Dynein, Dynactin, and Kinesin II’s Interaction with Microtubules Is Regulated during Bidirectional Organelle Transport

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Abstract. The microtubule motors, cytoplasmic dynein and kinesin II, drive pigmented organelles in opposite directions in *Xenopus* melanophores, but the mechanism by which these or other motors are regulated to control the direction of organelle transport has not been previously elucidated. We find that cytoplasmic dynein, dynactin, and kinesin II remain on pigment granules during aggregation and dispersion in melanophores, indicating that control of direction is not mediated by a cyclic association of motors with these organelles. However, the ability of dynein, dynactin, and kinesin II to bind to microtubules varies as a function of the state of aggregation or dispersion of the pigment in the cells from which these molecules are isolated. Dynein and dynactin bind to microtubules when obtained from cells with aggregated pigment, whereas kinesin II binds to microtubules when obtained from cells with dispersed pigment. Moreover, the microtubule binding activity of these motors/dynactin can be reversed in vitro by the kinases and phosphatase that regulate the direction of pigment granule transport in vivo. These findings suggest that phosphorylation controls the direction of pigment granule transport by altering the ability of dynein, dynactin, and kinesin II to interact with microtubules.

Keywords: dynein • kinesin • melanophores • microtubules • phosphorylation

Introduction

The mechanism by which the direction of organelle transport along microtubules is controlled is not understood, although the microtubule motors, dynein and kinesin II, responsible for transporting organelles in opposite directions have been extensively studied. Two major models have been proposed to explain the direction of transport could be controlled. Motors might cyclically bind to and dissociate from organelles thereby dictating, by their presence or absence, the direction of organelle transport. Alternatively, motors might remain on organelles during bidirectional transport, but their activity, i.e., their ability to generate force along a microtubule, may be enhanced or inhibited to regulate the direction of transport (Sheetz et al., 1989; Hirokawa et al., 1990; Hirokawa, 1996). Strong support for one or the other of these models is lacking.

Evidence that motor–organelle interactions may be regulated is provided by various studies. Vesicles that contain dynein but not kinesin move towards the minus ends of microtubules, whereas those that contain both kinesin and dynein move towards the plus ends of microtubules (Hirokawa et al., 1990, 1991; Dahlstrom et al., 1991; Muresan et al., 1996), suggesting that vesicles acquire and lose kine-
Materials and Methods

The immortalized Xenopus melanophores were cultured at 25°C in L-15 medium (Sigma-Aldrich) containing 15% heat-inactivated FBS (GIBCO BRL), 2 mM L-glutamine, 100 μM penicillin, and 0.1 mg/ml streptomycin. Subconfluent cultures, grown in 15-cm plates, were transferred to serum-free medium overnight. To induce aggregation and dispersion of pigment granules, cells in serum-free medium were incubated in the dark in 10 μM melatonin or in 100 nM α-melanocyte stimulating hormone (MSH), respectively, for 1 h.

Cell Culture

Xenopus melanophores were washed twice with 0.7× PBS containing melatonin or MSH, respectively, then scraped with a rubber policeman into 1 ml (per 15-cm plate) cold lysis buffer containing melanotin or MSH and lysed by passage through a 25-gauge needle. Pigment granules were purified as described (Rogers et al., 1998) by centrifugation through 80% Percoll (Sigma-Aldrich) in BRB40. The pellet of purified pigment granules was either prepared for immunoblotting or resuspended in 100 μl cold lysis buffer supplemented with 1% Triton X-100 and extruded 10 times through a 25-gauge needle to shear the membrane and solubilize pigment granule–associated motors. Only a small fraction of the organelle motors could be extracted, possibly because melain is sticky. One or more granule membrane is solubilized, the motors and dynactin bind avidly to the pigment. The preparation was diluted to 40 μg/ml with 0.1 mM ATP for 20 min at 4°C in a 70.1 Ti rotor (Beckman) at 160,000 × g. The final aliquot used in experiments was centrifuged on 30% glycerol in lysis buffer containing 5 mM MgCl₂, pH 6.9, was assembled into microtubules by incubation at 37°C in the presence of 20 μM taxol. The resulting microtubules were used in microtubule cosedimentation and microtubule capture studies.

Abbreviations used in this paper: AMP-PNP, adenylyl imidodiphosphate; MSH, melanocyte stimulating hormone; PK, protein kinase; PP, protein phosphatase.

