Regulation of the intracellular distribution of cytoplasmic dynein by serum factors and calcium

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SUMMARY

Previous work has indicated that cytoplasmic dynein localizes primarily to lysosomes in cultured fibroblasts, consistent with a function for dynein in retrograde movement. We now show that dynein can be redistributed from a lysosome-associated pool to a more diffuse cytoplasmic pool upon shifting fibroblasts to culture medium lacking serum for several hours. The serum effect appears to be selective for dynein, in that the localization of kinesin and the overall morphology of intracellular organelles does not change. However, the distribution of kinesin-positive vesicles and lysosomes does appear to be altered during serum starvation, in that these organelles are located to greater extents in the peripheral regions of the cell. Dynein is also associated with the mitotic apparatus, but this localization does not change in response to serum starvation. Removal of calcium from the extracellular medium also results in the loss of punctate dynein staining, which can be recovered upon addition of calcium to calcium-free medium. The redistribution of dynein observed under these experimental conditions may reflect the activity of a regulatory process controlling the association of dynein with organelles, thereby providing one means of modulating intracellular transport.

Key words: cytoplasmic dynein, intracellular motility, lysosomes, serum depletion, calcium, retrograde transport

INTRODUCTION

Cytoplasmic dynein has been proposed to function as a microtubule-dependent retrograde organelle translocator (reviewed by Vallee and Shpetner, 1990; Schroer and Sheetz, 1991a). To determine whether dynein mediates transport in intact cells, one experimental approach is to determine whether the activity of this putative motor can be modified in such a way as to inhibit or promote vesicle movement. There are many models for how regulation may be carried out, such as modulation of ATPase activity, changes in coupling efficiency between ATP hydrolysis and force production, or through alterations in binding specificity and affinity for organelles and microtubules. There has been some recent progress made in identifying factors that affect dynein activity in vesicle motility assays (Schroer and Sheetz, 1991b). Dynein-organelle binding characteristics in isolated membrane preparations have also been examined as a first step toward understanding the nature of this interaction (Lacey and Haimo, 1992; Yu et al., 1992). As another approach, we have examined the subcellular distribution of dynein in cultured cells by immunocytochemistry. Previously, we showed that cytoplasmic dynein is associated with organelles in the endocytic pathway in fibroblasts and other cell types (Lin and Collins, 1992). We examined the dynein staining pattern in these cells following treatment with microtubule-active drugs and other agents which affect retrograde organelle traffic. Under these conditions, the organelle distribution in cells was disrupted, generally resulting in an accumulation of endosomes and lysosomes in the periphery of the cell. Dynein remained associated with these organelles, suggesting that dynein-organelle interactions can be maintained even in the absence of an active transport process.

We have extended this investigation to determine whether conditions could be found that would lead to loss of lysosomal dynein staining. In the present study we have identified cell culture conditions (serum starvation) under which dynein does not localize with discrete organelles, but appears to be distributed throughout the cytoplasm. As a first step in investigating the consequences of this change in dynein localization on vesicle trafficking, we have analyzed the distribution of lysosomes in serum-starved cells, and find alterations consistent with a decrease in retrograde motor activity.

MATERIALS AND METHODS

Cell culture

NRK cells (normal rat kidney, CRL 1570) were grown in DME
(Irvine Scientific, Santa Ana, CA) with penicillin/streptomycin (100 i.u./ml and 100 µg/ml, respectively) and 10% calf serum (HyClone Laboratories, Logan, UT). For serum starvation experiments, the cells were rinsed with warm DME several times and then incubated in this medium without serum for the indicated periods. For serum recovery experiments, the DME was supplemented with calf serum at the concentrations indicated for each experiment in the Figure legends. Several different batches of calf serum, including those of other suppliers, were tested, and all gave similar results. Normal calcium concentration in the culture medium (DME plus serum) is approximately 2 mM. For calcium depletion experiments, calcium-free DME was used in conjunction with calf serum dialyzed against PBS (50 mM NaPO₄, pH 7.4, 150 mM NaCl).

Immunofluorescence microscopy

Cells were prepared for fluorescence microscopy by methanol fixation at −20°C for 5 min, or by fixation in 3.75% formaldehyde in PBS for 5 min at room temperature, followed by methanol at −20°C for 2 min. Results using these protocols were indistinguishable.

