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Role of Dynactin in the Intracellular Localization and Activation of Cytoplasmic Dynein

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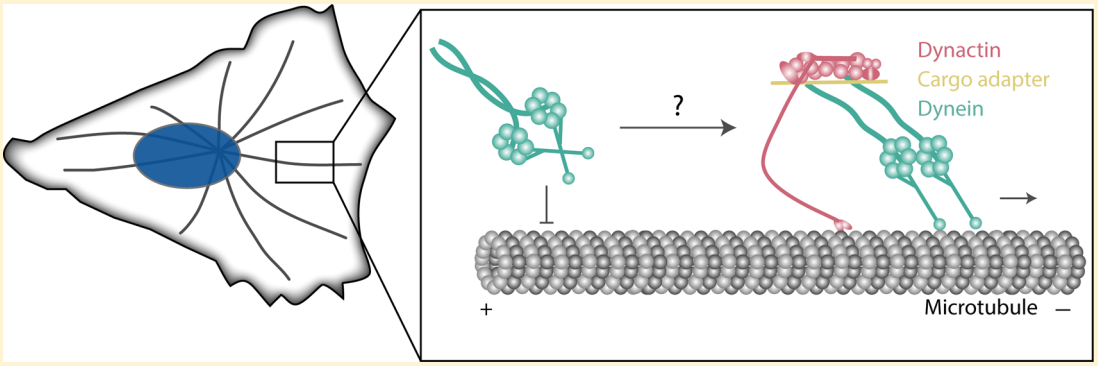

ABSTRACT: Cytoplasmic dynein, the major minus end-directed motor protein in several cell types, transports a variety of intracellular cargo upon forming a processive tripartite complex with its activator dynactin and cargo adaptors such as Hook3 and BicD2. Our current understanding of dynein regulation stems from a combination of *in vivo* studies of cargo movement upon perturbation of dynein activity, *in vitro* single-molecule experiments, and cryo-electron microscopy studies of dynein structure and its interaction with dynactin and cargo adaptors. In this Perspective, we first consolidate data from recent publications to understand how perturbations to the dynein−dynactin interaction and dynactin's *in vivo* localization alter the behavior of dynein-driven cargo transport in a cell type- and experimental condition-specific manner. In addition, we touch upon results from *in vivo* and *in vitro* studies to elucidate how dynein's interaction with dynactin and cargo adaptors activates dynein and enhances its processivity. Finally, we propose questions that need to be addressed in the future with appropriate experimental designs so as to improve our understanding of the spatiotemporal regulation of dynein's function in the context of the distribution and dynamics of dynactin in living cells.

Motor proteins convert the chemical energy of adenosine triphosphate (ATP) to mechanical work and thereby generate forces that are essential for processes such as cell propulsion,[1] cell division,[2] and long-range transport of intracellular cargo.[3,4] In eukaryotic cells, the cytoskeleton consists of actin, intermediate filaments, and microtubules (MTs).

The MT cytoskeleton is formed by α- and β-tubulin heterodimers. MT growth and shrinkage dynamics are faster on one end of the filament that is termed the plus end. The minus ends inside the cells are typically capped. In many cell types, the MTs form a radial array with the minus ends of individual MTs emanating from the MT organizing center (MTOC). The dynamic plus ends are present toward the periphery of the cell and undergo growth and shrinkage. MT-based transport is driven by cytoplasmic dynein and several members of the kinesin family of motor proteins[5] (Figure 1).

The kinesin family of motors consists of nearly 40 different kinesins organized into 14 families, several of which transport distinct cargo molecules within the cell.[6] For instance, the kinesin 3 family motors KIF1A and KIF1Bβ transport synaptic protein vesicles toward neuronal synapses,[7,8] whereas KIF5 plays an important role in mitochondrial transport.[9]

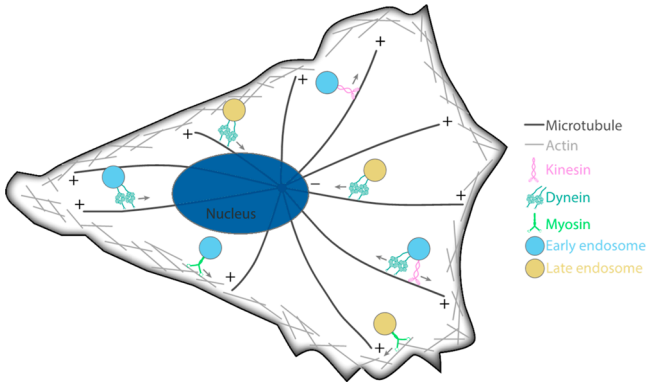

Figure 1. Intracellular cargo such as early and late endosomes is transported by kinesin and dynein motors on MTs and by myosin motors along the actin cytoskeleton.

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In contrast to the large family of kinesin motors, the dynein family of motors comprises only two classes, namely, axonemal and cytoplasmic dyneins.[10] Members of the axonemal dynein class of motors are expressed by ∼15 genes. They are found exclusively in cilia and flagella and, in conjunction with the axonemal MTs, are essential for ciliary force generation. The cytoplasmic dynein family consists of two motors, namely, cytoplasmic dynein-1 and -2. Cytoplasmic dynein-2 is responsible for intraflagellar transport along MTs in cilia and flagella.[11] Cytoplasmic dynein-1 (henceforth termed dynein) is ubiquitous across cell types and is the primary motor responsible for the transport of cargo toward the minus ends of MTs in the cytoplasm.[12]

Specifically, dynein is required for the orientation of the bipolar spindle,[13] kinetochore separation during cell division,[14] and long-range, minus end-directed intracellular transport during interphase.[15] In the crowded intracellular milieu, large, diffusionaly constrained cargoes[16] must be actively transported within the cell to be able to reach their intracellular destinations. In cells such as neurons, where the cell body can be >100 μm from synapses, transport of signaling molecules and vesicles is essential for proper communication and survival.

