Journal of Cell Science 107, 1557-1569 (1994)
Printed in Great Britain © The Company of Biologists Limited 1994

A cytoplasmic dynein motor in *Drosophila*: identification and localization during embryogenesis

Tom S. Hays[1,4,*], Mary E. Porter[2,4], Maura McGrail[1], Paula Grissom[4], Pam Gosch[1,4], Margaret T. Fuller[3] and J. Richard McIntosh[4]

[1]Department of Genetics and Cell Biology, University of Minnesota, St. Paul, MN 55108-1020, USA
[2]Department of Cell Biology and Neuroanatomy, University of Minnesota, Minneapolis, MN 55108-1020, USA
[3]Departments of Developmental Biology and Genetics, Beckman Center, B300, Stanford University School of Medicine, Stanford, CA 94305-5427, USA
[4]Department of Molecular, Cellular, and Developmental Biology, University of Colorado, Boulder, CO 80309, USA

*Author for correspondence

SUMMARY

We have characterized a cytoplasmic dynein motor isoform that is present in extracts of *Drosophila* embryos. A prominent high molecular weight (HMW) polypeptide (>400 kDa) is enriched in microtubules prepared from nucleotide-depleted embryonic extracts. Based on its ATP-sensitive microtubule binding activity, 20 S sedimentation coefficient, sensitivity to UV-vanadate and nucleotide specificity, the HMW polypeptide resembles cytoplasmic dyneins prepared from other organisms. The *Drosophila* cytoplasmic dynein acts as a minus-end motor that promotes microtubule translocation in vitro. A polyclonal antibody raised against the dynein heavy chain polypeptide was used to localize the dynein antigen in whole-mount preparations of embryos by immunofluorescence microscopy. These studies show that the dynein motor is associated with microtubules throughout embryogenesis, including mitotic spindle microtubules and microtubules of the embryonic nervous system.

Key words: dynein, microtubule motor, *Drosophila*

INTRODUCTION

Microtubule-mediated motile processes influence numerous aspects of cell behavior, including chromosome segregation, cell division, organelle transport and changes in cell shape. These processes are critical to the life and growth of both individual cells and multicellular organisms. In *Drosophila* development, the distributions and contributions 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; reviewed by Fyrberg and Goldstein, 1990). After fertilization, microtubules are involved in the fusion of the male and female pronuclei and in the subsequent rapid and nearly synchronous mitotic divisions that occur in the interior of the syncytial embryo. After the first seven of these nuclear divisions, the centrosomes and associated nuclei begin a microtubule-dependent migration to the surface of the syncytial embryo and are positioned in a cortical layer of cytoplasm that is packed with cytoskeletal filaments. Following the thirteenth nuclear division, the syncytial blastoderm is transformed into the cellular blastoderm. During this time the elongation of nuclei and extensive saltatory transport of membranous organelles also appear to be dependent on the microtubule cytoskeleton (Fullilove and Jacobson, 1971; Foe and Alberts, 1983).

The motors that associate with cytoplasmic microtubules are likely to power many of these motile processes in *Drosophila*. Microtubule motors are energy-transducing enzymes that convert the energy derived from nucleotide binding and hydrolysis into the sliding of microtubules and/or the movement of organelles or vesicles relative to the microtubule (reviewed by Gibbons, 1988; McIntosh and Porter, 1989; Vallee and Shpetner, 1990; Bloom, 1992; Skoufias and Scholey, 1993). To date, two major classes of microtubule motors have been characterized, the kinesins and the dyneins. In most cases, kinesins translocate along the microtubule lattice toward the plus end, while the dynein motors that have been assayed to date move toward the minus end of the microtubule.

Drosophila offers several advantages for the study of cytoplasmic dynein and its role in development. Using methods applied to the characterization of microtubule-associated motors in other organisms and tissues (Vale et al., 1985; Scholey et al., 1985; Lye et al., 1987; Paschal et al., 1987; Bloom et al., 1988; Porter et al., 1988; Collins et al., 1989), cytoplasmic motors can readily be purified from *Drosophila* embryos in quantities sufficient for biochemical and in vitro motility assays. For example, kinesin has previously been purified and characterized from embryos (Saxton et al., 1988). With regard to the study of dynein motors in *Drosophila*, it is important to note that the embryo apparently lacks ciliated

tissues (Smith, 1968; Kiefer, 1973) and therefore cytoplasmic dynein isoforms can be purified in the absence of axonemal precursors. However, axonemal dyneins are present in the sperm cells of male adult flies (Hardy et al., 1981; Gepner and Hays, 1993) and so will allow for a comparison between dynein isoforms. These comparisons may shed light on domains of the dynein polypeptides that account for their functional specificity; for example, the targeting of the dynein motors to their proper positions in the axoneme or to specific cytoplasmic cargoes. In this study, we have identified and purified a cytoplasmic dynein from *Drosophila* embryos. We demonstrate that the *Drosophila* cytoplasmic dynein acts as a minus-end-directed MT motor and we show that the dynein heavy chain polypeptide is found in association with multiple microtubule arrays including mitotic spindle and axonal microtubules.

MATERIALS AND METHODS

Protein purification

Embryos (0-24 hour) were harvested from an *Oregon-R* strain of *Drosophila melanogaster* and washed three times in PMEG (0.1 M PIPES, pH 6.9, 5 mM EGTA, 0.9 M glycerol, 5 mM $MgSO_4$, 0.1 mM EDTA plus 10 μg/ml aprotinin, leupeptin and pepstatin, 0.1 mg/ml soybean trypsin inhibitor, *n*-tosyl-L-arginine methylester, benzamidine and 0.5 mM dithiothreitol (DTT)). The embryos were resuspended in 1.5 volumes PMEG and disrupted on ice using a Wheaton glass homogenizer. The homogenization step was monitored by light microscopy to ensure that the majority of the embryos had been disrupted. All subsequent steps were carried out on ice or at 4°C, except where noted. The embryo homogenate was spun at 57,000 ***g*** for 45 minutes to pellet the insoluble material. A top layer of lipid was removed and the remainder of the supernatant was collected and further clarified at 140,000 ***g*** for 45 minutes. Microtubules were polymerized by adding 20 μM taxol (Natural Products Branch, Division of Cancer Treatment, National Cancer Institute) and 1.0 mM GTP to the supernatant and incubating the extract for 15 minutes at 25°C, and 30-45 minutes at 4°C (Vallee, 1982). For preparing dynein, the yield was enhanced by the removal of ATP, so the supernatant was adjusted to a final concentration of 100 mM glucose and 10 units/ml hexokinase (Sigma, St Louis, MO) and further incubated for 45 minutes. The extracts were layered onto a 2 ml 15% sucrose cushion and then centrifuged in a Sorvall HB-4 swinging bucket rotor at 23,000 ***g*** for 60 minutes at 4°C to pellet the assembled microtubules and their associated proteins. The resultant microtubule protein was washed by resuspension in PMEG plus taxol and recentrifuged. The microtubule protein was then extracted in 0.5-1.0 ml PMEG buffer containing 10 mM MgATP and 20 μM taxol, and pelleted by centrifugation as above to obtain a supernatant referred to as the 'ATP extract'. This ATP extract was either fractionated immediately by centrifugation on 5% to 20% sucrose gradients, or stored at –80°C after freezing in liquid N_2.

Rebinding of ATP-extracted proteins to microtubules

The microtubule-associated proteins (MAPs) that were extracted with ATP were dialyzed overnight at 4°C to remove ATP and then clarified by centrifugation. MAP-free phosphocellulose-purified bovine brain tubulin was prepared according to the method of Williams and Detrich (1979). The clarified supernatant was incubated with taxol-preassembled, phosphocellulose-purified brain tubulin (1 mg/ml) in the absence or presence of 10 mM MgATP. Microtubules and MAPs were collected by centrifugation at 100,000 ***g*** for 2 minutes in a Beckman airfuge using an A100 rotor. The resulting supernatants and pellets were analyzed by SDS-PAGE.

Sucrose gradient purification and NTPase measurements

A 0.5-1.0 ml sample of ATP extract was fractionated by centrifugation through a 5% to 20% sucrose gradient (11.6 ml) in PMEG at 125,000 ***g*** for 16 hours at 4°C in a Beckman SW41 rotor. ATP extract frozen at –80°C was first clarified by centrifugation at 100,000 ***g*** for 20 minutes before use. The gradients were collected in twenty 0.6 ml fractions, which were analyzed by standard SDS-PAGE (see below). Protein concentration was determined by the method of Bradford (1976). ATPase and CTPase activities in gradient fractions were analyzed by a modification of the method of Cohn et al. (1987) as described by Grissom et al. (1992).

Vanadate cleavage

Samples of the ATP extract or 20 S dynein fractions were cleaved in the presence of vanadate as described by Lee-Eiford et al. (1986). Drops (50-100 μl) of samples in the appropriate experimental buffers were supplemented with vanadate, placed on parafilm, and irradiated on ice for 45 minutes using a long wavelength UV lamp (model EN-28, Spectronics Corp., Westburg, NY). For cleavage at the V_1 site the dynein samples were dialyzed into V_1 buffer containing 0.45 M sodium acetate, 0.1 mM EDTA, 7 mM β-mercaptoethanol, 10 mM HEPES, pH 7.5, and supplemented with 100 μM sodium vanadate, 0.5 mM ATP, and 1 mM magnesium acetate. For cleavage at the V_2 site the dynein samples were dialyzed into V_2 buffer containing 0.45 M sodium acetate, 0.1 mM EDTA, 1 mM Na_2SO_4, 5 mM glutathione, 10 mM HEPES, pH 7.5, and supplemented with 100 μM sodium vanadate, 0.5 mM ATP and 1 mM manganese acetate. The irradiated samples were subsequently analyzed by SDS-PAGE.

Gel electrophoresis and immunoblot procedures

SDS-polyacrylamide gel electrophoresis was on 0.75 mm thick slab gels using the buffer system of Laemmli (1970). All polyacrylamide gels shown were stained by Coomassie Blue. The bis/acrylamide ratio was 1/100 (w/w) to allow better visualization of high molecular weight polypeptides (Porter and Johnson, 1983). Higher resolution of the dynein heavy chain region was achieved by electrophoresis on 3% polyacrylamide gels with a 0 to 8 M urea gradient and a borate-sulfate buffer system as described by Neville (1971). Proteins were blotted to nitrocellulose (Towbin et al., 1979) and probed with blot affinity-purified antibodies (Olmsted, 1981), using either the immunoperoxidase or alkaline phosphatase reaction for detection.

