JCB Comment

Dynactin polices two-way organelle traffic

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How is the bidirectional motion of organelles controlled? In this issue, Deacon et al. (2003) reveal the unexpected finding that dynactin (previously known to control dynein-based motility) binds to kinesin II and regulates anterograde movement of *Xenopus* melanosomes. This result suggests that dynactin may be a key player in coordinating vesicle traffic in this system.

The movement of intracellular cargo is essential for cell survival. In animal cells, membranous organelles are propelled through the cytoplasm by microtubule-based motor proteins. Anterograde movement toward microtubule plus ends at the cell periphery is driven by motor proteins of the kinesin superfamily, whereas retrograde movement toward minus ends at the cell center is largely accomplished by cytoplasmic dynein. In most cells, organelles do not travel smoothly in one direction but frequently switch between plus and minus end–directed travel. The net time spent traveling in the plus versus the minus end direction determines the steady-state distribution of an organelle population within a cell. A long-standing question for those studying organelle transport is how this bidirectional trafficking is coordinated. Is the binding of kinesin and dynein to vesicles mutually exclusive, or are these motors bound at the same time but with their activities coordinately regulated? What molecule(s) might be responsible for linking kinesin and dynein activities? In this issue, Vladimir Gelfand's group (Deacon et al., 2003) addresses these questions by studying the motor proteins kinesin II and cytoplasmic dynein that move pigment granules in *Xenopus* melanophore cells. Their results are surprising; the dynactin complex, previously known to bind to cytoplasmic dynein and anchor it to organelles, also interacts with kinesin II and is necessary for plus end–directed motion. The ability of dynactin to physically interact with these two opposite polarity motors suggests that it may be the long sought-after molecular switch that coordinates bidirectional movement in this system.

Previous studies hinted that the actions of dynein and kinesin may be controlled via dynactin. Dynactin is a large, multimeric protein complex. Its p150^{Glued} subunit has binding sites for both microtubules and the intermediate chain of dynein and is thought to be responsible for the association of dynein with many of its cargo organelles (Karki and Holzbaur, 1995; Vaughan and Vallee, 1995; Waterman-Storer et al., 1995). Curiously, the treatment of extruded squid axoplasm with antibodies against p150^{Glued} inhibited both the anterograde and retrograde movement of organelles along microtubules (Waterman-Storer et al., 1997). These antibodies were known to inhibit the interaction of dynactin with dynein, but their effect on anterograde movement was more difficult to explain. However, genetic studies yielded similar results. Martin et al. (1999) found that mutations in either p150^{Glued}, the cytoplasmic dynein heavy chain, or kinesin I inhibited both retrograde and anterograde fast axonal transport in *Drosophila* larvae. This phenotype potentially could be explained by stalled retrograde vesicles sterically blocking the movement of anterograde cargo, but the authors also suggested the possibility of a physical linkage between kinesin, dynein, and dynactin. This theory was further tested by tracking the movement of lipid droplets in *Drosophila* embryos (Gross et al., 2002b). A mild defect in the dynein heavy chain impaired several aspects of minus end–directed transport of lipid droplets: run lengths, velocities, and the opposing optical trap force required to halt droplet movement were all decreased. Surprisingly, this mutation produced similar effects on droplets moving toward the microtubule plus ends. Embryos expressing a mutant p150^{Glued} protein that partially impaired dynactin function also exhibited impaired movement in both the plus and minus end directions. Collectively, these results suggested that dynactin might be involved in coordinating the bidirectional movement of organelles. However, these studies did not provide a molecular explanation of how this mechanism might work.

To study the mechanism of coordination of bidirectional vesicle movement, Deacon et al. (2003) used *Xenopus* melanophores due to the unique ability to experimentally control the directional movement of their pigmented melanosomes (Daniolos et al., 1990). Upon treatment of melanophores with melatonin, the cAMP concentration in the cytoplasm drops and the melanosomes move with a net minus end–directed bias and aggregate toward the cell center. Treatment with melanocyte-stimulating hormone (MSH)* restores cAMP levels, and the melanosomes exhibit a plus end–directed bias and disperse throughout the cell. Aggregation

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*Abbreviations used in this paper: KAP, kinesin-associated protein; MSH, melanocyte-stimulating hormone.

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is accomplished by cytoplasmic dynein (Nilsson and Wallin, 1997), whereas dispersion requires the combined actions of kinesin II and the actin-based motor myosin V (Rogers and Gelfand, 1998; Tuma et al., 1998; Gross et al., 2002a). Kinesin II is a heterotrimeric protein consisting of two motor subunits and a third nonmotor subunit known as kinesin-associated protein (KAP) (Cole et al., 1992). KAP is thought to be involved in binding kinesin II to its cargo, although the mechanism for this interaction is not known.

