

Pushing for answers: is myosin V directly involved in moving mitochondria?

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In budding yeast, the actin-based class V myosin motors, Myo2 and Myo4, transport virtually all organelles from mother to bud during cell division. Until recently, it appeared that mitochondria may be an exception, with studies showing that the Arp2/3 complex is required for their movement. However, several recent studies have proposed that Myo2 has a direct involvement in mitochondria inheritance. In this issue, Altmann et al. (Altmann, K., M. Frank, D. Neumann, S. Jakobs, and B. Westermann. 2008. *J. Cell Biol.* 181:119–130) provide the strongest support yet that Myo2 and its associated light chain Mlc1 function directly and significantly in both mitochondria–actin interactions and in the movement of mitochondria from mother to bud. The conflicting functions of Arp 2/3 and Myo2 may be reconciled by the existence of multiple pathways involved in mitochondrial transport.

Mitochondria are essential organelles that are critical in several cellular processes including calcium homeostasis, respiration, metabolism, and apoptosis. They are extremely dynamic and constantly fuse, divide, and move along cytoskeletal elements within the cell (Okamoto and Shaw, 2005; Chan, 2006; Hoppins et al., 2007). Regulated movement and anchoring place mitochondria in intracellular locations that transiently require their function. For example, in neurons, mitochondria are transported from the cell body to sites of high energy requirement such as active growth cones and the synapse (Hollenbeck and Saxton, 2005). Mitochondrial movement and anchoring also ensure proper partitioning of the organelle between mother and daughter cells during cell division (Boldogh et al., 2005). Because mitochondria cannot be made de novo, proper inheritance of mitochondria during cell division is crucial for survival of the daughter cell (Warren and Wickner, 1996).

Interaction of mitochondria with cytoskeletal elements appears to be critical for all mitochondrial processes (Boldogh and Pon, 2007). In higher eukaryotes, long-range mitochondrial movements occur along microtubules and are mediated by kine-

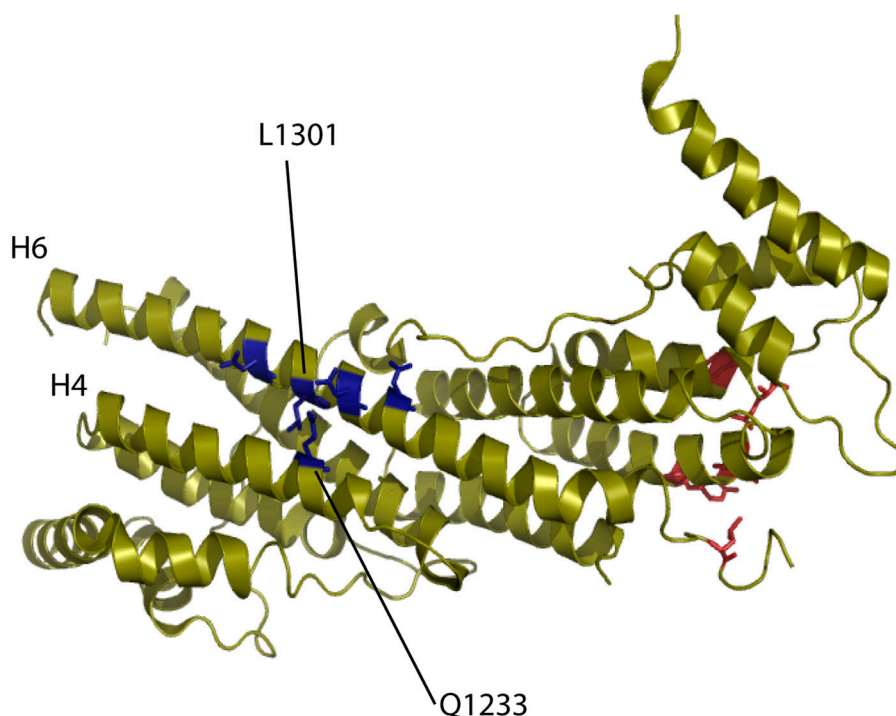
sin and dynein motors (Hollenbeck and Saxton, 2005). In contrast, in budding yeast, mitochondria predominantly interact with the actin cytoskeleton and use actin tracks for both anterograde and retrograde movement during cell division (Fehrenbacher et al., 2004; Boldogh and Pon, 2006).

Retrograde movement toward the base of the mother cell occurs passively through the retrograde flow of actin (Fehrenbacher et al., 2004). Conversely, anterograde movement toward the bud/daughter cell requires force generation mechanisms to overcome the inherent retrograde flow (Fehrenbacher et al., 2004). Although some studies have implicated the actin-based myosin V motor Myo2 in anterograde movement, other studies have suggested that Myo2 is not directly involved in mitochondrial motility (Boldogh and Pon, 2007; Frederick and Shaw, 2007).