Cytoplasmic dynein-1 is a large homodimeric complex consisting of ∼530 kDa dynein heavy chains (DHCs) that associate with accessory proteins comprising dynein intermediate chains (DIC), multiple light chains (LCs), and light intermediate chains (LICs). The N-terminus of DHC binds to the DICs and LICs. The DHC contains a C-terminal ring of six AAA+ sites with AAA1 serving as the primary site of ATP hydrolysis.[17−19] DHC's C-terminal half contains a MT-binding stalk. During the first step of dynein's mechanochemical cycle, binding of ATP to the motor domain detaches dynein from the MT. Furthermore, the linker domain that is normally docked to AAA5 adopts a bent conformation. This conformational change induced by ATP binding increases the range of diffusional search of dynein's MT-binding stalk. Upon ATP hydrolysis and release of P_i, the MT binding affinity of the stalk is restored, and as a result, it binds strongly to the MTs.[20] This is followed by the powerstroke, in which the linker domain returns to its original position close to the MTBD, thereby exerting force on the cargo that is bound to dynein.[21−23] Alternating rounds of the mechanochemical cycle of both motor domains allow dynein to move processively, i.e., take several steps toward the minus ends of the MTs. Coordination between the motor domains enables yeast dynein to take multiple steps of either 8, 24, or 32 nm in a load-dependent manner.[24−27] The force exerted by a single dynein molecule varies across organisms. For example, human dynein has been reported to exert forces of up to ∼4.3 pN.[28] In contrast, force measurements on dynein-driven cargo in mouse macrophages indicate that single dynein motors exert forces of up to ∼1.2 pN.[29] On the other hand, forces of up to ∼7 pN have been reported for yeast dynein.[30] Dynactin is an ∼1.2 MDa, 23-subunit complex that increases dynein's processivity.[31]

Dynactin comprises a core formed by Arp1 subunits with distinct pointed and barbed ends, and a shoulder complex.[31] The pointed end complex of dynactin includes the p25, p27, and p62 subunits. The shoulder complex of dynactin includes the p150-Glued (p150 henceforth) subunit with an N-terminal MT-binding domain that establishes the link between dynactin and MTs. p150 also has a glycine-rich, basic domain (CAP-Gly) that can interact with MTs. The p24 and p50 subunits complete the shoulder complex.[31] The p150 subunit interacts with DIC and thus links dynein to dynactin.[32,33] We direct our readers' attention to refs 31 and 34−36 for excellent schematics of dynein and dynactin structure. Recent research suggests that dynactin, in conjunction with cargo adaptors such as protein bicaudal D homologue 2 (BicD2), Hook1−3, Spindly, and Rabll-FIP3, activates dynein.[37−42]

Dynein's cargo inside the cell includes early and late endosomes, lysosomes, peroxisomes, mitochondria, the Golgi complex, post-Golgi cargo, and kinetochores.[15] Therefore, unlike the highly specific kinesin family of motors, dynein interacts with multiple cargoes within the cell, and so, the spatiotemporal regulation of dynein dynamics and activity is an area of immense interest. In this Perspective, we elucidate dynactin's role in activating dynein and regulating its processivity and intracellular localization. Furthermore, we propose questions that need to be answered in the future to understand how dynein−dynactin and cargo form a processive complex inside the cell and enable organelle transport.

DYNACTIN REGULATES THE BEHAVIOR OF DYNEIN-DRIVEN TRANSPORT

Dynactin was first isolated as a cytosolic fraction that increased *in vitro* minus end-directed motility of vesicles from chick embryo fibroblasts.[37] Moreover, antibodies against the p150 subunit of dynactin reduced the motility of organelles obtained from the squid axoplasm.[43] Early experiments employing *in vitro* single-molecule bead assays revealed dynactin's effect on dynein's processivity at the single-molecule level. Specifically, 1 μm beads bound to single molecules of dynactin and dynein displayed significantly increased residence times and run lengths compared to those of beads bound to only dynein.[38] Abrogating the MT binding ability of dynactin removed this enhancement. Additionally, the basic domain of p150 was found to skate along the MTs and thereby enable longer interactions between dynactin-bound dynein and the MTs.[44] Therefore, the conclusion was that dynactin provided an additional point of attachment between cargo-bound dynein and MT, thereby enabling faster reattachment of dynein to MTs during its mechanochemical cycle.

Because of dynactin's role in enhancing the run lengths of dynein-driven cargo, the spatial distribution of dynactin and its effect on intracellular transport have been examined using a combination of *in vivo* expression and immunofluorescence localization of specific subunits. Overexpression of fluorescently tagged p150 revealed its strong MT binding affinity and its stabilizing effect on individual MTs.[45] Moreover, overexpression of the p50 (dynamitin) subunit of dynactin disrupted the dynactin shoulder complex−dynein motor interaction and interfered with dynein's *in vivo* function as indicated by a dispersed Golgi apparatus in live cells.[46] The latter also affected the localization of lysosome-targeted intracellular probes, indicating that intracellular transport was perturbed upon disruption of the dynactin complex. High-speed microscopy and automated particle tracking of endosomes in HeLa cells revealed that disruption of the dynactin complex by overexpression of the p50 subunit reduced the extent of long-range (>3 μm) motion of early endosomes.[47] The reduction in run lengths of cargo indicated that an intact dynactin complex is essential for the association of dynein with its cargo.

The MT plus ends inside a cell are bound by end-binding proteins such as EB1,[48] which additionally recruits the endosome−MT linker protein CLIP-170.[49] Dynactin was found to localize to plus ends of MTs in conjunction with CLIP-170, and a fraction of these plus end-localized dynactin

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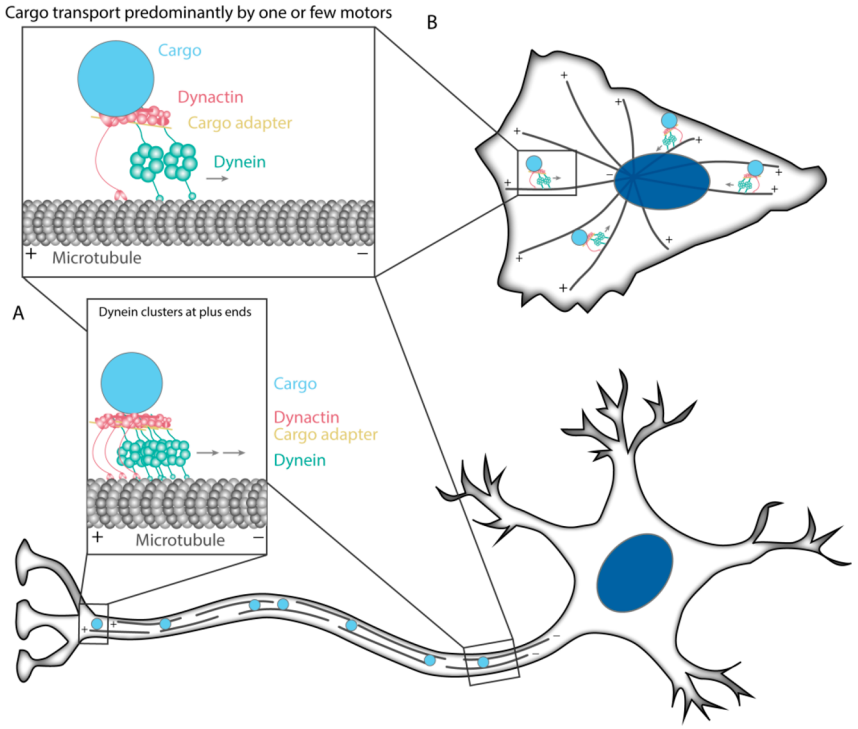

