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1 **Full Title: The regulation of mitochondrial homeostasis by the**
2 **Ubiquitin Proteasome System**

3

4 **Short Title: Mitochondria and UPS**

5

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14

1 ABSTRACT

2 From mitochondrial quality control pathways to the regulation of specific functions, the Ubiquitin
3 Proteasome System (UPS) could be compared to a Swiss knife without which mitochondria could not
4 maintain its integrity in the cell. Here, we review the mechanisms that the UPS employs to regulate
5 mitochondrial function and efficiency. For this purpose, we depict how Ubiquitin and the Proteasome
6 participate in diverse quality control pathways that safeguard entry into the mitochondrial
7 compartment. A focus is then achieved on the UPS-mediated control of the yeast mitofusin Fzo1
8 which provides insights into the complex regulation of this particular protein in mitochondrial fusion.
9 We ultimately dissect the mechanisms by which the UPS controls the degradation of mitochondria by
10 autophagy in both mammalian and yeast systems. This organization should offer a useful overview of
11 this abundant but fascinating literature on the crosstalks between mitochondria and the UPS.

12

1 INTRODUCTION

2 Mitochondria constitute an extended tubular network which is maintained by an equilibrium
3 between ongoing fusion and fission events of their outer and inner membranes [1]. These dynamics
4 are crucial for life since they allow mitochondrial morphology adapting to the energy needs of the cell
5 [2] as well as the maintenance of the redox potential, which is the main role of mitochondria [3]. The
6 mitochondrial network indeed provides the cell with ATP, a form of energy currency generated by
7 oxidative phosphorylation through complexes of the electron transport chain and the ATP synthase
8 lodged in the cristae of the mitochondrial inner membrane [4]. In addition to this oxidative
9 phosphorylation process and a myriad of biochemical pathways [5], mitochondria are involved in
10 ageing and developmental processes [6, 7]. Mitochondria are also semi-autonomous organelles as
11 they harbor their own DNA. However, mitochondria rely on nuclear DNA as the 13 mtDNA encoded
12 polypeptides are restricted to subunits of the oxidative phosphorylation system [8]. Nuclear encoded
13 mitochondrial proteins are synthesized as precursors on cytosolic ribosomes before entering
14 mitochondria through the TOM (Translocase of Outer Membrane) and TIM (Translocase of inner
15 membrane) complexes [9]. Consequently, the quality of these imported proteins is tightly regulated in
16 the cytosol to avoid any mitochondrial dysfunction.

17 The first line of defense to protect mitochondria in all eukaryotes are molecular chaperones,
18 such as HSP70 and HSP90, which usher import-competent unfolded proteins and protect them from
19 non-specific interactions [10-13]. Once they have reached their destination, proteins targeted to
20 mitochondria enter via TOM and TIM, the two main channels embedded in both mitochondrial
21 membranes [14]. Chaperones are also present inside mitochondria to ensure proper folding before and
22 after import or acute stress [15-18].

23 The second line of defense heavily relies on several mitochondrial proteases to sustain
24 mitochondrial homeostasis and prevent accumulation of damaged proteins that could lead to
25 mitochondrial dysfunction [19]. Whether in yeast or humans, all mitochondrial compartments have
26 their specific proteases with the exception of outer membranes for which protein degradation relies
27 almost exclusively on the cytosolic Ubiquitin Proteasome System (UPS) [20].

1 When all of these coping and defense mechanisms fail to preserve mitochondria and cellular
2 homeostasis is compromised, damaged mitochondria need to be eliminated. Some damaged
3 mitochondrial components can be eliminated by mitochondria-derived vesicles or MDVs in mammals
4 [21]. Nonetheless, a different death sentence awaits whole mitochondria in all kingdoms of life.
5 Impaired mitochondria are first “rejected” and separated from the rest of the mitochondrial network
6 by mitochondrial fission events [22]. These dissociated mitochondria will in turn be cleared by
7 mitophagy, a mechanism of autophagy dedicated to mitochondria that has strong links with the UPS
8 in metazoans but also in yeast.

9 The objective of this chapter will thus consist in reviewing the mechanisms that the UPS
10 employs to regulate mitochondrial function and efficiency. For this purpose, the review will be
11 organized in three main sections: In the first part, we will depict how Ubiquitin and the Proteasome
12 participate in diverse quality control pathways that safeguard entry into the mitochondrial
13 compartment in both vegetative and stress conditions. In the second part, we will focus on the
14 regulation of the yeast mitofusin Fzo1 by the UPS which will provide a clear example of the
15 complexity that underlies the function of this particular protein in mitochondrial fusion and its
16 intimate crosstalk with Ubiquitin. In the third and final section, we will dissect the mechanisms by
17 which the UPS controls mitophagy in both mammalian and yeast systems. This organization should *in*
18 *fine* offer a useful overview of these processes and will provide some clarification on this abundant
19 but altogether fascinating literature.

20

21 **THE UBIQUITIN PROTEASOME SYSTEM AND MITOCHONDRIAL QUALITY** 22 **CONTROL**

23 Ubiquitin is a small polypeptide of 76 amino acids that covalently links to other polypeptides
24 or to itself to form chains that can get disassembled, like “Lego” pieces. This process of ubiquitination
25 involves a multistep enzymatic cascade [23] where, at least, 3 distinct enzymes participate in
26 attaching one or more ubiquitin subunits to lysine residues of a target protein [24, 25]. The three types
27 of enzymes driving this reaction include a single ubiquitin activating enzyme E1, several ubiquitin-

1 conjugating enzymes E2s and even more ubiquitin ligase E3s which contribute to the broad substrate
2 specificity of ubiquitination (Figure 1). Once ubiquitin gets activated by the E1, it is next transferred
3 to the cysteine residue of an E2 through a thioester bond [26]. The E3 then facilitates the transfer of
4 the ubiquitin from the E2 to the lysine residue of the target substrate through an isopeptide linkage
5 (Figure 1). This transfer occurs directly from the E2 to the substrate with RING (Really Interesting
6 New Gene)-domain E3s. With HECT (Homologous to the E6-AP Carboxyl Terminus) or RBR (Ring
7 Between Ring)-domain E3s, ubiquitin is first conjugated to the catalytic cysteine of the E3 through a
8 thio-ester linkage before conjugation to the lysine of the target substrate [27]. Importantly, Ubiquitin
9 contains 7 lysine residues (K6, 11, 27, 29, 33, 48, 63) and an amino terminus which allows the
10 formation of polymeric ubiquitin chains [28]. A myriad of combinations of ubiquitin linkages are thus
11 possible, many of which leading to distinct outcomes [29, 30]. For instance, K63-linked poly-
12 ubiquitin chains can regulate the subcellular localization of target molecules, their affinity to partner
13 proteins and/or their activity [29, 31, 32]. In contrast, K48-linked ubiquitination leads to the
14 degradation of target substrates by the 26S proteasome, a multi-subunit enzyme complex that breaks
15 down peptide bonds in its proteolytic core [33]. Thus, the Ubiquitin proteasome system (UPS) is a
16 primary cytosolic “predator” of misfolded and damaged proteins.

17 The most thoroughly studied and best understood protein quality control by the UPS takes
18 place at the ER. In this ER Associated Degradation (ERAD) pathway found in both yeast and
19 mammalian systems, damaged proteins are retro-translocated from the ER and ubiquitinated thereby
20 allowing their extraction from the membrane and their ultimate degradation by the cytosolic
21 proteasome [34]. Mitochondrial Associated Degradation (MAD) is a similar quality control system
22 that takes place at the mitochondrial outer membrane [35] and its main actors are most often
23 conserved throughout evolution. MAD is thought to employ the same machinery as ERAD to extract
24 proteins from the outer membrane and trigger their degradation by the UPS [36]. This machinery
25 relies on the essential AAA-ATPase Cdc48, one of the most abundant cellular proteins, highly
26 conserved in all eukaryotes (p97 in mammals). In yeast, Cdc48 works in concert with several
27 identified co-factors including Ufd1, Npl4, Ubx2 and Doa1 in both ERAD and MAD [36-40] to

1 recognize poly-ubiquitinated substrates, dissociate them from their protein complexes or respective
2 membranes and allow their turnover by the proteasome [41, 42].

3 In vegetative conditions, yeast and mammalian chaperones (such as HSP70 and HSP90)
4 ensure correct folding and escort of proteins on their transit to mitochondria (Figure 2, Panel I). In
5 *Saccharomyces cerevisiae*, efficient mitochondrial import also relies on the continuous monitoring of
6 the TOM channel by a specific kind of MAD where the TOM channel constitutively interacts with the
7 Cdc48 adaptor Ubx2 [43]. In this mitochondrial Translocation Associated Degradation (mitoTAD)
8 pathway, proteins that clog the TOM import channel are ubiquitinated and recognized by a
9 mitochondrial pool of Ubx2 which promotes their extraction by the Cdc48-Ufd1-Npl4 complex and
10 their degradation by the proteasome (Figure 2, Panel I).

11 Given that the majority of mitochondrial proteins are synthesized by cytosolic ribosomes, it is
12 also important that the translation products remain under surveillance by a Ribosomal Quality Control
13 (RQC) system in order to maintain mitochondrial homeostasis [44, 45]. Consistent with this, Cdc48
14 and its cofactors Npl4 and Ufd1 are also involved in this process (Figure 2, Panel II). Upon translation
15 stalling, ribosomes split resulting in a 60S subunit, which remains bound to the nascent polypeptide
16 chain [46]. The RQC complex composed of Rqc1, Rqc2, Cdc48 and the Ubiquitin ligase Ltn1
17 (Listerin in mammals) [47] is then recruited (Figure 2, Panel II). Rqc2 binds charged t-RNAs that
18 induce the elongation of nascent polypeptide chains with C-terminal Alanine and Threonine residues
19 (CAT-tailing) [48]. This elongation exposes lysine residues of the incomplete chain that is
20 ubiquitinated by the Ltn1 ubiquitin ligase [49, 50]. The peptidyl-tRNA hydrolase Vms1 (ANKZF1 in
21 mammals) then triggers the release of the CAT-tailed peptide from the tRNA [51, 52] and Rqc1
22 recruits the Cdc48-Npl4-Ufd1 complex for extraction from the 60S ribosome and subsequent
23 degradation by 26S proteasomes [53]. Notably, the released polypeptides can be successfully
24 ubiquitinated and degraded by the 26S proteasome but they can also form CAT-tail dependent
25 aggregates in the cytosol [54, 55]. This RQC system has mainly been investigated in yeast but the
26 conservation of most RQC components in metazoans favors an overall similar quality control in
27 mammalian ribosomes (Izawa et al 2017).

1 Upon stress or when mitochondrial protein import is defective in *Saccharomyces cerevisiae*,
2 mitoprotein-induced stress responses are triggered (Figure 2, Panel III), all coordinated by a
3 transcriptional regulatory pathway [56]. The accumulation of mitochondrial protein aggregates in the
4 cytosol (mPOS, Mitochondrial Precursor Over-accumulation) reduces protein synthesis and import
5 [57]. This accumulation activates the UPRam (Unfolded Protein Response activated by the mis-
6 targeting of proteins) inducing the degradation of the protein aggregates by the 26S proteasome [58].
7 Ultimately, when the TOM channel is clogged by yeast protein aggregates, the Mitochondrial
8 Compromised Protein import Response (mitoCPR) is activated [59]. In this process, Cis1 recruits the
9 AAA-ATPase Msp1 (ATAD1 in mammals) to the TOM70 receptor in order to remove non-imported
10 precursor proteins that will subsequently be degraded by the proteasome (Figure 2, Panel III). While
11 all these processes have been identified in yeast, their conservation in mammals awaits clear analysis.
12 Whether ATAD1 has the same role as Msp1 in mitoCPR is for instance yet to be investigated.

13 However, ATAD1 in human and mouse seems to have a similar role as yeast Msp1 in the
14 Mitochondrial Associated Degradation of Tail Anchored proteins (MAD-TA). During MAD-TA
15 (Figure 2, Panel IV), tail anchored proteins that are mis-targeted to mitochondria are recognized by
16 Msp1/ATAD1 which allows their extraction and degradation in the cytosol [60-62]. In
17 *Saccharomyces cerevisiae*, Msp1 is thought to have the capacity to extract and transfer TA-proteins
18 from the outer membrane to the ER at mitochondria-ER contact sites while the ERMES complex, the
19 main ER-mitochondria tether in yeast [63], does not seem required for this function [64, 65]. Once
20 embedded in ER membranes TA-proteins are treated as ERAD substrates, which includes
21 ubiquitination by the ERAD E3 Doa10, extraction by the Cdc48-Ufd1-Npl4 complex and degradation
22 by cytosolic proteasomes [64, 65]. Whether ATAD1 promotes degradation of TA-proteins by ERAD
23 in mammals remains unknown.

