Kinesin Light Chains Are Essential for Axonal Transport in *Drosophila*

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Abstract. Kinesin is a heterotetramer composed of two 115-kD heavy chains and two 58-kD light chains. The microtubule motor activity of kinesin is performed by the heavy chains, but the functions of the light chains are poorly understood. Mutations were generated in the *Drosophila* gene *Kinesin light chain* (*Klc*), and the phenotypic consequences of loss of *Klc* function were analyzed at the behavioral and cellular levels. Loss of *Klc* function results in progressive lethargy, crawling defects, and paralysis followed by death at the end of the second larval instar. *Klc* mutant axons contain large aggregates of membranous organelles in segmental nerve axons. These aggregates, or organelle jams (Hurd, D.D., and W.M. Saxton. 1996. *Genetics.* 144: 1075–1085), contain synaptic vesicle precursors as well as organelles that may be transported by kinesin, kinesin-like protein 68D, and cytoplasmic dynein, thus providing evidence that the loss of *Klc* function blocks multiple pathways of axonal transport. The similarity of the *Klc* and *Khc* (Saxton et al. 1991. *Cell* 64:1093–1102; Hurd, D.D., and W.M. Saxton. 1996. *Genetics* 144: 1075–1085) mutant phenotypes indicates that KLC is essential for kinesin function, perhaps by tethering KHC to intracellular cargos or by activating the kinesin motor.

Intracellular transport requires the action of molecular motors that bind cargo and generate movement coupled to ATP hydrolysis along cytoskeletal filaments (Gibbons et al., 1994; Bloom and Endow, 1995; Mooseker and Cheney, 1995). One type of motor is exemplified by kinesin (Vale et al., 1985; Brady, 1985), which plays an integral role in intracellular transport along microtubules in many cell types (Goldstein, 1993; Vale and Fletterick, 1997). Native kinesin is a heterotetramer composed of two copies each of two polypeptide chains: kinesin heavy chain (KHC)[1] and kinesin light chain (KLC; Bloom et al., 1988; Kuznetsov et al., 1988; Johnson et al., 1990). KHC contains the motor domain (Penningroth et al., 1987; Bloom et al., 1988; Hirokawa et al., 1989; Scholey et al., 1989; Yang et al., 1989), which is sufficient to generate ATP-dependent forces along microtubules (Kuznetsov et al., 1989; Yang et al., 1990), whereas KLC is located in the non-motor tail domain of kinesin (Hirokawa et al., 1989; Gauger and Goldstein, 1990). Because KLC is located in the presumptive cargo-binding domain of kinesin, it has been suggested to play some role in mediating the interactions of the kinesin motor with its intended cargo. To date, however, little evidence to support this view has been obtained.

Secondary structure analyses predict that KLC participates in diverse protein–protein interactions. The amino terminal region of KLC is predicted to form an alpha-helical coiled coil that links KLC to KHC (Cyr et al., 1991; Gauger and Goldstein, 1993). The carboxy-terminal region of KLC is largely made up of six repeated units that are predicted to form tetratrico peptide repeat (TPR) domains (Gindhart and Goldstein, 1996) that are protein–protein interaction motifs identified in a diverse group of proteins (Lamb et al., 1995; Sikorski et al., 1990). Other proteins to which KLC might bind are not known, but a potential kinesin-binding protein is kinectin, the proposed kinesin receptor (Toyoshima et al., 1992; Futterer et al., 1995; Kumar et al., 1995; Yu et al., 1995).

Experimental evidence suggests that KLC may play one of two roles. One possibility is that KLC may be a positive factor necessary for kinesin–cargo binding (Cyr et al., 1991; Gauger and Goldstein, 1993; Stenoien and Brady, 1997). Alternatively, KLC might play a role in the negative regu-

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1. *Abbreviations used in this paper*: CSP, cysteine string protein; DHC, dynein heavy chain; KHC, kinesin heavy chain; KLC, kinesin light chain; TPR, tetratricopeptide repeat; KLH, keyhole limpet hemocyanin; SYT, synaptotagmin; TPR, tetratrico peptide repeat.

lation of kinesin activity, such that kinesin is inactive in the presence of KLC, but active in its absence or when its function is attenuated by cargo binding (Hackney et al., 1991, 1992; Matthies et al., 1993; Jiang and Sheetz, 1995). One recent attempt to test these hypotheses supported the view that KLC might be needed for cargo attachment of kinesin (Stenoien and Brady, 1997); however, additional data are clearly needed. To gain a better understanding of KLC function, we generated mutations in the *Drosophila* gene encoding KLC, studied the phenotypic consequences of loss of KLC function at the organismal and cellular level, and tested the positive and negative hypotheses of KLC function. We demonstrate that KLC is essential for axonal transport in *Drosophila* larva, and that locomotion defects associated with the loss of KLC function are a consequence of the disruption of multiple axonal transport pathways.

Materials and Methods

Identification of Klc Mutants

Identification of the P[*lacW*] (Bier et al., 1989) insertion *59A* in *Klc* intron 1 was described previously (Desai et al., 1996). The location of P[*lacW*] within *Klc* was determined by sequencing genomic DNA flanking the insertion site, examining the size of PCR-generated DNA fragments using combinations of primers hybridizing to P[*lacW*] and KLC cDNA sequences to amplify *59A* genomic DNA template, and by fine-structure restriction fragment length polymorphism mapping of *59A* genomic DNA using cloned KLC genomic sequences as probes. The lethal P-element insertion P[*lacW*]*l(3)A5-3-42* (Hartenstein and Jan, 1992) used for insertional mutagenesis of *Klc* was still present on the *59A* mutant chromosome. To remove P[*lacW*]*l(3)A5-3-42* from this chromosome, *w*; *59A*/*TM3* males were crossed to *w*; *Dr*/*TMS*, *Sb* Δ*2-3* females (Robertson et al., 1988). Dysgenic F_1 female progeny were crossed to *w*; *TM3, Sb*/*TM6B Hu Tb* males, and flies harboring the P[*lacW*] element in *Klc*, but not P[*lacW*]*l(3)A5-3-42*, were detected by alteration of the patterned *59A*-specific eye color. Putative *l(3)A5-3-42* revertants were crossed to P[*lacW*]*l(3)A5-3-42*/*TM3*, *Sb* to determine whether the P[*lacW*] element present in the *l(3)A5-3-42* complementation group had excised precisely. A *59A* derivative, Klc^1, lacks *l(3)A5-3-42*, but contains the P[*lacW*] insertion in *Klc*.

Small deletions of *Klc* as well as *Klc* revertants were generated by remobilizing the Klc^1 insertion, then screening for loss of the w^+ phenotype encoded by P[*lacW*]. These flies were backcrossed to Klc^1/*TM6B* for complementation tests. Deletion breakpoints were identified by Southern hybridization of genomic DNA from deletion mutants to cloned fragments of *Klc* genomic DNA and cDNA. Approximately 70% of the Klc^1 excision events tested (74/104) failed to complement Klc^1.

Transgenic Rescue Constructs

The transgenic construct GEN-KLC is composed of a 12.4-kb EcoRI-NotI fragment from KLC cosmid 8.1 (Fig. 1) subcloned into pCaSPeR4 (Pirrotta, 1988) cut with EcoRI and NotI. This DNA fragment contains the *Klc* coding region, ~5 kb of 5′ regulatory sequences, and 3 kb 3′ of *Klc* (see Fig. 1), but not *Ptp69D*. MYC-KLC was constructed by cutting pBS13a (Gauger and Goldstein, 1993) with DrdI and EcoRI, and then ligating a double-stranded linker DNA to the DrdI site at the 5′ end of the KLC cDNA (sequence of primer 1:5′AATTCCATGACGCAA3′; sequence of primer 2:3′GGTACTGCG5′). The linker DNA encodes amino acids 1–3 of KLC, and provides an EcoRI restriction site in the same translation frame as the EcoRI site in pWUM (Heck et al., 1993). The sequence tag amino-terminal of the KLC coding region in MYC-KLC is MEQKLISEEDLNS. This sequence is recognized by the anti-MYC antibody 9E10 (Evan et al., 1985). Fusion protein transcription is controlled by the *Drosophila* polyubiquitin promoter pUP2 (Lee et al., 1988), which ensures high-level expression in all cells. Rescue constructs were injected into *y* w^{1118} embryos with helper plasmid pπ25.7wc (Rubin and Spradling, 1982) using conventional techniques (Robertson et al., 1988).