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AMP-PNP at 100,000 g for 45 min at 4°C in an SW 50.1 rotor (Beckman). After centrifugation, the supernatant fraction was collected, and the surface of the glycerol cushion was washed twice with water. The glyceral cushion was then removed, and the microtubule pellet (normally not visible) was resuspended and prepared for gel electrophoresis.

Microtubule Capture Assays

Dynein, dynactin, and kinesin II were immunoprecipitated from aggregated and dispersed clarified lysates with the appropriate antibody immobilized on protein A-Sepharose 4B beads (Sigma-Aldrich). 20 μl of packed protein A-Sepharose 4B beads in 1 ml cold BRB40 was incubated with either 5 μg of 74.1 antibodies, 1.25 μg of p150 antibodies, or 10 μg of K2.4 antibodies for 1 h at 4°C. The beads with antibody were washed twice with BRB40 and then incubated in 1 ml of clarified lysate from aggregated or dispersed melanophores. After a 30-min incubation at 4°C, the beads were collected and washed three times with 1 ml cold lysis buffer containing 0.25% gelatin (Bio-Rad Laboratories). In some studies, the immunoprecipitants were then incubated for 30 min at 30°C in either 500 U of the catalytic subunit of PKA and 1 mM ATP; 117 μU of the catalytic subunit of PKC and 1 mM ATP; 50 U of PP2B and 0.2 μM free Ca^2+ and 0.5 μM calmodulin; 610 μU of PP2A; or 6.66 U of the recombinant catalytic subunit of PP1 in 1 ml BRB40 and again washed. Each immunoprecipitant was resuspended in 1 ml cold BRB40 and incubated with a final concentration of 0.2 mg/ml taxol-stabilized microtubules, 20 μM taxol, and 1 mM AMP-PNP for 30 min at 4°C. The beads were allowed to settle by gravity for 15 min, washed three times with 1 ml cold BRB40, washed once with 1 ml cold BRB40 containing 0.5 M NaCl and 0.05% Tween 20, washed again in 1 ml cold BRB40, and prepared for gel electrophoresis and immunoblotting. In studies to determine if microtubule capture by the motors/dynactin was ATP sensitive, AMP-PNP was omitted during the microtubule capture step and ATP, at a final concentration of 1 mM, was added to each immunoprecipitant-microtubule complex after capture. The beads were incubated for an additional 30 min at 4°C and then washed as described above.

Microscopy and Image Acquisition

Micrographs of melanophores aggregating and dispersing were obtained using bright field optics and acquired digitally using a Dage Newvicon camera. Immunoblots were digitally scanned. All figures were prepared using Adobe Photoshop® software.

Results

Microtubule Motors and Dynactin Cosediment with Pigment Granules during Bidirectional Transport

To determine if the direction of pigment granule transport in Xenopus melanophores is regulated by the cyclic association of microtubule motors with pigment granules, we isolated these organelles from cells that had either aggregated or dispersed pigment. Previous studies have demonstrated that cytoplasmic dynein and kinesin II are present on Xenopus pigment granules (Rogers et al., 1997). Here, we find these motors on the organelles, regardless of whether they have undergone aggregation or dispersion (Fig. 1). In addition, dynactin is also present and at equivalent amounts on granules isolated from melanophores with aggregated or dispersed pigment (Fig. 1). These findings suggest that net attachment or detachment of motors/dynactin to these organelles does not occur as a function of aggregation and dispersion and is not the mechanism regulating the direction of pigment granule transport.

Dynein, Dynactin, and Kinesin II on Pigment Granules Only Bind Microtubules Following One Direction of Transport

To determine if the direction of pigment granule transport in melanophores is regulated by controlling the activity of the microtubule motors, we examined the microtubule binding behavior of motors and dynactin extracted from pigment granules that had been transported in opposite directions. Pigment granules were isolated from melanophores that had either aggregated or dispersed pigment, and the motors and dynactin were extracted from these organelles by shearing in a buffer containing 1% Triton X-100. The extracts were incubated with microtubules under conditions that promote net attachment or binding, and the microtubules were collected by centrifugation through a glycerol cushion. The microtubules were collected from microtubule pellets which had been extracted from pigment granules that had undergone aggregation or dispersion (Fig. 2 A, a, lanes 1 and 2). Only the aggregated but not the dispersed pigment granules are capable of binding dynein and dynactin (Fig. 2 A, a, lanes 3 and 4). Dynactin, therefore, is preferentially associated with the aggregated pigment granules. The aggregated pigment granules are also capable of binding microtubules, while the dispersed pigment granules are not (Fig. 2 A, b, lanes 3 and 4).

