Paradigm lost: milton connects kinesin heavy chain to miro on mitochondria

Sarah E. Rice and Vladimir I. Gelfand

Department of Cell and Molecular Biology, Feinberg School of Medicine, Northwestern University, Chicago, IL 60611

The kinesin motor typically binds to cargo through its light chains. In this issue Glater et al. (p. 545) demonstrate a new type of linkage through the adapter protein, milton, and the mitochondrial membrane GTPase, miro. This is an important result because it represents a new mechanism of cargo binding and because miro's ability to bind GTP and calcium suggests that it is involved in the regulation of mitochondrial transport.

Mitochondrial transport: taking energy where it's needed, when it's needed

Polarized cells have an uneven distribution of active ATPases and they position their mitochondria so that ATP is produced close to where it is needed. Transportation of mitochondria to meet local energy needs is especially critical in neurons, where the site of mitochondrial production in the cell body (Davis and Clayton, 1996) can be centimeters away from a growth cone or synapse with high local ATP demand. Mitochondria move along both microtubules and actin, using microtubule-based molecular motors for long distance movements (Morris and Hollenbeck, 1995). They are among the most abundant and most mobile membrane-bound organelles and, thus, are a major cargo for microtubule motors. Conventional kinesin moves mitochondria to the plus ends of microtubules, while dynein moves them toward the minus ends (Tanaka et al., 1998; Pilling et al., 2006). Mutations in kinesin motors disrupt organelle transport causing mitochondria to bunch up in the axon or cell body, leading to neuronal dysfunction (Hurd and Saxton, 1996).

Mitochondria are distributed in cells with exquisite fine-tuning of both their location and number, and their transport is likely to be a very well-regulated process. Their distribution varies in response to multiple regulatory cues such as energy requirements, growth factors, or the membrane potential of the mitochondria (Morris and Hollenbeck, 1993; Chada and Hollenbeck, 2004; Miller and Sheetz, 2004). For example, Morris and Hollenbeck (1993) observed increased anterograde transport of mitochondria to active versus inactive growth cones, while Chada and Hollenbeck (2004) showed that mitochondria accumulate at local sites of nerve growth factor application.

Correspondence to Vladimir I. Gelfand: vgelfand@northwestern.edu

These experiments left two major questions: How do mitochondria connect to molecular motors? How is their movement by molecular motors controlled?

A key finding for addressing these two questions came from recent genetic screens in *Drosophila*. Defects in axonal transport were lethal at the embryonic or larval stages in previous screens, hampering the identification of proteins involved in axonal transport of mitochondria in *Drosophila*. However, Stowers and colleagues created mosaic flies whose eyes were homozygous for a mutant allele while the rest of the body was heterozygous (Stowers and Schwarz, 1999). Here, mutant flies were viable, but blind due to a loss of nerve excitation in the eye. Two independent screens performed by Stowers et al. and Guo et al. used this system to identify two distinct components important for transport; milton (Stowers et al., 2002), which co-immunoprecipitated with kinesin heavy chains, and miro (Guo et al., 2005), an integral mitochondrial membrane protein. Mutations in either of the genes appeared to abolish anterograde mitochondrial transport. The present paper by Glater et al. (2006) links these two results by showing both that kinesin, milton, and miro work together in anterograde transport and that milton attaches kinesin to mitochondria through miro.

Milton binds kinesin heavy chains in place of kinesin light chains

Glater et al. (2006) follow up on the original immunoprecipitation results showing interactions of milton with kinesin (Stowers et al., 2002). They demonstrate that milton forms a complex with the kinesin heavy chain and that no light chains could be found in the complex. This is different from most other known cargoes that bind to kinesin through the tetratricopeptide repeat (TPR) domains of the kinesin light chains (for review see Verhey et al., 2001). The only other example of cargo binding to the kinesin heavy chain is mRNP particles (Kanai et al., 2004; Ling et al., 2004); however, unlike mitochondria, the adaptor for RNA cargoes is yet unknown. These data are consistent with genetic experiments demonstrating that light chains are not required for transport of either mRNA or mitochondria by conventional kinesin (Palacios and St Johnston, 2002; Glater et al., 2006). Consistent with some cargoes binding the heavy chains directly, there is a small cellular pool of kinesin heavy chain dimers, which are not attached to kinesin light chains (Gyoeva et al., 2004). Milton not only interacts with kinesin heavy chains, but it competes directly with light chains to do so.

