Biochimica et Biophysica Acta xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbamcr

Review

# Mitophagy and mitochondrial dynamics in *Saccharomyces cerevisiae* ☆

Matthias Müller [a,b], Kaihui Lu [c], Andreas S. Reichert [a,b,c,*]

[a] Mitochondrial Biology, Buchmann Institute for Molecular Life Sciences, Goethe University Frankfurt am Main, Germany
[b] Mitochondrial Biology, Medical School, Goethe University Frankfurt am Main, Germany
[c] Institute of Biochemistry and Molecular Biology I, Medical Faculty, Heinrich Heine University, Düsseldorf, Germany

ARTICLE INFO

*Article history:*
Received 1 December 2014
Received in revised form 10 February 2015
Accepted 27 February 2015
Available online xxxx

*Keywords:*
Mitophagy
Mitochondrial dynamics
Atg32
Dnm1
Mgm1
Ubiquitination

ABSTRACT

Mitochondria fulfill central cellular functions including energy metabolism, iron-sulfur biogenesis, and regulation of apoptosis and calcium homeostasis. Accumulation of dysfunctional mitochondria is observed in ageing and many human diseases such as cancer and various neurodegenerative disorders. Appropriate quality control of mitochondria is important for cell survival in most eukaryotic cells. One important pathway in this respect is mitophagy, a selective form of autophagy which removes excess and dysfunctional mitochondria. In the past decades a series of essential factors for mitophagy have been identified and characterized. However, little is known about the molecular mechanisms regulating mitophagy. The role of mitochondrial dynamics in mitophagy is controversially discussed. Here we will review recent advances in this context promoting our understanding on the molecular regulation of mitophagy in *Saccharomyces cerevisiae* and on the role of mitochondrial dynamics in mitochondrial quality control. This article is part of a Special Issue entitled: Mitophagy.


## 1. Introduction

Mitochondria have a central role in cell survival and are involved in many essential cellular processes including generation of ATP by oxidative phosphorylation, the citric acid cycle, haem biosynthesis, formation of iron sulfur clusters, and β-oxidation of fatty acids. They are key regulators of programmed cell death pathways, and have crucial functions in multiple signalling pathways. Mitochondria are one main source of reactive oxygen species (ROS) which are generated as by-products by complex I and III of the electron transport chain [1,2]. Besides acting as important second messengers, ROS can lead to protein modifications, lipid peroxidation and DNA damage when produced in excess. Increased oxidative stress can lead to dissipation of the mitochondrial membrane potential and to cell death by the release of pro-apoptotic proteins [3].

Mitochondria are characterized by a double membrane architecture [4]. Two structurally and functionally distinct membranes, the mitochondrial outer membrane (OM) and the inner membrane (IM), separate the cytosol from the mitochondrial matrix [5,6]. Between both membranes a small aqueous space, the intermembrane space (IMS), exists. The inner membrane structure is highly diverse as it shows characteristic infoldings, termed 'cristae', which are highly variable in appearance [7]. The IM can be subdivided into the inner boundary membrane (IBM) that closely opposes the OM and the cristae membrane (CM) which represents the majority of the inner membrane surface in most cells. Both parts of the IM are connected by a small pore- or slot-like structure, the crista junction (CJ). CJs are proposed to restrict diffusion of molecules between intra-cristae compartments and the peripheral IMS and also within the IM [8]. This is supported by studies showing that the protein composition is different between the IBM and the CM and that it can adapt dynamically dependent on the physiological state of mitochondria [9–11].

The genome of mitochondria (mtDNA) is organized in circular double-stranded molecules, packed in compact particles, termed 'nucleoids' [12,13]. Human mtDNA is maternally inherited and contains 37 genes encoding for 13 subunits of complexes I, III, IV, and V; 2 ribosomal RNAs, and 22 tRNAs [14] while the majority of about 1000 – 2000 mitochondrial proteins is encoded in the nucleus. Thus, most mitochondrial

