

Drosophila Cytoplasmic Dynein, a Microtubule Motor That Is Asymmetrically Localized in the Oocyte

Min-gang Li, Maura McGrail, Madeline Serr, and Thomas S. Hays

Department of Genetics and Cell Biology, University of Minnesota, St. Paul, Minnesota 55108-1095

Abstract. The unidirectional movements of the microtubule-associated motors, dyneins, and kinesins, provide an important mechanism for the positioning of cellular organelles and molecules. An intriguing possibility is that this mechanism may underlie the directed transport and asymmetric positioning of morphogens that influence the development of multicellular embryos. In this report, we characterize the *Drosophila* gene, *Dhc64C*, that encodes a cytoplasmic dynein heavy chain polypeptide. The primary structure of the *Drosophila* cytoplasmic dynein heavy chain polypeptide has been determined by the isolation and sequence analysis of overlapping cDNA clones. *Drosophila* cytoplasmic dynein is highly similar in sequence and structure to cytoplasmic dynein isoforms reported for other organisms. The *Dhc64C* dynein

transcript is differentially expressed during development with the highest levels being detected in the ovaries of adult females. Within the developing egg chambers of the ovary, the dynein gene is predominantly transcribed in the nurse cell complex. In contrast, the encoded dynein motor protein displays a striking accumulation in the single cell that will develop as the oocyte. The temporal and spatial pattern of dynein accumulation in the oocyte is remarkably similar to that of several maternal effect gene products that are essential for oocyte differentiation and axis specification. This distribution and its disruption by specific maternal effect mutations lends support to recent models suggesting that microtubule motors participate in the transport of these morphogens from the nurse cell cytoplasm to the oocyte.

MICROTUBULES provide the architectural framework on which many cellular organelles are transported. The microtubule polymer is an intrinsically polar structure resulting from the asymmetric, head-to-tail polymerization of the $\alpha\beta$ tubulin heterodimer (Amos et al., 1976; Luduena et al., 1977). Consequently, the two ends of a microtubule can be distinguished by their tendency to gain (the plus end) or lose (the minus end) tubulin subunits. At a basic level the regulation of microtubule-based transport within cells is determined by the polarity of microtubules and their associated motor proteins. This has been emphasized in recent years by demonstrations that microtubule motors use the energy derived from ATP hydrolysis to translocate in a single direction along the microtubule lattice (for reviews see McIntosh and Porter, 1989; Goldstein, 1991; Vallee, 1993; Bloom, 1992; Walker and Sheetz, 1993). The depletion of endogenous nucleotides from cytoplasmic extracts increases the affinity between motors and microtubules and has allowed the purification of microtubule motors from a variety of organisms and cell types (McIntosh and

Porter, 1989). Two distinct families of microtubule motors, the dyneins and kinesins, have been identified. In combination with the assembly of polar arrays of microtubules within cells, these unidirectional cytoplasmic motors provide a mechanism for partitioning cellular organelles and molecules within the cytoplasmic compartment. For example, kinesins have been implicated in the anterograde transport of synaptic vesicles to the tips of nerve axons, the extension of the endoplasmic reticulum network out along the interphase microtubule network, and mitotic spindle assembly and maintenance (for reviews see Hoyt, 1994; Goldstein, 1993; Walker and Sheetz, 1993; Skoufias and Scholey, 1993). Similarly, cytoplasmic dynein motors have been implicated in retrograde axonal transport of vesicles, the perinuclear positioning of golgi apparatus, chromosome-to-pole movement on the mitotic spindle, separation of the mitotic spindle poles, and nuclear positioning during cell division (for reviews see Schroer, 1994; Vallee and Shpetner, 1990; Skoufias and Scholey, 1993).

In *Drosophila* development, the distributions of microtubules during early embryogenesis have been extensively characterized (Fullilove and Jacobson, 1971; Turner and Mahowald, 1976; Foe and Alberts, 1983; Walter and Alberts, 1984; Karr and Alberts, 1986; Warn et al., 1987; Kellogg et al., 1989; Theurkauf, 1992; Baker et al., 1993; for review see Fyrberg and Goldstein, 1990). In addition, mi-

M.-G. Li and M. McGrail have contributed equally to this work.

Address all correspondence to Dr. Tom Hays, Department of Genetics and Cell Biology, University of Minnesota, 1445 Gortner Avenue, St. Paul, MN 55108. Telephone: (612) 625-2226; FAX: (612) 625-5754.

croton organization during oogenesis appears to contribute to asymmetries that are required for the directional transport and localization of maternal mRNAs, protein, organelles, and other macromolecules from the nurse cell cytoplasm to the oocyte (Pokrywka and Stephenson, 1991; Theurkauf, 1993; discussed by Macdonald, 1992; Ruohola-Baker et al., 1994; Spradling, 1993; Ding and Lipshitz, 1993). This microtubule-dependent transport and localization of macromolecules during oogenesis is likely to participate in the specification of the embryonic axes.

The cytoplasmic motors that associate with microtubules are likely candidates to participate in the transport and localization of cytoplasmic components to and within the oocyte. In this paper, we report the molecular cloning of the complete 14.3-kb transcript encoding the cytoplasmic dynein heavy chain in *Drosophila*. The dynein transcript is expressed at high levels throughout development and is enriched in ovaries. In the developing egg chamber, the dynein gene is expressed in nurse cells, while the encoded dynein motor protein is differentially localized to the presumptive oocyte. This localization initiates at an early stage in the differentiation of the pro-oocyte and coincides with the establishment of a polar array of microtubules emanating from the presumptive oocyte. Dynein localization requires the function of genes in both the germline and somatic follicle cells. Our results suggest a model in which the minus-end dynein motor "walks" into the pro-oocyte along a polarized track of microtubules. We discuss the implications of this localization in terms of a dynein-based mechanism for the transport of informational molecules to the differentiating oocyte.

Materials and Methods

Isolation of cDNA and Genomic Clones

A λ ZAP cDNA expression library (Stratagene, La Jolla, CA), constructed by random priming from *Drosophila* 2–14 h embryo mRNA, was screened with a rabbit polyclonal antibody raised against the *Drosophila* dynein heavy chain polypeptide (Hays et al., 1994). The antibody was affinity-purified against each of two dynein polypeptide fragments produced from the cleavage of the intact heavy chain in the presence of vanadate and UV light (Hays et al., 1994). The library was plated and induced according to the supplier's instructions. Positive clones were detected with alkaline phosphatase-conjugated goat anti-rabbit secondary antibody (BioRad Labs., Hercules, CA) using 5-bromo-4-chloro-3-indolyl-phosphate (BCIP)¹ and nitro blue tetrazolium (NBT) substrate (Sigma Chem. Co., St Louis, MO), and recombinant plasmids were recovered by *in vivo* excision.

One positive clone, MS3, was shown by RNA blot analysis to hybridize to an ~14-kb RNA. Radiolabeled probes from this clone were used to extend the cDNA sequence in both directions by hybridization screens of the λ ZAP library and a 4–8 h embryo plasmid cDNA library in the vector pNB40 (Brown and Kafatos, 1988). Hybridization conditions were as described for Southern blots below. The identity of overlapping clones was confirmed by restriction analysis and sequence comparison using cDNA and genomic clones. Two genomic DNA libraries, a λ EMBL3A and a cosmid library, were both derived from an isogenic *Drosophila* stock (*iso-1* [Brizuela et al., 1994]) and were kindly provided by Dr. J. W. Tamkun, University of California (Santa Cruz, CA). Genomic clones were identified by hybridization to cDNA probes and subsequently purified. Genomic HindIII fragments were subcloned into pBluescript II (Stratagene) for se-

quence analysis. To isolate cDNA sequence from the 5'-most end of the transcript, primers based on the available cDNA sequence in the region were used to prime the synthesis of new cDNA from total embryonic RNA. Reverse transcription and initial amplifications were performed using a "5'RACE System" kit (GIBCO BRL, Gaithersburg, MD; Frohman et al., 1988) according to the supplier's instructions. One microgram of DNase-treated 0–24 h embryo RNA was reverse-transcribed with an antisense primer corresponding to either nucleotides 1834 to 1857 or alternatively nucleotides 1691 to 1713 (see Fig. 3). The RNA/DNA hybrids were digested with RNaseH, and the first strand cDNAs were tailed at the 3' end with dCTP using terminal deoxynucleotidyl transferase. First strand cDNAs were amplified sequentially, using a 5' RACE anchor primer and nested antisense primers from within clone ZL2 (primer 1, nucleotides 1598 to 1617; primer 2, nucleotides 1476 to 1494). The cDNA RACE products were subsequently amplified by the PCR with primers designed from the sequence of cDNA and genomic subclones. The PCR products were cloned into pBluescriptII, resulting in the subclones pTRI3 and pLRI52 (see Fig. 1). The primers used to recover the subclone pTRI3 were derived from known cDNA sequence. The 5' sense and the 3' antisense primers corresponded to nucleotides 539 to 558 and 1396 to 1416, respectively. The sense primer for pLRI52 was designed from genomic sequence corresponding to nucleotides 88 to 107, while the antisense primer was based on known cDNA sequence, nucleotides 565 to 584. The primers were selected in such a way that the genomic DNA regions amplified by these primers harbor introns, and, therefore, the PCR products could be easily confirmed to be derived from mRNA and not from contaminating genomic DNA.

The PCR reaction conditions for pTRI3 and pLRI52 were as follows: 5 μ l of 5' RACE product was amplified in a 50- μ l reaction containing 50 mM KCl, 10 mM Tris-HCl pH 9.0, 0.1% Triton X-100, 1.5 mM MgCl₂, 0.2 mM dNTP, 0.2 μ M of each primer, and 0.3 U of Taq polymerase. cDNA was denatured for 2 min at 95°C; and then the reaction was run for 30 cycles of 50°C for 2 min, 72°C for 3 min, and 94°C for 1 min. The extension was completed at 72°C for 10 min.

DNA Sequencing and Analysis

DNA sequencing was performed by the dideoxy chain termination method (Sanger et al., 1977) using Sequenase 2.0 polymerase (USB, Cleveland, OH) and plasmid vector primers. Both strands of the complete cDNA contained within overlapping clones, as well as selected genomic subclones, were fully sequenced using nested serial deletions generated by exonuclease III and S1 nuclease according to the method of Henikoff (1987). The template was a double-stranded plasmid prepared as described by Medberry et al. (1990). Sequencing reactions were electrophoresed on 6% denaturing polyacrylamide gels. The sequence data was assembled and analyzed using IntelliGenetics and UWGCG software on a Sun Microsystems 2/120 computer, and the MacVector Sequence Analysis Software package (International Biotechnologies, Inc., New Haven, CT). Predictions of coiled-coil domains were based on the statistical significance of the preferential distribution of different amino acids in each position of the heptad repeat (Lupas et al., 1991). A window size of 28 residues was used and scores of greater than 1.3 were taken to indicate a high probability of coiled-coil conformation (Lupas et al., 1991).

Primer Extension and RNase Protection

Primer extension was performed as described by Ausubel et al. (1987). Fifteen micrograms of total embryonic RNA were annealed to 5 ng ³²P-end-labeled primer and reverse transcribed by M-MLV reverse transcriptase (Life Sciences, Inc., St. Petersburg, FL). The antisense oligonucleotide primer was a 20mer, 5'-GTTCGCAATGGACAGGTTCA-3' (nucleotides 209 to 228 in Fig. 3). RNase protection was performed as described by Melton et al. (1984). The template was a genomic DNA subclone extending from nucleotides -923 to +330. The plasmid was linearized at a unique HindIII site downstream from the T3 phage promoter. ³²P-labeled antisense RNA was synthesized with T3 RNA polymerase *in vitro* (Stratagene). Fifteen micrograms of total embryonic RNA were annealed overnight at 45°C with labeled antisense RNA in the presence of 80% formamide, and then digested with a mixture of RNaseA and RNaseT1. The size of the products of both primer extension and RNase protection were determined on 6% acrylamide sequencing gels using genomic DNA as a size marker.

In Situ Hybridization

Digoxigenin-labeled probes were hybridized to whole-mount preparations

1. *Abbreviations used in this paper:* BCIP, 5-bromo-4-chloro-3-indolyl-phosphate; EST, expressed sequence tags; HS, hybridization solution; MTOC, microtubule-organizing center; NBT, nitro blue tetrazolium; ORF, open reading frame.

of ovaries according to the procedure described by Tautz and Pfeifle (1989) with minor modifications. Ovaries were dissected in EBR saline (130 mM NaCl, 5 mM KCl, 2 mM CaCl₂, 10 mM Hepes pH 6.9) and fixed for 20–25 min in 200- μ l PP (4% paraformaldehyde in PBS), 20- μ l DMSO, 600- μ l heptane, and then washed several times in PBT (PBS/0.1% Tween-20). Proteinase K treatment (30 min at 50 mg/ml in PBT) was stopped by incubation in 2 mg/ml glycine and repeated washes in PBT. Ovaries were refixed in PP and washed in PBT for 30 min with four changes. They were incubated 15 min in a 1:1 mixture of PBT and hybridization solution (HS), 10 min at room temperature in HS, then 1–2 h at 48°C in HS. Hybridization was for 20–30 h at 48°C using 0.5 mg/ml labeled cDNA probe in HS containing 10% dextran sulfate. Posthybridization washes were extended to 45–55 h at 48°C. Ovaries were moved stepwise into PBT at room temperature and washed 5 \times 10 min in room temperature PBT. They were incubated for 90 min with alkaline phosphatase-conjugated anti-digoxigenin antibody (previously preadsorbed against fixed ovaries) diluted 1:3,000 in PBT. After several brief rinses in PBT, the ovaries were washed overnight in PBT at 4°C. They were washed three times in alkaline phosphatase buffer at room temperature before staining with nitro blue tetrazolium (NBT) plus 5-bromo-4-chloro-3-indolyl-phosphate (BCIP). Color development was stopped with PBT washes, and ovaries were stored at 4°C in PBT until mounting in Immumount (Shandon, Pittsburgh, PA).

Probes were prepared using a Boehringer Mannheim "Genius" kit according to the supplier's instructions (Boehringer Mannheim Corp., Indianapolis, IN), except that final primer concentrations in the labeling reaction were increased to 1 mg/ml. *oskar* cDNA was kindly provided by Ruth Lehmann (Ephrussi et al., 1991). In situ hybridization to larval polytene chromosome squashes was carried out as previously described (Rasmusson et al., 1994). DNA probes were labeled with digoxigenin-dUTP using a Genius kit (Boehringer Mannheim) according to the manufacturer's directions.

Southern and RNA Blot Analyses

Genomic DNA for Southern analysis was prepared as previously described (Rasmusson et al., 1994) from an isogenized *Drosophila* stock, *iso-1* (Tamkun et al., 1991). Seven micrograms of DNA were digested with restriction enzymes, fractionated on a 0.9% agarose gel and transferred to Zeta-Probe nylon membrane (BioRad Labs.) by standard methods. Membranes were UV-cross-linked and dried in a vacuum oven at 80°C for 1.5 h. DNA probes were gel-purified and labeled with [³²P]dATP using random hexamer primers (Pharmacia LKB Biotechnology Inc., Piscataway, NJ) according to standard methods (Vogelstein and Gillespie, 1979). Hybridizations were carried out overnight at 42°C in 50% formamide, 10% dextran sulfate, 4 \times SSC (600 mM NaCl, 130 mM sodium citrate), 5 \times Denhardt's solution (0.02% each of BSA, Ficoll, and Polyvinylpyrrolidone), 100 μ g/ml sheared denatured salmon sperm DNA, and 20 mM KH₂PO₄. Final washes (3 \times 20 min) were at high stringency, in 0.2 \times SSC, 0.1% SDS at 65°C. Isolation of total RNA from Oregon-R flies and subsequent blot analyses were performed as previously described (Rasmusson et al., 1994). Ovaries and testes were dissected in *Drosophila* EBR saline, flash frozen, and stored at -80°C. Staged embryos were flash frozen, stored at -80°C, and ground with a mortar and pestle immediately before RNA extraction. Twenty-five micrograms of total RNA were run on 0.75% agarose-formaldehyde denaturing gels. The hybridization and washing conditions were the same as for Southern blots.

β -Galactosidase Reporter Construction and Expression

An \sim 1-kb BamHI fragment was isolated from the genomic subclone pCH81 and was inserted into the BamHI site of the P-element vector pCaSpeR- β gal (Thummel et al., 1988). The construct (600 μ g/ml) was coinjected with 50 μ g/ml helper plasmid p π 25.7w (Thummel et al., 1988) into embryos of the *Drosophila* strain Df(1)w-c (Lindsley and Zimm, 1992). Chromosomal location of insertion for each transformant was determined by segregation from balancer chromosomes. β -Galactosidase activity during oogenesis was assayed by the protocol of Cheung et al. (1992). Stained tissues were viewed under a Zeiss Axioskop 10 microscope using differential interference contrast optics. Images were captured on an IRIS Indigo Video board installed on a Silicon Graphics R3000 Indigo workstation. Hardcopy image prints were produced on a Tektronics Phaser IISDx dye sublimation printer.

Antibody Production and Purification

Polyclonal antibodies were elicited in rats against fusion proteins produced

in *E. coli*. The dynein cDNA fragments ZAP8, 822, 828 and 3100 (Fig. 8A) were cloned inframe into the expression vector pET5a (Novagen, Inc.; Madison, WI) and expressed in the bacterial strain BL21 (Novagen, Inc.; Studier et al., 1990). Cells were lysed by sonication and the insoluble inclusion bodies containing the fusion protein were pelleted by centrifugation. The inclusion bodies were washed three times in PBS (150 mM NaCl, 16 mM Na₂HPO₄, 4 mM NaH₂PO₄, pH 7.3) containing 1% Triton X-100, 1% deoxycholate, and 0.1% SDS, followed by solubilization in PBS containing 1% SDS, and fractionation on SDS-polyacrylamide gels (Laemmli, 1970). Gel slices corresponding to the fusion proteins were excised, washed in PBS to remove SDS, emulsified with an equal volume of complete Freund's adjuvant, and injected into rats. Subsequent boosts were done in incomplete Freund's adjuvant at 2-wk intervals. All injections were subcutaneous in a volume of 0.1–0.25 ml. The polyclonal sera obtained were separately affinity-purified against their corresponding peptides expressed as glutathione transferase fusion proteins in the appropriate pGEX vector (Smith and Johnson, 1988).

A 20-residue peptide, LGGSPFGPAGTGKTESVKAL, was synthesized at Mayo Clinic, Microchemistry facility (Rochester, MN) and used to immunize rabbits (Calico Biologicals, Reamstown, PA.). The peptide antibody (PEP-1) and the ZAP 8 antibody, were purified over an affinity column prepared with a ZAP8 fusion protein expressed in the vector pGEX1 (Smith and Johnson, 1988) in the bacterial strain HB101. The affinity column was generated by coupling the fusion protein to Actigel (Sterogene Bioseparations Inc., Arcadia, CA) resin according to the manufacturer's instructions. The antibody raised against the pET-3100 antigen was purified similarly against a pGEX1-3100 fusion protein. The 828 derived antibody was affinity purified according to the blot affinity method of Olmsted (1981). The affinity purified antibodies were stored at -20°C in 50% PBS/50% glycerol containing 1 mg/ml BSA.

Ovary MAP Preparation

Ovaries from well fed 3-d-old females were dissected in EBR, placed on ice until 100- μ l ovaries were obtained, quick frozen in liquid nitrogen, and stored at -80°C until use. Ovaries were rinsed three times in PMEG buffer (100 mM Pipes, pH 6.9; 5 mM MgOAc.4H₂O; 5 mM EGTA; 0.1 mM EDTA; 0.5 mM DTT; 0.9 M glycerol) and homogenized on ice in 1.5 vol PMEG plus protease inhibitors (10 μ g/ml aprotinin, 1 μ g/ml leupeptin, 1 μ g/ml pepstatin, 0.1 mg/ml each of soybean trypsin inhibitor, *n*-tosyl L-arginine methylester, and benzamide). Cytoplasmic dynein was isolated from ovary homogenates as described previously for *Drosophila* embryo homogenates (Hays et al., 1994).

Immunoblots and Immunolocalization

SDS-PAGE and immunoblotting were carried out as described by Laemmli (1970) and Towbin et al. (1979). Proteins were electrophoresed on 0.75-mm slab gels and electroblotted onto nitrocellulose. For the analysis of ovary MAP preparations, gels were prepared with a bis/acrylamide ratio of 1:100 (Porter and Johnson, 1983). Alkaline phosphatase-conjugated antibodies were diluted in PBS containing 0.05% Tween-20 and 5% BSA. Blots were developed with NBT plus BCIP (Sigma Chem. Co.) in alkaline phosphatase buffer (100 mM NaCl, 5 mM MgCl₂, 100 mM Tris, pH 9.5). Alternatively, blots were incubated with horseradish peroxidase-conjugated secondary antibodies (Amersham Corp., Arlington Heights, IL) and developed with the non-radioactive autoradiographic ECL method according to the suppliers' instructions (Amersham Corp.).