24 Similar to Npl4, Ufd1 or Ubx2, Doa1 is yet another Cdc48 cofactor previously implicated in
25 yeast ERAD that has been implicated in MAD. In the so called MAD-C pathway (Figure 2, Panel IV),
26 the Cdc48-Doa1 complex was shown to participate in the proteasomal degradation of diverse
27 substrates such as the yeast mitofusin Fzo1, the ERMES component Mdm34, the import complex
28 protein Tom70 and even the AAA-ATPase Msp1 [40]. Their extraction from the outer membrane and

1 direct degradation by the proteasome has been postulated but, as we will see later in this review, their
2 processing by a MAD-TA like pathway cannot be ruled out (Figure 2, Panel IV).

3 All together, we have listed the numerous UPS-dependent quality control and defense
4 mechanisms guarding mitochondrial homeostasis and integrity. The substrates of these mechanisms
5 are diverse, ranging from misfolded to transmembrane proteins located on the mitochondrial surface
6 but also within mitochondria, on the inner membrane and even the cristae [66]. However, within these
7 potential substrates for UPS-mediated degradation, Mitofusins represent a specific category of
8 mitochondrial proteins that stands out in most pathways described above.

9

10 **UBIQUITINATION OF THE YEAST MITOFUSIN FZO1**

11 **UPS-mediated control of Fzo1 by Mdm30**

12 Mitofusins are conserved throughout evolution and belong to the Dynamin family of large
13 GTPases [67-69]. Embedded in mitochondrial outer membranes, their primary role is to mediate
14 anchoring and initiate homotypic fusion of mitochondria (Figure 2, Panel V). Two mitofusins, MFN1
15 and MFN2, are expressed in mammals and both are essential for fusion of mitochondrial outer
16 membranes [70-73]. However, while mutation of MFN2 is causal in the Charcot Marie-Tooth Type
17 2A (CMT2A) disease, similar mutations in MFN1 are not [74-77]. UPS regulation of metazoan
18 mitofusins (MFN1 and MFN2) is fundamental in mitophagy (see section 3) whereas the sole yeast
19 mitofusin Fzo1 may be a degradation substrate in several of the pathways mentioned section 1 (see
20 below). Moreover, Mdm30-mediated ubiquitination of Fzo1 is central to the process of mitochondrial
21 fusion (Figure 2, Panel V).

22 Benedickt Westermann and colleagues identified the F-box protein Mdm30 as an important
23 regulator of mitochondrial fusion in yeast [78]. In this seminal study, deletion of *MDM30* was
24 demonstrated to inhibit mitochondrial fusion leading to a particular phenotype of mitochondrial
25 aggregation. Moreover, Fzo1 was found to accumulate in the absence of the F-box protein. Mdm30
26 was subsequently confirmed to promote degradation of Fzo1 [79]. However, while F-box domain
27 proteins were known to act as substrate recognition elements of multisubunit SCF ubiquitin ligases
28 [80-82], it was proposed that the SCF, Ubiquitin, or the proteasome were not involved in the Mdm30-

1 dependent turnover of the yeast mitofusin [79]. Two years later, this interpretation was radically
2 challenged as the SCF and the proteasome were shown to participate in the Mdm30-mediated
3 degradation of Fzo1 and the yeast mitofusin was unequivocally demonstrated to be ubiquitinated by the
4 SCFMdm30 ubiquitin ligase [83]. Ultimately, the UPS-dependent control of Fzo1 reached a
5 consensus [84, 85]. The straightforward conclusions of this set of studies was that Mdm30 is part of
6 an SCF E3 that promotes ubiquitination of Fzo1 and its subsequent degradation by the proteasome
7 [78, 83]. In this context, Fzo1 accumulation would block mitochondrial fusion resulting in the
8 mitochondrial aggregation phenotype seen in *MDM30* null cells [78, 79, 83].

9 In 2005, Albert Neutzner and Richard Youle discovered that Fzo1 is subject to proteasomal
10 degradation upon extended treatment with the alpha factor mating pheromone [86]. Mdm30 was
11 excluded from inducing this degradation. Moreover, Mdm30-mediated ubiquitination of Fzo1 is
12 detected in vegetative condition, indicating that this vegetative turnover of Fzo1 is very distinct from
13 that observed upon alpha-factor treatment.

14 Importantly, the Mdm30-dependent ubiquitination of the yeast mitofusin depends on the integrity of
15 the Fzo1 GTPase domain (Figure 3A). The V327T mutation in the GTPase domain of Fzo1, which is
16 analogous to the CMT2A I213T mutation in the GTPase domain of MFN2, was shown to decrease the
17 turnover of Fzo1 by Mdm30 [87]. Shortly after, a set of four additional mutations in the GTPase
18 domain of Fzo1 (K200A, S201N, T221S and D195A) was found to induce total abolishment of the
19 Mdm30-mediated ubiquitination and degradation of Fzo1 [84, 85]. Notably, full length Fzo1 K200A,
20 S201N and T221S do not bind Mdm30, which explains their stability and lack of ubiquitination [85].
21 Mdm30 was further demonstrated to bind the N-terminal half of Fzo1 whether or not the S201N
22 mutation was introduced in the GTPase domain of this N-terminal truncated portion [85]. In contrast,
23 Mdm30 did not bind the HR1-HR2 containing C-terminal half of Fzo1 (Figure 3B). This set of
24 observations pointed to a model in which the binding site of Mdm30 in the N-terminal half of full
25 length Fzo1 is hindered by the C-terminal half. The activity of the GTPase domain would then induce
26 a conformational switch of Fzo1 that would allow the recruitment of Mdm30 (Figure 3C).

27 The conformational switch of mitofusins has yet to be experimentally observed but it is
28 currently thought to take place during mitochondrial tethering, in the course of mitofusin auto-

1 oligomerization in *trans* [69, 88]. Consistent with this, multiple observations pointed to the
2 requirement of Mdm30-dependent ubiquitination and degradation of Fzo1 during mitochondrial
3 attachment [84, 85]. Furthermore, employment of the N-end rule pathway was used to bypass the
4 requirement of Mdm30 in mitochondrial fusion and Fzo1 degradation [85]. This allowed
5 demonstrating that it is the ongoing turnover of Fzo1 that is important to maintain efficient
6 mitochondrial fusion rather than the simple regulation of Fzo1 steady state levels.

7

8 **Role of the UPS in mitochondrial fusion**

9 When this set of studies was published, roughly ten years ago, it was difficult to conceive that
10 the multimeric SCFMdm30 ubiquitin ligase and the 3000 kDa 26S proteasome could access Fzo1
11 oligomers at the junction of anchored mitochondria. Since Fzo1 is essential for fusion of
12 mitochondrial outer membranes [89-91], it was also difficult to understand why the mitofusin needs to
13 be degraded for fusion to proceed. Nonetheless, recent structural observations of the mitofusin-
14 mediated membrane fusion process provide significant novel insights on these issues [69, 76, 92-96].

15 Mitochondrial tethering and ultimate fusion of outer membranes have been shown to occur
16 through successive steps [96]. It was shown that outer membranes of two attached mitochondria are
17 initially tethered by Fzo1-containing globular protein repeats (Figure 4A, Tethering stage). Successive
18 cycles of GTP hydrolysis by mitofusins then allow the fusion process to evolve towards a
19 mitochondrial docking step (Figure 4A, Docking stage). Docked intermediates are characterized by a
20 docking ring of protein densities that surrounds areas where outer membranes are separated by less
21 than 3 nm. The fusion of bilayers is then initiated by further GTP hydrolysis in the path of the docking
22 ring where the outer membrane curvature is presumably most pronounced (Figure 4A, Local
23 membrane fusion stage). This set of observations suggests that the formation of Fzo1 oligomers of
24 increasing sizes through successive cycles of GTP binding and hydrolysis progressively brings outer
25 membranes closer from each other and culminates in formation of the docking ring complex which
26 allows fusion at one critical point of membrane curvature. In this context, the requirement for
27 regulated assembly of Fzo1 oligomers by the UPS during formation of the docking ring becomes
28 amenable to speculation.

1 Fzo1 is proposed to dimerize in *cis* (on the same membrane) and the resulting *cis*-dimers to
2 engage in *trans*-oligomerization (from opposing membranes) to mediate mitochondrial attachment
3 [84]. Based on several structural insights on mitofusins and other dynamins, Fzo1 *cis*-dimers and
4 *trans*-oligomers were recently modelled [97]. It was thereby suggested that the docking ring could
5 form with *cis* dimers interacting through their GTPase domain (Figure 4B and 4C) and *trans*
6 oligomers assembling through the available trunks of the dimers (Figure 4C). GTP hydrolysis within
7 these oligomers would induce Fzo1 sliding along its trunk thus bringing opposing outer membranes
8 closer from each other. Additional cycles of *cis*-dimerization and *trans*-oligomerization around this
9 initial site would result in formation and expansion of the docking ring at the periphery of the
10 increasing contact area (Figure 4C). In this system, Fzo1 *cis*-dimers that did not engage in *trans*-
11 oligomerization at the initial site of tethering may instead nucleate additional sites of anchoring that
12 would perturb the proper assembly of the docking ring and result in abortive fusion (Figure 4D).
13 Consistent with this possibility, overexpression of Fzo1 increases mitochondrial tethering but
14 abrogates formation of the docking ring and inhibits outer membrane fusion [96]. In this context, free
15 Fzo1 dimers may be targeted for ubiquitination as their respective monomers have operated the
16 conformational switch required for Mdm30 binding [85]. These dimers would subsequently be
17 cleared by proteasomal degradation (Figure 4B). As outer membranes get closer together and the
18 docking ring assembles, the ongoing Mdm30-mediated proteasomal degradation of free Fzo1 dimers
19 would favor productive mitochondrial docking and ultimate fusion of outer membranes (Figure 4C).

20

21 **Regulation of Mdm30-mediated ubiquitination of Fzo1**

22 *Target lysines and ubiquitin proteases*

23 The proposed model of Fzo1 oligomerization and resulting role of Fzo1 degradation in
24 mitochondrial fusion above remains speculative, which warrants additional efforts and discoveries on
25 this topic. In parallel, whether Mdm30-mediated ubiquitination and degradation of Fzo1 is
26 constitutive or whether it is itself regulated needs to be addressed. In this regard, Fzo1 was shown to
27 be ubiquitinated on two distinct lysine residues (K464 and K398, Figure 3A) and proposed to be
28 regulated by two distinct ubiquitin proteases, Ubp2 and Ubp12 [98]. It was thereby suggested that

1 Ubp2 antagonizes the ubiquitination of Fzo1 promoted by an unknown ubiquitin ligase involved in
2 mitochondrial quality control whereas Ubp12 triggers the Mdm30-mediated ubiquitination on K464
3 and K398 of Fzo1 to regulate mitochondrial fusion [98]. The mutation of K398 and K464 into
4 Arginine (Figure 3A) led to the interpretation that K464 is essential for Fzo1 ubiquitination whereas
5 K398 is not essential but required for formation of the normal pattern of Mdm30-mediated
6 ubiquitination [98]. Consistent with these assumptions, respiration was strongly decreased by the
7 K398R mutation of Fzo1 but totally abolished by the K464R mutation. This led to propose that Fzo1
8 ubiquitination is primed on K464 and then transferred on K398 [98]. Yet, the possibility that K464R
9 alters Fzo1 structure and abolishes its function was not evaluated. K464 was later found to be
10 involved in the establishment of a disulfide bridge with D335 within the Fzo1 whole polypeptide [94].
11 In particular, swap charge mutations between the two residues demonstrated the importance of this
12 K464-D335 bridge for Fzo1 function [94]. These observations thus revealed that the K464R mutation
13 does not abolish Fzo1 function because of ubiquitination inhibition but does so because of Fzo1
14 structural defects.

15 Regarding the role of ubiquitin proteases, inactivation of Ubp2 induced very fast degradation
16 of Fzo1 and consequently very low levels of the mitofusin, which was accompanied with a phenotype
17 of high mitochondrial fragmentation and markedly decreased respiration [98]. In contrast, the
18 inactivation of Ubp12 had very weak phenotypes with a slight increase in Fzo1 levels and no effect on
19 mitochondrial fragmentation or respiration. Mutation of the Ubp12 catalytic site had no effect on the
20 ubiquitination pattern of Fzo1 besides a marginal increase in Fzo1 modified species that was
21 proportional to the slight increase of Fzo1 level in the absence of Ubp12 [98]. In face of this weak
22 impact of *UBP12* deletion on Fzo1 ubiquitination and mitochondrial fusion it was thus difficult to
23 understand how Ubp12 could antagonize the Mdm30-dependent regulation of Fzo1. In fact, this
24 function was later found to be carried out by Ubp2 [99]. More precisely, Ubp2 was shown to restrict
25 the length of K48-linked ubiquitin chains added on K398 of Fzo1, thereby slowing down the Mdm30-
26 dependent proteasomal degradation of the mitofusin [99]. These observations established Ubp2 as the
27 bona-fide antagonist of Mdm30-dependent ubiquitination on K398 of Fzo1 (Figure 5).

