The Journal of Cell Biology, Volume 146, Number 1, July 12, 1999 165–179

Drosophila roadblock and Chlamydomonas LC7: A Conserved Family of Dynein-associated Proteins Involved in Axonal Transport, Flagellar Motility, and Mitosis

Aaron B. Bowman,* Ramila S. Patel-King,§ Sharon E. Benashski,§ J. Michael McCaffery,‡ Lawrence S.B. Goldstein,* and Stephen M. King§

*Howard Hughes Medical Institute, Division of Cellular and Molecular Medicine, Department of Pharmacology, University of California San Diego, La Jolla, California 92039-0683; ‡Integrated Imaging Center, Department of Biology, Johns Hopkins University, Baltimore, Maryland 21218; and §Department of Biochemistry, University of Connecticut Health Center, Farmington, Connecticut 06032-3305

Abstract. Eukaryotic organisms utilize microtubule-dependent motors of the kinesin and dynein superfamilies to generate intracellular movement. To identify new genes involved in the regulation of axonal transport in Drosophila melanogaster, we undertook a screen based upon the sluggish larval phenotype of known motor mutants. One of the mutants identified in this screen, roadblock (robl), exhibits diverse defects in intracellular transport including axonal transport and mitosis. These defects include intra-axonal accumulations of cargoes, severe axonal degeneration, and aberrant chromosome segregation. The gene identified by robl encodes a 97–amino acid polypeptide that is 57% identical (70% similar) to the 105–amino acid Chlamydomonas outer arm dynein-associated protein LC7, also reported here. Both robl and LC7 have homology to several other genes from fruit fly, nematode, and mammals, but not Saccharomyces cerevisiae. Furthermore, we demonstrate that members of this family of proteins are associated with both flagellar outer arm dynein and Drosophila and rat brain cytoplasmic dynein. We propose that roadblock/LC7 family members may modulate specific dynein functions.

Key words: axonal transport • mitosis • dynein ATPase • nerve degeneration • flagella

Intracellular transport is facilitated by the movement of cytoplasmic dyneins and kinesins along ordered arrays of microtubules (Hirokawa, 1998). For example, plus end–directed kinesins move axonal cargo in the anterograde direction, whereas minus end–directed kinesins and cytoplasmic dynein generate the movement of retrograde axonal traffic. The motions of chromosomes in mitosis are also mediated by the actions of these motors on an ordered array of microtubules, the mitotic spindle apparatus. While multiple motors have been identified in both systems, and in some cases cargoes determined, little is known about the regulation of the movement they drive. Perhaps, the largest roadblock to answering these questions is the identification of proteins involved in these processes.

Dyneins, in particular, pose an important challenge because of the large numbers of associated proteins and diverse structural and functional roles (for review see Milisav, 1998). For example, in axonemes the outer arms and one class of inner arm contain two or more heavy chains (~530 kD) that form the globular heads and stems of the particle and provide the sites of ATP hydrolysis and microtubule motor activity. These heavy chains are tightly associated with one or more light chains that may directly regulate motor function (for example in response to Ca2+; King and Patel-King, 1995a). The base of the outer arm dynein particle consists of an additional subcomplex comprised of two closely related intermediate chains that contain WD repeats. At least one of these intermediate chains appears to be involved in cargo binding within the flagellum. Several additional light chains, some of which are shared between different dynein classes and are essential for dynein assembly, are found at the base of the outer arm dynein.

Cytoplasmic dynein is similarly complex and consists of a homodimer of heavy chains, a dimer of a WD repeat intermediate chains, four light intermediate chains (not...
present in the outer dynein arm), and several light chains. The 74-kD cytoplasmic dynein intermediate chain (IC74)\(^1\) has been shown to mediate the dynein–dynein interaction via direct association with p150\(^{glued}\) (Karki and Holzbaur, 1995). Dynactin is a multisubunit complex that may play a role in organelle transport and dynein subcellular localization (for review see Holleran et al., 1998). How- ever, the regulation and role of this dynein–dynein interaction within the context of dynein function is not clear.

Recent work suggests that dynein light chains (DLCs) may play crucial roles in dynein function and regulation. To date, two classes of cytoplasmic DLCs have been identified 1. including Tctex1 (and the homologous rp3) and the highly conserved 10 kD/LC8 DLC (previously called the M, 8,000 light chain). Both classes are also found associated with axonemal dynein. Tctex1 is a cytoplasmic and inner arm DLC thought to be involved in the meiotic drive of mouse t-haplotypes (King et al., 1996b; Harrison et al., 1998; K agami et al., 1998). Tctex1 also appears to associate with a subset of cytoplasmic dynein localized predomi- nantly to the Golgi apparatus, its tissue distribution is quite distinct from that of the related light chain rp3 (K ing et al., 1998; Tai et al., 1998). The 10 kD/LC8 DLC, associated with both cytoplasmic dynein and outer arm dynein (King and Patel-King, 1995b; King et al., 1996a), is also found associated with other enzyme systems such as myo- sin V (Espindola, F.S., R.E. Cheney, S.M. King, D.M. Suter, and M.S. Mosek er. 1996. A merican Society of Cell Biology, 372a (A brst.).), neuronal NOS (J affrey and Sny- der, 1996), and lBx (Crepieux et al., 1997). Mutations in Drosophila lead to altered axon trajectories, female sterility, morphogenetic defects, and apoptotic cell death (Dick et al., 1996; Phillips et al., 1996). This light chain is also essential for dynein heavy chain localization and nuclear migration in Aspergillus and for retrograde intraflagellar transport in Chlamydomonas (Beckwith et al., 1998; Pa- zour et al., 1998). A thorough basis for dynein’s require- ment for numerous associated light chains remains ob- scure, it has become apparent that these proteins play numerous roles in dynein function.

To identify novel modulators of kinesin and dynein mo- tor function, we took advantage of the observation that mutations in axonal transport motors of Drosophila share a common larval phenotype of posterior sluggishness and axonal cargo accumulation (Uurd and Saxton, 1996; Gindhart et al., 1998). Based upon these phenotypes we carried out a mutant screen in Drosophila melanogaster. Here we report the cloning of one such mutant identified in this screen, roadblock (robl). In a complementary ap- proach to understanding the regulation of dynein motors, we cloned the gene for the LC7 polypeptide associated with outer dynein arms from Chlamydomonas axonemes and found it to be highly homologous to robl. Together, these data identify a new family of dynein-associated pro- teins (both axonemal and cytoplasmic) with a role in the microtubule-based processes of axonal transport, flagellar motility, and mitosis.

**Materials and Methods**

**Axoneme and Dynein Purification**

Flagella were isolated from wild-type Chlamydomonas by standard methods (Wittman, 1986) and demembranated with NP-40. Dynein were extracted with 0.6 M NaCl and further purified by sedimentation through a 5–20% sucrose gradient (King et al., 1986). Flagellar axonemes were also prepared from mutants lacking outer (oda9) and various subsets of inner (ida1-4) dynein arms.