For immunofluorescence microscopy, ovaries were dissected from 2–4-d-old females in EBR and fixed for 5 min in 100- μ l devitelinizing buffer/600- μ l heptane using the method of Cooley et al. (1992). Devitelinizing buffer = 1 vol buffer B:1 volume 36% formaldehyde:4 vol H₂O. Buffer B contains 100 mM KH₂PO₄/K₂HPO₄, pH 6.8, 450 mM KCl, 150 mM NaCl, and 20 mM MgCl₂. After fixation, ovaries were rinsed three times with PBS, rinsed briefly three times with PBS/0.1% Triton X-100 (PBT), and then washed 1 h in PBT at RT on a rotating wheel (Cole Palmer, Inc., Chicago, IL). Ovaries were then dissected into individual ovarioles, washed for 1 h in PBT, and then blocked in PBT containing 1% BSA (Block buffer) at RT. Primary antibody incubations were carried out overnight at 4°C. The ovarioles were then washed for 2 h at RT with eight changes of Block buffer, and incubated with secondary antibody for 1.5–2 h at RT. Ovarioles were again washed with Block for 2 h with at least eight changes, rinsed 3 \times in PBT, and washed for 5 min in PBT, and mounted in a solution of 10% PBS, 90% glycerol containing 1 mg/ml *p*-phenylenediamine (Sigma Chem. Co.). All antibodies were diluted in Block buffer. Affinity-purified anti-dynein heavy chain antibodies were used at a dilution of 1:50. Fluores-

cein-conjugated goat-anti-rabbit and goat-anti-rat (Boehringer Mannheim Corp.) secondary antibodies were preabsorbed against 100- μ l embryos overnight at 4°C at a dilution of 1:10. Preabsorbed secondary antibodies were used at a final dilution of 1:100 in the presence of 5% normal goat serum. Ovarioles (egg chambers) were examined on either a Zeiss Axioskop 10 microscope with epifluorescence illumination using plan-neofluar 16 \times /0.5, 40 \times /0.75 lenses or a 63 \times /1.4 oil planapochromat lens, or a Nikon diaphot microscope with an MRC-600 confocal imaging system (Bio-Rad Labs.), using a 60 \times /1.4 planapochromat lens.

The wild-type *Drosophila* strain OregonR was used in all localization experiments unless otherwise noted. The alleles *BicD*^{P466}, *BicD*^{R26}, *egl*^{WU50}, *capu*^{RKI2}, and *spire*^{RP8} were provided by Dr. Trudi Schüpbach (Princeton University, NJ) and are described in Suter et al. (1989), Manseau and Schupbach (1989), and Schupbach and Wieschaus (1991). The deficiency *Df(2L)TW119* that removes the *BicD* gene was obtained from Ruth Steward and is described in Steward et al. (1987). Dynein localization was examined in *BicD/Df(2L)TW119* females that were recovered from the cross *BicD/CyO* \times *Df(2L)TW119/CyO*. The *stau*^{D3} allele was provided by Dr. Paul Macdonald (Stanford University, CA), and is described in St. Johnston and Nusslein-Volhard (1992). The *N^{tsl}/C(1)Dx* stock was obtained from the Bloomington Stock Center, (Bloomington, IN). The role of Notch during oogenesis is discussed in Ruohola et al. (1991) and Xu et al. (1992). An *N^{tsl}/FM7* stock was created by crossing *N^{tsl}/Y* males from the attached-X stock *N^{tsl}/C(1)Dx* to virgin *Tp(1:3)JCI35/FM7* females. From the progeny of this cross virgin, *N^{tsl}/FM7* females were collected and backcrossed to *N^{tsl}/Y* males from the attached-X stock. When maintained at the permissive temperature, *N^{tsl}/N^{tsl}* progeny survive. Temperature shift experiments were as described in Ruohola et al. (1991). 1-2-d-old female *N^{tsl}/N^{tsl}* and *N^{tsl}/FM7* siblings were incubated at 32°C for 30 h before processing ovaries for immunocytochemistry.

Results

Isolation of the Full-Length Dynein cDNA

The molecular characterization of the *Drosophila* cytoplasmic dynein heavy chain gene was initiated by isolating

cDNA clones encoding the full-length dynein polypeptide. An affinity-purified, COOH terminus specific, dynein heavy chain antibody (see Materials and Methods) identified two positive clones in immunological screens of a *Drosophila* embryo cDNA expression library. The putative dynein clones were plaque-purified and their cDNA inserts were used as probes for RNA blots to determine the size of the RNA transcript from which they were derived. SDS-PAGE analysis of the intact and UV-cleaved dynein polypeptide from *Drosophila* embryos predicts a heavy chain of >435 kD (Hays et al., 1994). Only one of the putative dynein cDNA clones, designated MS3, hybridized to an RNA transcript of sufficient size, 13-15 kb, to encode the dynein heavy chain. The 2.1-kb insert from MS3 is derived from position 10,945 bp to 13,115 bp near the 3' end of the dynein heavy chain gene (Fig. 1).

To isolate the full-length coding sequence of the dynein heavy chain, the MS3 insert was used to initiate a walk in cDNA libraries to isolate overlapping clones that extended toward both the 5' and 3' ends of the dynein transcript. Fig. 1 shows the final map of the overlapping cDNA clones spanning ~15 kb which were isolated, restriction mapped, and sequenced. Several independent clones representing the 3' end of the transcript were isolated. Sequence analysis indicated that each was derived from the same transcript and included a long polyadenine tail that initiated at the same position in the sequence of each clone. One of these clones, designated PL4, is shown (Fig. 1). Overlapping cDNA clones extending toward the 5' end of the transcript were obtained from a randomly primed λ ZAP cDNA library (Stratagene). The first nine clones from PL4 to ZAP22 each contain at least 190 bp of overlapping sequence and span 13.5

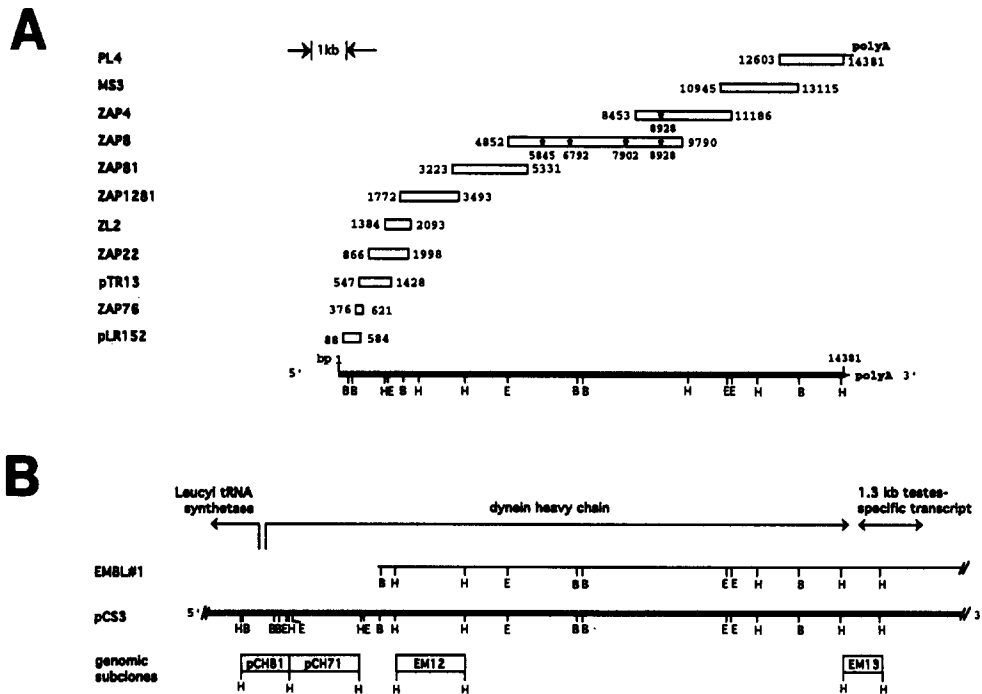


Figure 1. Molecular map of cytoplasmic dynein clones. (A) Overlapping cDNA clones are represented by open bars. Starting and ending nucleotide positions of each clone are indicated relative to the first nucleotide of the 14,381 nucleotide dynein transcript. Asterisks in the ZAP4 and ZAP8 clones indicate the positions of four phosphate binding loop (*P-loop*) motifs. A restriction map of the entire dynein cDNA is shown below the individual clones (*shaded bar*; B, BamHI; E, EcoRI; H, HindIII). (B) Genomic clones from the region of the dynein gene. Shaded bar at bottom shows the restriction map of a genomic cosmid clone (pCS3) that contains the entire dynein gene (B, BamHI; E, EcoRI; H, HindIII). Discrepancies between the genomic and cDNA restriction map reflect

the presence of a known polymorphism and 5' intron (see text). Thin line above the cosmid clone represents a genomic phage DNA clone (*EMBL#1*). The cytoplasmic dynein gene (*long arrow extending to the right*) is flanked on the 5' side by a putative leucyl-tRNA synthetase gene that is transcribed in the opposite direction, and on the 3' side by a testis specific transcript. The relative positions of selected genomic HindIII fragments used in the cloning and characterization of the dynein gene are shown at the bottom. Maps are drawn in scale as indicated.

kb of open reading frame (ORF) in the dynein transcript. cDNA clones extending further 5' to the end of the dynein transcript were recovered using a genomic probe to screen the cDNA library, and by priming the synthesis of new cDNA from embryonic mRNA (see Materials and Methods). The 5' coding sequence determined from PCR-derived cDNA clones was verified in the sequence of genomic clones.

Since the 5' most cDNA sequence did not contain stop codons in all three reading frames, primer extension and RNase protection experiments were conducted to determine the transcription start site and to confirm that the isolated cDNA clones contained the entire dynein transcript. Two extension products were revealed that correspond to start sites at positions 1 and 92, respectively (Fig. 2 A). To show that these two sites reflect the bonafide ends of transcripts and not premature termination due to secondary structure, RNase protection experiments were conducted. The protection of a genomic subclone extending from nucleotides -923 to 330 was analyzed. The sizes of the two fragments protected are in close agreement with the start sites indicated by primer

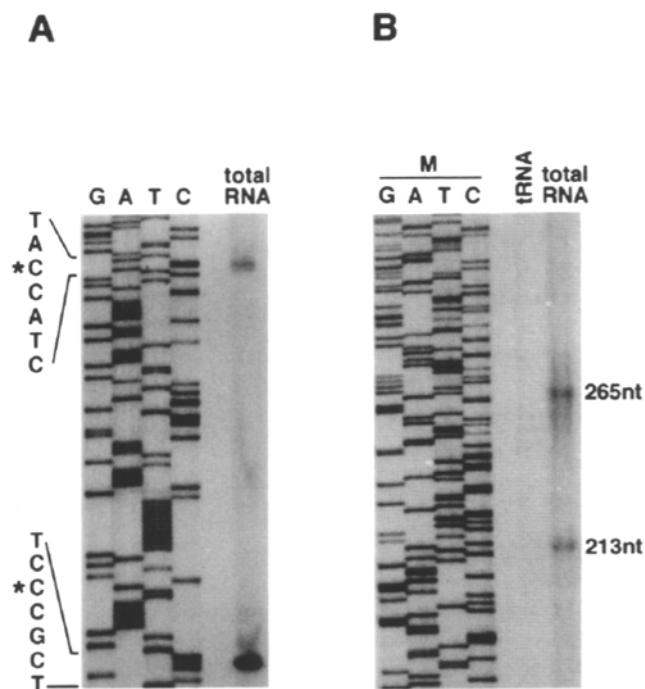


Figure 2. Determination of transcription start sites for the heavy chain mRNA. (A) Primer extension analysis. The DNA sequence is shown at left and serves as a size marker. The same antisense oligonucleotide primer (nucleotides 209 to 228) that was used for extending total embryonic RNA was used in the sequencing reactions. Two extension products were revealed, one of 228 bp and another of 136 bp, and the start site (asterisks) for each was identified from the sequence. (B) RNase protection experiments. A genomic fragment extending from nucleotides -923 to 330 was used as template. The two protected fragments are indicated and their sizes are in agreement with the predicted start sites determined by primer extension analysis. In the negative control lane, tRNA was used as template and no protected fragment was observed. The DNA sequence shown on the left was used as a molecular marker and the sequencing reactions were carried out with a primer starting 253 nucleotides upstream from the dynein heavy chain transcript.

extension (Fig. 2 B). A 582-bp genomic DNA fragment corresponding to the position of -341 bp to -921 bp, a position not expected to be transcribed, was used as a negative control and produced no protected fragment (data not shown). From these results, we conclude that the entire heavy chain transcript is cloned and that two sites are used to initiate the cytoplasmic dynein heavy chain transcript.

The first ATG codon in both transcripts initiates the same long ORF. If this ATG represents the translation start site in both transcripts, then the only difference between the two transcripts is in the length of the 5' untranslated leader sequences. One transcript is 14,381 nucleotides in length (starting at base pair 1) and the other transcript initiates 92 bp downstream. The 5' untranslated leaders are 162 and 70 nucleotides long, respectively. No TATA box was found by examination of the upstream sequence. The sequence flanking the first ATG (position 163 bp) differs from the consensus sequence (C/A AA A/C AUG) for translation initiation in *Drosophila* (Cavener, 1987). Additional ATG codons in the dynein ORF lie downstream and could also represent translation start sites. Like the first ATG, the second inframe ATG at position 499 bp in the cDNA does not show a consensus flanking sequence for translational initiation. However, the third inframe ATG at position 695 bp is flanked by a consensus sequence for translation initiation.

At the 3' end of the transcript, a 298 nucleotide noncoding sequence follows a TAA stop codon (position 14,080 bp) and shows an enrichment (74%) in AT dinucleotides. The consensus sequence (AATAAA) for polyadenylation (Proudfoot, 1989) does not appear in the 3' noncoding sequence. However, eighteen nucleotides upstream from the polyadenine tail there is a sequence of AATATA which has been found in the 3' noncoding sequence of other *Drosophila* genes (Berleth et al., 1988; Lehner and O'Farrell, 1989; Lantz et al., 1992) and may serve as a polyadenylation signal.

Structural Similarities among Dynein Heavy Chains

The nucleotide sequence and deduced amino acid sequence of the long ORF predicts a dynein heavy chain polypeptide containing 4,639-amino acid residues with a molecular mass of 530 kD and a pI of 5.9 (Fig. 3). The predicted amino acid sequence of the *Drosophila* dynein heavy chain was compared to the sequences for dynein heavy chains from sea urchin (Gibbons, et al., 1991; Ogawa, 1991), rat (Mikami et al., 1993; Zhang et al., 1993), slime mold (Koonce et al., 1992), and yeast (Li et al., 1993; Eshel et al., 1993). In each case, a high degree of sequence similarity over long stretches of the dynein polypeptides is apparent using a Pustell matrix comparison (Fig. 4 A). The *Drosophila* sequence was also compared to itself to reveal potential internal duplications. Other than the main diagonal resulting from self identity, no off-set diagonals were detected.

The *Drosophila* cytoplasmic dynein heavy chain is most similar to the predicted rat cytoplasmic dynein heavy chain polypeptide (Fig. 4 A and Table I; Mikami et al., 1993; Zhang et al., 1993). Overall the two sequences share 84% similarity and 72% identity at the amino acid level. As indicated in Fig. 4 A and Table I, the homology is distributed across the entire length of the polypeptides. By comparison, the overall similarity between the *Drosophila* dynein se-