Motility assays

The sucrose gradient fractions were analyzed for their ability to promote the movement of mcirotubules over a glass coverslip as described previously (Vale and Toyoshima, 1988). Samples (5-15 μl) of each fraction were allowed to adsorb onto the glass coverslip. Taxol-stabilized microtubules (5 μl of a 0.2 mg/ml soln in PMEG) prepared from MAP-free tubulin (Williams and Detrich, 1979) were supplemented with 2 mM ATP and added to the samples. Coverslips were inverted onto a glass slide and microtubule motility was assayed using video enhanced DIC. Alternatively, a perfusion chamber was used to assay microtubule motility in the presence of PMEG buffer containing 10 μM vanadate, 5 mM AMP-PNP, 0.5 mM ATPγS, 1 mM GTP, *N*-ethylmaleimide (NEM) or DTT. Three samples of the same fraction were sequentially drawn into the perfusion chamber and each sample was allowed to adsorb to the coverslip for 5 minutes. The unadsorbed protein was removed by perfusion with a solution of bovine serum albumin. Subsequently, taxol-stabilized microtubules in the experimental buffer of interest (see above) were perfused into the chamber and assayed for microtubule attachment and motility. Rates of microtubule movement were determined from the analysis of recorded video images as described previously (Porter et al., 1987).

To determine the polarity of motor activity, *Chlamydomonas* flagellar axonemes were utilized as described by Paschal and Vallee (1987). Samples (5-15 μl) of the fraction containing the peak of

dynein ATPase activity were perfused into a slide/coverslip chamber and allowed to adsorb onto a glass coverslip. Axonemes splayed at the distal ends were perfused into the chamber in PMEG buffer containing 2 mM ATP and the directionality of the movement of the axonemes was determined.

Preparation of dynein antibodies

Antibodies that recognize a *Drosophila* dynein heavy chain polypeptide were raised in two New Zealand white rabbits. ATP extracts (~1 mg/ml) containing the *Drosophila* cytoplasmic dynein were fractionated on 5% SDS-polyacrylamide gels and briefly stained in 0.1% Coomassie Blue, 50% methanol, 10% acetic acid. After destaining, the heavy chain polypeptide was excised from the gel, equilibrated with PBS (140 mM NaCl, 3 mM KCl, 5 mM $MgCl_2$, 1.5 mM KH_2PO_4, 8 mM Na_2HPO_4, pH 7.4), emulsified with complete Freund's adjuvant, and injected into the back and hindquarters of the rabbits. Rabbits were boosted at two-week intervals for 12 weeks with antigen in incomplete Freund's adjuvant. One of the rabbits produced high titer antiserum against the *Drosophila* dynein heavy chain polypeptide. At dilutions of 1:1000 the whole serum from this rabbit recognized predominantly the dynein heavy chain, but also showed minor reactivity to other lower molecular weight species. For this reason, all experiments reported in this study utilized antibodies that were affinity-purified against the the intact dynein heavy chain polypeptide, or one of the two V_1 vanadate cleavage fragments using the blot affinity purification method of Olmsted (1981).

Immunofluorescence

Embryos from Oregon R flies were collected, rinsed in 0.4% NaCl, 0.03% Triton X-100 and dechorionated in 50% bleach for 2 minutes. The dechorionated embryos were rinsed again in 0.4% NaCl, 0.03% Triton X-100, transferred to 100% heptane in a 50 ml conical tube, and vigorously shaken for 30 seconds to permeabilize the vitelline membranes. An equal volume of 33% formaldehyde was quickly added to the heptane and the mixture containing the embryos was incubated for 5 minutes with gentle shaking. Following fixation, the aqueous fixative phase was removed, an equal volume of methanol was added, and the tube vigorously shaken for 1 minute to disrupt vitelline membranes. Embryos with ruptured vitelline membranes were allowed to settle to the bottom of the methanol phase. The overlying methanol/heptane mixture and any floating embryos, whose vitelline membranes failed to rupture, were removed. The settled embryos were rinsed three times with fresh methanol to remove the remaining heptane and then rehydrated. Embryos were incubated for 5 minutes each in a methanol:PBS series (70:30, 50:50, 30:70), incubated for 10 minutes in PBS and rinsed 3 times with PBT (PBS + 0.1% Triton X-100). The collection, washing, fixation and permeabilization steps described above were all conducted at room temperature. Embryos were subsequently blocked in PBT containing 1% BSA (PBT-B) for 2 hours at room temperature before antibody incubations. For comparison, embryos were also fixed in methanol as previously described by Kellogg et al. (1988). In this procedure, the dechorionated embryos were transferred to a 50 ml conical containing 97% methanol/3% formaldehyde, 500 mM EGTA, pH 7.5, an equal volume of heptane was added, and the tube was vigorously shaken for 1 minute. Subsequent processing of the embryos was the same as in the formaldehyde fixation procedure. The pattern of antibody staining was comparable for both fixation methods.

Embryos were double-labelled with a mouse monoclonal antibody raised against alpha tubulin (Piperno and Fuller, 1985) diluted 1:50 in PBT-B, and the affinity-purified rabbit polyclonal antibody that is monospecific for the fly dynein heavy chain polypeptide diluted 1:1 in PBT-B. Goat anti-mouse Texas Red-conjugated (Jackson ImmunoResearch, West Grove, PA) and goat-anti-rabbit fluorescein-conjugated secondary antibodies (Boehringer Mannheim; Indianapolis, IN) were preabsorbed against fixed *Drosophila* embryos as described by Karr and Alberts (1986). Both secondary antibodies were used at a final dilution of 1:100 in PBT-B plus 5% normal goat serum. Incubations with primary antibodies were for 18-24 hours at 4°C on a rotating wheel (Rotator; Cole Palmer; Chicago, IL); secondary antibody incubations were for 2 hours at room temperature. Following the incubations, embryos were washed eight times for 15 minutes each in PBT-B. Stained embryos were counterstained in DAPI (1 μg/ml) in PBT to visualize DNA, and mounted in PBS containing 90% glycerol and 1 mg/ml ρ-phenylenediamine. Alternatively, stained and counterstained embryos were redehydrated in ethanol and mounted in benzyl benzoate:benzyl alcohol (1:2, v/v). Embryos were examined on either a Zeiss Axioskop 10 microscope with epifluorescence illumination using Plan-Neofluar 16×/0.5, 40×/0.75 lenses or a 63×/1.4 oil Planapochromat lens, or a Nikon Diaphot microscope with an MRC-600 confocal imaging system (Bio-Rad) using a 60×/1.4 Planapochromat lens.

Immunofluorescence controls

The specificity of the affinity-purified dynein antibody and its resultant staining pattern were examined in several control experiments. First, samples stained with secondary antibodies alone showed only a uniform, low level of background fluorescence relative to embryos incubated with both primary and secondary antibodies. Second, the whole rabbit serum was blot affinity-purified against a random 120 kDa polypeptide present in the ATP extracts. This antibody showed only a uniform, low level of fluorescence across the embryo even at lower dilutions than were used with the antibodies that were affinity-purified against the dynein polypeptide. In a third set of control experiments, the polyclonal dynein antibody was separately affinity-purified against each of vanadate-cleaved polypeptides. Antibodies purified against either of the cleavage fragments recognized only the expected dynein cleavage fragment on immunoblots of UV-cleaved ATP-MAPS (see Fig. 5A). Moreover, these antibodies produced the same staining pattern as that observed with dynein antibodies that were affinity-purified against the intact dynein heavy chain. The specificity of the affinity-purified dynein antibodies and their staining patterns was further tested by preincubating the antibodies with a strip of nitrocellulose to which the fly dynein heavy chain had been blotted. Embryos stained with the resultant dynein antibody-depleted serum showed no, or greatly reduced, staining of the mitotic spindle or nerve chord when used at the same dilution as affinity-purified antibody that had not been preincubated (see Fig. 9A).

The similarity between the staining pattern observed with the dynein antibodies and the pattern observed with tubulin antibodies is not due to fluorescence cross-over in the double-labelled samples. The same patterns are observed when specimens are labelled with each antibody separately. In this case, we observed no Texas Red fluorescence crossing into the fluorescein channel, and conversely no fluorescein signal in the Texas Red channel. Another possibility is that the affinity-purified dynein antibody might crossreact with tubulin. However, we found no evidence that the affinity-purified dynein antibodies crossreacted with tubulin on immunoblots of *Drosophila* microtubule protein (for example, see Figs 5B and 6B). Moreover, preincubation of the affinity-purified dynein antibody with native taxol-assembled *Drosophila* microtubules had no effect on the dynein staining pattern observed in embryos (see Fig. 9B). As a positive control for the preincubation method, two different monoclonal α-tubulin antibodies, 3A5 (Piperno and Fuller, 1985) and a commerical antibody (Amersham Corp; Arlington Heights, IL), were also preabsorbed against native microtubules. In both cases, microtubule staining in the embryos was completely eliminated or greatly reduced compared to the unabsorbed tubulin antibodies (see Fig. 9C). These controls demonstrate that the staining patterns observed with the dynein antibodies are due to the presence of a dynein antigen, and that the immunolocalization of dynein to microtubule arrays is not due to the crossreactivity of the dynein antibodies with tubulin.

RESULTS

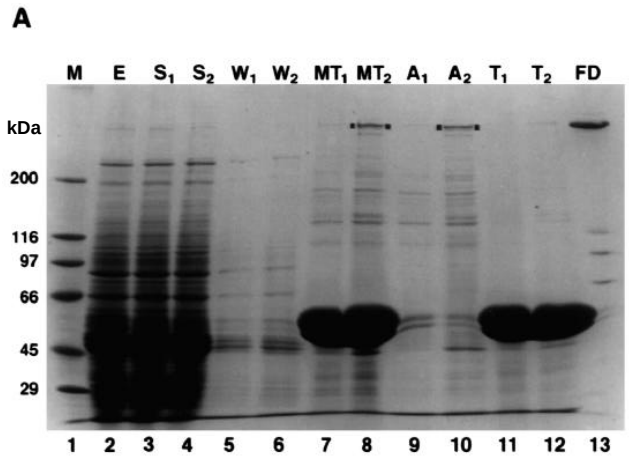
Nucleotide-sensitive binding of a *Drosophila* embryonic MAP

Microtubule-associated polypeptides (MAPs) that interact with microtubules (MTs) in a nucleotide-sensitive fashion are potential candidates for MT-based motors (Johnson et al., 1984; reviewed by Gibbons, 1988; McIntosh and Porter, 1989). We have identified a high molecular weight (HMW) MAP in *Drosophila* embryos that exhibits ATP-sensitive binding to MTs and resembles dynein in its properties. The HMW MAP was identified and purified by virtue of its nucleotide-dependent association with microtubules. Taxol-dependent assembly of MTs was induced in high-speed extracts of 0-24 hour *Drosophila* embryos in the presence or absence of endogenous nucleotides (Fig. 1). MTs assembled in extracts that were pretreated with hexokinase/glucose (or apyrase; data not shown) to reduce endogenous levels of nucleotide triphosphates were enriched for several MAPs when compared to MTs assembled in untreated extracts. The most prominent of the enriched polypeptides was a high molecular weight (>400 kDa) species that comigrates with heavy chain polypeptides of sea urchin flagellar dynein (Fig. 1A). The candidate *Drosophila* dynein heavy chain is enriched ~30-fold in MTs prepared from nucleotide-depleted embryonic extracts as compared to control extracts. This HMW *Drosophila* MAP was released from MTs by extraction of the MT pellet with 10 mM MgATP in a buffer containing taxol to prevent MT depolymerization.

