Cytoplasmic dynein-associated structures move bidirectionally in vivo

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Summary

Intracellular organelle transport is driven by motors that act upon microtubules or microfilaments. The microtubule-based motors, cytoplasmic dynein and kinesin, are believed to be responsible for retrograde and anterograde transport of intracellular cargo along microtubules. Many vesicles display bidirectional movement; however, the mechanism regulating directionality is unresolved. Directional movement might be accomplished by alternative binding of different motility factors to the cargo. Alternatively, different motors could associate with the same cargo and have their motor activity regulated. Although several studies have focused on the behavior of specific types of cargoes, little is known about the traffic of the motors themselves and how it correlates with cargo movement. To address this question, we studied cytoplasmic dynein dynamics in living Dictyostelium cells expressing dynein intermediate chain-green fluorescent protein (IC-GFP) fusion in an IC-null background. Dynein-associated structures display fast linear movement along microtubules in both minus-end and plus-end directions, with velocities similar to that of dynein and kinesin-like motors. In addition, dynein puncta often rapidly reverse their direction. Dynein stably associates with cargo moving in both directions as well as with those that rapidly reverse their direction of movement, suggesting that directional movement is not regulated by altering motor-cargo association but rather by switching activity of motors associated with the cargo. These observations suggest that both plus- and minus-end-directed motors associate with a given cargo and that coordinated regulation of motor activities controls vesicle directionality.

Introduction

Intracellular organelle transport is essential for many cellular processes. Motors that move along microtubules or microfilaments drive directional organelle transport. The minus-end-directed microtubule-based motor cytoplasmic dynein and the plus-end-directed microtubule-based motors of the kinesin family are believed to be responsible for minus-end-directed and plus-end-directed transport of intracellular cargoes along microtubules. Many vesicles display bidirectional movement along microtubules; however, the mechanisms regulating directionality are not well understood. Given that all of the motors discovered to date are unidirectional motors, two different mechanisms for controlling the directionality of vesicle transport have been proposed. In the first, the association model, directionality is achieved through specific association of different motors with the cargo, that is, cargo transported in the plus-end direction is associated with only plus-end-directed motors, whereas cargo transported toward the minus ends of microtubules binds only to minus-end-directed motors. The second mechanism, the regulation model, suggests that both plus-end- and minus-end-directed motors simultaneously associate with any given cargo and that the activities of the motors are regulated such that motors with the required directionality are dominant.

Previous evidence distinguishing between these models was based on immunohistochemistry studies of fixed cells and tissues. Several studies have indicated that for both dynein and kinesin-like motors, motor-cargo interaction could be regulated by phosphorylation (Dillman and Pfister, 1994; Lee and Hollenbeck, 1995; Lin et al., 1994; Marlowe et al., 1998; Niclas et al., 1996; Sato-Yoshitake et al., 1992), although it is not clear whether the cell actually uses this mechanism to regulate directional transport. Evidence for the regulation model came from comparing the distribution of motors and specific groups of membranous organelles. In neuronal axons, cytoplasmic dynein heavy chain antibody stained vesicles accumulated both proximal and distal to a ligation, suggesting that dynein associates with vesicles moving in both retrograde and anterograde direction (Hirokawa et al., 1990). In endoplasmic reticulum (ER)-Golgi membrane traffic, kinesin was immunolocalized not only to the membrane compartment destined for Golgi-to-ER transport but also to those in the reverse direction (Lippincott-Schwartz et al., 1995). Similar results have been shown by immunofluorescence localization of cytoplasmic dynein in the ER-Golgi system (Roghi and Allan, 1999). In melanocytes, both kinesin-like motors and dynein were detected by immunoblotting on aggregated and dispersed populations of melanophores (Nilsson et al., 1996; Reese and Haimo, 2000). These studies indicate that motors could localize to cargoes that potentially move in a direction opposite to their polarity, suggesting that motors do not dissociate from cargo moving in the wrong direction. However,
actual motor movement in both directions has not been directly visualized. Localization of both types of motors on a single vesicle could occur on static cargo instead of moving ones. Moreover, it is not clear how directional reversal occurs, what time scale it involves nor what the dynamics of the associated motors are. These questions cannot be easily addressed by static studies in fixed samples but rather require dynamics analysis in living cells.

