

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 on 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. Further, 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 future with appropriate experimental designs so as to better understand the spatio-temporal 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 which are essential for processes such as cell propulsion¹, cell division² and long-range transport of intracellular cargo^{3,4}. In eukaryotic cells, the cytoskeleton is made up 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 termed as 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 towards 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 proteins⁵ (Fig. 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 cell⁶. For instance, the kinesin 3 family motors, KIF1A and KIF1B β transport synaptic protein vesicles towards neuronal synapses^{7,8}, whereas KIF5 plays an important role in mitochondrial transport⁹.

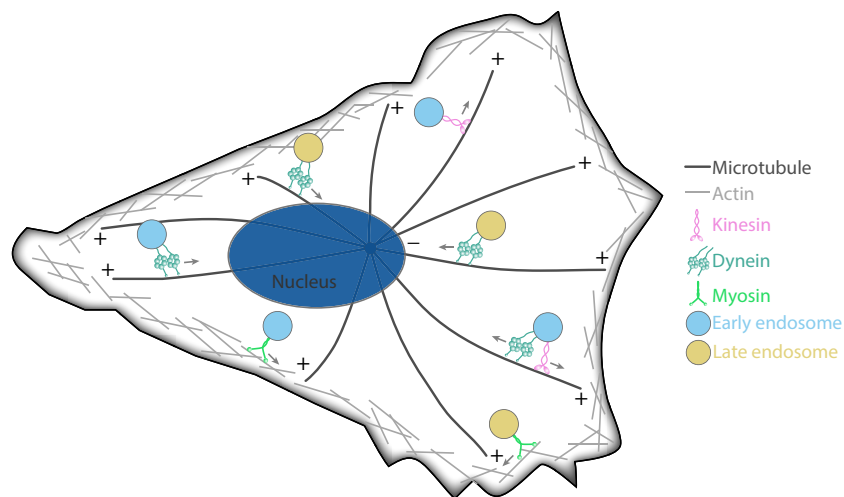


Figure 1. Intracellular cargo such as early and late endosomes are transported by kinesin and dynein motors on MTs, and myosin motors along the actin cytoskeleton.

In contrast to the large family of kinesin motors, the dynein family of motors comprises only two classes namely, axonemal and cytoplasmic dyneins¹⁰. 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 2 motors, namely cytoplasmic dynein-1 and 2. Cytoplasmic dynein-2 is responsible for intraflagellar transport along MTs in

cilia and flagella¹¹. Cytoplasmic dynein-1 (henceforth referred to as dynein) is ubiquitous across cell types, and is the primary motor responsible for the transport of cargo towards the minus ends of MTs in the cytoplasm¹².

Specifically, dynein is required for the orientation of bipolar spindle¹³, kinetochore separation during cell division¹⁴, and long-range, minus end-directed intracellular transport during interphase¹⁵. In the crowded intracellular milieu, large, diffusionally constrained cargo¹⁶ have to 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 more than 100 μm away 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 6 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, ATP binding to the motor domain detaches dynein from the MT. Furthermore, the linker domain which is normally docked to the 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 stalk is restored, and as a result it binds strongly to the MTs²⁰. 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 allows dynein to move processively, i.e. take several steps towards the minus ends of the MTs. Co-ordination between the motor domains enables yeast dynein to take multiple steps of either 8, 24 or 32nm 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 pN²⁸. In contrast, force measurements on dynein-driven

cargo in mouse macrophages indicate that single dynein motors exert forces of up-to ~ 1.2 pN²⁹. On the other hand, forces of up to ~ 7 pN have been reported for yeast dynein³⁰. Dynactin is a ~ 1.2 MDa, 23 sub-unit complex that increases dynein's processivity³¹.

Dynactin comprises a core formed by Arp1 sub-units with distinct pointed and barbed ends, and a shoulder complex³¹. The pointed end complex of dynactin includes the p25, p27 and p62 sub-units. The shoulder complex of dynactin includes the p150-Glued (p150 henceforth) sub-unit with an N-terminal MT-binding domain which establishes the link between dynactin and MTs. p150 also has a glycine-rich, basic domain (CAP-Gly) which can interact with MTs. The p24 and p50 sub-units complete the shoulder complex³¹. The p150 sub-unit interacts with DIC and thus links dynein to dynactin^{32,33}. We direct our readers' attention to Schroer, T. A. (2013), Chowdhury, S. et.al (2015), Carter, A. P. et.al (2016) and Ananthanarayanan (2016), for excellent schematics of dynein and dynactin structure^{31,34-36}. Recent research suggests that dynactin, in conjunction with cargo adaptors such as protein bicaudal D homolog 2 (BicD2), Hook1-3, Spindly, Rabll-FIP3, activate dynein³⁷⁻⁴².