The simplest explanation for the difficulty in determining whether or not Myo2 has a direct functional involvement in mitochondrial movement is the likely existence of multiple pathways that contribute to mitochondrial motility. Several lines of evidence show that Arp2/3-induced actin polymerization is important for anterograde mitochondrial movement along actin cables (Boldogh et al., 2005). Components of the Arp2/3 complex are found on mitochondria, where they are recruited by the RNA-binding, peripheral mitochondrial membrane protein Jsn1/Puf1 (Boldogh et al., 2001; Fehrenbacher et al., 2005). In contrast to molecular motors, Arp2/3-induced actin polymerization provides motility but not directionality. It appears that in this case, direction may be provided by parallel bundling of the F-actin array initiated by Arp2/3 with the actin cable and by the attachment of mitochondria to actin cables via a protein complex termed the mitochore (Boldogh et al., 2003; Boldogh and Pon, 2006). This complex is composed of three mitochondrial membrane proteins (Mmm1, Mdm10, and Mdm12) and is linked to mitochondria-associated Arp2/3 via the RNA-binding protein Puf3 (Garcia-Rodriguez et al., 2007). Mutants lacking components of either the mitochore or the Arp2/3 complex show decreased rates of mitochondrial movement and impaired mitochondrial inheritance (Boldogh and Pon, 2006). Also, when actin dynamics are inhibited by treating the actin depolymerization–impaired yeast mutant *act1-159* with Lat-A (which prevents G-actin polymerization), a 50% decrease in the proportion of moving mitochondria is observed (Boldogh et al., 2001). The mean rate of mitochondrial movement is also significantly reduced. Although this suggests that actin polymerization is important for mitochondrial motility, it also indicates that some

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Figure 1. **Structure of the Myo2 globular cargo binding domain (Pashkova et al., 2006), PDB ID: 2F6H, displayed using PyMOL.** Helices H4 and H6 contain the surface residues D1297, L1301, N1304, N1307, and Q1233 (shown in blue) that are important for vacuole movement as well as mitochondria morphology and distribution (Pashkova et al., 2006). Mutations in L1301 and Q1233 display the most severe mitochondrial defects. Residues shown in magenta are important for the movement of secretory vesicles but not the vacuole or mitochondria.



mitochondria can move even in the absence of actin dynamics. In support of the latter idea is the observation that phalloidin-stabilized actin can efficiently slide along immobilized mitochondria in vitro (Simon et al., 1995).

The first indication that myosin motors might function in mitochondrial distribution came from the observation that yeast actin mutants with point mutations under the myosin footprint display defects in mitochondrial organization (Drubin et al., 1993). Several years later, the identification of two Myo2 mutant alleles that specifically impair mitochondrial inheritance strengthened this postulate (Itoh et al., 2002; Boldogh et al., 2004). Moreover, two proteins were identified, Mmr1 and the Rab GTPase Ypt11, where absence of either protein results in a partial defect in mitochondrial inheritance (Itoh et al., 2002, 2004). Importantly, both Mmr1 and Ypt11 were shown to bind to the cargo-binding domain of Myo2 (Itoh et al., 2002, 2004). Also, when Mmr1, an outer mitochondrial membrane protein, is overexpressed, it causes mitochondria to accumulate at the mother-bud neck (Frederick et al., 2008). This further suggests a function for Myo2 because, at the end of the cell cycle, Myo2 concentrates in this region of the cell (Wagner et al., 2002). In addition to the above studies, cells depleted of Myo2 or its associated light chain Mlc1 exhibit severe defects in mitochondrial morphology and distribution (Altmann and Westermann, 2005).

Despite the above studies, other evidence does not fit the hypothesis that Myo2 has a major functional involvement in mitochondrial movement. For example, mitochondrial velocity is not affected in cells that express a mutant Myo2 motor missing portions of the lever arm domain (Boldogh et al., 2004). Further doubt for the direct involvement of Myo2 in mitochondrial movement was raised by the findings that Ypt11 is localized to the ER (Buvelot Frei et al., 2006) rather than to mitochondria

and that Ypt11 and Myo2 are required for immobilizing mitochondria at the bud tip rather than for movement of mitochondria from the mother to the bud (Boldogh et al., 2004).

In light of the ongoing questions of the role of Myo2 in moving mitochondria, the work by Altmann et al. (2008) in this issue (see p. 119) is particularly interesting in that it provides the most convincing evidence to date that Myo2 is directly involved in both mitochondria-actin interactions and anterograde mitochondrial movement. Using promoter shut-off strains that carry the essential *MYO2* and *MLC1* genes under the control of the Tet- O_7 promoter, Altmann et al. (2008) show using biochemical as well as visual in vitro assays that depletion of Myo2 or Mlc1 results in an ~50% reduction in ATP-sensitive binding of mitochondria to actin filaments. These findings indicate that Myo2 and Mlc1 have a significant involvement in mediating the interaction of mitochondria with actin. Importantly, this interaction is completely lost upon prior incubation of purified mitochondria with Myo2-specific antibodies. The authors also test known point mutations in the cargo-binding domain of Myo2 that either have a defect in binding to vacuoles or a defect in binding to secretory vesicles (Catlett et al., 2000; Pashkova et al., 2005, 2006). Although mutations that affect Myo2 binding to secretory vesicles have no effect on mitochondria, the surface residues that are required for vacuole movement are likewise required for the proper distribution of mitochondria to the bud (Fig. 1). Mutations in two of these residues (L1301P and Q1233R) display severe defects in mitochondrial morphology as well as in vitro binding of mitochondria to actin. In addition, *myo2(L1301P)* was further tested and found, using time-lapse imaging, to be impaired in anterograde transport of mitochondria from the mother to the bud. This last observation strongly indicates that Myo2 has a significant functional involvement in anterograde mitochondria movement rather than merely functioning

