

# Mitochondria use actin filaments as rails for fast translocation in *Arabidopsis* and tobacco cells

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**Abstract** Although mitochondria are known to move actively in plant cells, little is known about how they move. In higher plants, actin filaments have been reported to be involved in the movement of organelles, such as chloroplasts, peroxisomes, endoplasmic reticulum and Golgi apparatus. Mitochondria were visualized in living *Arabidopsis thaliana* plants using fluorescent proteins fused to a mitochondria targeting signal. To compare the movement of mitochondria to the movements of another organelle, we also examined peroxisomes because of their similarity in size. Velocities of individual mitochondria and peroxisomes, as measured by time-lapse laser scanning microscopy, varied, although the average velocities of the two organelles were similar. Latrunculin B, an actin-depolymerizing drug, stopped movement of mitochondria and peroxisomes, demonstrating that movement of these organelles depends on actin filaments. On the other hand, propyzamide, a microtubule-depolymerizing drug, did not affect the movement of mitochondria and peroxisomes. In living *Arabidopsis* plants in which mitochondria or peroxisomes are visualized by red fluorescent protein and cytoskeletal elements are simultaneously visualized by green fluorescent protein, both organelles moved using actin filaments as rails. In contrast, their movement was not related to arrays of microtubules. We also examined mitochondrial movement in tobacco cultured cells visualizing mitochondria and cytoskeletal element simultaneously. Mitochondrial movement was often seen along actin filaments but not along microtubules. These findings suggest that mitochondria move along actin filaments rapidly and over long distances in higher plants.

**Key words:** Cytoskeleton, mitochondrial movement.

Intracellular translocations of mitochondria enable them to move to areas in a cell that have a high energy demand (Hollenbeck and Saxton 2005). In budding yeast, mitochondrial distribution and motility depend on actin cables (Drubin et al. 1993; Fehrenbacher et al. 2004; Lazzarino et al. 1994). On the other hand, microtubules are known as a major apparatus for mitochondrial long-distance movement in mammalian nerve cells. Members of kinesins, microtubule-associated motors, mediate mitochondrial movement in the cells (Hollenbeck and Saxton 2005). There are much less reports about higher plant mitochondrial positioning and movement (Logan and Leaver 2000). In elongated cultured tobacco cells, mitochondrial motility was shown to depend on actin filaments through use of cytoskeleton-disrupting drugs (Van Gestel et al. 2002). *In vitro* and *in vivo* motility assays indicate that in the pollen tube of tobacco, microtubules cooperate with actin filaments in mitochondrial movement (Romagnoli et al. 2007). Mitochondria co-localized with actin filaments in fixed onion epidermal cells (Olyslaegers and Verbelen 1998)

and immunofluorescence studies using maize myosin XI show that colocalizes with mitochondria (Wang and Pesacreta 2004). These reports indicate that transport of mitochondria depends on actin filaments, as does the transport of other organelles such as chloroplasts, peroxisomes, the endoplasmic reticulum and Golgi stacks (Wada and Suetsugu 2004). However, no studies have simultaneously examined mitochondria and cytoskeletal elements to see if they interact. Here, to better understand how mitochondria use the cytoskeleton to move, we directly observed fluorescently labeled mitochondria and cytoskeletal elements in living *Arabidopsis* cells and tobacco cells.

## Materials and methods

### Plant materials

Bright yellow-2 (BY-2) tobacco (*Nicotiana tabacum* L.) cell suspension cultures were grown in modified Murashige and Skoog medium enriched with  $0.2 \text{ mg l}^{-1}$  2,4-D and were maintained as described (Nagata et al. 1992). *Arabidopsis thaliana* plants were grown in a growth chamber at 22°C under

a short day photoperiod (10-h/14-h light/dark cycle).

### Construction of plasmids

To visualize mitochondria, Green Fluorescence Protein (GFP) and DsRed1 (Clontech) (written as "Red Fluorescence Protein (RFP)" in this paper) were fused to the C-terminal 36 amino acids of the Arabidopsis mitochondrial ATPase  $\delta$  subunit as presequence (Sakamoto et al. 1996). Arabidopsis seeds with GFP for visualizing mitochondria were kindly provided by Dr. Sakamoto. Arabidopsis seeds with GFP and RFP for visualizing peroxisomes were kindly provided by Dr. Mano and Dr. Nishimura (Mano et al. 1999). Arabidopsis seeds with GFP-mTalin for visualizing actin filaments were kindly provided by Dr. Chua (Kost et al. 1998). Tobacco suspension cells BY-GF11 and BY-GT16 lines with GFP-fimbrin and GFP-tubulin for visualizing actin filaments and microtubules respectively were kindly provided by Dr. Hasezawa (Kumagai et al. 2001; Sano et al. 2005). Transgenic Arabidopsis seeds with GFP-TUA6 for visualizing microtubules were kindly provided by Dr. Hashimoto (Ueda et al. 1999). To visualize mitochondria and cytoskeletal filaments simultaneously, we obtained genetically crossed plants between two transgenic plants in which mitochondria and cytoskeletal filaments were visualized with RFP and GFP, respectively.