The ATP-extracted MAPs were dialyzed to remove ATP and tested for their ability to rebind to MTs. When taxol-stabilized MTs assembled from phosphocellulose-purified brain tubulin are added to the dialyzed MAP fraction, and subsequently pelleted, the HMW polypeptide is enriched in the MT pellet (Fig. 1B; compare lanes 4 and 5). In addition to the HMW polypeptide, several polypeptides of lower molecular weights are present in the MT pellet. The rebinding of the HMW polypeptide and the lower molecular weight species is inhibited by the presence of 10 mM ATP (Fig. 1B; lanes 6 and 7). We have not characterized the lower molecular weight components that may be associated with the HMW polypeptide as part of a dynein complex. However, several of the lower molecular weight polypeptides are similar in mass to polypeptides reported to associate with cytoplasmic dyneins prepared from other sources (Schroer et al., 1989; Paschal et al., 1987). In particular, a 150 kDa polypeptide appears enriched in *Drosophila* MT pellets prepared from 0-24 hour embryos. Moreover, immunoblot experiments (M. McGrail, A. Silvanovich and T. S. Hays, unpublished data) suggest that this 150 kDa polypeptide is related to the glued polypeptide, a homolog of the 150 kDa subunit of dynactin (Gill et al., 1991; Holzbaur et al., 1991).

The *Drosophila* HMW MAP is a cytoplasmic dynein motor

The characterization of axonemal dyneins from cilia and flagella has established a set of criteria by which to identify dyneins involved in microtubule-based motility (reviewed by Pallini et al., 1982; Porter and Johnson, 1989; McIntosh and Porter, 1989; Witman, 1992). In addition to ATP-sensitive binding to MTs and component heavy chain polypeptides of high molecular weight (>400 kDa), other characteristics of multisubunit dynein complexes include: (1) a large native particle size with a sedimentation coefficient of ~20 S; (2) an



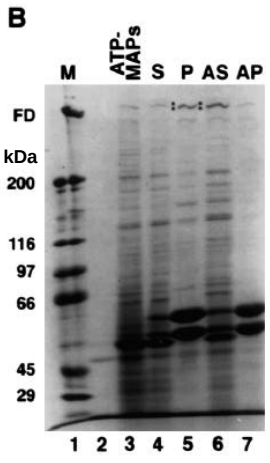


Fig. 1. Nucleotide sensitive binding of the fly HMW MAP to microtubules. (A) Microtubule assembly was induced in high-speed embryonic extracts (E, lane 2) by the addition of 1 mM GTP and 30 μM taxol. The extract was then divided into two aliquots, and hexokinase (7.5 units/ml) and 100 mM glucose were added to the second aliquot to reduce the ATP concentration. The two samples were then subjected to differential centrifugation to yield two microtubule pellets and two microtubule-depleted embryonic extracts (S_1 and S_2, lanes 3 and 4). The two microtubule pellets were resuspended, washed in extraction buffer without nucleotide and recentrifuged to yield two wash supernatants (W_1 and W_2, lanes 5 and 6) and two microtubule pellets (MT_1 and MT_2, lanes 7 and 8). The HMW MAP is greatly enriched in the hexokinase/glucose-treated sample (MT_2, lane 8). The pellets were extracted with 10 mM MgATP/ 0.1 M KCl and recentrifuged to yield ATP extracts (A_1 and A_2, lanes 9 and 10) and tubulin pellets (T_1 and T_2, lanes 11 and 12). Dots indicate the high molecular weight dynein heavy chain polypeptide. Molecular weight markers (M; lane 1) and a sea urchin flagellar dynein sample (lane 13) are indicated. (B). *Drosophila* dynein binds to preassembled microtubules in an ATP-sensitive fashion. A 10 mM MgATP extract of taxol-assembled microtubules was exhaustively dialyzed to remove ATP and then clarified by centrifugation to yield a pellet (lane 2) and a supernatant (lane 3) of ATP-MAPS. This material was then incubated with phosphocellulose-purified, taxol-assembled, bovine brain tubulin in the absence and presence of 10 mM MgATP. Microtubules and MAPs were collected by centrifugation in a Beckman airfuge, and the resulting supernatants and pellets (S and P) were analyzed by SDS-PAGE (lanes 4-7). The HMW MAP rebound to microtubules in the absence of ATP (lane 5, upper pair of dots), but did not rebind to microtubules in the presence of ATP (lane 7). Several lower molecular weight polypeptides also exhibit ATP-sensitive binding to microtubules. Sea urchin flagellar dynein and molecular weight markers were electrophoresed as standards in lane 1.

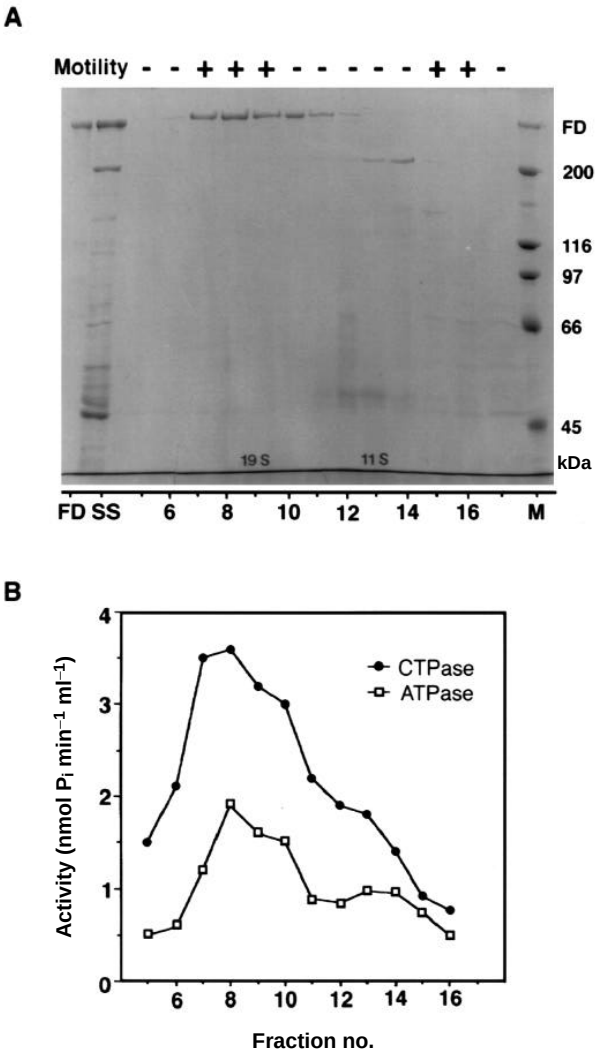


Fig. 2. *Drosophila* cytoplasmic dynein sediments as a 20 S particle on sucrose density gradients. (A) The MgATP extract (SS) of hexokinase/glucose-treated microtubules was loaded onto a 5% to 20% sucrose density gradient in extraction buffer and centrifuged at 125,000 ***g*** for 16 hours. The resulting fractions were then analyzed by SDS-PAGE. The dynein heavy chain polypeptide sediments at ~19-20 S (lanes 7-11). The sucrose fractions were tested in vitro for their ability to promote microtubule translocation (top line). A peak of motility copurifies with the dynein complex on sucrose gradients. A second peak of motility in sucrose fractions (15-16) coincides with the sedimentation coefficient of kinesin. (B) The 20 S peak fractions containing the purified dynein complex are associated with a peak of ATPase and CTPase activity.

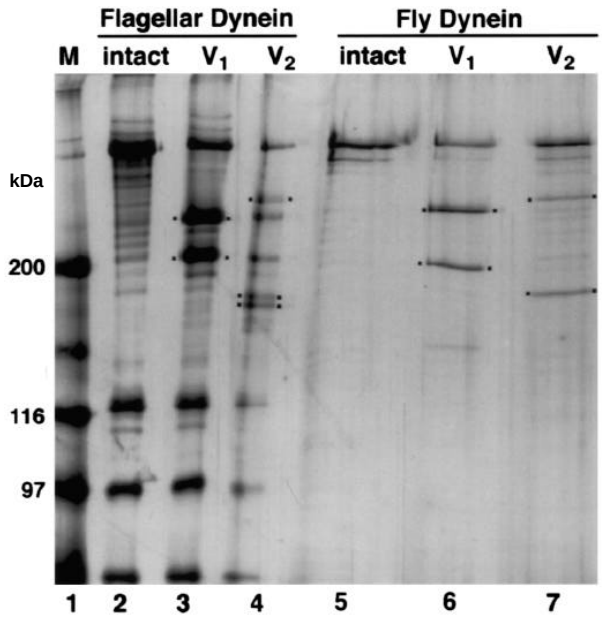


Fig. 3. Comparison of the vanadate-sensitive UV cleavage of sea urchin flagellar dynein (lanes 2-4) and *Drosophila* cytoplasmic dynein (lanes 5-7). Molecular weight markers (lane 1). Intact sea urchin flagellar dynein (lane 2) incubated on ice for 1 hour in 500 μM ATP, 100 μM V_i, 1 mM $MgC_2H_3O_2$ in V_1 buffer* (see below). Flagellar dynein irradiated for 1 hour with 500 μM ATP, 100 μM V_i and 1 mM magnesium acetate in V_1 buffer (lane 3). Flagellar dynein irradiated for 1 hour with 100 μM V_i, 1 mM manganese acetate in V_2 buffer** (lane 4). Nonirradiated *Drosophila* 20 S cytoplasmic dynein in 500 μM ATP, 100 μM V_i, 1 mM magnesium acetate in V_1 buffer (lane 5). *Drosophila* cytoplasmic dynein irradiated for 1.5 hours with 500 μM ATP, 100 μM V_i, 1 mM magnesium acetate in V_1 buffer (lane 6). *Drosophila* cytoplasmic dynein irradiated for 1.5 hours with 100 μM V_i, 1 mM manganese acetate in V_2 buffer (lane 7). *V_1 buffer: 0.45 M sodium acetate, 0.1 mM EDTA, 7 mM β-mercaptoethanol, 10 mM HEPES, pH 7.5. **V_2 buffer: 0.45 M sodium acetate, 0.1 mM EDTA, 1 mM Na_2SO_4, 5 mM glutathione, 10 mM HEPES, pH 7.5.

