Immunolocalization of kinesin in sea urchin coelomocytes

Association of kinesin with intracellular organelles

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Summary

We have recently used domain-specific monoclonal antibodies (mAbs) to immunofluorescently localize kinesin to vesicle-like structures in the cytoplasm of sea urchin coelomocytes. In order to characterize further these localization patterns we have examined the distribution of kinesin with respect to the arrangement of microtubules (MTs) and various organelles. In double-label experiments involving the immunofluorescent staining of kinesin (using a mixture of the mAbs SUK2, 4 and 5), MTs were labeled with an antiserum against sea urchin tubulin, the endoplasmic reticulum (ER) was labeled with an antiserum against a luminal calnexin-like protein, the Golgi apparatus was labeled with rhodamine-wheat germ agglutinin (WGA) or NBD-ceramide, mitochondria were labeled with rhodamine 123, endosomes were labeled with Texas Red-ovalbumin, and lysosomes were labeled with Lucifer yellow or acridine orange. Kinesin-labeled vesicle-like structures were found in the same regions of the cells as MTs and the ER, being widely distributed in motile cells, but restricted to the perinuclear regions of stationary cells. There also appeared to be a correlation between the distribution of endosomes and kinesin staining in a subpopulation of cells. The kinesin binding structures were found occasionally to align in linear arrays, consistent with the idea that kinesin may transport ER and endosomes along linear MT tracks. No clear correlations were observed between the kinesin staining and the distribution of mitochondria, the Golgi apparatus or lysosomes, suggesting that kinesin may specifically associate with only a subclass of organelles in coelomocytes.

Key words: kinesin, coelomocytes, organelles.

Introduction

Kinesin is a heterotetrameric, two-headed motor protein that uses microtubule (MT)-activated ATP hydrolysis to transport particles towards the plus ends of MTs in vitro (Vale et al., 1985a,b; Scholey et al., 1985; Brady, 1985; Kuznetsov and Gelfand, 1986; Porter et al., 1987; Cohn et al., 1987, 1989; Hackney, 1988; Wagner et al., 1989; Yang et al., 1989; Hirokawa et al., 1989; Scholey et al., 1989). Kinesin was first identified in neurons as a candidate motor for fast anterograde axonal transport (reviewed by Vale, 1987) and subsequent studies involving the reconstitution or disruption of kinesin-driven motility have supported a role for it in plus-end-directed membrane transport (Schroer et al., 1988; Brady et al., 1990; Hollenbeck and Swanson, 1990; Rodionov et al., 1991; Urrutia et al., 1991; Hirokawa et al., 1991; Saxton et al., 1991).

A growing body of evidence from immunocytochemical localization studies indicates that kinesin is associated with intracellular membranes. Anti-kinesins have been shown to stain detergent-extractable membranous vesicles, organelles and tubules in the cytoplasm of a variety of neuronal and non-neuronal cells (Hollenbeck, 1989; Pfister et al., 1989; Brady et al., 1990; Wright et al., 1991; Houliston and Elinson, 1991; Brady and Pfister, 1991; Hirokawa et al., 1991). The meaning of the results of other experiments, in which kinesin was immunolocalized to stress fibers (Okuhara et al., 1989) or spindle poles and primary cilia (Neighbors et al., 1988) in cultured cells, has not yet been resolved, but it is not unreasonable to speculate that these patterns also reveal sites of membrane-bound kinesin accumulation, or the presence of a cross-reactive kinesin-like protein (see Wright et al., 1991; Sawin et al., 1992, for discussion). For example, kinesin was first immunolocalized to mitotic appratuses in cells of the early sea urchin embryo (Scholey et al., 1985; Leslie et al., 1987), and subsequent studies with monoclonal antibodies that distinguish kinesin from kinesin-like proteins demonstrated an association with a membranous component of the mitotic apparatus (Wright et al., 1991). Immunolocalization studies further support the idea that kinesin-bound membranes are transported along MT tracks; kinesin-bound membranes have also been
been shown to exhibit MT-dependent organelle movements (Dabora and Sheetz, 1988; Vale and Hotani, 1988; Hollenbeck, 1989; Hollenbeck and Swanson, 1990; Rodionov et al., 1991). These organelles all exhibit a MT-dependent distribution pattern and plus-end-directed movement (Terasaki et al., 1986; Lee and Chen, 1988; Lee et al., 1989; Swanson et al., 1987; Heuser, 1989) consistent with the functioning of a kinesin-like motor. In addition, kinesin-driven movement of membranes on MTs can generate ER-like tubular networks in vitro (Dabora and Sheetz, 1988; Vale and Hotani, 1988), kinesin and the ER have been shown to codistribute during early embryogenesis in the sea urchin (Wright et al., 1991) and the frog (Houliston and Elinson, 1991), and the radial extension of tubular lysosomes in macrophages and pigment granules in melanophores has been shown to be inhibited by cellular incorporation of anti-kinesin antibodies (Hollenbeck and Swanson, 1990; Rodionov et al., 1991). In order to define better the membranous cargos transported along MTs, recent immunocytochemical studies have begun to make direct comparisons between the distributions of kinesin and various organelles (Brady and Pfister, 1991); however, much work remains to be performed in order to establish the identity of kinesin binding intracellular organelles.