Several recent studies have focused on the dynamic behavior of specific types of cargoes using GFP-fusions (Gross et al., 2000; Presley et al., 1997; Suomalainen et al., 1999; Wubbolts et al., 1999; Ye et al., 2000). These studies have revealed that many cargoes displayed bidirectional movement and could reverse direction of movement rapidly. However, the behavior of the motor has not been studied. To characterize dynein dynamics, we have analyzed the dynamics of a dynein intermediate chain (IC)-green fluorescent protein (GFP) fusion. We have generated stable cell lines that express the IC-GFP fusion in an IC-null background in Dictyostelium discoideum and have shown that the IC-GFP fusion functions normally. By time-lapse fluorescence microscopy, we observed that dynein travels bidirectionally along the microtubules and that dynein remains stably associated with the cargo during the rapid reversals of movement direction. Our results provide support for a model in which both minus- and plus-end-directed motors coexist on a given cargo and regulation of motor activities controls the directionality of transport.

Materials and Methods

Cell lines and constructs

To generate an IC-GFP expression construct, full-length dynein IC and GFP cDNAs were joined in frame at the IC C-terminus, with the stop codon of IC cDNA removed by PCR and inserted into Dictyostelium expression vector pBorp (Ostrow et al., 1994). The IC gene targeting construct consists of upstream (2.7 kb) and downstream (1.8 kb) dic targeting sequences surrounding the rhl1 gene (Hadwiger and Firtel, 1992). The upstream and downstream targeting sequences were originated from the Dictyostelium genomic sequence flanking the dic coding region, therefore should only target the endogenous dic locus but not the expression construct. For IC-GFP expression in the wild type, the fusion expression construct was transformed into Dictyostelium strain JH10 (Hadwiger and Firtel, 1992) by electroperoration (Pang et al., 1999). Stable transformants were screened for IC-GFP expression by immunoblotting with the IC antibody, and clones that express IC-GFP at a level similar to the endogenous IC were chosen as hosts for IC gene targeting. The linearized IC-targeting construct was then transformed into these IC-GFP expressing cell lines, and the resulting clones were screened for the loss of endogenous IC by immunoblotting.

Immunoblots and immunoprecipitations

The antibodies used in this study include: anti-Dictyostelium dynein IC, IC144; anti-Dictyostelium dynein heavy chain, NW127; and anti-Dictyostelium capping protein β. These antibodies have been previously described (Ma et al., 1999). To screen for protein expression in the transformants, protein samples were prepared from cells collected directly from the 96-well plates. For immunoblots, protein samples were separated on 7.5% SDS-polyacrylamide gels and transferred to PVDF membranes. Blots were blocked in 5% nonfat milk and incubated with the IC144 antibody against dynein IC (Ma et al., 1999) followed by an HRP-conjugated goat-anti-rat antibody. Blots were developed in Renaissance enhanced chemiluminescence reagent (NEN Life Science Products, Boston, MA) and exposed to X-ray film. For immunoprecipitations (IPs), 4×10⁷ cells were collected and washed twice with 15 mM Na-KPO₄ buffer, pH 6.5. Following resuspension in 1 ml IP buffer (50 mM PIPES, pH 6.8, 5 mM EDTA, 0.1% NaN₃, 25 mM Na pyrophosphate, 2.5 mM DTT, 1 mM PMSE, 50 μg/ml leupeptin, 50 μg/ml pepstatin, 1 mM ATP) cells were lysed by sonication. The cell lysate was cleared by centrifugation at 38,000 g for 30 minutes at 2°C. Protein-A sepharose preincubated with the IP antibody was added to the cell lysate and the mixture incubated while rocking for at least 2 hours at 4°C. Dynein heavy chain antibody NW127 was used to IP the dynein complex, whereas affinity-purified capping protein β antibody R18 was used to IP the dynactin complex. Sepharose-head-bound immune complexes were collected by centrifugation and washed four times with IP buffer. The final pellets were resuspended in 30 μl 2×SDS sample buffer (125 mM Tris, pH 6.8, 4% SDS, 10% 2-mercaptoethanol, 20% glycerol), boiled for 5 minutes, centrifuged and the supernatant collected and analyzed by immunoblot.

Immunofluorescence staining

For tubulin staining, cells plated on coverslips were fixed in 1.85% formaldehyde in 15 mM Na-KPO₄, pH 6.5 for 5 minutes at room temperature, then extracted in –15°C methanol for 5 minutes. The coverslips were then stained with a rat anti-α-tubulin monoclonal antibody (Serotech, Ltd.) followed by a lissamine-conjugated goat-anti-rat secondary antibody.