Figure 2. Dynactin's role in dynein-driven transport varies with cell type and intracellular location. (A) Dynactin bound to the MT plus ends enhances dynein clustering. In Cos-7 cells and distal neurites, minus end-directed transport is initiated when the growing MT plus ends encounter cargo. (B) In HeLa cells, dynactin's MT-binding domain and dynein's clustering at MT plus ends are not required for cargo transport.

spots were associated with cytoplasmic dynein.[50] In fact, the 74 kDa dynein intermediate chain IC 74 was observed to track the growing plus ends of MTs in live HeLa cells.[51] In Cos-7 cells, at low expression levels, p150 was observed to track the growing plus ends of the MTs, and minus end-directed motility of the Golgi complex was initiated upon contact with a growing MT plus end containing p150.[52] Taken together, these results suggest that in Cos-7 cells, minus end-directed cargo transport is initiated when growing MT plus ends containing dynein and dynactin encounter cargo in the periphery of the cell.

On the contrary, abolishing the MT plus end targeting of p150 in HeLa cells had no effect on the intracellular transport of endocytosed transferrin or acidic endosomal compartments.[53] Additionally, siRNA-mediated knockdown of CLIP-170 and EB1 in HeLa cells revealed a sequential mechanism for targeting of p150 to the plus ends of MTs. EB1 was found to bring CLIP-170 first to the plus ends and, subsequently, p150 bound to the latter. In mouse hippocampal neurons, MT plus end tracking of p150 was essential specifically for the initiation of transport from the distal regions of neurites, whereas p150 lacking the MT-binding domain could rescue minus end-directed transport in the mid-axon.[54] Interestingly, p135, an isoform of p150 that lacks the MT-binding domain, is found predominantly in the brain, indicating that the MT-binding domain of p150 might not be necessary for transport in some instances,[55] as in the case of mid-axon.[54] In HeLa cells, expression of p135 fully substituted the function of p150 as indicated by the rescue of Golgi dispersion in cells lacking endogenous p150.[56]

In essence, the enhancement of processivity of dynein-driven transport requires an intact dynactin complex that can associate with dynein. Moreover, in certain cells like Cos-7 cells and hippocampal neurons, dynactin-mediated MT plus end targeting of dynein plays an important role in cargo transport. It is possible that this difference in the requirement for plus end targeting of dynein depends on the MT architecture, which in turn sets the limit on the range of distances the cargo is transported within the cell[57] (Figure 2). For instance, transport of cargo over long distances requires multiple motors, in which case groups of dyneins targeted to the MT plus end can effectively initiate long-range cargo transport as observed in Cos-7 and hippocampal neuron cells.[54,58] On the other hand, in HeLa cells, most cargo runs are short,[47,59] and therefore, dynactin-mediated MT plus end targeting of dynein might be inconsequential.

Studies of dynein purified from *Saccharomyces cerevisiae* suggested that single molecules of dynein might be auto-inhibited, which prevented them from being processive.[60] Specifically, single molecules of *S. cerevisiae* dynein bound to the p150 homologue Nip100 lacking the MT-binding domain had a longer average run length compared to that of single dynein motors, which were almost stationary. Additionally, Pac1/Lis1-mediated inhibition of dynein was relieved by Num1, which activated dynein's processive motion.[61] Finally, in *Schizosaccharomyces pombe*, the anchor protein Mcp5 activated dynein by binding to its N-terminus.[62−66] In *in vitro* stepping assays, single molecules of the reconstituted complete human dynein complex remain statically bound to the MTs,[67] whereas groups of dynein motors participated effectively in MT gliding assays. These results indicated that dynein is perhaps activated by proteins binding to its tail domain.

DYNEIN AUTOINHIBITION AND PROCESSIVITY ARE REGULATED BY DYNACTIN AND CARGO ADAPTOR BINDING

In the context of intracellular transport, cargo adaptors such as BicD2, Hook3, and Spindly bind to dynein's N-terminal tail domain and recruit dynein to specific cargoes and regulate their

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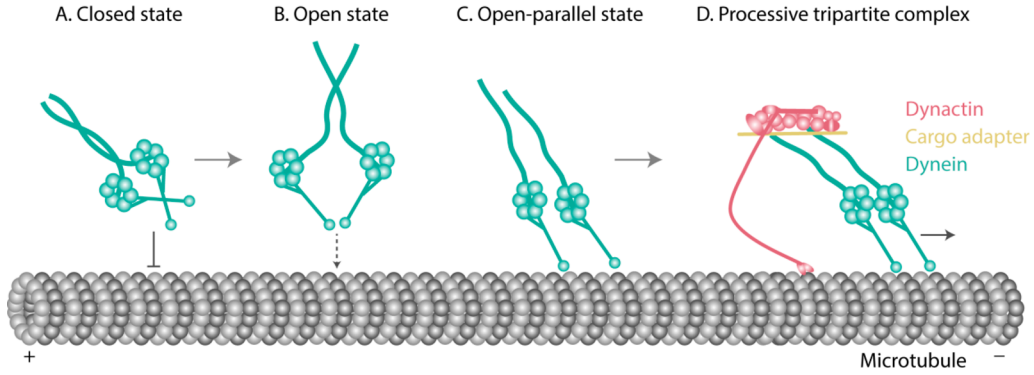