Rat brain cytoplasmic dynein was isolated by a AP-dependent microtubule affinity (Paschal et al., 1991) and was further purified by sucrose den- sity gradient centrifugation. A Alternatively, cytoplasmic dynein, dynactin, and kinesin were obtained directly from rat brain homogenates by immu- noprecipitation using the 74-1, 50-1, and H-2 mAbs, respectively, as de- scribed previously (Dillman and Pfister, 1994; King et al., 1996a). These samples were provided by Dr. Kevin Pfister (University of Virginia Health Science Center).

Drosophila dynein was immunoprecipitated from 0–15 h embryo ho- mogenates with the 74-1 antibody using a method similar to the one above. In brief, 0.6 g (wet weight) of dechorionated embryos were homog- enized in 1 ml of lysis buffer (25 mM Tris-Cl, pH 8.0, 50 mM NaCl, 0.5% Triton X-100, 2 mM EDTA, 1 mM PM SF) contained with 10 mM aprotinin, 40 mM/ml bestatin, and 1 mM/ml leupeptin. The homogenate was split into two 400 pl aliquots to which 2.5 mM of 74-1 antibody was added to one sample (dynein immunoprecipitate), the other was mock-immunoprecipi- tated without antibody (bead control). Precipitation was performed with 10 pl of protein A-Sepharose 4B (Zymed Labs, Inc.) preblocked with 5% BSA in lysis buffer. The beads were washed five times with 20 vol of lysis buffer and the final immunoprecipitate was resuspended in 50 pl of SD-PA GE loading buffer. 20 pl of each pellet were analyzed by Western blot as described below.

Chlamydomonas axoneme and rat brain dynein samples were electro- phoresed in 5–15% acrylamide gradient gels. Drosophila samples were electrophoresed with tricine buffer in 10% acrylamide gels. The gels were either stained with Coomassie blue or blotted to nitrocellulose and probed with the 74-1 mAb to detect IC74 (Dillman and Pfister, 1994); Chlamy- domonas and rat samples were also probed with R7178 rabbit polyclonal antibody anti-LC7 (1:50), see below, whereas Drosophila samples were probed with 68B3 rabbit polyclonal antibody anti-robl (1:500), see below, or with the anti-tubulin mAb a 3A 5 (Piperno and Fuller, 1985). Immunoblotting conditions were as previously described (King et al., 1996a).

**Analysis of Peptides from LC7**

Purified outer arm dynein was concentrated in a Centricon 30 ultrafiltra- tion unit (A micon) that had been previously treated with 5% Tween 20 in TBS to reduce nonspecific protein binding. The sample was electrophoresed in a 5–15% acrylamide gradient gel and blotted to a polyvinylidene difluoride membrane (Immobilon P; Millipore Corp.). The LC7 band was excised and treated with trypsin in situ. Peptides eluting from the membrane were purified by reverse-phase chromatography using a C18 column and peptide masses determined by matrix-assisted laser desorp- tion/ionization time-of-flight mass spectrometry. Two peptides of suffi- cient purity were obtained and sequenced at the Protein Micssequencing Facility, University of M assachusetts M edical School.

**Molecular Analysis of LC7**

A portion of the LC7 coding region (~450 bp) was originally obtained from the first strand of cDNA made from RNA enriched for flagellar se- quences using PCR. The forward primer 5’-GGCGCA AT TCA AGA GAC CGA GAT Y TA G-3’ was designed from the peptide sequence (K)KHEIM using the Chlamydomonas codon bias and incorporated an EcoRI site and GC clamp at the 5’ end. The oligo (dA) 5’-GGCGGCTGCACTGATG-3’ was employed as the reverse primer. The reaction was performed using Pfu DNA polymerase and standard buffer conditions with the following thermal profile: 96°C for 1 min, 50°C for 1 min, and 72°C for 1 min for 40 cycles followed by a final 10 min at 72°C. This PCR product was used to isolate a full-length clone from a λ ZapII Chlamydomonas cDNA library. Multiple clones were obtained

---

\(^1\) Abbreviations used in this paper: BDGP, Berkeley Drosophila Genome Project; bxd, late RNA encoded bithoraxoid protein; ChAT, choline acetyltransferase; DLC, dynein light chain; EMS, ethyl methanesulfonate; EST, expressed sequence tag; IC74, 74-kD cytoplasmic dynein intermedia- te chain; khc, kinesin heavy chain; NCBI, National Center for Biotechnol- ogy Information; robl, roadblock; SYT, synaptotagmin; V G, ventral ganglion.
and the longest sequenced on both strands using Sequanase v2.0 and a 7-deaza dGTP sequencing kit (U.S. Biochemical Co.). Southern and Northern blots were prepared and probed using standard methods.

**LC7 Fusion Protein and Antibody Preparation**

The LC7 coding region was subcloned into the pMAL-c2 vector by PCR-based cloning (New England Biolabs Inc.). This resulted in the COOH-terminal fusion of LC7 to the maltose-binding protein via a hydrophilic linker containing a Factor Xa cleavage site. Expressed protein was purified by amylose affinity chromatography and the entire fusion protein was used to raise antisera in rabbit R7178. Subsequently, electrophoretically isolated recombinant LC7 was used to blot purify the antisera using the minor adoptions to the method of OImsted (1986) described by King et al. (1996a).

**Identification of the roadblock Mutant**

F 3 lethal balanced ethyl methanesulfonate (EMS) mutant lines (cn bw I(2)E M S/CyO) were obtained from the laboratory of Dr. Charles Zucker (University of California San Diego). The mutant larvae were examined 5 to 6 d after egg laying for sluggish crawling behavior. Roadblock was identified as a posterior larval sluggish mutant with a late third instar larval lethal phase (robl allele). Preliminary studies identified an absence of imaginal tissue and extreme posterior paralysis in which larvae become completely paralyzed in the posterior, whereas the anterior remained noticeably mobile.