Fixed cells were incubated with primary antibody for 1 h, and secondary antibody for 20 min, in a humidified chamber at 37°C. Dynein polyclonal antibody (Lin and Collins, 1992) was used as either whole antisera or affinity-purified antibody at a dilution of 1:50 in PBS, and goat anti-rabbit secondary antibodies conjugated to either fluorescein or rhodamine (Jackson ImmunoResearch Laboratories, West Grove, PA) were used at a concentration of 45 µg/ml, also in PBS. Affinity-purified antibody was prepared as previously described (Lin and Collins, 1992) by acid elution from a column containing purified dynein bound to Affi-Gel 10 (Bio-Rad Laboratories, Richmond, CA). Mock affinity-purified antibody was prepared by binding and eluting the immune rabbit serum from Affi-Gel to which serum albumin had been coupled. For some studies, kinesin monoclonal antibody H1 at 20 µg/ml (Pfister et al., 1989) was used along with goat anti-mouse secondary antibody. Polyclonal antibody to rat cathepsin D (Sensibar et al., 1990) was used as a marker for lysosomes. Following antibody incubations, coverslips were mounted with gelvatol (Monsanto, St. Louis, MO) containing 100 mg/ml 1,4-diazabicyclo[2.2.2]octane and viewed using a Carl Zeiss Inc. (Thornwood, NY) axioskop microscope with either a ×63 plan-apochromat or a ×40 plan-neofluar objective. Photomicrographs were taken with T-MAX 400 film (Eastman Kodak Co., Rochester, NY) and processed with T-MAX developer. All photographs and prints of dynein immunofluorescence were made using a timed exposure optimized for visualization of the control dynein staining pattern.

Analysis of lysosome distribution

Control, serum-starved, or serum-recovered cells were fixed and stained with antibody to cathepsin D, and photographed as described above. Cell staining patterns on the photographic negatives were analyzed using a laser densitometer (Ultrascan SL, Pharmacia LKB, Piscataway, NJ). Densitometric scans included the region of most intense vesicular staining in the perinuclear region, and extended from the center of the nucleus to the outer margin of the cell. The slit width was set to 1 mm, compared to an average nuclear diameter of 2 mm on the negatives. The perinuclear region of highest staining intensity generally extended out from the nucleus for 1-2 mm. For purposes of calculation, the limit of this region was defined as that which showed a decrease of > 65% from the peak intensity. To determine the extent of peripheral staining, the summed area of the peaks of staining intensity outside of the perinuclear region was divided by the total area. The numbers were not corrected for differences in cell area between perinuclear and peripheral regions, and are therefore an underestimate of the organelles in the periphery. The data were analyzed by Student’s t-test modified for unpaired data of unequal variances.

Other methods

SDS-PAGE was performed using 7% acrylamide in the separating gel and 3% acrylamide in the stacking gel. Transfer conditions and western immunoblot analyses were carried out as previously described (Collins and Vallee, 1989). Dynein content in whole cell extracts was determined by laser scanning densitometry of immunoblots. The intensity of the alkaline phosphatase color reaction was determined to be linear with respect to dynein concentration in the ranges used by comparison with serial dilutions of a dynein standard. Protein analysis was performed using BCA reagents (Pierce, Rockford, IL).

RESULTS

Cytoplasmic dynein localization is altered in cells incubated in the absence of serum

We have previously determined that dynein co-localizes with lysosomes and that certain agents that disrupt the distribution of lysosomes in cultured fibroblasts do not affect this association (Lin and Collins, 1992). Fig. 1a shows a typical field of NRK cells grown under control conditions, stained for cytoplasmic dynein. We determined by double-labeling with fluorescent dextrans and wheat germ agglutinin that the majority of organelles in this cell type which are labeled with the anti-dynein antibody are lysosomes, as had been shown previously for 3T3 and BHK cells (Lin and Collins, 1992). As found in our previous study, the dynein-associated organelles clustered in the perinuclear region of the cell, in close apposition to the microtubule-organizing center.