Lethal Phase Analysis

To determine the lethal phase of *Klc* mutant and control larvae, flies of the appropriate genotypes were placed in 8-oz. egg collection chambers. Egg collections (0–22h) were made on Karo-agar (9.5% Karo corn syrup; Best Foods, Englewood Cliffs, NJ, 3.4% agar) plates supplemented with yeast paste. All mutants were balanced by the chromosome *TM6B*, *Hu Tb*, which permits discrimination of short *Tubby* heterozygous larvae from longer non-*Tubby* larvae. 2 d after egg laying, mutant and control second instar larvae were placed in groups of 50 onto 60-mm Karo-agar plates supplemented with yeast paste. 2 d later, the numbers of surviving and dead larvae for each genotype were noted, and survivors were placed onto fresh plates to limit bacterial contamination. This procedure was repeated until pupation. Larvae were kept in a humid chamber at 25°C.

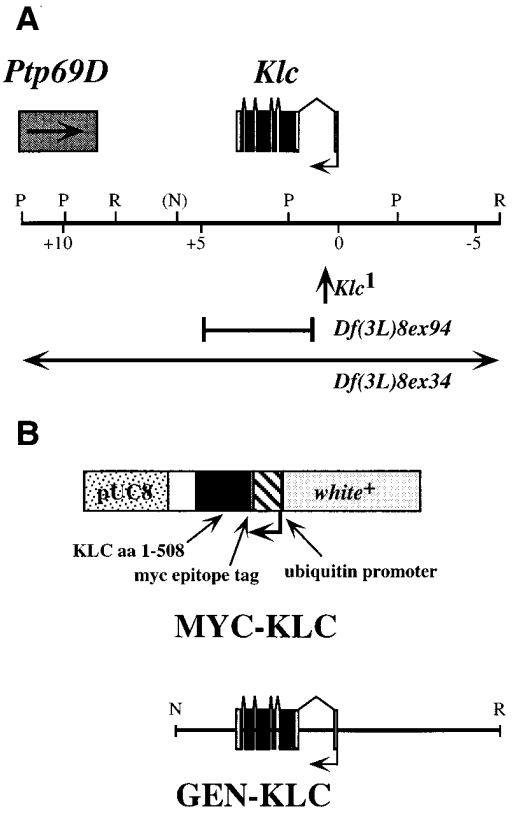

Figure 1. (*A*) Map of *Klc* genomic interval and location of *Klc* mutants used in this analysis. The *Klc* transcription unit is composed of six exons, and is transcribed from right to left in this diagram. *Ptp69D* (Desai et al., 1996) is located 3′ of *Klc*, and is transcribed toward *Klc*. Map coordinates are shown in kb. Restriction sites are shown as follows: *P*, PstI; *R*, EcoRI; *N*, the NotI site in cosmid 8.1 used for subcloning GEN-KLC (see Materials and Methods). The location of P[*lacW*] in *Klc* intron 1 is shown as an upward pointing arrow. Sequences deleted by the lesions *Df(3L)8ex94* and *Df(3L)8ex34* are shown as solid lines. The endpoints of *Df(3L)8ex94* are shown; the endpoints of *Df(3L)8ex34* are unknown. (*B*) Maps of KLC transgenic constructs MYC-KLC and GEN-KLC. The MYC-KLC transgene is composed of KLC amino acids 1–508 fused to a 13–amino acid epitope tag recognized by the anti-MYC monoclonal antibody 9E10 (Evan et al., 1985). This fusion protein is under the transcriptional control of the Drosophila polyubiquitin promoter, which ensures high levels of transgene expression in all tissues (Lee et al., 1988). The map position of the NotI-EcoRI genomic DNA fragment included in GEN-KLC is also shown.

Antisera Production and Immunoblotting

Polyclonal rabbit antisera were generated against the peptide sequence CLTRAHEKEFGK (KLC-LG3), corresponding to amino acids 381–390

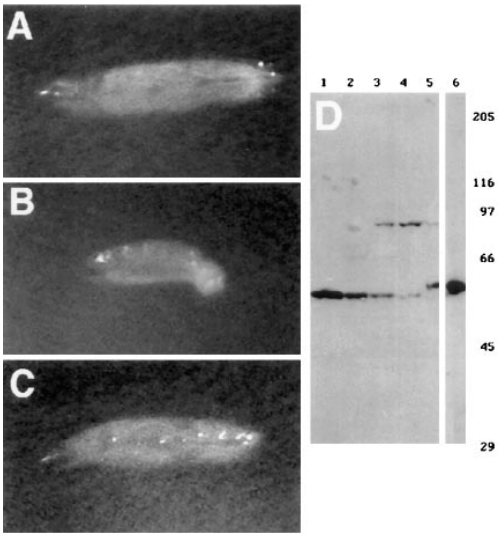

Figure 2. (*A–C*). *Klc* mutant larvae have locomotion defects that are rescued by GEN-KLC. Anterior is to the left, and the dorsal surface of the larva is shown. (*A*) Wild-type and *Df(3L)8ex94*/+ (shown) larvae crawl using peristaltic waves of muscle contraction. Most if not all of the ventral surface remains on the medium. (*B*) *Klc* mutant larvae of the genotype *Df(3L)8ex94*/*Klc*^{1} (shown) and other hypomorphic *Klc* mutant combinations flip their posterior end off the surface during crawling. This phenotype is quite similar to the tail flipping phenotype observed in certain *Khc* mutant combinations (Hurd and Saxton, 1996). (*C*) GEN-KLC rescues the tail-flipping phenotype. GEN-KLC; *Df(3L)8ex94*/*Klc*^{1} larvae exhibit normal larval crawling behavior (compare wild-type [*A*] to [*C*] rescued larvae). The MYC-KLC transgene also rescues the *Klc*-dependent tail flipping phenotype (not shown). (*D*) Characterization of KLC antisera at different *Drosophila* life stages. Total protein was extracted from wild-type individuals at the following life stages: 0–6 h embryos (lane *1*); third instar larvae (lane *2*); adult females (lane *3*); adult males (lane *4*); and adult males transformed with MYC-KLC (lanes *5* and *6*). Lanes *1–5* were incubated with affinity-purified KLC antisera, and lane *6* was incubated with an antibody recognizing the epitope tag of MYC-KLC. The epitope tag causes MYC-KLC to have an apparent molecular weight slightly larger than 58 kD, the molecular weight of *Drosophila* KLC (Gauger and Goldstein, 1993). Lane *6* shows that the epitope-specific antibody recognizes a protein of the same apparent molecular weight as KLC (lane *5*) in MYC–KLC transformants. The adult-specific 91-kD protein may not be a KLC isoform, as it is recognized by antisera specific to KLC-LG3. peptide coupled to KLH, but not other KLC antisera (see Materials and Methods). Molecular weight standards are shown in kD (*right*).

of *Drosophila* KLC (Gauger and Goldstein, 1993). This region is highly conserved among KLCs cloned from diverse species (Cyr et al., 1991; Gauger and Goldstein, 1993; Wedaman et al., 1993; Beushausen et al., 1993; Cabeza et al., 1993; Fan and Amos, 1994; Chernajovsky et al., 1996). The peptide was linked to BSA or keyhole limpet hemocyanin (KLH). Antisera production was provided by BAbCO (Richmond, CA). Affinity purification of antisera KLC-LG3-BSA and KLC-LG3-KLH was performed by linking the NH_2-terminal cysteine of KLC-LG3 to a SulfoLink™ column (Pierce Chemical Co., Rockford, IL), and then purifying KLC-LG3-specific antisera according to the manufacturer's recommendations. Working dilutions of KLC-LG3 antisera are 1:100 for immunoblotting and immunohistochemistry (Fig. 2 *c*).