-921	AGCTCTTTTT	CCACCTTGGC	GCCCGACCGC	CAGGAACAGC	COTTTGGCGT	CACCTGCTCA	TCTCGGAGCA	CGGTCTTATC	AACGGGATCC	CAGTTAACAA	GAGCCTCGTT	CTGGTAGGCC	-802
-801	AATCATGCG	GATGAGCAT	TAGGAACAGA	TGCTGGCTCC	ACTTGTAGTA	TTTGGGACTG	CAGTGGAGCA	ATTCATGGTT	CCAGTCAAG	GAGCAACCAA	GTCCCTTGGG	TTGTCTCTTC	-682
-561	ATCTGGGCAA	TGTTCTGGCA	GCTCCGAGAC	GGGGCTTCCA	CCCCAGCGTC	ATTGGGGGCG	TTCTCGGCGG	CGACGGCCAA	GGAAATCCCA	CCCATTTGGT	GAACACATCT	CTGTCGGCAC	-562
-561	ATCTGGGCAA	ACGAGGCTCC	CGAATCGGCT	ATGGTATAGA	CGCGCAGCAT	CCGCATGTCG	AGATATCCCG	AGAGATCGAG	GAACATCGAG	AGCAGCTAGT	AGACTCTCTG	CGATCCCTTG	-442
-441	GGATGAACT	GCCTCTCACT	GAGCTGCTCC	CCGCAATAGC	CCTCAATCCG	GTCTGTGACA	TCACGTGTTA	GCCTCGAATT	CTGGAATTTA	ACAAAGGTCT	TATTTGCTTT	CTCGGATATA	-322
-321	TTATGACCC	ACATCATCTC	GGTCCAGCT	TTGGTATAGC	AGGCGCCGCT	AGCTTATCCA	GTAGACAGCT	CCCCAAGGTC	GTGTTCTTCT	CAGTGCCTTC	ATTATACCGG	TTTAAATGAT	-202
-201	<u>GGAAATGAC</u>	<u>ATTTCAGAT</u>	<u>TATTAATTA</u>	<u>CAAAATAGA</u>	AGAAACCACT	GCNCTATCCA	ATFCGACAGC	TTTCTCTCTG	CATCGAGTTG	CCCAATCGCT	CCAGTATGAT	CCCAATGCTC	-82
-81	TAGAAATTT	TGGCGGAG	CATTCCTGAC	TGACTGGTAA	CATTCGCCCC	AGTCTGTGAT	TCTGATATCT	GCCGCAACTA	TGTTAGCACT	GGCTTTTTTG	CTTTGCAATT	TCAAATGAGT	39
40	GCGAGGGCG	TTTACATTTT	CGCAAAAAA	AAACTCAAGT	AATTTTTCAC	AGGCGGAGC	ATTGTGAAAA	ATTAATTTGT	ACACCTTTTG	AGGAGTATTT	AATACCGAAA	ACCTTTGTGG	159
160	GACATGGDT	ACTCCGTTGA	GAACCCCGAT	ACATCGGTGG	ATCCAATCGT	GAACCTGTCC	ATTGCGAGT	ATGATGCATT	TOCCAATTTAT	CTTCGGAAGG	CGGTGACCAT	TTTGTCCGP	279
280	GAGGACGAT	TGOTTCCCGC	ATCGCTGAAC	GATGCTCTGG	ACGATCCCGT	CAACCAAGAG	ACCATCCGGA	AGTTCCTCTC	GGATCCCGAC	GTCCAGGCGC	TTTACGTCCA	ACGCAATTCG	399
400	ATTAAGAGG	ACGATCTCTA	CGACGGCGCG	GAGGCGGAGG	ACGAGAAGGA	CGAGGTAACC	TACCAAATCA	GCAACAGATG	TCACCTTACC	AACAGTCCGA	TGCACTCTTT	GGCCTGCATC	519
520	AAGCGCGCG	TGTTCTGGGA	GCGCGACAGT	TGATATCATT	CGCAGCTCGC	CGTATCAATC	TTCTCGGAGC	GATCTCCCTA	CGAGACGCTG	CACGTCTTCA	TCAGCAAGTC	CGTGGCGCCT	639
640	TACTTCAAGT	CGTACGTCAA	GGAGTGGGTT	CGTCTGATC	CGGATGTGTA	CAAAATGGCT	CCCTCGTGGG	AGAAGAAGCT	GGCCGAGCTG	GAGATGGGAC	TGCTGCACTC	CGACAAAAAC	759
760	ATCGACATAC	CGGAGATCAC	GCTGACCGCT	CACCAACCGC	TCAACATATG	GATCCGTAAG	TGCGCCGAGG	AGAACCAGAA	GGCCAAAGTG	GCCGATTTTG	CGCACAAGT	GGAGATTTCT	879
880	TCITTTCTGA	ATCTGCTGCA	GAATGGTGT	AACCGCTGGA	TCGCTGAGAT	CAAGAAGGTT	ACCAAGCTGA	ACCCGAGTCC	AGGCTCCGGA	ACTTCGACTG	AGGAATTTTC	CTTCTGGCTC	999
1000	AACTCGAGC	GGGCTTGTGA	TGCAATTCAG	GAAAAGCCCG	AGTCCGCGGA	GGTGGCTTTA	TCTTGTGATA	TCGTGAAGCA	TGAAAACCGT	TTCCATGCCA	CCGTGTCTCT	TGATTCAGAT	1119
1120	ACCGACTAA	AACGAGCTCT	ACTTACAGTA	GCCGACTACA	ATCCTCTGAT	GAAGGATTTT	CCTATTAAAT	ATTTACTTTT	CGCTACCGAG	CTGGAGAAGA	TCCGATCCAG	TGTCCAGAGC	1239
1240	ATCTTCGCTC	ATCTACGAAA	GOTCCGCAAC	ACCAAGTACC	CAATCCAGCG	GTCCCTCAAG	CTTATCGAGC	CCATATCGCG	GGATCTGTGG	CAGCAACTGC	TTAAGTGTCT	GGCCACCGCA	1359
1360	CGCTTAATG	ACATTCCTTT	CGATTCATTC	GAGCGCTGCA	TGAACCAAGT	CTTCAAGGTC	TTTCACTGCT	GGGATGATGA	GTACGACAAG	TTCCAGGAGT	TGCTCGGAGA	CATTGTGAAG	1479
1480	AAGAGCGCG	ACGAGCATCT	TAAAGTGGTG	TGGCTGTTTT	CTCCGGCCCA	TAAAGAGGTC	CAAATCCGCA	TGGAGCAGAT	CGCCAGTTTC	CGAGCCGAGC	ACGAAACAGT	CGCCACCGTC	1599
1600	ATCTCGGCTG	TGCTCGGCGC	CACCAAGCGG	GCTGTGGCG	ATGACGCGAA	CGTCTGGAAA	ACCAAGGAGC	CCTACAGTCC	GGACCGTGGC	GATGCTAAGC	CTATCGAAGA	GCTCAACTVA	1719
1720	CGCTACGAGA	ACGTCAAGGA	AGTGGACTGT	CTGGATATCA	CCAAGGAGGT	ATCCGAGGCG	TGAGGCGCG	CCGTCAAGCG	CTATGAGGAG	AGATTTGATC	CGGTGAGAGC	TGCAATTAAT	1839
1840	GCTCATCTAC	GTGATCAGCT	GGAAACAGCC	AAGAAGCCCA	ACGAGATGTT	CCGATTTTTC	TGCGGTTTCA	ATGCGCTCTT	CGTCCGTCGG	CACATCCGTA	CGCCTATCCG	GGATATCCAG	1959
1960	ACCGAGCTGA	TTCAAGCGGT	CAAGGATGAT	ATCGAGCGCT	TGCAATGAAA	GTTTAAAGTT	CAGTATCCGC	AGAGCAAGAG	CTCCGCTTTG	TCTTCTGTGC	GAGATCTGCC	CGCGTGTGCC	2079
2080	GGCTCCATCA	TTTGGCGCGC	TCAGATTGAC	AACCAACTGA	CGATGTACTT	GAAGCGGCTC	GADGATVTL	TGGGCAAGGG	CTGGGAGACC	CATATCGAGG	GCCAGAGCTT	GAGGCGKAG	2199
2200	GGTATAGCT	TCAGAGCCAA	GCTTTCCATT	TCGGATGTGT	TCCATGAGTG	GGCCCGCAAG	GTCCAGGAGT	GCAACTTTTG	CAGCACTGGC	CGTATCTTTA	CCATCGAGCT	AACCGCTTCC	2319
2320	CGCATGGAC	GTGGCAATCT	TCCTCGTTTT	CGTGTCAACT	TTTTTCCGGA	GATCATCACT	CCGCCCAAGG	AAGTCCGCAA	CATCAAGAAT	CTGGGCTTTA	GAGTTCOACT	GACCATTTGT	2439
2440	AACAGGCGG	ATCAAGCGAA	CCAGATTAT	CCTTATGCTA	TATCATTTGAT	TGAGAGTGT	CGCAGCTATG	AGCOTACATT	GGAAAAGATC	GAGATCGTGG	CTAGCATTTG	CGCCCTTTGT	2559
2560	GCTGAGCTG	GGAAAGATCT	ACTTAACTGG	GTCTCCGAGG	GCATCCGCGT	CATCTGGGAG	TCATATAGC	TGATCCGCTA	CGTGTCCCG	CTGTCCGAGT	GTGTCAACCA	GTCTCAGGAG	2679
2690	AAGTTCGAG	ATTTGCTGT	TGTTGAAGAG	CAACTTGDV	TGGATGTGGC	CTGCTGGAAA	ACCTTGGCCG	ACAGTGCAGC	CACCTTCTGT	GAGATTTCTT	CGAAGATTTA	GCATCCCGTT	2799
2800	GAGATCTCT	CGCTCGCTCA	GTACTTCAAC	TGAGATTTCT	GGGTACTCTG	ACTCGATGAA	GAGGTGTGGA	AGAAGCTGGC	CCTCCGCTTG	CAGCGCGGTA	TTCAAGGCTG	GACGAGGCTT	2919
2920	CTCACCGCA	ATRAAGAGGA	GGTGTACACC	TCTATGGACA	CTGATGCGCC	CGCTCAAGCC	ACTCACAGC	TGGAGGAGA	CCCCGAGATT	CAGAACCGTG	TGCATGAGAT	TCGTATCACC	3039
3040	AACGAGCAGA	TGATCTGTTA	CCCATCCATT	GAGGAAGCCC	GTTTCCAGAT	CATCGACAGC	TTTTTCCGCT	GGCAGCGGAT	CGTCACTCCG	CAAGTGGCTC	HGAAGATC	CCGCTACCCAG	3159
3160	GTGGATQW	YLYLPSI	BEAR	FQIQ	MQQ	PFAW	QAI	VTS	QVRL	QST	RYQ	3279	
3280	TACGTGGATG	AGTGGCTGGC	CTATGAGAGC	TTGTGGGATC	TCCAGCTGTA	TATGTGTE	GGACGCTTGG	GTGAGGATGT	GAACTTGTGG	ATCAAGTGT	TGAATGACAT	CAAGCAATCG	3399
3400	CGCCACCACT	TTGATCGCTC	TGATACTGCG	AGAGCCATG	GTCCCATTTAT	TATTTAGT	GCCAAAGTGC	AGGCTAAGGT	CACCTTGA	TACGATTTCT	GGCAAGAGGA	GGCATTTGGC	3519
3520	AACTTTGGCA	CCCTCTGGG	AACCGAGATG	ACAAGCTTCC	ACAGCAAGGT	GTCCCAAGCT	CGTACCGTCA	TGAGAGATGCA	GAGCATCGAG	GCCCGCAGTA	CATCGAGTGC	CGTGAAGCTT	3639
3640	KFCFTLLG	TEMTSFH	SKVSKS	RTDGL	EMQ	SI	EAS	STSD	AVSF	3759			
3760	CATGTGAGCA	ACATTTAAGG	CGAGTGGCTC	GCCTTTAAGC	AGATTATCAA	GGCGAAGC	ACGCGCATAC	AGACACAGGT	TGCCAGTTTG	CAGGCGAAGA	TTTGTGGCGA	GGATTAAGGCT	3879
3880	GTGAGAGCAG	GCATCTGAGA	CTTCTTAAAC	GATTTGGAGA	AGACCAAGCC	ACAGCGAGCG	AAAGTTCGAC	CAGATGATGC	CCTACAACAG	CTGAGATCT	TTGAGAGCAA	GTACTCCGCT	3999
4000	TTGAGGAGAG	AGCGGACAAA	CGTGTCTAAG	GCCAAAGGAG	CACTGAGCTC	GCAGAATGCA	GCCTTCCGGA	ACAACAGTGC	TGAGGCTATG	AAGTGGCTTC	TCGAGAGGCT	ACAAGATCTG	4119
4120	CGCGGCTCT	GGAGCGATTT	ATCCAAGGTT	TGAGACAGTA	TTGATGAGAC	ACCGGACAAA	CCCTGGCTTT	CAGTGCAGCC	CGGTAAGTTG	CTGCAAGC	TGAGAGCCAT	GATGCTCAA	4239
4240	CTCAAGGAG	TGCCAGCTCG	TCCTCGTATG	TATGAATCGT	ATGATGACTG	GAAGAAGTTG	ATCCAGAGCT	ACATCAAGGT	GAACATCGTG	ATTGTGGAGC	TAAAGTCGGA	TGCTCTTAAAG	4359
4360	GAGCGCAAT	GGAAAGCAAT	GACCAAGCAG	CTGCGGTTA	ATTGGGTTAT	ATCCGATTTT	TCCTCCGCGC	AGGTGTGGGA	TGTGAATCTA	CAGAAAAGCG	AGGATATCGT	CAAGGATATT	4479
4480	ATCTTAGTGG	CCCAAGGCGA	GATGGCCTTG	GAGGAGTTCC	TCAAGCAGGT	CGTGAAGTCC	TGGCAGACT	ATGAGCTGGA	CTTGATCAAC	TACCAGAACA	AGTCCCGCAT	CATCCCGGCT	4599
4600	TGGACDATT	TGTTCAACAA	GGTGAAGGAG	CACATTAACT	TGTGGCGCCG	ATGAAGCTST	CTCCCTATTA	CAAGGCTTCT	GAGGAGGAGG	CTCCTTCCGG	TGGAGACTTC	CGGTTCCAG	4719
4720	ATTAAGCGCC	TGTTTGTAT	ATGGAATGAT	GTGCAAGAGC	GTGGGTTCTA	CTTGGAGTCA	ATCTCTCTG	GCAGCGCTGA	TATTAAGAGC	CTCCTTCCGG	TGGAGACTTC	CGGTTCCAG	4839
4840	AGCATCAGCT	CCGAAATCTC	TGGGTTGATG	AAGAAGGTGA	CCAAAATCGCC	CAAGGTTGTC	GATGTCTGTA	ACATCCCTGC	TGTGACGCGA	TCTTTGGAGC	GCCTCCGCGA	TTTGTGGGA	4959
4960	AAATCCAGAA	AGGCTCTGGG	CGAGTATCTG	GAGCGCGAGA	GAACCTCATT	CCCTCGTCTC	TACTTCTGGG	GAGATGAAGA	TCTTTTGGAA	ATAATTGGCA	ACAGCAAGAA	TATTCACAGT	5079
5080	CTCGAGAGAG	ATTTCAKAA	AATGTTTCTG	GGTGTAGCTG	CCATCTGTTT	GAACGAGAAA	AACAAGCTTA	TTCTGGGTTAT	TTCATCCGCG	GAAGGAGGAG	AGGTTGCACTT	CATGAANCTP	5199
5200	GTCTTCCAGC	TGAGACATCC	CAAGATCAAC	GAGTGGCTGT	CGCTGTGGGA	GAAGCAAAAT	CGAATCACGC	TGGCTTCACT	TTTGGCCGAG	GCTGTGCGAG	ATATCAAGCA	GTTCGCGAT	5319
5320	GGAAAGATCG	ACCCCCAGCG	CTACATGGAG	TGGTGTGACA	AGTACCAAGC	CCAGATTTGA	GTCTCGGCGC	CACAAATCTT	GTGGTCCGAG	GATGTGGAGT	CGCCCTTCCA	CGAGCTTCT	5439
5440	GAGAACAAAC	AATCTTAAGC	GATGCAAGCG	GTTTTGGGTA	ACGTAGAGAG	CACTTCAAC	GTCTTGGGCT	ATTCGCTCTT	GCAGGAGCAG	CCGCTCTCC	GTCTGTGTA	GCTCCAGCAC	5559
5560	CTTATAAAGC	AGTTTGTGCA	CAAGCGAAGC	GTAGCCCGAG	GACTCTTAAA	CAACGAGGTT	ACCTCGCCCA	AGTCCCTTCA	ATGCGTGTGC	GAGATCGAGT	TCTACTTTGA	TCCACGTCAA	5679

Figure 3. Complete sequence of the cytoplasmic dynein heavy chain mRNA and protein. Nucleic acid sequence of cDNA and 5' flanking genomic DNA, and the deduced amino acid sequence is shown. Two vertical arrows indicate transcription initiation sites for the heavy chain mRNA. The first start site is numbered as position 1. The termination codon is indicated by an asterisk. Four phosphate-binding loop motifs are underlined. The sequence of a 20-amino acid synthetic peptide used to elicit a rabbit antibody (PEP-1) is framed. The horizontal arrow upstream from the heavy chain transcript indicates the transcriptional start site and direction of transcription for a putative *Drosophila* leucyl tRNA synthetase gene. These sequence data are available from Genbank under accession number L23195.

5690 AC09AAGTCC TC0ACCAACT GACCATTAC AT09GTAACG CACGATTCTT CTAT0GCTTT GAGTATCTGG OT0TTCAGA TCGATT00TG CAGACT0CTC TA0CGGACCC CT0CTATTGG 5799
TEV L Q Q L TI H M A N A R F P Y G F E Y L G V Q D R L V Q T P L T D R C Y L
5800 ACAATGACAC AG0CTT0TGA AT0CAC0GCTT G0G0G0TAC C0T1T0G0CC T0C0G0TACT G0A0A0A0CAG AAT0C0T0CA G0C0AT0G0T A0C0A0C0AG C0G0AT0T0C T0C0G0TATC 5919
T M T Q A L R S R L G G G S P F G P A G T G K T E S V K A L G M Q L G R F V L V F L V
5920 AAT0CGATGG AG0CTT0TGA TT0TCA0G0C AT0G0C0GAA T0T1T0T0CG T0T1T0T0CG OT0G0G0CAT G0G0G0T0CT C0G0G0G0TCT AAT0G0ACTG AGG0AG0TAT G0C1T0C0AT 6039
N C D E T F D F Q A H G R I F V G L C Q V Q A M G C F D E F M R L E E R M L S A
6040 C0T0C0A0C0 AAT0C0A0C0 CATT0C0AGG C0C0T0G0A0T AC0G0AT0GA C0C0A0C0A0G G0G0AT0TCA C0T0T0G0A0T G0T0G0C0A0A C0A0T0C0C0C0 T0C0C0C0A0 C0T0G0TATC 6159
C S Q Q Q I Q T I Q E A L K Y E M D S N K E S I T V E L V G K Q V R V S F D M A I
6160 TTTATPACCA T0AAT0C0T0G C0T0T0C0G0C CATT0C0A0T T0C0G0ATAA CTT0G0A0A0A CTT0T1T0AG0T C0C0T0G0C0AT G0C0C0A0C0CA C0C0G0C0AGC T0A0T0T0C0GA G0T0G0AT0TGG 6279
F I T C T M N P Q Y A G H S N L P D N L K K L F R S L A M T T P D R Q L I A E V M L
6280 TTT0C0CAAG G0AT0C0G0C C0C0G0A0A0A C0T0G0T0C0A AAT0T0G0CC CTT0C1T0A0G C1AT0T0G0AC AGC0A0C0T0C C0A0C0A0G0C C0C0T0C0G0 T0G0T0C0G0A T0C0T1T0G0A 6399
F S Q T Q F R S A E K L A C K I V P F F X L C D E Q L S N Q S H Y D F G L R A L K
6400 TCC0T0TGA T0C0C0G0T AA0C0T0A0G C0G0AT0C0A T0AT0A0AG0T C0A0G0A0C0G AT0A0A0A0C AG0C0G0C0A A0A0C0T0C0G A0A0C0T0C0G T0G0C0G0A0A C0T0C0C0G0A 6519
S V L I S A G N V K R D R I M K I K E Q M K Q R G D E N I D E A S V A E N L P E
6520 Q E I L I Q S V C E T M V P K L V A E D I P L L P S L L S D V P P N V G Y T R A
6640 G0G0AT0A0G OT0TGA0GA AG0AT0C0CG AAG0T0T0CC AG0AG0ACTA C0T0G0T0C0C AGC0G0G0T G0C0G0C0T0G AT0G0A0A0G T0C0G0C0A0C T0C0G0C0A0C C0C0C0A0C0T 6759
E M K G L K E E I R K V C Q E D Y L V C G E G D E Q G A A W M E K V L Q L Y O I
6760 T0C0A0C0TAA ACC0AT0G0T T0T0G0T0C G0C0C0A0C0A G0T0C0C0C0A T0G0A0G0C TTT0T0A0G0C T0T0G0A0C0C T0C0G0G0C0G T0C0G0G0C0G T0G0C0A0C0T G0C0T0G0T 6879
S N L N H G L M M V G P S G S G K E T A W K T L L K A L E R F E G V E G V A H V
6880 AT0G0T0CCA AG0C0C0A0T C0A0G0A0C0C CTTT0T0G0T T0C0T0G0A0C G0A0T0C0C0C G0A0T0G0A0C0C AT0G0T1T0T G0L F T H I L R K I I D N V R G
7000 G0G0T0A0A C0G0C0A0T G0T0C0T0C0T G0C0G0T0G0 T0G0T0C0GA G0T0G0T0A G A0C0T0G0A C T0T0T0C0T0G T0A0C0A0C0A C0C0T0G0C0T T0C0T0G0C0T 7119
E I N K R Q W I I F G0G0T0G0T G0G0T0C0G0 G0G0T0G0T G0G0T0C0G0 A0C0T0G0A G T0G0T0C0G0 C0C0T0G0C0G C0A0T0G0C0T T0C0T0G0C0T 7239
7120 T0C1T0G0C0T C0A0C0A0T G0T0G0T0C0 G0G0T0C0G0 G0G0T0C0G0 A0C0T0G0A G T0G0T0C0G0 C0C0T0G0C0G C0A0T0G0C0T T0C0T0G0C0T 7359
7240 AT0T0C0A0G AC0T0T0C0T C0G0T0C0G0T AC0T0T0C0T T0G0A0G0T AG0C0A0G0C T0C0T0G0C0T T0C0T0G0C0T T0C0T0G0C0T T0C0T0G0C0T 7479
7360 G0C0G0C0G0 AT0T0C0T1T G0C1T0G0C0T C0A0T0C0T1T C0A0C0G0A0T C0A0T0T0G0C0T C0C0A0G0A0C C0A0C0A0T0G C0A0C0A0T0G C0A0C0A0T0G 7599
7490 C0T0G0T0C0T T0C1T0C0A0T G0C0C0A0C0G C0C0C0T0A0A AT0T0T0T0C TTT0C0A0T0C C0A0C0T0C0A ATTT0C0C0T T0C0C0T0G0T C0A0T0G0A0C C0A0C0A0T0G C0A0G0C0T0G 7719
7600 G0T0A0C0T0G T0C1T0G0C0T A0T0T0C0C0G G0T0C0A0A0T T0A0G0T0C0G C0A0T0T0T0G G0G0A0C0T0G T0C0C0A0G0T G0A0C0C0T0G C0A0C0C0G0C G0A0C0C0G0C T0C0C0A0T 7839
7720 AT0C0A0C0T AG0T0A0A0T G0G0T0G0A0C T0G0T0C0C0T G0A0C0A0A0 G0T0A0C0A0T AT0C0A0C0T0G A0A0C0C0A0 G0T0G0C0A0T C0C0G0A0T0G T0T0C0C0A0T AT0G0A0T0C 7959
7840 G0T0C0T0C0G AG0C0T0C0T G0T0A0C0T0G C0T0G0T0A0C A0A0A0C0A0T G0T0G0C0T0G G0C0C0A0C0T G0C1T0G0T0A G0A0T0G0A0C C0T0T0C0G0C C0C0T0C0G0C T0C0C0G0A0T 8079
7960 AT0G0A0G0T AG0C0C0T0A TTT0C0C0A0T TTT0C0C0A0T G0C0T0C0A0C C0G0A0C0T0C G0C1T0A0G0C TTT0G0A0C0T A0C0T0G0A0T C0C0A0C0A0 G0A0C0A0C0A C0C0A0C0A0 T0T0C0T0C0C C0C0A0C0A0T 8199
8080 AT0C0G0A0G0T G0C0T0G0T0T G0T0C0G0A0T G0A0T0C0A0T T0C0C0A0C0T G0A0C0A0C0T G0G0C0C0A0C G0T0T0A0T0C G0T0C0C0A0T G0T0C0C0A0T C0A0C0A0G0C C0T0C0T0C0C 8319
8200 G0C0A0C0A0T AG0C0T0G0T T0C0C0T0G0A G0C0A0T0C0A0T T0T0G0G0T0C T0T0A0T0C0A C0C0A0T0C0A C0C0G0C0T0G C0A0T0G0C0T0G C0A0C0A0G0C G0C0A0T0C0T 8439
8320 T0C0G0A0T T0C0T0G0A G0A0C0T0C0T A0C0G0C0A0T A0C0G0C0A0T C0C0G0C0A0T A0C0G0C0A0T T0C0T0G0T0C T0C0T0G0T0C T0C0T0G0T0C C0T0C0A0C0A G0C0A0T0G0T 8559
8440 G0G0T0T0C T0G0A0C0A0C0A G0A0C0C0T1T T0C0C0C0G0A T0A0T0C0T0C T0C0C0A0C0T0G AG0A0G0C0C0A AG0A0G0C0C0A G0C0A0T0C0G C0C0T0G0A0T 8679
8560 T0C0C1T0C0G T0G0A0G0A0T G0T0C0A0C0T S L P V E G L V R L W A H E A L R L F Q D R L V D D S E R R W T N E N I D L V G
8680 C0A0A0G0A0C T0C0T0G0A0C C0A0C0A0A0A A0C0T0C0G0A0T C0T1T0C0A0C A0A0C0G0T0A G0A0A0G0A0T A0A0C0G0G0T A0A0C0G0G0T A0A0C0G0G0T A0A0C0G0G0T A0A0C0G0G0T 8799
8800 Q K H F P G I N Q E E A L Q R P I L Y S N W L S K D Y M P V N R E E L R E Y V H
8920 G0C0C0G0T0A AG0C0T0C0T A0G0A0A0G0C C0T0G0T0C0C C0A0C0T0C0C G0T1T0T0G0C C0T0G0A0A0T G0C0T0T0C0A G0C0T0T0C0A T0A0T0C0A0C A0A0A0G0T0A C0A0C0A0G0A C0T1T0G0A0G 9039
9040 G0T1T0G0T0T G0C0T0C0G0C C0C0T0C0G0C T0C0A0G0A0C A0A0A0G0T0C TTT0C0T1T0G G0T0A0G0T0C G0T0A0G0T0C G0T0A0G0T0C G0T0A0G0T0C G0T0A0G0T0C G0T0A0G0T0C G0T0A0G0T0C 9159
9160 G0A0G0A0G0T G0C0T0G0T0T C0A0G0T0G0C G0A0T0C0A0C C0T0C0A0C0T T0C0A0G0T0C G0A0G0T0C0C A0G0C0G0A0G A0C0G0C0A0G T0C1T0T0T0G G0A0C0C0A0G C0A0C0G0T0A C0A0G0T0T0C 9279
9280 ACC0A0C0A0G T0A0T0C0A0A T0C0A0C0T0G G0T0C1T0C0A T0A0T0C0T0C C0C0A0G0T0A C0C0A0G0T0C G0T0C0C0A0C C0T0T0A0C0T G0T0T0G0T0T A0A0T0G0T0T 9399
9400 G0C0A0C0T0G C0A0C0T0C0C T0C0T0C0A0G G0T0G0C0A0G A0T0T0C0A0C T0G0T0G0A0C T0G0A0A0A0C C0A0A0T0G0A T0C0C0C0G0A T0C0C0C0G0A T0C0T0C0A0C C0G0T0T0G0C A0C0T0G0C0A 9519
9520 G0C0A0T0C0A C0A0C0T0C0A T0C0G0T0C0C A0A0C0T0C0C T0T0T0T0C0A C0C0A0C0A0C C0C0A0G0C0A C0C0A0G0C0A G0C0C0T0C0A C0A0T0C0A0C G0C0C0T0C0A 9639
9640 H Y L D F I H K L Y N E K R S D L E E Q Q L H L N V G L N K I A D V E Q
9760 G0T0G0A0G0A T0C0A0A0A0T G0C0G0C0T0G A0A0G0C0A0G A0T0T0C0A0C C0A0A0C0A0G G0C0C0A0C0G C0A0A0C0T0A G0A0T0G0T0C C0A0A0C0A0G A0A0G0C0A0G A0A0G0A0G 9879
9880 AT0C0A0T0C0 AG0A0A0T0C G0T0A0C0T0G C0G0C0A0A0 C0G0T0A0A0T C0A0A0G0A0A C0C0A0A0T0C T0A0T0G0C0A T0T0G0C0A0G T0T0G0C0A0G T0T0G0C0A0G T0T0G0C0A0G T0T0G0C0A0G 9999
10000 G0C0T0C0A0G C0A0T0C0C0A G0A0C0A0C0T G0T0A0G0T0C G0A0C0A0T0C T0A0T0C0C0A T0C0G0T0C0A A0A0T0G0T0C T0A0T0G0T0C T0A0T0G0T0C T0A0T0G0T0C T0A0T0G0T0C T0A0T0G0T0C 10119
10120 T0G0A0A0T0C G0T0C0C0C0T G0A0T0G0C0C G0A0A0T0T0C T0A0A0T0C0A T0T0A0T0C0A T0C0G0T0C0A G0A0A0C0A0C C0A0T0G0T0T G0A0A0C0A0C C0G0C0A0G0A T0A0T0G0T0C G0C0C0A0G0A T0A0T0G0T0C 10239
10240 AAT0C0G0A0C AT0A0C1T0C0A G0A0G0T0A0T C0C0C0C0A0T G0C0C0C0A0T T0C0T0T0G0 T0C0T0T0G0T A0A0T0G0C0C A0T0G0C0A0T C0A0G0C0A0G T0C1T0T0T0G G0A0C0C0A0G A0C0T0T0G0A G0C0T0T0C0C 10359
10360 G0A0G0A0C0T G0T0C0C0T0G0A G0A0C0A0G0C G0A0T0G0A0T G0G0C0A0T0C T0G0C0A0T0C C0C0A0A0C0C A0A0G0C0T0G T0G0A0C0A0T A0T0G0C0C0A0T A0T0G0C0C0A0T A0T0G0C0C0A0T A0T0G0C0C0A0T 10479
10480 CTT0A0T0T0C0 AG0C0T0A0G0C AAT0C0A0A0C G0A0T0C0A0G A0T0C0A0G0C C0A0G0T0G0A0T C0C0T0C0A0T0G C0C0T0C0A0T0G C0C0T0C0A0T0G C0A0T0G0A0C A0T0G0A0C0C0A0T A0T0G0C0C0A0T A0T0G0C0C0A0T 10599
10600 G0A0C0A0T0C0A G0T0C0A0C0A0T G0C0A0C0A0T T0T0T0C0T0C C0C0T0C0T1T A0C0C0C0A0T G0T0G0T0A0C G0A0T0G0T0C G0A0T0G0T0C G0A0T0G0T0C G0A0T0G0T0C G0A0T0G0T0C 10719
10720 C0A0G0A0C0T AG0C0G0C0G C0A0T0C0A0T C0C0G0C0A0T C0C0G0C0A0C T0A0A0T0C0T T0C0A0T0C0G T0C0A0T0C0G T0C0A0T0C0G T0C0A0T0C0G T0C0A0T0C0G T0C0A0T0C0G T0C0A0T0C0G 10839
10840 ACC0G0A0G C0A0T0A0T0T G0A0G0C0T0C C0G0T0A0C0T T0A0T0C0T0G T0A0T0C0T0G G0T0C0A0G0C0A C0A0A0G0C0A C0A0A0G0C0A T0A0T0G0A0A T0A0T0G0A0A T0A0T0G0A0A T0A0T0G0A0A 10959
10960 TTT0T0G0A0T A0T0G0T0C0G C0A0G0A0T0T G0A0T0C0G0C C0A0G0A0T0T G0A0T0C0G0C C0A0G0A0T0T G0A0T0C0G0C C0A0G0A0T0T G0A0T0C0G0C C0A0G0A0T0T G0A0T0C0G0C C0A0G0A0T0T 11079
11080 G0T0C0T0A0G G0T0A0C0T0G G0T0A0T0C0C C0T0G0C0A0C AG0A0C0A0C0A TTT0T0G0C0G T0C0T0T0G0C T0C1T0C0T0C T0A0T0G0A0C T0C1T0C0T0C T0A0T0G0A0C T0C1T0C0T0C T0A0T0G0A0C 11199
11200 T0T0G0G0T0A C0A0T0C0T0A CTT0A0C0T0C A0C0T0G0A0C C0T0C0A0C0T C0C0A0G0T0C G0A0C0G0T0C T0A0A0G0C0T0A A0C0T0C0G0A0T A0T0G0A0C0A G0C0C0C0A0G A0C0C0C0A0G 11319
11320 C0T0G0A0G0T A0T0C0T0C0T G0C0C0T0C0C C0A0T0G0A0A G0A0G0T0T0T G0C0C0C0A0C G0C0C0C0A0C G0C0C0C0A0C G0C0C0C0A0C G0C0C0C0A0C G0C0C0C0A0C G0C0C0C0A0C 11439
11440 A0A0G0A0G0C C0T0A0T0A0T A0A0T0G0A0G G0T0G0C0A0A C0A0A0A0G0T A0T0G0C0G0A G0C0C0C0A0C G0C0C0C0A0C G0C0C0C0A0C G0C0C0C0A0C G0C0C0C0A0C G0C0C0C0A0C 11559
11560 ACC0T0G0A0C G0T0C0A0A0C G0T0C0A0C0T C0T0A0C0A0T A0C0T0C0A0A G0A0T0T0C0T G0A0T0T0C0T G0A0T0T0C0T G0A0T0T0C0T G0A0T0T0C0T G0A0T0T0C0T G0A0T0T0C0T 11679
11680 G0A0C0T0G0C G0A0T0G0T0C C0A0G0A0C0T C0C0A0G0T0T G0C0T0A0G0C A0T0A0G0C0C G0A0T0G0C0C G0A0T0G0C0C G0A0T0G0C0C G0A0T0G0C0C G0A0T0G0C0C 11799
E R L G I V T R D L F P Q Q Y E R V A R G M I H N D R L T F A L L M C K I H L K