We have previously utilized domain-specific monoclonal antibodies (mAbs) against the heavy chain of sea urchin kinesin to immunofluorescently localize kinesin to Triton-extractable vesicle-like structures in the cytoplasm of sea urchin coelomocytes (Wright et al., 1991). Coelomocytes are terminally differentiated, nonmitotic cells which can be isolated from the sea urchin’s coelomic fluid and maintained in short-term culture (Edds, 1977). These cells have been shown to exhibit MT-dependent organelle movements (Henson et al., 1990b). In the present study, we have examined the distribution of kinesin with respect to the arrangement of MTs, the ER, the Golgi apparatus, mitochondria, endosomes and lysosomes. The results indicate a correlation between the distribution of kinesin and the distribution of MTs, the ER and endosomes, but not between kinesin, mitochondria, the Golgi apparatus or lysosomes. These results support the hypothesis that kinesin specifically associates with only a subclass of the organelles in coelomocytes; a fact which may have relevance in terms of cellular functions.

Materials and methods

Materials

Sea urchins of the species Strongylocentrotus droebachiensis were collected from the near-shore waters surrounding the Mt. Desert Island Biological Laboratory, Salisbury Cove, ME. The well-characterized monoclonal antibodies SUK2, 4 and 5 (Ingold et al., 1988), previously mapped to the stalk, head and tail (respectively) domains of the sea urchin kinesin heavy chain (Schloley et al., 1989; Wright et al., 1991), were used in this study. A previously characterized polyclonal antiserum against a sea urchin calcequin-staining protein (SCS) found in the ER lumen (see Oberdorf et al., 1988; Henson et al., 1989; Henson et al., 1990a; Terasaki et al., 1991) was provided by Dr. Benjamin Kaminer (Boston University School of Medicine, Boston, MA). An acetone powder extract of S. purpuratus eggs was supplied by Dr. David Begg (University of Alberta, Edmonton, Alberta, Canada).

The following purchases were made: antiserum against sea urchin egg tubulin from Polysciences Inc. (Warrington, PA); rhodamine 123, a monoclonal antibody against &-tubulin and Lucifer yellow from Sigma Chemical Co. (St. Louis, MO); rhodamine-conjugated wheat germ agglutinin (WGA), NBD-ceramide, and Texas Red-labeled ovalbumin from Molecular Probes Inc. (Eugene, OR); fluorophore and alkaline phosphatase-conjugated secondary antibodies from Hyclose Inc. (Logan, UT); the majority of other chemicals were from either Sigma Chemical Co. or Fisher Scientific (Pittsburgh, PA).

Immunoblotting and immunofluorescent staining

General gel electrophoresis and immunoblotting procedures followed those described by Henson et al. (1989). For western blots, the kinesin SUK2, 4 and 5 monoclonal antibodies were used as a mixture at a total IgG concentration of approximately 20 μg/ml, and the SCS antiserum was used at a 1:1000 dilution. Primary antibody binding to the nitrocellulose was visualized with alkaline phosphatase-conjugated secondary antibodies.

