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The antiapoptotic OPA1/Parl couple participates in mitochondrial adaptation to heat shock☆

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ABSTRACT

The mitochondria-shaping protein optic atrophy 1 (OPA1) has genetically distinguishable roles in mitochondrial morphology and apoptosis. The latter depends on the presenilin associated rhomboid like (PARL) protease, essential for the accumulation of a soluble intermembrane space form of OPA1 (IMS-OPA1). Here we show that OPA1 and PARL participate in the heat shock response, a stereotypical cellular process of adaptation to thermal stress. Upon heat shock, long forms of OPA1 are lost and mitochondria fragment. However, mitochondrial fusion is dispensable to maintain viability, whereas IMS-OPA1 is required. Upon conditioning—a process of mild heat shock and recovery—IMS-OPA1 accumulates, OPA1 oligomers increase and mitochondria release less cytochrome *c*, ultimately resulting in cellular resistance to subsequent apoptotic inducers. In *Parl*$^{-/-}$ cells accumulation of IMS-OPA1 is blunted and conditioning fails to protect from cytochrome *c* release and apoptosis. Thus, the OPA1/PARL dependent pathway of cristae remodeling is implicated in heat shock. This article is part of a Special Issue entitled: 17th European Bioenergetics Conference (EBEC 2012).

1. Introduction

Mitochondria are versatile and dynamic organelles that play a key role in the regulation of metabolism, cellular signaling and apoptosis, during which they release cytochrome *c* and other cofactors that once in the cytosol contribute to the activation of the effector caspases required to demolish the dying cell [52]. The process of mitochondrial permeabilization is controlled by the Bcl-2 family of oncogenes: the so called BH3-only members (like BID and BIM) transduce private apoptotic signals to the organelle, activating the multidomain proapoptotic proteins of the family (that include BAX and BAK) responsible for the permeabilization of the outer mitochondrial membrane. The anti-apoptotic members like BCL-2 itself regulate this process, preventing at multiple points the activation of the proapoptotic multidomains [48].

Morphological and ultrastructural alterations accompany the recruitment of mitochondria by the cell death pathway, including fragmentation of the network [17,30] and remodeling of the cristae [43,54] in order to allow the complete release of cytochrome *c*. Mitochondrial shapes vary depending on the organism, the cellular type, the metabolic state and the environmental conditions [4]. A growing family of mitochondria-shaping proteins controls the morphology of the organelle. In mammals, Fission 1 (FIS1), mitochondrial fission factor (MFF) and dynamin related protein 1 (DRP1) regulate mitochondrial fission [24,35,44,55]; mitofusins (MFN) 1 and 2 and optic atrophy 1 (OPA1) control the fusion process [8,40,41]. Interestingly, OPA1 has genetically distinguishable functions in mitochondrial fusion and release of cytochrome *c* during apoptosis [18]. The function of OPA1 is tightly controlled at the genetic and post-translational level: OPA1 gene undergoes alternative splicing and the protein is proteolyzed, leading to the generation of several forms with different electrophoretic mobilities. Under normal conditions, in most tissues 2 long and 3 short forms of the protein can be distinguished; both long and short OPA1 are required to maintain mitochondrial fusion [46]. Several proteases have been found to be involved in the generation of the short forms of OPA1, including the matrix AAA protease paraplegin and AFGL3 and the intermembrane space AAA protease YME1 [16,21,23]. Following mitochondrial dysfunction, an additional cleavage by the ATP independent protease OMA1 inactivates the long forms of OPA1 leading to an accumulation of short forms of OPA1 [16] and to segregation of fragmented mitochondria from the network

Abbreviations: BAK, Bcl-2 associated killer; BAX, Bcl-2 associated protein X; BCL-2, B-cell lymphoma 2; DRP1, dynamin related protein 1; FCCP, cyanide m-fluorophenylhydrazone; FIS1, fission 1; HSP, heat shock protein; IMM, inner mitochondrial membrane; IMS, intermembrane space; MEFs, mouse embryonic fibroblasts; OMM, outer mitochondrial membrane; OPA1, optic atrophy 1; MFN, mitofusin; PARL, presenilin associated rhomboid like

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[14]. In addition, the short forms of OPA1 constitutively produced by the AAA proteases seem also to be the substrate of a mitochondrial rhomboid protease called presenilin associated rhomboid like (PARL). PARL was originally discovered in a yeast two hybrid screening for presenilin interactors. It then turned out to be a mitochondrial enzyme that in yeast (where it is christened Pcp1p) and in *Drosophila melanogaster* cleaves the orthologs of OPA1 [31,32]. Considerable confusion has emerged on the role of PARL, based on our early report that it is required for the accumulation of a soluble form of the OPA1, essential for apoptosis but not for mitochondrial fusion [9]. This report ingenerated the idea that the generation of the short forms of OPA1 depended on PARL (see for example the introduction in [15,29]). Conversely, we ourselves introduced the possibility that in analogy with other intramembrane proteolytic cascades such as that of Notch [53], PARL acts downstream of other protease(s) [9]; despite our words of caution, the dependence of the accumulation of the soluble form of OPA1 on PARL has been equaled to a broader role for the protease in the constitutive generation of the short forms of OPA1. In conclusion, our current understanding of OPA1 cleavage is certainly increasing, yet several areas remain obscure: for example, it is still largely unknown how the activity of the different proteases is controlled; whether they operate in parallel or in series (with the remarkable exception of Parl that seems to operate only on the lower MW forms of OPA1); which are the domains implicated in substrate recognition by the proteases, as well as their exact cleavage site in OPA1. Altogether, these black boxes bamboozle our interpretation of how these proteases participate in the regulation of mitochondrial morphology and apoptosis. In particular, in the case of Parl it is unclear if the proposed role in apoptosis mediated by OPA1 can be extended to stimuli other than drugs activating the intrinsic pathway of cell death; and whether it participates in cellular adaptation.

