

# Regulation of Mitochondrial Outer Membrane Fusion by the Mitofusin Fzo1

## Abstract

Mitochondria are essential organelles in all eukaryotic cells forming a tubular and dynamic network undergoing constant fusion and fission events. Both processes depend on dynamin-related proteins (DRPs), a special class of GTPases, which provide the mechanical forces necessary for membrane remodeling. Mitofusins, (called Fzo1 in yeast) are the DRPs mediating fusion of mitochondrial outer membranes (OM). In yeast, OM fusion depends on two additional proteins, the integral OM protein Ugo1 and the cytosolic F-Box protein Mdm30, which mediates the turnover of Fzo1.

To understand the mechanisms underlying Fzo1 mediated OM fusion, the role of Fzo1 ubiquitylation, oligomerization and GTPase activity and the function of Fzo1 interacting partners was analyzed. The obtained results allowed to propose a first comprehensive model of the OM fusion process. First, Fzo1 homo-dimerizes in the mitochondrial OM depending on GTP binding and its interaction partner Ugo1. This dimerization protects Fzo1 against Mdm30-independent degradation. Next, Fzo1 dimers interact in *trans* with Fzo1 molecules in the opposing membrane, leading to mitochondrial tethering. GTP hydrolysis by Fzo1 presumably induces a conformational change, allowing Mdm30-dependent ubiquitylation of Fzo1. Finally, Fzo1 is degraded allowing completion of fusion, suggesting a non-cycling mechanism for mitofusin-mediated fusion. Ubiquitylation at a distinct and conserved lysine is essential for Fzo1 function in this process. The deubiquitylating enzymes (DUBs) Ubp2 and Ubp12 were identified as novel regulators of Fzo1 ubiquitylation. Each DUB recognizes distinct ubiquitylated forms of Fzo1. Ubp2 rescues Fzo1 from proteasomal degradation, thereby supporting mitochondrial fusion. Ubp12 limits the rate of fusion by cleaving pro-fusion and Mdm30-dependent ubiquitylated forms of Fzo1. Moreover, distinct ubiquitin ligases target Fzo1 to different pathways, supporting either mitochondrial fusion or proteasomal degradation. The present study shows that Fzo1 mediated fusion can be dissected into distinct steps and that Fzo1 ubiquitylation is highly regulated by a complex machinery.

