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Review

Mitochondrial morphology in mitophagy and macroautophagy ☆

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ABSTRACT

Mitochondria are critical organelles in energy conversion, metabolism and amplification of signalling. They are however also major sources of reactive oxygen species and when dysfunctional they consume cytosolic ATP. Maintenance of a cohort of healthy mitochondria is therefore crucial for the overall cell fitness. Superfluous or damaged organelles are mainly degraded by mitophagy, a selective process of autophagy. In response to the triggers of mitophagy, mitochondria fragment: this morphological change accompanies the exposure of "eat-me" signals, resulting in the engulfment of the organelle by the autophagosomes. Conversely, during macroautophagy mitochondria fuse to be spared from degradation and to sustain ATP production in times of limited nutrient availability. Thus, mitochondrial shape defines different types of autophagy, highlighting the interplay between morphology of the organelle and complex cellular responses. This article is part of a Special Issue entitled: Mitochondrial dynamics and physiology.

1. Introduction

The word "autophagy", derived from the Greek and meaning "self-eating", was originally proposed by Christian de Duve more than 40 years ago to describe a catabolic process conserved from lower to higher eukaryotes [1]. Autophagy is essential for recycling energy sources when cells deal with challenging conditions, such as nutrient depletion or hypoxia, or during development [2]. Additionally, autophagy plays a key role in cellular quality control processes, being essential for the degradation of superfluous or damaged organelles and oxidized proteins [3].

Autophagy broadly refers to any process of degradation of cytosolic components by the lysosome, but it can be more precisely subdivided in 3 types identified based on the different cargo delivery to the lysosome — macroautophagy, microautophagy and chaperone-mediated autophagy (CMA) [4]. During macroautophagy, a "phagophore" expands into a double membrane organelle that engulfs cytosolic components (proteins, ribosomes and organelles), giving rise to the autophagosome. The external membrane of the autophagosome fuses with the lysosomal membrane, the inner vesicle and its cargo being therefore degraded. The ensuing nutrients are recycled back to the cytosol via membrane permeases [5]. Macroautophagy, the main focus of this review, will be hereafter referred as autophagy. Microautophagy differs from macroautophagy in that cytosolic components are directly sequestered by the lysosome through invaginations of the lysosomal membrane. In CMA, a form of autophagy described only in mammals, soluble proteins are delivered to the lysosome by crossing its membrane in a complex with chaperones.

Initially, autophagy was believed to be a non-selective process, meaning that cytosolic components would be randomly surrounded by the autophagosome. Albeit de Duve in 1966 suggested that autophagy could be selective, data supporting this hypothesis were lacking at the time [6]. Later on, under specific conditions, certain macromolecular components were found to be preferentially delivered to the lysosome [7–9]. Several examples of selective degradation have been then revealed, including the specific break down of aggregated proteins [10], the selective removal of superfluous or damaged organelles — like mitochondria (mitophagy) [11], peroxisomes (pexophagy) [12] and endoplasmatic reticulum (ER-phagy) [13] — and the specific degradation of invading bacteria (xenophagy) [14]. Selectivity in cargo targeting to the autophagosome is mediated by autophagy receptors, proteins that simultaneously interact with specific cargoes and with autophagy modifiers conjugated to the autophagosomal membrane, like yeast Atg8 and the mammalian homologues LC3/GABARAP proteins [15,16].

Abbreviations: ATG, autophagy-related genes; CCCP, cyanide m-chlorophenylhydrazone; CMA, chaperone-mediated autophagy; CsA, cyclosporine A; $\Delta\psi_m$, mitochondrial membrane potential; IMM, inner mitochondrial membrane; IMS, intermembrane space; OMM, outer mitochondrial membrane; PAS, phagophore assembly site; PTP, permeability transition pore; ROS, reactive oxygen species; RTG, retrograde signalling pathway

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The term "mitophagy" was introduced by Lemasters in 2005 [17], even if the first descriptions of mitochondria inside lysosomes date from circa 40 years before. Engulfment of mitochondria together with other organelles by lysosomes in rat hepatocytes exposed to glucagon was described in 1962 [18]. Moreover, in 1977, Beaulaton and Lockshin described that during metamorphosis of silkmoth muscles, autophagy targeted almost exclusively mitochondria, the first example of selective mitochondrial autophagy [8]. In the last few years, mitophagy has been intensively studied. Accumulating evidence indicates that mitochondria can be selectively removed by autophagy and the signals that specifically target mitochondria to autophagy have started to be unravelled.

Mitochondria are dynamic organelles that continuously fuse and fragment during cell life, appearing in situ as short round-shaped or elongated organelles, with a major axis that can reach 5 μm [19]. On the other hand, autophagosomes are globular organelles with a diameter of approximately 1 μm [3], posing a sterical problem to mitochondrial engulfment by autophagosomes. Indeed, it has been suggested that mitochondrial fragmentation precedes mitophagy [20–23]. Conversely, when massive autophagy is induced in the cells by nutrient depletion, for instance, mitochondria elongate [24,25]. Elongated mitochondria are spared from autophagy and optimize ATP production in times of starvation [24].

In this review, we provide an overview of the molecular mechanisms of mitophagy, in yeast and mammals, focusing on the relationship between autophagy and mitochondrial dynamics and on the different features of mitochondrial shape during mitophagy and macroautophagy.