Figure 3.

11800 GGCACCTCGG AGTCTAATT GGATGCCGAG TTCAACTTCT TCTCGGAGG CCGCGAGGOT CTCTAGACAA ATCCACGCC COTAGAAGGA CTTTCTCCG AGCAGATTGA GAGTGTGAAC 11919
G T S E S N L D A E F N P F L R S R E G L L A N P T P V E G L S A E Q I E S V W
11920 CGCGTGGCCC TTGCTCTTCC CATCTTCCGC AAGCTTCTTG AGAAGGTACG ATCCATCCC GACTGGGTG CCGTGGTGA CCGTGAACAGG TTGTACCCCA GCTGTGGAC 12039
R L A L R L P I F R K L L E K V R S I P E L G A W L Q Q S S P E Q V V F Q L W D
12040 GAAATCAAGG CTCTCAATCC GATTCGACGC TCGTTCACC AGCTCTGCT TATCCAGGCC TTCCGAGGCC ATCCGCTTAT CCGCCCGCT CACAATGTG TCACACTGT GCTCGGAGAA 12159
E S K A L S P I A S G V H Q L L L I Q A F R P D R V I A A A H N V V N T V L G E
12160 GACTTATGC CCAACGCCGA AGAGGAATG SACTCACTT CTGTGTGGA AACTGCAACA CTCTGCGCT GCTGTCTCG GTGCCCGTT TCGATGCAT CGGCCGATG 12279
D F M P N A E Q E L D F T S V V D K Q L N C N T P A L L C S V P G F D A S G R V
12280 GATGACTGG CAGCGAGCA AATAAACAA ATTTCCAGCA TTGCTATCG TTACGAGAA GACTGAGCG AGCTATTAAC AGACTGTGCG AGACTGTGCG CTGGCTGCTC 12399
D D L A A E Q N K Q I S S I A I G S A E G F N Q A E R A I N M A C K T G R W V L
12400 TTGAAGAGG TCCACTATG TCCCAATG CTGTGACG TGGAGAAGAA GATGACTCC CTGACGCCCT ATTCGCGCTT CCGCTGTGTC CTCAGATG AGATTAAATC AAAGTCCG 12519
L K N V H L A P Q W L V Q L E K K H S B L Q P H S G F R L F L T N S I N P K V P
12520 GTTAACTTAC TGCCTCTG TGCTGCTT GTTTCGAG CACCACCAG CATCAGACT AATCTGCTCG GCACCTTCTC TACGGTACCG CCGGACCGCA TGATGAAGAC TCAAGTGAA 12639
V N L R A G R I F V F E P P P G I R A N L L R T F S T V P A A R M M K T P S E
12640 AGAAGTCCG TTTACTTCT GCTGGCTGG TTCCACGCCA TGTCACAGG GCGACTCCGA TATGTGCTC TTGCTGGCC CAAGAAGTAC GAGTTTAAAG AATCGAATC CGCGTGGCT 12759
R A R L Y F L L A W F H A I V Q E R L R Y V P L G W A K K Y E F N E S D L R V A
12760 TCGACACAT TCGACACTG GATCGACACC ACGCCATGG GCCGCACCA TCTGCCACA GAGAAGTTC CATGGATGC CCGTGCACC CTGCTCTCG AGTCCATTA COGAGAAAG 12879
C D T L D T W I D T A M G R T N L P P E K V P W D A L V T L L S Q S I Y G G K
12880 ATCGATAAG ATTTGATCA GCGCCTTTG ACTTCTTCT TGAAGAAGT CTTCACCGCA CCGACTTTC AGCGGACTT TGCCCTGGT GCTAACGTTG ATGCCCTC CGAGGACTC 12999
I D N D F D Q A R L L T S F L K K L P T A R S F E A D P A L V A N V D G A S G G L
13000 COTCACATTA CCAATCCCGA TCGACCCCT CCGATCAT TCTTGAATG GATTCAGAAC TTGACTGACC GCGACACTC GTCTGTGCTT GGATTACCGA ACAATCCCGA GAAGTGTG 13119
R H I T N P D G T R R D H F L K W I E N L T D R Q T P S W L G L P N H A E K V L
13120 CTTACACCC GTGTATAG TTGTGCACT AAGCTCTCA AGATGCAACA CCGTGAAGAC GAGCATGAT TGCTTACAG CCGTGAAGAT CAATCGAGC AATCTGCAAT GGCTCGCGC 13239
L T T R G T D L V S K L L K M Q Q L E D D D E L A Y S V E D Q S E Q S A V G R G
13240 GAGGATGAC GTCCATCAT GATGAAGCA CTTCAACTC CAGTACTG CTGTGTGAA CTCTCCCA AGAATCTGCA AGTGTCAAG COTACTGTG AGACATCAA GGATCGGTG 13359
E D G R P S W M K A L H N S A T A W L E L L P K N L Q V L K R T V E N K R P L
13360 TACGATACT TCGACCGCA GGTGCAAGT GGTTCGCTC TGTGCAAC AGTATAGT GAGCTCGAG ATGTGCTCT AATTTCCAG CCGGAGAAGA ACAACACCA CCACATCTG 13479
Y R Y F S R E V T S G S R L Q T V I L D V V L L Q D I C Q G E K K Q T W H H R
13480 TCCATGTTT CCGACTGAT TCGCGTATA ATTCGAGG GTTGAAGCG GTATACATTT CCGTCAAGT CCGACTGAT CCAATGATC ACGACTTCA CCAACCTGT CCAAGACTG 13599
S M L S E L V R G I I P K G Y T V F A G C T V I Q W I T D F S N R V Q Q L
13600 CAGAAGTGT CCAACTTGT TTCCAGGCT GCGCCCAAG AGCTGACGG CTCCCGCTG TGCTCGGTG GTTTGCTCAA TCCGAGGCC TACATCACG CACACGACA GTGTGTGCC 13719
Q K V S Q L V S Q A G A K E L Q G F P V W L G L L N P E A Y I T A T R Q C V A
13720 CAGCGAATA GTTGTGCTC AGAGAGCTC GCGCTGATG TGACAATAC GATGCTGGG CTAAGAAGC ATCAAAAGA TTGTTGCTT GGGGTACTG CCGACTGCA GGTCAAAAT ACAAGGCC 13839
Q A N S W S L E E L A L D V T I T D A G L K N D Q K D C C F G V T G L K L Q G A
13840 CAGTCAAGA ACAATGAAT GCTCTGCTC TCCACTATCA TGATGATCT ACCTGTACC ATTCTCAAGT GGATAAAGT TTCCCTGGAG CACACGATCA CCAAGTTGAC ATTCCGCTT 13959
Q C K N H E L L A S T I M D L P V T I L K W I K I S S E P R I S K L T L P V
13960 TACTTGAAT CCAACGCTAC AGAATACTT TCACTGTTG ACTTGGCCCT TCGCGCTGG CAGGAATCC ATGACTTCTA CCAAGAGGC GTGGCAATTT TGACCTTCAAC 14079
Y L N S T R T E L L F T V D L A V A A G Q E S H S F Y E R G V A V L T S T A L
14080 TAACTGTTA CCAAGATA TCCATATAT CCAATATTT AGTCCAAAT ATTTCTCAA AGCTTTTACC TAAAGTATP AAAAGTTAA TCTAAAGTA TATCTGACT CTTTCACT 14199
*
14200 AAAAAACAT AGAATTTAC AAAATGTAAT TTCAAGATT AAAATGAAT ACTTTAACT TGTAGATT ATTTTAAAA TATTTCAAT TCTGTGATA GCATGATTT GTGAAAGTA 14319
14320 TTTTAAATC TGTTCACCAT TCCGATAAA GCTTACTAAA TATATATACT CATAGCAAC CCA(n) 14381

Figure 3.

quence and the *Dictyostelium* (Koonce et al., 1992), *Aspergillus* (Xiang et al., 1994), yeast (Li et al., 1993; Eshel et al., 1993), sea urchin (Gibbons, et al., 1991; Ogawa, 1991), and *Chlamydomonas* dynein (Mitchell and Brown, 1994; Witman et al., 1994) polypeptides is substantially lower. As shown by the matrix comparisons, this reduced similarity is most evident over the NH₂-terminal one third of the polypeptides. Further comparisons between the sequences were made using NH₂-terminal (residues 1-1500), Central (resi-

dues 1500-3000), and COOH-terminal (residues 3000-4639) segments from the *Drosophila* sequence. Each segment was aligned to the other published dynein sequences to determine the % identity and % similarity in each of the segments (Table I). The sequence comparisons demonstrate that the NH₂-terminal one third of the dynein polypeptides is most divergent, and that a central domain is the most highly conserved in all cases (Fig. 4 A and Table I). Within the more conserved central domain (residues 1500 to 3000; see Table I), the percentage of identity between the *Drosophila* and other published dynein heavy chain polypeptides is: rat (79%), *Aspergillus* (62%), *Dictyostelium* (61%), yeast (61%), sea urchin (39%), *Chlamydomonas* α (31%), *Chlamydomonas* β (33%), and *Chlamydomonas* γ (33%).

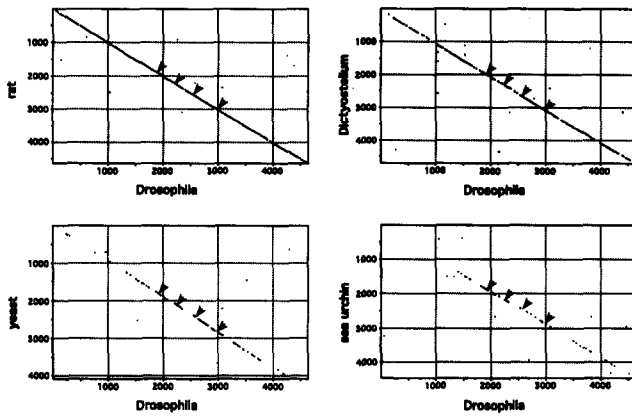
The conserved central domain encompasses a cluster of four phosphate-binding loop (P-loop) motifs with the consensus sequence of GXXGXGKT/S (Fig. 4 B). The positions of these four P-loops in the central domain of the dynein polypeptide, the spacing of the sites relative to one another, as well as the amino acid residues that comprise each site, are highly similar in all reported dynein sequences (Fig. 4 B). The predicted sequence of the first P-loop (residues 1895-1902) in *Drosophila* is totally conserved among all dynein sequences. The sequence of the second *Drosophila* P-loop (residues 2210-2217) is conserved in the rat cytoplasmic sequence (Fig. 4 B) and in a cytoplasmic dynein sequence from sea urchin (not shown; see Gibbons et al., 1992). The third P-Loop sequence (residues 2580-2587) is identical in cytoplasmic dynein isoforms from *Drosophila*, rat, *Dictyostelium*, sea urchin, *Aspergillus*, and yeast, but differs from the sea urchin β axonemal dynein sequence, as well as the α, β, and γ axonemal sequences reported for *Chlamydomonas* (e.g., see Fig. 4 B; see also Gibbons et al., 1991; Mitchell and Brown, 1994; Witman et al., 1994). The

Table I. Dynein Heavy Chain Sequence Similarities

	Drosophila Dhc64C			
	Full length	Residues 1-1500	Residues 1500-3000	Residues 3000-end
Cytoplasmic				
Rat	72 (84)	67 (82)	79 (88)	70 (81)
Aspergillus	51 (69)	40 (60)	62 (78)	49 (68)
Dictyostelium	52 (70)	41 (62)	61 (77)	52 (69)
Yeast	30 (55)	20 (46)	39 (62)	31 (56)
Axonemal				
Sea urchin	26 (51)	19 (44)	33 (58)	27 (51)
Chlamy. β	26 (50)	19 (45)	33 (57)	26 (49)
Chlamy. γ	26 (50)	19 (45)	33 (56)	26 (49)
Chlamy. α	NA	NA	31 (55)	NA

Amino acid sequence comparisons of *Drosophila* cytoplasmic dynein with dynein heavy chains from other species. Numbers in bold indicate percent identical residues. Numbers in parentheses indicate percent similar residues. NA, not available. Alignments and comparisons were made using the UWGCG BESTFIT program using the default settings. The dynein sequences used for rat, *Aspergillus*, *Dictyostelium*, yeast, sea urchin and *Chlamydomonas* are Genbank sequences from, respectively, Mikami et al. (1993); Koonce et al. (1992); Eshel et al. (1993); Gibbons et al. (1991); Mitchell and Brown (1994); and Witman et al. (1994).

A



B

P-loop₁

dmc ^{dhc}	1872	PLTDRCYL/TMTQALERSLGGSPFPGAGTKTESVKALGNQLGRFVLVFNCF
rnc ^{dhc}	1881A.....H.....
ddc ^{dhc}	1946L.....M.N.....T.....S.....C.
enc ^{dhc}	1910F.L.....CO.....Y.....L.....T.....C.
scd ^{dhc}	1773LLIGFA.L.DS.HQKY..CP.....T..F.QN..V.V.....
su ^β dhc	1829I.L.S.HLVMS.A.A.....TT.D.RA..IM.Y.....
cr ^β dhc	1896FI.L..QRLV..A.A.....TT.D.ARA..IQCY.....
cr ^γ dhc	1796I..I.LS...GMF..A.A.....TT.D..T..KY.V.....
cr ^α dhc	552I.I.A...CVLS..TA.A.....TT.D.SA..KS.Y.....