Dynein's cargo inside the cell include early and late endosomes, lysosomes, peroxisomes, mitochondria, Golgi complex, post-Golgi cargo and kinetochores¹⁵. Therefore, unlike the highly specific kinesin family of motors, dynein interacts with multiple cargoes within the cell, and so, the spatio-temporal 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 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 fibroblasts³⁷. Moreover, antibodies against the p150 sub-unit of dynactin reduced the motility of organelles obtained from the squid axoplasm⁴³. Early experiments employing *in vitro* single-molecule bead assays revealed dynactin's effect on dynein's processivity at the single-molecule level. Specifically, 1 μ m-sized

beads bound to single molecules of dynactin and dynein displayed significantly increased residence times and run lengths compared beads bound only to dynein³⁸. 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 MTs⁴⁴. Therefore, it was concluded that dynactin provided an additional point of attachment between cargo-bound dynein and MT, thereby enabling faster reattachment of dynein to MTs during its mechano-chemical cycle.

Owing to 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 immuno-fluorescence localization of specific sub-units. Overexpression of fluorescently-tagged p150 revealed its strong MT-binding affinity and its stabilizing effect on individual MTs⁴⁵. Moreover, overexpression of the p50 (dynamitin) sub-unit 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 cells⁴⁶. 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 sub-unit reduced long range (>3 μm) motion of early endosomes⁴⁷. 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 EB1⁴⁸, which additionally recruits the endosome-MT linker protein CLIP-170⁴⁹. Dynactin was found to localize to plus ends of MTs in conjunction with CLIP-170, and a fraction of these plus end-localized dynactin spots were associated with cytoplasmic dynein⁵⁰. In fact, the 74 KDa dynein intermediate chain IC 74, was observed to track the growing plus ends of MTs in live HeLa cells⁵¹. 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 p150⁵². 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 compartments⁵³. Additionally, siRNA-mediated knockdown of CLIP-170 and EB1 in HeLa cells revealed a sequential targeting mechanism 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-axon⁵⁴. 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 instances⁵⁵, as in the case of mid-axon⁵⁴. In HeLa cells, expression of p135 fully substituted the function of p150 as indicated by the rescue of Golgi dispersion in cells lacking endogenous p150⁵⁶.

In essence, the enhancement in processivity of dynein-driven transport requires an intact dynactin complex that can associate with dynein. Moreover, in certain cells like Cos-7 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 cell⁵⁷ (Fig. 2). For instance, transport of cargo over long distances requires multiple motors, in which case groups of dyneins targeted to 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.