Rabbit polyclonal anti-KHC (Saxton et al., 1988) was affinity-purified (Olmsted, 1981) against bacterially expressed full-length KHC as follows: BL21(DE3) cells (Novagen, Inc., Madison, WI) containing pET-KIN were induced, harvested, lysed, and centrifuged according to Yang et al. (1990). A portion of the insoluble pellet containing high levels of KHC was analyzed by SDS-PAGE and then transferred to polyvinylidine difluoride membrane (Bio-Rad Laboratories, Hercules, CA). The band corresponding to KHC was excised from the blot, and then incubated with 1 ml of a 1:100 dilution of anti-KHC crude sera in TBST (15 mM Tris-Cl, 150 mM NaCl, 0.1% Tween-20, pH 7.5) for 2 h at room temperature. After washing, KHC-specific antibodies were eluted with 100 mM glycine, pH 2.5. The antibody solution was neutralized with 1 M Tris-Cl, pH 8.8, and FCS was added to 5%. The affinity-purified antibody was dialyzed overnight at 4°C with PBS in 50% glycerol. Affinity-purified anti-KHC was used at 1:10 for immunostaining.

Western blot analysis of KLC antisera on total fly protein (Fig. 2 *c*) was performed as follows: *Drosophila* embryos (10 μl), two third instar larvae, two adult females, three adult males, and three MYC-KLC transformant males were homogenized in 40 μl 5× protein gel loading buffer (Laemmli, 1970) and boiled for 3 min. Next, 10 μl of each homogenate were loaded onto a 10% SDS-PAGE gel, and then electrophoresed 4 h at 25 mA. Western transfer and detection were performed according to Barton et al. (1995). KLC antisera was used at a dilution of 1:100, and anti-MYC monoclonal antibody 9E10 (Evan et al., 1985) was used at a 1:1,000 dilution. Goat anti-mouse-HRP and goat anti-rabbit-HRP secondary antibodies (Cappel, Durham, NC) were used at a 1:20,000 dilution. The ECL kit (Nycomed Amersham Inc., Princeton, NJ) was used for secondary antibody detection.

Immunochemistry

Wild-type and mutant larvae were stained according to Hurd and Saxton (1996), with a few modifications. Larvae were dissected in dissection medium (64 mM NaCl, 2 mM $CaCl_2$, 2 mM $MgCl_2$, 1 mM KCl, 2.5 mM Hepes, pH 7.2, 18 mM sucrose) by pinning the anterior and posterior ends of the larvae with stainless steel pins onto a Sylgard™ (Precision Instruments, Sarasota, FL)-coated 35-mm petri plate, and then cutting along the dorsal surface with retinal scissors. Gut and fat body were removed, and the dorsal cuticle was pinned to the Sylgard plate. Larvae were fixed with 4% formaldehyde (Ted Pella, Inc., Redding, CA) in dissection buffer. Fixation conditions were 30 min at room temperature with five buffer changes. Fixed larvae were washed with antibody incubation buffer (PBS, 0.1% Triton X-100, 2% FCS) for 40–60 min at room temperature. All primary antibody incubations were performed overnight at 4°C. Secondary antibody incubations were 1–2 h at room temperature. Antibody washes were 40–60 min at room temperature in antibody incubation buffer. Primary antibodies used in this analysis include: affinity-purified rabbit polyclonal anti-KLC at 1:100, affinity-purified rabbit polyclonal anti-KHC at 1:10 (Saxton et al., 1988), rabbit polyclonal antisynaptotagmin at 1:500 (Littleton et al., 1993), mouse monoclonal anti-cysteine string protein at 1:20 (Zinsmaier et al., 1994), affinity-purified rabbit polyclonal anti-KLP68D at 1:20 (Pesavento et al., 1994), and mouse monoclonal anti–dynein heavy chain (DHC) at 1:1,000 (McGrail and Hays, 1997). FITC and Texas Red–conjugated goat anti–rabbit and goat anti–mouse secondary antibodies (Cappel) were used at a 1:200 dilution. All secondary antibodies were tested for cross-reactivity with fixed Drosophila tissue. Stained larvae were mounted in 90% glycerol, 100 mM Tris-Cl, pH 9.5, 2% *N*-propyl gallate.

Confocal and Video Microscopy

Samples were examined using a MRC 1000 confocal microscope (Bio-Rad Laboratories). Images were observed with a 40× oil immersion objective on an Optiphot™ (Nikon, Inc., Melville, NY) inverted microscope. The iris setting was 3, and the zoom setting was 3.5. Crawling behavior of wild-type and *Klc* mutant larvae was videotaped at 20× using a video camera mounted on a dissection microscope. Images were captured from videotape using a Snappy™ video frame grabber (Minolta, Ramsey, NJ).

Image files were prepared using Photoshop v.3.0 and 4.0 (Adobe Systems, San Jose, CA) and Canvas v.3.5 (Deneba Software, Miami, FL) on various Apple Macintosh systems. Images were printed on a Phaser IISDX™ printer (Tektronix. Inc., Beaverton, OR).

Results

KLC Function is Essential for Drosophila Development

To generate mutations in the previously cloned *Klc* gene at chromosome band 69D (Gauger and Goldstein, 1993), we screened for the insertion of a nearby *P* transposable element into the *Klc* interval. This approach, known as

P-element–directed mutagenesis (Cooley et al., 1988; Tower et al., 1993; Zhang and Spradling, 1993; Dalby et al., 1995), is a powerful tool for identifying mutations in a previously cloned gene without regard for its mutant phenotype. Two insertions were identified in a 15-kb region containing *Klc* and *Ptp69D*, a receptor tyrosine phosphatase required for motor axon guidance during embryonic development (Desai et al., 1996, Desai et al., 1997; Fig. 1). One of these insertions, 59A (subsequently renamed Klc^1), is located in the first intron of the *Klc* transcription unit (Fig. 1). Although this 12-kb insertion greatly reduces the amount of KLC protein synthesized within cells, some KLC protein can be detected by Western analysis (data not shown).

To generate small deletions that wholly or partially remove the *Klc* transcript, a second round of P-element mutagenesis was performed. Excision of a P-element, while sometimes causing mutant reversion by restoring the gene to its original structure, often results in deletion of sequences flanking the insertion site (O'Hare and Rubin, 1983; Searles et al., 1986; Tsubota and Schedl, 1986). Several imprecise excisions were identified; the most informative are shown in Fig. 1. *Df(3L)8ex34* is one of several deletions that remove *Klc* and flanking DNA sequences, as detected by Southern hybridization of cloned DNA from the *Klc* interval to genomic DNA extracted from *Klc* mutant individuals (see Materials and Methods). In contrast, *Df(3L)8ex94* appears to remove only the *Klc* transcription unit, as the 5′ breakpoint maps near the *Klc* transcription start site, and the 3′ breakpoint is between *Klc* and *Ptp69D* (Fig. 1; Desai et al., 1996). Both Klc^1 and *Df(3L)8ex94* complement lethal mutations in *Ptp69D*, suggesting that these mutations do not affect the function of *Ptp69D*. Reduced levels of KLC accumulation are observed in *Df(3L)8ex34* and *Df(3L)8ex94* heterozygotes (data not shown), thereby confirming data suggesting that these deficiencies disrupt *Klc*.