P-loop₂

dmc ^{dhc}	2187	GAANREKVLQLYQISLNHGLMNVGPGSGKSTAMKTLKALERFEGVGS
rnc ^{dhc}	2199	GM.V.....TQI.....M.RV.....L.....
ddc ^{dhc}	2248	KQE.V.I...H.L.I...V.....G.T.S.EVY.E.Y.GVDNIRS
enc ^{dhc}	2204	SEM.IT.I...QSH.V.....K.....S...I..Q..G.Y.
scd ^{dhc}	2051	SEEFLLK.CM.F.YMQRTQA.TL.KA.C.TAT...VID.MAI.D.HAN
su ^β dhc	2110	KDSFVL.V..KELLAVR.SVTVI.NA.T...QVL.V.N.TYSMMKPKV
cr ^β dhc	2179	DDQPLL.YSHVRELFVVRMSVFL.AA.C.TAV.R...R.QNSSGKTI
cr ^γ dhc	2075	HPTM.N.CI...EYLVV..I.L.....AICBC.AA..YELGTRIV
cr ^α dhc	855	KDDFVLR.VDFSELLAIR.CVFLM..T.T.RTECYRV.A..ITKCGNPF

P-loop₃

dmc ^{dhc}	2559	TVRHESLLYTWLAENKPLVLCGPGSGKTMFLSALRALPDMEVVGLNFS
rnc ^{dhc}	2572A.....
ddc ^{dhc}	2648	T.VDV.HA..S.R.I.....T.T..F.F..S.....
enc ^{dhc}	2571DV..S.....L.....A..K.N.....
scd ^{dhc}	2397	..IK..KIF.DL.NSKRGIT.....IMN...NSSLVD..I..
su ^β dhc	2439	T.VRPFMDLLMERGR.VM.V.NA.L..SVLVGDK.SN.GEDS..AMVFP
cr ^β dhc	2509	T.LTYF.DSLVSNIGYAMFV.NT.T..SAINVMK..NMDTETMSFYTIN
cr ^γ dhc	2404	S..YGA..NLSYNVD.ATL.V.G..TA..N.INQFISKFNATETANKTIT
cr ^α dhc	1207	..SSLRFF.DMVDLR..IMFV.GA.V...QLVRGK.GS.NEEQISLSISF

P-loop₄

dmc ^{dhc}	2902	DHVLRIDRIFRQPGHLLIGVSGAGKTTLSRFVAMNGLSIFQIKVHNK
rnc ^{dhc}	2915VY.....R.....
ddc ^{dhc}	2991I.....V.....A.....G.SV.....YT..N.N
enc ^{dhc}	2912	E.A.....V.....I.....S.....KV.....G.
scd ^{dhc}	2739I.....ALK.V..MM..A.RT...I.T...L..K.V.P.I.RH
su ^β dhc	2785	Q..C.N..LES.R.NA..V.G.S..QS.A.LASYISS.EV..TLRKG
cr ^β dhc	2859	E..T.A..IDL.R.NAM.V..G.S..QS.A.LASYIC.YEYV..S.SST
cr ^γ dhc	2783	T.LM.T.LLAW..LG..V.G.S..QS..LS.YIA.PTFYIT.TY.V
cr ^α dhc	1560	K..C..S..VSN.S.A..V..G.S..QS.A.LA.HIC.YATQM.VISGS

Figure 4. Structural similarities among dynein heavy chain polypeptides. (A) Dot plots show protein sequence matrices between two dynein heavy chains. X-axes for all plots represent the *Drosophila* cytoplasmic dynein heavy chain. Y-axes are: rat brain cytoplasmic dynein (top left, *Rattus norvegicus*; Mikami et al., 1993; Zhang et al., 1993), slime mold cytoplasmic dynein (top right, *Dictyostelium discoideum*; Koonce et al., 1992), yeast cytoplasmic dynein (bottom left; *Saccharomyces cerevisiae*; Eshel et al., 1993; Yeh et al., 1993), sea urchin flagellar β axonemal dynein (bottom right; *Tripneustes gratilla*; Gibbons et al., 1991). Numbers indicate amino acid positions. The PAM250 scoring matrix (Pearson,

fourth *Drosophila* P-loop (residues 2922-2929) is again identical in sequence only to the corresponding P-loops found in the rat and sea urchin cytoplasmic dynein sequences (Fig. 4 B; Gibbons et al., 1992). Thus, in the case of cytoplasmic dynein sequences, all four P-loop motifs are identical in the *Drosophila*, rat, and sea urchin isoforms, but only the first and third P-loop sequences are totally conserved in all cytoplasmic dynein isoforms, including the *Dictyostelium*, *Aspergillus*, and yeast cytoplasmic dyneins. Considering the residues adjacent to the P-loop motifs, the yeast cytoplasmic dynein is clearly the most divergent (Fig. 4 B).

The secondary structure prediction for the *Drosophila* cytoplasmic dynein heavy chain is similar to those obtained for other dynein sequences and shows that α helix, β sheet, and β turn conformations are predicted along the entire length of the protein. This prediction is consistent with the predominant globular shape of native dynein complexes as determined by electron microscopy (for review see Vallee, 1993; Schroer, 1994). We examined the propensity of the α helical domains to form coiled-coil conformations using an algorithm developed by Lupas et al. (1991) to identify characteristic 4-3 hydrophobic heptad repeats (Hodges et al., 1972; McLachlan and Stewart, 1975; McLachlan and Karn, 1983). Five potential heptad repeat regions were identified in the heavy chain polypeptide sequence. For all five regions the amino acid distributions within the heptad repeats yielded scores (>1.5 , except in terminal residues of final repeat; Lupas et al., 1991) that strongly suggest their coiled-coil conformations. Of these, three are short with only five heptad repeats (35 residues) and two are relatively long with eleven and twelve heptad repeats (77 and 84 residues), respectively. Relative to the central cluster of nucleotide-binding motifs, two of the short repeats are NH₂-terminal, while the third short repeat and both long repeats are located COOH-terminal. The first short repeat region is at residues 530 to 564, the second occurs at residues 1266 to 1300, the two long repeat regions are at residues 3186 to 3262 and 3398 to 3481, and the third short repeat region is at residues 3752 to 3786.

1990), a window size of 15 residues, and a minimum score of 60% were used for the analysis. Arrows identify positions of the four P-loop motifs. (B) Sequence alignment of amino acid residues surrounding the four conserved P-loop motifs in cytoplasmic and axonemal dynein heavy chains. The *Drosophila* sequence is shown on the upper line of the comparisons. Dots represent identical amino acids. A bar above each alignment indicates the position of the P-loop. Sequence identities within the P-loop are indicated by asterisks. Species source of dynein sequences is shown at left (dmc^{dhc}, *Drosophila melanogaster* cytoplasmic dynein heavy chain; rnc^{dhc}, *Rattus norvegicus* cytoplasmic dynein heavy chain, Mikami et al., 1993; ddc^{dhc}, *Dictyostelium discoideum* cytoplasmic dynein heavy chain, Koonce et al., 1992; enc^{dhc}, *Emmericella (Aspergillus) nidulans* cytoplasmic dynein heavy chain, Xiang et al., 1994; scd^{dhc}, *Saccharomyces cerevisiae* cytoplasmic dynein heavy chain, Yeh et al., 1993; Eschel et al., 1993; su^βdhc, sea urchin β axonemal dynein heavy chain, Gibbons et al., 1991; cr^βdhc, *Chlamydomonas reinhardtii* β axonemal dynein heavy chain, Mitchell and Brown, 1994; cr^αdhc, *Chlamydomonas reinhardtii* α axonemal dynein heavy chain, Mitchell and Brown, 1994; cr^γdhc, *Chlamydomonas reinhardtii* γ axonemal dynein heavy chain, Witman et al., 1994).

Eight human cDNA sequences (accession numbers: T07853, T06228, T03672, T09530, T05469, T05294, M62078, and M85915) present in the collection of expressed sequence tags (EST; Adams et al., 1991, 1992) show significant similarity to the *Drosophila* dynein heavy chain sequence. Each of the EST clones shares at least 40% identical residues in overlaps of 54–109 amino acids. In addition to these EST clones, Vaisberg et al. (1993) have recently recovered a PCR-derived human dynein cDNA clone that contains the first P-loop motif. Besides the identification of other dynein sequences, our searches of the sequence databases revealed no other similarities of clear significance. Direct comparisons between the predicted *Drosophila* dynein heavy chain sequence and the predicted amino acid sequences of other known microtubule-associated proteins, including kinesin isoforms, MAP2, and tau, also failed to reveal any striking similarities.

Genomic Organization of the Cytoplasmic Dynein Heavy Chain Transcription Unit

Genomic DNA blot experiments show that the cytoplasmic dynein heavy chain is encoded by a single copy gene (Fig. 5 A). In addition, in situ hybridization to polytene chromosomes from larval salivary glands also reveals a single cytological map position for the cytoplasmic dynein gene (Fig. 5 B). Labeled fragments from the inserts of the MS3, ZAP4, and ZAP8 cDNA clones each hybridized to a single site located at position 64C on the third chromosome (Fig. 5 B). During the course of this work, partial sequences of seven dynein-related heavy chain genes were identified in *Drosophila* genomic DNA by PCR strategies (Rassmusson et al., 1994). This family of dynein heavy chain genes includes genes that appear to encode several axonemal dynein isoforms, as well as the cytoplasmic dynein isoform. One of the

partial (~400 bp) dynein clones recovered by PCR also maps to position 64C. The complete cDNA sequence reported here encompasses the partial sequence of the dynein gene previously designated *Dhc64C* according to its cytological map position.

To recover a functional transcription unit of the dynein heavy chain gene, we have analyzed genomic clones encompassing the dynein coding sequence (Fig. 1 B). A 320-bp restriction fragment from the 5' end of the MS3 cDNA clone was used to probe a λ EMBL3A library (Tamkun et al., 1992). Three genomic clones were isolated and their restriction enzyme sites were mapped. As shown in Fig. 1 B, the phage clone EMBL#1 contains the 3' end of the dynein heavy chain transcript and extends ~14 kb towards the 5' end of the gene. We subsequently screened a cosmid library (Tamkun et al., 1992) with a 2.0-kb HindIII insert from the genomic subclone EM12 (Fig. 1 B). Two of ten positive clones, pCS3 and pCS14, included the entire coding sequence of the dynein heavy chain gene. The clone pCS3 extends ~5 kb upstream from the predicted translational start site and includes a portion of each of two additional transcription units located 5' and 3' to the dynein gene. The positions of selected restriction enzyme sites in both the phage and cosmid genomic clones are shown in Fig. 1 B. The sequences of both ends of contiguous subclones of genomic DNA fragments spanning the dynein gene were determined. Both these end-run sequences and the restriction maps of the genomic clones matched with the overlapping cDNA clones, with the exception of one HindIII restriction site polymorphism toward the 3' end of the cDNA and the altered restriction map of genomic clones towards the 5' end of the gene due to the presence of a relatively large intron. The HindIII restriction site polymorphism and the boundaries of the large 5' intron

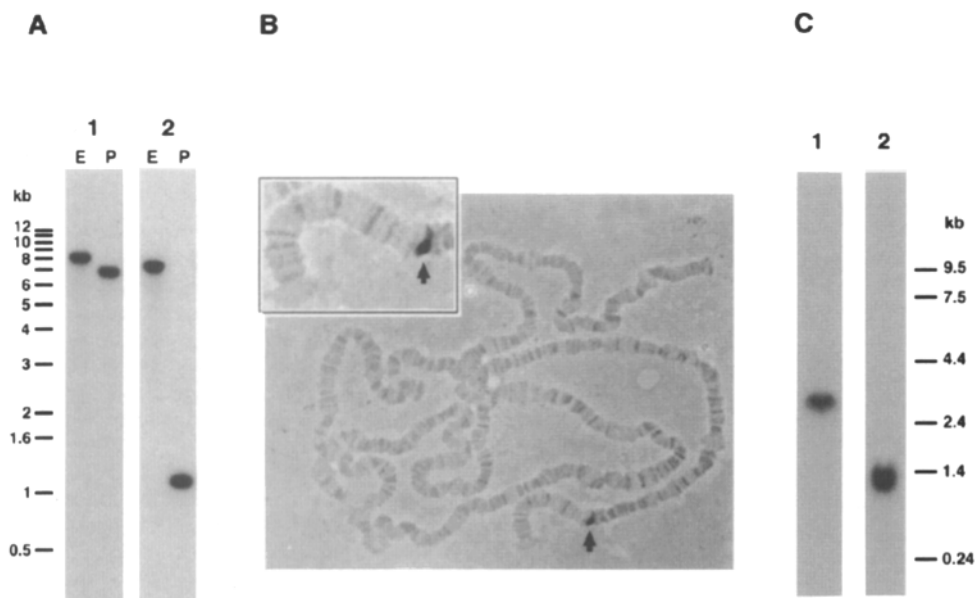


Figure 5. Copy number and chromosomal location of the *Drosophila* cytoplasmic dynein gene. (A) Genomic DNA blots. DNA was digested with restriction enzymes as indicated (E, EcoRI; P, PstI). (Panel 1) DNA was probed with a 0.4-kb internal DNA fragment that spans bp 5770–6177, and includes the first P-loop motif. (Panel 2) DNA was hybridized to a ~1-kb BamHI–HindIII fragment at the 3' end of the dynein gene (see Fig. 1). Neither probe contained internal restriction sites for EcoRI or PstI, and both probes hybridized to a single, unique restriction fragment. Molecular size markers are shown at left. (B) In situ localization of the dynein gene on polytene chromosomes. The insert of the ZAP4 cDNA was labeled using digoxi-

genin-dUTP and hybridized to polytene squashes. Detection of the hybridized probe was by anti-digoxigenin coupled to alkaline phosphatase. Inset shows an enlargement of the tip of chromosome 3L and the location of the heavy chain gene at position 64C is indicated by an arrow. (C) RNA blots probed with genomic DNA extending 5' and 3' outside the dynein gene. Two additional transcription units were identified. (Lane 1) A probe extending 9 kb upstream recognizes a 3-kb transcript in RNA from ovaries; and all other tissues examined (data not shown). (lane 2) A probe extending into the 3' region beyond the dynein gene hybridizes with a 1.3-kb transcript which is only detected in RNA from testes.

were confirmed by sequence analyses and comparison of genomic and cDNA clones. The restriction map of the cDNA differs from the genomic map in the 5' region that is included in the subclones pCH71 and pCH81 (Fig. 1). The 3.6 kb of genomic DNA contained in these subclones was sequenced completely and comparison to the cDNA sequence revealed the presence of a ~1-kb intron and a smaller intron of 62 bp.

As another verification of the limits of the dynein transcript, RNA from various tissues was probed with genomic DNA extending 5' and 3' from the gene. We identified two transcription units that flank the 5' and 3' ends of the dynein heavy chain gene (Fig. 1 B). RNA blot analysis shows that the *Drosophila* gene located 5' to dynein encodes a 3-kb transcript that is present in all tissues examined (Fig. 5 C). No additional 5' transcripts were detected on RNA blots with a probe that extends 9 kb upstream of the dynein gene (Fig. 5 C). Adjacent to the 3' end of the dynein coding sequence, we identified a testes-specific transcript. RNA blots were probed with the genomic subclone EM13, which contains the last 34 bp of the dynein cDNA sequence and ~1.3 kb of additional downstream sequence. A 1.3-kb transcript was detected only in total RNA prepared from testes.

Two cDNA clones containing a portion of the 5' flanking transcript were isolated with a probe derived from pCH81. Both cDNA clones initiate at position -171 and are transcribed from the DNA strand opposite to that of the cytoplasmic dynein gene. Analysis of the sequence derived from pCH81 and the two cDNA clones predicts an ORF that begins at position -305 bp and extends through position -921 bp. The partial sequence determined for the ORF predicts 200-amino acid residues that share 72% similarity and 59% identity with leucine-tRNA ligase precursor in fungi (Chow et al., 1989), yeast (Herbert et al., 1988) and *E. coli* (Hartlein and Madern, 1987).

Our analyses suggest that a genomic fragment extending from position ~-924 bp upstream of the dynein coding sequence to position ~15,681 bp that lies ~100 bp downstream of the cDNA transcript is likely to contain a complete and functional genomic transcription unit for the cytoplasmic dynein gene.

The Cytoplasmic Dynein Transcript Is Abundantly Expressed in Ovaries

Expression of the *Dhc64C* dynein gene was examined throughout development (Fig. 6). RNA blots were probed with subcloned genomic and cDNA fragments that span the entire dynein gene. All cDNA probes derived from the dynein heavy chain coding sequence recognized a transcript of ~14.5 kb in all stages and tissues examined. The cytoplasmic dynein heavy chain gene is differentially expressed during development, with the highest levels of transcripts observed in ovaries and 0–2-h embryos. The level of the dynein transcript falls dramatically in RNA prepared from 2–4-h embryos, and then increases in later staged embryos. The decrease in dynein transcript at 2–4 h is likely due to the turnover of maternally supplied transcript before the initiation of zygotic transcription. The cytoplasmic dynein transcript is also readily detected in larval and pupal stages, as well as in adult tissues including heads and testes. A second transcript of slightly larger size is also detected in relatively low levels in adult ovary, larval, and pupal RNA (Fig. 6). Probes

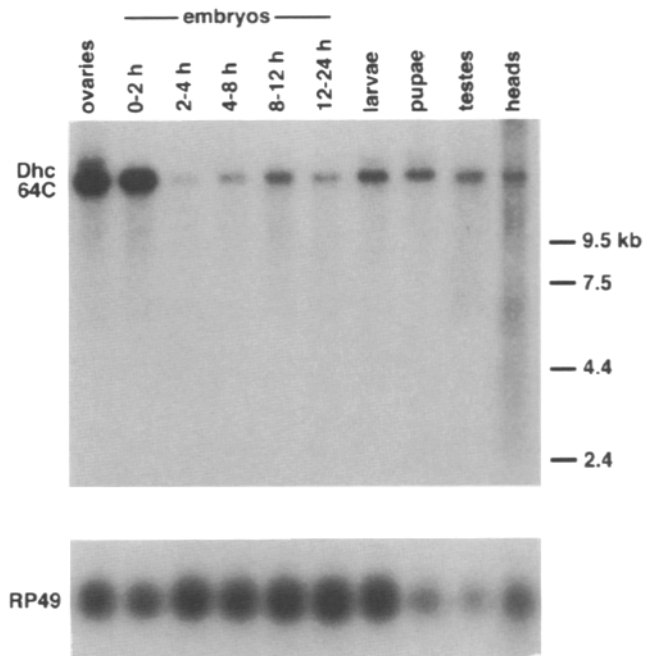


Figure 6. Developmental profile of the dynein mRNA. RNA blot contains total RNA isolated from ovaries; embryos (0–2, 2–4, 4–8, 8–12, and 12–24 h); larvae (mixed instars); pupae; testes; adult heads. The blot was hybridized to a radiolabeled probe from the region surrounding P-loop₁. The same expression profile was observed with probes derived from the 5' and 3' ends of the dynein cDNA. To control for equivalent loading and the integrity of the RNA the blot was later hybridized to a probe, RP49, derived from a ribosomal protein gene (lower panel; Vaslet et al., 1980). RNA size markers are indicated in kb at right side.

derived from the cDNA clones pTR13, ZAP8, and PL4 lie, respectively, at the 5' end, middle, and 3' end of the *Dhc64C* gene. Each of these probes detects both transcripts on RNA blots. Since our probes are specific to *Dhc64C* on genomic DNA blots, the two transcripts may result from the alternate splicing of exons. Alternatively the larger transcript may represent an immature mRNA that retains unspliced introns. A probe derived from the 1-kb intron at the 5' end does not hybridize to either transcript on RNA blots (data not shown). However, we have not mapped the complete intron/exon organization of the genomic clones and therefore cannot exclude other introns that could be present in a larger immature dynein mRNA.

The abundant expression of cytoplasmic dynein in ovaries suggests a role for the microtubule motor in oogenic transport. To examine this possibility, we have compared the spatial distribution of the dynein transcript with that of the encoded heavy chain polypeptide. To detect dynein transcript, ovaries were separately hybridized with digoxigenin-labeled DNA probes from several regions across the dynein coding sequence. All probes revealed a similar pattern of dynein transcript distribution. Low levels of the transcript are detected in the germarium and early stage egg chambers, while an elevation in the amount of dynein transcript in the nurse cell complex is apparent in later stage 8–10 egg chambers (Fig. 7 A). No enrichment of the transcript in the oocyte is observed, indicating that the dynein transcript is not accu-

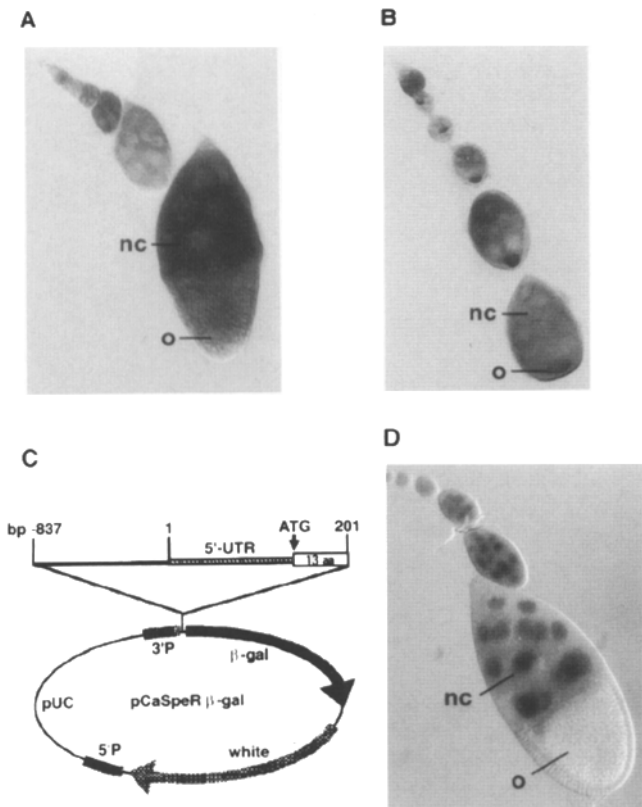


Figure 7. Spatial distribution of *Dhc64C* expression in ovaries. (*A* and *B*) In situ hybridization to wild-type ovaries. Ovaries were hybridized with either a *Dhc64C* dynein probe (*A*), or an *oskar* probe (*B*). Low levels of the dynein transcript are detected throughout the cytoplasm of the early stage egg chambers. Elevated levels of dynein transcript are apparent in the nurse cell complex (*nc*) in later egg chambers. No enrichment of the dynein transcript in the presumptive oocyte is observed. In contrast, the control *oskar* probe (*B*) shows the posterior localization of the *oskar* transcript in the oocyte. (*C*) Reporter construct. A DNA fragment carrying the dynein promoter was inserted into the BamHI site of the P-element vector pCaSpeR β gal. The transcription start site of the larger dynein transcript is marked as number 1. The hatched box in the insert represents the 5' untranslated region of the dynein gene and the open box in the insert represents the region encoding the first 13 aa of the dynein polypeptide. The first ATG in the dynein gene is indicated. (*D*) Expression of reporter construct. A single ovariole from a transformed female stained with X-gal. Intense staining is observed in the nurse cell nuclei. (*nc*, nurse cell complex; *o*, oocyte).

mulated in the oocyte. In contrast, a control DNA probe for *oskar* mRNA shows the typical enrichment of *oskar* transcript in the oocyte that has been previously described (Fig. 7 *B*; Kim-Ha et al., 1991; Ephrussi et al., 1991). We also used a transcriptional reporter gene to monitor the expression of the *Dhc64C* gene during oogenesis. A translational fusion between the dynein gene and the β -galactosidase coding sequence was introduced into flies using P-element mediated germline transformation. The reporter construct contains β -galactosidase fused in frame to the first 13 amino acids of the dynein heavy chain coding sequence. The sequence upstream of the first methionine residue extends to position -837 bp (Fig. 7 *C*). As shown in Fig. 7 *D*, β -galac-

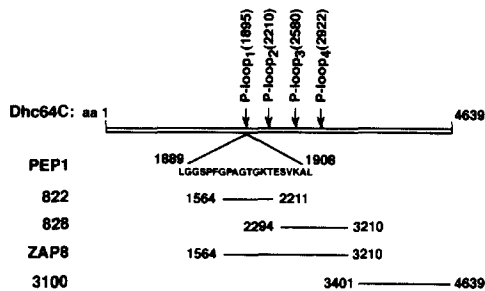
tosidase activity is detected throughout oogenesis beginning in the germarium, and is observed in both the nurse cell cytoplasm and nurse cell nuclei. As has been noted previously by Cheung et al. (1992) for an *fs(1)* K10- β -galactosidase transcriptional reporter, we also detect β -galactosidase activity in oocyte nuclei (Fig. 7 *D*). After stage 10b, a high level of β -galactosidase activity is detected in the oocyte cytoplasm presumably due to the rapid transport of the cytoplasmic contents of the nurse cells into the oocyte. The pattern of expression observed for the dynein-Lac-Z reporter construct was identical for five independent transformants, and non-transgenic flies showed no β -galactosidase activity under the conditions used.