**Cloning of the roadblock Gene**

The robl gene was mapped approximately to cytological position 54 on the second chromosome of Drosophila by meiotic recombination. Screening of nearby lethal alleles obtained from the lab of Dr. Gerry Rubin (University of California Berkeley), identified I(2)10408 as a robl allele (robl10408). A additionally a P-element mobilization screen with other nearby insertions generated another robl allele (robl2). Genomic sequence was rescued off the ends of robl10408 and robl2 by inverse PCR (BDGP protocol; http://www.fruitfly.org/gp/desrupt/) and used to identify Drosophila genomic clones from the Berkeley Drosophila Genome Project (BDGP) using the PI filter blot purchased from Genome Systems, Inc. The P1 clones in the robl genomic region (D02233 and D02859) were sequenced using a B1 377 DNA sequencer. A analysis revealed large deletions in robl10408 and robl2 that were partially overlapping, thus, identifying the robl genomic interval. Homozygous robl10408 and robl2 genic DNA were made from third instar larvae and used to confirm both deficiencies by PCR and Southern analysis. Sequencing and PCR analysis of robl10408 and robl2 DNA revealed a 193-bp deletion identifying the robl genomic region (BDGP). BLAST analysis using the BDGP database identified a full-length genomic sequence number AI292590) by BDGP from a head cDNA library. Roadblock/LC7 family members have been identified for late RNA-encoded bithoraxoid protein (bdx) (G1H0635; accession number A1113381) and robl62A (GH15530; accession number A1292590) by BDGP from a Drosophila head cDNA library. The proteins T24H 10.6 (accession number 998537) and bxd (accession number 290293) were identified from dbNR using BLASTP. Mouse, rat, and human ESTs identified, were compared by nucleotide sequence using BLASTA STN against the species-specific NCBI GenBank dbEST to identify ESTs from identical genes; two different genes were identified in all three species (accession numbers from representative ESTs are given in Fig. 4). The predicted translation of all mammalian ESTs was determined using DNA Strider (CEA); the small size of the genes meant that almost all ESTs translated into full-length protein. Protein comparison was done using the GCG PILEUP command to generate the dendrogram; the output MFF file was run through the BOX SHADE SERVER (http://www.isrec.isb-sib.ch/software/BOX_form.html) and the output EPS file was imported into Adobe Illustrator 6.0.

**Larval Segmental Nerve Immunostaining**

Larval segmental nerve immunostaining was done as described by Hurd and Saxton (1996). A anti-synaptotagamin (DSY T2) (Littleton et al., 1993) was used at 1:500. A α-choline acetyltransferase (4B 1) (Yasuyama et al., 1995) was used at 1:2,000. Immunostained larvae were observed using a Bio-Rad MR C1024 confocal microscope as previously described (Gindhart et al., 1998).

**Electron Microscopy of Larval Segmental Nerves**

The method below is a hybrid of our standard protocol (McCaffery and Farquhar, 1995) with a previously described Drosophila method (Hurd and Saxton, 1996). Drosophila larvae were dissected and pinned open to expose the segmental nerves and muscles. The larvae were fixed for 1 h in 100 mM cacodylate buffer, pH 7.4 at room temperature, containing 3% freshly prepared formaldehyde, 1.5% glutaraldehyde, and 2.5% sucrose. The larval segmental nerves were washed in buffer containing 10 mM cacodylate, pH 7.4, containing 2.5% sucrose and subsequently fixed in Palade’s osmium (1% OsO4, prepared in Kellenberger’s buffer, pH 6.8) for 1 h on ice. The larvae were en bloc stained overnight at room temperature in 2% Kellenberger’s uranyl acetate, subsequently dehydrated through a graded series of ethanol, and embedded in Epon. Larvae were flat embedded and oriented to permit cross-sectioning and visualization of the larval segmental nerves. 80-nm sections were cut on a Leica Ultratome E ultramicrotome, collected onto 400 mesh nickel, high transmission grids, poststained in 2% uranyl acetate and lead citrate, and observed in a J EM 1200 EX II transmission electron microscope.

**Larval Mitotic Brain Squash Analysis**

U ntrated third instar larval brain squash analysis was done as previously described (Gonzalez and Glover, 1993). A iso, brains from robl homoyzogous larvae were analyzed after colchicine (0.5 × 10–10 M for 105 min) and hypoxic treatment as previously described (Gonzalez and Glover, 1993). The mitotic index was determined by counting the number of prometaphase, metaphase, and anaphase mitotic structures seen in a significant number of defined microscope fields (6× objective with 1.6× ocular).

**Results**

**Identification and Cloning of the roadblock**

roadblock (robl) was identified in a screen for novel ax-
onal transport mutants in Drosophila melanogaster. The rob\textsuperscript{l} EMS mutant allele is recessive lethal, dying at the third larval instar. The rob\textsuperscript{l} homozygous larvae show a progressive posterior sluggish phenotype leading to complete posterior paralysis, a common phenotype of axonal transport mutants in Drosophila (Hurd and Saxton, 1996; Gindhart et al., 1998). Further characterization of rob\textsuperscript{l} revealed a complete absence of imaginal tissue, indicating a possible strong mitotic defect as well. To obtain rob null alleles, deletions were generated from flanking P-elements that mapped near rob. Homozygous null and rob\textsuperscript{l}/null (rob\textsuperscript{l} hemizygote) animals die as late pupae; they also demonstrate a posterior larval sluggishness, a peculiar tail flipping phenotype, and accumulations of axonal cargo within their segmental nerves, as has been described for other axonal transport mutants in Drosophila (Hurd and Saxton, 1996; Gindhart et al., 1998). Aditionally, the reduced size of imaginal tissue, rough pupal eyes, and missing bristle phenotypes seen in these animals are characteristic of mitotic mutants in Drosophila. Thus, the rob mutants phenotypes suggest roles for this gene in both axonal transport and mitosis.

Two overlapping deficiencies, rob\textsuperscript{1(2)k10408} and rob\textsuperscript{C}, identify the genomic interval encoding rob\textsuperscript{l} (Fig. 1 A). Sequencing of the entire genomic interval identified five putative gene candidates that may be affected by both deficiencies. To identify which gene encoded rob\textsuperscript{l}, we sequenced rob\textsuperscript{l} and discovered a 193-bp deletion in the middle of a small transcription unit in the interval that we believe to be rob for several reasons. First, a 5-kb segment of this region that contains only rob\textsuperscript{l}, and one adjacent gene, was found to fully rescue all above-mentioned phenotypes in rob\textsuperscript{l} hemizygotes. Second, this gene adjacent to rob\textsuperscript{l} was sequenced from rob\textsuperscript{l} and found to be unaltered from the wild-type parental chromosome. In fact, this gene appears to be a rob pseudogene because it lacks any identifiable start codon. Third, rob\textsuperscript{1(2)k10408} hemizygotes are fully rescued by the genomic rescue construct that indicates that other genes in this interval are not essential and the observed phenotypes are rob\textsuperscript{l}-dependent.