The loss of *Klc* function causes lethality during the larval and pupal stages of development, depending upon the level of residual *Klc* activity in the allelic combinations tested. For example, null alleles such as *Df(3L)8ex94* die at the boundary of the second and third larval instars, whereas *trans*-heterozygous combinations such as Klc^1/*Df(3L)8ex94* or Klc^1 homozygotes live until the late third larval instar and the pupal stages, respectively (Table I). Two lines of evidence suggest that the observed lethality is due to loss of *Klc* function. First, chromosomes from which the P[*lacW*] element has been precisely excised from *Klc* fully complement *Klc* mutations. Second, transgenic rescue constructs containing either *Klc* genomic DNA or cDNA sequences provide full or partial rescue of *Klc* mutations, depending on the allelic combinations used. The genomic rescue construct GEN-KLC contains 12.4 kb of genomic DNA, including 5 kb 5′ of the *Klc* transcription start site and 3 kb 3′ of *Klc* (Fig. 1). GEN-KLC rescues much of the lethality associated with *Df(3L)8ex94* and *Df(3L)8ex94*/Klc^1, but rescues Klc^1 lethality only from midpupal stage to late pupal stage (Table I). The minimal rescue of Klc^1 by GEN-KLC is puzzling because we expected that Klc^1 would be rescued by GEN-KLC. The failure of GEN-KLC to rescue Klc^1 lethality suggests that other lethal mutations on the Klc^1 mutant chromosome may exist. However, additional data suggest that the lethality of Klc^1 is dependent upon P[*lacW*] insertion into *Klc*. First, Klc^1 revertants in which P[*lacW*] has been excised complement Klc^1. Second, the Klc^1 chromosome was generated multiple times by precise excision of a second lethal P[*lacW*] element from the 59A mutant chromosome; all isolates of Klc^1 have the same recessive lethal phenotype alone or in combination with other Klc^1 mutant chromosomes. Finally, attempts to separate secondary lethal mutations on the Klc^1 chromosome from *Klc* by meiotic recombination were unsuccessful (data not shown).

If *Klc* is nested within the regulatory region or transcription unit of a nearby gene, insertional mutations in *Klc* may alter the function of both *Klc* and its encompassing gene. Precise removal of the P[*lacW*] element from *Klc* would restore the function of both *Klc* and its nearby gene. Imprecise excisions such as *Df(3L)8ex94*, in contrast, restore the function of the nearby gene, but not *Klc*. This hypothesis predicts that *Df(3L)8ex94*/Klc^1 individuals can be rescued by GEN-KLC, because *Df(3L)8ex94* lacks the secondary lethality associated with Klc^1. This prediction is indeed the case (Table I), as GEN-KLC rescues the heteroallelic combination *Df(3L)8ex94*/Klc^1 to adulthood. Therefore, we are able to conclude that the P[*lacW*] insertion associated with Klc^1 may affect the function of a nearby gene, but other *Klc* mutations such as *Df(3L)8ex94* complement any secondary lethality that may be associated with Klc^1.

The cDNA rescue construct MYC-KLC encodes a myc-epitope tag-KLC fusion protein under control of the polyubiquitin promoter, which directs high levels of expression in all tissues (Figs. 1 and 2; Lee et al., 1988). The MYC-KLC transgene provides partial rescue of both the *Klc* insertion and deletion mutants (Table I). Some mutant combinations, such as *Df(3L)8ex94*/Klc^1, are rescued to adulthood, providing strong evidence that the phenotypes associated with this mutant combination are *Klc*-dependent. Like GEN-KLC, MYC-KLC incompletely rescues Klc^1, which again suggests that the P[*lacW*] insertion in *Klc* may also affect a nearby gene. Perhaps the most interesting MYC-KLC partial rescue phenotype is observed for the null mutant *Df(3L)8ex94*. Individuals completely lacking *Klc* function die at the boundary between the second and third larval instars; however, MYC-KLC facilitates survival of *Klc* null individuals to the end of the third larval instar, with apparently normal crawling behavior and activity levels. Intriguingly, larvae harboring MYC-KLC in a *Klc* null background live several days as late third instar larvae, but never enter the wandering third instar stage that foreshadows pupariation. A striking aspect of this phenotype is that MYC-KLC; *Df(3L)8ex94* larvae remain alive in the food after all of their wild-type siblings become adults. The wanderer phenotype is rescued by GEN-KLC and is not observed in *Df(3L)8ex94*/Klc^1 individuals (Table I), suggesting that the wanderer phenotype is caused by defects in a gene deleted by *Df(3L)8ex94*, but present within GEN-KLC and unaffected by the Klc^1 insertion mutant. A second possibility is that MYC-KLC, because it encodes only one KLC isoform, may not encode alternate, minor KLC isoforms necessary for pupariation. Although the presence or absence of splice variants in *Drosophila* KLC has not been closely examined (Gauger et al., 1993), KLC homologs in other organisms undergo extensive alternative splicing to

Table I. Complementation and Lethal Phase Analysis of Klc Mutants

Genotype*	*Klc*^{1}	*Df (3L)8ex94*	MYC-KLC, *Klc*^{1}	MYC-KLC, *Df (3L)8ex94*	GEN-KLC; *Klc*^{1}	GEN-KLC, *Df (3L)8ex94*
Klc^1	0% (315)	0% (612)	0% (494)	24% (185)	1.2% (956)	40% (302)
	MP	L3-EP	LP	ND	ND	ND
Df(3L)8ex94		0% (223)	16% (281)	0% (712)	25% (471)	40% (128)
		L2-L3	ND	L3	ND	ND
MYC-KLC, *Klc*^{1}			2.5% (364)			45% (266)
			ND			ND
MYC-KLC, *Df(3L)8ex94*						82% (761)
						ND
GEN-KLC; *Klc*^{1}					2.8% (1579)	
					ND	

Flies of the genotypes shown were mated, the numbers of mutant and nonmutant progeny were noted, and the stage of development at which mutant progeny died was determined. The percentages shown for each mating is the percentage of mutant^1/mutant^2 progeny observed relative to the expected number of mutant^1/mutant^2 progeny. The number in parentheses represents the total number of progeny scored for each mating. The lethal phase for each mating is shown as: L2, second larval instar; L3, third larval instar; EP, early (nonpigmented) pupa; MP, midpupa; i.e., approximately half the pupae are pigmented, whereas the other half are nonpigmented. *Balancer chromosomes are omitted from the genotypes shown.

generate different KLC isoforms (Cyr et al., 1991; Beushausen et al., 1993; Wedaman et al., 1993). If other *Drosophila* KLC isoforms have unique functions, these functions will not be rescued by MYC-KLC, but may be rescued by GEN-KLC, which has the potential to encode multiple KLC isoforms, and by *Klc*^{1}, which synthesizes small amounts of wild-type protein. Nevertheless, MYC-KLC rescues many facets of the *Klc* mutant phenotype, thereby strengthening our assertion that phenotypes observed in *Klc* mutants result from loss of *Klc* function.

KLC Mutations Exhibit Phenotypes Reminiscent of KHC Mutations

The phenotypes of mutations in Drosophila *Khc* have been extensively studied (Saxton et al., 1991; Gho et al., 1992; Hurd and Saxton, 1996; Hurd et al., 1996). The *Khc* mutant phenotype is characterized by locomotion defects, progressive paralysis, and death during the larval stage of development. Paralysis is more severe at the distal (posterior) end of the larva, suggesting that the long motor axons innervating the posterior body wall muscles are more severely affected than the shorter axons of anterior segments. These phenotypes are caused by impairment of neuron function, as evidenced by electrophysiological defects including reduction of compound action potentials and the amplitude of excitatory junction currents, as well as reduction of the number of boutons at the neuromuscular junction. Light and electron microscopic analysis suggested that transport of a variety of cargos, both anterograde and retrograde, is blocked in *Khc* mutant larvae, leading to the formation of axonal swellings that accumulate diverse cargos.