28

1 *An unexpected link with the desaturation of fatty acids*

2 The role for Ubp2 capacity to tune Fzo1 turnover required being clarified. A first clue in this
3 regard, came from the unexpected finding that, together with Fzo1, Mdm30 can also target Ubp2 for
4 ubiquitination and degradation by the proteasome [99]. In the absence of Mdm30, both Fzo1 and
5 Ubp2 are thus stabilized (Figure 5). Notably, the natural stabilization of Ubp2 was found contributing
6 to the defects in respiration and mitochondrial fusion seen in cells lacking Mdm30 [99]. Consistent
7 with this, Ubp2 is an important antagonist of the HECT domain ubiquitin ligase Rsp5 [100] and one
8 main function of Rsp5 is to activate the synthesis of the $\Delta 9$ -fatty acid desaturase Ole1 in the so called
9 Ole1 pathway [101]. Ubp2 stabilization in *MDM30* null cells was shown to antagonize this pathway
10 thereby resulting in decreased synthesis of Ole1 [99]. This work thus revealed that lack of Mdm30 not
11 only induces stabilization of Fzo1 and Ubp2 but also decreased desaturation of fatty acids.

12 Importantly, mitochondrial fusion defects in *mdm30* or *ubp2* null cells, in which Fzo1 is
13 either stabilized or rapidly degraded, were rescued by increased or decreased desaturation of fatty
14 acids, respectively [99]. In particular, addition of oleate in wild type cells induced a natural increase
15 of Fzo1. This natural increase of Fzo1 maintained efficient mitochondrial fusion upon high
16 desaturation of fatty acids but was also shown to depend on both Mdm30 and Ubp2 [99]. These
17 observations [99] indicate that the Mdm30-mediated degradation of Fzo1 is not constitutive but
18 tightly controlled by Ubp2, according to the activation status of the Ole1 pathway (Figure 5). More
19 precisely, Mdm30-mediated degradation of Fzo1 becomes essential for mitochondrial fusion upon
20 low desaturation of fatty acids, but dispensable upon high expression of Ole1 (Figure 5).

21 While the precise purpose of this balance remains to be investigated, it is possible to speculate
22 on its function. In this regard, the desaturation status of fatty acids is established to be intimately
23 linked to the representation of phospholipids in the cell [102]. For instance, low desaturation of fatty
24 acids induces a drastic increase in cellular amounts of phosphatidic acid (PA) whereas the overall
25 amount of phosphatidyl choline or phosphatidyl serine decreases [102]. Intriguingly, derivatives of PA
26 have been shown to participate in mitofusin-dependent membrane fusion in mammalian systems [103,
27 104]. In this context, PA may facilitate outer membrane fusion after formation of a smaller

1 Mitochondrial Docking ring Complex (MDC) thus requiring increased degradation of Fzo1 upon low
2 fatty acids desaturation. Conversely, higher fatty acids desaturation resulting in decreased synthesis of
3 fusogenic lipids would impose stabilization of Fzo1. These higher levels of mitofusin would then
4 allow assembly of an MDC of a size appropriate to trigger fusion of outer membranes (Figure 5).

5

6

7 **Alternative pathways of Fzo1 degradation**

8 Numerous other factors have been shown to be implicated in the UPS-mediated degradation
9 of Fzo1. Among those, inactivation of Vms1, Cdc48, Ubx2, Doa1 or Ubp3 have all been shown to
10 impact Fzo1 levels. Whether these factors control the Mdm30-mediated degradation of Fzo1 or
11 whether they control turnover of the mitofusin through other pathways and other ubiquitin ligases has
12 not always been necessarily considered. In particular, it is striking that most if not all of these factors
13 have established roles in quality control mechanisms and especially ER-Associated Degradation
14 (ERAD), which contrasts with the apparent dedicated role of Mdm30-mediated degradation of Fzo1
15 in the regulation of mitochondrial fusion.

16

17 *Vms1*

18 Vms1 was identified in 2010 as a factor required for protein quality control at the
19 mitochondria [105]. This factor comprising a ring finger domain, an ankyrin repeat region and a
20 characteristic Vms1-like domain, all conserved in other species, was shown to interact specifically
21 with the AAA-ATPase Cdc48 [105]. While Cdc48 interacts with the heterodimer Npl4-Ufd1 in
22 ERAD, Vms1 was shown to bind Npl4 but not Ufd1 [105]. It was thus proposed that the Cdc48-Npl4-
23 Vms1 complex could ensure a protein quality control function at the mitochondria similar to the
24 function of the Cdc48-Npl4-Ufd1 complex at the ER. Consistent with this, Vms1 and Cdc48 were
25 found to be implicated in Fzo1 degradation [105]. Importantly, this degradation did not depend on
26 Mdm30 and exclusively occurred in stress conditions [105]. In agreement with this observation, Fzo1
27 turnover in the absence of stress was only marginally affected by the inactivation of Cdc48 or Vms1
28 [106], which drastically contrasts with the strong stabilization of Fzo1 in the absence of Mdm30.

1 Notably, a more precise function in Ribosome Quality Control (RQC) was later attributed to Vms1
2 (see section 1- Figure 2, Panel II). In 2017, Vms1 was found to bind 60S ribosomes at the
3 mitochondria and shown to participate in CAT-tailing inhibition to prevent import of CAT-tailed
4 polypeptides in mitochondria [45]. Shortly after, Vms1 was characterized as a peptidyl-tRNA
5 hydrolase that promotes release of the nascent chain thus facilitating its subsequent extraction and
6 degradation [51, 52]. This set of findings thus suggest that Cdc48 and the RQC pathway (Figure 2,
7 Panel II) could trigger Fzo1 for proteasomal degradation under stress conditions.

8

9 *Cdc48*

10 Cdc48 has also been found to inhibit the degradation of Fzo1 in vegetative growth [107]. In
11 this work, specific inactivation of the ATPase was found to induce faster degradation of the mitofusin.
12 This counterintuitive result was explained by the possibility that Cdc48 could promote the
13 degradation of Ubp12 [107]. In this context, Ubp12 would be stabilized upon inactivation of Cdc48,
14 which would induce faster turnover of Fzo1. Yet, lack of Ubp12 did not induce stabilization of Fzo1
15 upon Cdc48 inactivation [107]. Consequently, the unexpected impact of Cdc48 on Fzo1 in vegetative
16 growth remains to be explained. In this regard, Cdc48 is a key factor in the Ole1 pathway that is
17 essential to activate synthesis of the fatty acid desaturase [101, 108]. Inactivation of Cdc48 thus
18 induces decreased desaturation of fatty acids [108]. Taking into consideration that decreased
19 desaturation of fatty acids promotes increased degradation of Fzo1 [99], this would very well explain
20 the decreased level of Fzo1 upon inactivation of Cdc48 (Figure 5, low UFA).

21

22 *Ubx2*

23 Another regulator of Fzo1 degradation is Ubx2, the ER-resident co-factor of Cdc48. First
24 identified for its role in ERAD [39, 109, 110], Ubx2 was also demonstrated to perform a key function
25 in the Ole1 pathway [102]. Consistent with this, lack of Ubx2 was shown to decrease desaturation of
26 fatty acids and to induce remodeling of the cellular phospholipidome [102]. This may explain the
27 intriguing observation that Fzo1 oligomerization properties could be regulated by Ubx2 [111], which
28 may link Ubx2 to the Mdm30-mediated control of Fzo1 [99]. However, in contrast to Cdc48 [107],

1 inactivation of Ubx2 does not increase degradation but induces a weak stabilization of Fzo1 under
2 vegetative growth [112]. Ubx2 interacts with the mitofusin [112], suggesting its direct involvement in
3 Fzo1 turnover. Yet, stabilization of Fzo1 upon inactivation of Ubx2 is not as strong as upon
4 inactivation of Mdm30. In particular, Fzo1 is not stabilized when Ubx2 and Ubp2 are co-inactivated
5 [112], which drastically contrasts with the total inhibition of Fzo1 degradation upon co-inactivation of
6 Mdm30 and Ubp2 [99]. This demonstrates that Ubx2 controls Fzo1 turnover through a pathway that is
7 distinct from Mdm30.

8 Importantly, a small pool of Ubx2 localizes on outer membranes (Figure 2, Panel I) to
9 promote the mito-TAD quality control process [43]. Which ubiquitin ligase acts in this pathway
10 remains to be identified (Figure 2, Panel I). Nonetheless, Vms1 was functionally linked to this mito-
11 TAD pathway [43], suggesting that RQC and Ltn1 could be involved in the ubiquitination of stalled
12 mitochondrial substrates. Alternatively, the ubiquitin ligases Ubr1 and San1 were recently found to
13 participate in a MAD pathway involving Ubx2, Cdc48, Doa1, Proteasomes as well as the Hsp70
14 chaperones Sis1 and Ssa1-4 [113]. In this study, Fzo1 was confirmed to be stabilized upon
15 inactivation of Ubx2 but the mitofusin was not affected in the absence of Doa1 nor upon inactivation
16 of the Hsp70 machinery [113]. It remains thus possible but yet to be proven that Ubx2-mediated
17 degradation of Fzo1 involves ubiquitination by Ltn1, Ubr1 or San1.

18

19 *Doa1*

20 The Cdc48 co-factor Doa1 has recently been involved in MAD but it was also clearly
21 excluded from impacting Fzo1 turnover in vegetative growth [113]. This notably contrasts with
22 previous work showing that Doa1 recruits Cdc48 to promote degradation of the mitofusin in the
23 absence of stress [40]. Here, co-inactivation of Cdc48 and Mdm30 led to additive stabilization of
24 Fzo1 as compared to single impairments of the ATPase or the E3 [40]. This strongly suggests that
25 Cdc48 promotes degradation of Fzo1 through a pathway that is distinct from the Mdm30-mediated
26 turnover of the mitofusin. Yet, the Doa1-Cdc48 complex was also shown to interact with
27 ubiquitinated species of Fzo1 in the presence but not in the absence of Mdm30 [40]. These Fzo1
28 ubiquitinated species migrated higher than those usually added by Mdm30 [40]. It is thus possible that

1 aggregation of Fzo1 in the absence of Mdm30 (Figure 4D), precluded pull-down of Fzo1 molecules
2 modified by a ligase distinct from Mdm30. As mentioned earlier for Ubx2, the ubiquitin ligases Ltn1,
3 Ubr1 or San1 could therefore also be involved in this Doa1-Cdc48 pathway (Figure 2, Panel IV,
4 MAD-C). Nonetheless this interesting study [40] opened yet another possibility involving the AAA
5 ATPase Msp1. Inactivation of both Cdc48 and Doa1 were found to induce strong stabilization of
6 Msp1 [40], which is well established to control the mis-localization of tail-anchored proteins [61, 62,
7 114]. In particular, Msp1 has the ability to induce translocation of substrates from mitochondria to the
8 ER [64, 65]. The ERAD E3 Doa10 then promotes ubiquitination of the translocated proteins followed
9 by their Cdc48-mediated extraction and ultimate proteasomal degradation (Figure 2, Panel IV, MAD-
10 TA). Whether Doa1 could promote Fzo1 turnover through this Msp1-Doa10-Cdc48 axis is thus an
11 exciting possibility that should not be excluded (Figure 2, Panel IV, MAD-C). This possibility also
12 applies to Ubx2-mediated degradation of Fzo1 as the MITO-TAD pathway has been functionally
13 linked to Msp1 [43].

14 While Doa1 clearly promotes degradation of Fzo1 [40], the controversy on its involvement in
15 vegetative growth [113] has also been nicely resolved [115]. The steady state level of Fzo1 was
16 shown to drastically drop down in old cells but also in young cells treated with concanamycin A, a
17 specific inhibitor of the vacuolar ATPase which mimics age-related modifications on mitochondria
18 [115]. This decrease was shown to be proteasome dependent and inactivation of Doa1 strikingly
19 blocked this specific turnover of Fzo1 in old cells [115]. Taken together, the data presented in this
20 interesting study were rather consistent in proving that Mdm30 and Rsp5 have no involvement in this
21 degradation [115]. It will thus be very interesting to check whether Doa1-mediated degradation of
22 Fzo1 in old cells involves Ltn1, Ubr1, San1 or Doa10 ubiquitin ligases (Figure 2, Panel IV, MAD-C).