### Drug treatment

Stock concentrations of 2 mM Latrunculin B (Calbiochem) and 2 mM propyzamide (Wako) were made in dimethyl sulfoxide (DMSO, Wako). In the case of Arabidopsis experiments, stock solutions (and DMSO alone for controls) were dissolved in milliQ water (Millipore) and 0.002% Tween20 (Sigma Aldrich) to the appropriate concentrations. 15-day-old transgenic Arabidopsis plants were immersed in the drug solution. To depolymerize actin filaments or microtubules, Arabidopsis plants were treated with 10  $\mu$ M Lat-B for 1 h or 100  $\mu$ M propyzamide for 8 h. BY-2 cells used for visualizing actin filaments or microtubules with GFP were incubated in culture medium of BY-2 containing Lat-B, propyzamide at a final concentration 10  $\mu$ M, respectively. BY-2 medium containing 0.5% (v/v) DMSO was used as a control. Incubation times were 1 h for Lat-B treatments and 5 h for propyzamide treatments. To visualize mitochondria in BY-2 cells, MitoTracker Orange (Molecular Probes) was added to a final concentration of 50 nM.

### Microscopic observations

Living plant cells were observed using a fluorescence microscope (TE2000-U, Nikon). Fluorescence of GFP and RFP were imaged using the 488 nm argon or 543 nm helium/neon laser of a confocal laser scanning microscopic unit (Micro Radian MR/AG-2, Bio-Rad) equipped with a 100 $\times$ object lens (N.A.1.3, Nikon). To take time-lapse images, 15 images were serially scanned 15 times on the same region and same plane of transgenic Arabidopsis epidermal cells at 2.2-second intervals (at 4.4-second intervals in the case of BY-2 cells). The images were then superimposed onto one image. Micrographs in Figure 2A and Figure 3A are z-series projected images of every 1  $\mu$ m serial section. These images were processed with Adobe Photoshop 7.0 software (Adobe Systems, Mountain

View, CA).

### Measurement of Velocity

To measure velocity, Arabidopsis leaf epidermal cells were scanned at 1-second intervals. Movements of individual mitochondria and peroxisomes were quantified using Image-Pro Plus software (Media Cybernetics). Mitochondria moving at less than 0.3  $\mu$ m s<sup>-1</sup> were excluded from the analyses because it is difficult to distinguish moving and non-moving mitochondria at these speeds, 0–0.3  $\mu$ m s<sup>-1</sup>. We measured 785 mitochondria and 455 peroxisomes in more than 30 cells.

## Results

In Arabidopsis, we measured the velocity of mitochondria and peroxisomes in leaf epidermal cells using a confocal laser scanning microscope. Our observations were limited to the X-Y plane because the focal plane was narrow and because the cytoplasm was compressed to a thin layer between large vacuoles and the plasma membrane.

To determine whether mitochondrial movement was different from the movement of other organelles, we also examined the movement of peroxisomes, which are as large as mitochondria (about 1  $\mu$ m in diameter). Peroxisomes have been reported to move along actin filaments in plant cells (Jedd and Chua 2002; Mano et al. 2002; Mathur et al. 2002). Mitochondria and peroxisomes were visualized with red fluorescent protein (RFP) and GFP, respectively. The average velocity of moving mitochondria was 1.44  $\mu$ m s<sup>-1</sup> (S.D.  $\pm$ 0.89) and the fastest velocity was 5.21  $\mu$ m s<sup>-1</sup> (n=785) (Figure 1).

Many mitochondria (and peroxisomes) moved actively and some were static. The velocities of individual

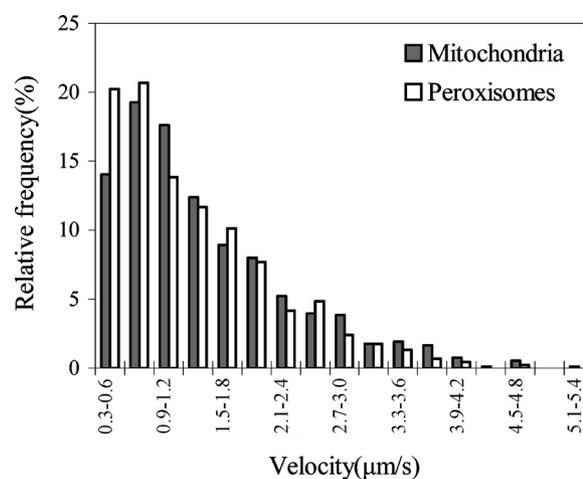


Figure 1. Relative frequency of mitochondrial velocities in Arabidopsis leaf epidermal cells. Mitochondrial velocities are designated as cross-hatched bars and peroxisomal velocities as white bars. Average velocity of moving mitochondria is 1.44  $\mu$ m s<sup>-1</sup> (S.D.  $\pm$ 0.89, n=785). That of peroxisomes is 1.31  $\mu$ m s<sup>-1</sup> (S.D.  $\pm$ 0.81, n=455). Velocities were determined from the distance moved by individual mitochondria or peroxisomes in 1 second.