The Soluble Pool of Dynein, Dynactin, and Kinesin II Is Also Regulated during Bidirectional Transport

To determine if dynein, dynactin, and kinesin II on the pigment granules are specifically targeted for regulation, we assessed the ability of the soluble pool of these proteins to bind to microtubules as a function of the direction of pigment granule transport. Melanophores with aggregated or dispersed pigment were lysed, and a high speed super-
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The natural fraction containing the soluble proteins was prepared and mixed with microtubules, which were then collected by centrifugation, as described above, and blotted for dynein (Fig. 2 B, a), or simultaneously for dynactin and kinesin II (Fig. 2 B, b). More dynein and dynactin in an aggregated cell lysate cosediment with microtubules than in a dispersed cell lysate (Fig. 2 B, a and b, lanes 3 and 4). Conversely, more kinesin II cosediments with microtubules when this motor is derived from a dispersed cell lysate than from an aggregated cell lysate (Fig. 2 B, b, lanes 3 and 4). Conventional kinesins are present in Xenopus melanophores but are not on pigment granules and does not drive their transport (Rogers et al., 1997; Tuma et al., 1998), and this kinesin exhibits no differential binding to microtubules as a function of the direction of organelle transport (Fig. 2 B, c). These findings indicate that those motors involved in driving pigment transport are specifically targeted by the regulatory system in melanophores. Furthermore, the entire pool of these motors, not just those on the pigment granules, is regulated. Accordingly,
because it is regulated in concert with the pigment granule–associated motors/dynactin and is much more abundant, we use the soluble pool as the source of dynein, dynactin, and kinesin II in all subsequent studies. We refer to dynein, dynactin, or kinesin II that is competent to bind microtubules as active and that which is incompetent to bind microtubules as inactive.

**The Pool of Active Motor/Dynactin Rises and Falls during Bidirectional Transport**

Pigment granules saltate during both directions of transport in *Xenopus* melanophores, and isolated pigment granules show biased rather than unidirectional movements on microtubules (Rogers et al., 1997). These observations suggest that dynein and kinesin II might be active during both directions of movement. To determine if there is opposing motor activity during aggregation or dispersion, we examined the microtubule binding behavior of dynein, dynactin, and kinesin II obtained from melanophores in the process of aggregating and dispersing pigment. Cells with dispersed pigment were induced to undergo pigmentation aggregation and redispersion (Fig. 3). At various times during transport, cells were lysed, and the motors/dynactin were examined for their microtubule binding activity (Fig. 3). As the pool of active dynein and dynactin increases (Fig. 3, lane 2) as the pool of active kinesin II decreases (Fig. 3, lane 1). In cells with fully aggregated pigment, the size of the active pool of dynein and dynactin is at a maximum whereas that of kinesin II decreases (Fig. 3, lane 3). During redispersion of pigment, the active pool of dynein and dynactin falls as that of kinesin II increases (Fig. 3, lane 4) until, in fully dispersed cells, the size of the active pool of kinesin II is at a maximum whereas no active dynein or dynactin is detected (Fig. 3, lane 5). These observations suggest that some opposing motor is active during each direction of transport, a finding that may explain why pigment granules saltate during aggregation and dispersion. Therefore, change in the net direction of pigment granule transport likely occurs when the ratio of active dynein to active kinesin II reaches some critical threshold.

**Immunoprecipitated Motors/Dynactin Differentially Capture Microtubules**

The differential binding to microtubules is either an inherent property of the motors/dynactin or is due to some other factor in the pigment granule extract or the cell lysate. Therefore, we sought to determine if purified motors/dynactin retain their differential microtubule binding behavior. To conduct these studies, we developed a “microtubule capture” assay. Dynein, dynactin antibody–bead complexes (left) are then challenged to bind microtubules (MTs). Microtubules are captured by active dynein, dynactin, or kinesin II immobilized on the beads (right). (B) Dynein (a), dynactin (b), and kinesin II (c) from aggregated (A) and dispersed (D) melanophore lysates were immunoprecipitated, incubated with taxol-stabilized microtubules, and probed either for motors/dynactin (top) or for tubulin (bottom). Heavy and light chains of the immunoprecipitating antibodies are also detected in the top panels. DIC, intermediate chain of cytoplasmic dynein; 150, p150glued subunit of dynactin; 85, 85-kD motor subunit of kinesin II; tub, tubulin. Dynine and dynactin from aggregated cells and kinesin II from dispersed cells capture microtubules. (C) Microtubule capture by motors is ATP sensitive. Dynine and dynactin immunoprecipitated from aggregated melanophore extracts and kinesin II immunoprecipitated from dispersed extracts were incubated with taxol-stabilized microtubules, washed, and incubated in the presence (+) or absence (−) of 1 mM ATP. Immunoblotting for tubulin reveals that microtubules dissociate from dynein and kinesin II, but not from dynactin, in the presence of ATP.

