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Minireview

Plant mitochondrial fission and fusion

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Abstract Single plant cells have hundreds of mitochondria that move around and change their shape through the processes of fission and fusion. The *Arabidopsis* genome has genes for two dynamin-related proteins, DRP3A and DRP3B, that are similar to genes involved in mitochondrial fission in yeasts. DRP3A and DRP3B were localized to mitochondrial constricted sites and ends. Over-expression of DRP3A or DRP3B with point mutations caused severe elongation of mitochondria. These results show that DRP3A and DRP3B are functional homologues of the dynamin-related protein for mitochondrial fission in yeasts. On the other hand, *Arabidopsis* appears to have no genes similar to those involved in yeast mitochondrial fusion. Little has been written about mitochondrial fusion in plants. Using a novel photoconvertible fluorescent protein, we have recently shown that mitochondria in live plant cells rapidly undergo fusion and fission on a time scale of seconds. In this mini-review, we focus on plant mitochondrial fission and fusion, and compare them with mitochondrial fission and fusion in yeasts and animals, about which much has been learned in recent years.

Key words: Aberrant mitochondrial morphology, dynamine related protein.

Mitochondria, which are found in almost all eukaryotic cells, have roles not only in metabolism and ATP synthesis, but also in cell death (e.g. aging or apoptosis, reviewed in Hales 2004). Mitochondrial morphology is polymorphic and dynamic. It is affected by the balance between mitochondrial fission and mitochondrial fusion (Sesaki and Jensen 1999, Bleazard et al. 1999). In budding yeast *Saccharomyces cerevisiae*, mitochondrial fission mutants have a single reticular mitochondrion in a cell, compared with from 5 to 10 tubular-reticular mitochondria in wild-type cells (Sesaki and Jensen 1999, Bleazard et al. 1999). On the other hand, mitochondrial fusion mutants have many small particle mitochondria in a cell. These mutants cannot grow in non-fermentable media, because they are incapable of respiration. For unknown reasons, these mutants lack their mitochondrial DNA. Interestingly, double mutants, in which both mitochondrial fission and fusion are disrupted, can grow on non-fermentable media (Sesaki and Jensen 1999, Bleazard et al. 1999). Furthermore, mitochondria in the double mutant have an almost normal shape, but no longer undergo fission or fusion.

Mitochondrial fission

Because mitochondria are considered to be the remnants of free-living alpha-proteobacteria (Margulis and Bermudes 1985), the fission mechanism of mitochondria may be similar to the cell-division mechanism of bacteria. In fact, orthologues of the alpha-proteobacterial cell division gene *ftsZ* were found from two algae, *Cyanidioschyzon merolae* and *Mallomonas splendens* (Takahara et al. 2000; Beech et al. 2000). However, the *Arabidopsis* genome, like the genomes of yeasts and animals, doesn't have *ftsZ* orthologues.

Dynamin-related proteins were identified in yeasts and animals as the factors responsible for mitochondrial fission (Sesaki and Jensen 1999; Bleazard et al. 1999; Labrousse et al. 1999). Dynamins are large GTPase proteins that wrap around the neck of endocytotic vesicles, and are believed to tighten or pinch off (or recruit the factor to pinch off) the vesicles from the plasma membrane (Sweitzer and Hinshaw 1998; Hinshaw 2000). Dynamins and dynamin-related proteins have been found in many organisms (van der Bliek 1999). Among these proteins are mitochondrial fission-related dynamin-related proteins (MtDRPs hereafter) that have also been found in a variety of organisms (including algae, plants, protozoa, yeasts and mammals). A phylogenetic analysis has shown that MtDRPs cluster in a single lineage, indicating that they are strongly conserved.

We found two *Arabidopsis* MtDRPs, DRP3A and DRP3B (formerly named ADL2a and ADL2b, respectively) (Arimura and Tsutsumi 2002, Arimura et al. 2004a). A GFP-DRP3B fusion protein localized to the ends and constricted sites of mitochondria (Arrows and arrowheads respectively in Figure 1). These sites were

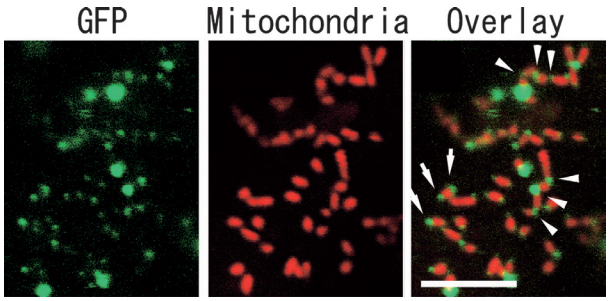

Figure 1. Localization of GFP-DRP3B within tips and constriction sites of mitochondria. BY-2 cells transiently expressing GFP-DRP3B with the mitochondrial marker MitoTracker, were examined with a confocal laser scanning microscopy. The panels show high-magnification images of a part of a single BY-2 cell. Left and middle panels are separate images obtained with the GFP and MitoTracker of the right images, respectively. GFP-DRP3B (green) was located in small punctate structures, many of which were localized in tips and constricted sites of mitochondria (red). GFP-DRP3B spots are localized to places where the mitochondria were constricted. GFP signals are localized at the tips of mitochondria. Scale bar, 5 μm. Reproduced from Arimura and Tsutsumi (2002).