23

24 *Ubp2, Ubp12 and Ubp3*

25 Altogether, it is without a doubt that the yeast mitofusin Fzo1 could be a natural substrate for
26 distinct mitochondrial quality control pathways. Nonetheless deep uncertainty remains regarding the
27 ubiquitin ligases that could target Fzo1 for degradation in these situations. In parallel, the involvement
28 of Mdm30 in the regulation of mitochondrial fusion is well established. Several factors involved in

1 quality control processes could participate in this specific process. Yet, this might take place through
2 an indirect impact on the Ole1 pathway rather than through a direct action on Fzo1 ubiquitinated
3 species. The ubiquitin protease Ubp2, as both the antagonist of Mdm30-mediated ubiquitination of
4 Fzo1 and a substrate for Mdm30-dependent degradation [99], is also absolutely central in the
5 regulation of mitochondrial fusion (Figure 5). On the other hand, the other ubiquitin protease, Ubp12,
6 might have a more indirect effect on Fzo1 [98, 107]. In this regard, *UBP12* inactivation has been
7 proposed to induce stabilization of Ubp2 [107]. As the antagonist of Mdm30-mediated degradation of
8 Fzo1 [99], this stabilization of Ubp2 (Figure 5, high UFA) would be consistent with the weak increase
9 in mitofusin levels seen upon *UBP12* inactivation [98]. Nonetheless, how Ubp12 could favor the
10 degradation of Ubp2 remains unclear. Intriguingly, inactivation of a third ubiquitin protease, Ubp3,
11 was recently shown to perturb mitochondrial dynamics [111]. Absence of *UBP3* induced stabilization
12 of both Ubp2 and Ubp12 but had surprisingly no effect on Fzo1 degradation [111]. While these
13 results are difficult to reconcile, one cannot exclude that the three UBPs could be involved in other
14 pathways involved in mitochondrial homeostasis. In fact, this is even likely as Ubp3 and Rsp5, a
15 ligase antagonized by Ubp2 [100], have been implicated in mitophagy, the degradation of
16 mitochondria by autophagy [116, 117].

17

18 **UBIQUITINATION AND MITOPHAGY**

19 **The main principles of mitophagy**

20 Mitophagy is a selective form of autophagy that leads to the clearance of unnecessary or
21 damaged mitochondria by the lysosomal/vacuolar compartment. This specific type of mitochondrial
22 degradation occurs through either macromitophagy or micromitophagy depending on how
23 mitochondria or mitochondrial fragments are targeted to the lysosome. During macromitophagy,
24 mitochondria destined to be cleared are recognized by the autophagic machinery and subsequently
25 enveloped by a double membrane structure called the autophagosome. The autophagosome then fuses
26 with the lysosome before its mitochondrial content gets degraded by lysosomal proteases [118].
27 Micromitophagy is less described and does not involve the formation of an autophagosome. Instead,

1 vesicles that bud from the mitochondria are then targeted to the lysosome [119]. In this section, we
2 will mainly focus on macromitophagy, hereafter referred as mitophagy.

3 The autophagic machinery relies on Atg8 (LC3 and GABARAP in mammals), an ubiquitin
4 like protein that has the exceptional ability to conjugate itself to lipids through a thioester cascade that
5 involves E1-like, E2-like and E3-like enzymes [120]. This allows Atg8/LC3 to be lipidated to the
6 membrane of the autophagosome where it is exposed. There, Atg8/LC3 can interact with autophagy
7 receptor proteins that contain a specific WXXL sequence called the AIM/LIR (Atg8 Interacting
8 Motif/LC3 Interacting Region) domain [121]. This receptor-mediated mitophagy coexists with
9 ubiquitin-dependent mitophagy where specific adaptor proteins with a ubiquitin binding domain and
10 an AIM/LIR domain connect Atg8/LC3 to ubiquitinated proteins on mitochondrial outer membranes
11 (Figure 6 and 8).

12 Atg32 (Autophagy related protein 32), the receptor involved in yeast mitophagy (Figure 8), is
13 a single spanning transmembrane protein with its N-terminal domain facing the cytosol and harboring
14 the AIM/LIR domain [122, 123]. After induction of mitophagy, Atg32 is phosphorylated and interacts
15 with the soluble protein adaptor Atg11 [124]. Atg11 targets the complex to the pre-autophagosomal
16 assembly site where Atg32 can interact with lipidated Atg8 that is anchored to the membrane of the
17 autophagosome [123].

18 In mammalian cells, several mitophagy receptors with their AIM/LIR motif have been found
19 on mitochondrial outer membranes. These include NIX/BNIP3L (BCL2 interacting protein 3 like)
20 involved in mitophagy during erythrocyte or neuronal differentiation, FUNDC1 (Fun14 domain
21 containing 1) and BNIP3 for hypoxia induced mitophagy, BCL2L13 (Bcl2 Like Protein 13) a
22 proposed functional homolog of Atg32, FKBP8 (FKBP prolyl isomerase 8) or AMBRA1 [125-132].
23 Even though receptor-mediated mitophagy is distinct from ubiquitin-dependent mitophagy, its
24 regulation by ubiquitin has been clearly demonstrated. Ubiquitination of FUNDC1 by the RING
25 domain ligase MITOL/MARCH5 occurs at the very beginning of hypoxia to induce proteasomal
26 degradation of FUNDC1, resulting in decreased mitophagy [133]. This process was proposed to
27 protect the cell at the beginning of hypoxia from improper mitophagy [133]. PHB2 (Prohibitin2) is yet
28 another peculiar mitophagy receptor [134]. Embedded in the mitochondrial inner membrane in a large

1 heterodimeric complex with PHB1, PHB2 becomes accessible to LC3 after rupture of the outer
2 membrane consecutive to ubiquitination and proteasomal degradation of outer membrane proteins.
3 Notably, such regulation of mitophagy by ubiquitin is distinct from ubiquitin-dependent mitophagy
4 which holds a significant importance in metazoan.

5 A main feature of ubiquitin-dependent mitophagy, is that the modifier, ubiquitin, is itself
6 modified by phosphorylation [135-137]. This phospho-ubiquitination process involves the
7 mitochondrial targeted protein kinase PINK1 (Phosphatase and Tensin homolog (PTEN)-Induced
8 putative Kinase 1) and the ubiquitin ligase PARKIN (Figure 6, Panel I). Following induction of
9 mitophagy, the two enzymes trigger the phospho-ubiquitination of numerous proteins from the
10 mitochondrial outer membrane. The phospho-ubiquitin tag is then recognized and bound by ubiquitin
11 binding adaptors such as OPTN (optineurin) and NDP52 (nuclear domain 10 protein 52) that are the
12 primary receptors for mitophagy [138] or p62, TAX1BP1 (Tax1 binding protein 1) and NBR1
13 (neighbor of BRCA1 gene 1) [139]. These adaptor proteins contain an ubiquitin interacting motif and
14 an AIM/LIR motif that connect ubiquitinated proteins from the outer membrane to Atg8-like proteins
15 on the autophagosomal membrane. Damaged mitochondria are then associated to autophagosomes
16 before disposal in the lysosome (Figure 6, Panel I).

17

18 **The mitochondrial kinase PINK1 and the ubiquitin ligase PARKIN**

19 Under normal conditions, the serine/threonine kinase PINK1 is targeted to mitochondria and
20 degraded very rapidly (Figure 7A). After import through the TOM and TIM complexes, the
21 mitochondrial targeting peptide of PINK1 is cleaved by MPP (mitochondrial processing peptidase),
22 before its IMM (Inner Mitochondrial Membrane) spanning domain gets further cleaved by PARL
23 (Presenilin-associated rhomboid-like) [140, 141]. This cleavage induces the retrotranslocation of
24 PINK1 to the cytosol and its subsequent degradation by the UPS through the N-end rule pathway
25 (Figure 7A). As a result of this turnover, the level of PINK1 is very low on healthy mitochondria with
26 an efficient membrane potential ($\Delta\Psi_m$) [142]. However, upon treatment with mitochondrial
27 uncouplers such as CCCP (Carbonyl Cyanide 3-Chlorophenylhydrazine), the $\Delta\Psi_m$ drops down and
28 the import of PINK1 is inhibited. In these depolarized mitochondria, the kinase is trapped on the outer

1 membrane where it forms a dimer in association with the TOM complex (Figure 6, Panel I). At this
2 stage, PINK1 activates and induces its autophosphorylation [143-145]. A cascade of PINK1-
3 dependent phosphorylation then targets mono and poly-ubiquitin on the conserved Serine 65 residue
4 of ubiquitin but also the ubiquitin ligase PARKIN on its Ubl (Ubiquitin like) domain [135, 137]. This
5 induces the transfer of PARKIN from the cytosol to depolarized mitochondria where the E3
6 ubiquitinates a massive amount of substrates on outer membranes [146-152].

7 PARKIN, is a RING/HECT hybrid E3 of the RBR family of ubiquitin ligases (Figure 7B-C).
8 Its distinct domains include the Ubiquitin-like (Ubl) domain that contains the S65 phosphorylation
9 site for PINK1, a Repressor (REP) domain as well as four Zinc-coordinating RING-like domains,
10 RING0, RING1, IBR (In between Ring), and RING2. RING1 is the binding site for the E2 whereas
11 RING2 contains the catalytic cysteine (Cys431) that binds ubiquitin through a thioester bond before
12 transfer to the substrate (Figure 7B). PARKIN is located in the cytoplasm in a tightly packed
13 autoinhibited state [153-155]. The Ubl domain is bound to RING1, rendering S65 hardly accessible
14 for phosphorylation by PINK1 whereas the REP domain interacts with RING1, thereby masking the
15 binding site for the E2 (Figure 7C, Inactive form). In parallel, RING0 obstructs the ubiquitin acceptor
16 cysteine 431 on RING2 [154-157]. Interestingly, PARKIN begins its activation after binding to
17 phospho-ubiquitin which acts as an allosteric modulator. The interaction of the phosphate with His302
18 and Arg305 on RING1 induces the dissociation of the Ubl domain that becomes accessible for
19 phosphorylation by PINK1 (Figure 7C, Active form). Subsequent conformational changes promote
20 the release of RING2 from RING0, converting PARKIN to its fully active state before targeting to
21 mitochondria [154, 156]. The mechanism of this translocation from the cytosol to mitochondria after
22 loss of $\Delta\Psi_m$ has been extensively debated. PINK1 was initially proposed to be the receptor for
23 PARKIN on depolarized mitochondria [150]. The employment of ubiquitin mutants demonstrated
24 nonetheless that poly-phosphorylated ubiquitin is the genuine PARKIN receptor on damaged
25 mitochondria [158, 159]. Consistent with this, the activation and mitochondrial recruitment of
26 PARKIN was recently found to be delayed upon inactivation of the RING domain ligase
27 MITOL/MARCH5 [160]. In this context, the primary ubiquitination step would be mediated by
28 MITOL/MARCH5 which is localized at the outer membrane [160]. PINK1-dependent

1 phosphorylation of ubiquitin and PARKIN activation would then trigger the ubiquitination of
2 numerous substrates on outer membranes (Figure 6, Panel I). As shown previously, the resulting
3 ubiquitin chains are subsequently phosphorylated by PINK1 which enhances the recruitment of
4 PARKIN molecules to mitochondria in a feed-forward amplification process [161].

5 As opposed to other ubiquitin ligases, PARKIN does not seem to recognize a specific amino
6 acid sequence on its substrates but was proposed to ubiquitinate outer membrane proteins in a non-
7 selective manner [160]. Among these hundreds of PARKIN substrates, including the voltage anion
8 channels VDAC1, 2, 3, the GTPases MIRO1 and 2 or the translocase of the outer membrane TOM70,
9 mitofusins (MFN1 and 2) were the first substrates to be identified approximately a decade ago [162].
10 In addition to the decoration of outer membrane proteins with phospho-ubiquitin and subsequent
11 autophagosomal recruitment, PARKIN-dependent ubiquitination was shown to induce the
12 proteasomal turnover of numerous proteins of the outer membrane. For instance, proteasome-
13 dependent degradation of factors such as TOM components leads to the rupture and permeabilization
14 of the outer membrane in parallel to mitophagy [163] (Figure 6, Panel III). MFN1 and 2 were shown
15 to be ubiquitinated by PARKIN and degraded by the proteasome in a p97 dependent manner leading
16 to reduced mitochondrial fusion followed by segregation of defective mitochondria [147] (Figure 6,
17 Panel IV). More recently, this degradation of mitofusins was linked to the dissociation of
18 mitochondria from the ER before engulfment by the autophagosome [164] (Figure 6, Panel II).

19

20 **Mitochondria-ER contact sites and mitophagy**

21 Contact sites between mitochondria and the ER were first described by electron microscopy
22 in the middle of the 20th century [165]. The later purification of MAM (Mitochondria Associated
23 Membranes), and their biochemical analysis demonstrated their involvement in lipid biosynthesis
24 [166]. Soon after it was proposed that MAM are sites of Ca²⁺ transfer, a process that was subsequently
25 shown to rely on VDAC1 on mitochondria and the Ca²⁺ release channel IP3R on the ER [167, 168]
26 (Figure 6, Panel II). In the last decade, the concept of MERC (Mitochondria ER Contact sites) has
27 emerged as multi-tasking platforms involved in many processes such as lipid transfer, mitochondrial
28 fission or mitophagy [169, 170]. A main characteristic of MERC relies on the physical connection of

1 their membranes by protein tethers [171]. On the outer membrane, these tethers include PACS-2,
2 VDAC1, PTPIP51 or the mitofusins MFN1 and MFN2 whereas IP3R, VAPB and MFN2 act on the
3 ER membrane [172]. The landmark discovery that MFN2 also localizes on the ER led to the finding
4 that mitofusins are enriched at MERC and involved in the regulation of these contact sites [173].
5 Whether MFN2 increases or diminishes the distance between the ER and mitochondria is subject to
6 controversy [173-176]. Nonetheless, its involvement as a regulator of MERC association and function
7 is widely accepted and was shown to be regulated by ubiquitination (Figure 6, Panel II).