The phenotype of null *Klc* mutations is quite similar to the *Khc* null mutant phenotype. KLC protein accumulation is observed at all stages of development; a single predominant species of 58 kD is observed (Fig. 2). The presence of KLC at all stages of development suggests that KLC function may be required for developmental processes. However, embryonic development appears normal, and first instar larvae hatch apparently unhindered by the loss of *Klc* function. The lack of zygotic KLC accumulation during embryogenesis and the first larval instar is presumably compensated by a maternally supplied pool of KLC protein. However, the second larval instar is characterized by a progressive loss of vigor as the larvae exhibit increasing amounts of paralysis, eventually resulting in complete paralysis and death near the end of the second larval instar. Occasionally a *Klc* null individual can proceed to the third larval instar, but these escapers often lack the strength to shed their second instar cuticle. Similar to *Khc* mutants, progressive paralysis associated with loss of *Klc* function begins at the posterior end of the larva and proceeds anteriorly. It is thus likely that paralysis of *Klc* mutants, like *Khc*, reflects the differential requirement for kinesin-based transport in the longer axons of the posterior segments relative to the shorter axons innervating the anterior segmental muscles.

Combinations of less severe *Klc* mutations live to the late third larval instar or pupal stage of development (Table I). Like the null *Klc* mutants, the terminal phenotype of *Klc* hypomorphic alleles is larval paralysis, with the exception of weak mutants that pupate but fail to eclose. In addition, partial loss of *Klc* function often results in unusual locomotion defects. Wild-type larvae move along a surface by rhythmic contractile waves that originate at the posterior end and move in a concerted fashion toward the anterior. These contractile waves are facilitated by subcuticular body wall muscles controlled by motor axons whose cell bodies are located in the larval CNS. Normally, the dorsal and ventral muscles of each segment contract in unison, ensuring that the larva maintains contact with the surface. However, *Klc* mutations that allow survival to late third larval instar cause the posterior end of the larva to lift its tail off the medium (Fig. 2). Severely affected individuals can be observed lifting the posterior 40% of their body. This tail-flipping phenotype is also observed in certain *Khc* mutant combinations (Hurd and Saxton, 1996). It has been proposed that the tail-flipping observed in *Khc* mutants is the result of a temporal gradient of paralysis such that the ventral body wall muscles lose muscle tone before dorsal body wall muscles. Contraction of the dorsal body wall muscles in the absence of counterbalancing ventral muscle contraction then causes the tail to flip upward. The tail-flipping and paralysis phenotypes observed in *Klc* mutant larvae are quantitatively rescued by the transgenic

constructs GEN-KLC and MYC-KLC (Table I; Fig. 2), providing strong evidence that the neuromuscular defects of *Klc* mutants result from the loss of *Klc* function.

Loss of Klc Function Disrupts Multiple Pathways of Axonal Transport

Macromolecular structures required for synapse function, such as membranous vesicles, neurotransmitters, and the machinery controlling synaptic vesicle fusion and recycling, must be transported great distances from the point of synthesis in the cell body to the synapse. Similarly, for chemical signals received at the synapse to be acted upon by the neuron, the signals must be transported from the synapse to the cell body. Microtubule motors such as kinesin are necessary for these transport phenomena. Perhaps the paralytic and tail-flipping phenotypes associated with loss of *Klc* function are the result of a defect in axonal transport owing to mislocalization of kinesin cargoes. In fact, loss of *Khc* function is known to cause massive disruption of fast axonal transport, resulting in focal swellings packed with many different types of membrane-bound organelles, including mitochondria, prelysosomal mutivesicular bodies, and synaptic vesicle precursors. Loss of *Khc* function does not disrupt slow axonal transport, however, as molecules that undergo slow axonal transport, such as tubulin, are not observed in *Khc* organelle jams (Hurd and Saxton, 1996).

To determine if loss of *Klc* function results in formation of axonal organelle jams similar to those observed in *Khc* mutants, we studied the cellular phenotype of *Klc* mutant axons from tail-flipping *Klc* mutant larvae. Biochemical analyses in mammalian systems suggest that kinesin is associated with many different types of membranous vesicles, including synaptic vesicles and mitochondria (Hollenbeck, 1989; Pfister et al., 1989; Dahlstrom et al., 1991; Hirokawa et al., 1991; Leopold et al., 1992; Burkhardt et al., 1993; Vallee and Sheetz, 1996). Therefore, we studied the transport of proteins such as the synaptic vesicle components synaptotagmin (SYT) and cysteine string protein (CSP), which undergo high levels of microtubule-based axonal transport (Littleton et al., 1993; Zinsmaier et al., 1994; Parfitt et al., 1995). Segmental nerves from control *Klc*/+ larvae exhibit punctate but relatively uniform CSP and SYT staining in the segmental nerves (Fig. 3, *a* and *b*). However, large immunoreactive clusters of CSP and SYT are observed in the segmental nerves of *Klc* mutant larvae (Fig. 3, *c* and *d*). These clusters appear to represent organelle jams similar to those observed in *Khc* mutant larvae (Hurd and Saxton, 1996). Small clusters of immunoreactivity are sometimes observed in control larvae, but their size and frequency are greatly reduced in comparison with *Klc* mutant segmental nerves. Immunostaining of *Khc* mutant larvae exhibiting the tail-flipping behavioral phenotype reveals similar staining patterns for SYT and CSP (Hurd and Saxton, 1996; our unpublished results). These results suggest that, in addition to sharing behavioral phenotypes, *Khc* and *Klc* mutations appear to cause a similar disruption of fast axonal transport. Furthermore, the similar phenotype of *Klc* and *Khc* mutants suggests that both the light chains and heavy chains are necessary for kinesin function, and that the light chains may have a positive role in kinesin function (such as cargo binding) instead of being a negative regulator of kinesin activity.

The observation that organelle jams from *Khc* mutant larvae contain a variety of membranous organelles, including both anterograde and retrograde cargoes (Hurd and Saxton, 1996), led us to test directly the hypothesis that