The role of dynactin in melanosome transport was investigated by disrupting dynactin function via the overexpression of dynamitin (Echeverri et al., 1996), a crucial subunit that holds the dynactin complex together. To ensure that all observed melanosome movement occurred on the microtubule cytoskeleton, actin filaments were depolymerized with latrunculin B. Here, the authors report that melanosome movement to both the plus and minus ends of microtubules was inhibited by dynamitin overexpression, suggesting a role for dynactin in coordinating bidirectional movement. They considered whether this result might be explained if both kinesin II and dynein bound to dynactin and thereby docked onto membranes. To test this idea, kinesin II was immunoprecipitated with a series of antibodies, and the authors found that dynactin was pulled down along with this kinesin motor in all cases. The reverse experiment of immunoprecipitating with $p150^{Glued}$ antibodies also brought down kinesin II. Blot overlays of purified melanosomes with $p150^{Glued}$ detected an interaction with a 115-kD protein, the expected size of *Xenopus* KAP. Subsequent overlay and affinity pull-down experiments with purified proteins confirmed the direct binding of $p150^{Glued}$ to KAP. By constructing a series of GST fusion proteins, Deacon et al. (2003) were able to map the site of this interaction to residues 600–811 of $p150^{Glued}$ and the COOH-terminal domain of KAP. Interestingly, this region of $p150^{Glued}$ also interacts with the dynein intermediate chain, which raised the question of whether kinesin II and dynein might compete for binding to dynactin. Using a blot overlay competition assay, the authors found that the COOH-terminal KAP domain blocked the binding of $p150^{Glued}$ to the dynein intermediate chain, whereas the NH_2-terminal KAP domain, used as a control, did not. This result confirms that the two motors cannot bind dynactin simultaneously.

If these biochemical results are relevant to melanosome movement, then overexpression of KAP should inhibit both anterograde and retrograde traffic. Indeed, overexpession of *Xenopus* KAP or just its COOH-terminal fragment inhibited bidirectional melanosome movement. As a control, NH_2-terminal KAP had no effect on retrograde movement and only a small effect on anterograde movement, perhaps due to interactions with the kinesin II motor subunits. Together, the results of Deacon et al. (2003) demonstrate that kinesin II, via its KAP subunit, binds to the $p150^{Glued}$ subunit of dynactin and that this interaction is important for kinesin II–mediated movement of melanosomes.

Although the authors identify the $p150^{Glued}$ subunit of dynactin as a key player in coordinating the bidirectional movement of melanosomes, the mechanism is still unclear. Their biochemical results showing competitive binding to dynactin suggest that binding of kinesin II and dynein to melanosomes may be mutually exclusive events; however, previous work has shown that this is not the case. In a recent paper from the same authors (Gross et al., 2002a), as well as an earlier study from Reese and Haimo (Reese and Haimo, 2000), the relative amounts of kinesin II and dynein bound to purified melanosomes did not change when cells were treated with melatonin to stimulate aggregation or with MSH to stimulate dispersion. Thus, it is possible that proteins other than dynactin might bind kinesin II and dynein to melanosomes. This question also could be addressed by determining if motor binding to melanophores is diminished in cells overexpressing KAP or dynamitin. Unfortunately, Deacon et al. (2003) were not able to answer this question by biochemical isolation of melanosomes and motor quantitation because transfected cells were only a small percentage of the total population. Another possible model is that dynactin is not needed for recruiting kinesin II and dynein to melanosomes but is somehow involved in regulating the activation or organization of motors already bound to the membrane.

Future studies will no doubt explore whether dynactin is involved in bidirectional transport in systems other than melanophores. In intraflagellar transport, kinesin II and cytoplasmic dynein 2 are involved in moving nonmembranous particles between the cell body and the tip of the flagella or cilia (Rosenbaum and Witman, 2002). It will be interesting to determine whether dynactin plays a role in this type of cargo transport. In neurons, kinesin I is responsible for moving organelles from the cell body to the axon terminal. As discussed above, Martin et al. (1999) found that mutations in either kinesin I heavy chain, dynein, or $p150^{Glued}$ all produced the same phenotype in *Drosophila* larvae neurons, suggesting that dynactin may play a role in coordinating bidirectional movement in this system as well. Immunoprecipitation of neuronal $p150^{Glued}$, however, brought down only dynein but not kinesin I. This finding may result from the fact that kinesin I, which possesses a light chain unrelated to the KAP subunit, could be linked indirectly to dynactin by another protein. Thus, this study by Deacon et al. (2003) has opened up a new area of exploration and dynactin will undoubtedly receive closer scrutiny from kinesin researchers in the future.

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