![Figure 4. Microtubule capture by immobilized dynein, dynactin, and kinesin II varies as a function of the direction of pigment transport. (A) Model for microtubule capture assay. Protein A–antibody beads with bound antibodies (primary antibodies 18ab 74.1, p150, or K2.4) are incubated with aggregated or dispersed *Xenopus* melanophore lysates to immunoprecipitate the appropriate motor/dynactin. Motor/dynactin antibody–bead complexes (left) are then challenged to bind microtubules (MTs). Microtubules are captured by active dynein, dynactin, or kinesin II immobilized on the beads (right). (B) Dynein (a), dynactin (b), and kinesin II (c) from aggregated (A) and dispersed (D) melanophore lysates were immunoprecipitated, incubated with taxol-stabilized microtubules, and probed either for motors/dynactin (top) or for tubulin (bottom). Heavy and light chains of the immunoprecipitating antibodies are also detected in the top panels. DIC, intermediate chain of cytoplasmic dynein; 150, p150glued subunit of dynactin; 85, 85-kD motor subunit of kinesin II; tub, tubulin. Dynine and dynactin from aggregated cells and kinesin II from dispersed cells capture microtubules. (C) Microtubule capture by motors is ATP sensitive. Dynine and dynactin immunoprecipitated from aggregated melanophore extracts and kinesin II immunoprecipitated from dispersed extracts were incubated with taxol-stabilized microtubules, washed, and incubated in the presence (+) or absence (−) of 1 mM ATP. Immunoblotting for tubulin reveals that microtubules dissociate from dynein and kinesin II, but not from dynactin, in the presence of ATP.](image-url)
that tubulin may be modified by the kinesin II immunoprecipitate. It appears as a doublet (c), and see also in Figs. 4 and 6), suggesting PKA or PP2A treatment. Note that tubulin captured by kinesin II. The ability of dynactin to capture microtubules is unaffected by inhibition of microtubule capture by kinesin II from dispersed cells, whereas PP2A enhances microtubule capture by kinesin II from aggregated cells, whereas PP2A enhances microtubule capture by kinesin II from aggregated cells. PKA inhibits microtubule (MT) capture by dynein and enhances microtubule capture by kinesin II from aggregated cells, whereas PP2A enhances microtubule capture by kinesin II. The ability of dynactin to capture microtubules is unaffected by PKA before addition of microtubules. PKA-treated dynein–microtubule interactions so that minus end–directed transport is favored. PP2A has the opposite effect on the motors’ microtubule binding behavior of motors/dynactin, we incubated the immunoprecipitated proteins with PP2A before microtubules captured by untreated motors from dispersed cells (Fig. 5 a and c, compare tubulin in lane 6 with lane 2) and diminishes for motors/dynactin from dispersed cells (Fig. 5 a and c, compare tubulin in lane 6 with lane 2). In contrast, PKA-treated kinesin II from dispersed cells is unaltered by PKA treatment (Fig. 5, a and c, compare tubulin in lane 3 with lane 1), as expected if these motors have already been affected by PKA during dispersion in vivo. Unlike dynein and kinesin II, dynactin’s ability to capture microtubules is unchanged by treatment with PKA (Fig. 5 b, compare tubulin in lanes 3 and 4 to lane 1 with lane 2). These findings suggest that phosphorylation mediated by PKA induces dispersion in vivo by inactivating dynein–activating kinesin II–microtubule interactions so that plus end–directed microtubule transport is favored.

