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RESEARCH HIGHLIGHT

Backseat drivers: Regulation of dynein motility

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Control of the activity of the microtubule motor cytoplasmic dynein 1 is essential for its function in intracellular transport. A recent paper by McKenney *et al*. published in *Science* shows that activation of processive dynein motility requires the formation of cargo adaptor-dynein-dynactin complexes.

Cells rely on their intracellular components being in the right place at the right time. In eukaryotic cells, microtubule-based transport by motor proteins belonging to the dynein and kinesin families plays a crucial role in regulating the spatial-temporal distribution of a multitude of membrane bound organelles, protein complexes, and ribonucleoprotein complexes. Disruption of these transport functions can play a key role in pathological processes and the activities of microtubule motors are frequently usurped by both viral and bacterial pathogens to aid their replication [1]. Cytoplasmic dynein 1 is the predominant motor protein complex mediating transport towards the minus end of microtubules and it transports many different cargoes [2]. The dynein holoenzyme is composed of a dimeric heavy chain that contains the microtubule-binding and AAA-ATPase motor domains associated with a series of smaller accessory proteins implicated in regulation and cargo binding. Targeting of the motor to a specific cargo is often mediated by so-called 'adaptor proteins' that can associate with both the cargo (e.g., endosomes) and the motor complex itself.

Diverse functions and diverse cargoes necessitate a high level of cytoplasmic dynein regulation. Such regulation must limit motor activity to prevent wasteful ATP hydrolysis in the absence of cargo transport and prevent inappropriate movement of cargo-free motors on microtubules. Regulation must allow exquisite responses to dynamic spatial and temporal cues for cargo transport and allow for the selective recognition of a wide range of cargoes/adaptors that, ostensibly at least, may be quite different. It must also support bidirectional transport processes.

A second multiprotein complex, dynactin [3], helps to regulate cytoplasmic dynein 1. Indeed, dynactin is required for almost all known functions of cytoplasmic dynein. It is thought that dynactin plays a key role in attachment to cargo and promotes dynein activity. Despite this, its precise mechanism of action and the role of cargo attachment itself has remained unclear.

Recently, a study by McKenney *et al*. [4] in *Science* and a complimentary study by Schlager *et al*. [5] in *EMBO J*, have taken crucial steps forward, uncovering a role for tripartite cargo adaptor-dynein-dynactin complexes in directly promoting dynein activity (Figure 1). Both of these studies utilize elegant biochemical purification coupled with the technical feat of high-resolution, single-molecule, multicolor TIRF microscopy to examine the properties of these assemblies as they move on labelled microtubules *in vitro*.

McKenney *et al*. [4] begin by showing that cytoplasmic dynein purified from rat brain (that is free from both dynactin and cargo proteins) binds to microtubules but does not engage in the processive long distance movements characteristic of motility *in vivo*. This implies that dynein requires activation. To isolate transport-active complexes, the authors use an alternative approach — affinity purification (from RPE-1 cells) via the cargo adaptor BicD2 (that couples dynein to Rab6-containing organelles). This yields stable associations of BicD2, dynein and dynactin, which when examined in TIRF motility assays, exhibit speeds and run lengths approaching those observed *in vivo*. Importantly, the authors reveal that these complexes consist of a single copy of dynein, dynactin and BicD2 (a dimer), demonstrating that the intrinsic processivity of the holoenzyme is directly enhanced and ruling out effects from cooperation between motor complexes in this system.

The authors then ask which components of this tripartite complex are needed for dynein activation — is BicD2 required or is dynactin sufficient? They show that removal of BicD2 results in a loss of processive motility and dissociation of dynein from dynactin, demonstrating the importance of the cargo adaptor itself in formation of stable dynein-dynactin complexes and motility. Schlager *et al*. [5] come to similar conclusions in their study using recombinant human dynein produced in baculovirus (an achievement in its own right), showing that purified dynactin is unable to activate motility in the absence of BicD2.

