Current Biology, Vol. 13, R320–R322, April 15, 2003, ©2003 Elsevier Science Ltd. All rights reserved. DOI 10.1016/S0960-9822(03)00237-9

Dynactin: Coordinating Motors with Opposite Inclinations

Steven P. Gross

In eukaryotic cells, many organelles are transported bidirectionally along microtubules by kinesin and dynein. These opposite-polarity motors appear to be coordinated to avoid interfering with each other's function. New work has provided the first molecular insight into how such coordination might occur.

The organelles that inhabit a typical eukaryotic cell are restless bodies, usually in a state of motion from one part of the cell to another. One of the surprising things about this organelle motion is that, although the opposite-polarity molecular motors kinesin and dynein move unidirectionally with respect to their cytoskeletal tracks — towards the 'plus' or 'minus' end of a microtubule, respectively — many of the transported cargos move bidirectionally and frequently reverse course. Examples of such cargos include mRNA particles, mitochondria, endosomes, herpes virus particles and lipid droplets. This motion suggests that both classes of motors attach to the same organelle, and raises the question: how does a cargo that can go in either direction get to the right place? Obviously, net transport is controlled: 'on average' a nucleus-bound cargo spends more time employing a minus-end directed dynein than a plus-end kinesin.

This control might occur in two ways. First, opposite motors could be engaged in a tug-of-war, so that regulation could control the transport bias by determining who is likely to win the struggle. This could involve modulating either force production, by altering enzymatic activity, or the average number of active motor molecules of a particular type bound to the cargo. Second, motors might be coordinated, so that they do not interfere with each other's function. In this case, because each motor could be turned 'on' or 'off' independently, regulation would control net transport simply by keeping one set of motors 'on' longer.

Recent work on *Drosophila* showed that opposite polarity motors moving lipid droplets are indeed coordinated [1], but the mechanism by which this coordination is achieved was unclear. Deacon *et al.* [2] have now addressed this issue with a new study of bidirectional pigment granule motion in cultured *Xenopus* melanophores, cells which are specialized to aggregate or disperse pigment to allow the animal to rapidly change its coloration. They describe a molecular interaction that could lie at the heart of coordination between opposite polarity motors. Clarifying the ramifications of this interaction and establishing its generality should dramatically accelerate our understanding of bidirectional microtubule-based motion.

Department of Developmental and Cell Biology, University of California, Irvine, California. 92697, USA.
E-mail: sgross@uci.edu

Studies in diverse systems have provided evidence favoring the 'coordination' hypothesis. In cell culture systems and also in *Drosophila* neurons in the whole animal, function-blocking antibodies or genetic alterations affecting dynein or the dynein-regulatory complex dynactin were found to impair plus-end-directed, as well as minus-end-directed, motion [3–5]. Similarly, antibody or genetic impairment of the plus-end-directed motor kinesin affected minus-end-directed as well as plus-end-directed transport [3,6]. In the tug-of-war scenario, impairment of minus-end-directed motion should lead to enhanced plus-end-directed motion, given the reduced opposition to the plus-end motor activity. This was not observed.

Although these observations suggested motor coordination, an alternative interpretation was that the motors are not in fact coupled, but that the minus-end impairment indirectly causes plus-end impairment, and vice versa. For example, removal of motor function might cause a 'traffic jam', with the 'stuck' cargos blocking the motion of other cargos. Alternatively, the experimental manipulations might have led to aberrant motor function, with the 'impaired' motor(s) in a 'locked-up' state, tightly bound to the microtubules, and opposing motion in both directions.

These concerns have been addressed in a recent study which examined the bidirectional transport of lipid droplets in early *Drosophila* embryos [1]. The authors found that, not only did alteration of minus-end-directed motor activity alter plus-end-directed motion, under some circumstances it did so when the minus-end-directed stalling forces were normal, proving that the motors were not aberrantly 'locked-up'. Because only droplets not interacting with other cargos were studied, there was no issue of non-specific 'traffic-jam' inhibition. Further, these experiments showed that the $p150^{Glued}$ subunit of the dynactin complex plays a role in this coordination.