**Figure 1.** The rob\textsuperscript{l} genomic interval. (A) A diagrammatic map of the five genes identified in the genomic region around rob\textsuperscript{l} (accession number A F141921). The entire region has been sequenced and cDNA\textsuperscript{s} have been obtained for rob\textsuperscript{l} and genes 1 and 4. Gene 3 is a rob\textsuperscript{l}-like region, which is likely a pseudogene as it lacks any identifiable start codon. The two partially overlapping deficiencies rob\textsuperscript{1(2)k10408} and rob\textsuperscript{C} identify the rob\textsuperscript{l} genomic region, dotted lines correspond to regions missing in deficiencies. The EMS mutant rob\textsuperscript{l} deleted a small region in one of these genes allowing us to identify it as rob\textsuperscript{l}. The genomic rescue region shown completely rescues rob\textsuperscript{l}/rob\textsuperscript{C}. (B) The genomic sequence of the region encoding rob\textsuperscript{l} (corresponding to nucleotides 7,751–8,214 of genomic interval illustrated in A). Upper case characters show the protein coding sequence that is translated below for each codon; lower-case characters are used to show the 5'-UTR, introns 1 and 2, and 3'-UTR. The EMS mutant rob\textsuperscript{l} has a 193-bp deletion that is represented by bold characters. The deletion extends from intron 2 into the COOH terminus encoding exon 3, removing the intron's conserved branch point sequence that is underlined. Since rob\textsuperscript{l} is a recessive neomorphic allele, a partially functional or aberrant protein is likely made. Reverse transcriptase-PCR analysis of rob\textsuperscript{l} indicates that splicing of mutant intron 2 does not occur (data not shown). However, the mutant transcript maintains the correct reading frame through the remainder of intron 2 and exon 3. The resulting rob\textsuperscript{l} protein would have an internal deletion of 54 residues (IPVKST...HEIMVA replaced by a 12-residue insertion [GWFNCTSV-CA K]) from the remainder of intron 2.
Finally, an NH₂-terminal His-tagged robl cDNA construct under control of the hsp70Bb promoter fully rescues male robl hemizygotes if given daily heat shock. Reducing the frequency of heat shocks results in a restoration of the described robl phenotype. This cDNA construct does not rescue an apparent female sterility seen in the rescued robl hemizygotes, despite full rescue of all other observed robl phenotypes. Nevertheless, taken together, these data establish that the gene identified by the robl deletion is roadblock.

The genomic sequence of robl reveals a small three exon gene encoding a 97-residue polypeptide (Fig. 1 B). The 193-bp deletion found in robl removes portions of intron 2 and exon 3. Interestingly, this deletion results in a robl allele that is more severe than null alleles. The increased severity of robl homozgyous animals compared with robl homozgyous null animals suggests that this internal deletion is a recessive neomorphic allele that poisons intracellular transport. In fact, robl homozgyotes cannot be fully rescued by the genomic or cDNA rescue constructs. Thus, two copies of the robl mutation act in a dominant fashion to inhibit the action of wild-type robl. An alternative explanation for the inability to rescue robl homozygotes would be a secondary lethal lesion on the robl chromosome. However, we have confirmed the absence of any other lethal complementation groups on the robl chromosome by recombination mapping (data not shown).

Chlamydomonas LC7 Is an Outer Arm Dynein-associated Protein

The Chlamydomonas outer dynein arm contains eight distinct light chain components (Piperno and Luck, 1979; Pfister et al., 1982). Previously, we cloned and described all of these proteins except for LC7. To clone LC7, we purified and sequenced two tryptic LC7 peptides isolated from outer arm dynein (Fig. 2 A). Based upon this sequence, PCR primers were designed and an LC7 cDNA clone isolated. The largest cDNA clone was 864 bp in length (Fig. 2 B) and contained a single open reading frame of 105 residues with a predicted mass of 11,928 Da and a calculated pI of 7.85. Both peptide sequences obtained from purified LC7 were found in this clone (26/26 residues correct) and were both preceded by the predicted basic residue. Three in frame stop codons were present up-

**Figure 2.** Molecular analysis of LC7 from the Chlamydomonas outer dynein arm. (A) Two tryptic peptides from outer arm dynein LC7 were completely sequenced, yielding a total of 26 residue assignments. The actual mass of each peptide is in agreement with the calculated mass once methionine oxidation of the upper peptide is incorporated. (B) DNA and predicted protein sequence for the Chlamydomonas LC7 cDNA clone. Both peptide sequences are found in the coding region (26/26 residues correct). These sequences are indicated in bold type and are contiguous in the primary structure. The polyadenylation signal is underlined. This sequence is available in the NCBI GenBank (accession number AF140239). (C) Southern blot of genomic DNA from Chlamydomonas strain S1D2 digested with BamH I, PstI, PvuII, and Sma I and probed with the full-length LC7 cDNA. The data suggest that there is a single gene for LC7 in Chlamydomonas. (D) Northern blot analysis of RNA from nondeflagellated cells and from those actively regenerating flagella (30° postDF). A single message of ~0.95 kb that is induced in regenerating cells is evident.
stream of the first Met residue and a 489-bp 3' untranslated region, including a perfect copy of the Chlamydomonas polyadenylation signal, followed the stop codon.

Genomic Southern blot analysis revealed a single band in both BamH1- and SmaI-digested DNA, suggesting that there is a single LC7 gene in Chlamydomonas (Fig. 2C). A similar characteristic of flagellar proteins, Northern analysis revealed one message of ~0.95 kb that was greatly upregulated in cells that were actively regenerating their flagella (Fig. 2D).

The outer arm dynein samples used to obtain LC7 peptide sequences also contained inner dynein arm I1. This dynein partially cofractionates with the outer arm and is now known to contain light chain components (Harrison et al., 1998). To confirm that the LC7 protein is a component of the outer arm, axonemes were prepared from mutants lacking specific dynein structures including the outer arm (oda9), inner arm I1 (ida1, ida2, and ida3), and a subset of inner arms I2/3 (ida4). Immunoblot analysis of these samples using a polyclonal LC7 antiserum revealed that the LC7 polypeptide was present in the mutants lacking inner arms, but was drastically reduced in the strain lacking outer arms (Fig. 3A). Upon overexposure of the blot, a very small amount of LC7 could be detected in the outer armless axonemes. The origin of this minor fraction remains unclear as the LC7 protein could not be detected in sucrose gradient profiles of high salt extracts from outer armless strains (data not shown). Furthermore, sucrose gradient analysis of extracts from wild-type axonemes revealed that all the extracted LC7 comigrated with the outer arm at ~18 S (Fig. 3B).

A Family of robl/LC7 Proteins Is Conserved from Nematode to Man

The cloning of roadblock and LC7 revealed these proteins to be 57% identical and 70% similar. Additionally, both proteins are related to the predicted protein sequence from the late RNA of the Drosophila bithoraxoid complex (bxd); robl is 30% identical and 42% similar to bxd; LC7 is 26% identical and 39% similar to bxd. However, no known function has been attributed to this coding transcript from bxd (Lipshitz et al., 1987). The robl/LC7 similarity prompted us to look for additional robl-like genes in the NCBI GenBank. BLAST and comparative protein sequence analysis identified a large family of robl-like proteins conserved in Drosophila, nematode, Chlamydomonas, and three mammalian species (Fig. 4A and B). Four other robl-like genes, in addition to the bxd late RNA, were identified in Drosophila and are designated here by their cytological location: robl62A, robl37BC, robl22E, and robl60C. In mammals, two classes of robl/LC7-like genes were identified by homology (Fig. 4A). However, C. elegans apparently has only a single robl-like gene in its genome (Fig. 4A). The differences between robl/LC7-like family members may suggest a possible functional distinction between the various members within an organism.