Figure 3. Formation of processive tripartite complex requires conformational changes in dynein. (A) In wild type cells, 74% of dynein exists in the autoinhibited ϕ particle form.[76] The ϕ particle has low affinity for MTs and cannot form a processive complex. (B and C) Compared to the ϕ particle, the open form of dynein has enhanced interaction with MTs and can bind to dynactin and cargo adaptors. (D) In the processive complex, dynein's MT-binding domains are parallel to each other. How and where dynein, dynactin, and the cargo come together inside the cell is currently not understood. Note that the dynein complex consists of two identical DHCs that are typically dimerized at the N-terminal tail region by the binding of DICs, DLICs, and DLCs; however, we have omitted these additional subunits in this schematic for the sake of clarity.

movement. For instance, BicD2 recruits dynein onto Rab6-positive cargo and thereby regulates Golgi−endoplasmic reticulum (ER) transport.[68] The cargo adaptor Hook1 is essential for dynein-driven transport of BDNF signaling molecules in the axons of primary hippocampal neurons.[69] Cargo adaptors are homodimeric proteins with multiple coiled-coil (CC) domains. Current research suggests that the N-terminal half of the CC domain can bind to dynein−dynactin only when the adaptor is attached to the cargo through its C-terminal half.[42] For instance, the N-terminal CC1-CC2 domain of BicD2 binds to dynein−dynactin, and its C-terminal CC3 domain binds to Rab6-positive cargo.[70] Similarly, the C-terminal CC domain of Hook3 interacts with the Golgi complex.[71] The CC domain of Spindly recruits dynein−dynactin to kinetochores in HeLa cells.[72]

In the past decade, an improved understanding of dynein-driven motility has been made possible by studying dynein, dynactin, and cargo adaptor machinery together. Dynein and dynactin were found to have a very low affinity for each other inside cells, as indicated by the low level of co-precipitation in a pull-down assay.[73] This could be artificially increased by overexpressing the N-terminal fragment of BicD2.[73] Moreover, Rab6a-positive vesicles that were constitutively attached to BicD2-N displayed an increased level of motion toward the minus ends of MTs. Therefore, the N-terminus of BicD2 links endosomal cargo to dynein and dynactin, thereby enabling their processive motion inside the cell. In other studies in which dynactin was added to dynein stepping assays, only a fraction of dynactin in solution co-localized with dynein, indicating that additional factors are required to induce the interaction between dynein and dynactin.[74,75] Addition of the BicD2-N fragment increased the level of co-localization of dynactin and dynein, and these three components formed a processive complex. Moreover, complexes formed by dynein−dynactin and other cargo adaptors (DDC), Hook3 and Spindly, were also found to be processive *in vitro*/[75] This confirmed that dynein exists in a default autoinhibited state, and simultaneous binding of dynactin to DIC and a cargo adaptor to DLIC on DHC's tail domain activates the dynein complex for processive motion.

Cryo-electron microscopy structures of pig brain dynein demonstrated that a native configuration of dynein, termed the ϕ particle, has weak MT and dynactin binding affinity.[76] MT binding was enhanced by disrupting the self-dimerization of dynein's motor domains, thereby converting it to an "open" form. The "open" form of the ϕ particle required dynactin binding to rearrange the MT-binding stalks to a "parallel" configuration that was then capable of processive movement when it formed a complex with dynactin and BicD2 (Figure 3). In contrast to the cargo adaptor BicD2 that recruits a single dynein molecule into the processive complex with dynactin, dynein−dynactin−cargo adaptor complexes consisting of Hook3 and BICDR1 could potentially recruit two dynein complexes into the complex.[77,78] This processive complex could exert stronger forces and move faster along the MT network in an *in vitro* single-molecule assay.

Recent studies have also focused on uncovering the link between MT plus end binding of dynein and formation of processive DDC complexes. In *in vitro* assays, dynein binds to growing MT plus ends in an EB1−dynactin-dependent manner.[79] Addition of BicD2-N to the solution initiated minus end-directed runs of the DDC complex from a region close to the growing MT plus ends. Therefore, cytoplasmic dynein could potentially exist as two distinct populations within the cell: one at the growing MT plus ends and the other as part of the DDC processive complex, both of which depend on dynactin.[79]

THE *IN VIVO* FORMATION AND DYNAMICS OF THE TRIPARTITE COMPLEX ARE CURRENTLY UNKNOWN

Our current understanding of dynein-driven intracellular transport suggests that dynactin might play a dual role in controlling dynein's function *in vivo*. First, dynactin targets dynein to MT plus ends, and in certain cell types, this is essential to initiate minus end-directed runs of cargo driven by clusters of dynein. Second, as confirmed by multiple studies, dynactin and cargo adaptors form a tripartite complex to convert dynein from a motor with weak MT binding affinity to a processive motor capable of transporting cargo.[69,73−75]

However, studies in HeLa and Arpe-19 cells suggest that early endosomal cargoes move in short processive runs of approximately 1−2 μm punctuated with frequent pauses.[59] While organelle crowding within the cytoplasm and on the MT might be one of the reasons for interruptions in cargo transport, it could also indicate cargo motion driven by one or a few dynein

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motors, thereby eliminating the role of dynactin-mediated MT plus end targeting of dynein in these cells.

It is now well established that a dynein−dynactin−cargo complex (mediated by adaptors like BicD2 and Hook3) is essential for processive motion. As evinced by low levels of co-precipitation in pull-down assays, the interaction between dynein and dynactin is weak, and when DHC is overexpressed in HeLa cells, it remains predominantly cytosolic despite the presence of a MT-binding domain.[73] Moreover, all studies employing the processive complex have resorted to using a molar excess of the N-terminal fragment of BicD2 to increase the level of complex formation.[75,74] Taken together, the interaction among the three components of the processive complex is dynamic and the *in vivo* location and time scales of interaction between the members of the processive complex are not known.

Furthermore, while the processive motion of the DDC complex reported in single-molecule studies is under zero-load conditions,[74,75] motors exhibit altered run times and processivities under load in living cells.[29,80−82] These constraints necessitate understanding the regulation of dynein-driven transport by dynactin under cellular conditions.

FUTURE DIRECTIONS

The primary questions that future experiments should tackle are the *in vivo* localization of the components of the dynein−dynactin−cargo adaptor complex, how they come together to initiate dynein-based motility, and the dynamics of their interactions. This will also provide insight into how dynein-driven motility is terminated.