8 In PARKIN KO cells, the level of MFN2 at MERC increases, the mitochondria-ER distance
9 as well as contact sites are affected and the transfer of Ca^{2+} from the ER to mitochondrial is perturbed
10 [177, 178]. This effect is specific of MFN2 since a mutation in its HR1 domain (K416R) blocks its
11 PARKIN-mediated ubiquitination and decreases ER-Mito contacts as well as Ca^{2+} transfer [178]. The
12 separation of mitochondria from the ER is also required for efficient mitophagy [164].
13 PINK1/PARKIN induce phospho-ubiquitination of MFN2 leading to its extraction from outer
14 membranes by p97 which promotes dissociation of the ER from mitochondria (Figure 6, Panel II)
15 prior to further phospho-ubiquitination and processing for mitophagy [164]. After CCCP treatment
16 (30-60 min), MFN2 is ubiquitinated rapidly, ahead of other substrates such as TOM20 or MIRO that
17 are modified hours later [164]. This indicates that ubiquitination of MFN2 is an early event in the
18 process of mitophagy.

19 In addition to PARKIN, MUL1 and MITOL/MARCH5 are two other ligases that regulate
20 MERC through ubiquitination of MFN2. MUL1, also denominated MAPL (Mitochondrial-Anchored
21 Protein Ligase), MULAN (Mitochondrial Ubiquitin Ligase Activator of NF- κ B) or GIDE (Growth
22 Inhibition and Death E3 ligase), is a mitochondrial outer membrane anchored protein. Besides its role
23 as an ubiquitin ligase, MUL1 preferentially conjugates the ubiquitin-like protein SUMO (Small
24 Ubiquitin Modifier) to its substrates. One of its targets is the mitochondrial fission Dynamin DRP1.
25 Once sumoylated, DRP1 induces mitochondrial fission and stabilizes MERCs which is a prerequisite
26 for Ca^{2+} flux and induction of apoptosis [179, 180]. MUL1 also binds and ubiquitinates MFN2 to
27 regulate the function of the mitofusin negatively [181]. This MUL1-dependent ubiquitination of
28 MFN2 regulates mitophagy before PARKIN during mild stress or in parallel to PARKIN during high

1 stress [181, 182]. The ubiquitin ligase MITOL promotes K63 polyubiquitination of mitochondrial
2 MFN2 on the lysine 192 localized in the GTPase domain. This ubiquitination does not induce
3 proteasomal degradation or mitophagy but was proposed to stimulate oligomerization of
4 mitochondrial and ER-resident MFN2 thereby improving the formation of MERCs [183]. MITOL
5 may thus have a dual role in promoting MERCs and in initiating PARKIN/PINK1 feed forward
6 process (Figure 6, Panel I) required for ubiquitin-dependent mitophagy [160].

7 Notably, other ubiquitin ligases including SMURF1 (SMAD Ubiquitination Regulatory
8 Factor 1), Gp78, HUWE1 or MGNR1 have been involved in mitofusins ubiquitination and mitophagy
9 but their mechanism of action remains to be fully described [184-187]. MFN1 has also been shown to
10 be ubiquitinated by HUWE1 to induce apoptosis [188]. In parallel, several deubiquitination enzymes
11 of the USP (Ubiquitin Specific Protease) family have been implicated in the regulation of MFN2 and
12 Mitophagy. USP8 was shown to antagonize ubiquitination by PARKIN and counteract mitophagy
13 [189] whereas USP15, USP30 and USP35 were found to deubiquitinate MFN2 and other outer
14 membrane proteins initially modified by PARKIN [190-192].

15 PARKIN which is undoubtedly the main ligase involved in macromitophagy has also been
16 implicated in other forms of mitophagy. In piecemeal mitophagy, the accumulation of misfolded
17 protein aggregates in the mitochondrial matrix induces the segregation of mitochondrial portions, in a
18 DRP1-dependent mechanism, and their degradation by PARKIN/PINK1-dependent mitophagy [22].
19 PARKIN was also shown to induce the sequestration of mitochondria in early endosomes before
20 fusion with lysosome [193]. In another form of micromitophagy, that also requires PARKIN and
21 PINK1, budding of vesicles from the mitochondrial outer membrane generates mitochondria derived
22 vesicles (MDVs) independent of Drp1. These MDVs subsequently fuse with endosomes before
23 merging with the lysosome [194-196]. In yeast, Mitochondria Derived Compartments (MDCs) have
24 been described in old cells [197]. Their formation requires components of the autophagy and
25 mitochondrial fission machineries. MDCs then carry a specific set of outer and inner membrane
26 proteins that are degraded after transport to the vacuole. No role for ubiquitin has been described in
27 this pathway and whether MDCs correspond to membrane vesicle compartments remains to be
28 confirmed.

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Ubiquitin and mitophagy in yeast

As opposed to mitofusins from metazoans, the yeast mitofusin Fzo1 has never been involved in mitophagy nor in the establishment of ER-Mitochondria contact sites. However, the yeast mitofusin was recently found to participate in the tethering of mitochondria to peroxisomes [198]. This function was deduced from the observation that overexpression of Fzo1 increases PerMit (Peroxisome-Mitochondria) contacts [198]. The functional significance of these PerMit contacts mediated by Fzo1 remains to be elucidated and, while a potential role for ubiquitination has been suggested, the precise mechanism of Fzo1 involvement in PerMit contacts needs to be further investigated.

While Fzo1 is not involved in the establishment of MERCs in yeast, this function is widely accepted to be established by the multi-protein complex ERMES (ER-Mitochondria Encounter Structure) (Figure 8) which includes Mmm1, an integral membrane protein of the ER, Mdm34 and Mdm10, two integral proteins of the mitochondrial outer membrane as well as the soluble protein Mdm12 [63]. Similar to MERCs in mammalian cells, ERMES-mediated contacts are essential for mitochondrial dynamics or ca^{2+} and phospholipid exchange but have also been implicated in mitophagy [199]. This involvement in mitophagy was first evidenced by the observation that ERMES colocalizes with components of the autophagosome [200]. Since Mdm34, Mmm1 and Mdm12 contain SMP (Synaptotagmin-like Mitochondrial-lipid binding Protein) domains that allow the binding of glycerophospholipids and especially phosphatidylcholine [201-203], this led suggesting a role for ERMES in the transfer of lipids from the ER to the growing autophagosome during mitophagy [200].

Shortly after, the first clue for an involvement of ubiquitin in yeast mitophagy came with the employment of a synthetic quantitative array (SQA) technology [116]. In this form of high throughput screen, an enzymatic reaction was used for the detection of general autophagy and specific mitophagy defects in the yeast knock-out strain collection. Cells were treated with Rapamycin for induction of mitophagy and the deubiquitinating enzyme Ubp3 as well as its cofactor Bre5 [204, 205] were identified both as inhibitors of mitophagy and activators of general autophagy. It was confirmed that deletion of either *UBP3* or *BRE5* leads to an increased induction of mitophagy upon treatment with Rapamycin but also upon other mitophagy inducing conditions. In addition, Ubp3 and Bre5 were

1 shown to be recruited to mitochondria after induction of mitophagy, thus suggesting a direct role of
2 the deubiquitination complex on mitochondrial proteins. These results are reminiscent of ubiquitin-
3 mediated mitophagy in metazoans. However, while Ltn1 and Rsp5 are two E3s that were previously
4 known to be counteracted by Ubp3/Bre5 in either ribophagy for Ltn1 or autophagy of misfolded
5 cytosolic proteins for Rsp5 [206], the substrates of the Ubp3/Bre5 complex and the ligase(s) that are
6 involved in yeast mitophagy remained unknown.

7 Mdm34 and Mdm12 were subsequently found to be subject to ubiquitination by Rsp5 [40,
8 117] (Figure 8). Both proteins contain L/PPXY (PY) motifs that are recognized by the WW domains
9 of Rsp5 [40, 117]. Mutagenesis of the Mdm34 PY motif not only inhibited Rsp5-mediated
10 ubiquitination of both Mdm34 and Mdm12 but was also found to induce defects in mitophagy [117].
11 Similar to metazoans, yeast mitophagy thus depends on a link between MERCs and ubiquitination
12 (Figure 8). While ubiquitinated by Rsp5, Mdm34 was highly stable and still detected after four hours
13 of cycloheximide (CHX) treatment [117]. However, in a distinct genetic background, Mdm34 was
14 rapidly degraded and no longer detected after 30 min of CHX chase [40]. This degradation was
15 dependent on Rsp5, Doa1, Cdc48 and the proteasome. Rsp5-mediated ubiquitination of Mdm34 is
16 thus implicated in both regulation of mitophagy but also in MAD of Mdm34 (Figure 8). This ability
17 of Rsp5 to favor proteasomal degradation of Mdm34 or to facilitate efficient mitophagy could relate
18 to distinct lysines that are targeted for ubiquitination within Mdm34 or, more likely, to the nature of
19 ubiquitin linkages that are used to modify the ERMES component.

20 Rsp5 conjugates monoubiquitin and short K63 linked chains on Mdm34 and Mdm12 [117].
21 This type of ubiquitination is preferred by Rsp5 but is a poor trigger for proteasomal degradation as
22 opposed to K48-linked chains composed of four ubiquitin moieties or more [207]. However, it has
23 been shown that Rsp5 is also able to catalyze the formation of K11, K33 and K48 chains *in vitro*
24 [208]. Moreover, editing of ubiquitin chains through trimming by DUBs and re-extension has been
25 described. For instance, K63 linked chains added on RNA polIII by Rsp5 are trimmed by Ubp2 to
26 leave only monoubitin that is further elongated with K48-linked ubiquitin chains by another E3 ligase
27 (elongin/cullin complex) before ultimate targeting to the proteasome [209]. In addition, after heat
28 shock, K63 linked ubiquitin chains added by Rsp5 are not only trimmed by Ubp2 and Ubp3 but the

1 increase in temperature also induces Rsp5 to catalyze K48-ubiquitination on trimmed chains to
2 promote degradation of the substrates by the proteasome [210, 211]. It is thus highly conceivable that
3 specific DUBs involved in the editing of ubiquitin chains made by Rsp5 dictate the fate of Mdm34
4 toward induction of mitophagy or proteasomal degradation. Ubp3 is a very good candidate for this
5 function given its ability to negatively regulate mitophagy and its involvement in the editing of K63
6 chains made by Rsp5.

7 Notably, whether Mdm34 is engaged in the formation of the ERMES complex or whether
8 Mdm34 is free of any interaction with ERMES components could also drastically modify the effect of
9 its Rsp5-mediated ubiquitination. In this regard, the ubiquitination of both Mdm34 and Mdm12 upon
10 induction of mitophagy [117] indicates that Rsp5 modifies both proteins while associated in the
11 ERMES complex (Figure 8). Similar to mammalian mitophagy, this ubiquitination may induce the
12 dissociation of MERCS and facilitate the engulfment of defective mitochondria in autophagosomes. A
13 non-mutual exclusive possibility is that Rsp5-mediated ubiquitination of ERMES could be recognized
14 by yeast autophagy receptors such as CUET proteins that act as ubiquitin-Atg8 adaptors [212]. In this
15 context, ubiquitin-dependent mitophagy in yeast would also share some very strong similarities with
16 metazoans. However, if yeast ubiquitin has been shown to be phosphorylated on Serine 37 and this
17 phosphorylation has been implicated in membrane trafficking [213], the kinase involved in this
18 phosphorylation remains unknown and the involvement of phospho-ubiquitin in yeast autophagy is
19 not established. The role of the UPS in yeast mitophagy thus holds yet to be discovered features
20 (Figure 8) that will reveal the extent of homology between yeast and mammalian systems.