Taken together, these studies make a strong case for a model in which there is coordination between opposite motors on the same cargo. How such coordination could be achieved at a molecular level has, however, been an entirely open question. One possibility was that the two classes of motors might not bind to a single cargo at the same time, for instance, if there were a single receptor that can tether either kinesin or dynein to the cargo, but not both simultaneously. Then, the motors could not interfere with each other, because they would not be on the cargo at the same time.

While this would be an elegant way of achieving coordination, evidence from a number of systems points against it. First, using fluorescence microscopy to observe GFP-tagged dynein in *Dictyostelium*, Ma and Chisholm [7] found that, when bidirectional cargos reverse course, the dynein stays bound.

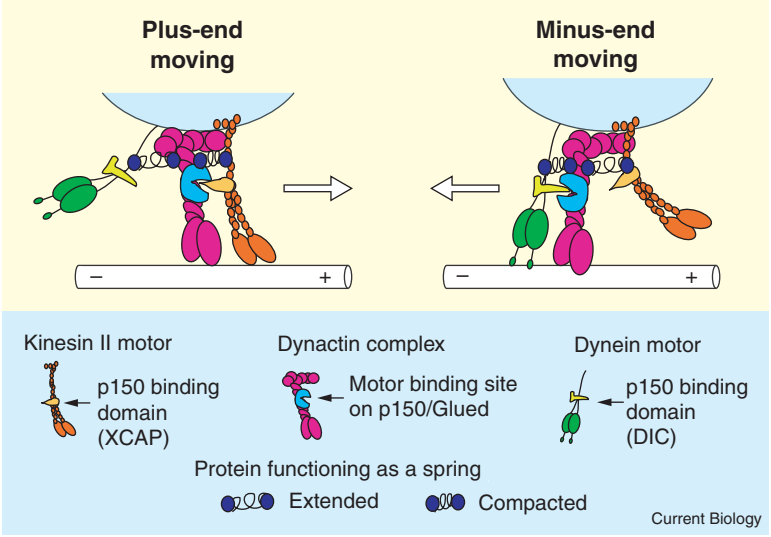

Figure 1. A model for dynactin action in coordinating motor proteins.

Left: kinesin-II binding to the dynactin complex both activates kinesin-II and inactivates dynein, resulting in plus-end-directed cargo motion. Right: DIC binding to the dynactin complex activates dynein and inactivates kinesin-II. Both motors remain cargo-bound, independent of their interaction with the KAP/DIC binding site on p150^{Glued}. When not held in place by the dynactin complex — through the KAP/DIC binding site — the motors are effectively turned off, perhaps by being pushed away from the microtubule by hypothesized 'spring' proteins. Such spring proteins might bind to either the cargo or to the dynactin complex (as indicated). The length of a plus-end-directed (or minus-end-directed) run is determined by how long XCAP (or DIC) remains bound to p150^{Glued}. Reversals in motion occur when XCAP is released, and DIC binds, or vice versa.

Second, biochemical quantification in melanophores showed that the amount of plus-end-directed versus minus-end-directed motors bound to the pigment granules does not change, regardless of whether they are moving on average towards the plus or minus end [8]. Finally, in *Drosophila* the biophysical experiments of Gross *et al.* [1] indicated both motors are functionally present simultaneously, because mutations in one set of motors were found to alter opposite-polarity motion. Thus, both classes of motors are present on the cargo, regardless of its direction of travel, but their relative activities are regulated so that they usually do not interfere with each other's functions.

Deacon *et al.* [2] investigated the molecular mechanism of coordination in the context of pigment-granule transport in cultured *Xenopus* melanophore cells. They have established a molecular link between the opposite polarity motors kinesin-II and dynein, through the dynactin complex. Dynactin's importance for correct dynein function is well established, as are molecular interactions between dynein and dynactin (reviewed in [9]). For instance, dynactin's p150^{Glued} subunit binds the dynein intermediate chain (DIC). In contrast, the molecular details of how dynactin might play a role in plus-end motor function has been unclear.

In melanophores, plus-end pigment granule motion is driven by kinesin II, a heterotrimer composed of two motor subunits and a non-motor subunit called KAP. By immunoprecipitation against a variety of targets, Deacon *et al.* [2] confirmed the DIC–p150^{Glued} interaction but also showed that KAP can bind directly to p150^{Glued}. This interaction was found to be exclusive: either KAP can bind, or DIC, but not both. Further, using a blot overlay assay, they demonstrated that KAP and DIC bind to approximately the same region of the p150^{Glued} protein (residues 600–811). In this assay, binding was competitive: the amount of p150^{Glued} binding to immobilized DIC decreased in proportion to the amount of carboxy-terminal KAP that was present together with the p150^{Glued}.