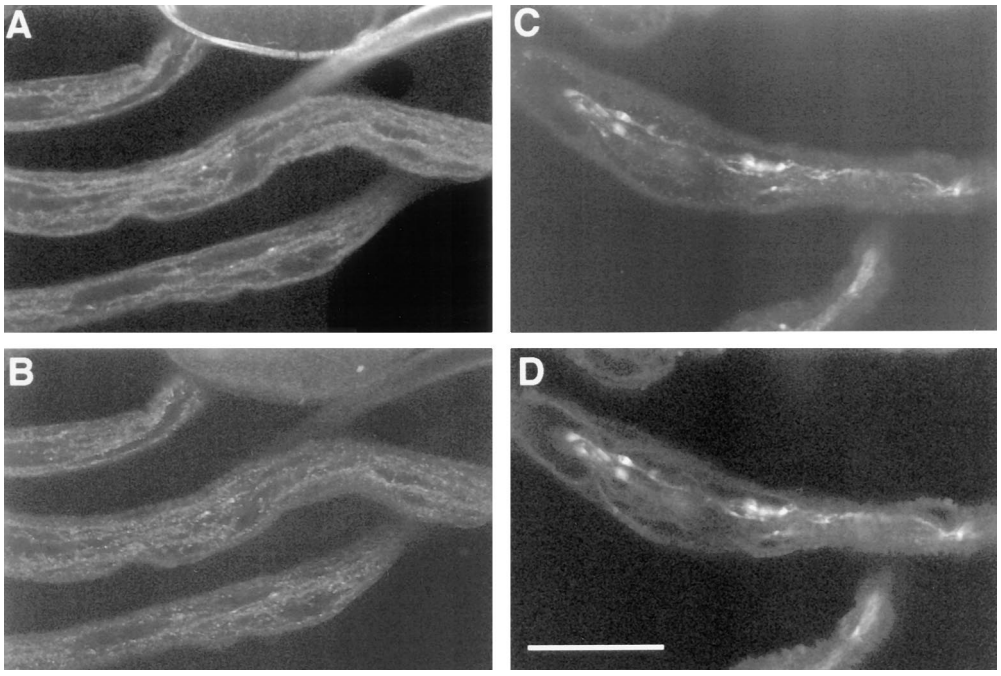

Figure 3. Loss of *Klc* function causes disruption of axonal transport. Larvae heterozygous for *Klc* (*A*, *B*) or Klc^{1}/*Df(3L)8ex94* mutants (*C*, *D*) were simultaneously incubated with antibodies specific for cysteine string protein (*A*, *C*) and synaptotagmin (*B*, *D*) to compare the accumulation patterns of these synaptic vesicle components in wild-type and *Klc* mutant backgrounds. Although accumulation of CSP and SYT is punctate but fairly uniform in the segmental nerves of control larvae (*A*, *B*), large aggregates of CSP and SYT immunoreactivity are observed in *Klc* mutants (*C*, *D*), suggesting that normal axonal transport of these proteins are blocked. Furthermore, the pattern of CSP (*C*) accumulation completely overlaps SYT (*D*), suggesting that these proteins are found in the same subset of organelle jams. Individual organelle jams are presumed to be confined to a single axon (Hurd and Saxton, 1996). *Bar*, 25 μM.

failure of kinesin transport in *Klc* mutants interferes with the ability of other molecular motors to transport their cargoes. To determine if *Klc* mutations interfere with transport pathways of other motors, we examined immunolocalization of two other motor proteins that are likely to play important roles in fast axonal transport. The first of these motors is KLP68D (Pesavento et al., 1994), which is a plus-end directed neuronal motor homologous to members of the murine KIF3 family and the sea urchin KRP85/95 family (Scholey, 1996; Yamazaki et al., 1995). The second motor we examined is DHC, which is the heavy chain of the retrograde fast axonal transport motor cytoplasmic dynein (McGrail and Hays, 1997). KLP68D is obviously localized to large aggregates in *Klc* mutant larvae (Fig. 4 *b*), whereas its localization in *Klc*/+ control segmental nerves is fairly uniform (data not shown), suggesting that the presence of KLP68D aggregates is a direct consequence of loss of *Klc* function. Some of these aggregates colocalize with CSP (compare Fig. 4, *a* and *b*), demonstrating that KLP68D protein is present in organelle jams. Identification of organelle jams that contain KLP68D but not CSP suggests that the composition of the membranous organelles in jams is heterogeneous. This heterogeneity may reflect the stochastic nature of organelle jam formation, or could be due to the presence of both sensory and motor axons in segmental nerves, each accumulating different ratios of intracellular cargos. While the degree of impairment of KLP68D-dependent transport in *Klc* mutant axons cannot be directly measured, the presence of high levels of KLP68D immunoreactivity in organelle jams suggests that loss of kinesin function causes a pleiotropic block of anterograde fast axonal transport.

To test whether the organelle jams found in *Klc* mutant axons may impede retrograde fast axonal transport, immunolocalization of DHC in control and *Klc* mutant larvae was compared. DHC is observed in large aggregates in *Klc* mutants (Fig. 4 *d*), but not in *Klc*/+ control larvae, and the DHC aggregates sometimes colocalize with SYT (compare Fig. 4, *c* and *d*). However, many DHC-labeled organelle jams do not colocalize with SYT-labeled organelle jams, suggesting again that the distribution of intracellular cargos within organelle jams is heterogeneous. This result demonstrates that cytoplasmic dynein is also present in organelle jams, and supports the hypothesis that loss of kinesin function inhibits multiple pathways of motor-driven fast axonal transport. However, we cannot conclude that *Klc* mutations disrupt fast retrograde axonal transport. Cytoplasmic dynein is a fast retrograde motor in axons, but much cytoplasmic dynein in axons may be in an inactive state, transported by anterograde fast axonal transport to the synapse (Hirokawa et al., 1990; Dillman et al., 1996). Because we cannot determine whether the DHC in organelle jams is in an active state, additional experiments are required to determine unequivocally the relationship between loss of *Klc* function and disruption of cytoplasmic dynein-dependent transport pathways. In conclusion, these results strongly suggest that behavioral defects observed in *Khc* and *Klc* mutant larvae are not solely due to a failure to localize specific kinesin cargos, but result from a general failure of several pathways of motor-mediated axonal transport (Hurd and Saxton, 1996).

KLC May Be Required for KHC Attachment to Cargoes In vivo

An important question is whether the kinesin light chains are necessary for kinesin binding to its intracellular cargoes. Whereas some results suggest that the KHC tail domain is sufficient for kinesin–cargo interaction (Skoufias et al., 1994; Bi et al., 1997), other experiments demonstrate that KLC function may be necessary for a subset of kinesin binding to intracellular cargoes (Stenoien and Brady, 1997). We attempted to test directly the role of KLC in cargo binding by studying immunolocalization of KHC in

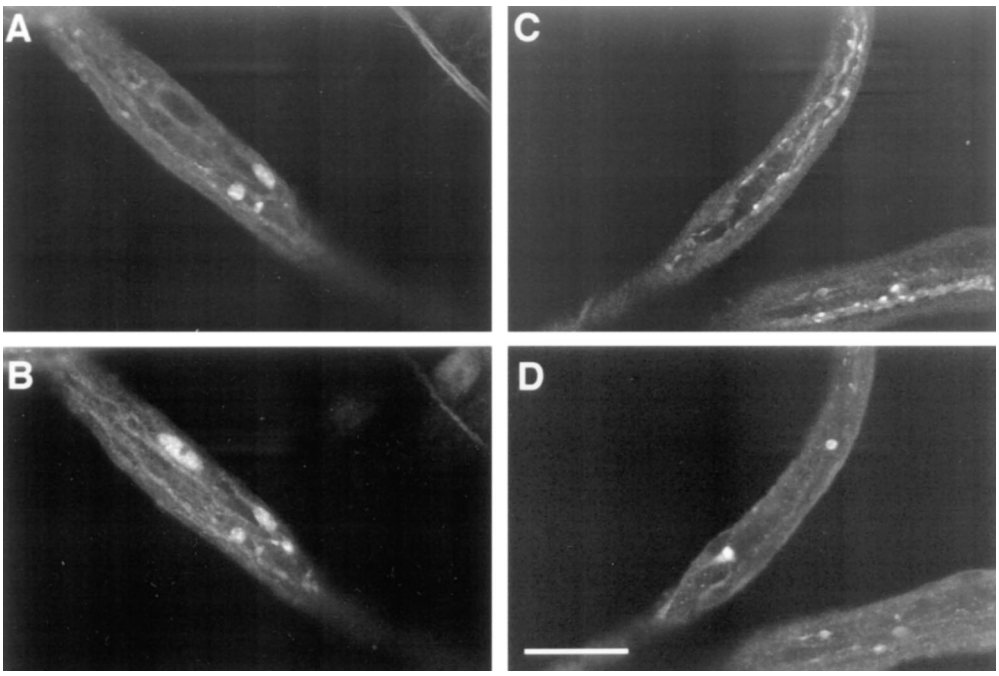

Figure 4. KLP68D and cytoplasmic dynein are found in organelle jams. Segmental nerves of *Df(3L)8ex94*/*Klc*[1] larvae were incubated with antibodies to CSP (*A*) and KLP68D (*B*), or SYT (*C*) and DHC, the motor component of cytoplasmic dynein (*D*). Both KLP68D and DHC immunoreactivity are observed in organelle jams. *Bar*, 20 μM.