S. droebachiensis coelomocytes were collected and isolated according to the methods of Edds (1977). Cells were suspended in coelomocyte culture medium (CCM is 0.5 M NaCl, 5 mM MgCl₂, 1 mM EGTA, 20 mM HEPES, pH 7.4), allowed to settle onto naked or polylysine-coated coverslips for 1-2 hours, and then fixed in 90% methanol plus 50 mM EGTA at either –20°C or –80°C for 1-2 hours. Fixation at the lower temperature was suggested by Dr. Mark Terasaki (NINDS, NIH) as a means of reducing methanol-induced vesiculation of the ER. Staining for kinesin was accomplished using a mixture of the monoclonal antibodies SUK2, 4 and 5 (at 200 μg/ml total IgG) and followed the procedures described for coelomocytes by Wright et al. (1991). The working dilutions of the other antibodies used in double-labeling experiments were as follows: SCS antiserum 1:500; monoclonal α-tubulin antibody 1:300; sea urchin tubulin antiserum 1:300; fluorophore-conjugated secondary antibodies (in some cases preabsorbed with an acetone powder extract of S. purpuratus eggs) 1:50 to 1:300. Fluorescence microscopy was performed with a Nikon Optiphot 2 microscope using a 60× (NA 1.4) Planapo phase-contrast objective lens. Photographs were taken on Kodak TriX 400 ASA or Ektar 1000 ASA 35 mm film.

Double labeling of kinesin and organelles

For double labeling of kinesin and mitochondria, cells were set onto a coverslip which had been etched with a gridwork pattern. The cells were then incubated in CCM plus 10 μg/ml of rhodamine 123 (prepared as a 1 mg/ml stock in ethanol) according to Chen (1989). The position of a few cells within the grid work was noted, their rhodamine 123 staining pattern was photographed, and the cells were then fixed in cold methanol. The fixed cells were then processed for kinesin staining, the original cells were relocated and rephotographed.

For double labeling of kinesin and the Golgi apparatus, cells were fixed in cold methanol and processed for kinesin immunofluorescent staining. The secondary antibody staining step was supplemented with a 1:1000 dilution of rhodamine-conjugated WGA (prepared as a 0.5 mg/ml stock in PBS), a lectin which has

For double labeling of kinesin and endosomes, cells on etched coverslips were incubated for 1 hour at 18-20°C in 100-200 µg/ml of Texas Red-conjugated ovalbumin in CCM. After a wash with CCM, a small number of cells were viewed and their fluorescent endosome pattern was photographed. The cells were then fixed in cold methanol, processed for kinesin labeling, and the original cells were relocated and photographed again.

For labeling late endosomes/lysosomes, cells were incubated for 6-8 hours at 18-20°C in CCM plus 2 mg/ml Lucifer yellow, followed by a 1 hour wash in CCM (see Swanson, 1989). The labeling of intracellular acidic compartments, including lysosomes, was accomplished by treating cells with CCM plus 10 µg/ml acridine orange (after Hart and Young, 1975). Acridine orange has been previously shown to stain the identical structures labeled by internalized fluorescent markers and anti-lysosomal protein antibodies (Matteoni and Kreis, 1987). However, this vital stain must be used with caution when examining lysosomal morphology, since prolonged exposure to acridine orange has been shown to convert tubular lysosomes to spherical structures in some cells (Knapp and Swanson, 1990).

Results

Immunoblotting of coelomocyte samples with anti-kinesins and anti-SCS

A mixture of the SUK2, 4 and 5 anti-kinesin antibodies reacted specifically with the kinesin heavy chain (M_r ≅ 130×10^3) on immunoblots of coelomocyte lysates (Fig. 1, lane a; see also Wright et al., 1991). Utilization of a mixture of the mAbs allowed for amplification of the signal in immunoblotting and immunolocalization experiments. These mAbs are kinesin-specific and do not bind to other sea urchin kinesin-related proteins or any bacterially expressed kinesin-like proteins that we have tested (Wright et al., 1991; Cole et al., 1992, and unpublished observations). Blotting of the coelomocyte samples with the SCS antiserum resulted in a single immunoreactive species migrating at the appropriate molecular mass of 63 kDa (Fig. 1, lane b; see also Oberdorf et al., 1988; Henson et al., 1989).