ATPase activity that is inhibited by low concentrations of vanadate; (3) a minus-end motor activity that promotes MT translocation in vitro; and (4) the cleavage of dynein heavy chains at specific sites (V_1 or V_2) when irradiated with near-UV light in the presence of nucleotide and vanadate.

The HMW, ATP-sensitive MAP from *Drosophila* embryos shares these properties and resembles cytoplasmic dyneins isolated from other sources. When ATP extracts prepared as described above are fractionated on sucrose density gradients, the *Drosophila* HMW MAP sediments as a 20 S particle, and is associated with a peak of ATPase activity (Fig. 2A and B). The ATPase activity is significantly inhibited by 10 mM vanadate (data not shown). The substrate specificity of axonemal dynein (ATP > GTP > CTP; see Shimizu and Johnson, 1983) differs from the cytoplasmic forms of dynein (CTP > GTP > ATP; see Shpetner et al. 1988; Pallini et al., 1982) that have been tested and appears to distinguish between the two isoforms of the enzyme. The *Drosophila* MAP, like other cytoplasmic dyneins, is also associated with a prominent peak of CTPase activity (Fig. 2B).

One of the most striking features of dyneins is the cleavage of the heavy chain polypeptides at a single site (V_1) by UV-irradiation in the presence of ATP, Mg^{2+} and vanadate (Lee-Eiford et al., 1986; Gibbons et al., 1987). When Mn^{2+} is substituted as the divalent cation, axonemal β heavy-chain dyneins are cleaved at a second unique site (V_2 site) (Gibbons et al., 1987; Mocz et al., 1988), while the α and γ heavy chain axonemal dynein polypeptides of *Chlamydomonas* are cleaved at two or three sites (King and Witman, 1987, 1988). Vanadate-mediated photolysis under the conditions used appears to be diagnostic for dyneins. Other ATPases, including myosins and

kinesins, are not cleaved under these same conditions (Gibbons et al., 1987; Lye et al., 1987). As shown in Fig. 3, the *Drosophila* cytoplasmic dynein heavy chain is cleaved at a single site (V_1) in the presence of ATP, Mg^{2+} and vanadate. The *Drosophila* cytoplasmic dynein is cleaved at a different site (V_2) in the presence of Mn^{2+}. The polypeptide fragments produced by cleavage at the two sites are similar in size to the V_1 and V_2 cleavage fragments reported for other dynein heavy chains. The cleavage of the dynein heavy chain at either the V_1 or V_2 sites did not occur in the absence of vanadate, ATP or exposure to UV light. These results indicate that structural features characteristic of the dynein heavy chain polypeptides from other organisms are conserved in *Drosophila*. This conservation in structure has been subsequently confirmed by the sequence analysis of the *Drosophila* cytoplasmic dynein heavy chain gene and its comparison to other published dynein sequences (Rasmusson et al., 1994).

The *Drosophila* cytoplasmic dynein translocates MTs in vitro (Fig. 4). To assay for motor activity, individual protein fractions were adsorbed onto a glass coverslip (Sale and Fox, 1988; Vale and Toyoshima, 1988) and subsequently perfused with MTs in the presence of 2 mm ATP. Two peaks of MT gliding were observed from the ATP MAPs fractionated on sucrose gradients. One peak of motor activity was associated with the 20 S region of the gradient and was correlated with the presence of dynein heavy chain polypeptide and a peak of ATPase activity (Fig. 2). MT gliding promoted by the 20 S fraction was unidirectional, required the presence of millimolar ATP, and exhibited rates comparable to those observed for other cytoplasmic dyneins at room temperature (range: 0.5-1.5 μm/s). As expected for a dynein motor, the MT gliding associated with the 20 S peak fractions was inhibited ~90% by 25 μM vanadate, or millimolar *N*-ethylmaleimide. MT motility was not observed in the presence of GTP, CTP or AMP-PNP. Motor activity was also observed in fractions from the 10 S region of the gradient and was associated with a second peak of lower ATPase activity. The 10 S peak of motor activity has previously been characterized as kinesin activity (Saxton et al., 1988). Both kinesin and contaminating myosin present in this region of the gradient may contribute to the level of ATPase activity observed. The two peaks of motor activity were separated by protein fractions that exhibited no motor activity, consistent with the presence of two separate motor protein complexes.

To determine the polarity of force production for the *Drosophila* dynein motor we used *Chlamydomonas* flagellar axonemes in the in vitro gliding assay as described by Paschal and Vallee (1987). The plus end of these axonemes is frayed during preparation, providing a marker for polarity that can be seen in the light microscope. Axonemes glided with their frayed plus ends leading on coverslips that were first adsorbed with the 20 S fraction from sucrose gradients (Fig. 4B). Coverslips adsorbed with the 10 S fraction of the gradient were previously shown to promote movement of the opposite polarity (Saxton et al., 1988). These results demonstrate that the *Drosophila* cytoplasmic dynein has mechanochemical

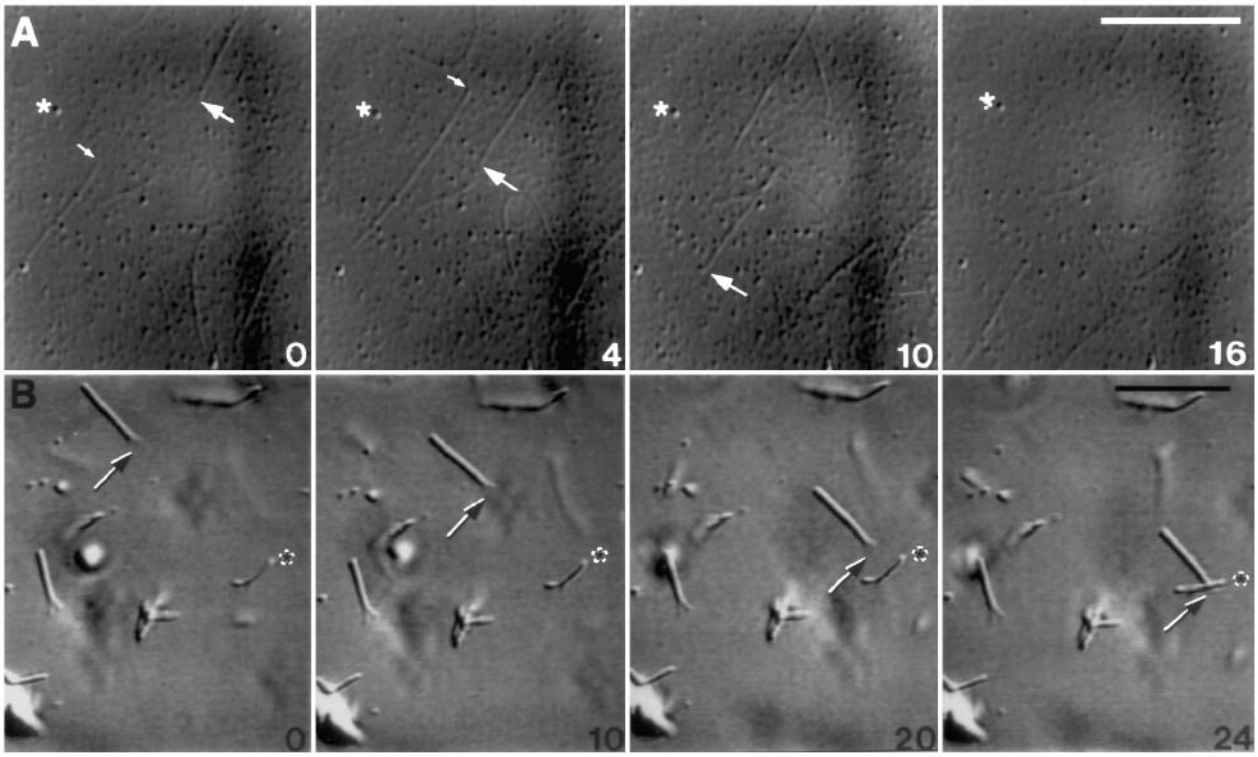


Fig. 4. The *Drosophila* dynein is a minus-end microtubule motor. Single microtubules (A panels) and demembranated *Chlamydomonas* axonemes (B panels) actively translocate on coverslips preincubated with the 20 S sucrose fractions (see Fig. 2A) that are enriched for *Drosophila* dynein. Elapsed time indicated in seconds. The rates of movement varied from 0.5 to 1.5 μm/s. Arrows mark the translocation of single microtubules and axonemes. Asterisks indicate a stationary marker for reference. Axonemes moved with the frayed or plus ends leading, consistent with the dynein motor walking towards the minus end of the axoneme.

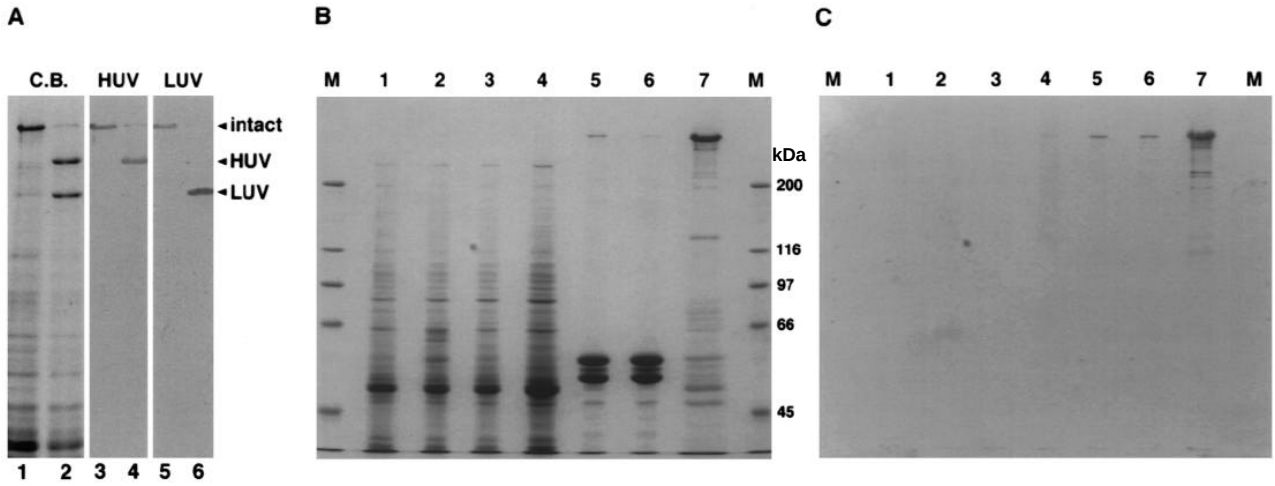


Fig. 5. Specificity of dynein antibody. (A) A rabbit polyclonal dynein antibody was purified against the high molecular weight (HUV) or low molecular weight (LUV) vanadate cleavage fragments of the *Drosophila* dynein. Replicate samples of intact (lanes 1,3,5) and UV-cleaved (lanes 2,4,6) *Drosophila* ATP MAPs were electrophoresed and stained with Coomassie Blue (C.B.; lanes 1-2) or blotted to nitrocellulose and probed with antibodies purified from either the HUV (lanes 3-4) or LUV (lanes 5-6) cleavage fragment. (B) Coomassie Blue-stained SDS-PAGE of samples from a typical preparation of *Drosophila* ATP MAPs. Approximately 15 μg of protein from each purification step was loaded on a 7.5% polyacrylamide gel. Fly embryo homogenate (lane 1), low-speed supernatant (57,000 ***g***; lane 2), high-speed supernatant (140,000 ***g***; lane 3), microtubule-depleted supernatant (lane 4), taxol-assembled microtubule pellet (lane 5), MgATP-extracted tubulin pellet (lane 6), MgATP-extracted MAP supernatant (lane 7). Molecular weight markers (M) are indicated. (C) Immnunoblot of gel identical to that shown in B probed with dynein antibody affinity-purified against the intact heavy chain. The *Drosophila* cytoplasmic dynein heavy chain polypeptide is enriched in the MgATP MAP fraction. The presence of crossreacting lower molecular weight species in lane 7 (B and C) is variable between samples and appears to reflect proteolysis that occurs despite the presence of inhibitors. Results using antibodies purified against either the HUV or LUV cleavage fragments are identical.