robl Mutants Have a Distal Biased Axonal Transport Defect

Mutations in robl cause phenotypes similar to other axonal transport mutants in Drosophila. Previous analysis of kinesin heavy chain (khc) and kinesin light chain mutants demonstrated massive accumulations of axonal cargo and motors distributed randomly along the entire length of the larval segmental nerves. These accumulations were shown to be massive local axonal swellings that fill with organelles and vesicles (Hurd and Saxton, 1996; Gindhart et al., 1998). The accumulation phenotype correlates with the other common axonal transport phenotypes in Drosophila, tail flipping and posterior paralysis. It was proposed that these mutants disrupt the processive movement of their cargo.
A ROADBLOCK/LC7 FAMILY DENDROGRAM

Drosophila rob-like (rob162A)
Drosophila bithoraxoid at 89E (bxd)

Rat rob-like Class 1 ESTs (e.g. AA801427)
Mouse rob-like Class 1 ESTs (e.g. w98641)
Human rob-like Class 1 ESTs (e.g. hum424e02b)

Rat rob-like Class 2 ESTs (e.g. A1407980)
Mouse rob-like Class 2 ESTs (e.g. w98780)
Human rob-like Class 2 ESTs (e.g. AA446298)
Drosophila roadblock at 54C (robI)

Chlamydomonas LC7 (LC7)

Drosophila rob-like (rob122E)
C. elegans predicted gene (T244110.6)
Drosophila rob-like (rob1378C)
Drosophila rob-like (rob160C)

MAMMALIAN ROB/LC7-LIKE CLASS 1

MAMMALIAN ROB/LC7-LIKE CLASS 2

B ROADBLOCK/LC7 FAMILY ALIGNMENT

 rob162A 1 HSLKTVKRVAVYQRDTRTELNLQSKQGGFYSHSNSIYRLDVRDEEIEEHP
 bxd 1 HSLKTVKRVAVYQRDTRTELNLQSKQGGFYSHSNSIYRLDVRDEEIEEHP
 Rat Class 1 1 HSLKTVKRVAVYQRDTRTELNLQSKQGGFYSHSNSIYRLDVRDEEIEEHP
 Mouse Class 1 1 HSLKTVKRVAVYQRDTRTELNLQSKQGGFYSHSNSIYRLDVRDEEIEEHP
 Human Class 1 1 HSLKTVKRVAVYQRDTRTELNLQSKQGGFYSHSNSIYRLDVRDEEIEEHP
 robI 1 HSLKTVKRVAVYQRDTRTELNLQSKQGGFYSHSNSIYRLDVRDEEIEEHP
 rob1378C 1 HSLKTVKRVAVYQRDTRTELNLQSKQGGFYSHSNSIYRLDVRDEEIEEHP
 rob160C 1 HSLKTVKRVAVYQRDTRTELNLQSKQGGFYSHSNSIYRLDVRDEEIEEHP

 rob162A 66 HSLKTVKRVAVYQRDTRTELNLQSKQGGFYSHSNSIYRLDVRDEEIEEHP
 bxd 66 HSLKTVKRVAVYQRDTRTELNLQSKQGGFYSHSNSIYRLDVRDEEIEEHP
 Rat Class 1 54 HSLKTVKRVAVYQRDTRTELNLQSKQGGFYSHSNSIYRLDVRDEEIEEHP
 Mouse Class 1 54 HSLKTVKRVAVYQRDTRTELNLQSKQGGFYSHSNSIYRLDVRDEEIEEHP
 Human Class 1 54 HSLKTVKRVAVYQRDTRTELNLQSKQGGFYSHSNSIYRLDVRDEEIEEHP
 robI 55 HSLKTVKRVAVYQRDTRTELNLQSKQGGFYSHSNSIYRLDVRDEEIEEHP
 rob1378C 57 HSLKTVKRVAVYQRDTRTELNLQSKQGGFYSHSNSIYRLDVRDEEIEEHP
 rob160C 105 HSLKTVKRVAVYQRDTRTELNLQSKQGGFYSHSNSIYRLDVRDEEIEEHP

Figure 4. A large family of rob/LC7-like proteins. BLAST analysis has identified several mammalian ESTs, Drosophila genes, and a gene from C. elegans that are highly homologous to rob/LC7. (A) A dendrogram of the rob/LC7-like family members identified is shown. This dendrogram was generated using the GCG PILEUP command. We identified at least five Drosophila roadblock-like genes by searching the BDGP-derived ESTs and genomic sequences. Previously unidentified genes have been designated by their cytological location determined by BDGP. Also, two classes of rob/LC7-like genes have been identified as mammalian ESTs (identified by a representative EST accession number). (B) An alignment of the protein family is shown. The alignment was generated by the same GCG PILEUP command as an MSF file. BOX SHADE was used to illustrate aligned amino acid identity (dark shaded residues) and similarity (light shaded residues).
within the axon, causing the axons to swell, filling with transported axonal material. Immunostaining of rob\textsuperscript{I}/null hemizygous and rob\textsuperscript{I} null homozygous larvae reveals frequent accumulations of synaptotagmin (SYT) (Fig. 5, C and I) and choline acetyltransferase (ChAT) (Fig. 5, D and J) in the larval segmental nerves. In contrast, SYT (Fig. 5 A) and ChAT (Fig. 5 B) show only a low background level staining in wild-type segmental nerves. Additionally, axonal transport motors (of the kinesin I and kinesin II family), cysteine string protein, and a marker for endocytic traffic are also observed to accumulate in the axons of rob\textsuperscript{I} mutants (data not shown). Thus, rob\textsuperscript{I} mutants have a gross phenotype similar to that previously described for axonal transport mutants in Drosophila; a progressive larval posterior paralysis, tail flipping, and segmental nerve axonal cargo accumulation.

In rob\textsuperscript{Iz} mutants, unlike previously described axonal transport mutants, there is a strong tendency for the synaptic cargo to accumulate at the distal regions of axons with only infrequent proximal accumulations. This distal bias can be inferred from the organization of the Drosophila larval nervous system. The larval segmental nerves are anti-parallel bundles of mostly cholinergic sensory neuron axons and noncholinergic motor neuron axons. The (ChAT and SYT expressing) sensory neurons project axons from peripheral cell bodies towards the anterior into the ventral ganglion (VG), whereas the (SYT expressing but ChAT lacking) motor neurons project axons in the opposite direction from cell bodies in the VG towards the posterior and peripherally where they form neuromuscular junctions.

In rob\textsuperscript{Iz} hemizygous larvae, ChAT accumulations were found predominantly in the distal portions of the sensory axons (the anterior region of the larval segmental nerves) as seen by comparing staining at the anterior VG (Fig. 5 F) with staining observed in segmental nerves in the posterior of the larvae (Fig. 5 H). SYT shows a gradual increase in the frequency of accumulations toward the distal portions of the motor axons (the posterior region of the larval segmental nerves) as seen by comparing the staining at the anterior VG (Fig. 5 E) with staining observed in segmental nerves at the posterior region of the larvae (Fig. 5 G). Thus, the frequency of ChAT accumulations is inversely correlated with the distance from the VG, whereas SYT accumulations show the opposite correlation.