The Cytoplasmic Dynein Motor Encoded by the *Dhc64C* Transcript Accumulates in the Pro-oocyte

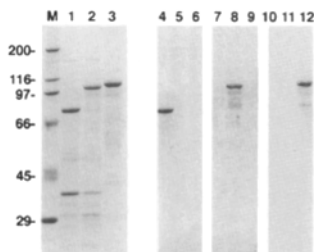
To examine the distribution of the cytoplasmic dynein heavy chain polypeptide during oogenesis, we generated polyclonal antisera against three distinct dynein epitopes contained within the non-overlapping cDNA subclones pET822, pET828, and pET3100 (Fig. 8, *A* and *B*, lanes 1–3; and Materials and Methods). The antisera were subsequently affinity-purified and shown to be specific for the dynein domain against which they were raised (Fig. 8 *B*, lanes 4–12). Furthermore, each antiserum can detect the enrichment of the dynein heavy chain as a microtubule-associated protein. As shown in Fig. 8 *C*, the heavy chain polypeptide prepared from ovaries is enriched in microtubules assembled in the absence of ATP, and can be eluted from microtubule pellets in the presence of 10 mM MgATP. We have previously demonstrated that cytoplasmic dynein can be purified from embryos in this same manner and have directly demonstrated its minus-end motor activity in vitro (Hays et al., 1994). These results suggest that the *Dhc64C* dynein heavy chain in ovaries is also present in a functional dynein motor complex.

In striking contrast to the expression and distribution of the cytoplasmic dynein transcript in nurse cells, the encoded dynein heavy chain polypeptide is differentially localized to the oocyte. Each of the dynein antibodies was used to probe whole mount preparations of *Drosophila* ovarioles. As illustrated with the PEP1 antiserum in Fig. 9, each of the affinity-purified antibodies detects the differential accumulation of cytoplasmic dynein in the presumptive oocyte during oogenesis. Initially, dynein is uniformly distributed in the more anterior and mitotically active regions 1 and 2a of the germarium (Fig. 9 *A*; *bracket*). The elevated level of staining of a single cell in the 16-cell cyst is first detected in region 2b of the germarium (*arrowhead*), just anterior of where the egg chambers bud off from the germarium. As egg chamber development and migration down the ovariole proceeds, the cytoplasmic dynein antigen remains differentially localized to the presumptive oocyte (Fig. 9, *A* and *B*). Then, before the bulk transport of nurse cell cytoplasm to the oocyte, an increase is observed in the amount of dynein antigen present in the nurse cell cytoplasmic domain of stage 10 egg chambers (Fig. 9 *D*). Dynein is also detected at a low and uniform intensity in the follicle cells surrounding early egg chambers. At later stages this follicle cell dynein appears to concentrate to the apical region of each follicle cell that surrounds the oocyte (Fig. 9 *D*). This apical pattern of dynein distribution

A Peptides Used for Antibody Production



B



C

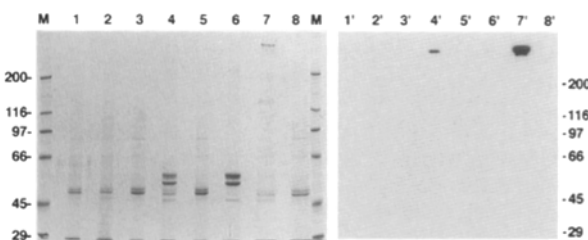


Figure 8. Antibodies specific for distinct dynein heavy chain epitopes. (A) A map of the position of peptides used in the production of dynein heavy chain antibodies is shown. (B) The antisera PEP1, 828, and 3100 define three distinct, non-overlapping epitopes in the *Drosophila* cytoplasmic dynein heavy chain and specifically recognize the pETS-822, pETS-828, and pETS-3100 bacterially expressed fusion proteins, respectively. Four replicate samples of pETS-822 (lanes 1, 4, 7, and 10), pETS-828 (lanes 2, 5, 8, and 11), and pETS-3100 (lanes 3, 6, 9, and 12) were run on 7.5% SDS-polyacrylamide gels. One set of the gel fractionated samples was stained with Coomassie blue (lanes 1–3). Molecular mass markers are shown at left (lane M). The remaining three replicate gels were blotted to nitrocellulose and separately probed with antiserum PEP1 (lanes 4–6), antiserum 828 (lanes 7–9), or antiserum 3100 (lanes 10–13). Approximately 1 μ g of fusion protein was loaded in each lane. (C) Cytoplasmic dynein is enriched in microtubules prepared from ovary homogenates. Duplicate sets of identical samples from the purification steps in a typical preparation of microtubule-associated proteins from *Drosophila* ovary extracts were fractionated on separate 7.5% SDS-polyacrylamide gels. (Lanes 1–8) One gel was stained in Coomassie blue. (Lanes 1–8) The replicate gel was blotted to nitrocellulose and probed with affinity-purified PEP-1 antibody. Approximately 1 μ g of protein from each purification step was loaded on a 7.5% polyacrylamide gel. (Lane 1) Ovary homogenate; (lane 2) high speed clarification pellet; (lane 3) high speed supernatant; (lane 4) taxol-assembled microtubule pellet; (lane 5) microtubule-depleted supernatant; (lane 6) MgATP extracted tubulin pellet; (lane 7) MgATP extracted microtubule associated protein supernatant; (lane 8) wash of taxol-assembled microtubule pellet before MgATP extraction. Lanes labeled M were

in follicle cells could reflect the role that these cells play in the polarized secretion of components required for the formation of several shell layers that encapsulate the mature egg (for review see Spradling, 1993).

Subcellular localizations of dynein within the oocyte cytoplasm are also observed. As shown in Fig. 9, a bright focus of dynein staining is closely apposed to the oocyte nuclei in early chambers (A; *small arrow*); and a perinuclear concentration of the dynein antigen is frequently apparent in stage 6–8 egg chambers (Fig. 9 A; *large arrowhead*). These patterns may reflect the early focus of the minus ends of microtubules at the single microtubule-organizing center (MTOC) of the oocyte, and a subsequent cage of microtubules that extends from the MTOC and encompasses the oocyte nucleus. During stage 9, the oocyte has grown to occupy approximately a third of the egg chamber volume (Fig. 9 C). At this stage, we detect an accumulation of dynein antigen at the posterior end of the oocyte, while a relatively lower level of dynein is still present throughout the oocyte cytoplasm. In some preparations, we can also observe slight accumulation of dynein in the anterior cortical regions of the oocyte that may represent intermediate steps in the path to posterior localization (Fig. 9 C). The posterior concentration of dynein appears to diminish in later stages, while an elevated level of dynein appears in the nurse cell cytoplasm (Fig. 9 D). Our results suggest that the cytoplasmic dynein motor is transported to and asymmetrically localized within the oocyte.

Maternal Effect Mutations Disrupt the Localization of Cytoplasmic Dynein

To examine the genetic requirements for dynein localization to and within the oocyte, we have asked whether the distribution of dynein is altered by several maternal effect mutations (Fig. 10). The gene products of the maternal effect loci *Bicaudal-D* (*Bic-D*) and *egalitarian* (*egl*) are the two earliest gene products known to be required for the specification of an oocyte within the syncytial 16-cell cyst. Loss of function mutations in either of these two genes disrupt oocyte differentiation and give rise to egg chambers in which each of the 16 cells develop as polyploid nurse cells (Schüpbach and Wieschaus, 1991). The differential localization of cytoplasmic dynein to the pro-oocyte appears to coincide with oocyte specification, suggesting that the *Bic-D* and *egl* products might be required for the accumulation of cytoplasmic dynein in the pro-oocyte. As shown in Fig. 10 A, *egl^{W50}* mutant ovaries show no accumulation of dynein in the oocyte. Dynein staining is uniformly distributed in the cystoblasts in the anterior most regions of the germarium, but rarely becomes localized to a single cell within the syncytial 16-cell cyst (compare Figs. 9 A and 10 A). Occasionally, we have observed a slightly elevated accumulation of dynein in a cell that is positioned toward the center of the cyst. In the later stage egg chambers, dynein staining is uniformly distributed throughout the 16 nurse cells. The localization of the dynein protein in sibling flies that are heterozygous for

loaded with molecular mass standards. A high molecular mass band present in the taxol microtubule pellet (lane 4 and 4') and enriched in the ATP eluted microtubule supernatant (lane 7 and 7') is specifically recognized by the PEP-1 dynein peptide antibody.

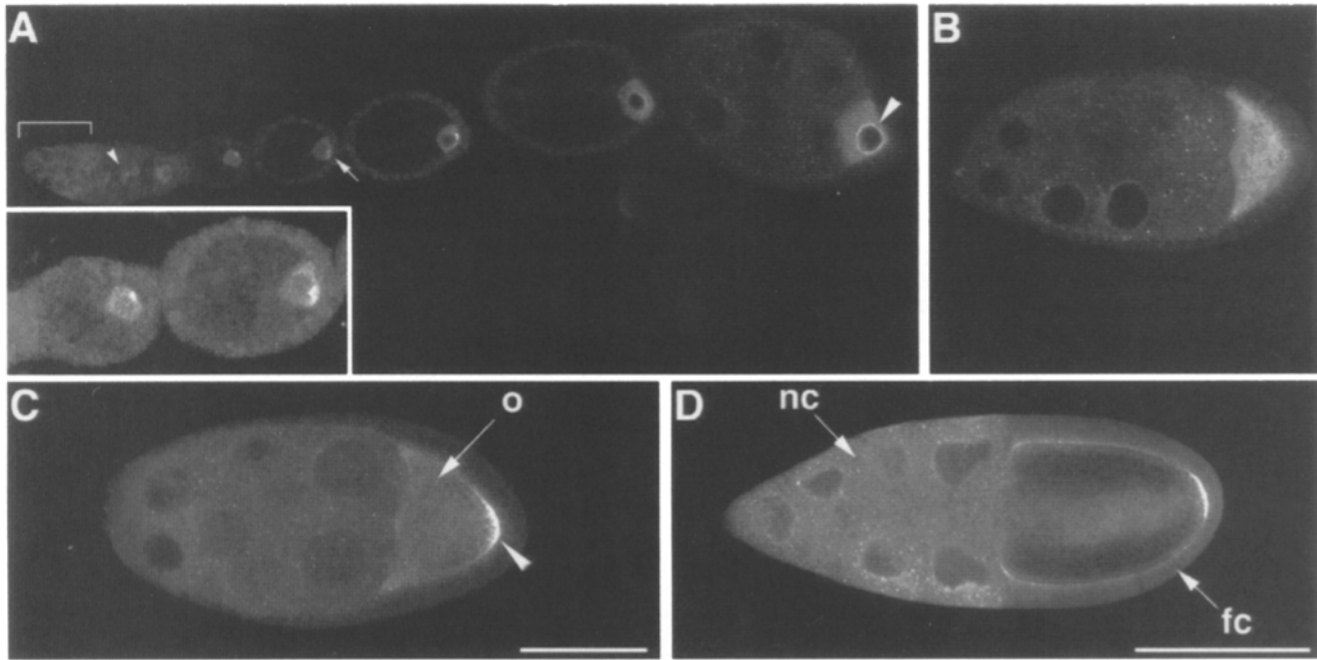


Figure 9. Asymmetric localization of dynein during oogenesis. Ovaries from 3-d-old females were dissected, processed for immunocytochemistry, and probed with the affinity-purified PEP-1 antibody. (A) The dynein polypeptide is detected uniformly throughout regions 1-2a of germarium (bracket). Dynein becomes concentrated in the presumptive oocyte as early as region 2b in the germarium (small arrowhead). As the egg chamber proceeds down the ovariole, the dynein antigen remains highly enriched in the developing oocyte relative to the nurse cells and gradually becomes concentrated to the posterior end of the oocyte (large arrowhead). In early egg chambers, a focus of staining is observed on the posterior side of the oocyte nucleus (see inset). (B) Early and late stage 9 (C) egg chambers showing the transition between the uniform (B) and posterior (C) accumulation of dynein in the growing oocyte. (D). In the stage 10 egg chamber the posterior focus of dynein staining is reduced, while a rim of dynein staining becomes apparent in the apical region of the follicle cells. The level of dynein staining observed in the nurse cell cytoplasm of both stage 9 and 10 egg chambers appears increased relative to earlier stages. The PEP-1 antibody, the antibodies elicited against the bacterial fusion proteins encoded by nonoverlapping cDNAs (3100, 828) and the ZAP8 cDNA all show the same pattern of dynein localization in ovaries. *fc*, follicle cells; *nc*, nurse cells; *o*, oocyte; magnification in A, B, and C are identical. Bar shown in C is 50 μm . Bar shown in D is 100 μm .

egl^{WU50} was indistinguishable from the pattern observed in the ovaries of wild-type flies.

The accumulation of dynein in the presumptive oocyte is also disrupted in ovaries derived from hemizygous *Bic-D^{R26}* mutant mothers (Fig. 10 B). In this case, dynein accumulation is initially observed in the pro-oocyte in region 2a-2b of the germarium, as well as in early stage 2 egg chambers. However, this accumulation in the single pro-oocyte is no longer observed in stage 4-5 egg chambers. The transient accumulation of dynein to the pro-oocyte in hemizygous *Bic-D^{R26}* ovaries probably reflects the partial function of the *Bic-D^{R26}* mutant protein (Ran et al., 1994). Moreover, this initial dynein accumulation in *Bic-D^{R26}* ovaries is not completely identical to that observed in wild-type ovaries. In ovaries from wild-type flies, a bright focus of dynein staining is normally situated posterior to the oocyte nucleus in stage 1-4 egg chambers (Fig. 9 A, see small arrowhead and inset). This focus of dynein staining most likely reflects the position of the MTOC where the minus ends of microtubules are focused. In the *Bic-D^{R26}* mutant ovaries the focus of dynein staining is instead located anterior to the nucleus (Fig. 10 B; see inset micrograph). This later result suggests that the *Bicaudal-D* protein may function in MTOC migration. Sibling progeny heterozygous for *Bic-D^{R26}* show a completely normal dynein distribution.

The posterior concentration of dynein during stage 9 of oogenesis is similar to the concentration of the pole plasm components *staufen* protein and *oskar* RNA. This suggests that the cytoplasmic dynein motor may play a role in the assembly of the pole plasm during stages 8 and 9 of oogenesis. To address this possibility, we localized dynein in ovaries from flies homozygous for the maternal effect mutations, *cappuccino* (*capu*) and *spire*. These genes act upstream of *staufen* and are required for the localization of *staufen* protein and *oskar* RNA to the posterior pole of the oocyte. In ovaries from *capu^{RK12}* flies, the pattern of dynein localization is unaffected until stage 9 of oogenesis (Fig. 10, C and D). As shown for ovaries derived from *capu* mutant flies, the enrichment of dynein at the posterior pole of the oocyte in stage 9 egg chambers is never achieved, and a uniform level of dynein is detected throughout the nurse cell and oocyte cytoplasm. Yet before stage 9, the initial accumulation of dynein to the presumptive oocyte is indistinguishable from that observed in wild-type ovaries (Fig. 10 C). The identical result was observed in oocytes from *spire^{RK48}* mutant flies (data not shown). Control flies heterozygous for either the *capu* or *spire* mutations show a dynein distribution in oogenesis that is completely wild type. We have also examined the localization of dynein in ovaries from flies carrying a mutation in *staufen* protein. In contrast to the *capu* and *spire* mu-

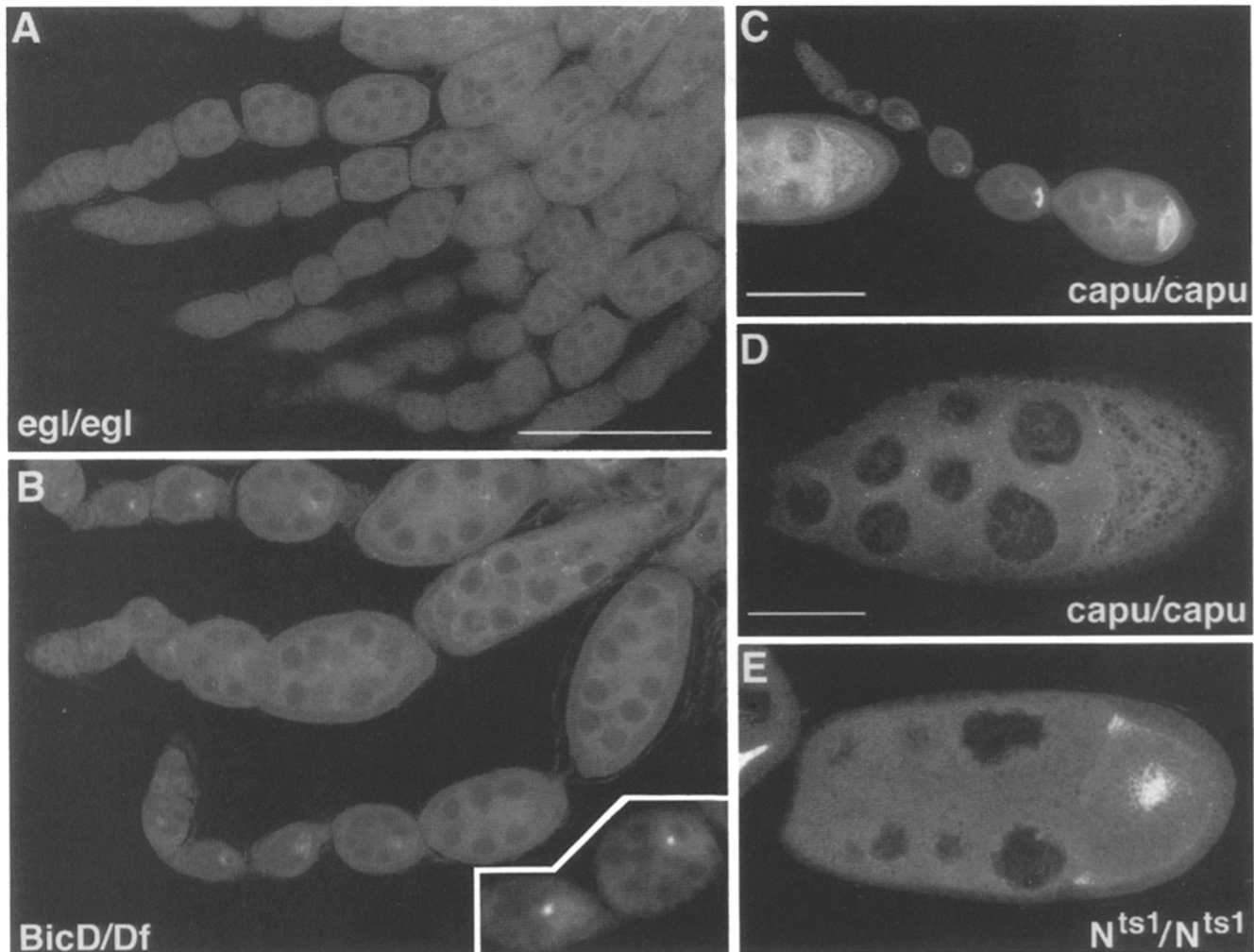


Figure 10. The effects of *egl*, *BicD*, *capu*, and *N* mutations on dynein localization in oogenesis. (A–E) Confocal images of ovarioles and egg chambers from ovaries dissected and processed for immunocytochemistry with the affinity-purified PEP1 antibody. The orientation of all egg chambers is with the anterior end to the left. (A) Ovarioles from an *egl^{WU50}/egl^{WU50}* female. Dynein does not accumulate in a single cell in the syncytial 16 cell cyst and remains uniformly distributed throughout all 16 nurse cells in the egg chamber. (B) Ovarioles from a *BicD^{R26}/Df(2L)TW119* female. A transient accumulation of dynein in the pro-oocyte is observed through stage 2–3 egg chambers. In contrast to wild-type egg chambers, the perinuclear focus of dynein staining in the pro-oocyte is located anterior to the oocyte nucleus (see inset). (C) Germarium and egg chambers from a *capu^{RKI2}/capu^{RKI2}* female. Dynein accumulates in the oocyte through stage 8 of oogenesis as in wild-type ovaries. The same result is observed with ovaries from the *spire^{RP48}* mutant (not shown). (D) A stage 9 egg chamber from a *capu^{RKI2}/capu^{RKI2}* female. Dynein is not enriched at the oocyte posterior, but is uniformly distributed throughout the nurse cell and oocyte cytoplasm. Like *capu^{RKI2}*, the *spire^{RP48}* mutant specifically disrupts the posterior localization of dynein in the stage 9 egg chamber. (E) A stage 9 egg chamber from a *N^{ts1}/N^{ts1}* female incubated at the restrictive temperature of 32°C for 30 h. Dynein is not enriched at the oocyte posterior, but is mislocalized to the center of the oocyte. A slight accumulation of dynein is detected at the anterior of the oocyte and in the cortical region of the oocyte extending along the anterior-posterior axis. Control *N^{ts1}/+* siblings from the *N^{ts1}/FM7* stock incubated at the restrictive temperature show normal dynein distribution throughout oogenesis. Magnification in A and B, and in D and E, are identical. Bars: (A and C) 100 μm; (D) 50 μm.

tations, flies homozygous for *staufer^{D3}* showed no effect on accumulation of dynein in the oocyte, or on the enrichment of dynein at the oocyte posterior (data not shown). These results suggest that dynein is not a component of the pole plasm, but that like *cappuccino* and *spire*, dynein might participate in the assembly of the pole plasm. To test this hypothesis further, we localized dynein in ovaries from females homozygous for a temperature-sensitive allele of *Notch*, *N^{ts1}*. *Notch* is required in the posterior follicle cells for the specification of polarity along the anterior-posterior axis in

the oocyte (Ruohola et al., 1991). In *N^{ts1}* females incubated at the restrictive temperature, the anterior determinant bicoid (*bcd*) RNA is localized to both the anterior and posterior of the oocyte, while *oskar* RNA and *staufer* protein are mislocalized to a spot in the center of the oocyte (Ruohola et al., 1991). As shown in Fig. 10 E, we observe a similar misplacement of cytoplasmic dynein to the center of oocytes derived from *N^{ts1}/N^{ts1}* flies incubated at the restrictive temperature of 32°C for 30 h. This mislocalization of dynein is restricted to stage 9 egg chambers, with no discernable

change in the accumulation of dynein during early stages of oogenesis. In heterozygous *N^{mi}/+* flies incubated at the restrictive temperature, cytoplasmic dynein distribution appears normal throughout oogenesis.