Fluorescently tagged DHC remains predominantly cytosolic when overexpressed in HeLa cells,[73] in contrast to p150, which marks the MT lattice.[45] This might indicate that dynein's interaction with MTs is dynamic and that a significant fraction of dynein inside cells is not bound to MTs. Single-molecule microscopy techniques, which have already provided great insight into the dynamics of single motors[65] and other proteins inside living cells,[83] can be used to visualize[84−87] and follow[88] single molecules of dynein and dynactin inside the cells as they stochastically interact with the cytoskeleton and other intracellular organelles. This will reveal the time scales of these interactions and their relation to cargo run times. Quantifying the kinetics of individual motors will allow us to verify if run times and/or lengths of intracellular cargo are a reflection of the stochastic binding and unbinding of the motors from the processive complex. Moreover, such studies can be easily repeated in cells devoid of dynactin or other specific dynein adaptors, thereby revealing the effect of these proteins under *in vivo* conditions. Because these interactions are essentially stochastic and involve small numbers of molecules, it is imperative that a large number of molecules be tracked, describing the behavior of the entire population.

In addition, quantifying the spatial inhomogeneity in the distribution of these components could provide insight into their role in cargo transport. For example, if the p150-mediated MT plus end clustering of dynein is the predominant mode of dynein−cargo interactions in specific regions of the cell, then cargoes in those regions are more likely to be driven by multiple dynein motors, resulting in longer run lengths.[89] In regions devoid of dynein clustering, cargo might be driven by fewer dyneins and these differences can be visualized using high-time resolution tracking and quantification of the movement of cargo across the cell.[90,91]

Finally, the effect of spatial variation and the kinetics of interaction among the components of the processive complex can be incorporated into computational models. For instance, computational models of the arrangement of motors on the cargo revealed that thermal fluctuations of the cargo could cause high loads on the motor leading to detachment.[92] Additionally, clustering motors on the cargo surface could potentially improve cargo run lengths. The movement of phagosomes was explained by a computational model that incorporated stochastic switching of activity between opposite polarity motors.[93] Taken together, deciphering dynactin's effect on dynein's *in vivo* activity requires the interpretation of results obtained using biochemical, microscopy, and computational experiments.

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Notes

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REFERENCES

(1) Gibbons, I. R. (1981) Cilia and flagella of eukaryotes. *J. Cell Biol. 91*, 107−124.

(2) Sharp, D. J., Rogers, G. C., and Scholey, J. M. (2000) Microtubule motors in mitosis. *Nature 407*, 41.

(3) Rosenbaum, J. L., and Witman, G. B. (2002) Intraflagellar transport. *Nat. Rev. Mol. Cell Biol. 3*, 813−825.

(4) Vale, R. D. (2003) The molecular motor toolbox for intracellular transport. *Cell 112*, 467−480.

(5) Caviston, J. P., and Holzbaur, E. L. F. (2006) Microtubule motors at the intersection of trafficking and transport. *Trends Cell Biol. 16*, 530−537.

(6) Hirokawa, N., Noda, Y., Tanaka, Y., and Niwa, S. (2009) Kinesin superfamily motor proteins and intracellular transport. *Nat. Rev. Mol. Cell Biol. 10*, 682−696.

(7) Hall, D. H., and Hedgecock, E. M. (1991) Kinesin-related gene unc-104 is required for axonal transport of synaptic vesicles in C. elegans. *Cell 65*, 837−847.

(8) Okada, Y., Yamazaki, H., Sekine-Aizawa, Y., and Hirokawa, N. (1995) The neuron-specific kinesin superfamily protein KIF1A is a uniqye monomeric motor for anterograde axonal transport of synaptic vesicle precursors. *Cell 81*, 769−780.

(9) Cho, K. I., Cai, Y., Yi, H., Yeh, A., Aslanukov, A., and Ferreira, P. A. (2007) Association of the kinesin-binding domain of RanBP2 to KIF5B and KIF5C determines mitochondria localization and function. *Traffic 8*, 1722−1735.

(10) Hook, P., and Vallee, R. B. (2006) The dynein family at a glance. *J. Cell Sci. 119*, 4369−4371.

(11) Witman, G. B., and Hou, Y. (2018) Dynein and intraflagellar transport. In *Dyneins*, pp 386−432, Elsevier.

(12) Allan, V. J. (2011) Cytoplasmic dynein. *Biochem. Soc. Trans. 39*, 1169−1178.