We further analyzed this distal enrichment of axonal accumulations by SYT–ChAT co-immunostaining analysis. Since ChAT is expressed only in sensory neurons, SYT–ChAT co-accumulations can only occur in sensory neuron axons. In addition, most (~95%) of ChAT accumulations along the length of the nerves co-immunostain with SYT, supporting a view that most ChAT negative SYT accumulations occur in motor axons. Co-immunostaining demonstrated that 71% of anterior SYT co-accumulations are ChAT positive. Thus, most anterior SYT accumulations are occurring in the distal regions of sensory axons and not the proximal region of motor axons. In contrast, only 16% of the posterior SYT accumulations are ChAT positive. Thus, most of the posterior SYT accumulations are likely occurring in the distal regions of motor axons and not the proximal regions of sensory axons. Therefore, the combined observations of an anterior–posterior accumulation...
frequency gradient, the majority of anterior SYT accumulations occurring in sensory axons, whereas the majority of posterior SYT accumulations occurring in motor axons, demonstrates that there is a strong propensity for synaptic axonal cargo accumulation to occur in the distal regions of axons in roadblock mutants.

Comparative analysis of robl null, robl1 hemizygous, and robl2 homozygous nerves revealed that as the number of robl2 alleles is increased, the number of observed SYT and ChAT accumulations decreased. The robl2 homozygous larvae have fewer axonal accumulations, ranging from ~1-5% than that observed for hemizygotes (data not shown). A similar distal enrichment in accumulations is observed for robl1 homozygotes, as has been described above for robl2 hemizygotes. Homozygous robl null larvae show a significant increase in axonal accumulations, ranging from ~200-400% than that observed for hemizygotes (data not shown). However, the ChAT accumulations in robl null homozygotes appear more uniformly distributed, despite obvious distal-enriched SYT accumulations. Perhaps, the large number of axonal accumulations observed in the robl nulls obscures the distal bias; alternatively, sensory neuron axons (ChAT positive axons) may be affected differently in robl nulls.

**robl Mutants Have Massive Axonal Loss and Nerve Degeneration**

We used EM to examine the morphology of the axonal swellings in segmental nerves from robl mutants. Previously, transmission EM of larval segmental nerves from khc mutants revealed that these massive axonal swellings are filled with all types of identifiable axonal cargo (Hurd and Saxton, 1996). The nerves of robl+/null (hemizygote) larvae also contain swollen axons that have become filled with axonal cargo (Fig. 6, A and D). These swollen axons are on average twice the diameter of the largest axon observed in wild type (Fig. 6 B). While the axonal swellings observed in khc mutants vary in size, their content characteristics are uniform, containing all observed membrane bound axonal content (Hurd and Saxton, 1996). In addition to these multicomponent axonal accumulations (Fig. 6 D), robl mutants also have a small subset of single component axonal accumulations (Fig. 6 C). These single compo-
component accumulations contain almost exclusively small clear vesicles and tend to be smaller on average than the multi-component accumulations. These small clear vesicles may represent a class of cargo that is particularly sensitive to retrograde transport failure in rob1 mutants. In support of this idea, when the synaptic area is examined by EM, there is an approximate twofold increase in the number of similar appearing small clear vesicles observed (data not shown).

The rob1 mutants also have severe axonal loss and nerve degeneration that is not observed in khc mutants, despite the fact that khc mutant axonal swellings are more numerous and on average twice the size of those observed in rob1 (Hurd and Saxton, 1996). All observed rob1 hemizygous larvae show at least mild axonal loss (Fig. 6A). When the segmental nerves from the most severely sluggish rob1 hemizygous larvae are analyzed by EM, extensive axonal loss and nerve degeneration is observed (Fig. 6E). Furthermore, the segmental nerves from rob1 homozygous larvae always show extensive axonal loss and nerve degeneration (Fig. 6F). The basis for this axonal loss and nerve degeneration is unclear; however, we have observed a few large multilamellae structures (~1/10 nerve diameter) indicating a possible phagocytic component to the axonal loss and nerve degeneration (data not shown).

**roadblock Is a Severe Mitotic Mutant and Female Sterile Mutation**

The first indication of a mitotic defect in rob1 mutants was the observation of a complete absence of the mitotically active tissues (imaginal tissues) in rob1 homozygous larvae. Additionally, rob1 hemizygous and rob1 null animals that survive into late pupal stages, demonstrate rough pupal eyes (Fig. 7L), missing bristles (data not shown), and reduced size of imaginal tissue (data not shown). These observations are consistent with a mitotic defect in *Drosophila*.

To examine the mitotic defect further, third instar untreated (no hypotonic or colchicine treatment) larval brain squashes were performed. This procedure permits observation of dividing neuroblasts within the larval central nervous system by staining with a fluorescent DNA dye and allows quantitation and characterization of the mitotic figures. The analysis revealed significant mitotic defects in rob1 hemizygous larvae. Numerous polyploid mitotic fig-
ures were observed (Fig. 7, D and E). Additionally, many of the polyplod figures showed hypercondensation of their chromosomes (Fig. 7 F). An abnormal anaphase figures were also observed with hypercondensed chromosomes and disorganization of the chromosomes around the pre- sumptive poles (Fig. 7 G). As anticipated, since the mutant survives until late pupal stages, apparently normal mitotic figures were also observed (not shown). The mitotic index in this mutant is fivefold higher than wild type (Fig. 7 K). This increased mitotic index is due to an increased number of figures from all mitotic phases counted (prometaphase, metaphase, and anaphase). A n elevated mitotic index for all phases, coupled with the variety of defective structures suggests defects in multiple stages of mitosis.

Larval brain squash analysis on the robl homozygotes also revealed a profound mitotic defect; in addition to the lack of imaginal tissue, there is a striking absence of prometaphase and metaphase mitotic figures. Only infrequent defective anaphase and telophase figures are seen. The few anaphase figures have severe bridging and lagging chromosomes (Fig. 7, H and I). In addition, we observe apparent telophase bridging in which DNA has become trapped between two dividing nuclei (Fig. 7 J). The failure to observe any prometaphase or metaphase figures prompted us to perform a larval brain squash on colchicine-treated brains. This procedure, which blocks cells in metaphase, resulted in an approximate doubling of the observed number of metaphase figures and a decrease in the observed frequency of postmetaphase figures in wild-type controls. However, in robl homozygotes, we observed a prometaphase or metaphase figure in treated third instar larval brains, yet the low frequency of observed defective anaphase and telophase figures remained unchanged from untreated brains. These data strongly suggest that third instar robl homozygote larvae lack cells capable of division and the few figures observed represent cells arrested in mitosis.