In the course of our studies on the effects of drugs and serum factors on retrograde transport, incubation of cells for short periods of time in the absence of serum was occasionally required. We observed that the punctate appearance of dynein immunofluorescence was altered after incubation of the cells in medium lacking serum for several hours. Fig. 1a-c shows a time-course of the loss of punctate dynein staining in NRK cells after shifting to culture medium lacking serum. Three to four hours were generally required in order to observe loss of the bright vesicular staining pattern (Fig. 1b). No further changes in dynein localization were detected following longer periods (up to 2 days) of serum starvation (not shown). The resulting staining pattern in serum-starved cells was faint diffuse fluorescence throughout the cytoplasm. No distinct vesicular structures appeared to be labeled. The diffuse immunofluorescence appeared to be brighter than background staining obtained using mock affinity-purified antibody on control cells (Fig. 1d). The effect of serum starvation on dynein distribution was not unique to NRK cells, as BHK and 3T3 cells also exhibited a change in dynein staining pattern similar to that observed for NRK cells (not shown). The redistribution observed was also not a unique property of our anti-dynein antibody, as similar results were obtained using...
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a monoclonal antibody against the dynein 74 kDa intermediate chain (J. F. Dillman and K. K. Pfister, unpublished; Paschal et al., 1992) (data not shown).

Dynein localizes to spindle microtubules in serum-starved mitotic cells

Serum starvation, depending on its duration, may affect growth and cell division. Long-term serum deprivation (12-24 hours) will cause cells to become quiescent, entering the Go-phase of the cell cycle (Brooks, 1976). In a typical experiment the initial loss of lysosomal dynein staining occurred following 4 hours of serum starvation, which is not a sufficient length of time to cause growth arrest of the entire population of cells. We observed no difference in the number of mitotic cells in short-term (4-6 hours) serum-starved subconfluent cultures, compared with those maintained in serum. Mitotic cells also lost their cytoplasmic punctate dynein pattern along with interphase cells upon serum starvation (Fig. 2a,c). However, the dynein distribution in the spindle in both cases was comparable to that found for tubulin (Fig. 2b), as had been shown previously (Lin and Collins, 1992). Unlike the lysosome-associated dynein, cytoplasmic dynein localized to the spindle did not appear to be affected by serum-starvation conditions (Fig. 2c). Our previous results (Lin and Collins, 1992) and those of other groups (Pfarr et al., 1990; Steuer et al., 1990) revealed staining of the mitotic apparatus, but this was observed in cells extracted with detergent prior to fixation. The cells shown in Fig. 2 were not detergent extracted, providing additional evidence that a mitotic spindle localization for this protein is not an artifact of prefixation treatment.

The effect of serum starvation on dynein distribution is reversible

The effect of serum starvation on dynein localization

**Fig. 1.** Time-course of disappearance of lysosomal dynein staining in NRK cells following serum starvation. Cells were cultured in complete control medium (DME plus 10% calf serum) (a) or shifted to medium which lacked serum for 3 h (b) or 6 h (c), then fixed and stained using affinity-purified dynein antibody. As a control for antibody specificity, NRK cells grown in the presence of serum were fixed and stained using mock affinity-purified antibody (d). Bar, 10 µm.
could be reversed upon restoration of serum to the medium. The results in Fig. 3 illustrate the time-course of recovery of a punctate dynein staining pattern in such an experiment. Recovery of punctate organellar staining was more rapid than that of the loss of such staining, with substantial return to a vesicular pattern within 10 minutes. The reappearance of punctate staining occurred throughout the cytoplasm, with small vesicles becoming labeled initially (Fig. 3b), and then with the appearance of larger and more brightly fluorescent vesicles in the perinuclear region of the cell (Fig. 3c,d). The length of the previous serum starvation period (from 4 to 20 hours) did not affect the rate or extent of recovery of the staining pattern when serum was added. No recovery of a punctate dynein pattern was observed in serum-starved cells shifted to fresh medium lacking serum. Nuclear spots were observed in a small number of cell preparations (Fig. 3a). However, these were also observed using preimmune serum and therefore do not reflect a specific antibody reaction.

Loss of punctate staining is not due to degradation of dynein or fragmentation of intracellular organelles

To determine whether recovery of dynein staining following serum starvation required new protein synthesis, cells were treated with 10 µg/ml cycloheximide during the last 90 minutes of a 5.5 hour period of serum starvation, and then incubated for an additional 2 hours with cycloheximide in the presence of serum. The punctate dynein staining pattern returned following addition of serum, indicating that this recovery of staining did not require synthesis of additional dynein, and that degradation of substantial amounts of dynein during the starvation period was unlikely (data not shown).