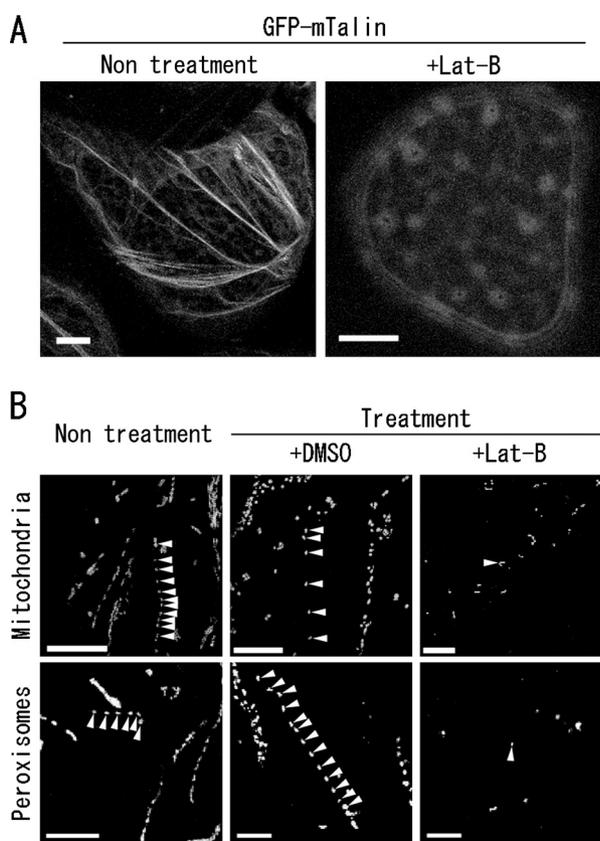


Figure 2. Effects of Lat-B treatments on mitochondrial and peroxisomal motilities. (A) Disruption of actin filaments by treatment of Lat-B. GFP-mTalin signals in Lat-B treated and non-treated Arabidopsis leaf epidermal cells. (B) Effects of Lat-B treatments on mitochondrial and peroxisomal motilities. Mitochondria and peroxisomes highlighted by GFP in Lat-B treated, DMSO treated and non-treated Arabidopsis leaf epidermal cells. DMSO is a solvent for Lat-B. Each panel was made by merging 15 serial photos at 1-second intervals in the same position. Arrowheads indicate the single mitochondrion or peroxisome in the image, showing movement during the observing time. Bars, 10  $\mu\text{m}$ .

mitochondria also varied (see also Figure 2B, 3B). The average velocity of moving peroxisomes was  $1.31 \mu\text{m s}^{-1}$  (S.D.  $\pm 0.81$ ) and the top speed was  $4.60 \mu\text{m s}^{-1}$  ( $n=455$ ) (Figure 1). These values (and their variances) are consistent with previously reported velocities of plant peroxisomes ( $0\text{--}6 \mu\text{m s}^{-1}$ ), although in cortical cells of Arabidopsis hypocotyls, it was as high as  $10 \mu\text{m s}^{-1}$  (Jedd and Chua 2002).

To understand how mitochondrial movement is related to cytoskeletal elements, the actin-depolymerizing drug Latrunculin B (Lat-B) and the microtubule-depolymerizing drug propyzamide were applied to Arabidopsis leaf epidermal cells and tobacco suspension cultured BY-2 cells. The minimum concentrations and times needed to completely disrupt the cytoskeleton were determined by using cytoskeleton-visualizing plants (Figure 2A, 3A, and see materials and methods). In the presence of Lat-B, mitochondrial movement in Arabidopsis leaf epidermal cells was initially sluggish