Female robl hemizygous flies rescued to adulthood by the 6XH is-tagged cDNA construct under heat shock promoter control show a female sterile phenotype. However, this same allelic combination is fully rescued by the robl genomic rescue construct, presumably under native robl promoter control. Female sterility is commonly observed in mutants of cytoplasmic dynein components in Drosophila (Phillis et al., 1996; McRae and Hays, 1997). Attempts to rescue the robl sterility phenotype by giving the cDNA-rescued females mild heat shock (to induce expression of robl) failed. Since the genomic construct fully rescues the female fertility defect, female sterility is likely a real robl mutant phenotype; robl cDNA under heat shock control is likely failing to provide appropriate levels of robl protein in the needed cells because of the inadequacy of non-native promoter control.

**Discussion**

We have identified a new family of axonemal- and cytoplasmic dynein-associated proteins. This family was identified by two independent means: the biochemical isolation and cloning of the Chlamydomonas outer arm dynein--associated LC7 polypeptide and the identification and cloning of a Drosophila axonal transport mutant, roadblock. Our discovery of a new family of DLCs with roles in axonal transport, flagellar motility, and mitosis has intriguing implications.

**The Structural Organization of Dyneins**

With this report, all the known components of Chlamydomonas outer arm dynein have now been sequenced and a complete list of the properties of outer dynein arm--associated DLCs can be made (Fig. 9 A). The outer dynein arm consists of three heavy chains that form the globular heads and stems of the particle. Each heavy chain is tightly associated with one or more light chains. Located at the...
base of the structure are two intermediate chains (IC1 and IC2) and several additional light chains including a member of the Tctex1 protein family (LC2) together with multiple copies of the LC8 polypeptide and its homologue LC6. The LC7 protein is not tightly associated with any heavy chain and appears to form part of the intermediate-light chain complex located near the base of the particle (Mitchell and Rosenbaum, 1986).

Examination of outer armless mutants revealed that a very small amount of the LC7 protein was still incorporated into the particle. A robl/LC7-like protein is present in cytoplasmic dynein. (A) Western blot analysis was performed on samples from the fractionation of a rat brain homogenate. Blots were probed with mAb 74-1 and the R7178 rabbit polyclonal to detect IC74 of cytoplasmic dynein and the M_r ~12,000 robl/LC7-like protein, respectively. (B) Rat brain proteins eluted from microtubules with ATP were sedimented in a 5–20% sucrose gradient. Samples were probed with the 74-1 and R7178 antibodies. The robl/LC7-like protein was detected only in the cytoplasmic dynein (IC74) sample. (C) Examination of outer armless mutants revealed that a very small amount of the LC7 protein was still incorporated into the cytoplasmic dynein (IC74) sample. (D) Drosophila embryo immunoprecipitates of cytoplasmic dynein (IC74) and the robl/LC7-like protein were precipitated with the 74-1 antibody and with 74-1 anti-IC74 antibody. The robl/LC7-like protein was only precipitated in the cytoplasmic dynein (IC74) sample. (E) Equally loaded Drosophila robl null and wild-type larval homogenates were probed with the 74-1 anti-IC74 antibody and with the 3A5 anti-tubulin antibody. The robl/LC7-like protein is detectable only in the robl null larvae, whereas tubulin is detected at about equal levels in null and wild-type larvae.

Figure 8. A robl/LC7-like protein is present in cytoplasmic dynein. (A) Western blot analysis was performed on samples from the fractionation of a rat brain homogenate. Blots were probed with mAb 74-1 and the R7178 rabbit polyclonal to detect IC74 of cytoplasmic dynein and the M_r ~12,000 robl/LC7-like protein, respectively. (B) Rat brain proteins eluted from microtubules with ATP were sedimented in a 5–20% sucrose gradient. Samples were probed with the 74-1 and R7178 antibodies. The robl/LC7-like protein was detected only in the cytoplasmic dynein (IC74) sample. (C) Examination of outer armless mutants revealed that a very small amount of the LC7 protein was still incorporated into the cytoplasmic dynein (IC74) sample. (D) Drosophila embryo immunoprecipitates of cytoplasmic dynein (IC74) and the robl/LC7-like protein were precipitated with the 74-1 antibody and with 74-1 anti-IC74 antibody. The robl/LC7-like protein was only precipitated in the cytoplasmic dynein (IC74) sample. (E) Equally loaded Drosophila robl null and wild-type larval homogenates were probed with the 74-1 anti-IC74 antibody and with the 3A5 anti-tubulin antibody. The robl/LC7-like protein is detectable only in the robl null larvae, whereas tubulin is detected at about equal levels in null and wild-type larvae.

Figure 9. Summary of dynein-associated proteins. (A) A complete table of outer dynein arm-associated light chains is shown. The nominal mass refers to the M_r determined by SDS-PAGE analysis; actual predicted mass of the proteins is given parenthetically. Previous work has elucidated biochemical interactions amongst these proteins. (B) A model of cytoplasmic dynein organization is shown. The cytoplasmic dynein particle is built around two heavy chains that form the stems and globular heads of the complex. Associated with the stems are a series of accessory proteins including: two IC74 intermediate chains that mediate dynein–dynactin interactions, two copies of the Tctex1 light chain (or of the related rp3 protein), one dimer of the highly conserved 10 kD/LC8 DLC (LC8 dimer), and a 22-kD polypeptide (the location of which is speculative). The present study indicates that cytoplasmic dynein contains a robl/LC7-like protein that, by analogy with flagellar outer arm dynein, is located at the base of the dynein particle. A table of known proteins associated with cytoplasmic dynein is given; the majority are conserved in axonemal dyneins.
rated into the axoneme. The origin of this pool remains unclear at present. It did not appear to derive from inner arm I1 as it could not be detected in salt extracts of axonemes lacking outer arms. It may represent a small pool of LC7 that is mistransported to the axoneme in the absence of the remainder of the outer arm. Alternatively, it may be associated with some other axonemal enzyme such as the DHC1b-like dynein that is responsible for retrograde intraflagellar transport (Pazour et al., 1999; Porter et al., 1999).

The cytoplasmic dynein particle is built around two ~520-kD heavy chains that form the stems and globular heads of the complex. A associated with the stems are a series of accessory proteins (Fig. 9 B). These are now known to include two IC74s, two copies of the Tctex1 light chain (or of the related rp3 protein), one dimer of the highly conserved LC8 protein, and perhaps a 22-kD polypeptide (the position of which is speculative). The present study indicates that cytoplasmic dynein also contains a robl/LC7-like protein. Since axonemal and cytoplasmic dyneins utilize homologous intermediate chain genes, it is likely that robl/LC7 associates with IC74. By analogy with flagellar outer arm dynein, we propose a cytoplasmic dynein organizational model where robl/LC7 is located at the base of the dynein particle (Fig. 9 B).