Discussion

The cytoplasmic dynein heavy chain gene, *Dhc64C*, encodes a dynein heavy chain that is similar to cytoplasmic dyneins characterized from other organisms. The predicted *Drosophila* dynein protein sequence shares 72% and 52% amino acid identity with the cytoplasmic dynein heavy chains reported for rat brain and *Dictyostelium*, respectively. In contrast only 26% of the *Drosophila* dynein amino acid residues are identical to those found in either the sea urchin or *Chlamydomonas* β -heavy chain axonemal dyneins that function to power flagellar motility. The greater similarity of the *Drosophila* dynein sequence to cytoplasmic isoforms is also observed in the conservation of residues that surround each of the four clustered P-loop motifs (Fig. 4 B). Interestingly, for all known cytoplasmic dynein sequences the amino acid residues of the first and third P-loops are totally conserved. In the case of the axonemal dyneins, the residues of the first and fourth P-loops are totally conserved. The conservation of amino acid residues in the first P-loop regions most likely reflects the participation of this domain in the binding and hydrolysis of ATP by the dynein motors. While the presence and positions of three additional phosphate-binding motifs in the central domain of the polypeptide is characteristic of all known dynein heavy chains, the contribution of these sites to motor function and/or structure remains to be determined.

In contrast to the P-loop regions and the central domain, the NH₂-terminal ~1500 residues of the dynein heavy chains are the most divergent. As viewed by electron microscopy, the native structures of cytoplasmic and axonemal dynein heavy chains are quite similar and appear to consist of a globular head domain and a short slender elongated stalk domain (for reviews see McIntosh and Porter, 1989; Vallee, 1993; Schroer, 1994). The short stalk domain is believed to provide the attachment of the dynein motor to the associated cargo. In the case of axonemal dyneins the cargo is the A-tubule of the outer doublet microtubule, while for cytoplasmic dyneins the cargo may include a variety of membranous organelles such as Golgi and synaptic vesicles (Vallee and Shpetner, 1990; Vallee, 1993; Schroer, 1994). The recent analysis of a β -axonemal dynein mutation in *Chlamydomonas* has shown that the NH₂-terminal domain of the dynein heavy chain is located within the stalk of the native dynein complex (Sakakibara et al., 1993). This work demonstrates that an NH₂-terminal 160-kD portion of the heavy chain is capable of stable assembly into the axoneme and is associated with a wild-type complement of intermediate and light chain polypeptides. Given these results, the sequence divergence in the NH₂-terminal domains of dynein heavy chains may reflect the varied attached cargoes of the different dynein heavy chains. Whether the NH₂-terminal sequence specifies and directly interacts with a cargo, or instead mediates the interaction through its association with intermediate and light chain polypeptides, is at present unknown.

In comparison to the *S. cerevisiae* dynein sequence, the *Drosophila* and all the other full-length dynein sequences

appear to have extended COOH-termini. The predicted cytoplasmic dynein heavy chain polypeptides encoded by the *Drosophila*, rat, *Dictyostelium*, and *Aspergillus* sequences, and the axonemal dyneins encoded by the *Chlamydomonas* β and γ , and sea urchin β heavy chain sequences, are respectively 4639, 4644, 4725, 4344, 4568, 4485, and 4466 residues in length. The reported full-length yeast sequence is only 4092 residues in length (Eshel et al., 1993). Multiple sequence alignment of the dynein sequences indicates that much of this length difference is accounted for by termination of the yeast sequence ~300 residues earlier than the other heavy chain polypeptides. While the location of the COOH terminus in the folded polypeptide remains to be determined, it does not appear to be required for the nucleotide-insensitive binding of axonemal dynein and therefore may contribute exclusively to the globular head domain (Sakakibara et al., 1993). No extended stretches of amino acid identities are observed in comparisons of the last 300 residues of the longer sequences and similar to the NH₂-termini these divergent COOH-termini may reflect heavy chain-specific functions. The cloning of the dynein heavy chains in a number of organisms will now permit the construction of truncated as well as chimeric dynein heavy chains. The reintroduction of these engineered hybrid dyneins using transformation methods should help to reveal the functional significance of the extended COOH-termini and other divergent and/or conserved domains.

The established globular head and flexible stem structural motifs of native axonemal and cytoplasmic dynein complexes are not mirrored by a bipartite secondary structure prediction. Dynein sequences do not predict an extended coiled-coil tail domain analogous to that observed for members of the myosin and kinesin superfamilies. β -sheets, β -turns, and α -helical domains are intermixed throughout the length of the heavy chain. The two longest α -helical domains are present in the COOH terminus of the *Drosophila* heavy chain polypeptide (residues 3158-3272 and residues 3397-3542). These domains appear to be conserved in their relative positions in both axonemal and cytoplasmic dynein isoforms (e.g., *Dictyostelium* residues 3253-3365 and residues 3492-3629; Koonce et al., 1992; sea urchin residues 2975-3223 and residues 3260-3314; Gibbons et al., 1991). In all cases they are predicted to form α helical coiled-coil domains and may participate in the folding of the globular domain of the heavy chain polypeptide or function in the binding of a separate component or regulator of the dynein complex. Recent work has described a "spring-loaded" mechanism for the conformational change of influenza hemagglutinin that is dependent on the dynamic interactions of coiled coils (Carr and Kim, 1993). Whether similar dynamics in the putative coiled-coil domains of the dynein globular head might contribute directly to motor function will require further investigation.

The *Drosophila Dhc64C* transcript is expressed throughout development and appears to encode the major cytoplasmic dynein present during oogenesis and embryogenesis (see also Rasmusson et al., 1994). This result suggests that the native *Drosophila* cytoplasmic dynein most likely contains a homodimer of the *Dhc64C* dynein heavy chain. Consistent with this interpretation, we previously showed that vanadate-mediated (V_i) cleavage of the *Drosophila* cytoplasmic dynein appears to occur at a single site in the heavy chain and

produces only two heavy chain polypeptide fragments (Hays et al., 1994). In comparison, similar cleavage of axonemal outer arm dynein produces multiple heavy chain fragments corresponding to the multiple distinct heavy chains known to be present in the outer arm complex (Mocz et al., 1988; King and Witman, 1987, 1988). Observations of a single major species of cytoplasmic dynein containing two identical dynein heavy chain polypeptides have been reported in rat brain, *Dictyostelium*, and rat testis (Mikami et al., 1993; Zhang et al., 1993; Koonce et al., 1992). However, in *Drosophila* we have detected minor levels of other dynein transcripts in embryos and ovaries, and we cannot rule out the possibility of additional dynein complexes containing other heavy chain polypeptides (Rasmusson et al., 1994).

The *Dhc64C* cytoplasmic dynein transcript is present in the nurse cell complex of the egg chamber throughout most stages of egg chamber maturation in the ovariole. In contrast, a striking differential localization of the dynein motor to the oocyte is observed with antibodies directed against three separate epitopes of the heavy chain polypeptide. Why does cytoplasmic dynein accumulate in the oocyte? One explanation is that the dynein motor is maternally supplied and stored in the oocyte for its later use in the syncytial embryo. The large size of the dynein heavy chain gene most likely precludes the synthesis of full length transcripts during the rapid nuclear division cycles of the syncytial blastoderm (Shermoen and O'Farrell, 1991). The high level of the dynein transcript detected in 0–2-h embryos is therefore presumed to represent the maternal supply that is rapidly transferred into the oocyte before nurse cell breakdown. The subsequent loss of the dynein transcript in 2–4-h old embryos suggests that this maternal endowment is degraded, and then later (4–8-h embryos) replaced by the zygotic transcription of the dynein heavy chain gene. We showed previously that the dynein heavy chain polypeptide is present throughout the cytoplasm in the syncytial blastoderm and appears to associate with mitotic and interphase microtubules during the rapid nuclear division cycles, as well as other microtubule arrays (e.g., CNS axons) at later stages (Hays et al., 1994). In association with these varied microtubule arrays, cytoplasmic dynein may power the transport of a variety of cellular components during embryonic development.

Does the early accumulation of dynein in the oocyte support a role for the dynein motor in the differentiation of the oocyte and the specification of embryonic axes? The distinct localization of the dynein heavy chain polypeptide in the oocyte, compared to the presence of the dynein transcript in the nurse cell complex, suggests that dynein may transport itself into the oocyte. An intriguing possibility is that the dynein motor also powers the transport of morphogenic molecules and signals from the nurse cell complex into the oocyte. In this regard, we have shown that the *Drosophila* cytoplasmic dynein motor can be purified from ovaries (Fig. 8 C) and embryos (Hays et al., 1994) by its increased affinity for microtubules in the absence of MgATP. This property reflects the enzymatic activity required for the minus-end directed microtubule translocation that we have previously shown for the *Drosophila* cytoplasmic dynein (Hays et al., 1994).

The movement of the dynein motor from the nurse cell cytoplasm where it is synthesized into the oocyte is likely to occur along a polarized array of microtubules. Recent evi-

dence supports the existence of a microtubule array with the necessary polarity to account for the translocation of dynein into the oocyte (Theurkauf et al., 1993; see also Pokrywka and Stephenson, 1991; Koch and Spitzer, 1983). The *Drosophila* oocyte develops within a cyst of 16 cells that are interconnected by cytoplasmic bridges or ring canals (King, 1970). Shortly after the formation of the syncytial 16 cell cyst in the germarium, the MTOC of the 15 cells destined to become nurse cells degenerate, or lose their association with the germline nuclei, and are transported through the ring canals to the single cell that will develop as the oocyte (Mahowald and Strassheim, 1970). The resulting single MTOC complex is first detected in region 2a-2b of the germarium and organizes a polar array of microtubules (Theurkauf et al., 1993). The minus ends of microtubules are apparently focused to the single MTOC located in the oocyte, while the distal plus ends of the microtubules extend through the ring canals and into the nurse cells. In the presence of this polar microtubule scaffold, the dynein motor is predicted to "walk" into the oocyte. Our results show that the differential accumulation of cytoplasmic dynein in the oocyte begins in region 2b of the germarium and so is temporally coincident with the establishment of microtubule polarity in the early egg chamber. The differential localization of the dynein heavy chain in the oocyte is maintained throughout oogenesis. Our analyses of ovaries from *Bic-D^{R26}* and *egl^{1WU50}* homozygous mutant flies indicate that the corresponding gene products are required for proper accumulation of dynein to the oocyte. This might readily be explained by the apparent participation of the *Bic-D* and *egl* gene products in the establishment and maintenance of the polarized microtubule array in the 16-cell syncytial cyst (Theurkauf et al., 1994). In the absence of this polar array of microtubules, the directed migration of the dynein motor into the oocyte would not occur. Previous studies have shown that the microtubule inhibitor colchicine, similar to mutations in *Bic-D* and *egl*, can disrupt the polarized microtubule array and block oocyte differentiation (Theurkauf et al., 1993; Koch and Spitzer, 1983). Preliminary data show that the accumulation of cytoplasmic dynein in the oocyte is also disrupted by colchicine (unpublished observations). Given that recessive mutations in *Bic-D* and *egl* block dynein transport to the oocyte and oocyte differentiation, our results are consistent with the view that dynein mediates the microtubule-based transport of cargoes that are required for oocyte differentiation.

A second pattern of dynein distribution is observed later in stage 8-10 egg chambers. In the somatic follicle cell epithelia that surrounds the oocyte, dynein is concentrated at the apical surface of the columnar cells. This polarized epithelium participates in the secretion of the multilayered egg shell and is known to provide signals that are involved in the specification of both the dorso-ventral and the anterior-posterior axes in the oocyte (for reviews see Schüpbach et al., 1991; Ruohola-Baker et al., 1994). It will be interesting to determine if dynein-mediated polarized secretion by the follicle cells is a component of the signaling mechanism between the soma and germline during oogenesis.

Within the oocyte, the dynein antigen becomes enriched at the posterior pole during stage 9 of oogenesis. These changes in dynein distribution may reflect a further reorganization of the oocyte microtubule cytoskeleton. In stage 7-8

egg chambers, the now posteriorly located MTOC degenerates and the apparent center and highest density of microtubule nucleation appears to shift to the anterior cortex of the oocyte (Theurkauf, 1993). Theurkauf and coworkers (1993) have shown that after brief treatments with microtubule-destabilizing agents, short microtubules remain at the anterior of the oocyte. Based on these results, the authors suggest that the more stable minus ends of the microtubules lie at the anterior cortex with the distal plus ends extending into the ooplasm. If the inference regarding microtubule polarity is correct, then it is not clear how, or if, the reorganization of microtubule polarity contributes to the apparent posterior enrichment of dynein. Given that dynein is a minus-end directed motor one might have predicted an anterior enrichment of dynein. One potential explanation is that the dynein motor may selectively bind and translocate along a subset of microtubules of opposite polarity to that inferred from the colchicine studies. Alternatively, the posterior enrichment of dynein may occur earlier in stages 2-7 as dynein actively translocates to and accumulates at the posterior MTOC. This earlier enrichment at the posterior pole might be masked by the elevated level of soluble dynein present throughout the ooplasm at these stages. Later during stages 9 and 10 the increased growth of the oocyte may effectively lower the concentration of soluble dynein and reveal the localized enrichment of dynein at the posterior pole. On this scenario, the gradual reorganization of microtubule polarity during stages 7-10 and the initiation of cytoplasmic streaming in stage 10b might contribute to the reduction in dynein localization at the posterior pole that we observe in oocytes after stage 10.

While we favor a model in which the dynein motor actively translocates to the posterior MTOC, we cannot eliminate alternative models. For example, the dynein motor could be complexed with a plus-end directed kinesin motor and so potentially be transported to the posterior pole as a cargo of the kinesin motor. However, to date, no native kinesin motor has been shown to accumulate at the posterior end of the oocyte. Yet, an ectopically expressed fusion protein in which β -galactosidase is attached to the motor portion of the *Drosophila* kinesin heavy chain has been reported to be transiently localized to the posterior end of the oocyte (Clark et al., 1994). The posterior localization of dynein could also result from the differential binding and stability of dynein at the posterior pole and the degradation of unbound dynein. Regardless of the mechanism of localization, the dynein motor must be added to the list of endogenous cytoplasmic components that are asymmetrically positioned to the posterior pole of the oocyte. Significantly, dynein is the only member of the list for which an intrinsic motor activity has been demonstrated.

The accumulation of dynein to the posterior pole plasm of stage 9 oocytes is distinct from the earlier accumulation of dynein to the pro-oocyte beginning in region 2b of the germarium. This is dramatically demonstrated in the ovaries of flies homozygous for the *capu*^{RK12} and *spire*^{RP48} mutations. In these ovaries the posterior accumulation of dynein fails to occur, while the earlier transport of dynein to the pro-oocyte occurs normally. Similarly, only the posterior localization of dynein is affected in *N^{ts1}/N^{ts1}* ovaries. The mislocalization of dynein to the center of the oocyte is similar to the mispositioning of *staufer* protein and *osk* RNA in *N^{ts1}/N^{ts1}* ovaries. Given the requirement for *capu*, *spire*, and *Notch* in the

proper assembly of the posterior pole plasm it is tempting to speculate that the dynein motor is also required for pole plasm assembly. In contrast dynein distribution is undisturbed in ovaries from homozygous *staufer*^{D3} flies, suggesting that dynein is not a component of the polar plasm itself, but a component of the localization machinery. Together with the disruption of dynein accumulation in the pro-oocyte by recessive mutations in *Bic-D* and *egl*, our observations suggest that dynein might participate in multiple steps during oogenesis. In early oogenesis, dynein might play an active role in some aspect of oocyte determination, while in later stages it might function in the correct spatial distribution of morphogens involved in the specification of the embryonic axis and pole cell formation. However, whether dynein motor activity is directly responsible for transport of any components of the posterior pole plasm remains to be determined.

In summary, while the role of cytoplasmic dynein in the transport of vesicles and organelles is well established, there is some evidence to support the speculation that dynein may also function to transport informational macromolecules in the form of mRNAs, RNP complexes, and proteins (for review see Wilhelm and Vale, 1993). Genetic studies have identified a number of genes (e.g., *Bic-D*, *egl*, *oskar*, *bicoid*, *staufer*, *cyclin B*, *nanos*, *swallow*, *orb*, and *fs(1)K10*) that participate in oocyte differentiation and/or the establishment of the embryonic axes in *Drosophila* (for reviews see Macdonald, 1992; Ruohola-Baker et al., 1994; Spradling, 1993; Ding and Lipshitz, 1993; St. Johnston and Nusslein-Volhard, 1992; Lasko, 1992). Several of these genes encode products (mRNAs and/or proteins) that show patterns of spatial distribution during oogenesis that are similar to those that we observe for cytoplasmic dynein. For example, *oskar*, *cyclin B*, *Bic-D*, *fs(1)K10*, *swallow*, and *orb* transcripts also appear to be synthesized in the nurse cells and accumulate preferentially in the presumptive oocyte (for reviews see Macdonald, 1992; Lasko, 1992; Ruohola-Baker et al., 1994; Spradling, 1993; Ding and Lipshitz, 1993; St. Johnston and Nusslein-Volhard, 1992). In addition, the posterior enrichment of *staufer* protein, as well as *oskar* and *cyclin B* mRNAs, is quite similar to the pattern seen for the dynein heavy chain polypeptide. In addition to describing the first evidence for the accumulation of an endogenous molecular motor protein in the *Drosophila* oocyte, we demonstrate several genetic requirements for proper dynein localization. The correlation between the requirement for the gene products *egl*, *Bic-D*, *capu*, *spire*, and *Notch* in oocyte determination and differentiation, and their requirement for the asymmetric localizations of cytoplasmic dynein in oogenesis, supports a model in which the motor protein is actively involved in the transport of morphogenetic determinants during oogenesis. The cloning and molecular characterization of the dynein heavy chain gene provides the necessary foothold to begin to understand the relationship between the structure of the *Drosophila* dynein motor and its function. Mutations in the dynein heavy chain gene are currently being characterized (Gepner, J., M.-G. Li, C. Kortas, S. Ludmann, M. McGrail, and T. S. Hays, manuscript in preparation) to establish the range of developmental processes that require dynein function and to directly address whether dynein-based microtubule transport is intimately coupled to oocyte differentiation and axis specification.

The authors thank Dr. Janice Gepner for contributing the figure of the polytene localization of *Dhc64C* by in situ hybridization, and for her critical reading of the manuscript. We acknowledge the technical assistance of Alison Cutlan in the analysis of the maternal effect mutations. We thank Drs. John Tamkun and Renate Deuring for the generous gift of libraries, Dr. Ruth Lehman for the *oskar* clone, and Dr. Carl Thummel for the pCaSpeR- β gal vector. We thank Drs. Trudi Schupbach, Ruth Steward, and Paul Macdonald for fly stocks. We acknowledge the contribution of Dr. Mark McNiven at the Mayo Clinic for his partial support of the synthesis of the PEP1 antibody. We also thank Drs. Mary Porter and Ann Rougvie for suggestions on the manuscript.

This work was supported by National Institutes of Health (NIH) grant GM-44757 to T. S. Hays. Additional support to T. S. Hays was provided by a March of Dimes Basil O'Connor award, and a grant from the Pew Charitable Trust. Support for M. McGrail was from NIH and National Science Foundation Research Training Grants.

Received for publication 16 May 1994 and in revised form 30 June 1994.