How can we interpret these observations? One possible model (Figure 1) is that motors have a hard time reaching the microtubule — for example, because of the hypothesized 'spring' proteins — so that the motor–microtubule interaction must be stabilized for efficient motor function. p150^{Glued} can independently bind microtubules, so the dynactin complex might facilitate the motor–microtubule interaction by holding the motor — through binding to KAP or DIC — close to the microtubule. However it works, the interaction of dynactin with KAP or DIC likely controls microtubule access rather than motor activity per se, because both motors have enzymatic activity without p150^{Glued}.

Finally, although the binding of p150^{Glued} to KAP or DIC is competitive *in vitro*, this binding is likely subject to additional regulation *in vivo*, because a simple competitive binding interaction would predict that increasing the KAP binding, and thereby increasing the length of plus-end-directed runs, would also allow KAP to more effectively displace bound DIC, shortening minus-end-directed runs. In a variety of systems, however, cells can alter plus-end-directed run lengths while leaving minus-end-directed run lengths unchanged [8,10,11]. The interaction of p150^{Glued} with KAP or DIC is likely to be at the heart of the regulation of bidirectional motion in cells, and provides the first foothold for taking apart the molecular mechanism of motor coordination.

References

1. Gross, S.P., Welte, M.A., Block, S.M. and Wieschaus, E.F. (2002). Coordination of opposite-polarity microtubule motors. J. Cell Biol. *156*, 715–724.
2. Deacon, S.W., Serpinskaya, A.S., Vaughan, P.S., Fanarraga, M.L., Vernos, I., Vaughan, K.T. and Gelfand, V.I. (2003). Dynactin is required for bidirectional organelle transport. J. Cell Biol. *160*, 297–301.
3. Martin, M., Iyadurai, S.J., Gassman, A., Gindhart, J.G., Jr., Hays, T.S. and Saxton, W.M. (1999). Cytoplasmic dynein, the dynactin complex and kinesin are interdependent and essential for fast axonal transport. Mol. Biol. Cell *10*, 3717–3728.
4. Valetti, C., Wetzel, D.M., Schrader, M., Hasbani, M.J., Gill, S.R., Kreis TE, Schroer TA. (1999). Role of dynactin in endocytic traffic: effects of dynamitin overexpression and colocalization with CLIP-170. Mol. Biol. Cell *10*, 4107–4120.

5. Waterman-Storer, C.M., Karki, S.B., Kuznetsov, S.A., Tabb, J.S., Weiss, D.G., Langford GM, Holzbaur EL. (1997). The interaction between cytoplasmic dynein and dynactin is required for fast axonal transport. Proc. Natl. Acad. Sci. U.S.A. *94*, 12180–12185.
6. Schroer, T.A., Schnapp, B.J., Reese, T.S. and Sheetz, M.P. (1988). The role of kinesin and other soluble factors in organelle movement along microtubules. J. Cell Biol. *107*, 1785–1792.
7. Ma, S. and Chisholm, R.L. (2002). Cytoplasmic dynein-associated structures move bidirectionally *in vivo*. J. Cell Sci. *115*, 1453–1460.
8. Gross, S.P., Tuma, M.C., Deacon, S.W., Serpinskaya, A.S., Reilein, A.R. and Gelfand, V.I. (2002). Interactions and regulation of molecular motors in Xenopus melanophores. J. Cell Biol. *156*, 855–865.
9. Karki, S. and Holzbaur, E.L. (1999). Cytoplasmic dynein and dynactin in cell division and intracellular transport. Curr. Opin. Cell Biol. *11*, 45–53.
10. Hollenbeck, P.J. (1996). The pattern and mechanism of mitochondrial transport in axons. Front. Biosci. *1*, d91–d102.
11. Welte, M.A., Gross, S.P., Postner, M., Block, S.M. and Wieschaus, E.F. (1998). Developmental regulation of vesicle transport in Drosophila embryos: forces and kinetics. Cell *92*, 547–557.