**PP2A Activates Dynein–Microtubule Interactions and Inhibits Kinesin II–Microtubule Interactions**

Aggregation of microtubules in *Xenopus* melanophores requires the participation of PKA (Reilein et al., 1995). Treatment of immunoprecipitated proteins with phosphatase alters the microtubule binding behavior of dynactin, we incubated the immunoprecipitated proteins with PP2A before microtubules captured by untreated motors from dispersed cells (Fig. 5 a and c, compare tubulin in lane 6 with lane 2). In contrast, PKA-treated motors from dispersed cells is equivalent to that captured by untreated motors from aggregated cells (Fig. 5, a and c, compare tubulin in lane 6 with lane 1). The microtubule binding behavior of dynein and kinesin II is not affected by treatment with PKA (Fig. 5 a and c, compare tubulin in lane 5 with lane 1), as expected if these motors have already been altered by PKA during aggregation in vivo. In contrast to dynein and kinesin II, dynactin’s microtubule binding behavior is unchanged by treatment with PP2A (Fig. 5 b). PP2A has the opposite effect on the motors’ microtubule binding properties as PKA, and dephosphorylation by PP2A may induce aggregation in vivo by activating dynein–activating kinesin II–microtubule interactions so that minus end–directed transport is favored.

**Dynactin–Microtubule Binding Is Inhibited by PKC**

Dynactin’s microtubule binding behavior is unaffected by treatment with either PKA or PP2A (Fig. 5 b). Nevertheless, because we observe that dynactin binds to microtubules differentially as a function of the direction of pigment granule transport in the melanophores from which the dynactin is isolated, dynactin’s behavior might be modified by other kinases or phosphatases in vivo. PKC induces partial dispersion in *Xenopus* melanophores (Reilein et al., 1995). Treatment of immunoprecipitated dynactin from aggregated cells with this kinase diminishes...
Dynactin’s ability to capture microtubules (Fig. 6 b, compare lane 3 with lane 1), converting its microtubule binding behavior to one mimicking dynactin in dispersed cells (Fig. 6 b, compare lane 3 with lane 2). Unlike dynactin, dynein from aggregated cells remains active after treatment with PKC (Fig. 6 a, compare lane 3 with lane 1). Dynactin isolated from dispersed cells is unaffected by PKC treatment (Fig. 6 b, compare lane 4 with lane 2), as expected if dynactin’s behavior has already been altered by PKC during dispersion in vivo. PKC may partially enhance dispersed dynein’s activity (Fig. 6 a, compare lane 4 with lane 2), but we have not observed this effect routinely. PKC also has little effect on the behavior of kinesin II (Fig. 6 c, compare lane 4 with lane 2). These findings suggest that dynactin’s ability to bind microtubules is more sensitive to calcium/calcmodulin and a functionally related phosphatase than is that of the other two molecules. Moreover, dynactin is affected by a distinct kinase.

Dynactin–Microtubule Binding Is Enhanced by PP1

Because dynactin–microtubule interactions can be inhibited by a kinase, dynactin–microtubule interactions should be enhanced by a phosphatase. PP2B (calcineurin) is required for aggregation in fish melanophores (Thaler and Haimo, 1990), but this phosphatase has no effect on Xenopus dynactin–microtubule binding or on dynein– or kinesin II–microtubule binding (Fig. 6, lanes 5–8). The phosphatase PP1 has not previously been implicated in regulating organelle transport in melanophores (Reilein et al., 1998), but has been implicated in other microtubule–dependent movements (Allan, 1995; Habermacher and Sale, 1996; Lindesmith et al., 1997). Unlike PP2A or PP2B, PP1 enhances the amount of microtubules captured by immobilized dynactin from dispersed melanophore extracts (Fig. 6 b, compare lane 12 with lane 10), converting the microtubule binding behavior of this dynactin to one mimicking that of dynactin isolated from aggregated cells (Fig. 6 b, compare lane 12 with lane 9). PP1 has no effect on the microtubule binding behavior of dynactin obtained from melanophores with aggregated pigment (Fig. 6 b, compare lane 11 with lane 9), as expected if a dephosphorylation event during aggregation in vivo has already altered dynactin’s behavior. Neither dynein– nor kinesin II–microtubule binding is affected by PP1 treatment (Fig. 6, a and c, compare lanes 11 and 12 with lanes 9 and 10). Dynactin’s microtubule binding ability, like that of dynein’s, is enhanced by a kinase and inhibited by a phosphatase, yet dynactin and dynein may be regulated independently as they are affected by different kinases and phosphatases.