References

- Adams, M. D., J. M. Kelley, J. D. Gocayne, M. Dubnick, M. H. Polymeropoulos, H. Xiao, C. R. Merrill, A. Wu, B. Olde, R. F. Moreno, A. R. Kerlavage, W. R. McCombie, and J. C. Venter. 1991. Complementary DNA sequencing: expressed sequence tags and human genome project. *Science (Wash. DC)*. 252:1651-1656.
- Adams, M. D., M. Dubnick, A. R. Kerlavage, R. Moreno, J. M. Kelley, T. R. Utterback, J. W. Nagle, C. Fields, and J. C. Venter. 1992. Sequence identification of 2,375 human brain genes. *Nature (Lond.)*. 355:632-634.
- Amos, L. A., R. W. Linck, and A. Klug. 1976. Molecular structure of flagellar microtubules. In *Cell Motility*. Vol. 3. R. Goldman, T. Pollard, J. Rosenbaum, editors. Cold Spring Harbor Laboratory Press, New York. 847-867.
- Ausubel, F. M., R. Brent, R. E. Kingston, D. D. Moore, J. A. Smith, J. G. Seidman, and K. Struhl. 1987. *Current Protocols in Molecular Biology*. 48(suppl.):1-5.
- Baker, J., W. E. Therkauf, and G. Schubiger. 1993. Dynamic changes in microtubule configuration correlate with nuclear migration in the preblastoderm *Drosophila* embryo. *J. Cell Biol.* 122:113-121.
- Berleth, T., M. Burri, G. Thoma, D. Bopp, S. Richstein, G. Frigerio, M. Noll, and C. Nuesslein-Volhard. 1988. The role of localization of *bicoid* RNA in organizing the anterior pattern of the *Drosophila* embryo. *EMBO (Eur. Mol. Biol. Organ.) J.* 7:1749-1756.
- Bloom, G. S. 1992. Motor proteins for cytoplasmic microtubules. *Curr. Opin. Cell Biol.* 4:66-73.
- Brizuela, B. J., L. Elfring, J. Ballard, J. T. Tamkun, and J. A. Kennison. 1994. Genetic analysis of the *brahma* gene of *Drosophila melanogaster* and polytene chromosome subdivisions 72AB. *Genetics*. 137(3):803-813.
- Brown, N. H., and F. C. Kafatos. 1988. Functional cDNA libraries from *Drosophila* embryos. *J. Mol. Biol.* 203:425-437.
- Carr, C. M., and P. S. Kim. 1993. A spring-loaded mechanism for the conformational change of influenza hemagglutinin. *Cell*. 73:823-832.
- Cavener, D. R. 1987. Comparison of the consensus sequence flanking translational start sites in *Drosophila* and vertebrates. *Nucleic Acids Res.* 15:1353-1361.
- Cheung, H. K., T. L. Serano, and R. S. Cohen. 1992. Evidence for a highly selective RNA transport system and its role in establishing the dorsoventral axis of the *Drosophila* egg. *Development*. 114:653-661.
- Chow, C. M., R. L. Metzberg, and U. L. Rajbhandary. 1989. Nuclear gene for mitochondrial leucyl-tRNA synthetase of *Neurospora crassa*: isolation, sequence, chromosomal mapping, and evidence that the *leu-5* locus specifies structural information. *Mol. Cell Biol.* 9:4631-4644.
- Clark, I., E. Giniger, H. Ruohola-Baker, L. Y. Jan, and Y. N. Jan. 1994. Transient posterior localization of a kinesin fusion protein reflects anteroposterior polarity of the *Drosophila* oocyte. *Curr. Biol.* 4:289-300.
- Cooley, L., E. Verheyen, and K. Ayers. 1992. *chickadee* encodes a profilin required for intercellular transport during *Drosophila* oogenesis. *Cell*. 69:173-184.
- Ding, D., and H. G. Lipshitz. 1993. Localized RNAs and their functions. *BioEssays*. 15:651-658.
- Ephrussi, A., L. K. Dickinson, and R. Lehmann. 1991. *Oskar* organizes the germ plasm and directs localization of the posterior determinant *nanos*. *Cell*. 66:37-50.
- Eshel, D., L. A. Urrestarazu, S. Vissers, J.-C. Jauniaux, J. C. van Vliet-Reedijk, R. J. Planta, and I. R. Gibbons. 1993. Cytoplasmic dynein is required for normal nuclear segregation in yeast. *Proc. Natl. Acad. Sci. USA*. 90:11172-11176.
- Foe, V. E., and B. M. Alberts. 1983. Studies of nuclear and cytoplasmic behavior during the five mitotic cycles that precede gastrulation in *Drosophila* embryogenesis. *J. Cell Sci.* 61:31-70.
- Frohman, M. A., M. K. Dush, and G. R. Martin. 1988. Rapid production of full-length cDNAs from rare transcripts: amplification using a single gene-specific oligonucleotide primer. *Proc. Natl. Acad. Sci. USA*. 85:8998-9002.
- Fullilove, S. L., and A. G. Jacobson. 1971. Nuclear elongation and cytokinesis in *Drosophila montana*. *Dev. Biol.* 26:560-571.
- Fyrberg, E. A., and L. S. Goldstein. 1990. The *Drosophila* cytoskeleton. *Annu. Rev. Cell Biol.* 6:559-596.
- Garnier, J., D. J. Osgunthorpe, and B. Robson. 1978. Analysis of the accuracy of simple methods for predicting the second structure of globular proteins. *J. Mol. Biol.* 120:97-120.
- Gibbons, I. R., B. H. Gibbons, G. Mocz, and D. J. Asai. 1991. Multiple nucleotide-binding sites in the sequence of dynein β heavy chain. *Nature (Lond.)*. 352:640-643.
- Gibbons, I. R., D. J. Asai, W. J. Tang, and B. H. Gibbons. 1992. A cytoplasmic dynein heavy chain in sea urchin embryos. *Biol. Cell*. 76:303-309.
- Goldstein, L. S. B. 1991. The kinesin superfamily: tails of functional redundancy. *Trends Cell Biol.* 1:93-98.
- Goldstein, L. S. B. 1993. With apologies to Scheherazade: tails of 1001 kinesin motors. *Annu. Rev. Genet.* 27:319-351.
- Hartlein, M., and D. Madern. 1987. Molecular cloning and nucleotide sequence of the gene for *Escherichia coli* leucyl-tRNA synthetase. *Nucleic Acids Res.* 15:10199-10210.
- Hays, T. S., M. E. Porter, M. McGrail, P. Grissom, P. Gosch, M. T. Fuller, and J. R. McIntosh. 1994. A cytoplasmic dynein motor in *Drosophila*: identification and localization during embryogenesis. *J. Cell Sci.* 107:1557-1569.
- Henikoff, S. 1987. Unidirectional digestion with exonuclease III in DNA sequence analysis. *Methods Enzymol.* 155:156-165.
- Herbert, C. J., M. Labouesse, G. Dujardin, and P. P. Slonimski. 1988. The NAM2 proteins from *S. cerevisiae* and *S. douglasii* are mitochondrial leucyl-tRNA synthetases, and are involved in mRNA splicing. *EMBO (Eur. Mol. Biol. Organ.) J.* 7:473-483.
- Hodges, R. S., J. Sodek, L. B. Smillie, and L. Jurasek. 1972. Tropomyosin: amino acid sequence and coiled-coil structure. *Cold Spring Harbor Symp. Quant. Biol.* 37:299-319.
- Hoyt, M. A. 1994. Cellular roles of kinesin and related proteins. *Curr. Opin. Cell Biol.* 6:63-68.
- Karr, T. L., and B. M. Alberts. 1986. Organization of the cytoskeleton in early *Drosophila* embryos. *J. Cell Biol.* 102:1494-1509.
- Kellogg, D. R., C. M. Field, and B. M. Alberts. 1989. Identification of microtubule-associated proteins in the centrosome, spindle, and kinetochore of the early *Drosophila* embryo. *J. Cell Biol.* 109:2977-2991.
- Kim-Ha, J., J. L. Smith, and P. M. Macdonald. 1991. *Oskar* mRNA is localized to the posterior pole of the *Drosophila*. *Cell*. 66:23-35.
- King, R. C. 1970. Ovarian Development in *Drosophila melanogaster*. Academic Press, New York.
- King, S. M., and G. B. Witman. 1987. Structure of the alpha and beta heavy chains of the outer arm dynein from *Chlamydomonas* flagella. Masses of chains and sites of ultraviolet-induced vanadate-dependent cleavage. *J. Biol. Chem.* 262:17596-17604.
- King, S. M., and G. B. Witman. 1988. Structure of the alpha and beta heavy chains of the outer arm dynein from *Chlamydomonas* flagella. Location of epitopes and protease-sensitive sites. *J. Biol. Chem.* 263:9244-9255.
- Koch, E. A., and R. H. Spitzer. 1983. Multiple effects of colchicine on oogenesis in *Drosophila*: induced sterility and switch of potential oocyte to nurse-cell developmental pathway. *Cell Tissue Res.* 228:21-23.
- Koonce, M. P., P. M. Grissom, and J. R. McIntosh. 1992. Dynein from *Dicystelium*: primary structure comparisons between a cytoplasmic motor enzyme and flagellar dynein. *J. Cell Biol.* 119:1597-1604.
- Laemmli, U. K. 1970. Cleavage of the structural protein during the assembly of the head of bacteriophage T4. *Nature (Lond.)*. 227:680-685.
- Lantz, V., L. Ambrosio, and P. Schedl. 1992. The *Drosophila orb* gene is predicted to encode sex-specific germline RNA-binding proteins and has localized transcripts in ovaries and early embryos. *Development*. 115:75-88.
- Lasko, P. F. 1992. Molecular movements in oocyte patterning and pole cell differentiation. *BioEssays*. 14:507-512.
- Lehner, C., and P. O'Farrell. 1989. Expression and function of *Drosophila cyclin A* during embryonic cell cycle progression. *Cell*. 56:957-968.
- Li, Y.-Y., E. Yeh, T. Hays, and K. Bloom. 1993. Disruption of mitotic spindle orientation in a yeast dynein mutant. *Proc. Natl. Acad. Sci. USA*. 90:10096-10100.
- Lindsley, D. L., and G. G. Zimm. 1992. The genome of *Drosophila melanogaster*. Academic Press, Inc., San Diego, CA. 1-1133.
- Ludueno, R. F., E. M. Shooter, and L. Wilson. 1977. Structure of the tubulin dimer. *J. Biol. Chem.* 252:7006-7014.
- Lupas, A., M. Van Dyke, and J. Stock. 1991. Predicting coiled coils from protein sequences. *Science (Wash. DC)*. 252:1162-1164.
- Macdonald, P. M. 1992. The means to the ends: localization of maternal messenger RNAs. *Semin. Dev. Biol.* 3:413-424.
- Mahowald, A. P., and J. M. Strassheim. 1970. Intercellular migration of centrioles in the germlinum of *Drosophila melanogaster*. *J. Cell Biol.* 45:306-320.
- Manseau, L. J., and T. Schupbach. 1989. *cappuccino* and *spire*: two unique maternal-effect loci required for both the anteroposterior and dorsoventral patterns of the *Drosophila* embryo. *Genes Dev.* 3:1437-1452.
- McIntosh, J. R., and M. E. Porter. 1989. Enzymes for microtubule-dependent motility. *J. Biol. Chem.* 264:6001-6004.

- McLachlan, A. D., and M. Stewart. 1975. Tropomyosin coiled-coil interactions: evidence for an unstaggered structure. *J. Mol. Biol.* 98:293-304.
- McLachlan, A. D., and J. Karn. 1983. Periodic features in the amino acid sequence of nematode myosin rod. *J. Mol. Biol.* 164:605-626.
- Medberry, A. L., B. E. L. Lockhart, and N. E. Olszewski. 1990. Properties of Commelina yellow mottle virus's complete DNA sequence, genomic discontinuities and transcript suggest that it is a pararetrovirus. *Nucleic Acids Res.* 18:5505-5513.
- Melton, D. A., P. A. Krieg, M. R. Rebagliati, T. Maniatis, K. Zinn, and M. R. Green. 1984. Efficient in vitro synthesis of biologically active RNA and RNA hybridization probes from plasmids containing a bacteriophage SP6 promoter. *Nucleic Acids Res.* 12:7035-7056.
- Mitchell, D. R., and K. S. Brown. 1994. Sequence analysis of the *Chlamydomonas* alpha and beta dynein heavy chain genes. *J. Cell Sci.* 107:635-644.
- Mikami, A., B. M. Paschal, M. Mazumdar, and R. B. Vallee. 1993. Molecular cloning of the retrograde transport motor cytoplasmic dynein (MAP1C). *Neuron.* 10:787-796.
- Mocz, G., W.-J. Y. Tang, and I. R. Gibbons. 1988. A map of photolytic and tryptic cleavage sites on the beta heavy chain of dynein ATPase from sea urchin sperm flagella. *J. Cell Biol.* 106:1607-1614.
- Ogawa, K. 1991. Four ATP-binding sites in the midregion of the β heavy chain of dynein. *Nature (Lond.)* 352:643-645.
- Olmsted, J. B. 1981. Affinity-purification of antibodies from diazotized paper blots of heterologous protein samples. *J. Biol. Chem.* 256:11955-11957.
- Pokrywka, N. S., and E. C. Stephenson. 1991. Microtubules mediate the localization of bicoid RNA during *Drosophila* oogenesis. *Development.* 113:55-66.
- Porter, M. E., and K. A. Johnson. 1983. Characterization of the ATP-sensitive binding of Tetrahymena 30S dynein to bovine brain microtubules. *J. Biol. Chem.* 258:6575-6581.
- Proudfoot, N. J. 1989. How RNA polymerase II terminates transcription in high eukaryotes. *TIBS (Trends Biochem. Sci.)* 14:105-110.
- Ran, B., R. Bopp, and B. Suter. 1994. Null alleles reveal novel requirements for Bic-D during *Drosophila* oogenesis and zygotic development. *Development.* 120:1233-1242.
- Rasmusson, K., J. Gepner, M. Serr, I. Gibbons, and T. S. Hays. 1994. A family of dynein motors in *Drosophila melanogaster*. *Mol. Biol. Cell.* 5:45-55.
- Ruohola-Baker, H., K. A. Bremer, D. Baker, J. R. Swedlow, L. Y. Jan, and Y. N. Jan. 1991. Role of neurogenic genes in establishment of follicle cell fate and oocyte polarity during oogenesis in *Drosophila*. *Cell.* 66:433-449.
- Ruohola-Baker, H., L. Jan, and Y. N. Jan. 1994. The role of gene cassettes in axis formation during *Drosophila* oogenesis. *Trends Genet.* 10:89-94.
- Sakakibara, H., S. Takada, S. M. King, G. B. Witman, and R. Kamiya. 1993. A chlamydomonas outer arm dynein mutant with a truncated β heavy chain. *J. Cell Biol.* 122:653-661.
- Sanger, F., S. Nicklen, and A. R. Coulson. 1977. DNA sequencing with chain-terminating inhibitors. *Proc. Natl. Acad. Sci. USA.* 74:5463-5467.
- Schroer, T. A. 1994. Structure, function and regulation of cytoplasmic dynein. *Curr. Opin. Cell Biol.* 6:69-73.
- Schüppbach, T., and E. Wieschaus. 1991. Female sterile mutations on the second chromosome of *Drosophila melanogaster*. II. Mutations blocking oogenesis or altering egg morphology. *Genetics.* 129:1119-1136.
- Shermoen, A. W., and P. H. O'Farrell. 1991. Progression of the cell cycle through mitosis leads to abortion of nascent transcripts. *Cell.* 67:303-310.
- Skoufias, D. A., and J. M. Scholey. 1993. Cytoplasmic microtubule-based motor proteins. *Curr. Opin. Cell Biol.* 5:95-104.
- Smith, D. B., and K. S. Johnson. 1988. Single-step purification of polypeptides expressed in *Escherichia coli* as fusions with glutathione S-transferase. *Gene (Amst.)* 67:31-40.
- Spradling, A. C. 1993. Developmental genetics of oogenesis. In *The Development of Drosophila melanogaster*. M. Bate and A. M. Arias, editors. Vol. 1. Cold Spring Harbor Laboratory Press, NY. 1-70.
- Steward, R., L. Ambrosio, and T. Schubach. 1987. Polarity in the oocyte and embryo of *Drosophila*. *UCLA Symp. Mol. Cell. Biol. New Ser.* 51:113-130.
- St. Johnston, D., and C. Nusslein-Volhard. 1992. The origin of pattern and polarity in the *Drosophila* embryo. *Cell.* 68:201-219.
- St. Johnston, D., D. Beuchle, and C. Nusslein-Volhard. 1991. *staufer*, a gene required to localize maternal RNAs in the *Drosophila* egg. *Cell.* 66:51-63.
- Studier, F. W., A. H. Rosenberg, J. J. Dunn, and J. W. Dubendorff. 1990. Use of T7 RNA polymerase to direct expression of cloned genes. *Methods Enzymol.* 185:60-89.
- Suter, B., L. Romberg, and R. Steward. 1989. *Bicaudal-D*, a *Drosophila* gene involved in developmental asymmetry: localized transcript accumulation in ovaries and sequence similarity to myosin heavy chain tail domains. *Genes Dev.* 3:1957-1968.
- Tamkun, J. W., R. A. Kahn, M. Kissinger, B. J. Brizuela, C. Rulka, M. P. Scott, and J. A. Kennison. 1991. The *arflike* gene encodes an essential GTP-binding protein in *Drosophila*. *Proc. Natl. Acad. Sci. USA.* 88:31208-3124.
- Tamkun, J. W., R. Deuring, M. P. Scott, M. Kissinger, A. M. Pattatucci, T. C. Kaufman, and J. A. Kennison. 1992. Brahma: a regulator of *Drosophila* homeotic genes structurally related to the yeast transcriptional activator SNF2/SWI2. *Cell.* 68:561-572.
- Tautz, D., and C. Pfeifle. 1989. A non radioactive in situ hybridization method for the localization of specific RNAs in *Drosophila* embryos reveals a translational control of the segmentation gene hunchback. *Chromosoma.* 98:81-85.
- Theurkauf, W. E., S. Smiley, M. L. Wong, and B. M. Alberts. 1992. Reorganization of the cytoskeleton during *Drosophila* oogenesis: implications for axis specification and intercellular transport. *Development.* 115:923-936.
- Theurkauf, W. E., B. M. Alberts, Y. N. Jan, and T. A. Jongens. 1993. A central role for microtubules in the differentiation of *Drosophila* oocytes. *Development.* 118:1169-1180.
- Thummel, C. S., A. M. Boulet, and H. D. Lipshitz. 1988. Vectors for *Drosophila* P-element-mediated transformation and tissue culture transfection. *Gene (Amst.)* 74:445-456.
- Towbin, H., T. Staehelin, and J. Gordon. 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proc. Natl. Acad. Sci. USA.* 76:4350-4354.
- Turner, F. R., and A. P. Mahowald. 1976. Scanning electron microscopy of *Drosophila* embryogenesis. 1. The structure of the egg envelopes and the formation of the cellular blastoderm. *Dev. Biol.* 50:95-108.
- Vallee, R. 1993. Molecular analysis of the microtubule motor dynein. *Proc. Natl. Acad. Sci. USA.* 90:8769-8772.
- Vallee, R. B., and H. S. Shpetner. 1990. Motor proteins of cytoplasmic microtubules. *Annu. Rev. Biochem.* 59:909-932.
- Vaisberg, E. A., M. P. Koonce, and J. R. McIntosh. 1993. Cytoplasmic dynein plays a role in mammalian mitotic spindle. *J. Cell Biol.* 123:849-858.
- Vaslet, C. A., P. O'Connell, M. Izquierdo, and M. Rosbash. 1980. Isolation and mapping of a cloned ribosomal protein gene of *Drosophila melanogaster*. *Nature (Lond.)* 285:674-676.
- Vogelstein, B., and D. Gillespie. 1979. Preparative and analytical purification of DNA from agarose. *Proc. Natl. Acad. Sci. USA.* 76:615-619.
- Walker, R. A., and M. P. Sheetz. 1993. Cytoplasmic microtubule-associated motors. *Annu. Rev. Biochem.* 62:429-451.
- Walter, J. E., and B. M. Alberts. 1984. In *Molecular Biology of Development*. R. Firtel and E. Davidson, editors. *UCLA Symp. Mol. Cell. Biol. New Series.* 19:263-272.
- Warn, R. M., L. Flegg, and A. Warn. 1987. An investigation of microtubule organization and functions in living *Drosophila* embryos by injection of a fluorescently labeled antibody against tyrosinated alpha-tubulin. *J. Cell Biol.* 105:1721-1730.
- Witman, G. B., C. G. Wilkerson, and S. M. King. 1994. Molecular analysis of the γ heavy chain of chlamydomonas flagellar outer-arm dynein. *J. Cell Sci.* 170:497-506.
- Wilhelm, J. E., and R. D. Vale. 1993. RNA on the move: the mRNA localization pathway. *J. Cell Biol.* 123:269-274.
- Xiang, X., S. Beckwith, and R. N. Morris. 1994. Cytoplasmic dynein is involved in nuclear migration in *Aspergillus nidulans*. *Proc. Nat. Acad. Sci. USA.* 91(6):2100-2104.
- Xu, T., L. A. Caron, R. G. Fehon, and S. Artavanis-Tsakonas. 1992. The involvement of the *Notch* locus in *Drosophila* oogenesis. *Development.* 115:913-922.
- Yang, J. T., R. A. Laymon, and L. S. B. Goldstein. 1989. A three-domain structure of kinesin heavy chain revealed by DNA sequence and microtubule binding analyses. *Cell.* 56:879-889.
- Zhang, Z., Y. Tanaka, S. Nonaka, H. Aizawa, H. Kawasaki, T. Nakata, and N. Hirokawa. 1993. The primary structure of rat brain (cytoplasmic) dynein heavy chain, a cytoplasmic motor enzyme. *Proc. Natl. Acad. Sci. USA.* 90:7928-7932.