Discoveries

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Mitochondrial Protein Quality Control: The Mechanisms Guarding Mitochondrial Health

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Abstract

Significance: Mitochondria are complex dynamic organelles pivotal for cellular physiology and human health. Failure to maintain mitochondrial health leads to numerous maladies that include late-onset neurodegenerative diseases and cardiovascular disorders. Furthermore, a decline in mitochondrial health is prevalent with aging. A set of evolutionary conserved mechanisms known as mitochondrial quality control (MQC) is involved in recognition and correction of the mitochondrial proteome. *Recent Advances:* Here, we review current knowledge and latest developments in MQC. We particularly focus on the proteolytic aspect of MQC and its impact on health and aging. *Critical Issues:* While our knowledge about MQC is steadily growing, critical gaps remain in the mechanistic understanding of how MQC modules sense damage and preserve mitochondrial welfare, particularly in higher organisms. *Future Directions:* Delineating how coordinated action of the MQC modules orchestrates physiological responses on both organellar and cellular levels will further elucidate the current picture of MQC's role and function in health, cellular stress, and degenerative diseases. *Antioxid. Redox Signal.* 22, 977–994.

Introduction

MITOCHONDRIA ARE DYNAMIC semiautonomous organelles present in virtually all eukaryotic cells and play fundamentally important roles in various aspects of cellular physiology. The vital functions of mitochondria include generation of ATP through respiration, integration of several key metabolic and cofactor-generating pathways, and regulation of ion homeostasis and apoptosis (141, 179). Perturbations of mitochondrial homeostasis and integrity lead to severe pathophysiological consequences and the onset of disease. Numerous studies implicate mitochondrial dysfunction as an underlying factor of multiple pathologies in humans, including cardiovascular disorders, myopathies, certain cancers, type II diabetes, and neurological and neurodegenerative diseases (23, 36, 50, 95, 104, 115, 130, 141, 149, 157, 179, 188). These maladies become particularly prevalent as people age and have been linked with age-associated decline in mitochondrial health (18, 95, 115, 157). Given the fundamental biological roles that mitochondria play in cellular physiology, it is crucial to sustain their wel-

fare. To this end, several interdependent mechanisms exist, from the molecular to the organellar level, to ensure mitochondrial homeostasis. These conserved mitochondrial quality control (MQC) mechanisms maintain the mitochondrial proteome to promote the organelle's normal function and thus cellular survival (7, 18, 21, 95, 157, 175).

The endosymbiotic origin of mitochondria defines several distinctive hallmarks of these organelles. Mitochondria, whose proteome accounts for 900–1500 polypeptides (146, 165), retained two membranes, the outer mitochondrial membrane (OM) and the inner mitochondrial membrane (IM), respectively. These phospholipid bilayers segregate two mitochondrial compartments—the matrix and the intermembrane space (IMS) (Fig. 1). The large matrix compartment houses multiple metabolic enzymes and the mitochondrion's own small circular genome (mtDNA) and the machineries necessary for its replication and expression. A recent study showed that ∼500 proteins reside in the matrix of mammalian mitochondrion (156). The IMS compartment is smaller and contains fewer (∼60) proteins (82, 183). The large portion of mitochondrial proteins—including the

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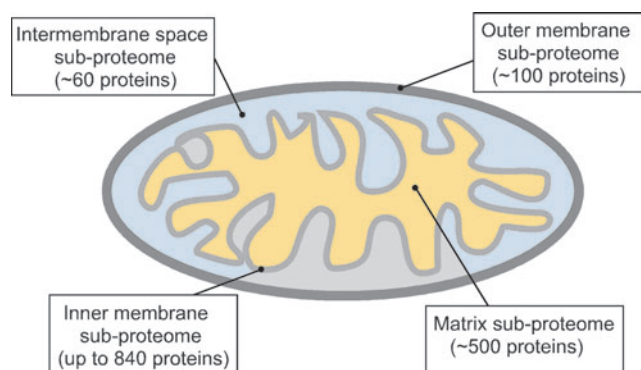
FIG. 1. Distribution of mitochondrial proteome throughout the organelle. The vast majority of mitochondrial proteins reside in the matrix and inner mitochondrial membrane (IM) subcompartments. The approximate numbers of polypeptides in each subproteome are calculated based on available data (82, 146, 156, 165, 185, 198). To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars

electron transport chain and F_1F_O ATPase multiprotein complexes (collectively known as the oxidative phosphorylation [OXPHOS] system)—localizes to the IM. The IM proteome is particularly enriched in the specific invaginations of the membrane termed cristae. The OM subproteome is estimated to contain ~ 100 polypeptides (198). Another