properties analogous to axonemal and cytoplasmic dyneins from other sources. Dynein 'walks' towards the minus end of a MT and consequently when tethered to a glass coverslip, dynein pushes MTs in the opposite direction with their plus ends leading.

Distribution of the dynein motor during embryogenesis

To gain insight into the potential function(s) of cytoplasmic dynein during embryogenesis, we have examined the temporal and spatial distribution of the dynein motor in embryos. A rabbit polyclonal antibody that reacts with the heavy chain polypeptide was affinity-purified against the intact heavy chain, or separately against each of the two polypeptide fragments produced by photocleavage at the V_1 site. The affinity-purified antibodies recognize the intact dynein polypeptide, as well as the respective UV-cleavage fragments against which they were affinity purified (Fig. 5A). These results indicate that the affinity-purified antibodies are monospecific and recognize the dynein heavy chain polypeptide, rather than a different polypeptide that comigrates with the dynein heavy chain polypeptide on SDS-PAGE. The affinity-purified antibodies detect only minor amounts of the dynein polypeptide in high-speed extracts of embryos, but show the enrichment of dynein that occurs during the MT-affinity purification (Fig. 5B and C).

Immunoblot analysis indicates that cytoplasmic dynein is present throughout embryonic development, with higher levels present in the first 8 hours of development compared to later stages. We monitored the presence of the dynein polypeptide in preparations of MTs from unfertilized embryos and staged collections of fertilized embryos (Fig. 6A and B). The *Drosophila* dynein can also be purified from unfertilized embryos, indicating that dynein is maternally deposited into the developing oocyte (Fig. 6A and B). As shown in Fig. 6A and B, there are multiple HMW bands that migrate close together and that are recognized by the affinity-purified dynein antibody. This heterogeneity is variable, dependent on the preparation, and is likely due to the proteolysis of a single dynein heavy chain polypeptide during its purification, despite the presence of protease inhibitors. The high molecular weight MAP polypeptides from a 0-24 hour embryo collection were analyzed on high-resolution urea gradient gels (Fig. 7). Only a single prominent HMW band is observed in samples of the high speed embryonic extracts or taxol-assembled MTs from a typical preparation of *Drosophila* cytoplasmic dynein. This is in contrast to the 7-8 HMW bands observed in a sample of whole testis from *Drosophila*, or a sample of sea urchin flagellar dynein.

The cytoplasmic dynein appears to be associated with MTs in *Drosophila* embryos. The spatial distribution of dynein was determined by immunofluorescence microscopy in whole-mount preparations of *Drosophila* embryos using affinity-purified dynein antibodies. The dynein antigen is enriched in mitotic spindles in both the syncytial blastoderm and cellularized blastoderm (Fig. 8D,E,J,K). In the cellularized embryo, this spindle localization reveals the domains of mitotic cells (Fig. 8B,E) that arise in a specific temporal and spatial pattern during gastrulation (Foe, 1989). The pattern of mitotic spindle staining observed with the dynein antibodies follows the known distribution of spindle MTs. The dynein antigen is localized at the spindle poles and along the spindle micro-

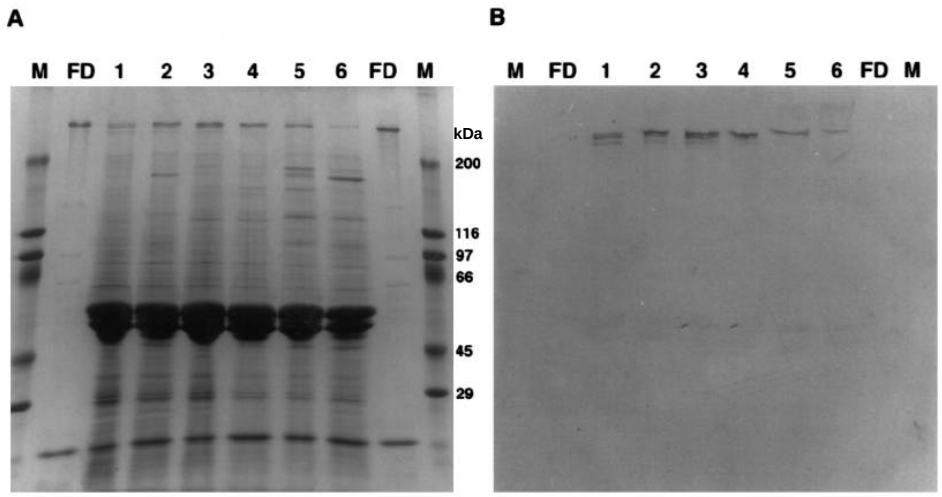


Fig. 6. Developmental profile of cytoplasmic dynein during *Drosophila* embryogenesis. (A) 5% to 15% gradient SDS-PAGE of microtubule protein prepared from unfertilized (lane 1) and staged collections of embryos: 0-2 hour (lane 2); 2-4 hour (lane 3); 4-8 hour (lane 4); 8-12 hour (lane 5); 12-24 hour (lane 6). MTP samples were prepared in the presence of hexokinase glucose to deplete ATP (see Materials and Methods: protein purification). Equal amounts of protein were loaded for each developmental stage. Molecular weight markers (M) and sea urchin flagellar dynein (FD) are indicated. (B) Western blot of gel identical to A in the samples loaded. The Western blot was probed with dynein antibodies purified against the intact dynein heavy chain. The antibody recognizes a high molecular weight polypeptide in all the MTP samples.

tubules during prometaphase (Fig. 8J; also Fig. 9B). In anaphase, the dynein antigen appears to redistribute out along the astral microtubules (Fig. 8K). However, the dynein staining pattern appears discontinuous and punctate, and is distinct from the more fibrous pattern observed with tubulin antibodies. Control experiments demonstrated that the pattern of staining observed with the dynein antibody is specific and not due to a crossreaction with tubulin (Fig. 9; see Materials and Methods for details).

The dynein antigen is not exclusively associated with MTs of the mitotic spindle, but appears to associate with all microtubular arrays examined (see Fig. 8J and K). The particulate dynein staining pattern colocalizes with the cytoplasmic MTs that originate from the interphase centrosomes during each of the rapid nuclear divisions of the syncytial blastoderm. In the later nuclear division cycles that immediately precede cellularization, the dynein antigen is localized near the centrosomes on the apical surfaces of interphase nuclei and extends basally along the cage of MTs that surround each nucleus (Fig. 8I,L). Dynein staining is also present at reduced levels throughout the cytoplasm of the syncytial embryos (Fig. 8J,K,L). This cytoplasmic staining is also greatly reduced following immunoabsorption with the dynein antigen (Fig. 9), suggesting that the staining does reflect a bona fide dynein complex present throughout the cytoplasm.

In the cellularized embryo, the cytoplasm within all cells appears to label with the dynein antibody in a pattern that is similar to that seen for tubulin staining (Fig. 8B,E). At 8-13 hours into development, the embryonic nervous system becomes established. The dynein antigen is concentrated in the cells and axons of the ventral nerve cord and the peripheral nervous system of the embryonic brain (Fig. 8M,N). During this time period, the level of dynein staining observed in the overlying epidermal tissue appears slightly reduced compared to earlier stages. The reduced staining observed by immunofluorescence is consistent with the reduced amount of the dynein heavy chain that copurifies with MTs from these older embryos (Fig. 6A,B).

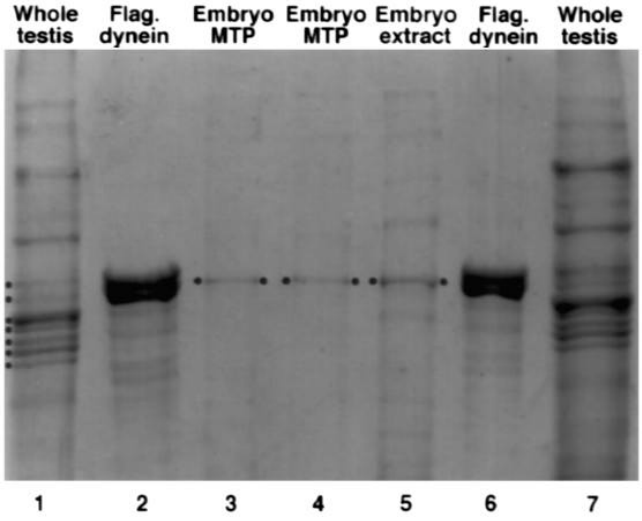


Fig. 7. Comparison of the *Drosophila* embryonic MAPs with dynein heavy chains from *Drosophila* testes and sea urchin flagella. A single high molecular weight polypeptide (dot) is present in high-speed embryonic extracts (lane 5) and taxol-assembled microtubules (lanes 3 and 4). In contrast, multiple HMW polypeptides are present in samples of sea urchin flagellar dynein (lanes 2 and 6) and *Drosophila* testes (lanes 1 and 7). Samples were electrophoresed on 0 to 8 M urea, 3.2% polyacrylamide gradient gels.