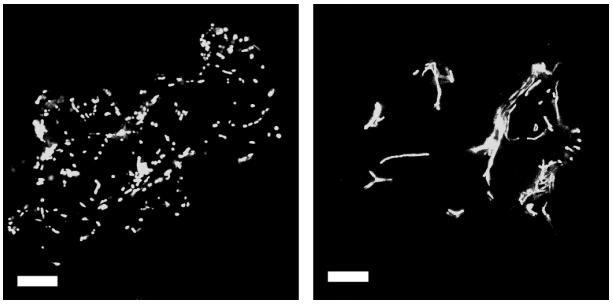

Figure 2. Mutagenized *Arabidopsis* DRP3 has dominant-negative effects on mitochondrial morphology. Confocal laser scanning microscopy images show *Arabidopsis* epidermal cells transformed with wild-type DRP3B (left) or mutagenized DRP3B (right). At left, mitochondria have normal morphology like wild type (data not shown) (small spherical or peanut-shaped particles). At right, mitochondria are fewer and longer due to the blocking of fission. Scale bars, 10 μm. Revised from Arimura and Tsutsumi 2002.

probably sites at which fission had just been completed or was still in progress. Similar results were obtained with DRP3A. Over-expression of DRP3A or DRP3B with a point mutation in its GTPase domain caused severe elongation of mitochondria (Figure 2). Similar results were observed in yeast and animals with mutated MtDRPs (Sesaki and Jensen 1999; Bleazard et al. 1999). MtDRPs are thought to be cytosolic proteins, but occasionally some MtDRPs aggregate at the fission sites of mitochondria (Nishida et al. 2003).

Another factor involved in mitochondrial fission is Fis1p. Fis1p is an approximately 17 kDa protein with a trans-membrane domain at its C-terminus. Fis1p is required for the proper localization of MtDRPs in yeast (Mozdy et al. 2000; Tieu and Nunnari 2000). A Fis1 homologue was found in mammals (Mozdy et al. 2000). We also found a Fis1 homologue in *Arabidopsis*. Therefore, plants, yeast and animals would share a system that uses Fis1 and MtDRP1 for mitochondrial fission.

Mitochondrial fusion

Fzo1 (Fuzzy Onion) has been identified as a gene responsible for male sterility in Drosophila (Hales and Fuller 1997). In wild-type sperm, mitochondria fuse to form two interdigitated mitochondria. However, in the sperm of *fzo1* mutants, many mitochondria do not fuse but gather into a fuzzy aggregate. Therefore, fzo1 was concluded to be a factor in mitochondrial fusion. Fzo1 contains a GTPase domain, and has weak homology to dynamin-related proteins. Fzo1 localizes to the outer-membrane by two trans-membrane domains near its C-terminus, and both the C- and N-termini project into the cytosol.

Homologues of Fzo1 have been found in yeast and mammals. Disruptions of these homologues inhibit mitochondrial fusion and cause mitochondrial fragmentation due to ongoing fission (Rapaport et al. 1998; Hermann et al. 1998; Santel and Fuller 2001). Subsequently, two other factors,. Mgm1 and Ugo1, were found to be involved in mitochondrial fusion in yeast (reviewed in Hales 2004).

The *Arabidopsis* genome does not appear to have any sequences homologous to these genes. Plant mitochondria are usually smaller particles whose shapes are very different from those of yeasts and animals. Do plant mitochondria actually fuse? Belliard et al. (1979) reported the occurrence of plant mitochondrial fusion based on the finding that plants regenerated from the fusion of protoplasts had a novel mitochondrial-DNA restriction pattern, as if the DNA was recombined from the DNAs of the parental cells. Although this result implies that the mitochondria of the parental cells fuse, it could be an artifact due to the method of protoplast fusion, which can affect membranes. To more directly test whether plant mitochondria fuse, we prepared single cells that simultaneously have green- and red-labeled mitochondria (Arimura et al. 2004b). Then, fusion of a red mitochondrion and a green mitochondrion would result in a yellow mitochondrion. To prepare these cells, we used a fluorescent protein, Kaede, which is an orthologue of green fluorescent protein that was cloned from stony coral (Ando et al. 2003). The color of Kaede's fluorescence can be irreversibly changed from green to red by irradiation of UV-purple light. We made an expression vector of Kaede with a mitochondrial pre-sequence, and introduced it to plant cells. Half of a cell was irradiated by 400 nm light, resulting in a single plant cell in which half the mitochondria were green and the other half were red.