21

22 **CONCLUSION**

23 We have seen multiple facets of mitochondrial homeostasis regulation by the UPS. From
24 mitochondrial quality control pathways to the regulation of specific functions, the UPS could be
25 compared as a Swiss knife without which mitochondria could simply not maintain its integrity in the
26 cell. Nonetheless, what is now evident was not so obvious fifteen years ago when mitochondria,
27 despite all its complexity, was already known to be devoid of ubiquitin. It was then conceived that the
28 surface of the organelle could be subject to a UPS-mediated regulation similar to that taking place on

1 the ER membrane. The Mdm30-mediated control of Fzo1, the PARKIN-dependent regulation of
2 mitophagy and, more recently, the successive discovery of diverse MAD pathways, not only
3 confirmed this initial view but also overtook all expectations. The next challenges will now consist in
4 taking a step backward and identify the ligases involved in each MAD pathway as well as their
5 intrinsic relationships. Regarding mitochondrial fusion, it will be informative to investigate its control
6 in mammalian cells as deeply as it has been carried out in yeast over the last twelve years. As for
7 mitophagy, the legacy of all discoveries achieved in metazoans will undoubtedly benefit dissecting
8 the role of the UPS in yeast mitochondrial clearance. The UPS and mitochondria still have a lot of
9 new gifts to offer for the next decade.

10

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17

18 **AUTHOR CONTRIBUTIONS**

19 CA wrote the introduction and section 1. MMC wrote section 2. NBT wrote section 3. MMC edited
20 the whole manuscript. CA generated Figures 1, 2, 3 and 5. LC generated Figure 4. OO generated
21 Figures 6 and 8. NBT generated Figure 7 and supervised the design of Figures 6 and 8. MMC
22 supervised the design of all Figures.

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1 FIGURE LEGENDS

2 **Figure 1:** The Ubiquitination cascade. An ubiquitin activating enzyme E1, promotes a thioester bond
3 between the C-terminus of ubiquitin and the catalytic cysteine of a conjugating enzyme E2. The
4 ubiquitin ligase then links the loaded E2 to a specific substrate. The direct (RING E3s) or sequential
5 (HECT or RBR E3s) transfer of ubiquitin from the E2 to the substrate then induces the formation of
6 an isopeptide bond between the C-terminus of ubiquitin and a target lysine of the substrate. With its
7 seven lysines or its N-terminal methionine, ubiquitin can itself be the target of the ubiquitination
8 cascade. This results in the formation of chains with diverse ubiquitin linkages that can trigger very
9 distinct functions.

10

11 **Figure 2:** UPS-mediated degradation at the mitochondrial outer membrane. Mitochondrial
12 morphology is maintained by ongoing events of fusion and fission and intimate contacts with the ER.
13 The UPS, through E3s that are known (Ltn1, Doa10, Mdm30) or yet to be identified, safeguards the
14 transport of proteins inside the organelle but also specific proteins embedded in outer membranes. (I)
15 In the absence of cellular stress, proteins encoded by the nuclear genome are synthesized, folded and
16 escorted to mitochondria. Import through the TOM complex is continuously monitored by the TOM
17 Associated Degradation (mitoTAD) pathway. (II) Upon ribosomal stalling, the Ribosomal Quality
18 Control (RQC) pathway is activated which also prevents clogging of the TOM channel. (III) When
19 the level of stress increases and the mitochondrial import is inhibited, the mitochondrial Precursor
20 Over-accumulation Stress (mPOS) initiates the Unfolded Protein Response Activated by the mis-
21 targeting of proteins (UPRam) which allows clearance of protein aggregates. If clogging of the TOM
22 channel persists, the Mitochondrial Compromised Protein import Response (mitoCPR) is activated.
23 (IV) Two Mitochondrial Associated Degradation (MAD) pathways that may share some common
24 features also target mis-targeted Tail Anchored proteins (MAD-TA) and C-terminally anchored outer
25 membranes proteins (MAD-C). (V) The yeast mitofusin Fzo1 is regulated by the UPS during Outer
26 Mitochondrial Membrane (OMM) fusion.

27

1 **Figure 3:** The yeast mitofusins Fzo1 and its binding to Mdm30. (A) Fzo1 includes a bipartite
2 transmembrane domain (purple) that allows exposure of its N- and C-terminal extremities in the
3 cytoplasm. The GTPase domain (yellow-orange) and Heptad Repeat regions HRN, HR1 (blue) and
4 HR2 (green) are indicated. All point mutations mentioned in the main text are also indicated. The
5 scale at the bottom indicates the precise location of each domain. (B) Capacity of Mdm30 to bind
6 distinct versions of Fzo1. When the GTPase domain is mutated (red cross), Mdm30 does not bind
7 full-length but does bind the N-terminal half of Fzo1 as shown in [85]. This suggests a conformational
8 switch where the GTPase domain promotes displacement of the C-terminal half which allows access
9 of Mdm30 to the N-terminal half of Fzo1. (C) From left to right: Topology of Fzo1 on the
10 mitochondrial outer membrane; Structural model of Fzo1 as described in [94]; The colors indicate the
11 positions of the HRN, HR1, HR2, TMs and GTPase domains. The four-helix bundle and the trunk of
12 Fzo1 formed in the model are highlighted.; Schemes of closed and opened conformations of Fzo1
13 based on the structural model. The GTPase domain would induce displacement of the four-helix
14 bundle relative to the trunk to yield the opened conformation of Fzo1. Mdm30 would bind the opened
15 but not the closed conformation of Fzo1.

16

17 **Figure 4:** A mechanistic model of outer membrane fusion and its regulation by the UPS. (A) *In vitro*
18 mitochondrial attachment and fusion as observed by cryo-ET (see main text). 3D renderings of
19 attached outer membranes (in red and yellow) at distinct stages of the fusion process are shown.
20 Protein densities are depicted in blue. These data are from Brandt et al. [96]. (B) Cis-dimerization of
21 Fzo1 and ubiquitination by Mdm30. Fzo1 is depicted as the 3D model described in DeVecchis et al.
22 [94] Its conformational switch and cis dimerization is depicted as in Brandner et al. [97]. Mdm30
23 would promote ubiquitination and degradation of free cis-dimers in which Fzo1 has undergone its
24 conformational switch. (C) Cis-dimerization of Fzo1 after GTP binding would induce membrane
25 tethering through the formation of Fzo1 trans-oligomers as described in Brandner et al. [97]. GTP
26 hydrolysis within the oligomers would bring membranes closer together followed by the dissociation
27 of Fzo1 molecules that would redistribute at the edge of this region of close apposition. Successive

1 cycles of GTP binding and hydrolysis would culminate in the extension of the surface of apposition
2 surrounded by the docking ring composed of a macromolecular Fzo1 trans-oligomer. The ultimate
3 cycle would induce fusion where membrane curvature is most pronounced concomitant with
4 dissociation of the docking ring. (D) In the absence of Mdm30, Fzo1 cis-dimers would be stabilized
5 which would perturb the regulated assembly of trans-oligomers thereby resulting in abortive
6 complexes that inhibit formation of the docking ring and block outer membrane fusion as upon Fzo1
7 overexpression in Brandt et al. [96].

8

9 **Figure 5:** Mitochondrial fusion is regulated by a balance between Fzo1 degradation and the
10 desaturation of fatty acids. When the desaturation of fatty acids is low (low UFA), mitochondrial
11 fusion is efficient if the degradation of Fzo1 is high. For this purpose, Ubp2 is ubiquitinated by
12 Mdm30, which induces its degradation by the proteasome. Mdm30-mediated ubiquitination of Fzo1 is
13 not antagonized which allows extension of ubiquitin chains and efficient proteasomal degradation of
14 the mitofusin. When the desaturation of fatty acids is high (high UFA), mitochondrial fusion is
15 efficient if the level of Fzo1 is also high. For this purpose, the ubiquitination of Fzo1 by Mdm30 is
16 antagonized by Ubp2 which limits the extension of ubiquitin chains and decreases the degradation of
17 the mitofusin. At the bottom, efficient mitochondrial outer membrane (MOM) fusion is symbolized
18 by formation of the Docking ring.

19

20 **Figure 6:** Ubiquitin-dependent mitophagy in metazoans. (I) In damaged mitochondria that have lost
21 their membrane potential ($\Delta\Psi_m$), the import of PINK1 is blocked which induces its accumulation on
22 the mitochondrial outer membrane. Autophosphorylation, dimerization and association with the TOM
23 complex activate PINK1 which phosphorylates ubiquitin either free or already conjugated to
24 substrates by MITOL. Parkin activation through binding of phospho-ubiquitin and phosphorylation by
25 PINK1 leads to its recruitment to the mitochondria where it associates with phospho-ubiquitinated
26 substrates and induces massive ubiquitination of proteins from the outer membrane. Phospho-
27 ubiquitinated substrates associate with ubiquitin binding adaptors that themselves bind to LC3 on pre-
28 autophagosomal membranes. After full engulfment in the autophagosome that fuses with the

1 lysosome, mitochondria are degraded and their components recycled in the cytosol. (II) This process
2 of mitophagy requires dissociation of mitochondria from the ER that are normally connected by
3 several tethers. One of these tethers, MFN2, is a target of phospho-ubiquitination by PINK1/PARKIN
4 which induces its p97/VCP-mediated extraction from the outer membrane and subsequent degradation
5 by the proteasome, resulting in dissociation of mitochondria from the ER. (III) After mitochondrial
6 damage and in parallel to mitophagy, numerous proteins of the outer membrane (TOM20, TOM40...)
7 are ubiquitinated by PARKIN, extracted by p97/VCP and degraded by the proteasome leading to
8 permeabilization and rupture of the outer membrane. (IV) After loss of $\Delta\Psi_m$, sequential
9 ubiquitination of MFN1 and MFN2 by PARKIN, extraction by p97/VCP and degradation by the
10 proteasome also leads to a decrease in mitochondrial fusion which participates in segregating
11 damaged mitochondria for subsequent degradation by mitophagy.

12

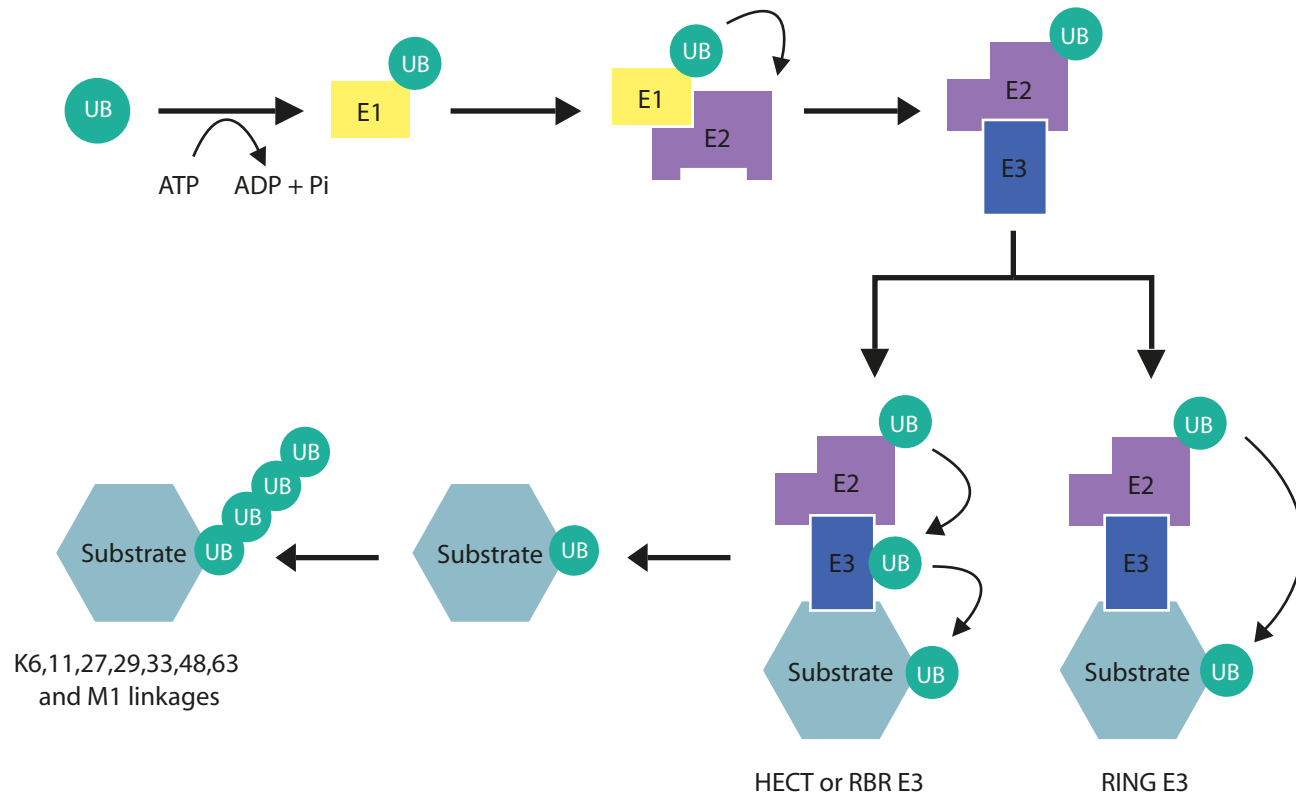
13 **Figure 7:** PINK1 targeting to mitochondria and conformations of the RBR ubiquitin ligase PARKIN.