DISCUSSION

We have identified and purified a cytoplasmic dynein motor present in *Drosophila* embryos. The native *Drosophila* dynein molecule shares several properties in common with other dyneins including a sedimentation coefficient of approximately 20 S and a minus-end-directed, ATP-dependent, motor activity along MTs *in vitro*. The *Drosophila* dynein isoform contains a large heavy chain polypeptide of greater that 400 kDa that is cleaved at specific sites (V_1 or V_2) in the presence of vanadate and UV light. The large size of the heavy chain and its ability to be cleaved in the presence of vanadate is a well characterized

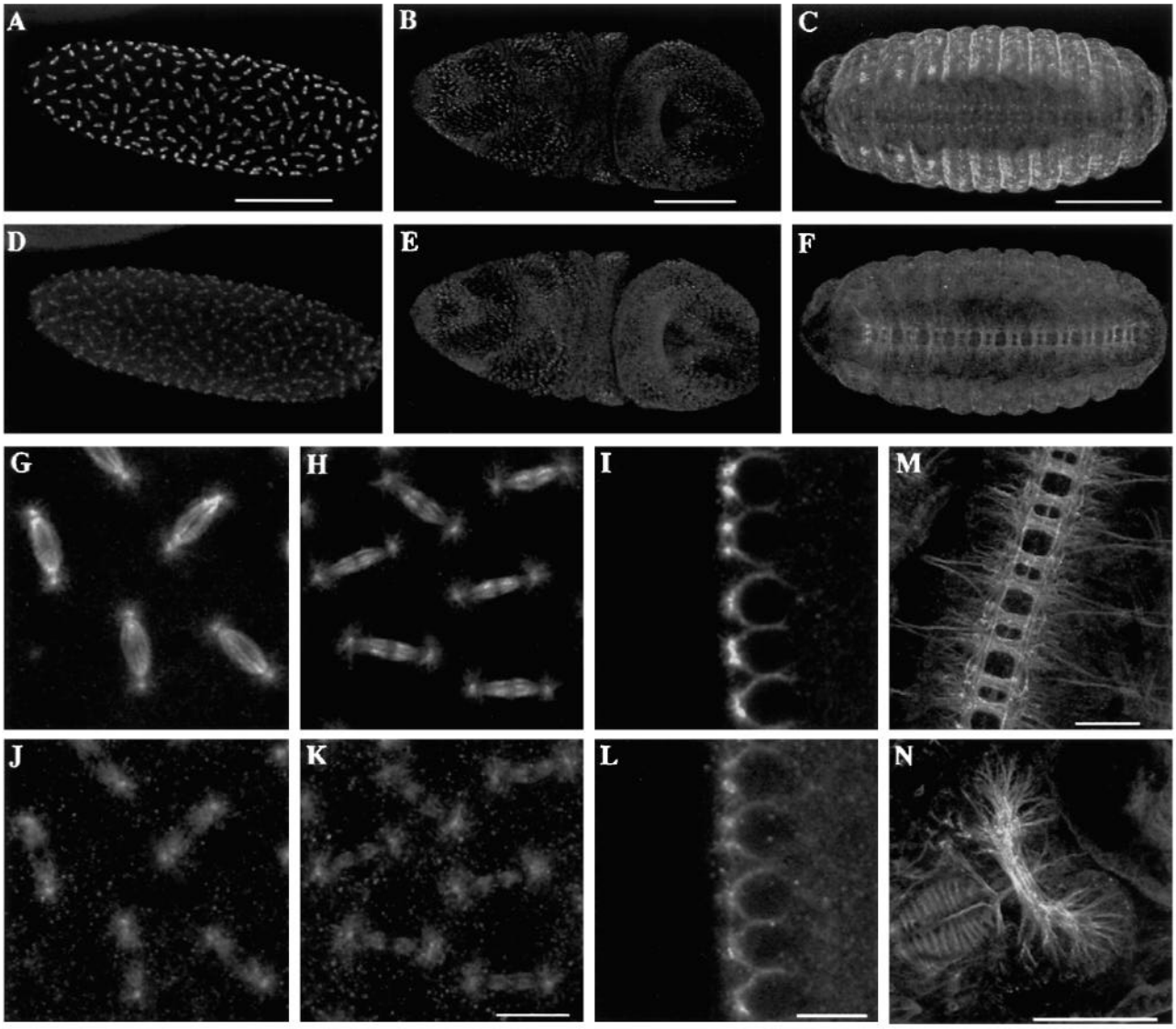


Fig. 8. Localization of cytoplasmic dynein during embryogenesis. (A-C) Whole-mount preparations of *Drosophila* embryos were double-labelled with both affinity-purified dynein antibodies and a α-tubulin monoclonal antibody. Top row (A-C): tubulin antibody. Second row (D-F): dynein antibody. Low-magnification views of a late syncytial blastoderm stage (A, D), an early germ band elongation stage (B, E), and a 12 to 13-hour-old embryo with established segments and a ventral nerve chord (C, F). Dynein is associated with microtubules throughout development, including mitotic spindle microtubules in the syncytial blastoderm (D) and mitotic domains of postblastoderm embryos (E), as well as the developing nervous system (F). Bars (A-C), 100 μm; and apply to the corresponding image in (D-F). High-magnification views of metaphase and anaphase mitotic spindles in the syncytial embryo illustrating tubulin (G,H) and dynein (J, K) staining patterns. Micrographs (G,H,J,K) are at identical magnification. Bar (K), 10 μm. Dynein is associated with the centrosomal microtubules throughout the nuclear cycle. In nuclear cycles 10-14, tubulin localization (I) reveals the pair of centrosomes at the apical surface of the cortical nuclei and the basket of microtubules extending basally around each nucleus. The dynein antigen is colocalized with the tubulin staining (L). Magnification in (I) and (L) is the same; bar (L), 5 μm. Dynein localization in the commissures of the ventral nerve chord (M) and the peripheral central nervous system of the embryonic brain (N) including the supraoesophageal commissure and ganglia, and the motor axons innervating the pharnyx. Bars (M) and (N), 20 μm and 50 μm, respectively.

feature of dynein motors. Both the minus-end-directed motor activity and the ATPase activity consistently cofractionate with the dynein heavy chain polypeptide on sucrose density gradients.

The dynein isoform isolated from *Drosophila* embryos is a soluble dynein that is most likely involved in cytoplasmic microtubule-based motility, as opposed to axonemal motility. Unlike mammals with ciliated epithelial tissues or sea urchins with a ciliated embryonic blastula stage, insects do not appear to possess motile cilia in their somatic tissues (Smith, 1968; Kiefer, 1973). This lack of motile axonemes outside of the testis, eliminates the potential for copurification of ciliary dynein precursors with the embryonic cytoplasmic dynein isoform. The presence of the *Drosophila* dynein as a microtubule-associated protein in extracts of unfertilized eggs and/or embryos, the CTPase activity of the purified protein, and the localization of the corresponding antigen to cytoplasmic MTs,

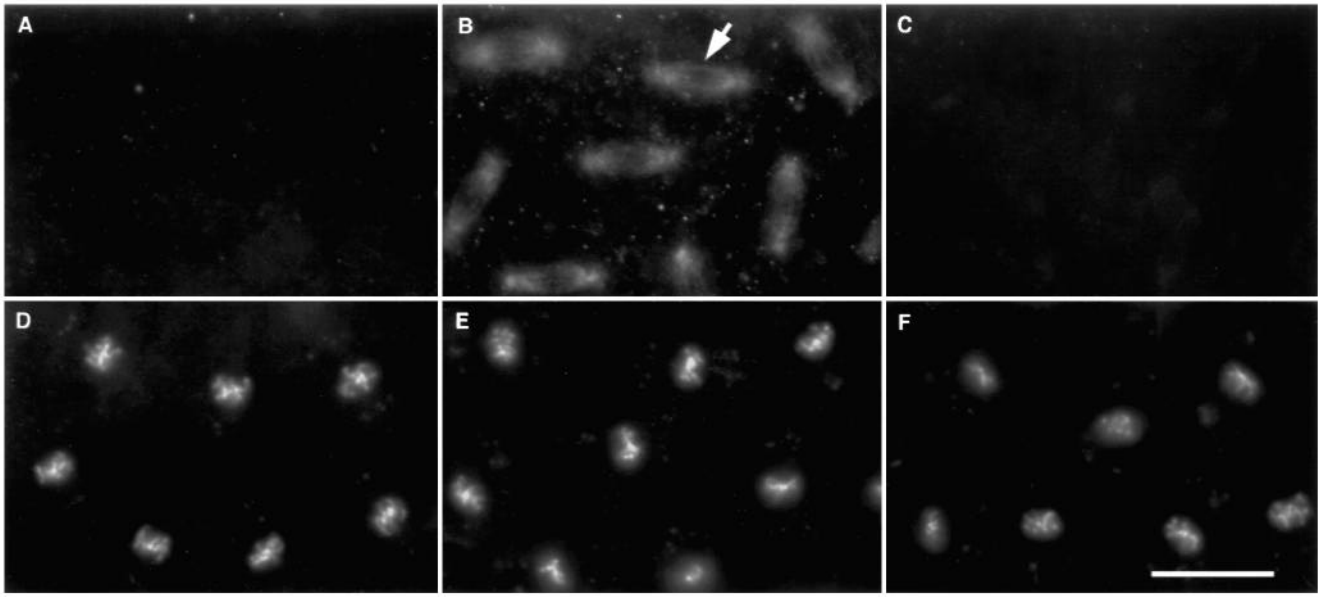


Fig. 9. Control for dynein localization in mitotic spindles. (A) Spindle staining is eliminated when the dynein antibody is preincubated against the dynein antigen. (B) Spindle staining is retained when the dynein antibody is preincubated against MAP-free microtubules prepared from *Drosophila* embryos. Dynein appears concentrated at the spindle poles, but is also associated with spindle fibers (small arrow) that extend between the two spindle poles. (C) The preincubation of the monoclonal α-tubulin antibody with microtubules does eliminate spindle microtubule staining. Without preincubation, the tubulin antibody readily stains spindle microtubules (Fig. 8G,H). Micrographs in D-F are the corresponding DAPI images for A-C. Bar, 10 μm.

support the conclusion that the purified dynein represents a cytoplasmic isoform. This conclusion has recently been confirmed by the isolation and sequence analysis of the full-length cDNA that encodes the *Drosophila* cytoplasmic dynein heavy chain (M.-G. Li, M. McGrail, M. Serr and T. S. Hays, unpublished). This dynein heavy chain sequence shares extensive amino acid identity throughout its length with the cytoplasmic dynein sequences published for *Dictyostelium* and rat (Koonce et al., 1992; Mikami et al., 1993).