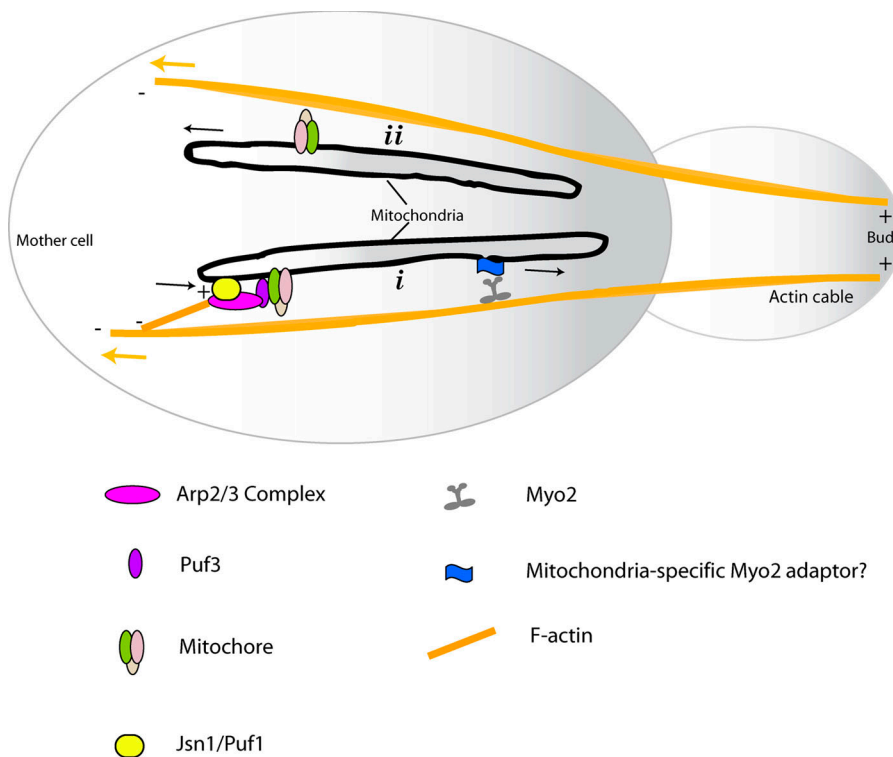


Figure 2. **Model showing mitochondria movement during cell division in budding yeast.** Mitochondria may use Arp2/3- and/or Myo2-based mechanisms for anterograde movement (i). Retrograde movement (ii) requires mechanisms that link mitochondria to actin cables but does not require force generation. Yellow arrows indicate the direction of retrograde actin flow and black arrows indicate the direction of mitochondria movement.

to retain mitochondria at the bud tip, as has been previously suggested. Further support for the direct involvement of Myo2 in mitochondrial movement would be provided by identification of an adaptor protein that directly links Myo2 to the mitochondrial membrane.

In an effort to reconcile the observations of Altmann et al. (2008) with the evidence for the function of Arp2/3 in mitochondrial motility, it is tempting to speculate that both mechanisms are important in moving mitochondria either simultaneously or, under different circumstances, that they move different pools of mitochondria (Fig. 2). Note that mutations in either pathway result in a partial loss of mitochondrial inheritance. If both pathways are involved in mitochondrial movement, one would expect that a combination of a mutation in the mitochondria or Arp2/3 complex with one of the *myo2* point mutants that affects mitochondrial movement would result in a more severe and possibly lethal defect. Also, at least one recent study has revealed that there might be other yet-to-be-identified pathways that regulate mitochondrial movement and inheritance (Frederick et al., 2008). This study shows that the Rho GTPase Gem1, which functions in the control of mitochondrial morphology, also functions, independently of Mmr1 and Ypt11, in mitochondrial inheritance. Gem1 is the yeast orthologue of Miro, an adaptor protein that links mitochondria to kinesin-1 motors in higher eukaryotes (Fransson et al., 2006; Glater et al., 2006). Determination of the function of Gem1 in yeast may provide insight into yet another potential mechanism for mitochondrial movement that appears to have been conserved through evolution.

Although budding yeast can survive in the absence of mitochondrial respiration, they absolutely require mitochondria for viability. Because mitochondria cannot be made de novo,

there is likely a selective pressure in favor of organisms that have multiple pathways to ensure that these organelles are faithfully transferred to daughter cells during cell division. We anticipate that, in the future, many more players in mitochondrial dynamics will be revealed. The challenge will then be to understand how the different pathways are coordinated to ensure efficient organelle function and inheritance.

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