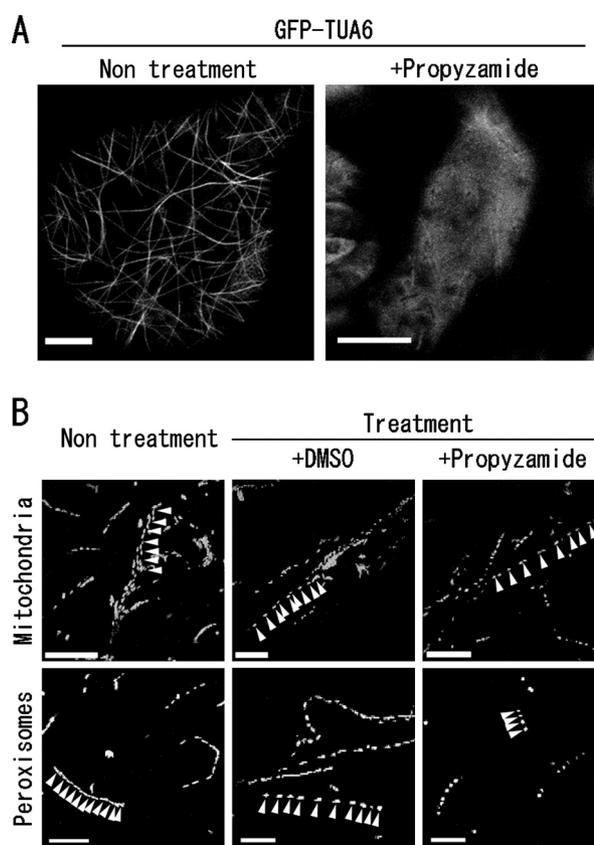


Figure 3. Effects of propyzamide treatments on mitochondrial and peroxisomal motilities. (A) Disruption of microtubules by treatment of propyzamide. GFP-TUA6 signals in propyzamide treated and non-treated Arabidopsis leaf epidermal cells. (B) Mitochondria and peroxisomes highlighted by GFP in propyzamide treated, DMSO treated and non-treated Arabidopsis leaf epidermal cells. DMSO is a medium for propyzamide. Each panel is made by merging 15 serial photos (1-second interval) at the same position. Arrowheads indicate the single mitochondrion or peroxisome in the image, showing movement during the observation. Bars, 10  $\mu\text{m}$ .

and was eventually reduced to just Brownian motion (Figure 2B). Lat-B also stopped the movement of peroxisomes (Figure 2B), as was previously described in onion and Arabidopsis (Jedd and Chua 2002; Mano *et al.* 2002; Mathur *et al.* 2002). Non-treatment and treatment with DMSO (Control; the solvent for Lat-B) resulted in no apparent inhibition of mitochondrial motility (Figure 3B). When using BY-2 cells, we got the same results that we obtained with Arabidopsis plants. BY-2 cells treated with  $10 \mu\text{M}$  Lat-B for 1h also showed disappearance of filamentous actin and global arrest of mitochondrial motility (Figure 5). After removal of the drug, mitochondrial motility was gradually restored (data not shown). By contrast, treatments of propyzamide didn't appear to affect mitochondrial motility (Arabidopsis; Figure 3B, BY-2 cells; Figure 5). Peroxisomal motility was also not inhibited by propyzamide treatments (Figure 3B). These data are compatible with previous observations of mitochondrial movement in tobacco suspension cells (Van Gestel *et al.* 2002). These data