As a further test of protein stability under serum-starvation conditions, whole cell extracts were prepared from control and serum-starved cells by lysis in SDS-containing gel sample buffer. Following SDS-PAGE and transfer to nitrocellulose, antibody was used to detect the presence of cytoplasmic dynein in the samples. Fig. 4 shows the results of this analysis. As found previously (Lin and Collins, 1992), the antibody reacted primarily with the 74 kDa subunit of cytoplasmic dynein in both cell extracts, and with purified dynein from calf brain. Results from eight independent experiments examining control, serum-starved, and serum-restored conditions have shown no effect of these treatments on dynein content in cell extracts, quantitated as described in Materials and Methods. No reactivity with protein species other than dynein in either the control or serum-starved cell extracts was observed, demonstrating that the presence and absence of punctate staining under different culture conditions was not due to antibody recognition of serum constituents taken into the cell. The lack of reactivity against calf serum components was also tested directly by immunoblot analysis of calf serum using dynein antibody. No reactive species were detected (data not shown).

An important concern was whether the loss of punctate dynein staining was secondary to a disruption of the lyso-
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somal compartment in NRK cells cultured without serum. The lysosomal compartment in serum-starved cells, as determined by localization of cathepsin D in fixed cells (see Fig. 7) and confirmed by acridine orange labeling of live cells (data not shown), appeared unchanged from that of controls grown in serum, both in the number of lysosomes and their morphology. The microtubule and actin networks, and the overall cell shape were not appreciably altered in cells serum-starved for up to 12 hours (data not shown).

To examine whether the effect of serum starvation was selective for dynein redistribution, we examined the staining pattern of kinesin in serum-starved and serum-refed cells. In a preliminary study we found that dynein and kinesin appeared to associate with distinct organelle populations in cultured cells (Lin et al., 1991). Although punctate dynein staining was lost during serum starvation (Fig. 5a), the pattern observed for kinesin in serum-starved cells remained vesicular (Fig. 5b), and similar to that of cells to which serum had been restored (Fig. 5d). However, the kinesin-labeled vesicles in serum-starved cells appeared more evenly distributed in the cytoplasm compared with cells to which serum had been added, where they were more concentrated in the perinuclear region.

Maintenance of lysosomal dynein staining relies on extracellular calcium
Calcium has been implicated in the regulation of vesicle motility in some systems (Breuer and Atkinson, 1988; Oshima et al., 1988; McNiven and Ward, 1988; Thaler and Haimo, 1990). To investigate the signalling pathways involved in the alteration of dynein distribution upon serum starvation, we examined the requirement for calcium in this process. We found initially that recovery of punctate dynein staining upon addition of serum to serum-starved cells was blocked in the absence of extracellular calcium (data not shown). We next tested whether calcium depletion alone could induce the loss of lysosomal staining in cells grown in the presence of serum. As shown in Fig. 6a, cells cultured in calcium-free medium for 4 hours do not retain

Fig. 3. Time-course of reappearance of punctate dynein staining upon return to serum. Cells were serum-starved for 12 h and then fixed and stained with affinity-purified dynein antibody (a) or returned to medium containing 1% calf serum for 10 min (b), 30 min (c), or 1 h (d) prior to fixation. Bar, 10 µm.
punctate dynein fluorescence. At this time point there were no obvious changes in cell shape, attachment to the sub-stratum, or organization of actin stress fibers. Recovery of lysosomal dynein staining occurred upon addition of 2 mM calcium to the medium (Fig. 6b).

**Lysosomes become peripherally distributed in serum-starved cells and recover a perinuclear localization following serum recovery**

To examine whether serum starvation disrupted the normal distribution of lysosomes, we evaluated the degree to which lysosomes exhibited a peripheral localization in control, serum-starved, and serum-recovered cells. Fig. 7 shows control, serum-starved and serum-refed cells stained with antibody to cathepsin D to label the lysosomes. Densitometric scans of photographic negatives were used to quantify the intensity of immunofluorescence in the perinuclear and peripheral regions as a percentage of total staining intensity, as described in Materials and Methods. These data for control and treated cells are shown in Table 1. Though

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**Fig. 4.** Reactivity of dynein subunits in NRK cell extracts. Whole cell extracts were prepared by scraping cells from confluent 100 mm dishes into SDS-containing gel sample buffer. Following SDS-PAGE and electrophoretic transfer to nitrocellulose, the blot was reacted with whole dynein anti-serum and visualized using alkaline-phosphatase-conjugated secondary antibody. Lane 1, sucrose-gradient-purified calf brain dynein; lane 2, extract from cells grown in control medium containing serum; lane 3, extract from cells serum-starved for 12 h. Equivalent amounts of cell protein were loaded. The position of the dynein 74 kDa intermediate chain subunit is indicated.