The *Drosophila* cytoplasmic dynein appears to contain a single heavy chain polypeptide species. High-resolution urea-gels detect only a single high molecular weight species in taxol-assembled MTs prepared from embryonic extracts. In contrast, multiple heavy chain polypeptides are resolved in preparations of *Drosophila* testes. Consistent with these observations, we have recently shown that a single member of a dynein heavy chain gene family is abundantly expressed in embryos, while at least five other heavy chain genes are expressed at high levels in testis (Rassmusson et al., 1994). In addition, as shown in the present study, the cleavage of the *Drosophila* cytoplasmic dynein heavy chain polypeptide under conditions that promote cleavage at either the V1 or V2 site results in only two lower molecular weight polypeptide fragments. Thus, similar to the predicted native structure of other cytoplasmic dyneins (Vallee et al., 1988; Neely et al., 1990), the predominant embryonic *Drosophila* cytoplasmic dynein complex most likely contains a homodimer of a single heavy chain polypeptide. However, we cannot rule out the possibility of the presence during embryogenesis of minor amounts of additional dynein motor complexes that are composed of distinct dynein heavy chain polypeptides (Rassmusson et al., 1994).

In addition to the dynein heavy chain, multiple polypeptides of lower molecular weights have been characterized as components of cytoplasmic dyneins in other organisms (Paschal et al., 1987; Schroer et al., 1989). We have not characterized other components of the native *Drosophila* cytoplasmic dynein complex present in our preparation. Polypeptide species that migrate at approximately 150, 66 and 47 kDa appear to rebind to MTs in the absence of ATP. While similar in molecular weight to previously characterized dynein subunits, the identification of these and/or other polypeptides as true subunits of the *Drosophila* dynein complex, or as dynein-associated regulatory polypeptides, remains to be determined. Future developmental analyses of the composition of the dynein motor complex and its associated polypeptides may help to reveal components that target the motor to specific cellular cargoes and macromolecules.

Proposed functons for dynein motors have included mitotic force production and the transport of chromosomes during mitosis, retrograde transport of membrane-bound organelles in nerve axons, the perinuclear positioning of the Golgi apparatus, transport of lysosomal vesicles and the transport of mRNAs (reviewed by Vallee and Shpetner, 1990; Bloom, 1992; Skoufias and Scholey, 1993; Wilhelm and Vale, 1993). Our studies indicate that the dynein antigen is associated with MT arrays throughout embryogenesis and therefore support the hypothesis that the cytoplasmic dynein motor is utilized in multiple MT-dependent processes in *Drosophila* development. We have shown that cytoplasmic dynein is also present in unfertilized embryos and may therefore participate in microtubule-based transport during oogenesis. Indeed, subsequent studies have characterized an assymmetrical localization of the dynein motor to the presumptive oocyte at a very

early stage in oogenesis (M. McGrail and T. S. Hays, unpublished data).

The colocalization of the dynein antigen with mitotic spindle MTs raises the question of whether cytoplasmic dynein serves as a motor for nuclear division. The pattern of dynein distribution within the spindle appears to reflect its association with spindle MTs and is similar to previous observations of dynein localization in the spindles of mammalian tissue culture cells (Pfarr et al., 1990; Steuer et al., 1990) and sea urchin cells (Hisanaga and Sakai, 1980; Piperno, 1984). In the mammalian studies, however, the dynein antigen also appeared to be concentrated at the kinetochore regions of chromosomes as early as late prophase. Despite careful examination, we have not observed a similar kinetochore localization of dynein in *Drosophila* mitotic chromosomes. Given the small size of the kinetochores on *Drosophila* mitotic chromosomes, this apparent discrepancy might result from the elevated staining of the spindle, which may hinder the detection of dynein at the kinetochore. Another explanation is that our fixation methods do not preserve dynein association with *Drosophila* kinetochores. An alternative possibility is that cytoplasmic dynein does not function as a chromosome-associated motor during mitosis. Instead, it may provide the forces required for the partitioning of cytoplasmic organelles during nuclear and cell division, for the positioning of the nuclei and/or spindles, or for the morphogenesis of the spindle and the separation of the spindle poles during mitosis. Recent evidence suggesting that cytoplasmic dynein function is not required for chromosome transport comes from the identification and mutational analysis of a cytoplasmic dynein gene in *Saccharomyces cerevisiae* (Li et al., 1993; Eshel et al., 1993). A deletion disruption mutation of the yeast dynein gene shows no impact on chromosome segregation. Instead, positioning of the spindle apparatus into the daughter bud appears to be delayed in the dynein mutants, resulting in occasional bi- and multinucleate mother cells. Moreover, in mammalian tissue culture cells, proper spindle pole separation and the morphogenesis of the bipolar spindle are prevented when cells are microinjected with antibodies to the dynein heavy chain (Vaisberg et al., 1993). During the syncytial blastoderm stage in *Drosophila* embryos, the dynein motor could participate in the proper positioning and/or migration of the multiple centrosomes and nuclei that occupy a single cytoplasm. Analysis of the maternal effects of dynein mutations should help to clarify the role of the dynein motor in the early nuclear division cycles of the syncytial embryo.

Another striking pattern of dynein localization is observed between 8 and 12 hours during *Drosophila* embryonic development. During this period, dynein appears to accumulate throughout the axons of the central and peripheral nervous system. In contrast, dynein staining in most of the surrounding embryonic tissues appears reduced, while tubulin staining is maintained at high levels. This observation suggests that dynein is differentially lost and/or retained in these tissues. We have shown that a single gene encodes the predominant dynein transcript expressed in embryos (Rasmusson et al., 1994). Comparable levels of expression for the other members of the dynein gene family are seen only in testis. Very low levels of these other transcripts, however, do appear to be expressed in embryos, and we cannot rule out the possibility of differential expression of specific dynein isoforms in cells of the embryonic nervous system. Regardless of these uncertainties, the accumulation of dynein in the nervous system is consistent with the proposed role of cytoplasmic dynein as a retrograde motor involved in axonal transport (Vallee et al., 1989; Schnapp and Reese, 1989).

Our results indicate that *Drosophila* cytoplasmic dynein is associated with several microtubule arrays and is likely to power the cytoplasmic transport of organelles and/or other cargoes in multiple cells and tissues of the embryo. Future genetic analyses of cytoplasmic dynein function in *Drosophila* should reveal the range of developmental processes that require dynein function and may provide insights into the molecular regulation of directed cytoplasmic transport.

The authors thank Madeline Serr for her help in the preparation of figures, and the critical reading and editing of the manuscript. We also thank Dr Cathy Regan for providing samples of *Drosophila* testis in the early stages of this work. This work was supported by NIH grants GM-44757 to T.S.H, HD-18127 to M.T.F, and GM-36663 to J.R.M., and NSF grant DCB9005079 to M.E.P. Additional support was provided by an ACS JFRA and a MOD Basil O'Connor award to T.S.H. Support for M.M. was from Reseach Training Grants from NIH and NSF.

REFERENCES

Baker, J., Theurkauf, W. E. and Schubiger, G. (1993). Dynamic changes in microtubule configuration correlate with nuclear migration in the preblastoderm Drosophila embryo. *J. Cell Biol.* **122**, 113-121.

Bloom, G. S., Wagner, M. C., Pfister, K. K. and Brady, S. T. (1988). Native structure and physical properties of bovine brain kinesin and identification of the ATP-binding subunit polypeptide. *Biochemistry* **27**, 3409-3425.

Bloom, G. S. (1992). Motor proteins for cytoplasmic microtubules. *Curr. Opin. Cell Biol.* **4**, 66-73.

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **72**, 248-254.

Cohn, S. A., Ingold, A. L. and Scholey, J. M. (1987). Correlation between the ATPase and microtubule translocating activities of sea urchin egg kinesin. *Nature* **328**, 160-163.

Collins, C. A. and Vallee, R. (1989). Preparation of microtubules from rat liver and testis: Cytoplasmic dynein is a major microtubule associated protein. *Cell Motil. Cytoskel.* **14**, 491-500.

Eshel, D., Urrestarazu, L. A., Vissers, S., Jauniaux, J.-C., van Vliet-Reedijk, J. C., Planta, R. J. and Gibbons, I. R. (1993). Cytoplasmic dynein is required for normal nuclear segregation in yeast. *Proc. Nat. Acad. Sci. USA* **90**, 11172-11176.

Foe, V. E. and Alberts, B. M. (1983). Studies of nuclear and cytoplasmic behavior during the five mitotic cycles that precede gastrulation in *Drosophila* embryogenesis. *J. Cell Sci.* **61**, 31-70.

Foe, V. E. (1989). Mitotic domains reveal early commitment of cells in *Drosophila* embryos. *Development* **107**, 1-22.

Fullilove, S. L. and Jacobson, A. G. (1971). Nuclear elongation and cytokinesis in *Drosophila montana*. *Dev. Biol.* **26**, 560-571.

Fyrberg, E. A. and Goldstein, L. S. B. (1990). The *Drosophila* cytoskeleton. *Annu. Rev. Cell Biol.* **6**, 559-596.

Gepner, J. and Hays, T. S. (1993). A fertility region on the Y chromosome of *Drosophila melanogaster* encodes a dynein microtubule motor. *Proc. Nat. Acad. Sci. USA* **90**, 11132-11136.

Gibbons, I. R., Lee-Eiford, A., Mocz, G., Phillipson, C. A., Tang, W. J. Y. and Gibbons, B. H. (1987). Photosensitized cleavage of dynein heavy chains. *J. Biol. Chem.* **262**, 2780-2786.

Gibbons, I. R. (1988). Dynein ATPases as microtubule motors. *J. Biol. Chem.* **263**, 15837-15840.

Gill, S. R., Schroer, T. A., Szilak, I., Steuer, E. R., Sheetz, M. P. and Cleveland, D. W. (1991). Dynactin, a conserved, ubiquitously expressed component of an activator of vesicle motility mediated by cytoplasmic dynein. *J. Cell Biol.* **115**, 1639-1650.

Grissom P. M., Porter, M. E. and McIntosh, J. R. (1992). Two distinct isoforms of sea urchin egg dynein. *Cell Motil. Cytoskel.* **21**, 281-292.

Hardy, R. W., Tokuyasu, K. T. and Lindsley, D. L. (1981). Analysis of spermatogenesis in *Drosophila melanogaster* bearing deletions for Y-chromosome fertility genes. *Chromosoma* **83**, 593-617.

Hisanaga, S-I. and Sakai, H. (1980). Cytoplasmic dynein of the sea urchin egg. I. Partial purification and characterization. *Dev. Growth Differ.* **22**, 373-384.

Holzbaur, E., Hammarback, J. A., Paschal, B. M., Kravit, N. G., Pfister, K. K. and Vallee, R. B. (1991). Homology of a 150K cytoplasmic dynein-associated polypeptide with the *Drosophila* gene glued. *Nature* **351**, 579-583.

Johnson, K. A., Porter, M. E. and Shimizu, T. (1984). Mechanism of force production for microtubule-dependent movements. *J. Cell Biol.* **99**, 132s-136s.

Karr, T. L. and Alberts, B. M. (1986). Organization of the cytoskeleton in early *Drosophila* embryos. *J. Cell Biol.* **102**, 1494-1509.