Discussion

The Microtubule Binding Activity of Dynein, Dynactin, and Kinesin II Is Regulated by Specific Kinases and Phosphatases during Bidirectional Organelle Transport

Our results support the hypothesis that motor–microtubule–cargo interactions are regulated to control the direction of pigment granule transport in melanophores. Dynein and dynactin bind microtubules when these proteins are isolated from melanophores with aggregated, but not dispersed, pigment. Kinesin II binds microtubules when it is isolated from melanophores with dispersed, but not aggregated, pigment. Given that dynein drives aggregation (Nilsson and Wallin, 1997), whereas kinesin II drives dispersion (Tuma et al., 1998), the in vitro microtubule binding properties of each of these proteins correlates with its expected in vivo activity. Moreover, conventional kinesin, which does not drive pigment granule transport (Tuma et al., 1998), is not regulated in concert with aggregation and dispersion. Therefore, control of the direction of pigment granule transport appears to be accomplished by specifically regulating the microtubule binding activity of the molecules that drive this transport, dynein, and dynactin, and kinesin II.

The microtubule binding behavior of the soluble pool of dynein, dynactin, and kinesin II mimics that of the pigment granule–associated proteins (see Fig. 2), suggesting that the regulatory system does not specifically target the motors/dynactin on these organelles, but, instead, modifies the entire pool of these proteins. Because pigment granule–associated motors cannot be extracted in quantity, we have used the soluble pool in several of our studies. We infer a mechanism underlying regulation of the direction of pigment granule transport, the accuracy of which depends on whether the behavior of dynein, dynactin, and kinesin...
II in the soluble pool reflects that of the cognate pigment granule–associated proteins. Nevertheless, these findings reveal an important feature of dynein, dynactin, and kinesin II that has not been recognized previously: their ability to interact with microtubules can be regulated.

Protein phosphorylation and dephosphorylation control the direction of pigment granule transport in melanophores (Rozdzial and Haimo, 1986). We find that the microtubule binding behavior of the motors can be reversed in vitro by treatment with PKA and PP2A, the kinase and phosphatase involved in regulating opposing directions of pigment granule transport in *Xenopus* melanophores in vivo (Reilein et al., 1998). Dynein’s microtubule binding activity is activated by PP2A and inhibited by PKA, whereas kinesin II’s is inhibited by PP2A and activated by PKA. Our findings suggest that PKA induces dispersion by activating kinesin II’s ability to interact with microtubules, whereas simultaneously inhibiting dynein’s. Conversely, PP2A induces aggregation by inhibiting kinesin II’s ability to interact with microtubules, while simultaneously activating dynein’s. Dynactin, like dynein, is activated by phosphatase treatment and inhibited by kinase treatment, but a distinct kinase and phosphatase, PKC and PP1, respectively, are responsible for its modulation. PKC induces partial pigment granule dispersion in vivo, but a role for PP1 in regulating pigment granule transport in melanophores has not been recognized previously (Reilein et al., 1998).

We believe that the change in motor/dynactin–microtubule binding behavior that we observe upon treatment of these molecules with specific kinases or phosphatases reveals the underlying mechanism controlling pigment transport in vivo for the following reasons. Dynein’s microtubule binding properties of each protein, the kinase and phosphatase that do act on each protein, and the kinase and phosphatase that do not act on each protein, suggest that this protein has two functional states. Dynein is active when isolated from aggregated cells and that PP2A converts inactive dynein from dispersed cells to active dynein. (c) PKA, PKC, and PP2A have been demonstrated to regulate pigment granule transport, and PP1 has been implicated in other microtubule-dependent movements, suggesting that the alterations in behavior conferred on the motors/dynactin by these enzymes are not an in vitro artifact. (d) These enzymes only alter the behavior of the motors or dynactin from melanophores that have transported pigment in one direction and not the other. For example, PKA inhibits the microtubule binding activity of dynein that has been isolated from aggregated cells. Dynein that is isolated from dispersed cells is already in its inactive state and is not further inhibited by PKA. (e) Finally, when motors are isolated from cells with aggregated or dispersed pigment, one motor is active, whereas the other is not. PKA and PP2A, respectively, reverse the behavior of the motors in vitro and retain this feature. One motor is active, whereas the other is inactive, after treatment with either of these enzymes. These findings suggest that cyclic phosphorylation and dephosphorylation induce dispersion and aggregation in melanophores (Rozdzial and Haimo, 1986) by cyclically activating and inhibiting motor/dynactin–microtubule interactions, thereby controlling the direction of transport; this system is modeled in Fig. 7.