Image capturing and analysis

Cells expressing IC-GFP in an IC-null background were used for GFP imaging. Living cells were flattened by agar overlay (Yumura et al., 1984) and imaged in a humid chamber. GFP signals were observed by fluorescence microscopy with a 100× 1.4 N.A. PlanNeoFluar oil objective. Images were captured using Micromax 512 BFT cooled CCD camera (Princeton Instruments). Time-lapse sequences were captured using the streaming mode, which allows 302 msec intervals with 300 msec exposures. Metamorph 4.0 (Universal Imaging) was used for image capturing and data analysis.

Results

IC-GFP expression in an IC-null background in Dictyostelium

To study dynein dynamics, we sought to express an IC-GFP fusion in an IC-null background in Dictyostelium discoideum so that the only IC present would be the tagged version. Because dynein is essential for Dictyostelium viability (Koonce and Knecht, 1998; Ma et al., 1999), it was not possible to generate an IC-null cell line. Instead, we first produced stable cell lines that express IC-GFP in a wild-type background and selected clones that expressed the tagged IC at a level similar to that of the endogenous IC (Fig. 1a). Then, we disrupted the endogenous IC locus by gene targeting using IC gene flanking sequences not present in the expression construct (Fig. 1b,c). Because of the size-shift caused by the GFP-tag, it was possible to easily distinguish the IC-GFP fusion from the endogenous IC and screen for IC-GFP expression as well as the loss of endogenous IC by immunoblotting with an IC antibody (Fig. 2a). We have identified four positive clones from a total of 90 clones screened, with the average homologous recombination efficiency of 4.2%. This two-step procedure effectively replaced the endogenous IC with IC-GFP expressed at wild-type IC levels.
IC-GFP is functionally comparable to wild-type IC

IC plays an important role in cytoplasmic dynein function. A major function of dynein IC is to link dynein to its regulatory partner, the dynactin complex (Karki and Holzbaur, 1995; Ma et al., 1999; Vaughan and Vale, 1995). It has previously been demonstrated that the N-terminal domain of IC is critical for interacting with dynactin, and the C-terminal region is essential for dynein association (Karki and Holzbaur, 1995; Ma et al., 1999; Vaughan and Vale, 1995). To directly compare the ability of IC-GFP and native IC to associate with dynein and dynactin, we performed immunoprecipitations using cells that express both proteins. The ratio of fusion to native protein in either dynein or dynactin immunoprecipitates was similar to their ratio in cell lysates, suggesting that the IC-GFP fusion efficiently associates with both complexes (Fig. 2b). Moreover, cells expressing only IC-GFP were phenotypically indistinguishable from wild-type cells with respect to growth rate, microtubule and spindle morphology and multicellular development, indicating that IC-GFP is physiologically functional. Since it is known that over 90% of the IC in Dictyostelium associates with the dynein complex (Ma et al., 1999), the IC-GFP signal in these cells represents dynein’s normal intracellular localization. The IC-GFP distribution agrees with that previously reported for the dynein heavy chain (Koonce and McIntosh, 1990).

Dynein-containing structures move bidirectionally along microtubules in living cells

IC-GFP expression allowed us to study dynein dynamics in living cells by following GFP signals with time-lapse fluorescence microscopy. In still images, dynein typically appeared as puncta distributed throughout the cytoplasm, with a strong perinuclear accumulation that coincided with the microtubule organizing center (MTOC) (Fig. 2c; Fig. 3). In living cells, the highly dynamic dynein typically moved rapidly over long distances along linear tracks extending radially from the MTOC, moving both towards and away from the MTOC. The linear tracks for dynein traffic most probably represent microtubules for several reasons. First, in cells fixed and stained to display microtubules, there is significant colocalization of dynein puncta and microtubules (Fig. 3). Second, dynein moves along microtubules in vitro. Third, living cells imaged with long exposures (2 seconds or greater), or when a time series of images was superimposed, showed dynein averaged into linear tracks closely resembling the pattern of interphase microtubules (Fig. 2d). It is unlikely that the movement of dynein was driven by microtubule dynamics because the microtubule polymerization rate (0.05-0.35 μm/second; (Cassimeris et al., 1988; Sammak and Borisy, 1988)), depolymerization rate (0.07-0.6 μm/second; (Cassimeris et al., 1988; Mitchison and Kirschner, 1984; Sammak and Borisy, 1988)) and treadmilling rate (50-60 μm/hour; (Farrell et al., 1987)) are too slow to account for the observed speed of 1-2 μm/second. Also, microtubule dynamics cannot explain several dynein puncta traveling along the same track. Therefore, the linear movements probably represent dynein, which is likely to be associated with the surface of cargo, moving along microtubules.

