Abstract

Mitochondria, often described for their ATP production via the OYPHOS system, additionally generate iron-sulfur-clusters, essential for protein stability. They are dynamic organelles constantly undergoing fusion and fission, forming an interconnected network, dynamically reshaped due to environmental conditions. Several quality control mechanisms regulate mitochondrial proteostasis, biogenesis, dynamics and turnover, supporting their function. Additionally, mitochondria play a crucial role in cellular redox signalling. Fusion of the outer mitochondrial membrane is controlled by dynamin-related GTPases (DRPs), called mitofusins, highly conserved among several species. Moreover, post-translational modifications regulate the fusion capacity of mitofusins. The yeast mitofusin Fzo1 is ubiquitylated at K398. Crystal structures of the homologue bacterial dynamin like protein (BDLP) suggested stretched and bent conformations of mitofusin. Based on this the mammalian mitofusin MFN1 and MFN2 were recently crystallized allowing detailed analysis of the mitofusin protein structure. Nevertheless, the precise role and mechanism how Fzo1 complexes are stabilized within conformational changes remains elusive. As dynamcis are essential for mitochondrial maintenance they also play a major role in oxidative signaling and quality control. However, the regulation of mitochondrial morphology and dynamic proteins under oxidative stress conditions remain elusive.

The aim of this study was to examine how the redox state affects Fzo1, in either promoting or inhibiting the fusion process. Therefore, first, cysteine residues, often described for their role in protein interactions, were analyzed. Their function on the cascade of events enabling Fzo1 to merge between two outer mitochondrial membranes was elucidated. Second, we investigated how oxidative stress, known to inhibit fusion, regulates Fzo1. Novel players inducing or repressing Fzo1 and its fusogenic properties were identified.

We could show that two cysteine residues, namely C381 and C805, are critical for mitochondrial fusion. C381 is required for the formation of the *trans*-tethering complex, before GTP hydrolysis. C805 allows stabilizing ubiquitylated forms of Fzo1 molecules within higher oligomeric complexes, prior to fusion. Fzo1^{C805S} variants are degraded by the proteasome, preventig fusion. Strickingly, in absence of the E4 ligase Ufd2, Fzo1 levels and mitochondrial fusion are rescued. This reveals for the first time a role of Ufd2 on mitochondria. Furthermore, we showed that oxidative

stress regulates mitochondrial morphology and Fzo1 in a p-Ub-dependent manner. We identified a novel E3 ligase, Mar5, MARCH5 in mammals, acting on Fzo1 upon oxidative stress. Moreover, the fission adaptor protein Mdv1 affected Fzo1 under oxidative stress. This suggests a novel mechanism regulating the interplay of fusion and fission dynamics under oxidative stress conditions.

The results of this study identified the importance of cysteine residues within the fusion cascade of mitochondria. Both cysteine residues mediating fusion are conserved in higher eucaryotic systems. Our analyses shed new light on how the interplay between conformational changes and ubiquitylation allow the membrane-embedded version of dynamin-related proteins to promote fusion events. Furthermore, we identified novel quality ubiquitin ligases acting on mitochondria. Finally, an unexpected connection between regulation of fusion and fission was identified, opening up a magnitude of potential new research directions.

All in all, these results lead to a major understanding of mitochondrial dynamics and give new insights that help to understand defects in mitochondrial morphologies and dysfunctions, in constitutive conditions and upon oxidative stress. Our findings could provide knowledge about how defects in mitochondrial dynamic proteins affect the onset of neurodegenerative diseases.