in microtubule affinity-purified cytoplasmic dynein. Because the function of cytoplasmic dynein is essential during early development, the viability of the null *Dlc90F* mutant demonstrates that the dynein motor complex can assemble and function in the absence of the 14-kDa light chain. On the other hand, the robust expression of this light chain and its assembly into the 19S complex suggest that an additional, nonessential dynein regulatory function is yet to be understood.

The *Drosophila* 14-kDa LC subunit may not always associate with the dynein motor complex. Our immunoblot analyses of fractionated whole ovary and embryo extracts reveal that the majority of the 14-kDa LC subunit does not cosediment with the 19S complex and is present in a “free” pool of light chain. Previously, Tai et al. (1998) reached a similar conclusion based on immunocytology and immunoprecipitation experiments in mammalian cells. The free pool of the 14-kDa LC may represent the dynamic assembly of the dynein complex. Alternatively, the 14-kDa light chain may function in other cellular processes outside the dynein motor pathway and in doing so interact with and regulate other proteins. This possibility was previously demonstrated for the LC8 dynein light chain in its role as a component of both the myosin V and nitrous-oxide synthase complexes. Nonetheless, the limited phenotype of the 14-kDa LC null argues against this polypeptide acting in many places outside the dynein motor in *Drosophila*.

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insert into the nuclear envelope to become the basal body is surrounded by cytoplasmic microtubules. We speculate that the nuclear-associated dynein cap interacts with the cytoplasmic microtubules to facilitate the nuclear attachment and morphogenesis of the basal body. This hypothetical mechanism is supported by the observation that the nuclear cap of dynein localization and the nuclear attachment of basal bodies is greatly diminished or lost in the 14-kDa LC mutant background. Our experiments do not test whether the 14-kDa LC is directly involved in tethering dynein to the spermatid nucleus or, alternatively, affects expression of other dynein components during spermiogenesis. However, given the requirement for other dynein subunits throughout development, such an impact on expression would have to be unique to spermiogenesis. The association of cytoplasmic dynein and dynactin with the nuclear envelope of developing spermatids in mammalian cells has also been described and may be important in nuclear morphogenesis (Yoshida et al., 1994; Fouquet et al., 2000). Indeed, recent work has indicated that cytoplasmic dynein can associate with nuclei in a variety of cell types, and it has been implicated in nuclear migration, centriolar migration, and nuclear envelope breakdown (Xiang et al., 1994; Reinsch and Karsenti, 1997; Gonczy et al., 1999; Robinson et al., 1999; Salina et al., 2002). If the 14-kDa LC is involved in the targeting of cytoplasmic dynein in the Drosophila spermatid, then the viability and restricted phenotype of the Dic90F mutant suggest that other mechanisms can regulate the nuclear localization of dynein outside the testis.

**REFERENCES**


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