Have you seen?

Traffic control: adaptor proteins guide dynein–cargo takeoff

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The precise movement of intracellular components requires active transport by molecular motors along the filamentous tracks of the cytoskeleton. While yeast cytoplasmic dynein can walk for some distance along microtubules, mammalian dynein is non-processive. This has raised the question of how this motor can transport cargo. In two recent papers by the Carter, Bullock and Vale labs, mammalian dynein processivity has now been successfully reconstituted \textit{in vitro} in the presence of adaptor proteins.

See also: MA Schlager \textit{et al} and RJ McKenney \textit{et al}

Since kinesin and dynein serve as major players in cytoskeletal trafficking, elucidating how the activity of these molecular motors is modulated during cargo binding and transport is central to understanding cellular organization. While much of our knowledge of the \textit{in vitro} behavior of dynein comes from studies of \textit{Saccharomyces cerevisiae} dynein (Reck-Peterson \textit{et al}, 2006), studies of the mammalian motor have revealed major differences in its behavior. For example, single-molecule experiments of native mammalian dynein either attached to beads under force traps or analyzed using total internal reflection fluorescence microscopy revealed only diffusion (Mallik \textit{et al}, 2005; Miura \textit{et al}, 2010) or the ability to walk for very short distances (<1 \textmu m) on microtubules (King & Schroer, 2000; Ross \textit{et al}, 2006; Ori-McKenney \textit{et al}, 2010). This is in contrast to the processive movement of yeast dynein \textit{in vitro} (Reck-Peterson \textit{et al}, 2006) and the long-range intracellular movements of mammalian cargo \textit{in vivo} (Ori-McKenney \textit{et al}, 2010). The non-processive behavior of mammalian dynein was also confirmed for human dynein reconstituted from baculovirus and \textit{E. coli} overexpression; this motor only showed diffusive interactions with the microtubule (Troktet \textit{et al}, 2012). These and other studies have begged the question: Can mammalian dynein walk processively? Schlager, Hoang, McKenney and colleagues addressed this question in papers recently published in \textit{The EMBO Journal} and \textit{Science} (McKenney \textit{et al}, 2014; Schlager \textit{et al}, 2014).

Schlager, Hoang and colleagues established a robust system to overexpress human dynein using baculovirus, expressing all six subunits of the human dynein complex from a single virus. The purified recombinant human dynein complex had properties similar to those of the native mammalian motor, including an expected molecular weight of 1.40 MDa. Electron microscopy and image processing showed that recombinant human dynein appears indistinguishable from native dynein purified from pig brain. Finally, the recombinant human dynein only diffuses on microtubules \textit{in vitro}, as it had been previously reported (Troktet \textit{et al}, 2012).

Next, Schlager, Hoang \textit{et al} added dyactin, a dynein processivity factor, to their purified dynein (Schroer, 2004). As the universal dynein–cargo adaptor, dyactin has previously been shown to increase the run length of both chick embryo (King & Schroer, 2000) and yeast dynein (Kardon \textit{et al}, 2009) \textit{in vitro}. Unexpectedly, native dyactin purified from pig brain did not bind dynein, nor did it have any effect on processivity \textit{in vitro} (Fig 1A). These results suggested that an additional factor—required to induce processive dynein movement—might be missing.

BicaudalD-2 (BICD2), a well-characterized dynein adaptor, was identified by the authors as a potential processivity factor for the motor. Previous work had shown that an N-terminal truncation of BICD2 efficiently co-precipitated with dynein–dynactin complexes (Splinter \textit{et al}, 2012). Although addition of this N-terminal fragment of BICD2 (BICD2N) to recombinant dynein \textit{in vitro} had no effect on processivity, addition of dynactin to the mix changed this dramatically: The recombinant human dynein now became highly processive, with excursions exceeding 5 \textmu m (Fig 1B).

How does BICD2N induce processivity in dynein? While this adaptor could directly alter dynein’s mechanical properties, it was also possible that the increased processivity resulted from oligomerization of dynein–dynactin complexes, with any given individual dynein remaining non-processive. To test this possibility, the authors prepared a 50:50 mixture of dynein labeled with two different fluorophores. They then added BICD2N and dynactin and monitored the colocalization of the fluorescence signal from the two different dyes as they moved along microtubules. The fact that the processive runs contained a single dye shows that BICD2 does not change oligomerization of dynein but rather that it alters its processivity by acting on the motor itself.

Since the addition of BICD2N results in a stable ternary complex with dynein and dynactin, the authors characterized the morphology of the complex using negative stain electron microscopy and data processing. The image analysis showed a large...
Adaptors increase dynein’s processivity

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additional density that aligned with the tail domain of dynein, suggesting that dynactin may have an extended interaction interface with dynein that is mediated by BIC2DN.

This work by Schlager and Hoang et al. may represent a new paradigm for mammalian dynein-mediated transport, where dynein only exhibits high levels of processivity upon the combined interaction with dynactin and a cargo adaptor. Indeed, McKenney and coworkers very recently reported the same effect and extended the list of adaptors capable of increasing the run length of mammalian dynein to include Rab11-FIP3, Spindly and Hook3 (McKenney et al., 2014) (Fig 1B). These two papers provide a framework for further dissection of the ability of dynein to be targeted and activated at specific cargos in vivo. In particular, structural and functional experiments that parse the interplay between dynein, dynactin and cargo adaptors stand to illuminate the molecular decisions responsible for mammalian cargo transport.

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References


Figure 1. BicaudalD-2 promotes the formation of a processive dynein-dynactin complex. Recombinant human dynein alone is not processive in vitro. The addition of either dynactin or BicD2N does not stimulate processivity. The combined presence of BicD2N, dynactin and dynein results in a highly processive dynein molecule. Other adaptors, such as Spindly, Rab11-FIP3, and Hook3, also increase dynein’s processivity in the presence of dynactin.

Recombinant human dynein

HIGHL Y PROCESSIVE

DIFFUSIVE, NON-PROCESSIVE

BicD2N

Recombinant human dynein

Dynein cargo adaptors
- BicD2
- Hook3
- Rab11-FIP3
- Spindly