Immunolocalization of kinesin and microtubules

During the course of the present study, it became clear that two separate and previously unappreciated subpopulations of petaloid coelomocytes could be distinguished, based on the relative degrees of motility, their morphological appearance in phase-contrast, the distribution of their actomyosin (unpublished observations) and their pattern of kinesin staining. The motile cell type was irregular in shape with elongated lamellipodia, and had numerous phase-dense granules spread throughout the cytoplasm (Fig. 2A). The stationary cell type had the overall shape of a fried egg, did not exhibit phase-dense cytoplasmic granules, but did contain numerous vacuoles around the nucleus (Fig. 2A). Edds et al. (1983) previously reported the existence of motile coelomocytes, and presented preliminary evidence for the existence of subpopulations, based on separation on sucrose step gradients (Edds, 1991).

Staining for kinesin revealed that the motile cells displayed an extensive array of punctate cytoplasmic staining, while in the stationary cells the labeling was restricted to the perinuclear region (Fig. 2B). On occasion the kinesin pattern appeared to align into linear arrays (Fig. 2B, arrows). The kinesin staining patterns in both cell types persisted following saponin extraction (0.02%, after Hollenbeck, 1989), but was abolished in cells extracted with the detergent Triton X-100 (1%) prior to fixation (data not shown, see Wright et al., 1991). The apparent nuclear staining seen with anti-kinesin may be indicative of kinesin’s presence on the nuclear envelope. However, we believe that this staining is largely nonspecific in origin, based on the following observations: (1) we found that although different batches of SUK2, 4, 5 mAbs gave identical results on western blots, they varied in their level of nuclear staining (compare Figs 2B and 3D; see also Fig. 11 of Wright et al., 1991); (2) control staining with some secondary antibodies alone also displayed labeling of the nucleus (data not shown).

We reasoned that the differences in the kinesin patterns apparent in the two cell types were indicative of differential MT distributions. Labeling of cells with anti-tubulin revealed that motile cells contain an extensive cytoplasmic array of MTs, while MTs in the stationary cells are restricted to the perinuclear region (Fig. 3A,B). Double labeling of cells for kinesin and tubulin demonstrated a discrete correlation between the two staining patterns, with extensive distributions of MTs and kinesin in the motile cells and only a juxtanuclear arrangement of the two patterns in the stationary cells (Fig. 3C,D). Codistribution of kinesin and MTs has been previously reported for vertebrate cell types (Hollenbeck, 1989; Pfister et al., 1989; Brady and Pfister, 1991) and sea urchin embryos (Wright et al., 1991). The reason that MTs are excluded from the peripheral regions of many of the coelomocytes is due to steric exclu-
Fig. 2. (A) Phase-contrast image showing disc-shaped stationary cells (s) and the more elongate motile cells (m). The cytoplasm of the motile cells contains numerous granules while the cytoplasm of the stationary cells is relatively clear. (B) Kinesin staining reveals punctate arrays in the cytoplasm of the motile cells, with staining being confined to the perinuclear region of the stationary cell cytoplasm. Linear arrays of kinesin labeling are present in some motile cells (arrows in B). Bar, 10 µm.
Association of kinesin with organelles

Immunolocalization of kinesin with respect to cellular organelles

Double labeling of cells for kinesin and the ER luminal protein, SCS, revealed a close correlation between the two staining patterns (Fig. 4). The ER and kinesin patterns proved to be well spread in the cytoplasm of the motile cells and perinuclear in the stationary cells. Close examination of individual ER tubules compared with the kinesin pattern does underscore the relatedness of the distributions, but also indicates that they are not absolutely superimposable throughout the entire cytoplasm (Fig. 4). A similar