Figure 3 shows the time-course of a single onion

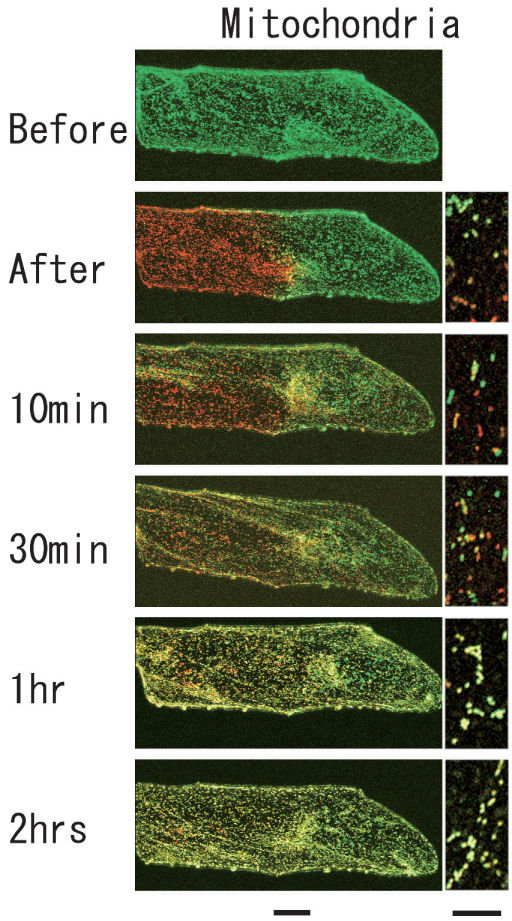

Figure 3. Mitochondrial fusion in onion bulb epidermal cells. Time-course observation of an onion bulb epidermal cell transformed with mitochondria-localized Kaede fusion proteins. A portion of each cell was exposed to 400 nm light to photoconvert some of the mitochondria to red. Highly magnified images of mitochondria are shown at right. Both green and red Kaede in mitochondria were completely mixed and redistributed to all mitochondria in the cell within 2 h. Scale bars indicate 50 μm for whole cell images and 10 μm for high magnification images. Revised from Arimura et al. (2004b).

epidermal cell, containing more than 15,000 mitochondria. All green and red mitochondria in the cell were changed into uniform yellow mitochondria in only one or two hours. This result shows that plant mitochondria also fuse frequently. Because the average planar area of the mitochondria didn't change during the time course, not only mitochondrial fusion but also mitochondrial fission should have occurred in about the same frequency. Figure 4 shows a mitochondrial fusion event between single green and red mitochondria in an onion epidermal cell. The Mitochondrial fused without quite changing their morphology. The mixing between two mitochondria was accomplished within only three seconds. Then, the fused mitochondria occasionally underwent fission at the same site where fusion occurred. Figure 4b shows that mitochondria don't always fuse with their neighbors.

We applied the same method to plastids and

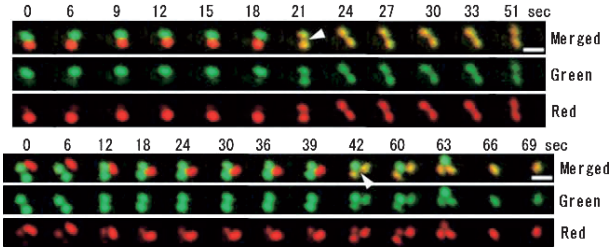

Figure 4. Time-course observations of red and green mitochondria. Images were acquired at 3 s intervals. Arrowheads indicate constrictions at the point of fusion. Scale bars, 2 μm. Revised from Arimura et al. (2004b).

peroxisomes. In these cases, green or red organelles remain even a day after the photoconversion. Therefore, mitochondrial fusion occurs very frequently, but plastids and peroxisomes don't fuse as often as mitochondria (Arimura et al. 2004b).

The genes and mechanisms for plant mitochondrial fusion are still unknown. We (Feng et al. 2004) and another group (Logan et al. 2003) have screened and mapped mutants with aberrant mitochondrial morphology. We believe that these approaches will enable us to find novel plant-specific genes, or unknown common genes for mitochondrial dynamics and morphology.

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