**Dynactin Regulation May Not Be Required for Bidirectional Transport**

The biological function of dynactin is not well understood. Dynactin is required by dynein to transport organelles (Gill et al., 1991). It may do so by enhancing dynein’s processivity and thereby prevent organelles from diffusing away from the microtubule (King and Schroer, 2000). Dynactin may also control dynein’s phosphorylation state, and thereby its activity (Kumar et al., 2000). We show here not only that the interaction of motors with microtubules can be regulated, but also that the interaction of dynactin with microtubules can be similarly regulated. Accordingly, studies seeking to elucidate the role of dynactin will need to consider that this protein has two functional states. Dynactin is in its active state when dynactin drives pigment granule aggregation. How, then, does net displacement of the granules occur if active dynactin tethers them to the microtubules in a non–ATP-dependent manner? We observe that relative to dynactin both dynein and kinesin II become concentrated on microtubules in cosedimentation assays.

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**Figure 7.** Model for the bidirectional transport of pigment granules along microtubules. Protein phosphorylation and dephosphorylation control the direction of transport in melanophores. A pigment granule at the minus end of a microtubule (left) is bound to the microtubule by active dynein and dynactin (filled heads), whereas inactive kinesin II (clear heads) is unable to bind to the microtubule. PKA and PKC, activated upon stimulation of melanophores with MSH, convert dynein and dynactin to their inactive forms (clear heads), whereas PKA converts kinesin II to its active form (filled heads), allowing it to bind to microtubules. Active kinesin II transports pigment granule towards the plus end of the microtubule, and aggregation of pigment granule transport corresponds to the minus end of a microtubule (right) is converted to its inactive form (clear heads) by PP2A and PKC, activated upon stimulation of melanophores with MSH, whereas inactive kinesin II (clear heads) is unable to bind to the microtubule by active dynein and dynactin (filled heads), whereas inactive kinesin II (clear heads) is unable to bind to the microtubule by active dynein and dynactin (filled heads). PP1 may enhance dynein-mediated aggregation by anchoring pigment granules at the minus ends of microtubules after their transport.
(see Fig. 2), suggesting that dynactin’s affinity for microtubules may be significantly weaker than the motors’. Thus, dynactin might contribute to dynein’s processivity by stochastically tethering the granules to the microtubules, yet not interfere significantly with force generation if it also dissociates frequently from the microtubules. Activated dynactin’s affinity for microtubules needs to be measured to determine if this proposal is viable. Because dynactin’s behavior is modified by a different kinase and phosphatase than is dynein’s, these two proteins may, in any case, behave independently during aggregation and dispersion in vivo. Dynactin is converted into its active microtubule binding form by treatment with PP1, yet aggregation can be induced in Xenopus melanophores under conditions in which PP1 is inhibited (Reilein et al., 1998). Given that aggregation can occur in the absence of PP1 activity, then dynein apparently is capable of driving aggregation without dynactin being in its active microtubule-binding form. Active dynactin might serve only a modulatory role in pigment aggregation, facilitating longer excursions by the pigment granules towards the minus ends of microtubules. Alternatively, active dynactin might anchor pigment granules at the minus ends of microtubules only after aggregation, mediated by active dynein, has been completed.

Partial dispersion can be induced in Xenopus melanophores by PKC without simultaneous activation of PKA (Sugden and Rowe, 1992; Reilein et al., 1998). We find here that PKC alters the behavior of dynactin, but not dynein or kinesin II, converting dynactin to its inactive form. The extent of dispersion induced by PKC (Reilein et al., 1998) appears similar to the reported “relaxation” of the aggregated pigment mass after microtubules are depolymerized in melanophores of an antibody that inactivates kinesin V (Rogers et al., 1998). Dynactin keeps organelles tethered at the minus ends of microtubules, then if PKA is activated it result in release of the granules. In a current experiment, we are testing whether PKC is sufficient to drive partial dispersion of pigment granules away from cell center. If not, we can infer that basal amounts of active dynactin are sufficient to drive partial dispersion of granules dynein is not activated. Inactivation of dynactin, however, is apparently not a prerequisite for pigment to disperse. Complete dispersion can be induced by PKA even when PKC activity is inhibited (Reilein et al., 1998). Thus, although dynactin regulation clearly occurs during aggregation and dispersion, this regulation may enhance, but not be an essential component of, bidirectional transport.