It has been well established that Dictyostelium interphase microtubules, like in most other eukaryotic cells, are organized in a radial array with their minus ends focusing on the...
microtubule organizing center (MTOC) in the center of the cell and their plus ends extended to the cell periphery. These established microtubule polarities were used to determine direction of dynein movement. Because the central mass of dynein-GFP colocalizes with the MTOC (Fig. 3) and because dynein moves along the radial array of microtubules, we refer to movements toward the central dynein mass as traveling towards microtubule minus ends, and movement away from the central mass as traveling towards microtubule plus ends. There are a small number of MTs that are curved back towards the center of the cell, resulting in the plus ends being away from the cell periphery. We have taken care to avoid using such MTs in our analysis.

As a minus-end-directed microtubule-based motor, dynein-engaged cargo would be expected to travel towards the MTOC. Indeed, we frequently observed dynein moving towards the central dynein mass along linear tracks (Fig. 4a). However, we also observed, almost equally, as many examples of dynein moving away from the central mass towards the cell periphery (Fig. 4b). The microtubule plus-end-directed movement of dynein is most probably a result of dynein being passively moved through its association with a cargo carried by a plus-end-directed motor. Dynein-associated structures moving along microtubules in the plus-end direction suggests that motors of opposing polarities co-exist on these cargoes.

The average velocity of dynein movement in either direction is similar, with minus-end-directed movement occurring at 1.8±0.3 μm/second (n=65) and plus-end-directed movement at a rate of 1.7±0.4 μm/second (n=55) (Fig. 5a). This result is consistent with previously measured microtubule-dependent vesicle movements and in vitro motility of dynein and kinesin-like motors in Dictyostelium (1.4-2.8 μm/second) (McCaffrey and Vale, 1989; Pollock et al, 1999; Pollock et al., 1998; Roos et al., 1987). The normal distribution of minus-end-directed velocities suggests that the movement is driven predominantly by a single motor, most probably dynein. Dynein movements were sometimes interrupted by short pauses, which occur more frequently in plus-end-directed rather than minus-end-directed runs. Movement usually resumed on the same track following the pause, suggesting that the cargo did not fall off the track in the process. It should be noted that this analysis is greatly facilitated by the relatively small numbers of microtubules in the cytoplasm of Dictyostelium cells. This reduces the likelihood of switching of cargo from one microtubule to another. In both directions, dynein-associated structures can usually travel continuously along the same track over a distance of several microns (Fig. 5b), indicating that movement in either direction is quite processive.

Dynein remains stably associated with cargo that reverses direction of movement

Although most dynein travels unidirectionally over a long
distance (up to 7 μm), some dynein-containing vesicles occasionally reverse the direction of their movement along the same track (Fig. 6a). The properties of the plus-end-directed and minus-end-directed elements of bidirectional movements are similar to those in unidirectional movements described above. A direction reversal is usually preceded by a very short pause (<1 sec) or no pause at all (Fig. 6b). The intensity of the corresponding dynein-GFP signal did not change during direction reversal. The stable association of dynein with the cargo during the swift reversal of direction argues against motor dissociation/association as a cause for change in direction. Instead it suggests a rapid and coordinated switch between opposing motor activities on the same cargo.

Dynein moves rapidly into and out of structures at both ends of the microtubule

In addition to dynein traffic along the microtubule tracks, we also observed interesting dynein dynamics at both the minus and plus ends of microtubules. As mentioned above, cytoplasmic dynein was most concentrated around the MTOC (Fig. 3), where the minus ends of microtubules meet. This seems to be the result of dynein accumulating at the ends of the microtubule tracks, as there is constant flow of dynein into the MTOC. Probably because of the high microtubule density, dynein dynamics were most active in this region. This central pool of dynein was constantly exchanging with the rest of the cytoplasm, with punctate dynein signals flowing into and out of the MTOC (Fig. 7a). Some puncta fluctuated near the MTOC, whereas others ran long distances.