2. Mitophagy

Mitochondria are crucial organelles for energy production, regulation of cell signalling and amplification of apoptosis [26–28]. At the same time however, they are the major source of reactive oxygen species (ROS) that may oxidize mitochondrial own lipids, proteins and DNA [29]. Therefore, mechanisms of mitochondrial quality control have evolved to avoid cell damage and to maintain the overall fitness of the cell. Mitophagy has emerged as a key mechanism in this quality control, responsible of the elimination of superfluous or damaged mitochondria [30]. The critical role of autophagy in the maintenance of a "healthy" cohort of mitochondria was shown both in yeast [31] and mammals [23]. Yeast strains carrying deletions in autophagy-related genes (*ATG*) are unable to degrade mitochondria during the stationary phase, display growth defects in non-fermentable carbon sources and accumulate dysfunctional mitochondria. Accordingly, *ATG* mutants show lower oxygen consumption rates, decreased mitochondrial membrane potential and higher ROS levels [31]. Similarly, maximal respiration is reduced in mammalian cells deficient for ATG5 or treated with a pharmacological inhibitor of autophagy [23]. These genetic evidences support an essential role for mitophagy in the maintenance of mitochondrial and therefore cellular health. We will now overview our current knowledge of how mitophagy is triggered in yeast and mammalian cells, highlighting the relationship between this degradation process and the shape of the organelle.

2.1. Mitophagy in yeast

2.1.1. When?

The first studies in *Saccharomyces cerevisiae* provided evidence that in order to be targeted to autophagy, mitochondria need to be dysfunctional [32,33]. Priault and colleagues found increased mitophagy under anaerobic conditions in a *FMC1* null mutant, where the mitochondrial ATPase is dysfunctional. During anaerobiosis, the ATPase by operating in reversal (i.e., by hydrolyzing ATP) maintains mitochondrial membrane potential ($\Delta\psi_m$), and, consequently, mitochondrial ion homeostasis and biogenesis. In *FMC1* null strain during anaerobiosis, $\Delta\psi_m$ collapses since mitochondria cannot use glycolytic ATP to maintain $\Delta\psi_m$. The authors proposed that this defect targets mitochondria to autophagy [33]. The idea that mitochondrial dysfunction leads to the removal of the organelle was further supported by Nowikovsky and colleagues [34]. Shutting-off the expression of *MDM38* leads to loss of mitochondrial K^+/H^+ exchange, osmotic swelling, reduction of $\Delta\psi_m$, mitochondrial fragmentation, and, mitophagy. Even though mitochondrial dysfunction targets the organelle to autophagy, treatment of yeast with the uncoupler carbonyl cyanide m-chlorophenylhydrazone (CCCP), that dissipates $\Delta\psi_m$ is not sufficient to induce mitophagy [35,36], suggesting that an additional yet unidentified factor is required [37].

Induction of non-selective macroautophagy also leads to mitophagy in yeast [36,38,39]. Indeed, mitophagy can be induced by nitrogen starvation or by the Tor kinase inhibitor rapamycin in yeast previously grown in a non-fermentable carbon source that induces mitochondrial proliferation. Nevertheless, macroautophagy and mitophagy appear to be differentially regulated: nitrogen-starvation in the presence of a non-fermentable carbon source induces macroautophagy, but not mitophagy. It should be stressed that under these metabolic conditions mitochondria are essential for energy production [39], highlighting the existence of signalling cascades that can spare mitochondria from autophagic degradation. One interesting possibility, as we will discuss later, is that sterical hindrance of elongated organelle from engulfment by the autophagosomes prevents mitophagy when mitochondria are required for energy production. Along this line, superfluous mitochondria in yeast are also removed by mitophagy: mitochondrial removal is induced during stationary phase in a non-fermentable carbon source [38–40], when energy requirements are reduced.

2.1.2. How?

The search of a specific signal targeting mitochondria to autophagosomes has been intensive and led to the description of the first mitochondrial "eat me" signal in yeast in 2004. Uth1, an outer mitochondrial membrane protein, has been found to be essential for mitophagy induced by rapamycin or nitrogen starvation, without affecting per se the autophagic machinery [35]. Few years later, in a screen for functional interactors of Atg1, the mitochondrial protein Aup1 was identified to be essential for efficient mitophagy during stationary phase. Under these conditions, and in disagreement with the study of Kissova and co-workers, mitophagy played a pro-survival role, since deletion of Aup1 was lethal [38]. It has been recently suggested that Aup1 regulates mitophagy by controlling the retrograde signalling pathway (RTG) [41]. Nevertheless, the function of both Uth1 and Aup1 in mitophagy has been challenged by Kanki and colleagues [42]. In their hands, lack of these proteins did not block mitophagy, possibly as a consequence of the differences in the background of the yeast strains used or in the detection methods.

Recently, in a genomic screen for yeast mutants defective in mitophagy, two other mitochondrial proteins have been identified, named Atg32 and Atg33 [36,40,42]. Atg32 is an outer membrane protein, essential for mitophagy, but not for macroautophagy or other types of selective autophagy. Selective autophagy in yeast requires a receptor and an adaptor protein: Atg32 acts as a receptor protein that interacts with the adaptor protein Atg11, most likely to sequester mitochondria to the phagophore assembly site (PAS) [40,42]. In addition, Atg32 possesses an evolutionary conserved motif (WXXI/L) critical for the interaction with Atg8, an ubiquitin-like protein essential for autophagosomal membranes growth. The interaction between Atg32 and Atg8 is required for mitochondrial recruitment by the phagophore [40]. Atg32has been the first protein described to be required to mitophagy and to interact with the autophagic machinery. Nonetheless, important questions remain open: what is the physiological significance of Atg32-induced mitophagy? In other words, what happens in the absence of Atg32? Surprisingly enough, no differences