Fig. 3. (A and B) Anti-tubulin labeling indicates that motile cells (at left) contain well-spread cytoplasmic arrays of MTs while stationary cells (at right) contain only focal, perinuclear staining. (C and D) Double label for tubulin (C) and kinesin (D). The punctate kinesin staining is widely distributed in the motile cells (m), which contain an extensive array of MTs. In the stationary cells (s) both the kinesin and MT patterns are perinuclear. Within the motile cells the kinesin distribution appears to be restricted to the region of the cell which contains MTs. Bars, 10 µm.
Fig. 5. Phase-contrast (A) and fluorescence (B) images of live coelomocytes stained with rhodamine 123 to label the mitochondria. Mitochondria appear extensively distributed in the cytoplasm of the motile cells (M) as opposed to the stationary cells (S). Double labeling for kinesin (C, E) and mitochondria (D, F) in motile cells. There appears to be no codistribution between the kinesin and mitochondrial staining patterns. Bars, 10 µm.
Association of kinesin with organelles

A relationship between kinesin and ER distribution was seen when live cells were labeled with the ER marker dye DiOC$_6$ followed by kinesin staining (data not shown). Labeling of cells with DiOC$_6$ followed by SCS staining indicated that methanol fixation does not lead to a gross redistribution of the ER.

**Fig. 6.** Phase-contrast (A) and fluorescence (B) images of NBD-ceramide-stained live cells; and phase-contrast (C) and fluorescence (D) images of rhodamine-WGA-stained fixed cells. Both fluorescent probes give a characteristic Golgi-like perinuclear staining pattern. Double labeling of cells with kinesin (E) and rhodamine-WGA (F) indicates that there is no apparent codistribution between the two patterns. Bars, 10 µm.
Fig. 7. Phase-contrast (A,C) and fluorescence (B,D) images of live (A,B) and fixed (C,D) motile cells following incubation in Texas Red-labeled ovalbumin (TRO). The majority of endosomes labeled by the TRO appear as punctate fluorescent sources that are widely distributed in the cytoplasm and are arrayed in patterns which resemble the kinesin distributions. Double labeling for TRO-labeled endosomes (E) and kinesin (F) shows that there is a significant degree of overlap in the two staining patterns. Bars, 10 µm.
Staining mitochondria in live cells with rhodamine 123 indicated that the motile cells contained a more widespread array of mitochondria than that seen in the stationary cells (Fig. 5A,B), consistent with the MT and ER patterns. Staining of cells for mitochondria followed by kinesin immunofluorescence indicated a regional overlap but no apparent association between the two patterns (Fig. 5C-F). Concentrations of mitochondria were observed in regions of cells which did not contain any detectable kinesin staining. It is possible that mitochondria have been redistributed between the photographing of the live cells and the fixation step. However, our unpublished observations indicate that mitochondrial movements in these cells do not occur in a short enough time-scale to effect their distribution drastically prior to fixation.

NBD-ceramide staining of live cells and rhodamine-WGA staining of fixed cells indicated that the Golgi apparatus was in the characteristic perinuclear position in coelomocytes (Fig. 6A-D). Double labeling of cells for kinesin and rhodamine-WGA indicated no apparent codistribution between the two patterns, although there was limited regional overlap (Figs 6E,F).

Labeling of endosomes in cells incubated in Texas Red-labeled ovalbumin (TRO) demonstrated that small endosomes were widely distributed in the cytoplasm of motile cells (Fig. 7A-D) in a pattern reminiscent of that seen in the kinesin immunofluorescent staining (see Fig. 2). Double labeling of cells with TRO and kinesin indicated a large degree of codistribution between the two patterns in the cytoplasm of a subpopulation of motile cells (Fig. 7E,F). Note that some TRO-containing endosomes do not stain for kinesin and that some kinesin-stained structures do not contain incorporated TRO.

Labeling of lysosomes by acridine orange staining, or via long-term labeling with either lucifer yellow or TRO, indicated that they exist as large, spherical, perinuclear structures in the coelomocytes (Fig. 8). This pattern of lysosomal distribution does not correlate with the pattern of kinesin labeling.

