Old dog, new tricks: Arf1 required for mitochondria homeostasis

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The small GTPase Arf1 that is classically required for the budding of COPI-coated vesicles from the Golgi membrane is now proposed to have novel and conserved roles in the morphological and functional maintenance of mitochondria: It functionally localizes to ER/mitochondria contact sites; it allows for the recruitment of a degradation machinery to mitochondria to remove toxic mitofusin/Fzo1 clusters; and it allows the extension of autophagy sequestration membranes needed for mitophagy to clear damaged mitochondria.

See also: **KB Ackema et al** (November 2014)

Arf1 is classically known to play critical roles in membrane traffic by initiating the recruitment of the COPI coat proteins to the Golgi membrane and by modulating the activity of lipid-modifying enzymes. As a small GTPase, Arf1 exists in a GDP-bound form that is mostly cytosolic, and a GTP-bound form that is associated with membranes. The exchange of GDP for GTP on Arf proteins is catalyzed by the guanine nucleotide exchange factors ArfGEF (Donaldson & Jackson, 2011). Like Arfs, ArfGEFs exist in a dynamic equilibrium between membrane-bound and cytosolic pools, and as such, they can spatially and temporally dictate Arf activation. In this regard, recently, Arf1 has been shown to mediate COPI vesicle formation on lipid droplets to allow lipid droplet-ER interactions (Wilfling et al, 2014).

In this issue of *The EMBO Journal*, the Spang group describes novel and conserved roles for Arf1 and its ArfGEF (Gbf1) in mitochondria homeostasis that are independent of COPI-coated vesicle budding and

membrane traffic through the secretory pathway (Ackema et al, 2014). *C. elegans* muscle cells depleted of Gbf1 or Arf1 by RNAi exhibit an altered morphology and function of mitochondria. Similar defects are observed in yeast *arf1/2* double mutants and *gea1/2* (ArfGEF) mutants, as well as in mammalian cells in culture depleted of Gbf1. This is not due to impairment in cytoskeletal dynamics and in the balance between mitochondria fission and fusion. Instead, Arf-GTP is proposed to have a direct role in mitochondria homeostasis, as it is found associated to mitochondria and/or ER/mitochondria contact sites, a novel localization for this Golgi GTPase (Fig 1).

Arf1 appears to be required for three aspects of mitochondria homeostasis (Fig 1).

(1) Arf1 contributes to ER/mitochondria contact sites independently of the known ERMES complex. Upon reduction of Arf1 activity, contact sites still exist but might be functionality impaired:

The authors show that removing a known ERMES component, Miro/Gem (known to be required for maintenance of ER/mitochondria contact sites in yeast, Kornmann et al, 2011), results in the same mitochondria phenotype as *arf1/2* mutant cells. Furthermore, by combining *arf1/2* and *gem1* mutations, the mitochondrial morphology is very severely compromised, more than in the individual mutants. This suggests the existence of two non-redundant complexes required for ER/mitochondria contact sites, the known ERMES and a novel Arf1-dependent complex. In yeast, the removal of either leads to the impairment of the ER/mitochondria contact site function and drastic change in mitochondrial morphology. This new Arf1 based complex

might also explain the presence of ER/mitochondria contact sites in organisms that are devoid of ERMES.

(2) Arf1 allows the targeting of a mitochondria degradation pathway (MAD) to mitochondria where it removes deleterious clusters of mitofusin Fzo1. In the absence of Arf1 activity, Fzo1—potentially toxic—clusters accumulate and lead to impairment of mitochondrial function and morphology.

Arf1-GTP is shown by Ackema et al to interact, whether directly or not, with the triple A-ATPase Cdc48/p97 that forms a key part of the MAD, a degradation pathway dedicated in removing misfolded proteins from mitochondria for subsequent degradation by the proteasome (Heo et al, 2010).

One of the MAD substrates is the mitofusin Fzo1, a GTPase embedded in the mitochondrial outer membrane and involved in their fusion (Escobar-Henriques & Langer, 2014). Arf1 loss of function could result in a less efficient targeting of CDC48 to the mitochondrial membrane and a reduction of MAD activity, thus leading to an elevated Fzo1 level. However, Fzo1 levels are not strongly affected in the yeast *arf1/2* mutant when compared to wild-type. Surprisingly, though, Fzo1 localization is drastically modified. In wild-type cells, Fzo1 is localized to the whole outer mitochondrial membrane, whereas it forms bright foci in the *arf1/2* mutant, possibly causing mitochondrial damage. Interestingly, upon Cdc48 overexpression in *arf1/2* mutant cells, these Fzo1 foci are resolved, suggesting that the role of Cdc48 is to remove deleterious foci.

Taken together, it seems that Arf1 drives Cdc48-MAD to remove mitofusin/Fzo1 clusters that may occasionally form, thereby most likely maintaining both mitochondria shape and function. In absence of Arf1

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Figure 1. Proposed function of Arf1 in mitochondria homeostasis.

Arf1 appears to be required for functional ER-mitochondrial contact sites (1), Cdc48 recruitment to mitochondria resulting in mitofusin Fzo1 homeostasis by MAD (2) and mitophagy (3). Arf1 loss of function leads to mitochondrial fragmentation and hyperconnectivity in yeast and *C. elegans*, respectively, when these events are compromised.

function, Cdc48 is not targeted or recruited to the Fzo1 foci that accumulate, leading to mitochondrial defects.

(3) Arf1 contributes to autophagy/mitophagy resulting in the removal of damaged mitochondria. In absence of Arf1, damaged mitochondria accumulate:

The authors show that Arf1 promotes mitophagy. In the absence of Arf1 function, it is shown that mitophagy (and perhaps autophagy in general) is impaired, thus leaving the cells with more aberrant and

dysfunctional mitochondria. The role of Arf1 in mitophagy could either be non-specific by promoting the expansion of autophagosomal membranes during autophagy (van der Vaart *et al*, 2010) or via its localization to ER/mitochondria contact sites in a similar fashion to the ERMES complex, which has been previously involved in mitophagy (Böckler & Westermann, 2014).

Of course, many issues remain to be clarified, including urgent ones: What is the proportion of Arf1 on mitochondria? How

is Arf1GEF targeted and localized to mitochondria in the first place? What are the components of the complex recruited by Arf1 to ER/mitochondria contact sites? Why does the morphological phenotype in yeast (fragmented mitochondria upon Arf1/Gea1/2 loss of function) appear to be the opposite of the one observed in *C. elegans* muscle cells (hyperconnected mitochondria)? These issues need to be addressed in future work. The work by the Spang's group outlines an essential role of Arf1 in recruiting complexes—COPI and an hypothetical novel complex mediating ER/mitochondria contact sites—as well as in mediating organellar contacts, such as ER/lipid droplets or ER/mitochondria. As such, Arf1 might also act at other cellular sites.

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