**Fig. 5.** Kinesin distribution in serum-starved cells. NRK cells were double-labeled with antibodies to dynein (a,c) and kinesin (b,d). The cells were serum-starved for 14 h (a,b) or starved for 12 h and then incubated with 10% calf serum for an additional 2 h (c,d). Bar, 10 µm.
there was considerable variability from cell to cell, there was a clear and statistically significant trend toward dispersion of lysosomes following long-term (16 h) serum starvation, compared with lysosomes in cells to which serum was restored. Control cells exhibited a distribution that was more similar to the perinuclear pattern of serum-recovered cells, consistent with the lysosome distributions described by others (Herman and Albertini, 1984; Matteoni and Kreis, 1987; Heuser, 1989). Cells serum-starved for 4-6 hours, and those which were calcium-deprived for 3-4 hours, exhibited no obvious redistribution of lysosomes compared with controls.

**DISCUSSION**

Cytoplasmic dynein has been considered to function as a retrograde organelle translocator. Based on our previous work (Lin and Collins, 1992) and that of other groups...
Table 1. Distribution of lysosomes in NRK cells

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<tr>
<th>Condition</th>
<th>Lysosomes outside perinuclear region* (percentage of total)</th>
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<tbody>
<tr>
<td>Control</td>
<td>14.5±8.8</td>
</tr>
<tr>
<td>Serum-starved</td>
<td>50.6±20.0</td>
</tr>
<tr>
<td>Serum-refed</td>
<td>7.3±6.6</td>
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*NRK cells were fixed and stained for cathepsin D immunofluorescence. Photographic negatives were scanned and the fluorescence signal due to lysosomes in the periphery of the cell was calculated as a percentage (±s.d.) of total immunofluorescence in the scanned area, as described in Materials and Methods. The lysosome distribution in serum-starved cells (n=11) was significantly different (P<0.01) from that of control or refed cells (n=7).

(Gilbert and Sloboda, 1989; Hirokawa et al., 1990; Koonce and McIntosh, 1990; Lacey and Haimo, 1992), evidence for membrane association of dynein both in vivo and in vitro has been obtained.

Reconstitution assays examining directed organelle transport in cell lysates have provided additional evidence that dynein and associated proteins are involved in retrograde translocation (Schnapp and Reese, 1989; Schroer et al., 1989). However, the question of regulation of dynein activity is only starting to be addressed. It is likely that the regulation of organelle motility takes place at several levels. These may include the reversible association of dynein (and kinesin) with specific organelles, and for modulation of the activity of the enzyme once bound. There may also be regulation at the level of association of organelles with microtubules, either mediated directly by the motor molecule itself, or by accessory proteins. In addition, in extremely polarized cells such as neurons, there must be some means of transporting a retrograde motor in inactive form to the nerve terminal in order for it to associate with organelles to be transported back to the cell body.

Because of the relatively stable dynein staining patterns we have found previously (Lin and Collins, 1992), it seemed likely that dynein remains bound to its target organelles and that motility is controlled at other levels. The altered localization of cytoplasmic dynein in response to brief serum starvation in the current study was completely unexpected. The most straightforward explanation for the loss and reappearance of dynein staining on lysosomes is that the dynein is released from the organelle membrane into a cytoplasmic pool in the absence of serum, and rebinds the lysosomal membrane upon restoration of serum. The serum effect was dependent on the presence of extracellular calcium, and depletion of calcium was sufficient to induce the loss of lysosomal dynein staining in the continued presence of serum. The reversible interaction between dynein and membranous organelles likely reflects the activity of a regulatory pathway involved in intracellular vesicle transport, pushed to extremes under the serum- or calcium-depletion conditions described here.