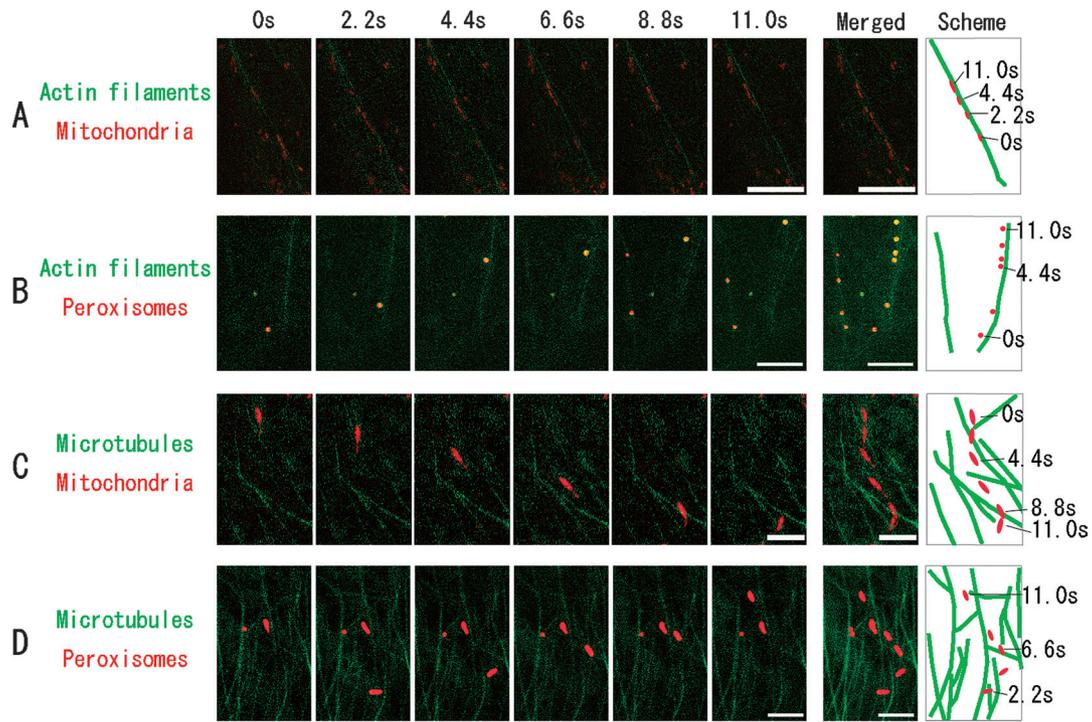


Figure 4. Simultaneous visualization of organelle movement and cytoskeletons in *Arabidopsis* leaf epidermal cells. (A) Mitochondria (red) and actin filaments (green), (B) Peroxisomes (red) and actin filaments (green), (C) Mitochondria (red) and microtubules (green), (D) Peroxisomes (red) and microtubules (green). (A, C) Scale bars, 10  $\mu\text{m}$ . (B, D) Scale bars, 5  $\mu\text{m}$ . Each image was taken at 2.2-second intervals. "Merged" images are superimposed ones of the time-lapse images at their left. Schemes were made from "merged" images. Red ellipses represent mitochondria (A, C) and peroxisomes (B, D) and green lines represent actin filaments (A, B) and microtubules (C, D).

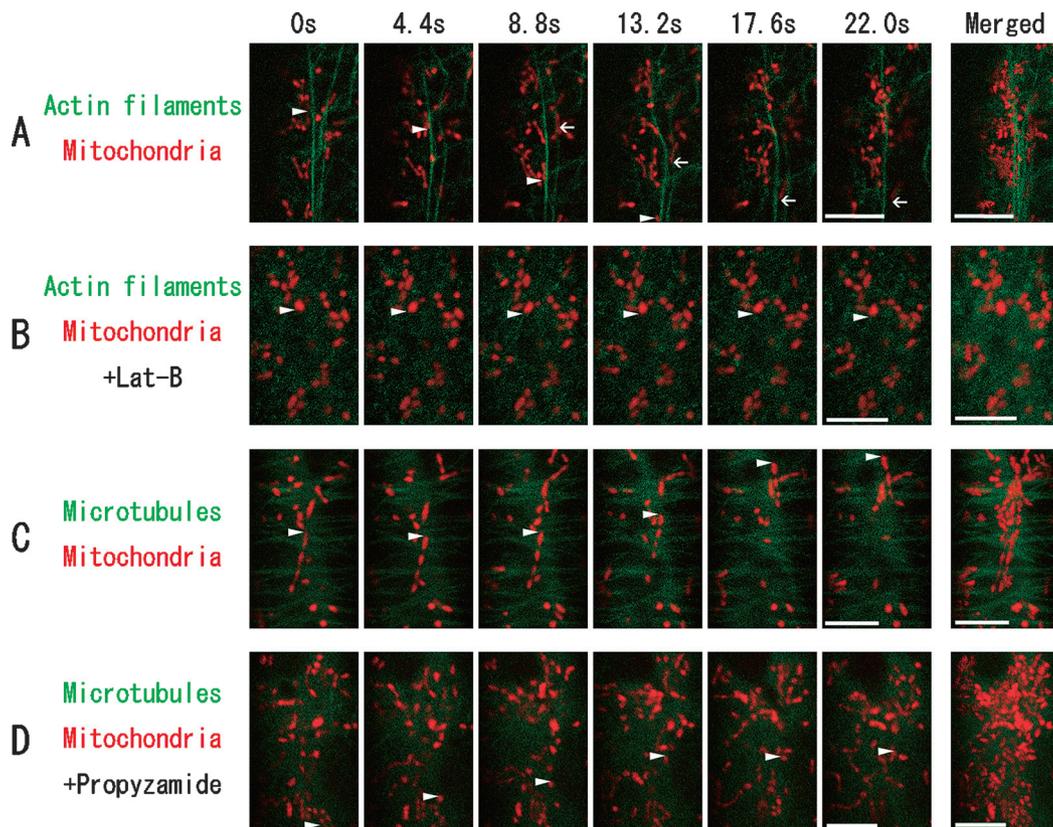


Figure 5. Simultaneous visualization of mitochondrial motility and cytoskeletons in BY-2 cells. (A, B) Mitochondria (red; stained with MitoTracker) and actin filaments (green; visualized with GFP-FIM1) without (A) and with (B) Lat-B. (C, D) Mitochondria (red; MitoTracker) and microtubules (green; GFP-tubulin) treated without (C) and with (D) propyzamide. Scale bars, 5  $\mu\text{m}$ . All images were taken at 4.4s intervals. "Merged" images were made by merging 15 serial photos at left. Arrowheads and an arrow indicate the single mitochondrion in the images, showing movement during the observation.

indicate that transport of mitochondria depends on filamentous actin, as does the transport of peroxisomes.