When cells are exposed to stressful stimuli that results in the inhibition of protein synthesis, such as mRNA translation inhibitors and UV irradiation, mitochondria undergo hyperfusion [50]. During starvation a similar process of mitochondrial elongation occurs, and it is mirrored at the ultrastructural level by an increase in the surface of the cristae where the ATP synthase oligomerizes to maximize its efficiency; and depends on a signaling cascade triggered by a rise in cyclic AMP levels and impinging on Drp1 to cause unopposed mitochondrial fusion [20]. Exposure to stressful conditions results in different responses at the cellular level that stereotypically follow a scheme where the attempt of compensation precedes suicide of the stressed cell. This paradigm is best evident in the case of ER stress, when the activation of the unfolded protein response first tries to compensate the organellar damage, then signals the activation of the apoptotic pathway. Mitochondrial elongation during starvation (and other stressful conditions) seems to be another example of this paradigm of compensation/suicide, where changes in the shape of the organelle regulate the continuum between survival and death of the cell.

In addition to nutrient availability, cells can respond efficiently also to changes in temperature by activating the so called heat shock response. In response to thermal stress, cells synthesize and accumulate heat shock proteins (HSPs), chaperones or proteases that protect the cell in response to potentially damaging conditions [38]. Of note, chaperones are key in the mitochondrial import of proteins, highlighting a cross-talk between thermal adaptation and the organelle [45]. In addition, if the temperature surpasses a duration or intensity threshold, the cell is committed to death, via activation of the mitochondrial pathway of apoptosis [38]. HSP 27 and 70, two main players in the heat shock response, block apoptosis also at a post-mitochondrial level, by interacting with cytochrome *c* and with the other released cofactor AIF in the cytosol [5,39].

One of the most remarkable aspects of the heat shock response is that upon transient exposure of cells to mild heat stress, it renders cells resistant to further cellular insults. This might be linked to the ability of overexpressed HSPs to block apoptosis. HSP27 and HSP70, two main heat shock response proteins, prevent apoptosis at a post-mitochondrial level, by interacting with cytochrome *c* and AIF cofactor released in the cytosol [5,38]. HSPs might prevent apoptosis not only by interfering with the activation of the apoptosome, but also by interfering with key molecules of the extrinsic pathway such as DAXX and JNK [38]. Whether HSPs can also interfere with mitochondrial permeabilization and release of apoptotic cofactors is still a matter of debate; in particular, whether mitochondrial changes induced by heat stress directly participate in the acquisition of cellular resistance against apoptotic stimuli remains unclear [3].

Here we analyzed whether OPA1 participates in the heat shock response. We show that upon heat shock the antiapoptotic soluble form of OPA1 accumulates in mitochondria and that this accumulation favors heat stress-induced tolerance against subsequent apoptotic cell death. A genetic analysis proved that the rhomboid protease PARL participates in cellular adaptation to heat shock by impinging on soluble OPA1. Thus, our data suggest a role for OPA1 in heat stress-induced cytoprotection against apoptotic stimuli.

2. Methods

2.1. Cell culture

Mouse embryonic fibroblasts (MEFs) of the indicated genotype were cultured at 37 °C in 5% CO_2 as previously described [20]. For heat shock treatment, cells were brought to a confluence of around 85% at 37 °C and then cultures in a 5% CO_2 incubator at 42 °C (for mild heat stress) or at 45 °C (for severe heat stress) for the time indicated. For conditioning, cells were exposed to mild heat shock followed by recovery at 37 °C.

2.2. Mitochondrial morphology analysis

8×10^4 MEFs were seeded in plastic dishes containing a glass coverslip. After 24 h cells were transfected with mitochondrial yellow fluorescent protein (mtYFP) for imaging of live cells or immunostained with a FITC-conjugated anti TOM20 antibody. Heat shock treatment of cells started 20 h post-transfection. Images of mtYFP or FITC were acquired using a Leica TCS SP5 inverted confocal microscope equipped with a 63×, 1.3 NA PLANApo objective (Leica) using the appropriate excitation lasers and emission filters, as previously described [20]. Morphometric analysis was performed as previously described [8].

2.3. Cell viability assays

1.2×10^5 MEF cells were seeded in 12 well plastic plates, after 24 h cells were exposed to heat shock treatment for the indicated time points (42 °C for mild heat shock; 45 °C for severe heat shock) and cell viability was determined by flow cytometry. For co-transfection experiments, 7×10^4 MEFs were seeded on plastic 12-well plates, after 18 h of growth cells were co-transfected with Transfectin (Biorad) and 24 h post transfection cell viability was determined by flow cytometry. Cells were harvested and stained with propidium iodide (PI) and Annexin-V-FITC (Bender) according to manufacturer's protocol. Flow cytometry analyses were performed using a FACS Calibur cytometer (Becton-Dickinson). Viability was measured as the percentage Annexin-V, PI negative cells.

2.4. Mitochondrial biochemistry

Mitochondria were isolated from MEFs by differential centrifugation as described previously [19]. For sub-fractionation of mitochondria, isolated mitochondria were hypotonically swollen in 10 mM KPi (pH 7.4), centrifuged for 10 min at 12,000×*g* and the pellet