endo- and exonuclease pair? Is there an entirely different apparatus to identify aberrant 50S subunits? Or are they spared quality control? One can argue that aberrant 30S subunits are the more dangerous for the cell, since they could potentially sequester scarce initiation factors or messenger RNAs (mRNAs).

It is interesting to compare the observations of Jacob et al. (2013) with the handling of nonfunctional ribosomes by eukaryotic cells, studied mostly in yeast (reviewed in Lafontaine, 2010). Defective small (40S) and large (60S) ribosomal subunits are removed by quite distinct mechanisms. Yeast 40S subunits made inactive by a single-nucleotide mutation of 18S rRNA are subject to degradation only if they are allowed to translate. Intriguingly, key factors in such degradation are Ski7 and Dom34, the same as those involved in resolving "No-Go" translation, in which a translating ribosome has reached an impasse on the mRNA (Cole et al., 2009). Thus, the decay of such 40S subunits may be the direct result of inactive translation. However, the turnover is slow compared to the rate of translation. Perhaps a nonfunctioning 40S subunit is given many opportunities to prove its mettle.

By contrast, for an inactive yeast 60S subunit, degradation is initiated not by a nuclease, but by an E3 ubiquitin ligase, subsequent polyubiquitination of many ribosomal proteins, and, finally, proteasomal degradation (Fujii et al., 2012). Proteasomal activity is essential for the degradation of inactive 25S rRNA, although the mechanistic details are yet to be worked out. In neither case does the experimental system allow a direct test of whether it is the inactive subunit alone or an 80S couple that is degraded.

Cells depend on ribonucleoprotein (RNP) complexes for translation, splicing, RNA modification, and so on. RNPs defective due to improper RNA processing, or to mutant or deficient proteins, must often be deleterious to the cell. Thus, it seems inevitable that there are many ways in which aberrant RNPs can be detected and degraded. Quality control of RNPs is a fertile area of research for both prokaryotes and eukaryotes, its surface only scratched by the papers referred to above.

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Activation and Degradation of Mitofusins: Two Pathways Regulate Mitochondrial Fusion by Reversible Ubiquitylation

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Mitofusins are large GTPases essential for mitochondrial fusion. In this issue, Anton et al. (2013) report that two independent pathways of ubiquitylation/deubiquitylation control activation and degradation of mitofusins, revealing a sophisticated mechanism of regulating mitochondrial dynamics.

According to the endosymbiont hypothesis, mitochondria are derived from a prokaryotic cell that was taken up by a eukaryotic host cell. For a long time, it has been assumed that mitochondria function as semiautonomous organelles whose main function is to provide ATP for the rest of the cell. Studies in recent years revealed, however, that mitochondria are deeply embedded in the signaling network of eukaryotic cells. Mitochondria are not only crucial for cellular energy