*Abbreviations:* AIM, Atg8 family interacting motif; ALP, alkaline phosphatase; Ape1, aminopeptidase I; ATG, autophagy-related; CCCP, carbonyl cyanide m-chlorophenylhydrazone; CJ, crista junction; CM, cristae membrane; CMA, chaperone-mediated autophagy; Cvt, cytoplasm to vacuole targeting; cytALP, cytosol-localized ALP; ER, endoplasmic reticulum; ERMES, ER mitochondria encounter structure; GFP, green fluorescent protein; GSH, reduced glutathione; GTP, guanosine triphosphate; IBM, inner boundary membrane; IM, inner membrane; IMS, intermembrane space; LIR, LC3 interacting region; MAPK, mitogen-activated protein kinase; mtALP, mitochondria-localized ALP; mtDNA, mitochondrial DNA; NAC, N-acetylcysteine; OM, outer membrane; PAS, phagophore assembly site; PE, phosphatidylethanolamine; PINK1, PTEN-induced putative kinase 1; PKA, protein kinase A; ROS, reactive oxygen species; rRNA, ribosomal RNA; *S. cerevisiae*, *Saccharomyces cerevisiae*; SQA, synthetic quantitative array; TMD, transmembrane domain; TOR, target of rapamycin; TORC1/2, target of rapamycin complex 1/2

☆ This article is part of a Special Issue entitled: Mitophagy.

* Corresponding author at: Institute of Biochemistry and Molecular Biology I, Medical Faculty, Heinrich Heine University, Düsseldorf, Germany.
*E-mail address:* reichert@hhu.de (A.S. Reichert).

http://dx.doi.org/10.1016/j.bbamcr.2015.02.024
0167-4889/

proteins are synthesized in the cytosol and are subsequently imported into the organelle by distinct protein translocases ensuring proper targeting to the different mitochondrial subcompartments [15,16].

Mitochondria are highly dynamic as they constantly undergo balanced fusion and fission events [17,18]. The dynamics of mitochondria is important for various cellular functions such as mtDNA inheritance and intracellular distribution [19,20]. The opposing action of fission and fusion needs to be tightly regulated in order to adapt mitochondrial morphology to altered physiological needs. In *Saccharomyces cerevisiae*, at least three large GTPases and their interaction partners ensure these processes. The dynamin-related large GTPase Mgm1 is responsible for inner membrane fusion. The large GTPase Fzo1 mediates docking and fusion of the mitochondrial outer membranes. Mitochondrial fission is executed by the dynamin-related GTPase Dnm1 which binds to the outer membrane via Fis1 and Mdv1 [21]. Dnm1 assembles into higher oligomers at the mitochondrial surface promoting the formation of rings and spirals around the organelle. These oligomers divide the organelle in a GTP-dependent manner presumably in a similar way as classical dynamins act during endocytosis [21,22]. For most of these core components required for fusion and fission of mitochondria orthologs have been identified ensuring mitochondrial dynamics in mammals [23].

Since mitochondria display a key role in maintaining cellular homeostasis it is not surprising that mitochondrial dysfunction is associated with many pathological conditions including neurodegenerative diseases, cancer, diabetes and obesity [24–26]. Human mitochondrial disorders arise from mutations in mitochondrial and/or nuclear DNA [27]. Mitochondrial damage and mtDNA mutations are causally linked to the ageing process in eukaryotic cells [28,29]. Post-mitotic cells such as neurons and muscle cells strongly depend on mitochondrial function and are in particular susceptible to the deleterious consequences of pathogenic mutations as they usually cannot be replaced by neighbouring cells. Mitochondrial dysfunction in neurons can lead to several neurodegenerative disorders, such as Parkinson's and Alzheimer's disease [30]. Some of these neuropathies are associated with mutations in genes affecting mitochondrial dynamics. For example, autosomal dominant optic atrophy type I, a quite common neuropathy affecting retinal ganglion cells of the optic nerve, is caused by mutations in the gene encoding OPA1, the mammalian ortholog of the fusion factor Mgm1 from baker's yeast [31,32].