To see the relationships between mitochondrial movement and the orientations of cytoskeletal filaments in living *Arabidopsis* cells, we produced transgenic plants in which mitochondria and cytoskeletal filaments were simultaneously visualized by RFP and GFP, respectively. In plants in which actin and mitochondria could be visualized, the mitochondria moved along actin filaments. To our knowledge, this is the first such observation of plant mitochondria. Figure 4A shows three mitochondria moving along actin filaments, each at a variable velocity. On the other hand, in *Arabidopsis* with fluorescently-labeled microtubules and mitochondria, the mitochondria seemed to be moving where microtubules were not seen, and many of the moving mitochondrial tracks didn't match the orientation of the microtubules (Figure 4C). We also observed that the tips of mitochondria attached to microtubules and resisted floating into the cytoplasmic streaming (data not shown).

We also examined mitochondrial movement in BY-2 cells using GFP to label actin filaments (Kumagai *et al.* 2001) and MitoTracker to stain mitochondria. In this case, mitochondria also move along actin filaments (Figure 5A) in directions orthogonal to microtubule arrays (Figure 5C). As previously shown in this paper, peroxisomes appeared to move along actin filaments in our conditions, too (Figure 4B, D). These observations demonstrate that mitochondria moved along actin filaments as if the actin filaments were rails, and that they did not move along microtubules.

## Discussion

We examined mitochondria and peroxisomes in *Arabidopsis* leaf epidermal cells and tobacco suspension cultured cells to see how they moved. The movements of mitochondria and peroxisomes were quite similar with respect to velocity, variance of speed, dependence on actin filaments and independence of microtubules, whereas we observed that many mitochondria did not move while peroxisomes moved actively in the same cell (data not shown). These results suggest that mitochondria and peroxisomes use similar molecular-mechanisms for movement but its determination of when to move depends on the necessity of each organelle. In higher plants, actin filaments are involved in the movements of other organelles, such as chloroplasts, endoplasmic reticulum and Golgi apparatus (reviewed in (Wada and Suetsugu 2004)), although the molecular mechanisms involved are unclear.

Romagnoli *et al.* (2007) observed that tobacco pollen tube mitochondria moved rapidly along actin filaments *in vitro* ( $1.73 \pm 0.73 \mu\text{m s}^{-1}$ ), which is similar to our *in vivo*

value of  $1.44 \pm 0.89 \mu\text{m s}^{-1}$ . However, they also observed mitochondria moving slowly along microtubules ( $0.22 \pm 0.05 \mu\text{m s}^{-1}$ ), while in our study, mitochondria movement along microtubules was less than our limit of detection ( $0.3 \mu\text{m s}^{-1}$ ). In any case, our *in vivo* study shows that mitochondria and peroxisomes in *Arabidopsis* and tobacco move quickly and over long distances and that the movement depends on actin. Our results are consistent with the *in vivo* results of Van Gestel *et al.* (2002) and the *in vitro* results of Romagnoli *et al.* (2007).

At least four types of actin-based intracellular organelle motility have been described: (1) Motility using a comet tail structure. In this type, polymerization of actin monomers on the tail region of pathogenic bacteria is used to power strokes in eukaryotic host cells (Dramsai and Cossart 1998). The motility of mitochondria in *Drosophila* spermiogenesis is reported to be similar to "comet tail" motility (Bazinet and Rollins 2003). (2) Motility using a basket-shape structure. In this type, random fine actin filaments around chloroplasts may contribute to their movements in response to light (Kandasamy and Meagher 1999). (3) Drift on the cytoplasmic stream. In this type, cytoplasmic streaming in plants depends on actin filaments (Bradley 1973; Shimmen and Yokota 2004). Some organelles may drift only on the cytoplasmic streaming. (4) Motility using actin filaments as rails. In this type, budding yeast mitochondria are in traction by an actin motor protein, myosin, when they are transferred from mother to daughter cells (Boldogh *et al.* 2004; Itoh *et al.* 2002).

The present results clearly show that mitochondrial motility is not due to two of these mechanisms, Comet tail and Basket-shape. Our results do not show whether mitochondria interact directly with actin filaments or are passively propelled by cytoplasmic streaming, because cytoplasmic streaming is also inhibited by the actin-disrupting drug (Bradley 1973). Romagnoli *et al.* (2007) clearly showed that isolated mitochondria could move along both cytoskeletons without cytoplasmic streaming. However, it is possible that mitochondrial movement *in vivo* is affected by cytoplasmic streaming.

It is possible that myosin molecules are involved in mitochondrial and peroxisomal movement in *Arabidopsis*. Phylogenetic analysis has shown that myosins cluster into two plant-specific subfamilies, Myosin VIII with 4 members and Myosin XI with 13 members (Berg *et al.* 2001; Reddy and Day 2001). The latter myosin XI are more closely related to animal and fungal myosin Vs, which move multiple cargoes, for example, vacuoles, secretory vesicles, late Golgi, peroxisomes and mitochondria in budding yeast than to other myosins (reviewed in Weisman 2006). A member of the myosin XI family is reported to be involved in organelle transport from observations of the *mya2* mutant (Holweg and Nick 2004), suggesting that

functional myosins exist in higher plants and play a vital role in the movement of mitochondria and other organelles. Moreover, maize myosin XI was found to colocalize with mitochondria (Wang and Pesacreta 2004) and 170 kDa myosin XI was found to be associated with the surface of mitochondria (Romagnoli et al. 2007). Members of myosin XI family may directly bring about mitochondrial long-distance movement along actin filaments (i.e., rail type motility) as shown in budding yeast.