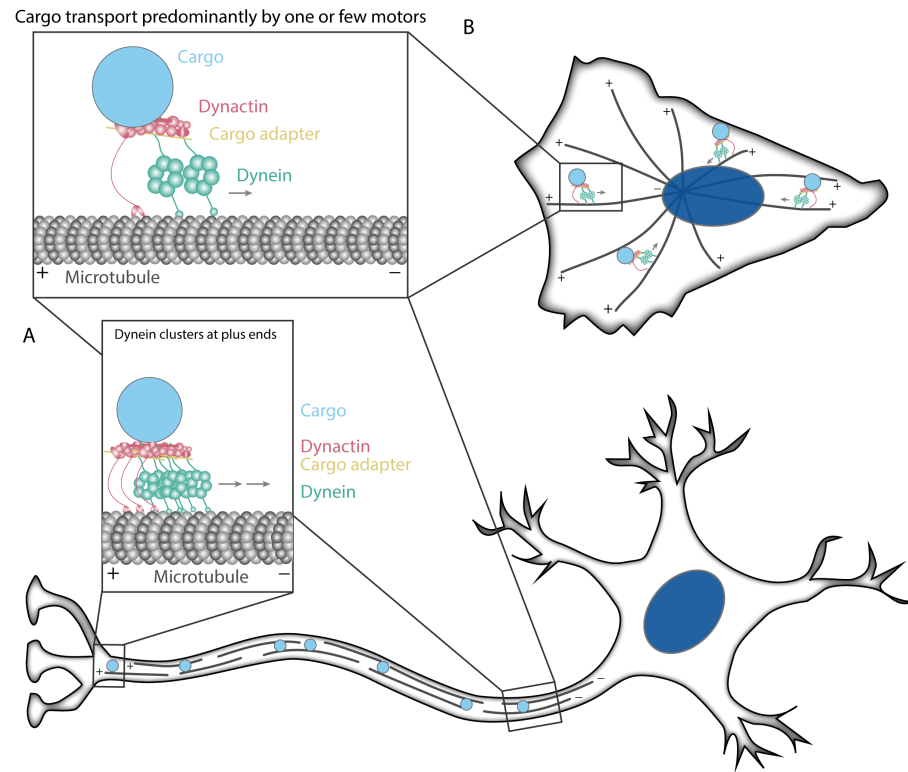


Figure 2. Dynactin's role in dynein-driven transport varies with cell type and intracellular location. (A) Dynactin bound to the MT plus ends enhances dynein clustering. In Cos-7 cells and distal neurites, minus end-directed transport is initiated when the growing MT plus ends encounter cargo. (B) In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

Studies on dynein purified from *S. cerevisiae* suggested that single molecules of dynein might be auto-inhibited which prevented them from being processive⁶⁰. Specifically, single molecules of *S. cerevisiae* dynein bound to the p150 homolog Nip100 lacking the MT-binding domain had a longer average run length compared to single dynein motors, which were almost stationary. Additionally, Pac1/Lis1 mediated inhibition of dynein was relieved by Num1, which activated dynein's processive motion⁶¹. Finally, in *S. pombe*, the anchor protein Mcp5 activated dynein by binding to its N-terminus^{62–66}. In *in vitro* stepping assays, single molecules of reconstituted complete human dynein complex remain statically bound to the

MTs⁶⁷, 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 auto-inhibition and processivity are regulated by dynactin and cargo adaptor binding. In the context of intracellular transport, cargo adaptors such as BicD2, Hook 3 and Spindly bind to dynein's N-terminal tail domain and recruit dynein on to specific cargo and regulate their movement. For instance, BicD2 recruits dynein onto Rab6-positive cargo and thereby regulates Golgi-endoplasmic reticulum (ER) transport⁶⁸. The cargo adaptor Hook1 is essential for dynein-driven transport of BDNF signaling molecules in the axons of primary hippocampal neurons⁶⁹. 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 half⁴². For instance, the N-terminal CC1-CC2 domain of BicD2 binds to dynein-dynactin, and its C-terminal CC3 domain binds to Rab6-positive cargo⁷⁰. Similarly, the C-terminal CC domain of Hook 3 interacts with the Golgi complex⁷¹. The CC domain of Spindly recruits dynein-dynactin to kinetochores in HeLa cells⁷².

In the past decade, an improved understanding of dynein-driven motility has become 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 low coprecipitation in a pull-down assay⁷³. This could be artificially increased by overexpressing the N-terminal fragment of the BicD2⁷³. Moreover, Rab6a-positive vesicles that were constitutively attached to BicD2-N displayed increased motion towards 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 where dynactin was added to dynein stepping assays, only a fraction of dynactin in solution colocalized 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 colocalization of dynactin and dynein, and these three components formed a processive complex. Moreover, complexes formed by dynein-dynactin and other cargo adaptors (DDC), Hook3/Spindly were also found to be processive *in vitro*⁷⁵. This confirmed that dynein exists in a default auto-inhibited state,

and simultaneous binding of dynactin to DIC and cargo adaptor to DLIC on DHC's tail domain, activates the dynein complex for processive motion.

Cryo-EM structures of pig brain dynein demonstrated that a native configuration of dynein, termed the phi-particle, has weak MT- and dynactin-binding affinity⁷⁶. 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 phi-particle required dynactin binding to rearrange the MT-binding stalks to a 'parallel' configuration which was then capable of processive movement when it formed a complex with dynactin and BicD2 (Fig. 3). In contrast to the cargo adaptor BicD2 which 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 higher 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 manner⁷⁹. 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 dynactin⁷⁹.