Discussion

In recent studies antibodies generated against brain kinesin have been utilized to immunolocalize the protein to detergent-extractable membranous structures in the cytoplasm of vertebrate cells (Hollenbeck, 1989; Pfister et al., 1989; Brady and Pfister, 1991). We subsequently reported similar kinesin staining patterns in the cytoplasm of sea urchin cells using immunolabeling with domain-specific mAbs raised against the heavy chain of sea urchin egg kinesin (Wright et al., 1991). These mAbs react specifically with conventional kinesin (Wright et al., 1991), and we consider it to be extremely unlikely that a member of the superfamily of eukaryotic kinesin-like proteins is being detected in our experiments (Enos and Morris, 1990; Meluh and Rose, 1990; Hagan and Yanagida, 1990; Endow et al., 1990; McDonald and Goldstein, 1990; Zhang et al., 1990; Stewart et al., 1991; Cole et al., 1992; Sawin et al., 1992). The actual identity of the kinesin-associated membrane-bound structures in cells has not been fully elucidated. In the present study we have extended our previous work by comparing the distribution of kinesin with that of various intracellular organelles. The results suggest that in coelomocytes kinesin may specifically associate with only a subclass of organelles, namely the ER and endosomes.

A large number of studies have indicated a role for kinesin in the anterograde transport of organelles along MTs in the cytoplasm of neuronal and non-neuronal cells. This role would supposedly require that kinesin be found associated with the organellar membranes which were in the vicinity of the cell’s MT array. Previous kinesin localization studies in cells have indicated a regional codistribution between MTs and kinesin (Hollenbeck, 1989; Pfister et al., 1989; Brady and Pfister, 1991; Wright et al., 1991; Houlston and Elinson, 1991). In this study we clearly demonstrate a relationship between the distributions of kinesin and microtubules in coelomocytes (Figs 2, 3). In addition, we indicate a differential distribution pattern of...
MTs and kinesin in motile vs. stationary cells; motile cells containing extensive cytoplasmic arrays of MTs and kinesin, whereas in stationary cells the MTs and kinesin are restricted to area surrounding the nucleus (Fig. 3). The extensive distribution of kinesin in motile cells may be based on the need to distribute organelles during movement; a process which may have functional significance. As a speculative example, the ER may need to be widely distributed during motility to provide calcium sequestration and release capabilities (inferred from the presence of the luminal SCS protein; see Henson et al., 1989, 1990a) to various regions of the cell.

It has been speculated that kinesin plays a role in MT-based ER movements. In vitro studies using cell extracts have shown that a combination of kinesin, MTs and membranes can give rise to ER-like tubulovesicular networks (Dabora and Sheetz, 1988; Vale and Hotani, 1988). A preliminary immunolocalization study on tissue culture cells indicated a similarity between kinesin patterns in fixed cells and the patterns seen with live cells stained with the ER probe DiOCC (Hering and Borisy, 1988). Hollenbeck (1989) argued for an association between kinesin and the ER in chick cells, based on the codistribution of the kinesin and MT patterns (similar to the codistribution seen between ER and MTs; see Terasaki et al., 1986) and the finding that both the kinesin staining and the ER were resistant to saponin extraction but susceptible to Triton extraction. Kinesin and resident ER proteins have been demonstrated to codistribute in the mitotic apparatus of early sea urchin embryos (Wright et al., 1991) and in the cortex of frog embryos (Houliston and Elinson, 1991). In addition, a 160 kDa kinesin-binding membrane protein, isolated from chick brain microsomes using kinesin immunomobilized on a SUK4 column (Toyoshima et al., 1990), has been preliminarily localized to the ER in chick embryo fibroblasts (Toyoshima et al., 1991). In the present study we demonstrate a regional codistribution between the ER and kinesin (Fig. 4), suggesting that a subfraction of the kinesin within the cell may be associated with ER membranes. The punctate nature of the kinesin staining may be indicative of methanol-induced vesiculation of the ER and/or a patchy distribution of kinesin along the ER membrane. Studies on ER movement in vitro and in vivo (Lee and Chen, 1988; Dabora and Sheetz, 1988; Vale and Hotani, 1989; Lee et al., 1989) indicate that ER can be translocated along a MT via only a few attachment points. Previous observations on coelomocytes labeled for ER and MTs indicated that the ER exhibits plus-end-directed movement in spreading cells (Henson et al., 1990b), a movement pattern consistent with kinesin function.