While known cellular responses to serum/amino acid starvation include increased autophagy (Hendil et al., 1990), the rapid time-course of recovery, the finding that inhibition of protein synthesis by cycloheximide does not prevent recovery, and the detection of dynein on immunoblots under all treatment conditions, indicate that selective proteolysis and resynthesis are not responsible for loss and recovery of vesicle-associated dynein. It is possible, however, that the more diffuse cytoplasmic staining pattern obtained in serum-starved cells does not reflect a true solubilization of dynein. The dynein that is released from the lysosomes may be associated with other structures or protein complexes in the cytoplasm. Our attempts to fractionate NRK cell homogenates have revealed no clear differences in the distribution of dynein among soluble and membrane pools from control and serum-starved cells. This may reflect the ability of dynein to rebind membranes in a non-specific manner once the cells are lysed (V. M. Delgado-Partin and C. A. Collins, unpublished). Based on immunostaining with cathepsin D antibody and fluorescent markers for endocytic compartments, there does not seem to be any change in the morphology of the lysosomes themselves. However, the redistribution of lysosomes to the periphery of the cells under serum starvation conditions shown here (Fig. 7), and also described by Herman and Albertini (1984), indicates that dynein may be involved in maintaining the normal perinuclear location of these organelles. A similar dispersion of kinesin-associated vesicles was found under serum starvation conditions (Fig. 5). We have not observed fragmentation of the Golgi apparatus in serum-starved cells, and the redistribution of lysosomes and kinesin-labeled vesicles required longer term (12-16 hours) culture in the absence of serum. However, additional non-motor proteins may be involved in maintenance of intracellular distributions of various organelles in relationship to the cytoskeleton once they have been translocated into place, as have been implicated for endosomes, lysosomes and the Golgi apparatus (Bloom and Brashear, 1989; Mitieux and Rousset, 1989; Scheel and Kreis, 1991; Goltz et al., 1992; Karecla and Kreis, 1992). Further work will be required to examine the consequences of dynein redistribution on endocytosis and vesicle trafficking in serum-starved or calcium-depleted cells.

Though cytoplasmic dynein and kinesin are proposed to act as a mechanochemical enzyme along microtubules, we and others have no evidence for their localization along interphase microtubules except in association with membranous organelles (Hollenbeck, 1989; Pfister et al., 1989; Koonce and McIntosh, 1990; Henson et al., 1992; Lin and Collins, 1992). However, dynein does localize to the mitotic apparatus in addition to organelles in dividing cells. Though the vesicular staining pattern was altered by serum starvation, dynein localization in the spindle was unaffected by this treatment. This result suggests that the binding of dynein to spindle components is regulated by a pathway distinct from that of cytoplasmic organelles. Dynein has also been localized to the kinetochore region in isolated chromosomes (Pfarr et al., 1990; Steuer et al., 1990), but the function of dynein in the spindle, whether as a mitotic motor or in some other role, has yet to be determined. It is interesting that dynein remains associated with vesicles in mitotic cells. The inhibition of vesicle transport observed during mitosis cannot, therefore, be explained by the lack of an association of dynein with mitotic organelles (c.f. Allan and Vale, 1991).

Cellular responses to serum factors and calcium are numerous and well documented (reviewed in Deuel, 1987; Tsien and Tsien, 1990; Herschman, 1991). Phosphorylation...
pathways utilizing calcium- or cAMP-dependent protein kinases and phosphatases have been proposed to be involved in regulation of vesicle movement in fish scale chromatophores and other cell systems (Rozdial and Haimo, 1986; McNiven and Ward, 1988; Oshima et al., 1988; Palazzo et al., 1989, Thaler and Haimo, 1990; Ohashi and Ohnishi, 1991; Sammak et al., 1992; Davidson et al., 1992). There is evidence from antibody microinjection experiments that kinesin is involved in dispersion of pigment granules in melanophores (Rodionov et al., 1991), and phosphorylation of kinesin, in vitro, has been shown to decrease its binding to synaptic vesicles (Sato-Yoshitake et al., 1992). However, it is not known whether cytoplasmic dynein is also involved in granule movements in chromatophores, and regulation of motility by phosphorylation of these motor proteins has not yet been demonstrated. It is also possible that calcium itself or calcium together with calmodulin may have a direct effect on the association of dynein (or kinesin) with organelle membranes and on ATPase and motor activities. Clearly, an important area for further work will be the dissection of the mechanisms involved in localization and activation of these motor proteins in order to carry out directed transport of specific organelle populations.

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REFERENCES


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