Kellogg, D. R., Mitchison, T. J. and Alberts, B. M. (1988). Behavior of microtubules and actin filaments in living *Drosophila* embryos. *Development* **103**, 675-686.

Kellogg, D. R., Field, C. M. and Alberts, B. M. (1989). Identification of microtubule-associated proteins in the centrosome, spindle, and kinetochore of the early Drosophila embryo. *J. Cell Biol.* **109**, 2977-2991.

Kiefer, B. I. (1973). In *Genetic Mechanisms of Development* (ed. F. H. Ruddle), pp. 47-102. Academic Press, New York.

King, S. M. and Witman, G. B. (1987). Structure of the alpha and beta heavy chains of the outer arm dynein from Chlamydomonas flagella. *J. Biol. Chem.* **262**, 17596-17604.

King, S. M. and Witman, G. B. (1988). Structure of the gamma heavy chain of the outer arm dynein from Chlamydomonas flagella. *J. Cell Biol.* **107**, 1799-1808.

Koonce, M. P., Grissom, P. M. and McIntosh, J. R. (1992). Dynein from Dictyostelium: primary structure comparisons between a cytoplasmic motor enzyme and flagellar dynein. *J. Cell Biol.* **119**, 1597-1604.

Laemmli, U. K. (1970). Cleavage of structural proteins during assembly of the head of bacteriophage T4. *Nature* **227**, 680-685.

Lee-Eiford, A., Ow, R. A. and Gibbons, I. R. (1986). Specific cleavage of dynein heavy chains by ultraviolet irradiation in the presence of ATP and vanadate. *J. Biol. Chem.* **261**, 2337-2342.

Li, Y.-Y., Yeh, E., Hays, T. and Bloom, K. (1993). Disruption of mitotic spindle orientation in a yeast dynein mutant. *Proc. Nat. Acad. Sci. USA* **90**, 1-8.

Lye, R. J., Porter, M. E., Scholey, J. M. and McIntosh, J. R. (1987). Identification of a microtubule-based cytoplasmic motor in the nematode C. elegans. *Cell* **51**, 309-318.

McIntosh, J. R. and Porter, M. E. (1989). Enzymes for microtubule-dependent motility. *J. Biol. Chem.* **264**, 6001-6004.

Mikami, A., Paschal, B. M., Mazumdar, M. and Vallee, R. B. (1993). Molecular cloning of the retrograde transport motor cytoplasmic dynein (MAP1C). *Neuron* **10**, 787-796.

Mocz, G., Tang, W.-J. Y. and Gibbons, I. R. (1988). A map of photolytic and tryptic cleavage sites on the β heavy chain of dynein ATPase from sea urchin sperm flagella. *J. Cell Biol.* **106**, 1607-1614.

Neely, M. D., Erickson, H. P. and Boekelheide, K. (1990). HMW-2, the sertoli cell cytoplasmic dynein from rat testis is a dimer composed of nearly identical subunits. *J. Biol. Chem.* **265**, 8691-8698.

Neville, D. M. (1971). Molecular weight determination of protein-dodecyl sulfate complexes by gel electrophoresis in a discontinuous buffer system. *J. Biol. Chem.* **246**, 6328-6334.

Olmsted, J. B. (1981). Affinity-purification of antibodies from diazotized paper blots of heterogeneous protein samples. *J. Biol Chem.* **256**, 11955-11957.

Pallini, V., Bugnoli, M., Mencarelli, C. and Scapigliati, G. (1982). Biochemical properties of ciliary, flagellar and cytoplasmic dyneins. In *Prokaryotic and Eukaryotic Flagella* (ed. W. B. Amos and J. G. Duckett), pp. 339-352. London: Cambridge University Press.

Paschal, B. M., Shpetner, H. S. and Vallee, R. B. (1987). MAP1C is a microtubule-associated ATPase which translocates microtubules in vitro and has dynein-like properties. *J. Cell Biol.* **105**, 1273-1282.

Paschal, B. M. and Vallee, R. B. (1987). Retrograde transport by the microtubule-associated protein MAP1C. *Nature* **330**, 181-183.

Pfarr, C. M., Coue, M., Grissom, P. M., Hays, T. S., Porter, M. E. and McIntosh, J. R. (1990). Cytoplasmic dynein is localized to kinetochores during mitosis. *Nature* **345**, 263-265.

Piperno, G. (1984). Monoclonal antibodies to dynein subunits reveal the existence of cytoplasmic antigens in sea urchin egg. *J. Cell Biol.* **98**, 1842-1850.

Piperno, G. and Fuller, M. T. (1985). Monoclonal antibodies specific for an acetylated form of α tubulin recognize the antigen in cilia and flagella from a variety of organisms. *J. Cell Biol.* **101**, 2085-2094.

Porter, M. E. and Johnson, K. A. (1983). Characterization of the ATP-sensitive binding of Tetrahymena 30 S dynein to bovine brain microtubules. *J. Biol. Chem.* **258**, 6575-6581.

Porter, M. E., Scholey, J. M., Stemple, D. L., Vigers, G. P. A., Vale, R. D., Sheetz, M. P. and McIntosh, J. R. (1987). Characterization of the microtubule movement produced by sea urchin egg kinesin. *J. Biol. Chem.* **262**, 2794-2802.

Porter, M. E., Grissom, P. M., Scholey, J. M., Salmon, E. D. and McIntosh, J. R. (1988). Dynein isoforms in sea urchin eggs. *J. Biol. Chem.* **263**, 6759-6771.

Porter, M. E. and Johnson, K. A. (1989). Dynein structure and function. *Annu. Rev. Cell Biol.* **5**, 119-151.

Rasmusson, K., Serr, M., Gepner, J., Gibbons, I. R. and Hays, T. S. (1994). A family of dynein genes in *Drosophila melanogaster*. *Mol. Biol. Cell.* **5**, 45-55.

Sale, W. S. and Fox, L. A. (1988). Isolated β heavy chain subunit of dynein translocates microtubules in vitro. *J. Cell Biol.* **107**, 1793-1797.

Saxton, W. E., Porter, M. E., Cohn, S. A., Scholey, J. M., Raff, E. C. and McIntosh, J. R. (1988). *Drosophila* kinesin: Characterization of microtubule motility and ATPase. *Proc. Nat. Acad. Sci. USA* **85**, 1109-1113.

Schnapp, B. J. and Reese, T. S. (1989). Dynein is the motor for retrograde axonal transport of organelles. *Proc. Nat. Acad. Sci. USA* **86**, 1548-1522.

Scholey, J. M., Porter, M. E., Grissom, P. M. and McIntosh J. R. (1985). Identification of kinesin in sea urchin eggs, and evidence for its localization in the mitotic spindle. *Nature* **318**, 483-486.

Schroer, T. A., Steuer, E. R. and Sheetz, M. P. (1989). Cytoplasmic dynein is a minus end-directed motor for membranous organelles. *Cell* **56**, 937-946.

Shimizu, T. and Johnson, K. A. (1983). Kinetic evidence for multiple dynein ATPase sites. *J. Biol. Chem.* **258**, 13841-13846.

Shpetner, H. S., Paschal, B. M. and Vallee, R. B. (1988). Characterization of the microtubule-activated ATPase of brain cytoplasmic dynein (MAP 1C). *J. Cell Biol.* **107**, 1001-1009.

Skoufias, D. A. and Scholey, J. M. (1993). Cytoplasmic microtubule-based motor proteins. *Curr. Opin. Cell Biol.* **5**, 95-104.

Smith, D. (1968). *Insect Cells: Their Structure and Function.* R. and R. Clark, Ltd., Edinburgh.

Steuer, E. R., Wordeman, L., Schroer, T. A. and Sheetz, M. P. (1990). Localization of cytoplasmic dynein to mitotic spindles and kinetochores. *Nature* **345**, 266-268.

Theurkauf, W. E. (1992). Behavior of structurally divergent alpha-tubulin isotypes during Drosophila embryogenesis: evidence for post-translational regulation of isotype abundance. *Dev. Biol.* **154**, 205-217.

Towbin, H., Staehelin, T. and Gordon, J. (1979). Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: Procedure and some applications. *Proc. Nat. Acad. Sci. USA* **76**, 4350-4354.

Turner, R. R. and Mahowald, A. P. (1976). Scanning electron microscopy of Drosophila embryogenesis. I. The structure of the egg envelope and the formation of the cellular blastoderm. *Dev. Biol.* **50**, 95-108.

Vaisberg, E. A., Koonce, M. P. and J. R. McIntosh. (1993). Cytoplasmic dynein plays a role in mammalian mitotic spindle formation. *J. Cell Biol.* **123**, 849-858.

Vale, R. D., Schnapp, B. J., Reese, T. S. and Sheetz, M. P. (1985). Organelle, bead, and microtubule translocations promoted by soluble factors from the squid giant axon. *Cell* **40**, 559-569.

Vale, R. D. and Toyoshima, Y. Y. (1988). Rotation and translocation of microtubules in vitro induced by dyneins from Tetrahymena cilia. *Cell* **52**, 459-469.

Vallee, R. B. (1982). A taxol-dependent procedure for the isolation of microtubules and microtubule-associated proteins (MAPs). *J. Cell Biol.* **92**, 435-442.

Vallee, R. B., Wall, J. S., Paschal, B. M. and Shpetner, H. S. (1988). Microtubule-associated 1C from brain is a two-headed cytosolic dynein. *Nature* **332**, 561-563.

Vallee, R. B., Shpetner, H. S. and Paschal, B. M. (1989). The role of dynein in retrograde axonal transport. *Trends Neurosci.* **12**, 66-70.

Vallee, R. B. and Shpetner, H. S. (1990). Motor proteins of cytoplasmic microtubules. *Annu. Rev. Biochem.* **59**, 909-932.

Walter, M. and Alberts, B. M. (1984). Intermediate filaments in tissue culture cells and early embryos of Drosophila melanogaster. In *Molecular Biology of Development, UCLA Symposium on Molecular and Cellular Biology, New Series* (ed. R. Firtel and E. Davidson), pp 263-272. Alan R. Liss, Inc., New York.

Warn, R. M., Flegg, L. and Warn, A. (1987). An investigation of microtubule organization and functions in living *Drosophila* embryos by injection of fluorescently label antibody against tyrosinated α-tubulin. *J. Cell Biol.* **105**, 1721-1730.

Wilhelm, J. E. and Vale, R. D. (1993). RNA on the move: the mRNA localization pathway. *J. Cell Biol.* **123**, 269-274.

Williams, R. C. Jr and Detrich, H. W. III (1979). Separation of tubulin from microtubule-associated proteins on phosphocellulose. Accompanying alterations in concentrations of buffer components. *Biochemistry* **18**, 2499-2503.

Witman, G. (1992). Axonemal dyneins. *Curr. Opin. Cell Biol.* **4**, 74-79.

(Received 30 January 1994 - Accepted 3 March 1994)