Cell Cycle 12:19, 3131–3132; October 1, 2013; © 2013 Landes Bioscience

Participation of mitochondrial fission during mitophagy

Kai Mao and Daniel J Klionsky*

Life Sciences Institute; University of Michigan; Ann Arbor, MI USA

Due to the key role of mitochondria in cell metabolism, the quantity and quality of this organelle must be maintained properly, which is achieved by constitutive mitochondrial fusion and fission as well as biogenesis and degradation. A fission complex controls mitochondrial division, whereas degradation occurs primarily through autophagy. The latter can involve either nonselective or selective elimination of the organelle; the process of selective mitochondrial degradation by autophagy is referred to as mitophagy.

Mitophagy is used to remove damaged and superfluous mitochondria. Various studies have demonstrated physiological roles for mitophagy in higher eukaryotes. For example, mitophagy promotes cell survival in certain situations where the inability to remove the dysfunctional organelle leads to cell death and neurodegeneration, for example as seen in some types of Parkinson disease.[1] In addition, the clearance of mitochondria during the development of erythrocytes[2] and sperm-derived mitochondria after fertilization[3,4] are both controlled by mitophagy.

The molecular analysis of mitophagy started in the budding yeast *S. cerevisiae*, where it was clearly demonstrated to be a selective process requiring the scaffold protein Atg11.[5] In yeast, mitophagy appears to occur primarily in response to changing metabolic demands. For example, this process is induced when cells are shifted from nutrient-rich medium containing a non-fermentable carbon source to nitrogen starvation medium with a fermentable carbon source. Genomic screening led to the identification of *ATG32*, which encodes a mitophagy receptor, and established the first part of a molecular model for mitophagy in yeast.[6] Atg32 is a mitochondrial outer membrane protein, and it recruits Atg11 to mitochondria when mitophagy is induced. Atg11 links the cargo to other Atg proteins and promotes the formation of mitochondria-specific autophagosomes that ultimately lead to degradation of the organelle.

Another gene identified in our screen for mitophagy-defective mutants was *DNM1*, which encodes a dynamin-related GTPase controlling mitochondrial division. The mitochondrial localization of Dnm1 requires Fis1, an integral membrane protein, and Mdv1-Caf4, which redundantly bind to both Dnm1 and Fis1 to maintain the integrity of the complex. These 4 proteins constitute the mitochondrial fission complex.

Deletion of *DNM1* or *FIS1* strongly blocks mitophagy. In contrast, single deletion of the *MDV1* or *CAF4* genes has limited effects on mitophagy; this is due to the functional redundancy of Mdv1 and Caf4, as double deletion of both corresponding genes causes a dramatic decrease of mitophagy activity similar to that seen in the absence of Dnm1 or Fis1. Therefore, the intact fission complex is required for efficient mitophagy.

To specifically mark the degrading mitochondria on the mitochondrial network, we used the bimolecular fluorescence complementation (BiFC) assay, and showed that the fluorescent YFP puncta resulting from the interaction between Atg32 and Atg11 represent degrading mitochondria. We found that mCherry-tagged Dnm1, Mdv1 and Caf4 colocalize with the Atg32-Atg11 interacting puncta, suggesting that the fission complex is recruited to the degrading mitochondria when mitophagy is induced.

We propose that the cell contains at least 2 distinct complexes that are involved in mitochondrial division. One of these complexes acts constitutively to promote homeostatic mitochondrial fission, whereas there is also a mitophagy-specific fission complex—comprised of Atg32, Atg11, and the subunits of the homeostatic fission complex—that forms either in late log phase growth or following a shift from conditions that induce mitochondrial proliferation to one where mitochondrial respiration is no longer critical for energy production; these latter conditions drive the division of mitochondria that leads to subsequent degradation.

We confirmed the results from the BiFC analysis using co-immunoprecipitation. Together, the data suggest a direct interaction between Atg11 and Dnm1. Both Atg11 and Dnm1 are cytosolic proteins, and their recruitment to the mitochondria is dependent on Atg32 and Fis1, respectively. The BiFC puncta corresponding to the Atg11–Dnm1 interaction only constitute a small part of the total Dnm1 population, in agreement with our hypothesis that there are at least 2 separate populations of fission complexes. Further analyses enabled us to identify 2 Dnm1 mutants, $Dnm1^{E728R}$ and $Dnm1^{D729R}$, which retain normal (i.e., homeostatic) Dnm1 function but lose interaction with Atg11, and hence Atg32. As expected, these mutants show mitophagy defects, even though they allow cells to carry out homeostatic mitochondrial fission.

It has been suggested that mitochondrial fission occurs at contact sites with the endoplasmic reticulum (ER), referred to as the ER-mitochondria encounter structure (ERMES).[7] To examine the potential connection between the recruitment of

*Correspondence to: Daniel J Klionsky; Email: klionsky@umich.edu
Submitted: 08/29/2013; Accepted: 09/02/2013
http://dx.doi.org/10.4161/cc.26352
Comment on: Mao K, et al. Dev Cell 2013; 26:9-18; PMID:23810512; http://dx.doi.org/10.1016/j.devcel.2013.05.024

the fission machinery and the ERMES, we labeled mitochondria with a mitochondrially targeted BFP and the ER with HDEL–DsRed. We found that the Atg32-Atg11 BiFC puncta are also localized at the ERMES. In addition, we generated mCherry chimeras with Mdm12 and Mdm34, components of the ERMES,[8] and observed their colocalization with the Atg32-Atg11 BiFC puncta. Therefore, our results suggest that mitophagy-specific fission also involves the participation of the ER.

In this study, we revealed a direct link between mitochondrial fission and mitochondrial degradation. When mitophagy is induced, Atg11 is recruited to the mitochondria through interaction with Atg32. Atg11 further recruits the fission complex to promote the division of the reticular organelle. The resulting smaller fragments of mitochondria will subsequently be engulfed by phagophores to form mitophagosomes, mitochondria-specific autophagosomes, followed by fusion with, and then degradation within, the vacuole.

References

1. Narendra D, et al. J Cell Biol 2008; 183:795-803; PMID:19029340; http://dx.doi.org/10.1083/jcb.200809125
2. Schweers RL, et al. Proc Natl Acad Sci U S A 2007; 104:19500-5; PMID:18048346; http://dx.doi.org/10.1073/pnas.0708818104
3. Sato M, et al. Science 2011; 334:1141-4; PMID:21998252; http://dx.doi.org/10.1126/science.1210333
4. Al Rawi S, et al. Science 2011; 334:1144-7; PMID:22033522; http://dx.doi.org/10.1126/science.1211878
5. Kanki T, et al. J Biol Chem 2008; 283:32386-93; PMID:18818209; http://dx.doi.org/10.1074/jbc.M802403200
6. Kanki T, et al. Mol Biol Cell 2009; 20:4730-8; PMID:19793921; http://dx.doi.org/10.1091/mbc.E09-03-0225
7. Friedman JR, et al. Science 2011; 334:358-62; PMID:21885730; http://dx.doi.org/10.1126/science.1207385
8. Kornmann B, et al. Science 2009; 325:477-81; PMID:19556461; http://dx.doi.org/10.1126/science.1175088