Opposing Active Motors May Generate Saltatory Movements during Net Directional Transport

In Xenopus melanophores, pigment granules saltate during each direction of transport (Rogers et al., 1997), a feature shared by many other organelles in other cell types (Rebun, 1964; Pryer et al., 1986; Weiss et al., 1986). Saltations are characterized by short excursions of organelles in both directions along microtubules. Our findings that some active dynein and dynactin can be detected during dispersion and that some active kinesin II can be detected during aggregation suggest that a small pool of active motor may be able to drive these transient movements stochastically against the net direction of transport. Supporting this hypothesis is the observation that microtubules undergo reversals of direction in gliding assays performed in the presence of both active kinesin and cytoplasmic dynein (Vale et al., 1992). Thus, even if one motor overrides the other (Muresan et al., 1996), detachment of the overriding motor from the microtubule would provide the other motor an opportunity, if only briefly, to generate transport in the opposite direction. We propose that the size of each motor’s active pool determines the net direction of pigment granule transport along microtubules. As phosphorylation causes the pool of active dynein and dynactin to decrease and that of kinesin II to increase, net dispersion occurs. As dephosphorylation causes the pool of active dynein and dynactin to rise and that of kinesin II to fall, net aggregation occurs.

Regulating Transport of Different Organelles

Different organelles possess unique members of the kinesin superfamily (for reviews see Hirokawa, 1996; Goldstein and Philp, 1999) and may also possess unique isoforms of cytoplasmic dynein (Vaisberg et al., 1996). These motors likely vary in their sensitivity to regulation by particular kinases and phosphatases. For example, in the crayfish giant axon, activation of PKC inhibits vesicle, but not mitochondrial, transport (Okada et al., 1995). Changes in the distribution of pigment granules during aggregation, however, are blocked by the other kinase. Thus, not all organelles do not aggregate during aggregation and dispersion, but such dispersion may be accomplished by different mechanisms. Lysozymes, for example, aggregate during dispersion (Tuma et al., 1998). Conversely, pigment granules, are transported by conventional kinesin (Tuma et al., 1998), and we find that microtubule binding by this kinesin is not regulated by PKA. Aggregation and dispersion of pigment. An increase in cytoplasmic pH results in lysosomal clustering at the cell surface (Heuser, 1989), and an increase in pH has also been reported to inhibit conventional kinesin–microtubule binding (Verhey et al., 1998). This pH change may mimic a dephosphorylation event on conventional kinesin, but not on kinesin II, thereby altering the activity of kinesin. Differential regulation of various motors residing on different organelles would provide the cell the ability to control with precision the distribution of its organelles.

The Role of Myosin V in Regulation of the Direction of Pigment Granule Transport

Dispersion in Xenopus melanophores involves force generation mediated not only by kinesin II on microtubules, but also by myosin V on actin filaments (Rogers and Gelfand, 1998; Tuma et al., 1998; Rogers et al., 1999). Transport of pigment granules from microtubules onto actin filaments by myosin V may provide a mechanism for achieving a uniform distribution of pigment throughout the cytoplasm of dispersed cells (Tuma and Gelfand, 1999). Therefore, pigment granules might be expected to accumulate at the cell periphery during dispersion when myosin V cannot function. In fact, the reverse occurs in Xenopus melanophores, and pigment granules spontaneously aggregate in the absence of a functional actin–myosin V system (Rogers and Gelfand, 1998; Rogers et al.,
1999). Accordingly, it has been proposed that dynein activity may predominate over kinesin II activity in X. laevis melanophores (Tuma et al., 1998). This proposal is difficult to resolve with the current finding that dynein and dynactin are inactive, and kinesin II is active in dispersed cells. It is possible that signal transduction and, thus, the normal state of dynein, dynactin, and/or kinesin II activation is altered in the absence of a functional actin–myosin V system; therefore, pigment granules aggregate when they would be predicted to disperse. A complete understanding of the mechanism regulating the direction of transport will require that the activity of and relationship between these two motor systems be more fully elucidated.

In summary, we provide evidence that cytoplasmic dynein, dynactin, and kinesin II are cyclically activated and inactivated, either directly or via coinmunoprecipitating proteins, by phosphorylation and dephosphorylation to control their interaction with microtubules, and as a result the direction of organelle transport. It will be necessary to identify the targeted phosphorylation sites on these motors/dynactin or associated proteins and determine how their modification affects the ability of these proteins to interact with microtubules. In addition, it will be of great interest to determine if the transport of other organelles is also regulated by changes in the microtubule binding activity of their microtubule motors.

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Using the methods published in Reese and Haimo (2000), the author’s laboratory has been unable to reproduce the data in Figs. 2–6 of this article that demonstrate microtubule motor and dynactin regulation as a function of the direction of organelle transport in melanophores. The authors wish to correct the scientific record and retract the findings of this paper until such time as they are able to resolve the discrepancy.

A statement from each of the authors agreeing to the retraction of this article is on file at the Rockefeller University Press.