Based on our observations, the mean velocity of mitochondria and peroxisomes in *Arabidopsis* ( $1.44 \pm 0.89 \mu\text{m s}^{-1}$ ) was greater than that in budding yeast ( $49 \pm 21 \text{ nm s}^{-1}$  (Simon et al. 1997);  $27.5 \pm 4.7 \text{ nm s}^{-1}$  (Boldogh et al. 1998)). Tobacco cell myosin XI moves along actin in 35-nm steps at  $7 \mu\text{m s}^{-1}$ , which is the fastest known processive motion (Tominaga et al. 2003), and two yeast class V myosins (Myo2p and Myo4p) move along actin filaments *in vitro* at maximum speeds of  $4.5 \mu\text{m s}^{-1}$  and  $1.1 \mu\text{m s}^{-1}$ , respectively (Reck-Peterson et al. 2001). The very fast movement of mitochondria in higher plants may be at least partially caused by the very fast movement of myosin in higher plants.

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We thank Dr. Sakamoto for kindly providing transgenic *Arabidopsis* seeds with GFP for visualizing mitochondria. We thank Dr. Mano and Dr. Nishimura for kindly providing transgenic *Arabidopsis* seeds with GFP and RFP for visualizing peroxisomes. We thank Dr. Chua for kindly providing transgenic *Arabidopsis* seeds with GFP-mTalin. We thank Dr. Hashimoto for kindly providing transgenic *Arabidopsis* seeds with GFP-TUA6. We thank Dr. Hasezawa for kindly providing BY-GF11 and BY-GT16 cells.

## References

- Bazinet C, Rollins JE (2003) Rickettsia-like mitochondrial motility in *Drosophila* spermiogenesis. *Evol Dev* 5: 379–385
- Berg J, Powell B, Cheney R (2001) A millennial myosin census. *Mol Biol Cell* 12: 780–794
- Boldogh I, Vojtov N, Karmon S, Pon LA (1998) Interaction between mitochondria and the actin cytoskeleton in budding yeast requires two integral mitochondrial outer membrane proteins, Mmm1p and Mdm10p. *J Cell Biol* 141: 1371–1381
- Boldogh IR, Ramcharan SL, Yang HC, Pon LA (2004) A type V myosin (Myo2p) and a Rab-like G-protein (Ypt11p) are required for retention of newly inherited mitochondria in yeast cells during cell division. *Mol Biol Cell* 15: 3994–4002
- Bradley MO (1973) Microfilaments and Cytoplasmic Streaming—Inhibition of Streaming with Cytochalasin. *J Cell Sci* 12: 327–343
- Dramsi S, Cossart P (1998) Intracellular pathogens and the actin cytoskeleton. *Annu Rev Cell Dev Biol* 14: 137–166
- Drubin DG, Jones HD, Wertman KF (1993) Actin structure and function: roles in mitochondrial organization and morphogenesis in budding yeast and identification of the phalloidin-binding site.