In order to restrict mitochondrial damage and ensure organelle integrity, eukaryotic cells have evolved distinct quality control mechanisms acting at different levels [33]. First, intra-mitochondrial molecular protein quality control is exerted by highly conserved molecular chaperones and proteases [34]. They monitor the assembly of mitochondrial proteins and selectively remove misfolded, damaged or excess proteins from the organelle. In case mitochondrial damage cannot be controlled at this level, proper mechanistic steps are carried out at the organellar level to limit damage and improve function. Mitochondrial dynamics plays a pivotal role because continuous mixing of mitochondrial contents due to constant fusion and fission contributes to a homogenous inter-organelle complementation of damaged proteins and mtDNA molecules [35]. Mitochondrial fission has been proposed to separate dysfunctional mitochondria from the integral network in mammalian cells [36]. In yeast and mammals, those mitochondria incapable to re-fuse with intact organelles will be removed selectively via an autophagy-related process, termed 'mitophagy' [36–39]. Mitophagy is critical for the cellular regulation of steady-state mitochondrial turnover, and by that determines the amount of mitochondria in response to changing environmental conditions and during development [40–42]. PINK1 and PARKIN have been reported to cause early onset hereditary forms of Parkinson's disease [39,43]. Several studies propose that PINK1-/PARKIN-dependent mitophagy mediates the selective removal of damaged and dysfunctional mitochondria and by that ensures organelle quality control [36,44–47]. Another mechanistic link between mitochondrial dysfunction and autophagy, two processes well known to play pivotal roles in neurodegeneration and ageing, was proposed by a study in *S. cerevisiae* showing that mitochondrial respiratory dysfunction impairs induction of the cellular autophagic response in general [48]. Overall, all this clearly points to the possibility of modulating mitochondrial degradation as a potential therapeutic treatment of human neurodegenerative disorders [49,50]. However, the detailed mechanism and implication of mitophagy in mitochondrial quality control are still not fully understood. When damaged mitochondria cannot be effectively removed by autophagy, apoptosis is induced by the release of pro-apoptotic proteins from the intermembrane space as a third possibility of quality control occurring at the cellular level [51]. How the different levels of quality control are linked and coordinated will be a matter of future research.

## 2. Mechanisms and regulation of mitophagy

Mitochondria as well as other cytosolic constituents can be degraded by non-selective autophagy [52]. This occurs constitutively at a low basal level but is drastically induced upon starvation and other physiological signals including hormones, growth factors, and certain pathogens. In general three types of autophagy are described [see 53, 54]. (1) Macroautophagy (hereafter referred to as autophagy) defines the degradation pathway of cytoplasmic components involving their engulfment by autophagosomal membranes (Fig. 1). In this process the outer membrane of an autophagosome then fuses with the vacuole/lysosome and releases its remaining content into the acidic lumen where it is degraded by resident vacuolar/lysosomal hydrolases; (2) microautophagy defines a direct uptake of cytoplasmic material via invagination of the vacuolar/lysosomal membrane; and (3) chaperone-mediated autophagy (CMA) is found in higher eukaryotes and mediates the direct translocation of unfolded proteins across the lysosomal membrane requiring cytosolic and lysosomal chaperones [55]. More recently, the importance of selective forms of autophagy became evident. In yeast, several forms are well studied [56]. One example is the Cvt pathway, a biosynthetic process that occurs constitutively under nutrient-rich conditions. It mediates the selective transport of specific precursor enzymes into the vacuole and shares common mechanistic features with macroautophagy [57,58]. At least two vacuolar hydrolases, aminopeptidase I (Ape1) and α-mannosidase I (Ams1), synthesized as inactive proenzymes, assemble and form a large oligomeric complex, the 'Cvt complex', which serves as cargo in this selective type of autophagy.

The term 'mitophagy' was introduced by John Lemasters after first experiments in *S. cerevisiae* suggesting that mitochondrial degradation is a selective process [40,46]. This field has made considerable progress in the meantime improving our molecular understanding of mitophagy in yeast and higher eukaryotes. *S. cerevisiae* is employed as a highly suitable model to study the molecular mechanisms of mitophagy for various reasons including: i) there are numerous genes/proteins orthologous to human genes/proteins which fulfil essentially the same function; ii) it is probably the best characterized model organism with numerous whole-genome, transcriptome, and proteome data sets; iii) and finally, compared with other model systems, genetic manipulation and study of multiple intracellular processes in *S. cerevisiae* is easy, fast, and cheap.

More than 30 autophagy-related (*ATG*) genes have been identified in yeast and other fungi [54,59]. The majority of ATG genes is required for all forms of autophagy as they are necessary for induction, formation of the isolation membrane, assembly of the preautophagosomal structure (PAS), and maturation of autophagosomes (see Fig. 1). Still, some ATG genes are specific for selective forms of autophagy. Consequently, the same core ATG machinery is required for mitophagy as well as for non-selective autophagy [60]. Interestingly, mitophagy can in principle occur via micro- or macroautophagy which apparently depends on the mode of induction [38,47,56,61].

Please cite this article as: M. Müller, et al., Mitophagy and mitochondrial dynamics in *Saccharomyces cerevisiae*, Biochim. Biophys. Acta (2015), http://dx.doi.org/10.1016/j.bbamcr.2015.02.024