To determine whether this mechanism is unique to BicD2 or whether it holds for other cargo adaptors, McKenney *et al*. expand their study to include three other adaptors — Rab11-FIP3 (for recycling endosomes), Spindly (kinetochores) and Hook (early endo-

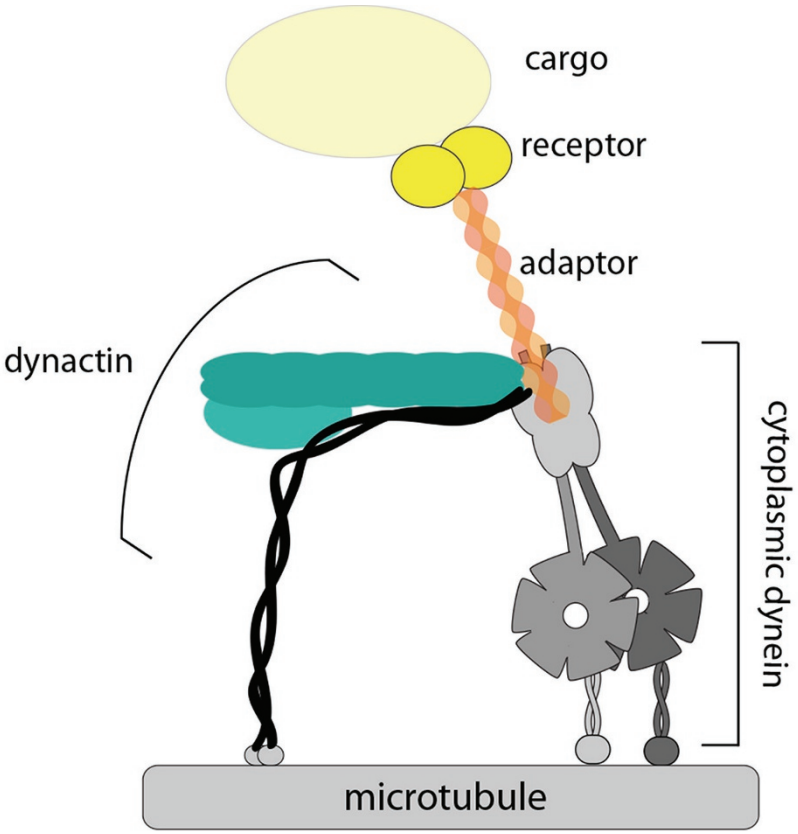

Figure 1 Schematic showing proposed organization of an active dynein-dynactin-cargo complex. Cargo (e.g., an endosome) couples to the motor complex via surface receptors (e.g., a Rab GTPases) that recruit specific adaptor proteins (e.g., BiCD2, Hook). These in turn recruit dynein/dynactin and stabilize their association, supporting the microtubule binding and processivity of the dynein-dynactin complex.

somes). They show that all three can be used to purify dynein-dynactin and that those complexes are capable of processive motility in a manner comparable to those derived from BicD2 affinity purification.

These studies thus highlight a crucial role for the cargo adaptor, coupled with dynactin, in dynein activation. This is somewhat reminiscent of several kinesin family proteins which exist in an inactive state in the absence of cargo [6]. In the future it will be important to understand why dynein is inactive in the absence of dynactin/cargo and what changes occur within the complex upon cargo adaptor/dynactin binding to cause its conversion to a processive motor. Clues may come from comparison with *S. Cerevisiae* dynein which appears constitutively active and may associate with dynactin in the absence of cargo [7]. It will also be important to determine the regulatory signals that control formation and dissociation of these active complexes, how they interact with other dynein regulators such as the Lis1-NudEL complex and how they are affected by the action of plus-end-directed motors associated with the same cargo.

Further progress should also come from understanding of the structural and biophysical characteristics of the motor-cargo interfaces that we now know must ultimately drive dynein activation. Indeed, the fact that four distinct cargo adaptors can promote formation of transport-active complexes may imply the existence of common features in dynein-cargo adaptor recognition mechanisms that support activation. Importantly, the establishment of these elegant *in vitro* systems that recapitulate many of the properties of cytoplasmic dynein *in vivo* will now allow for a full molecular dissection of this ubiquitous and fascinating process.

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