- Mol Biol Cell* 4: 1277–1294
- Fehrenbacher KL, Yang HC, Gay AC, Huckaba TM, Pon LA (2004) Live cell imaging of mitochondrial movement along actin cables in budding yeast. *Curr Biol* 14: 1996–2004
- Hollenbeck PJ, Saxton WM (2005) The axonal transport of mitochondria. *J Cell Sci* 118: 5411–5419
- Itoh T, Watabe A, Toh-e A, Matsui Y (2002) Complex formation with Ypt11p, a rab-type small GTPase, is essential to facilitate the function of Myo2p, a class V myosin, in mitochondrial distribution in *Saccharomyces cerevisiae*. *Mol Cell Biol* 22: 7744–7757
- Holweg C, Nick P (2004) *Arabidopsis* myosin XI mutant is defective in organelle movement and polar auxin transport. *Proc Natl Acad Sci USA* 101: 10488–10493
- Jedd G, Chua NH (2002) Visualization of peroxisomes in living plant cells reveals acto-myosin-dependent cytoplasmic streaming and peroxisome budding. *Plant Cell Physiol* 43: 384–392
- Kandasamy MK, Meagher RB (1999) Actin-organelle interaction: Association with chloroplast in *Arabidopsis* leaf mesophyll cells. *Cell Motil Cytoskelet* 44: 110–118
- Kost B, Spielhofer P, Chua NH (1998) A GFP-mouse talin fusion protein labels plant actin filaments *in vivo* and visualizes the actin cytoskeleton in growing pollen tubes. *Plant J* 16: 393–401
- Kumagai F, Yoneda A, Tomida T, Sano T, Nagata T, Hasezawa S (2001) Fate of nascent microtubules organized at the M/G1 interface, as visualized by synchronized tobacco BY-2 cells stably expressing GFP-tubulin: time-sequence observations of the reorganization of cortical microtubules in living plant cells. *Plant Cell Physiol* 42: 723–732
- Lazzarino DA, Boldogh I, Smith MG, Rosand J, Pon LA (1994) Yeast mitochondria contain ATP-sensitive, reversible actin-binding activity. *Mol Biol Cell* 5: 807–818
- Logan DC, Leaver CJ (2000) Mitochondria-targeted GFP highlights the heterogeneity of mitochondrial shape, size and movement within living plant cells. *J Exp Bot* 51: 865–871
- Mano S, Hayashi M, Nishimura M (1999) Light regulates alternative splicing of hydroxypyruvate reductase in pumpkin. *Plant J* 17: 309–320
- Mano S, Nakamori C, Hayashi M, Kato A, Kondo M, Nishimura M (2002) Distribution and characterization of peroxisomes in *Arabidopsis* by visualization with GFP: dynamic morphology and actin-dependent movement. *Plant Cell Physiol* 43: 331–341
- Mathur J, Mathur N, Hulskamp M (2002) Simultaneous visualization of peroxisomes and cytoskeletal elements reveals actin and not microtubule-based peroxisome motility in plants. *Plant Physiol* 128: 1031–1045
- Nagata T, Nemoto Y, Hasezawa S (1992) Tobacco By-2 Cell-Line as the Hela-Cell in the Cell Biology of Higher-Plants. *International Review of Cytology—a Survey of Cell Biology* 132: 1–30
- Olyslaegers G, Verbelen JP (1998) Improved staining of F-actin and co-localization of mitochondria in plant cells. *Journal of Microscopy-Oxford* 192: 73–77
- Reck-Peterson SL, Tyska MJ, Novick PJ, Mooseker MS (2001) The yeast class V myosins, Myo2p and Myo4p, are nonprocessive actin-based motors. *J Cell Biol* 153: 1121–1126
- Reddy AS, Day IS (2001) Analysis of the myosins encoded in the recently completed *Arabidopsis thaliana* genome sequence. *Genome Biology* 2: RESEARCH0024
- Romagnoli S, Cai G, Faleri C, Yokota E, Shimmen T, Cresti M

- (2007) Microtubule- and actin filament-dependent motors are distributed on pollen tube mitochondria and contribute differently to their movement. *Plant Cell Physiol* 48: 345–361
- Sakamoto W, Kondo H, Murata M, Motoyoshi F (1996) Altered mitochondrial gene expression in a maternal distorted leaf mutant of *Arabidopsis* induced by chloroplast mutator. *Plant Cell* 8: 1377–1390
- Sano T, Higaki T, Oda Y, Hayashi T, Hasezawa S (2005) Appearance of actin microfilament ‘twin peaks’ in mitosis and their function in cell plate formation, as visualized in tobacco BY-2 cells expressing GFP-fimbrin. *Plant J* 44: 595–605
- Shimmen T, Yokota E (2004) Cytoplasmic streaming in plants. *Curr Opin Cell Biol* 16: 68–72
- Shimmen T, Yokota E (1994) Physiological and biochemical aspects of cytoplasmic streaming. *Int Rev Cytol* 155: 97–139
- Simon VR, Karmon SL, Pon LA (1997) Mitochondrial inheritance: Cell cycle and actin cable dependence of polarized mitochondrial movements in *Saccharomyces cerevisiae*. *Cell Motil Cytoskelet* 37: 199–210
- Tominaga M, Kojima H, Yokota E, Orii H, Nakamori R, Katayama E, Anson M, Shimmen T, Oiwa K (2003) Higher plant myosin XI moves processively on actin with 35 nm steps at high velocity. *EMBO J* 22: 1263–1272
- Ueda K, Matsuyama T, Hashimoto T (1999) Visualization of microtubules in living cells of transgenic *Arabidopsis thaliana*. *Protoplasma* 206: 201–206
- Van Gestel K, Kohler RH, Verbelen JP (2002) Plant mitochondria move on F-actin, but their positioning in the cortical cytoplasm depends on both F-actin and microtubules. *J Exp Bot* 53: 659–667
- Wada M, Suetsugu N (2004) Plant organelle positioning. *Curr Opin Plant Biol* 7: 626–631
- Wang ZY, Pesacreta TC (2004) A subclass of myosin XI is associated with mitochondria, plastids, and the molecular chaperone subunit TCP-1 alpha in maize. *Cell Motil Cytoskelet* 57: 218–232
- Weisman LS (2006) Organelles on the move: insights from yeast vacuole inheritance. *Nature Rev Mol Cell Biol* 7: 243–252