Klc mutant axons. The absence of KHC from *Klc* mutant organelle jams would support a model in which KLC is necessary for cargo attachment. In contrast, the observation of KHC immunoreactivity in the absence of KLC would strongly suggest that some kinesin–cargo interactions are KLC-independent. Immunostaining of Klc^1/*Df(3L)8ex94* mutant larvae with antisera to KHC (Fig. 5 *b*) and CSP (Fig 5 *a*) demonstrates that KHC is present in *Klc* larval organelle jams. This result suggests that KLC is dispensable for kinesin–cargo interactions; however, it is possible that KHC is tethered to cargoes by residual light chains present in *Klc* mutant larvae. The allelic combination used for KHC immunolocalization, Klc^1/*Df(3L)8ex94*, makes low but detectable levels of KLC protein. Fig. 5 demonstrates that KLC protein, like KHC, is also observed in *Klc* mutant larvae, and that its immunoreactivity partially overlaps CSP (Fig. 5, *c* and *d*). Biochemical fractionation experiments also demonstrate that nearly all the residual KLC protein in light chain mutants is membrane-bound (our unpublished results), suggesting attachment to organelle cargoes. Although these results support a role for KLC in kinesin–cargo interactions, additional experiments such as immunoelectron microscopy will be required to define the relative contributions of KLC and KHC to cargo attachment at high resolution, and to demonstrate the direct association of KHC with membranous organelles in *Klc*-dependent organelle jams.

Discussion

Similar Axonal Transport Defects in Klc and Khc Mutants

The major conclusion of the experiments presented here is that the phenotype of *Klc* and *Khc* mutants are quite similar, especially with respect to disruption of fast axonal transport observed in both mutants (Saxton et al., 1991; Hurd and Saxton, 1996). *Klc* and *Khc* mutants exhibit paralysis that begins in the posterior end of the larva, which then proceeds anteriorly. This graded paralysis reflects the difference in the length of axons innervating the posterior segmental muscles in comparison to axons innervating more anterior segments (Hurd and Saxton, 1996). The cause of the locomotion defects and paralysis of *Klc* and *Khc* mutants may be formation of large aggregates of axonally transported organelles. These aggregations are similar to the clusters of anterograde and retrograde transports observed at the site of vertebrate nerve ligations (Smith, 1980; Tsukita and Ishikawa, 1980; Fahim et al., 1985; Hirokawa et al., 1990, Hirokawa et al., 1991). Immunostaining of organelle jams demonstrates that synaptic vesicle components, in addition to the neuronal motors KLP68D and cytoplasmic dynein, accumulate in *Klc* mutant axons. Electron microscopic analysis of *Khc* mutant axons demonstrate that many different types of membrane-bound organelles are present in organelle jams (Hurd and Saxton, 1996). Organelle jams may impair multiple anterograde and retrograde axonal transport and signaling pathways, resulting in a compromise of neuromuscular function similar to that observed in vertebrate neuropathies such as amyotrophic lateral sclerosis (Hurd and Saxton, 1996; for review see Bruijn and Cleveland, 1996).

Native kinesin is a heterotetramer composed of two heavy chains and two light chains (Bloom et al., 1988; Johnson et al., 1990; Kuznetsov et al., 1988). Although the high degree of light chain sequence conservation among higher eukaryotes (Cyr et al., 1991; Gauger and Goldstein, 1993; Wedaman et al., 1993; Beushausen et al., 1993; Cabeza et al., 1993; Fan and Amos, 1994; Chernajovsky et al., 1996) implies that the light chains are essential for kinesin function, their precise role is unclear. Existing suggestions for KLC function can be broken into two broad categories: positive and negative. If light chains are necessary for a positive function such as mediating kinesin–cargo interac-

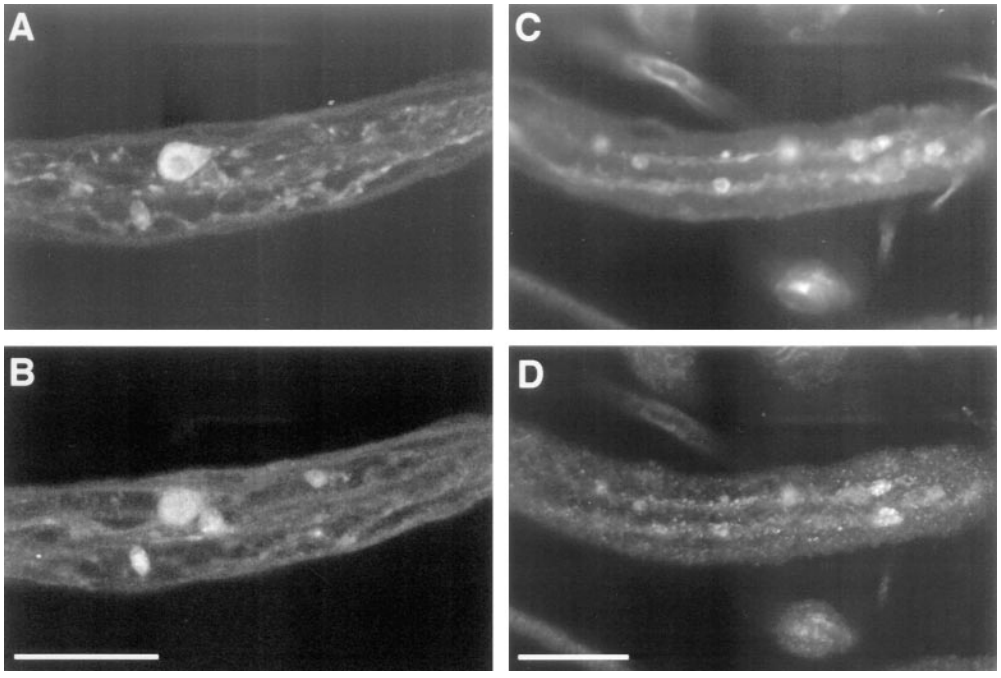

Figure 5. KHC and KLC accumulate in *Klc*-dependent organelle jams. *Df(3L)8ex94*/Klc^1 larvae were incubated with antibodies to CSP (*A*, *C*) and antibodies to KHC (*B*) and KLC (*D*). KHC aggregates are observed in the segmental nerves of *Klc* mutant larvae. This observation would suggest that KHC can bind and transport intracellular cargoes in a KLC-independent manner; however, KLC protein accumulation is also observed in *Klc* mutant segmental nerves (*D*). *Bar*, 20 μM.

tions, then light chain mutations may have a phenotype similar to mutations in *Khc* that result in loss of motor activity. Alternatively, if the light chains have a negative function, for example, to inhibit kinesin activity by repressing motor activity or cargo binding, then *Klc* mutants may exhibit a phenotype different than the *Khc* loss-of-function phenotype. The results presented here demonstrate that the phenotypic consequences of loss of *Klc* and *Khc* function are very similar. Although it is difficult to predict the phenotypic consequences, if any, of kinesin hyperactivity in the absence of light chain function, we propose that the underlying defect in both *Klc* and *Khc* mutants is the inability of kinesin cargos to participate efficiently in fast axonal transport. Thus, two hypotheses consistent with the observed *Klc* mutant phenotype are (*a*) the inability of the kinesin motor domain to interact with some or all of its intracellular cargoes, or (*b*) the inability of the kinesin motor domain to be properly activated. However, further analysis will be required to determine unequivocally the in vivo role of KLC in kinesin-dependent axonal transport.

Both KLC and KHC May Be Important for Cargo Binding

Analysis of kinesin light chain function in other systems supports the hypothesis that the light chains are important for cargo binding. Adding light chain antibody to purified brain microsomes reduces the amount of microsome-bound kinesin by one-third relative to untreated control vesicles (Yu et al., 1992). Similar results are obtained when a different KLC antibody, specific to the conserved 42–amino acid TPR region of KLC, is incubated with microsomes (Stenoien and Brady, 1997). In contrast, an antibody specific for the coiled-coil region of KLC, which is likely to mediate KLC–KHC interactions (Gauger and Goldstein, 1993), does not inhibit kinesin–vesicle interactions (Stenoien and Brady, 1997). These results suggest that the region of KLC containing six conserved 42–amino acid repeats may be necessary for some kinesin–cargo interactions, perhaps by binding to membrane-bound receptors on the cargo surface. The loss of processive kinesin-dependent cargo transport observed in *Klc* mutants is consistent with inhibition of vesicle binding seen in anti-KLC inhibition experiments.

