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Fuse or die

Shaping mitochondrial fate during starvation

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Mitochondria continuously change their shape and thereby influence different cellular processes like cell death or development. Recently, we showed that during starvation mitochondria fuse into a highly connected network. The change in mitochondrial shape was dependent on inactivation of the fission protein Drp1, through targeting of two different phosphorylation sites. This rapid inhibition of mitochondrial fission led to unopposed fusion, protecting mitochondria from starvation-induced degradation and enabling the cell to survive nutrient scarce conditions.

Key words: autophagy, starvation, mitochondria, fusion, fission, Drp1, PKA, Mfn1, Mfn2

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Mitochondria mediate a variety of different cellular functions like cell death, cell cycle regulation and cell migration.[1-5] Historically most of these roles were linked to mitochondrial energy production but the recognition of mitochondria's structural dynamism further extended the view on the regulatory potential of mitochondria.

Mitochondrial shape is determined through fission and fusion events leading to heterogeneous morphologies ranging from small, roundish individual elements to highly connected networks. Mitochondrial dynamics are tightly linked to cellular health status, in which mitochondria fragment when a cell undergoes programmed cell death.[1,2] Although it is relatively well understood how mitochondrial morphology changes during programmed cell death, the effect of milder stressors that cells can survive was unclear when we initiated our study.

Cells continuously adapt to changes in nutrient availability and can even survive complete starvation for several days. During those times cells become dependent on autophagy, a catabolic pathway used to recycle nutrients.[6] During starvation, autophagy is formally regarded 'non-selective', degrading cytoplasmic material en masse, even including mitochondria.[7] However, when we investigated mitochondrial fate during starvation, mitochondrial mass did not decrease as anticipated.[8,9] Instead, mitochondria became tubulated and formed extensive mitochondrial networks that were already visible 30 min after the onset of starvation.[8]

The rapid formation of mitochondrial networks led us to investigate how mitochondria reach that stage. First, we analyzed whether starvation-induced mitochondrial tubulation was dependent on the autophagosomal machinery. Mitochondria fused in the absence of ATG5 [10] (an essential component for autophagosomal biogenesis), suggesting that autophagy per se was not essential for mitochondrial elongation during starvation. We next focused on mitochondrial fusion proteins as potential mediators of starvation-induced fusion. Indeed, deficiencies in the fusion proteins Mitofusin (Mfn) 1 and 2 and the optic atrophic protein1 (Opa1) blocked mitochondrial network formation. This clearly established that mitochondrial inner- and outer-membrane fusion proteins are essential for starvation-induced network formation. Biochemical analysis of these fusion proteins, however, did not yield any obvious modifications explaining increased mitochondrial fusion.

Mitochondrial tubulation also can be mediated through inhibition of

mitochondrial fission.[11] We therefore analyzed the mitochondrial fission protein Drp1. We determined that two phosphorylation sites, Drp1^{S637} and Drp1^{S616}, regulate mitochondrial tubulation during starvation. While activation of Drp1 fission activity with a phospho-mimetic Drp1^{S616} mutation had relatively minor effects on mitochondrial fusion, manipulation of the Drp1^{S637} phosphorylation site strongly inhibited network formation during starvation. Furthermore, pharmacological results suggest that the Drp1^{S637} site is phosphorylated by PKA during starvation (unpublished results), leading to the cytosolic sequestration of Drp1,[8] as reported before in reference 11–13. While Drp1^{S637} is targeted by PKA, Drp1^{S616} can be regulated through the cell cycle dependent kinase Cdk1/CyclinB.[14] The time-restricted activity of Cdk1/CyclinB could explain why the PKA-regulated phosphosite affects fission more dramatically than the Drp1^{S616}-site. Dual activation of Drp1-activity (at the PKA and Cdk1/CyclinB site) blocked mitochondrial elongation most efficiently, suggesting that mitochondrial dynamics might be more sensitive to starvation during some cell cycle stages than others.

We determined that starvation-induced mitochondrial tubulation protects mitochondria from autophagosomal degradation. Previously, our group demonstrated that mitochondria serve as a membrane source for autophagosomes during starvation.[9] Fusion-dependent protection of mitochondria from autophagy, therefore, likely enables mitochondria to form autophagosomes leading to the replenishment of needed precursors. Is mitochondrial preservation for autophagosomal biogenesis the only reason why mitochondria tubulate during starvation? Would it also be beneficial for cells to maintain their most efficient means of producing ATP, the mitochondria, when nutrients are limited? Data from our lab (unpublished results) and evidence from Scorrano and colleagues[15] indicate that mitochondrial ATP production capacity is indeed enhanced during starvation. Mitochondrial networks, but not fragmented mitochondria, sustained ATP production during nutrient starvation. Additionally, mitochondrial fusion-capacity was essential for cellular survival: mitochondria deficient in fusion proteins Mfn1/2 or Opa1 died significantly faster during starvation compared with fusion-competent cells (unpublished data).

Mitochondrial fusion and increase in metabolism has been observed before in other scenarios,[5,16] suggesting that coupling of these processes is more widely applicable. But how does fusion induce changes in mitochondrial activity? Starvation tightly regulates the function of Drp1, but could this fission protein regulate mitochondrial metabolism? Some lines of evidence support a link between Drp1 inhibition and increased mitochondrial ATP output, while other results contradict this hypothesis.[5,17,18] Opa1 and Mfns might be capable of coupling mitochondrial function and morphology. Opa1, an inner membrane fusion protein, is a particularly promising candidate as it regulates mitochondrial inner membrane fusion and cristae structure.[19] The physical interaction of mitochondrial outer membrane proteins with Opa1 could couple cytosolic signals with changes in the mitochondrial interior. Whether Drp1, Opa1 or other mitochondrial fission/fusion proteins shape mitochondrial metabolism during starvation, and how their activity is integrated into starvation-induced signaling pathways remains to be established.

It is clear that we are only beginning to understand how mitochondrial dynamics are regulated during different cellular conditions. Our recent study emphasizes the role of mitochondrial dynamics under nutrient deprivation, in which mitochondrial fusion prevents mitochondria turnover by autophagy and enhances cell survival by providing energy and membranes for autophagosome biogenesis. Future research will determine if other organelles are also affected in their shape or activity during autophagy. The fact that the proteins controlling mitochondrial shape also affect the ER and peroxisomes indicates that mitochondrial morphology could be influential in many additional cell processes as well. We can thus expect many new discoveries as our understanding of the relationship between mitochondrial dynamics and cell physiology continues to grow.

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