DOI: 10.1021/acs.biochem.9b00772
Biochemistry 2020, 59, 156−162

(13) Vaisberg, E. A., Koonce, M. P., and McIntosh, J. R. (1993) Cytoplasmic dynein plays a role in mammalian mitotic spindle formation. *J. Cell Biol. 123*, 849−858.
(14) Dujardin, D. L., and Vallee, R. B. (2002) Dynein at the cortex. *Curr. Opin. Cell Biol. 14*, 44−49.
(15) Reck-Peterson, S. L., Redwine, W. B., Vale, R. D., and Carter, A. P. (2018) The cytoplasmic dynein transport machinery and its many cargoes. *Nat. Rev. Mol. Cell Biol. 19*, 382−398.
(16) Luby-Phelps, K. (1999) Cytoarchitecture and physical properties of cytoplasm: volume, viscosity, diffusion, intracellular surface area. *Int. Rev. Cytol. 192*, 189−221.
(17) Carter, A. P., Cho, C., Jin, L., and Vale, R. D. (2011) Crystal Structure of the Dynein Motor Domain. *Science 331*, 1159−1165.
(18) Roberts, A. J., Kon, T., Knight, P. J., Sutoh, K., and Burgess, S. A. (2013) Functions and mechanics of dynein motor proteins. *Nat. Rev. Mol. Cell Biol. 14*, 713−726.
(19) Reck-Peterson, S. L., Vale, R. D., and Gennerich, A. (2011) Motile properties of cytoplasmic dynein. In *Handbook of Dynein*, pp 145−172, CRC Press.
(20) Gibbons, I. R., Garbarino, J. E., Tan, C. E., Reck-Peterson, S. L., Vale, R. D., and Carter, A. P. (2005) The affinity of the dynein microtubule-binding domain is modulated by the conformation of its coiled-coil stalk. *J. Biol. Chem. 280*, 23960−23965.
(21) Burgess, S. A., Walker, M. L., Sakakibara, H., Knight, P. J., and Oiwa, K. (2003) Dynein structure and power stroke. *Nature 421*, 715−718.
(22) Schmidt, H., and Carter, A. P. (2016) Review: Structure and mechanism of the dynein motor ATPase. *Biopolymers 105*, 557−567.
(23) Carter, A. P. (2013) Crystal clear insights into how the dynein motor moves. *J. Cell Sci. 126*, 705−713.
(24) Reck-Peterson, S. L., Yildiz, A., Carter, A. P., Gennerich, A., Zhang, N., and Vale, R. D. (2006) Single-Molecule Analysis of Dynein Processivity and Stepping Behavior. *Cell 126*, 335−348.
(25) DeWitt, M. A., Chang, A. Y., Combs, P. A., and Yildiz, A. (2012) Cytoplasmic dynein moves through uncoordinated stepping of the AAA+ ring domains. *Science 335*, 221−225.
(26) Wang, Z., Khan, S., and Sheetz, M. P. (1995) Single cytoplasmic dynein molecule movements: characterization and comparison with kinesin. *Biophys. J. 69*, 2011−2023.
(27) Lippert, L. G., Dadosh, T., Hadden, J. A., Karnawat, V., Diroll, B. T., Murray, C. B., Holzbaur, E. L. F., Schulten, K., Reck-Peterson, S. L., and Goldman, Y. E. (2017) Angular measurements of the dynein ring reveal a stepping mechanism dependent on a flexible stalk. *Proc. Natl. Acad. Sci. U. S. A. 114*, E4564−E4573.
(28) Belyy, V., Schlager, M. A., Foster, H., Reimer, A. E., Carter, A. P., and Yildiz, A. (2016) The mammalian dynein-dynactin complex is a strong opponent to kinesin in a tug-of-war competition. *Nat. Cell Biol. 18*, 1018−1024.
(29) Rai, A. K., Rai, A., Ramaiya, A. J., Jha, R., and Mallik, R. (2013) Molecular adaptations allow dynein to generate large collective forces inside cells. *Cell 152*, 172−182.
(30) Gennerich, A., Carter, A. P., Reck-Peterson, S. L., and Vale, R. D. (2007) Force-Induced Bidirectional Stepping of Cytoplasmic Dynein. *Cell 131*, 952−965.
(31) Schroer, T. A. (2013) Dynactin. In *Encyclopedia of Biological Chemistry*, 2nd ed., pp 174−177, Elsevier.
(32) Vaughan, K. T., and Vallee, R. B. (1995) Cytoplasmic dynein binds dynactin through a direct interaction between the intermediate chains and p150Glued. *J. Cell Biol. 131*, 1507−1516.
(33) Vaughan, P. S., Leszyk, J. D., and Vaughan, K. T. (2001) Cytoplasmic Dynein Intermediate Chain Phosphorylation Regulates Binding to Dynactin. *J. Biol. Chem. 276*, 26171−26179.
(34) Carter, A. P., Diamant, A. G., and Urnavicius, L. (2016) How dynein and dynactin transport cargos: A structural perspective. *Curr. Opin. Struct. Biol. 37*, 62−70.
(35) Chowdhury, S., Ketcham, S. A., Schroer, T. A., and Lander, G. C. (2015) Structural organization of the dynein-dynactin complex bound to microtubules. *Nat. Struct. Mol. Biol. 22*, 345−347.
(36) Ananthanarayanan, V. (2016) Activation of the motor protein upon attachment: Anchors weigh in on cytoplasmic dynein regulation. *BioEssays 38*, 514−525.