A model proposing an exclusive role for KLC in mediating kinesin–cargo interactions is appealing, but other experimental evidence indicates that the COOH-terminal tail of KHC also participates in kinesin binding to intracellular cargoes. A bacterially expressed fragment of sea urchin KHC, including the stalk and tail domains, can bind to sea urchin egg microsomal membranes in a concentration-dependent and saturable manner, but the KHC stalk domain cannot (Skoufias et al., 1994), indicating that the COOH-terminal 200 amino acids of sea urchin kinesin has cargo-binding capacity. Injection of the sea urchin KHC stalk-tail domain into wounded sea urchin cells inhibits membrane resealing in a manner similar to that observed for a KHC motor domain–inhibitory antibody (Bi et al., 1997), providing in vivo evidence of a role for KHC in cargo binding. However, it is also possible that association of the injected KHC stalk-tail domain with an intracellular pool of KLC could result in inhibition of membrane resealing by a KLC-dependent mechanism.

The results of the experiments described above suggest that KLC is necessary for binding of some cargos, but not others. Individual cargos may contain distinct receptors, some of which interact with KLC, but others that bind KHC. Several lines of evidence support this model. First, purified KCl-washed vesicles bind kinesin in a saturable, concentration-dependent manner, and have the capacity to bind twice as much kinesin with light chains (10S native kinesin) as kinesin lacking light chains (7S kinesin; Skoufias et al., 1994). Second, purified vesicles bind twice as much nonphosphorylated kinesin as kinesin phosphorylated by A-kinase (Sato-Yoshitake et al., 1992), suggesting that some kinesin–cargo interactions, but not others, are blocked by phosphorylation. Third, washing synaptic vesicles with 1 M NaCl results in release of only 65% of membrane-bound kinesin (ibid.). Fourth and finally, incubation of purified microsomes with saturating amounts of anti-KLC inhibitory antibodies displaces only 30% of kinesin from the vesicle surface (Yu et al., 1992; Stenoien and Brady, 1997), demonstrating that, like incubation with NaCl, inhibition of KLC function does not result in quantitative release of kinesin from vesicles. These results all support a model in which kinesin can use multiple interaction pathways to bind intracellular cargoes. More speculatively, the cargo-binding domain of KLC (TPR motif) differs in proposed secondary structure from the putative KHC cargo-binding domain, which is predicted to be mostly alpha-helical coiled coil in structure (de Cuevas et al., 1992; Fan and Amos, 1994; our unpublished observations). Differences in the structure of the cargo-binding domains of KHC and KLC, as well as concomitant differences in the nature of their protein–protein interactions with intracellular cargos, may explain results suggesting multiple mechanisms of kinesin–cargo interactions.

Identification of kinesin homologs in the fungi *Ustilago maydis* (Lehmler et al., 1997), *Neurospora crassa* (Steinberg and Schliwa, 1995), and *Syncephalum racemosum* (Steinberg, 1997) further support this view of kinesin–cargo interactions. Like kinesins identified in higher organisms, fungal kinesins such as *Neurospora* Nkin and *Ustilago* Kin2 are necessary for intracellular transport events, including polarized secretion and hyphal extension (Lehmler et al., 1997; Seiler et al., 1997). However, biochemical purification of fungal kinesins demonstrate that they have no associated light chains. The absence of light chains from fungal kinesins may reflect less complex requirements for intracellular transport owing to simpler morphology, fewer cell types, and the less complex developmental profile of fungi relative to other organisms from which kinesin has been identified. Although the conservation of light chain structure throughout higher eukaryotes suggests that association of KLC with KHC is evolutionarily ancient, the light chains may have provided an additional level of complexity of the kinesin tail domain necessary during the evolution of increasingly complex pathways of intracellular transport. Interestingly, a KLC-like protein has recently been discovered in the cyanobacterium *Plectonema boryanum*, but a prokaryotic KHC was not identified (Celerin et al., 1997). Perhaps a protein similar to cyanobacterial KLC became associated with an ances-

tral KHC before the divergence of vertebrates and invertebrates. Higher vertebrates have added more layers of complexity by duplication and divergence of KHC and KLC (Aizawa et al., 1992; Niclas et al., 1994; Nakagawa et al., 1997; A. Rahman and L. Goldstein, unpublished results). Elaboration of kinesin tail structure and duplication of kinesin subunit-encoding genes facilitated an increase in the repertoire of intracellular cargoes with which kinesin can interact, and has enabled additional layers of regulatory complexity by cytosolic factors such as kinases and phosphatases (Sato-Yoshitake et al., 1992; Hollenbeck, 1993; Matthies et al., 1993; McIlvain et al., 1994; Lee and Hollenbeck, 1995; Okada et al., 1995; Lindesmith et al., 1997).

Organelle Jams Result from a Failure of Processive Kinesin-dependent Transport

An interesting property of *Klc* and *Khc* mutants is the stochastic distribution of organelle jams in larval segmental nerve axons. If kinesin cargos were unable to enter the axon when active kinesin levels are greatly reduced, then cargos would be concentrated in the cell body and proximal parts of the axon. Instead, organelle jams appear to be randomly distributed along the length of the axon (Hurd and Saxton, 1996; this analysis). In vitro experiments studying the movement of kinesin-coated latex beads along microtubules, or microtubules along a kinesin-coated glass coverslip, have demonstrated that the processivity of kinesin-dependent movement is directly proportional to the number of kinesin molecules on the latex bead or glass coverslip (Howard et al., 1989; Block et al., 1990). Similarly, the in vivo processive movement of kinesin cargos may also be dependent on the intracellular concentration of kinesin. In *Klc* and *Khc* mutants, the number of kinesin molecules on its intracellular cargoes may decrease as the concentration of maternally supplied kinesin is exhausted. Therefore, kinesin-dependent transport becomes less processive, resulting in nucleation of an axonal traffic jam (Hurd and Saxton, 1996). Comparative analysis of the amount of kinesin bound to wild-type and kinesin mutant organelles, as well as assays designed to study the processivity of kinesin-dependent transport in kinesin mutants will help us better understand the underlying mechanism of the organelle jam phenotype.

An unresolved issue is the nature of the cargos transported in the axon by kinesin, as well as how kinesin binds these cargoes. Presumably, *Khc* mutants result in failure to transport all kinesin cargos as the mechanochemical head domain is encoded by KHC. However, the loss of *Klc* function may inhibit the transport of only a subset of kinesin cargoes, given that the tail domain of KHC may be able to bind to vesicles in a KLC-independent manner. If this hypothesis is correct, why are the *Khc* and *Klc* phenotypes so similar? The underlying cause of the organelle jam phenotype may be the failure to transport processively a small percentage of kinesin cargoes. Perhaps the less processive kinesin cargos serve as nucleation points for organelle jams, and other axonal cargos such as those transported by KLP68D and cytoplasmic dynein become trapped in these kinesin-dependent organelle jams. We propose that the nature of the cargo precipitating the organelle jam itself may be irrelevant, and that many different kinds of membrane-bound organelles can potentially serve as nucleation points for organelle jams. Preliminary data suggest that mutations in several different complementation groups can result in formation of organelle jams (M. A. Martin, A. Gassman, and W. M. Saxton, personal communication; M. McGrail, A. Bowman, and L. Goldstein, unpublished results). Therefore, the presence or absence of organelle jams in the larval segmental nerve may provide a sensitive assay for identifying parallel pathways of fast axonal transport, as well as additional components necessary for kinesin-dependent transport.

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