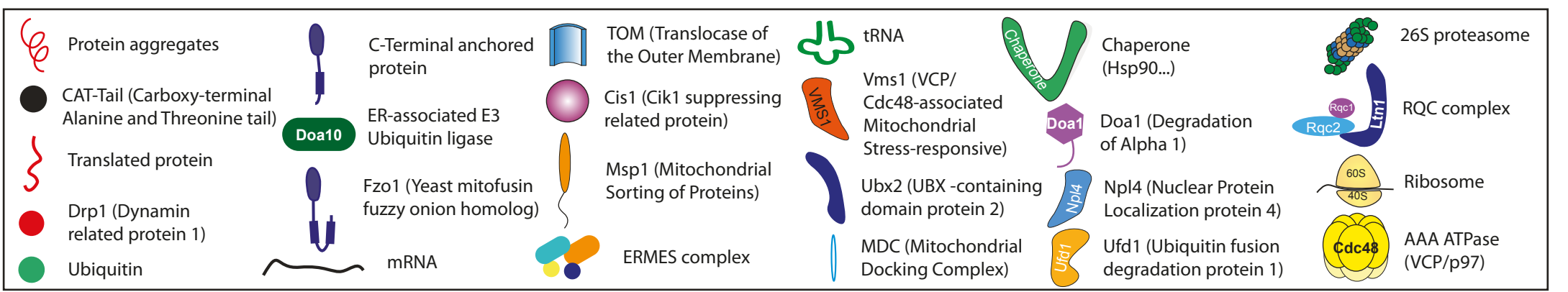
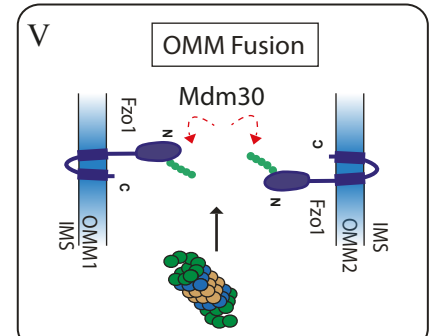
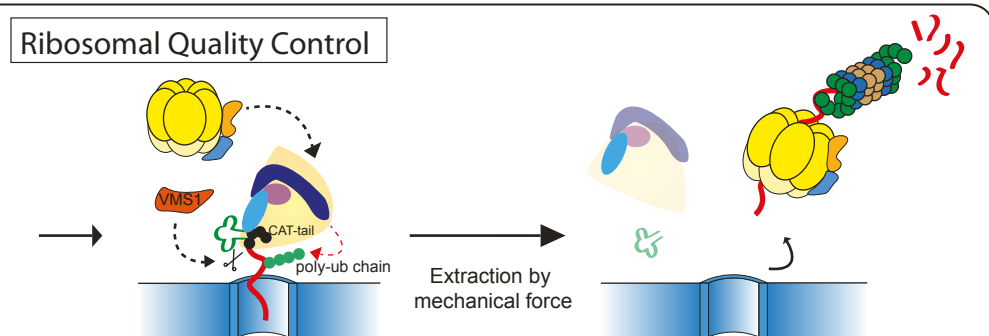
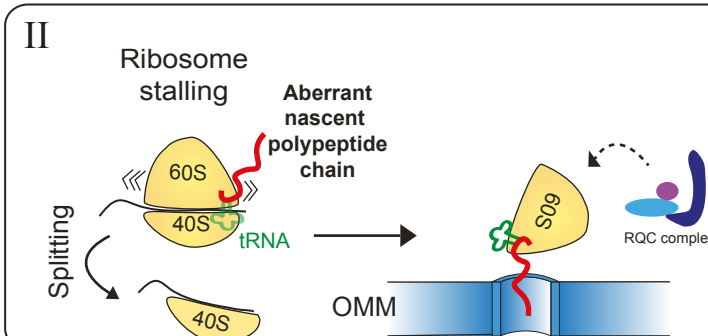
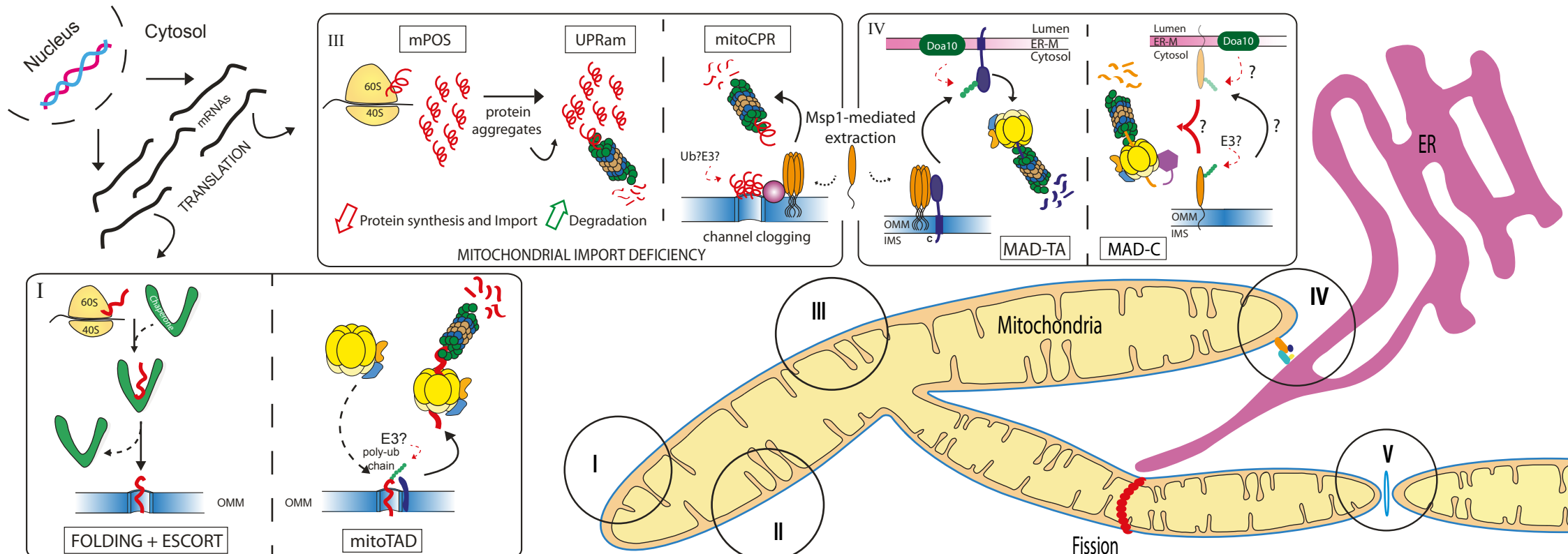
14 (A) In healthy mitochondria PINK1 levels are low. The kinase is imported into mitochondria and
15 retro-translocated to the cytosol to be degraded by the UPS N-end rule pathway. (B) The distinct
16 domains of PARKIN are indicated. His302 and Arg305 on RING1, Ser65 on the Ubl and Cys431 on
17 RING2 are the main residues implicated in the activation of the E3. The scale bar at the bottom
18 indicates the relative position of each domain. (C) Transition of PARKIN from the inactive to the
19 active state initiates with the binding of phospho-ubiquitin to His302 and Arg305 of RING1. This
20 displaces the Ubl that is phosphorylated on Ser65 by PINK1 which gives robust access to the E2
21 binding site on RING1. Since RBR ligases function like HECT E3s, ubiquitin from the loaded E2 can
22 be transferred to the catalytic Cys431 of RING2 that became accessible after its dissociation from
23 RING0.

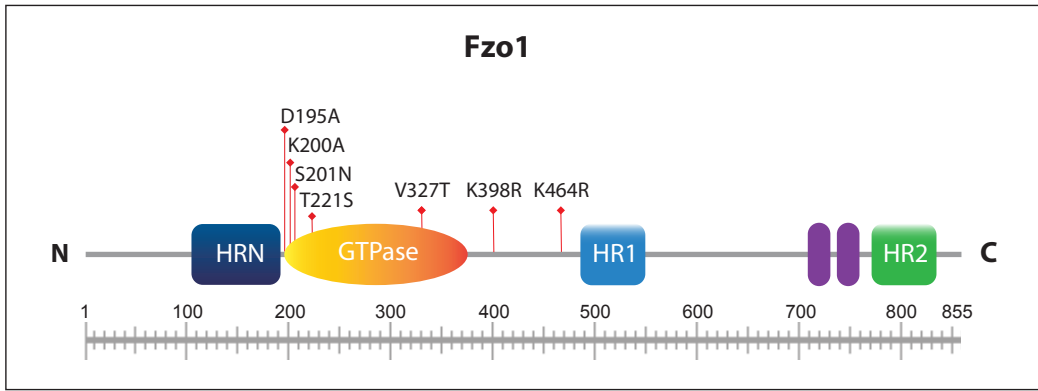
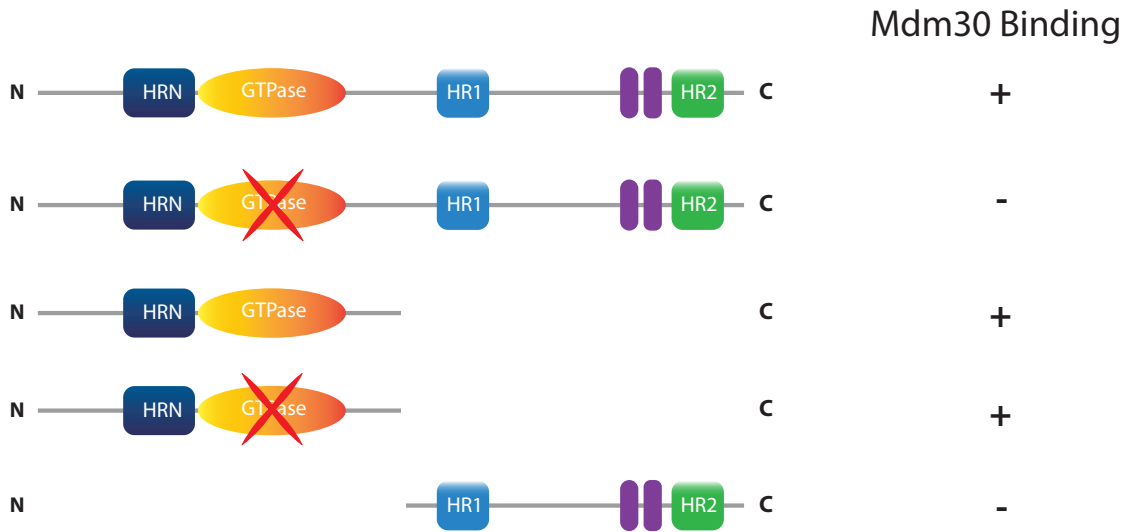
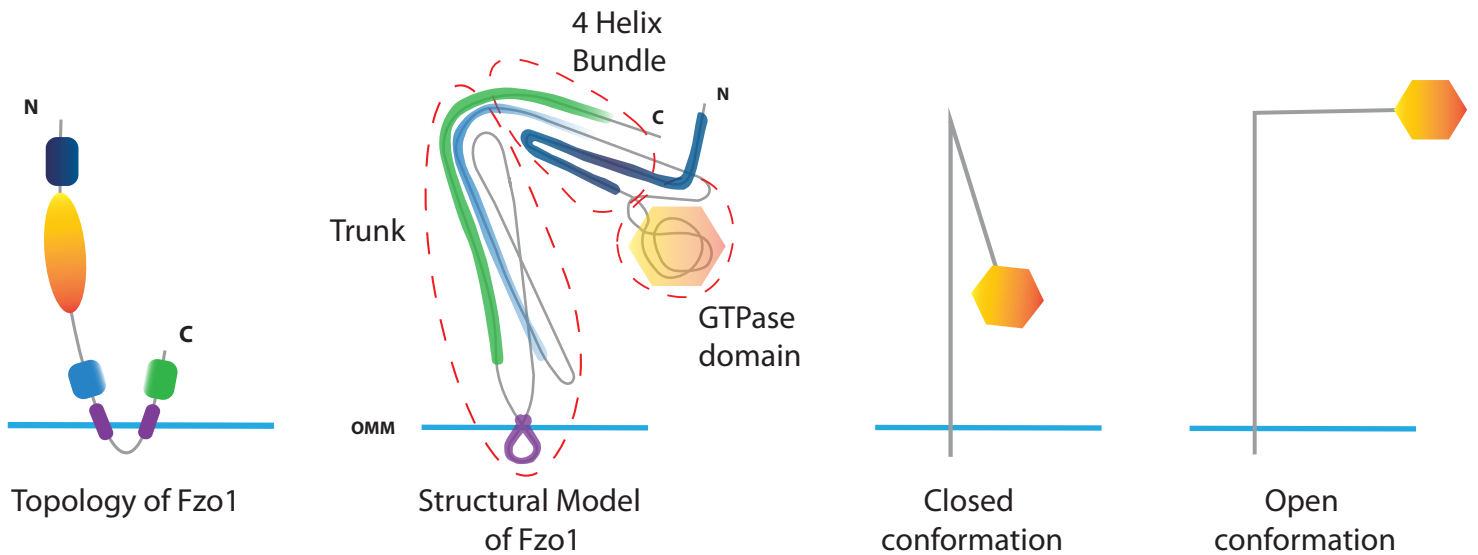
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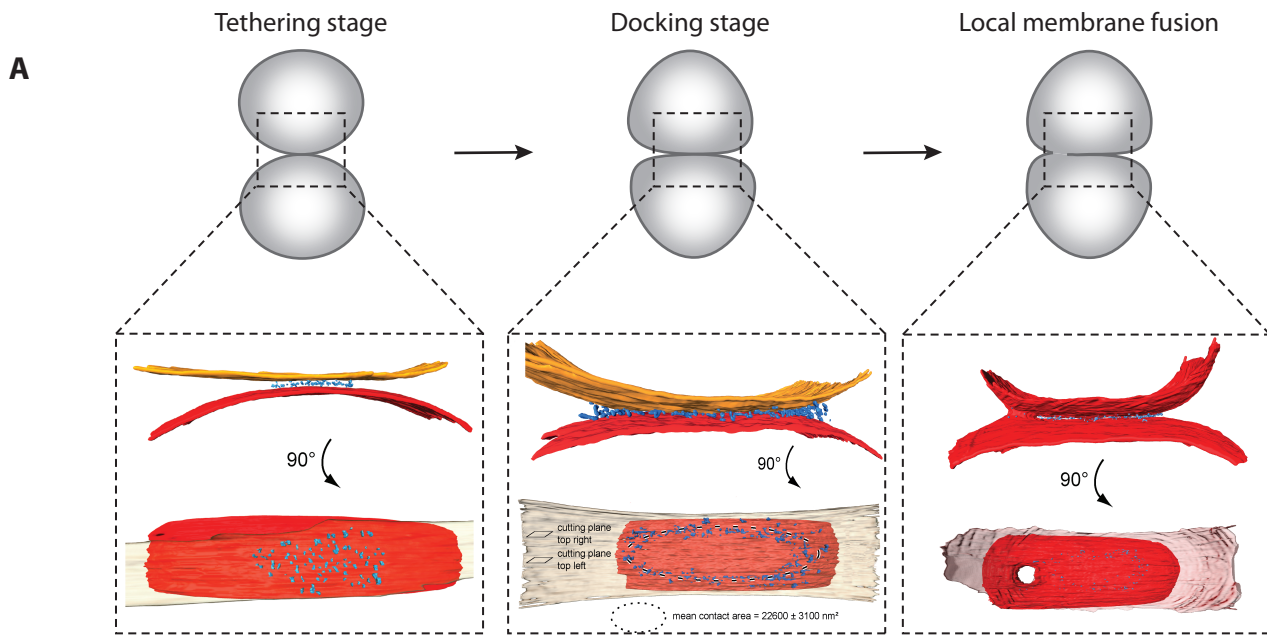
25 **Figure 8:** Mitophagy in yeast. Targeting to the autophagosome begins with phosphorylation of the
26 mitochondrial autophagy receptor Atg32. Phosphorylated Atg32 interacts with the protein adaptor
27 Atg11 and the complex is targeted to the pre-autophagosomal site assembly where Atg32 can interact
28 with Atg8 that is anchored in the membrane of the pre-autophagosome. Yeast mitophagy also