The *in vivo* formation and dynamics of the tripartite complex is 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}.

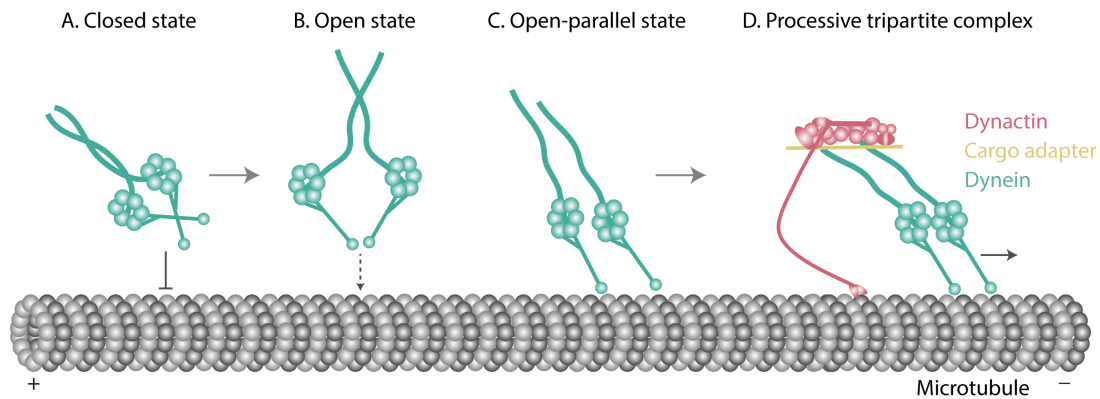


Figure 3. Formation of processive tripartite complex requires conformational changes in dynein. (A) In WT cells, 74% of dynein exists in the auto-inhibited phi-particle form⁷⁶. The phi-particle has low affinity for MTs and cannot form a processive complex. (B, C) Compared to the phi-particle, the open 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 cargo come together inside the cell is currently not understood. Please 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 sub-units in this schematic for clarity.

However, studies from HeLa and Arpe-19 cells suggest that early endosomal cargo move in short processive runs of about 1-2 μm punctuated with frequent pauses⁵⁹. 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 motors, thereby eliminating the role of dynactin-mediated MT plus end-targeting of dynein in these cells.

It is now well established that a complex of dynein-dynactin-cargo (mediated by adaptors like BicD2 and Hook3) is essential for processive motion. As evinced by low co-precipitation in pull-down assays, the interaction between dynein and dynactin is weak and when DHC is over expressed in HeLa cells, it remains predominantly cytosolic despite the presence of a MT-binding domain⁷³. Moreover, all studies employing the processive complex have resorted to using molar excess of the N-terminal fragment of BicD2 to increase complex formation^{75,74}. Taken together, the interaction between 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 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^{80,29,81,82}. These constraints necessitate understanding the regulation of dynein-driven transport by dynactin under cellular conditions.

Future directions. The primary question that future experiments should ascertain 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 cells⁷³, in contrast to p150 which marks the MTs lattice⁴⁵. 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 motors⁶⁵ and other proteins inside living cells,⁸³ can be used to visualize^{84–87} and follow⁸⁸ single molecules of dynein/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/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. Since, 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 cargo in those regions are more likely to be driven by multiple dynein motors resulting in longer run lengths⁸⁹. 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 cargo movement across the cell^{90,91}.

Finally, the effect of spatial variation and the kinetics of interaction between the components of the processive complex can be incorporated into computational models. For instance computational models of motor arrangement on the cargo revealed that thermal fluctuations of cargo could cause high loads on the motor leading to detachment⁹². 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 interpreting results obtained using biochemical, microscopy and computational experiments.

ACKNOWLEDGEMENTS

We thank L. A. Chacko and M. Shah for constructive comments on the manuscript.

FUNDING

This work was supported by the Science and Engineering Research Board, India Women Excellence Award, R. I. Mazumdar Young Investigator Award, and Wellcome Trust/DBT-India Alliance Fellowship (IA/I/18/1/503607) awarded to VA.

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