(37) Gill, S. R., Schroer, T. A., Szilak, I., Steuer, E. R., Sheetz, M. P., and Cleveland, D. W. (1991) Dynactin, a conserved, ubiquitously expressed component of an activator of vesicle motility mediated by cytoplasmic dynein. *J. Cell Biol. 115*, 1639−1650.
(38) King, S. J., and Schroer, T. A. (2000) Dynactin increases the processivity of the cytoplasmic dynein motor. *Nat. Cell Biol. 2*, 20−24.
(39) Cianfrocco, M. A., DeSantis, M. E., Leschziner, A. E., and Reck-Peterson, S. L. (2015) Mechanism and Regulation of Cytoplasmic Dynein. *Annu. Rev. Cell Dev. Biol. 31*, 83−108.
(40) Hoogenraad, C. C., and Akhmanova, A. (2016) Bicaudal D Family of Motor Adaptors: Linking Dynein Motility to Cargo Binding. *Trends Cell Biol. 26*, 327−340.
(41) Vallee, R. B., McKenney, R. J., and Ori-McKenney, K. M. (2012) Multiple modes of cytoplasmic dynein regulation. *Nat. Cell Biol. 14*, 224−230.
(42) Olenick, M. A., and Holzbaur, E. L. F. (2019) Dynein activators and adaptors at a glance. *J. Cell Sci. 132*, No. jcs227132.
(43) Waterman-Storer, C. M., Karki, S. B., Kuznetsov, S. A., Tabb, J. S., Weiss, D. G., Langford, G. M., and Holzbaur, E. L. F. (1997) The interaction between cytoplasmic dynein and dynactin is required for fast axonal transport. *Proc. Natl. Acad. Sci. U. S. A. 94*, 12180−12185.
(44) Culver-Hanlon, T. L., Lex, S. A., Stephens, A. D., Quintyne, N. J., and King, S. J. (2006) A microtubule-binding domain in dynactin increases dynein processivity by skating along microtubules. *Nat. Cell Biol. 8*, 264−270.
(45) Quintyne, N. J., Gill, S. R., Eckley, D. M., Crego, C. L., Compton, D. A., and Schroer, T. A. (1999) Dynactin is required for microtubule anchoring at centrosomes. *J. Cell Biol. 147*, 321−334.
(46) Valetti, C., Wetzel, D. M., Schrader, M., Hasbani, M. J., Gill, S. R., Kreis, T. E., and Schroer, T. A. (1999) Role of dynactin in endocytic traffic: Effects of dynamitin overexpression and colocalization with CLIP-170. *Mol. Biol. Cell 10*, 4107−4120.
(47) Flores-Rodriguez, N., Rogers, S. S., Kenwright, D. A., Waigh, T. A., Woodman, P. G., and Allan, V. J. (2011) Roles of Dynein and Dynactin in Early Endosome Dynamics Revealed Using Automated Tracking and Global Analysis. *PLoS One 6*, No. e24479.
(48) Morrison, E. E., Wardleworth, B. N., Askham, J. M., Markham, A. F., and Meredith, D. M. (1998) EB1, a protein which interacts with the APC tumour suppressor, is associated with the microtubule cytoskeleton throughout the cell cycle. *Oncogene 17*, 3471.
(49) Perez, F., Diamantopoulos, G. S., Stalder, R., and Kreis, T. E. (1999) CLIP-170 highlights growing microtubule ends in vivo. *Cell 96*, 517−527.
(50) Vaughan, K. T., Tynan, S. H., Faulkner, N. E., Echeverri, C. J., and Vallee, R. B. (1999) Colocalization of cytoplasmic dynein with dynactin and CLIP-170 at microtubule distal ends. *J. Cell Sci. 112*, 1437−1447.
(51) Kobayashi, T., and Murayama, T. (2009) Cell cycle-dependent microtubule-based dynamic transport of cytoplasmic dynein in mammalian cells. *PLoS One 4*, No. e7827.
(52) Vaughan, P. S., Miura, P., Henderson, M., Byrne, B., and Vaughan, K. T. (2002) A role for regulated binding of p150Glued to microtubule plus ends in organelle transport. *J. Cell Biol. 158*, 305−319.
(53) Watson, P., and Stephens, D. (2006) Microtubule plus-end loading of p150Glued is mediated by EB1 and CLIP-170 but is not required for intracellular membrane traffic in mammalian cells. *J. Cell Sci. 119*, 2758−2767.
(54) Moughamian, A. J., Osborn, G. E., Lazarus, J. E., Maday, S., and Holzbaur, E. L. F. (2013) Ordered Recruitment of Dynactin to the Microtubule Plus-End is Required for Efficient Initiation of Retrograde Axonal Transport. *J. Neurosci. 33*, 13190−13203.
(55) Tokito, M. K., Howland, D. S., Lee, V. M. Y., and Holzbaur, E. L. F. (1996) Functionally distinct isoforms of dynactin are expressed in human neurons. *Mol. Biol. Cell 7*, 1167−1180.
(56) Dixit, R., Levy, J. R., Tokito, M., Ligon, L. A., and Holzbaur, E. L. F. (2008) Regulation of dynactin through the differential expression of p150 Glued isoforms. *J. Biol. Chem. 283*, 33611−33619.