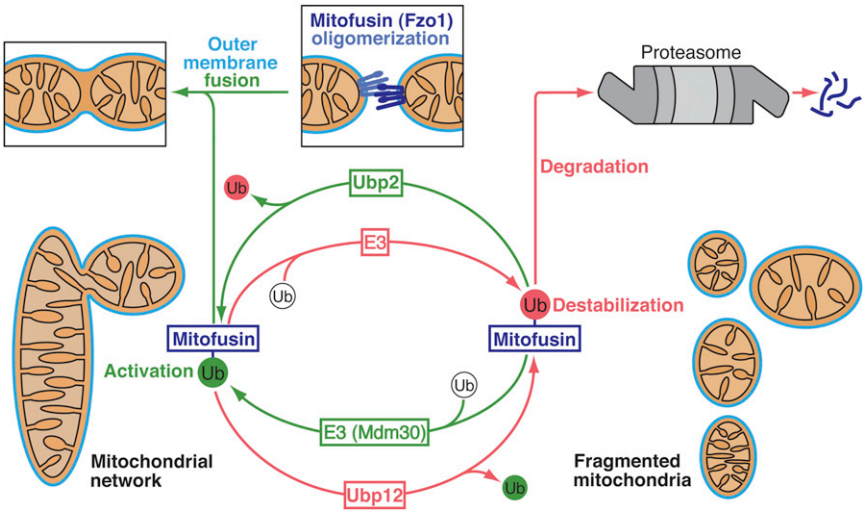

Figure 1. Regulation of Mitochondrial Fusion by Activation and Degradation of Mitofusins
Mitochondria form a dynamic network, which is regulated by fusion and fission. Outer membrane fusion is mediated by mitofusin, which is regulated by ubiquitylation. Conjugation with ubiquitin (Ub) either activates outer membrane fusion or targets mitofusin for proteasomal degradation. An E3 ubiquitin ligase containing the F-box protein Mdm30 activates mitofusin by attaching stabilizing ubiquitin chains. The deubiquitylase Ubp12 removes these activating ubiquitin chains and impairs outer membrane fusion, thus promoting the fragmentation of mitochondria. In contrast, a different E3 ligase attaches destabilizing ubiquitin chains to mitofusin. The deubiquitylase Ubp2 removes the destabilizing ubiquitin chains and thereby supports outer membrane fusion.

conversion and many metabolic pathways, they also play important functions in programmed cell death (apoptosis), developmental processes, and aging (Westermann, 2010; Cleland et al., 2011; Nunnari and Suomalainen, 2012). Disorders of mitochondrial function lead to a large variety of diseases that can manifest in many organs, particularly in neuronal cells that have a high energy demand. Mitochondria are not static organelles but typically form a dynamic network that is constantly remodeled by fusion and fission of the organelles (Westermann, 2010). Fusion promotes mixing of mitochondria, protects against loss of mitochondrial DNA, and supports an optimal bioenergetic activity. Fission promotes the distribution and inheritance of mitochondria and helps in the selective removal of damaged mitochondria (Escobar-Henriques and Anton, 2013).

A series of genetic and cell biological studies identified the machineries that mediate fusion and fission of the mitochondrial membranes. Two large GTPases related to dynamin, the outer membrane protein mitofusin (Fzo1) and the inner membrane protein OPA1 (Mgm1), mediate the fusion of outer and inner membranes, respectively (Westermann, 2010; Nunnari and Suomalainen, 2012; Escobar-Henriques and Anton, 2013). A further protein, Ugo1, interacts with both mitofusin and Mgm1 in yeast and may help to coordinate outer and inner membrane fusion processes. Here we focus on the function of mitofusin that is crucial for tethering of two mitochondria and the subsequent fusion of their outer membranes (Figure 1). Mitofusins are anchored in the outer membrane by two transmembrane segments and expose GTPase and coiled-coil domains to the cytosol. Mitofusins of one mitochondrion form dimers that oligomerize with mitofusin dimers of an adjacent mitochondrion, thus connecting both mitochondria and promoting outer membrane fusion. Studies in the past years showed that mitofusins are modified by ubiquitylation (Tanaka et al., 2010; Westermann, 2010; Leboucher et al., 2012; Escobar-Henriques and Anton, 2013). Typically, ubiquitin chains mark proteins for degradation, yet can also exert regulatory effects. E3 ligases are responsible for the attachment of ubiquitin to specific substrates. It has been shown that different E3 ligases, like Parkin and Huwe1 in mammals and SCF^{Mdm30} in yeast, are involved in ubiquitylation of mitofusins. The F-box protein Mdm30 is thought to function as a specificity-determining factor in the Skp1-Cullin-F-Box (SCF) ubiquitin ligase SCF^{Mdm30}. However, different views on the role of mitofusin ubiquitylation were discussed, ranging from destabilization to activation of mitofusins. In this issue of *Molecular Cell*, the groups of Escobar-Henriques and Langer report an elegant study that resolves the seemingly controversial effects of mitofusin ubiquitylation (Anton et al., 2013). They show that two independent pathways reversibly ubiquitinate the mitofusin of yeast mitochondria, termed Fzo1 (fuzzy onion). In one pathway ubiquitylation leads to an activation of mitofusin for fusion, whereas the other pathway marks mitofusin for degradation by the proteasome (Figure 1).