1 involves the contacts between the ER and the mitochondria that are mediated by the ERMES complex
2 composed of Mdm10, Mdm34, Mdm12 and Mmm1. In conditions of mitophagy induction, Mdm34
3 and Mdm12 are ubiquitinated by Rsp5. While inhibition of this ubiquitination affects mitophagy, its
4 precise function remains to be understood. ERMES has been proposed to provide lipids to the
5 growing autophagosome and ubiquitination could play a role in this phenomenon. Alternatively, Rsp5
6 could promote the targeting of mitochondria to the autophagosome, similar to PARKIN. In fact, in
7 analogy to PARKIN with metazoan mitofusins, Rsp5-mediated ubiquitination of Mdm34 is not only
8 involved in mitophagy but also promotes its Cdc48-dependent proteasomal degradation.

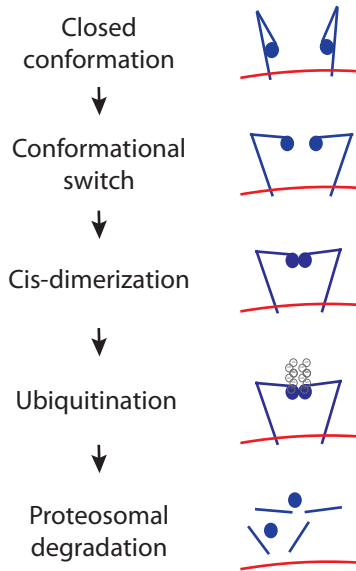




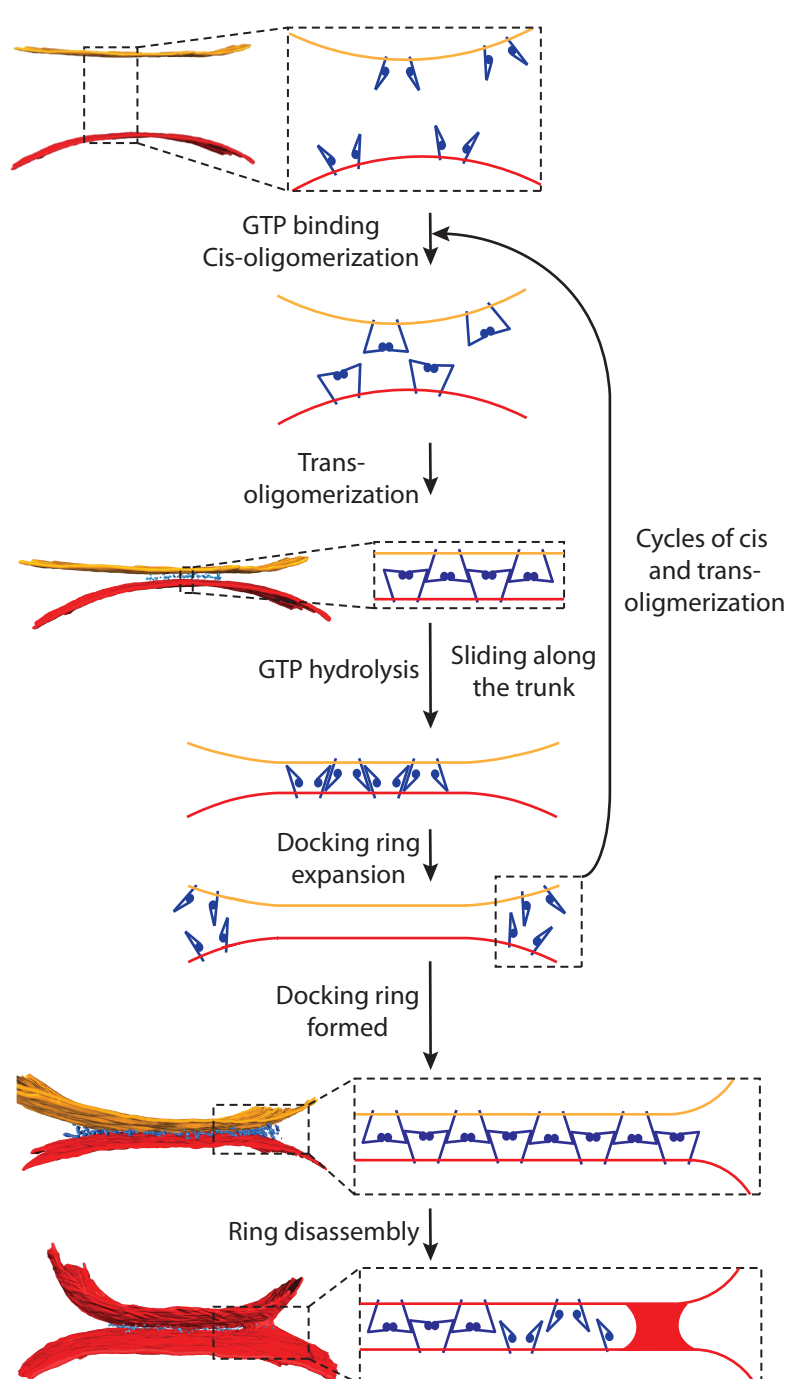
A**B****C**



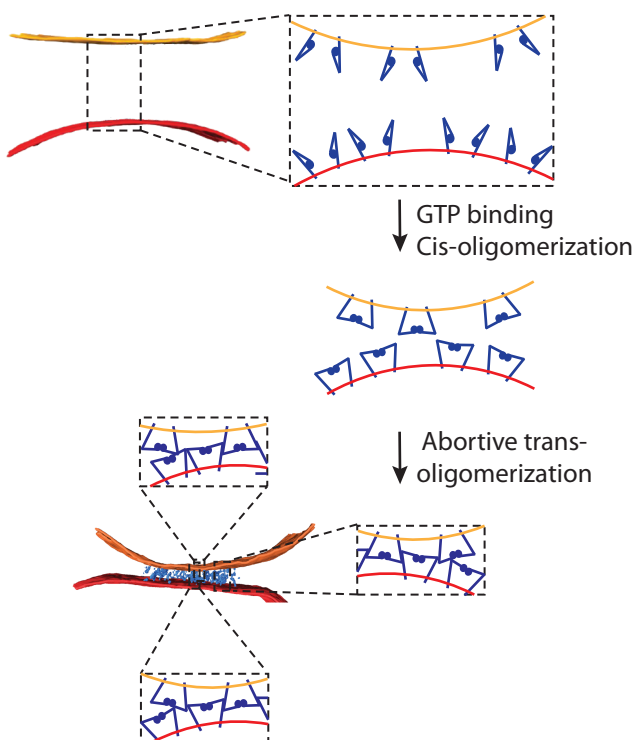
B Ubiquitination by Mdm30

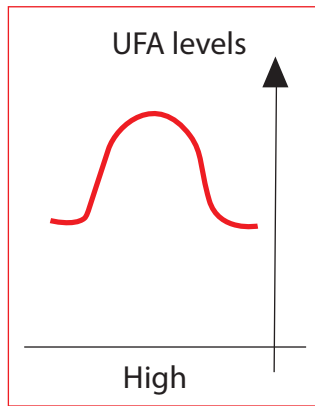
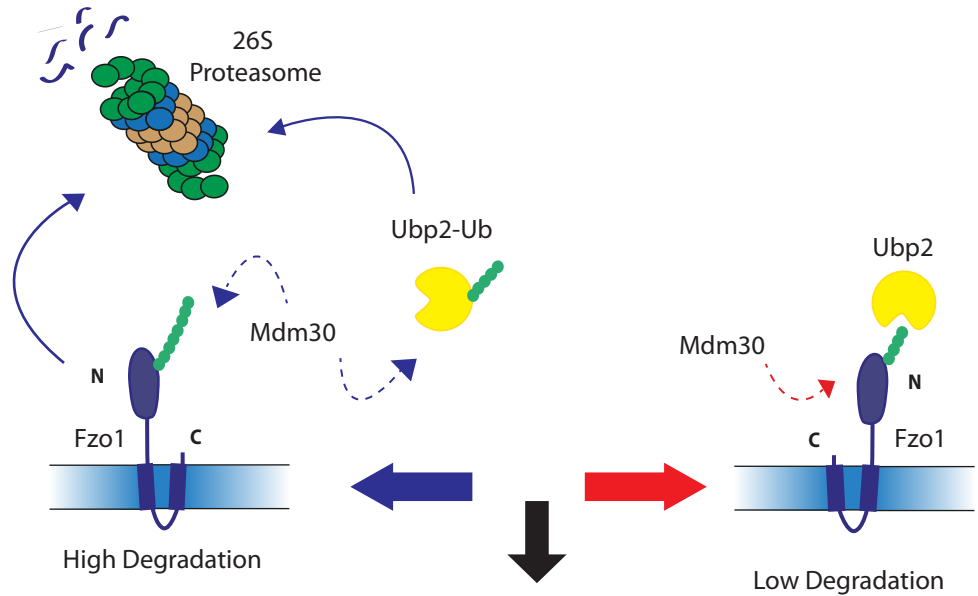
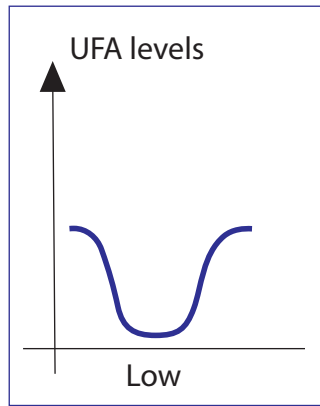


C With ubiquitination



D Without ubiquitination





Functional MOM fusion