Kinesin also appears to be associated with endosomes in a subpopulation of coelomocytes (Fig. 7). This may seem counter intuitive given the well-established net centripetal (minus-end-directed) movement of endosomes along MTs towards the perinuclear region of the cell (reviewed by Gruenberg and Howell, 1989). However, observations on living cells have indicated that endocytic vesicles move bidirectionally on MTs (Matteoni and Kreis, 1987; De Brabander et al., 1988). It is also clear that a significant amount of endosomes recycle to the cell surface and thus move in the centrifugal direction (Gruenberg and Howell, 1989) consistent with it being a cargo moved by the plus-end-directed sea urchin kinesin (Porter et al., 1987). In addition, recent studies employing cell-free assay systems report that the fusion of apically and basolaterally derived early endosomes with late endosomes requires the presence of kinesin as well as dynein (Bomsel et al., 1990), although the actual binding of endosomal carrier vesicles to microtubules has been suggested to be independent of motor proteins (Scheel and Kreis, 1991). Interestingly, cytoplasmic dynein has been recently found to be associated with lysosomes/late endosomes in cultured cells (Lin and Collins, 1992), and in a Meeting abstract a colocalization of kinesin and dynein on a subpopulation of membranous organelles in cells has been reported (see Lin et al., 1991). The potential association of both kinesin and cytoplasmic dynein with endosomes/lysosomes may help explain their demonstrated potential for bidirectional movement. Another more speculative possibility is that the directionality of microtubule motor proteins may be influenced by phosphorylation levels (see Rozdzial and Haimo, 1986) or by intracellular ionic conditions (see Naitoh and Kaneko, 1972). We are currently considering using in vitro and in vivo experimentation to address these possibilities with reference to sea urchin kinesin function in coelomocytes and embryos.

We did not see evidence for an association between kinesin and mitochondria, the Golgi apparatus or lysosomes. Similarly, kinesin does not appear to colocalize with mitochondria in early sea urchin embryos, where mitochondria appear to be excluded from the kinesin and ER-rich mitotic apparatus (Hinkley and Newman, 1988; Henson et al., 1989; Wright et al., 1991). Recent reports have indicated that kinesin may be associated with mitochondria, the Golgi and lysosomes in other cell types. Kinesin has been preliminary reported to bind to mitochondria isolated from bovine brain neurons (Leopold et al., 1989, 1990; Brady and Pfister, 1991), to Golgi in tissue culture cells (Murphy et al., 1991) and the incorporation of anti-kinesin antibodies has been shown to inhibit the radial extension of tubular lysosomes in cultured macrophages (Hollenbeck and Swanson, 1990). The discrepancies between the kinesin localization in coelomocytes and that seen in other cell types may be indicative of cell type-specific differences and/or a functional dependence of kinesin distribution. For example, the apparent lack of Golgi staining may be due to the relatively inactive secretory processes of coelomocytes when compared with certain tissue culture cells. In terms of lyosomal staining, it may be that kinesin-lysosome associations are present in only those cells which exhibit tubular lysosomes (Swanson et al., 1987; Robinson et al., 1986; Heuser, 1989). One likely possibility is that in coelomocytes other kinesin-like MT motor proteins are responsible for the movement and organization of mitochondria, the Golgi and lysosomes. We are now in a position to test this possibility using our peptide antibodies which cross-react with multiple members of the sea urchin kinesin superfamily (Cole et al., 1992).

The results of this study indicate that kinesin may associate with only a subclass of the organelles present in the coelomocyte. The ER and endosomes appear to be affiliated with kinesin and this specific association may have significance in terms of both organellar and overall cellular...
functions. Future studies will take a number of different approaches in order to examine these apparent kinesin-organelle associations more fully. These include the examination of the specific binding of kinesin to ER and endosomal membranes in vitro, the effects of antibody-mediated disruption of kinesin function on organellar distribution, and the immunoelectron microscopic localization of kinesin and organelles.

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References


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Fig. 4. Double labeling of kinesin (A, C) and the ER-resident protein SCS (B, D). The punctate kinesin pattern and the ER pattern appear regionally codistributed in some cells. Both are widely distributed in the motile cells (m) but only perinuclear in the stationary cells (s). Bars, 10 µm.