Anton et al. (2013) found that different lysine residues of mitofusin/Fzo1 are specifically modified with ubiquitin by two different E3 ligases, one of them being the SCF^{Mdm30} ligase, while the second E3 ligase has not been identified so far. (1) SCF^{Mdm30} functions in the activating pathway. Ubiquitylation of mitofusin occurs in a coordinated process that involves oligomerization of mitofusin and GTP hydrolysis (Amiott et al., 2009; Escobar-Henriques and Anton, 2013). Thereby mitofusin is activated for fusion and protected from proteasomal degradation. The coordinated oligomerization and ubiquitylation of mitofusin may sterically limit the further elongation of the ubiquitin chain and thus prevent access of the proteasomal degradation system (Anton et al., 2013). (2) The second (unknown) E3 ligase attaches ubiquitin chains to a different lysine residue of mitofusin, leading to destabilization of mitofusin by proteasomal degradation.

Anton et al. (2013) show that both mitofusin ubiquitylation pathways are reversible. They identified the specific deubiquitylases for each pathway (Figure 1). (1) The deubiquitylase Ubp12 acts at oligomers of mitofusin. Ubp12 selectively removes the activating ubiquitin chains, which were attached by the SCF^{Mdm30} ligase. These ubiquitin chains stabilize mitofusin and promote fusion. Thus, in cells lacking Ubp12, active mitofusin accumulates and an increased fusion of mitochondria is observed.

(2) The deubiquitylase Ubp2 removes destabilizing ubiquitin chains. These ubiquitin chains were attached to mitofusin independently of the SCF^{Mdm30} ligase. In cells lacking Ubp2, the levels of active mitofusin are reduced due to an increased degradation by the proteasome and mitochondrial fusion is decreased. Thus, Ubp2 protects mitofusin from proteasomal degradation. Selective ubiquitylation and deubiquitylation of mitofusins by two antagonistic pathways represents a new intricate mechanism for regulation of mitochondrial fusion. Anton et al. (2013) thus resolve the previous controversial views on the role of mitofusin ubiquitylation by revealing the function of different ubiquitylation sites. Ubiquitylation of mitofusin oligomers by SCF^{Mdm30} is critical for fusion of mitochondria. In contrast, ubiquitylation that leads to proteasomal turnover of mitofusin results in mitochondrial fragmentation.

Future studies will aim at the identification of the second E3 ligase that attaches the destabilizing Ubp2-sensitive ubiquitin chains. An exciting area will be the mechanistic and functional elucidation of further posttranslational modifications of mitofusins and their integration into regulatory networks in health and disease. Leboucher et al. (2012) reported that mammalian mitofusin 2 is phosphorylated in response to genotoxic stress, leading to the recruitment of the E3 ligase Huwe1 and proteasomal degradation of mitofusin 2. In contrast, oxidative stress was found to enhance mitofusin oligomerization through generation of disulfide bonds and thus promotes mitochondrial fusion (Shutt et al., 2012). Bcl-2 family members interact with mitofusins and exert regulatory effects on mitochondrial fusion upon induction of apoptosis, but also in healthy cells (Cleland et al., 2011). The E3 ligase Parkin, which is often mutated in Parkinson's disease, ubiquitinates mitofusins in depolarized mitochondria. Subsequent proteasomal degradation of mitofusins prevents fusion of damaged mitochondria and supports mitophagy (Tanaka et al., 2010). Though further work will be required to define the physiological and pathophysiological functions of mitofusins, the study by Anton et al. (2013) provides an important step forward in unraveling the molecular mechanisms that regulate mitochondrial dynamics. Recent studies revealed that the protein import machinery of the mitochondrial outer membrane is regulated via phosphorylation by several cytosolic kinases (Schmidt et al., 2011). It will be interesting to see if ubiquitylation and further posttranslational modifications will also play a role in regulating the protein import machineries of mitochondria.

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