Dynein also seems to accumulate at the plus ends of microtubules near the cell cortex. We frequently observed elongated dynein-GFP structures associated with microtubule plus ends in fixed cells (Fig. 3). Here, the dynein signal appeared as a comet-shaped structure, with an intensified end and a tapered tail. Similar structures of dynein-GFP were observed in living cells at the cell periphery. This localization resembles that of the endosome-microtubule linker protein CLIP-170, which preferentially binds to microtubule growing...
a single dynein IC gene, endogenous IC expression was disrupted the endogenous IC gene locus by gene targeting. We first selected cells expressing the IC-GFP endogenous cytoplasmic dynein localization, we functionally replaced the dynein. Interestingly, the microtubule tip decoration of dynein in Dictyostelium seemed to be quite dynamic, with dynein flow into and out of the end structure along the microtubule (Fig. 7b). The bidirectional flow at both ends of the microtubule is continuous with dynein traffic along the microtubules.

Discussion

We have characterized the dynamics of cytoplasmic dynein in living Dictyostelium cells using an IC-GFP fusion. Dynein is distributed in many punctate structures throughout the cytoplasm, at the ends of microtubules and is concentrated around the MTOC, consistent with previous immunofluorescence studies (Koonce et al., 1990). Dynein-associated structures constantly undergo fast linear movement along microtubules in both minus-end and plus-end directions and often reverse direction. Our data supports the idea that dynein remains associated with cargoes being transported by a plus-end-directed motor, as well as during rapid direction changes. These results strongly suggest that motors of different polarities coexist on the cargo and directional transport is controlled by coordinated regulation of motor activities (Hirokawa et al., 1990; Reese and Haimo, 2000; Rogers et al., 1997). To ensure that the IC-GFP signal represent normal cytoplasmic dynein localization, we functionally replaced the endogenous dynein IC with the IC-GFP fusion using a two-step approach. We first selected cells expressing the IC-GFP fusion at a level similar to the endogenous IC and then disrupted the endogenous IC gene locus by gene targeting. Because Dictyostelium is a haploid organism and there is only a single dynein IC gene, endogenous IC expression was completely eliminated. As a result, IC-GFP replaces the endogenous IC at wild-type levels. We compared IC-GFP with wild-type IC for association with the dynein and the dynactin complex by immunoprecipitation. IC-GFP associated with dynein and dynactin with the same efficiency as wild-type IC. This ensures that IC-GFP represents the dynein complex. Cells expressing only IC-GFP showed normal growth rates, spindle morphology, microtubule and Golgi organization and multicellular development, supporting the idea that the IC-GFP provides normal dynein function.

Cytoplasmic dynein is well known as a microtubule-based motor, the evidence for which mostly came from in vitro motility assays (Lye et al., 1987; Paschal et al., 1987; Schnapp and Reese, 1989; Schroer and Sheetz, 1991). Using the GFP-fusion, we have directly visualized dynein’s movement along the microtubules for the first time in living cells. Dynein travels along linear microtubules tracks for several microns. Dynein traveled along microtubules towards both microtubule plus ends and minus ends. Because cytoplasmic dynein is the only minus-end-directed microtubule motor identified in Dictyostelium so far and because the velocity of the minus-end-directed movement of dynein signal is consistent with that of dynein-dependent motility measured by in vitro motility assays, we infer that the minus-end-directed movement of dynein is powered by dynein itself. The plus-end-directed movement of the dynein signal, on the other hand, must be driven by a plus-end-directed microtubule motor. The velocity of the plus-end-directed dynein-associated cargo is consistent with that of the known plus-end-directed, kinesin-like motors in Dictyostelium. Multiple kinesin-like motors have been identified in Dictyostelium (de Hostos et al., 1998; McCaffrey and Vale, 1989; Pollock et al., 1999), and it is hard to predict at this point which ones are responsible for the plus-end-directed movement of dynein-associated structures.

The bidirectional movement of dynein in living cells strongly supports a model for the regulation of vesicle directionality in which both plus-end and minus-end-directed motors associate with a given vesicle. The fact that dynein can reside on a structure being transported by a plus-end-directed motor argues that motor dissociation is not necessary for the cargo to move in a direction opposite to the motor’s polarity. Further evidence for this comes from dynein’s stable association with structures that underwent several directional reversals. Our observations are consistent with previous immunohistochemistry of fixed samples showing that dynein or kinesin motors colocalized with membranous compartments that were thought to move in a direction opposite to the motor polarity (Hirokawa et al., 1990; Lippincott-Schwartz et al., 1995; Nilsson et al., 1996; Reese and Haimo, 2000; Rogers et al., 1997; Roghi, 1999). Our study complements these early studies by showing the dynamic association of dynein with cargo being transported by a kinesin-like motor and during the rapid reversal of direction. Together, these data strongly support a model in which directional movement is controlled by regulating motor activity instead of motor-cargo association.