DOI: 10.1021/acs.biochem.9b00772
Biochemistry 2020, 59, 156−162

(57) Nirschl, J. J., Ghiretti, A. E., and Holzbaur, E. L. F. (2017) The impact of cytoskeletal organization on the local regulation of neuronal transport. *Nat. Rev. Neurosci. 18*, 585−597.
(58) Yi, J. Y., Ori-McKenney, K. M., McKenney, R. J., Vershinin, M., Gross, S. P., and Vallee, R. B. (2011) High-resolution imaging reveals indirect coordination of opposite motors and a role for LIS1 in high-load axonal transport. *J. Cell Biol. 195*, 193−201.
(59) Zajac, A. L., Goldman, Y. E., Holzbaur, E. L. F., and Ostap, E. M. (2013) Local Cytoskeletal and Organelle Interactions Impact Molecular-Motor-Driven Early Endosomal Trafficking. *Curr. Biol. 23*, 1173−1180.
(60) Kardon, J. R., Reck-Peterson, S. L., and Vale, R. D. (2009) Regulation of the processivity and intracellular localization of Saccharomyces cerevisiae dynein by dynactin. *Proc. Natl. Acad. Sci. U. S. A. 106*, 5669−5674.
(61) Lammers, L. G., and Markus, S. M. (2015) The dynein cortical anchor Num1 activates dynein motility by relieving Pac1/LIS1-mediated inhibition. *J. Cell Biol. 211*, 309−322.
(62) Yamamoto, A., Tsutsumi, C., Kojima, H., Oiwa, K., and Hiraoka, Y. (2001) Dynamic Behavior of Microtubules during Dynein-dependent Nuclear Migrations of Meiotic Prophase in Fission Yeast. *Mol. Biol. Cell 12*, 3933−3946.
(63) Saito, T. T., Okuzaki, D., and Nojima, H. (2006) Mcp5, a meiotic cell cortex protein, is required for nuclear movement mediated by dynein and microtubules in fission yeast. *J. Cell Biol. 173*, 27−33.
(64) Yamashita, A., and Yamamoto, M. (2006) Fission Yeast Num1p Is a Cortical Factor Anchoring Dynein and Is Essential for the Horse-Tail Nuclear Movement During Meiotic Prophase. *Genetics 173*, 1187−1196.
(65) Ananthanarayanan, V., Schattat, M., Vogel, S. K., Krull, A., Pavin, N., and Tolić-Nørrelykke, I. M. (2013) Dynein Motion Switches from Diffusive to Directed upon Cortical Anchoring. *Cell 153*, 1526−1536.
(66) Thankachan, J. M., Nuthalapati, S. S., Addanki Tirumala, N., and Ananthanarayanan, V. (2017) Fission yeast myosin I facilitates PI(4,5) P 2 -mediated anchoring of cytoplasmic dynein to the cortex. *Proc. Natl. Acad. Sci. U. S. A. 114*, E2672−E2681.
(67) Trokter, M., Mucke, N., and Surrey, T. (2012) Reconstitution of the human cytoplasmic dynein complex. *Proc. Natl. Acad. Sci. U. S. A. 109*, 20895−20900.
(68) Matanis, T., Akhmanova, A., Wulf, P., Del Nery, E., Weide, T., Stepanova, T., Galjart, N., Grosveld, F., Goud, B., De Zeeuw, C. I., Barnekow, A., and Hoogenraad, C. C. (2002) Bicaudal-D regulates COPI-independent Golgi−ER transport by recruiting the dynein−dynactin motor complex. *Nat. Cell Biol. 4*, 986−992.
(69) Olenick, M. A., Dominguez, R., and Holzbaur, E. L. F. (2019) Dynein activator Hook1 is required for trafficking of BDNF-signaling endosomes in neurons. *J. Cell Biol. 218*, 220−233.
(70) Hoogenraad, C. C. (2001) Mammalian Golgi-associated Bicaudal-D2 functions in the dynein-dynactin pathway by interacting with these complexes. *EMBO J. 20*, 4041−4054.
(71) Walenta, J. H., Didier, A. J., Liu, X., and Krämer, H. (2001) The Golgi-Associated Hook3 Protein Is a Member of a Novel Family of Microtubule-Binding Proteins. *J. Cell Biol. 152*, 923−934.
(72) Gassmann, R., Holland, A. J., Varma, D., Wan, X., Civril, F., Cleveland, D. W., Oegema, K., Salmon, E. D., and Desai, A. (2010) Removal of Spindly from microtubule-attached kinetochores controls spindle checkpoint silencing in human cells. *Genes Dev. 24*, 957−971.
(73) Splinter, D., Razafsky, D. S., Schlager, M. A., Serra-Marques, A., Grigoriev, I., Demmers, J., Keijzer, N., Jiang, K., Poser, I., Hyman, A. A., Hoogenraad, C. C., King, S. J., and Akhmanova, A. (2012) BICD2, dynactin, and LIS1 cooperate in regulating dynein recruitment to cellular structures. *Mol. Biol. Cell 23*, 4226−4241.
(74) Schlager, M. A., Hoang, H. T., Urnavicius, L., Bullock, S. L., and Carter, A. P. (2014) In vitro reconstitution of a highly processive recombinant human dynein complex. *EMBO J. 33*, 1855−1868.
(75) McKenney, R. J., Huynh, W., Tanenbaum, M. E., Bhabha, G., and Vale, R. D. (2014) Activation of cytoplasmic dynein motility by dynactin-cargo adapter complexes. *Science 345*, 337−341.
(76) Zhang, K., Foster, H. E., Rondelet, A., Lacey, S. E., Bahi-Buisson, N., Bird, A. W., and Carter, A. P. (2017) Cryo-EM Reveals How Human Cytoplasmic Dynein Is Auto-inhibited and Activated. *Cell 169*, 1303−1314.
(77) Urnavicius, L., Lau, C. K., Elshenawy, M. M., Morales-Rios, E., Motz, C., Yildiz, A., and Carter, A. P. (2018) Cryo-EM shows how dynactin recruits two dyneins for faster movement. *Nature 554*, 202−206.
(78) Grotjahn, D. A., Chowdhury, S., Xu, Y., McKenney, R. J., Schroer, T. A., and Lander, G. C. (2018) Cryo-electron tomography reveals that dynactin recruits a team of dyneins for processive motility. *Nat. Struct. Mol. Biol. 25*, 203−207.
(79) Jha, R., Roostalu, J., Cade, N. I., Trokter, M., and Surrey, T. (2017) Combinatorial regulation of the balance between dynein microtubule end accumulation and initiation of directed motility. *EMBO J. 36*, 3387−3404.
(80) Mallik, R., Carter, B. C., Lex, S. A., King, S. J., and Gross, S. P. (2004) Cytoplasmic dynein functions as a gear in response to load. *Nature 427*, 649−652.
(81) Kunwar, A., Vershinin, M., Xu, J., and Gross, S. P. (2008) Stepping, Strain Gating, and an Unexpected Force-Velocity Curve for Multiple-Motor-Based Transport. *Curr. Biol. 18*, 1173−1183.
(82) Mallik, R., Rai, A. K., Barak, P., Rai, A., and Kunwar, A. (2013) Teamwork in microtubule motors. *Trends Cell Biol. 23*, 575−582.
(83) Okumus, B., Landgraf, D., Lai, G. C., Bakshi, S., Arias-Castro, J. C., Yildiz, S., Huh, D., Fernandez-Lopez, R., Peterson, C. N., Toprak, E., El Karoui, M., and Paulsson, J. (2016) Mechanical slowing-down of cytoplasmic diffusion allows in vivo counting of proteins in individual cells. *Nat. Commun. 7*, 11641.
(84) Tokunaga, M., Imamoto, N., and Sakata-Sogawa, K. (2008) Highly inclined thin illumination enables clear single-molecule imaging in cells. *Nat. Methods 5*, 159−161.
(85) Izeddin, I., Récamier, V., Bosanac, L., Cissé, I. I., Boudarene, L., Dugast-Darzacq, C., Proux, F., Bénichou, O., Voituriez, R., Bensaude, O., Dahan, M., and Darzacq, X. (2014) Single-molecule tracking in live cells reveals distinct target-search strategies of transcription factors in the nucleus. *eLife 3*, e02230.
(86) Coelho, M., Maghelli, N., and Tolić-Nørrelykke, I. M. (2013) Single-molecule imaging in vivo: The dancing building blocks of the cell. *Integr. Biol. (United Kingdom) 5*, 748−758.
(87) Ananthanarayanan, V., and Tolić, I. M. (2015) Single-molecule imaging of cytoplasmic dynein invivo. *Methods Cell Biol. 125*, 1−12.
(88) Krull, A., Steinborn, A., Ananthanarayanan, V., Ramunno-Johnson, D., Petersohn, U., and Tolić-Nørrelykke, I. M. (2014) A divide and conquer strategy for the maximum likelihood localization of low intensity objects. *Opt. Express 22*, 210.
(89) Shubeita, G. T., Tran, S. L., Xu, J., Vershinin, M., Cermelli, S., Cotton, S. L., Welte, M. A., and Gross, S. P. (2008) Consequences of Motor Copy Number on the Intracellular Transport of Kinesin-1-Driven Lipid Droplets. *Cell 135*, 1098−1107.
(90) Rogers, S. S., Flores-Rodriguez, N., Allan, V. J., Woodman, P. G., and Waigh, T. A. (2010) The first passage probability of intracellular particle trafficking. *Phys. Chem. Chem. Phys. 12*, 3753−3761.
(91) Rai, A., Pathak, D., Thakur, S., Singh, S., Dubey, A. K., and Mallik, R. (2016) Dynein Clusters into Lipid Microdomains on Phagosomes to Drive Rapid Transport toward Lysosomes. *Cell 164*, 722−734.
(92) Erickson, R. P., Jia, Z., Gross, S. P., and Yu, C. C. (2011) How Molecular Motors Are Arranged on a Cargo Is Important for Vesicular Transport. *PLoS Comput. Biol. 7* (5), e1002032.
(93) Sanghavi, P., D'Souza, A., Rai, A., Rai, A., Padinhatheeri, R., and Mallik, R. (2018) Coin Tossing Explains the Activity of Opposing Microtubule Motors on Phagosomes. *Curr. Biol. 28*, 1460−1466.

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Biochemistry 2020, 59, 156−162