**Cellular Functions that Require the robl/LC7 Family of Proteins**

Previous work has provided strong evidence that cytoplasmic dynein plays an important role in retrograde axonal transport (for review see Hirokawa, 1998). Recent work in Drosophila has directly demonstrated that cytoplasmic dynein and dynactin play essential roles in retrograde axonal transport (Martin, M.A., personal communication). The distal enrichment of axonal accumulations that we observe in robl mutants is consistent with a defect in axonal transport that initiates at the synapse. One intriguing explanation for the nerve degeneration seen in robl mutants is a failure of the retrograde transport pathway mediating the transport of neurotrophic signals from the synapse to the neuronal cell body (Johanson et al., 1995; Bhattacharyya et al., 1997). The massive axonal loss and degeneration seen in robl mutants, but not in anterograde axonal transport mutants (such as khc), may indicate a specific inability of these factors to reach the cell body in robl mutants. In this context, it is striking that a subset of axonal swellings in robl mutants are filled predominantly with small vesicles (Fig. 6 C). This observation is consistent with recent work suggesting a distinct class of small vesicles, resembling transport vesicles, may be the carrier of the retrograde neurotrophic signal of nerve growth factor-activated receptor tyrosine kinase, TrKA (Grimes et al., 1997). Together, these data demonstrate that dynein is required for retrograde axonal transport in vivo.

Dynein is also thought to play a role in chromosome alignment and mitotic spindle assembly (Eshel et al., 1993; Li et al., 1993; Merdes et al., 1996). Overexpression of the p50 subunit of dynactin disrupted chromosome alignment, causing cells to accumulate in a prometaphase-like state (Echeverri et al., 1996). Additionally, anti-dynein antibody injection experiments blocked the formation of spindles in prophase (Vaisberg et al., 1993). The excessive number of chromosomes observed in aneuploid mitotic figures from robl mutants suggests a role for dynein in ensuring proper chromosome inheritance. However, the lack of prometaphase and metaphase figures in robl mutants, and an accumulation of defective anaphase and telophase figures, suggests that robl-dependent dynein function in metaphase spindle alignment and assembly is not required for entry into anaphase. ZW10 mutants, which fail to localize dynein to the kinetochore, also exhibit anaphase defects, and has led to the suggestion that an absence of kinetochore-associated dynein function may allow a bypass of the wait anaphase checkpoint (Starr et al., 1998).

A role for dynein in the later stages of mitosis remains controversial. Cytoplasmic dynein heavy chain antibody injection experiments in mammalian cells failed to identify a role for dynein in anaphase chromosome movements (Vaisberg et al., 1993). Yet, dynein has been implicated in anaphase B spindle elongation in Saccharomyces cerevisiae (Saunders et al., 1995). The anaphase and telophase chromosome bridging and chromosome lagging in robl mutants suggests a role for dynein in anaphase chromosome segregation. A possible explanation for the apparent late mitotic phenotype observed when robl is disrupted may be redundant mitotic motors. In fact, it is only in the triple motor mutant (Cin8p Kip1p Dyn1p) of S. cerevisiae that the role of dynein in anaphase B spindle elongation is revealed (Saunders et al., 1995). Perhaps complete loss of dynein function (expected for heavy chain mutants and anti-heavy chain injection experiments) allows redundant motors to perform dynein’s role in chromosome segregation. However, a mutation of the dynnein-associated protein robl/LC7 may not abolish dynein function and instead result in aberrant dynein activity, which could interfere with the ability of redundant motors to compensate. Alternatively, the anaphase defects of robl mutants may result from pre-anaphase mitotic spindle assembly defects that are not detected by checkpoint controls.

ESTs belonging to two mammalian robl/LC7-like gene classes have been found from a wide assortment of embryonic, adult, and germline tissues (Fig. 4 A). We identified >100 independent human ESTs in dbEST that encode a robl/LC7-like gene belonging to the first class (e.g., accession number hum424E02B). These ESTs are found from a wide array of tissues with unique and heavy intracellular transport needs such as: neural tissues (fetal and adult brain and retina), tissues with a heavy transcytosis burden (liver, spleen, kidney, placenta, and breast), a tissue involved in pigment dispersion (melanocyte), and mitotically active tissues (fetal and tumor tissues). A iso, the rat robl/LC7-like gene from this first class was identified in the NCBI GenBank as being expressed in light-stimulated visual cortex (accession number 3288881). The robl/LC7-like gene identified by nine independent human ESTs of the second class (e.g., accession number AA446298) were found in a smaller subset of tissue types. This second class is found in human testes (6 of 9 clones) and tumor tissues (germ cell and kidney tumor tissues). Perhaps the testes expression may indicate a role for the second class with axonemal dynein, whereas the broad tissue expression may indicate a role for the first class with cytoplasmic dynein.

Together, the genetic and expression analyses suggest...
that the robl/LC7 family is important for many aspects of intracellular transport. In Drosophila, the mutant phenotypes found thus far suggest that the robl gene is required for axonal transport and mitosis. In addition, the female sterility defect seen in some genetic combinations suggests a role for robl in oocyte development. This finding is consistent with previous evidence that dynein plays a role in oocyte differentiation in Drosophila (McGraw and Hays, 1997). In Chlamydomonas, the presence of LC7 in outer arm axonemal dynein suggests a role in flagellar motility. Finally, the expression inferred from human EST tissue sources within non-neural quiescent adult human tissues suggests that robl/LC7-like proteins may have a wider role than has been suggested thus far by the Drosophila mutant phenotypes.

Possible Roles of the robl/LC7 Family in Dynein Function

Our work on robl/LC7 adds to a growing body of evidence supporting modulatory roles for DLC proteins in dynein-mediated movements. Specifically, the observation that DLC phenotypes are not as severe as dynein heavy chain phenotypes, the structural placement of DLCs at key positions in dynein, and the nonequivalent phenotypes among DLC mutants, supports this view. For example, other than female sterility, robl mutants have no apparent phenotypic similarities to the 10-kD/LC8 DLC (ddlc1) mutants in Drosophila (Dick et al., 1996; Phillips et al., 1996). In addition, some evidence suggests that Tctex1 associates with only a subset of cytoplasmic dynein, indicating it is used for only a subset of dynein’s functional roles (Tai et al., 1998). Intriguingly, the Tctex1-related protein rp3 may be associated with a cytoplasmic dynein population that does not contain Tctex1 (King et al., 1998). It is unclear whether each DLC plays a specific role in a subset of dynein functions or whether each DLC contributes generically to the functional roles of dynein.

In view of the dynein intermediate chains’ possible structural role in linking motor activity to cargo binding activity, they may be a key regulatory target of the dynein complex. For example, IC74 mediates the binding of dynein to dynactin via a direct interaction with the p150

References


Downloaded from jcb.rupress.org on December 23, 2017
Bowman et al. roblLC7: Dynein-associated Proteins