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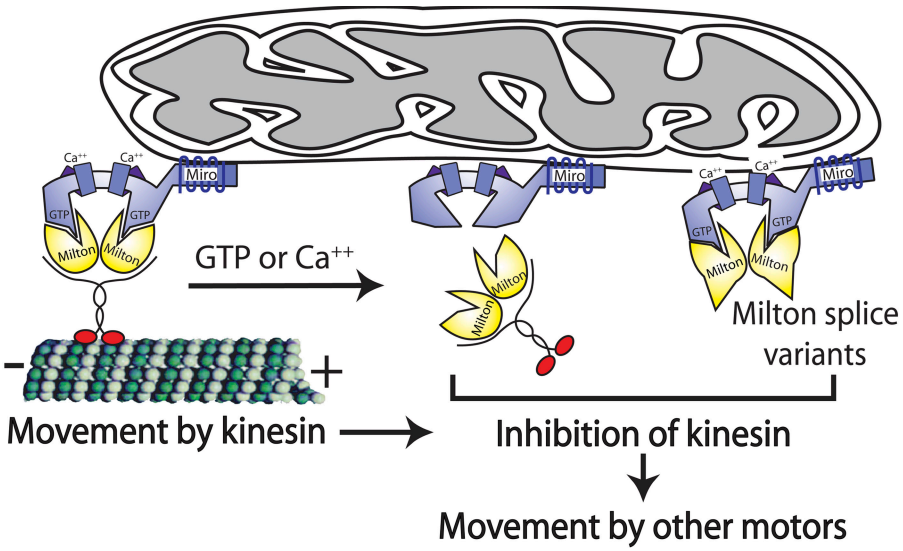

Figure 1. **Does miro regulate movement of mitochondria?** Left to right: milton connects kinesin (red) to miro on mitochondria. Miro has a GTPase domain, followed by two calcium-binding EF hand motifs, a second GTPase domain, and the transmembrane domain. GTP hydrolysis or changes in calcium binding by the EF hands may cause milton to dissociate from miro. Milton splice variants differ in their kinesin-binding properties (Glater et al., 2006). These inhibitory mechanisms may enable transport by other motors.

Thus, the kinesin light chains may simply be the most common of several cargo adaptor proteins, including milton, that bind to the same region of the kinesin heavy chain.

Milton connects kinesin to miro, an integral mitochondrial protein

Milton localizes to mitochondria but has no obvious sequence elements that would explain such localization. However, genome-wide two-hybrid screening identified miro as potentially interacting with milton (Giot et al., 2003). This was an exciting finding for two reasons. First, miro is an integral mitochondrial membrane protein found in all eukaryotes (Fransson et al., 2003; Aspenstrom et al., 2004; Frederick et al., 2004). Second, miro had previously been implicated in defects in axonal transport of mitochondria in *Drosophila* (Guo et al., 2005). Glater et al. (2006) confirmed these two-hybrid results by immunoprecipitation and functional experiments. Normally miro has a transmembrane domain that integrates it into mitochondria, but overexpression of miro protein lacking this domain resulted in mislocalization of both miro and milton to the cytoplasm.

Miro as an adaptor and possible regulator

It is very likely that miro is not only an adaptor for milton, but is also a critical regulator of kinesin-dependent mitochondrial transport. Potential mechanisms of regulation of transport by the miro–milton complex are shown in Fig. 1. Miro is a GTPase with both two GTP-binding domains and two EF hand domains that can potentially bind calcium. This means that either GTPase activity or calcium binding can regulate miro's conformation and, therefore, its ability to recruit milton or arrange the milton–kinesin complex at the surface of mitochondria. The existence of several splice variants of milton with different kinesin and miro binding properties implies that there might be several populations of mitochondria with different transport properties. None of these potential regulatory mechanisms has yet been tested, but some are very likely to occur.

In addition to recruiting kinesin via milton, miro may have other important mitochondrial functions. Miro is present in yeast, whereas milton is not, and it is known to play a role in maintaining normal mitochondrial morphology. Furthermore, yeast use actin rather than microtubules for mitochondrial transport, so the function of miro in yeast is clearly different. Could miro be a more general mitochondrial adaptor that binds to other motile complexes (such as Arp2/3, myosin V, or dynein) besides milton?

Recent observations of mitochondria transport in fly neurons in vivo demonstrated that there are two populations of mitochondria; one moves predominantly anterogradely while the other moves retrogradely (Pilling et al., 2006). Could it be that the GTPase or calcium "switch" on miro toggles between these two states or between microtubule- and actin-based transport? It will take time to determine the exact role of miro in motor-based mitochondrial transport, but in the short term it is reasonable to ask whether kinesin is bound to retrogradely transported neuronal mitochondria. If not, could it be dissociated by GTP hydrolysis of miro? This is an exciting and important area for further study because miro is likely to be the key universal adaptor and regulator for mitochondrial transport.

How to carry different cargoes on the same filament

The result by Glater et al. (2006) emphasizes one general principle of motor protein design: although the motor domains seem to be universal for a particular class of motor proteins, the cargo-binding parts are not. Motors of the same family are very divergent outside of the motor domain, and even a single motor can bind different classes of cargo using different parts of the molecule. This has been nicely demonstrated recently for cargoes of yeast class V myosin (myo2p) (Pashkova et al., 2006) and is further emphasized by the observation of Glater et al. (2006) that milton directly interacts with the kinesin heavy chain while most other known cargoes bind to the light chains. It is not surprising that the motor domains of molecular motors are so universal. After all, they bind to one of two filament types and all of them move by hydrolyzing ATP. However, motors such as conventional kinesin and cytoplasmic dynein

probably move dozens of different cargoes along microtubules, and each of them must be transported and regulated differently. The only way to accomplish this is to diversify motor–cargo interactions, as is nicely demonstrated by the present work of Glater et al. (2006).

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