When motors of opposing polarity are present on the same cargo, the direction of movement would depend on which of the motors is functional or exerts dominant function. This regulation could occur by regulating the force-generating function of the motor or by regulating the ability of the motor to interact with microtubules. The fast direction reversals suggest that such regulation must be able to rapidly coordinate activity of different motors on the same cargo, that is,
upregulating the activity of one group of motors while downregulating the opposite group of motors at the same time. Post-translational modification would be a convenient way to perform such regulation. Phosphorylation/dephosphorylation of motors or associated proteins, for example, could differentially regulate motor activity under various circumstances (Dillman and Pfister, 1994; McIlvan et al., 1994). Recently, Reese and Haimo suggested that the ability of dynein and kinesin II to bind to microtubules varies on aggregated or dispersed pigment granules in melanocytes. The microtubule-binding activity of these motors could be regulated in vitro by kinase and phosphatase (Reese and Haimo, 2000), suggesting that phosphorylation could regulate motor activity and thus control directional transport of pigments.

In interphase, dynein is associated mostly with membranous organelles including endosomes, lysosomes and intermediate compartments in the ER and Golgi complex (Hirokawa, 1998). Thus the majority of the dynein traveling along microtubules seems likely to be membranous vesicles. However, dynein may also associate with other types of cargo, such as nonmembranous protein particles. For example, neurofilaments display bidirectional movement along microtubules in neuronal axons (Prahлад et al., 2000; Roy et al., 2000; Wang et al., 2000). These movements may in part be mediated by cytoplasmic dynein (Dillman et al., 1996a; Dillman et al., 1996b). Thus, we cannot exclude the possibility that dynein may be associated with non-membranous particles. However, since Dictostelium appears not to contain intermediate filaments, it is unclear which proteins might reside in such particles.

The dynamics we observe for dynein also raise questions regarding the processivity of dynein-driven transport. In vitro, dynein’s processivity is limited (King and Schroer, 2000). Yet in living cells, as shown in Fig. 4b, dynein-containing structures frequently travel several microns in a single run without falling off the track. This indicates that, in vivo, dynein is capable of driving processive cargo movement. Several factors might contribute to dynein’s increased processivity in vivo. Dynactin has been shown to increase the processivity of dynein by two-fold (King and Schroer, 2000). In addition, multiple dynein molecules might be engaged on the same cargo to promote processive movement.

In addition to the bidirectional traffic of dynein along the microtubules, the two ends of microtubules are also points of active bidirectional traffic: dynein accumulated at, and appeared to move into and out of, the MTOC and the plus ends of microtubules. Microtubule minus ends meet at the MTOC, therefore dynein accumulation there is likely to be the result of motor accumulation at the end of the track. The microtubule minus end accumulation of dynein was first reported in Aspergillus by Xiang et al. (Xiang et al., 2000), and we observed a similar accumulation in Dictostelium. This pattern of dynein localization resembles that of the endosome-microtubule linker protein, CLIP-170, which preferentially binds to microtubule growing ends (Perez et al., 1999). It has been suggested that a specialized structure containing CLIP-170, dynein and dynactin might reside at the microtubule plus ends to help load endosomes to dynein motors (Valetti et al., 1999).

Dynein dynamics in living cells suggest a simplified view of motor traffic and recycling (Fig. 8). Dynein, as a minus-end-directed motor, transports cargo along the microtubule tracks towards the cell center and accumulates at the end of the tracks (MTOC). This central pool of dynein must somehow be recycled to the cell periphery. One efficient mechanism of recycling would be for dynein to ride along the same track back to the cell periphery by associating with cargo being transported by a plus-end-directed motor. This mechanism is supported by the observation that dynein travels toward the cell center with almost the same frequency as it travels toward the cell surface. This recycling mechanism would maintain a constant supply of the motor at places in need. Plus-end-directed motors might take a similar approach (i.e., utilizing dynein) to be recycled. It will be interesting to see if disruption of one motor would affect the recycling and thus the function of the opposite motor. Being an integral part of the motor traffic, the MTOC and the plus ends of microtubules seem to form a big central pool and many smaller peripheral pools of dynein in the cytoplasm. As much of the dynein movement either initiated or ended at the MTOC or microtubule plus ends, the ends might serve as switch stations to load cargo onto motors for a new round of transport and at the same time recycle the opposite motor back to its initiation station. The mechanisms responsible for loading and unloading of motors at the microtubule ends will be an important target for future study.

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References


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