hallmark of the mitochondrial proteome is its bigenomic nature. The vast majority of proteins comprising the mitochondrial proteome are encoded by nuclear genes, synthesized in the cytosol, and subsequently imported into the organelle, while only 13 of the ~ 1500 mitochondrial proteins are derived from mtDNA (18, 147, 179), These unique properties of mitochondrial architecture impose several significant challenges to mitochondrial functions and necessitate constant monitoring by MQC (Fig. 2). One challenge stems from the inherent generation of reactive oxygen species (ROS) by the electron transport chain complexes of OXPHOS (2, 9, 61) (Fig. 2A). During this process, some electrons may leak from the electron transport chain and rapidly react with molecular oxygen. Various reports estimate that 0.3%–2% of the mitochondrial O_2 consumption may be diverted toward ROS formation (2, 9, 61). Incomplete reduction of O_2 by escaping electrons leads to the formation of highly reactive superoxide anion, which can further promote formation of other ROS and reactive nitrogen species (RNS) radicals (161). While free radical-scavenging mechanisms are in place (82), they are not always sufficient to eliminate harmful ROS/RNS that are highly damaging to nucleic acids, proteins, and lipids (61, 161). In addition, many ROS are now recognized as important signaling molecules essential for intracellular communication and stress response (161). Therefore, as exemplified by a number of failed trials aiming to correct mitochondrial damage by antioxidant treatment (30, 29), massive neutralization of ROS may be equally detrimental for cells.
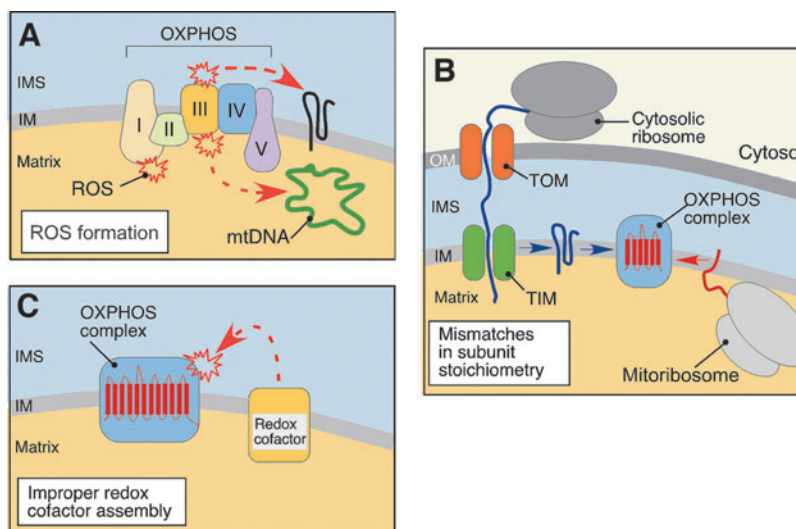

FIG. 2. Biochemical stresses that challenge normal mitochondrial function. Mitochondrial respiration is inherently linked to reactive oxygen species (ROS) production due to incomplete reduction of molecular oxygen by electron transport components of the oxidative phosphorylation system (OXPHOS system) **(A)**. Stalling the high-energy electrons at respiratory complexes I and III leads to generation of superoxide anion which—either directly or *via* subsequent ROS radicals—can damage biological molecules like mtDNA and propel additional damage. The biogenesis of OXPHOS complexes requires tight coordination between synthesis and assembly of the mitochondrial- and nuclear-coded proteins **(B)**. Polypeptides derived from the nuclear genome are translated on cytosolic ribosomes and imported in an unfolded state into the mitochondrion *via* presequence translocases of the outer (TOM) and inner (TIM) membranes. Imported polypeptides are inserted into the IM where they are joined with mitochondria-synthesized subunits. Mismatches in subunit stoichiometry can lead to accumulation of unfolded or unassembled proteins that can affect functional integrity of mitochondria. In addition, the electron transport chain units of OXPHOS contain redox-active cofactors poised for rapid electron exchange reactions **(C)**. When improperly assembled, these prosthetic groups can act as pro-oxidants through their inherent ability to generate ROS *via* Fenton-like reactions (61, 161). To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars

The other challenge arises from the dual genetic origin of several OXPHOS complexes (complexes I, III, IV, and V) (Fig. 2B). Biogenesis of these respiratory units requires tightly coordinated expression, sorting and folding of both mtDNA- and nuclear-coded polypeptides, and their subsequent assembly into stoichiometric complexes within the IM (18, 128). If such coordination fails, unfolded or orphaned subunits that are prone to misfolding and/or aggregation may accumulate. Finally, the electron transport chain complexes contain multiple redox cofactors, which when improperly assembled or stalled in the assembly process, can act as pro-oxidants and further contribute to the challenging biochemical environment in the organelle (31, 99, 100, 132) (Fig. 2C). If unopposed, these challenges can distort mitochondrial protein homeostasis and propel progressive mitochondrial failure. The acute failure at one of the aforementioned risk sites may trigger a so-called vicious cycle—a series of deleterious events leading to the gradual increase of mitochondrial damage. For instance, accumulation of unassembled or misfolded polypeptides can impede biogenesis and/or function of the OXPHOS, which in turn will produce excessive ROS. These can further disrupt the mitochondrial proteome through induction of mutations in mtDNA and additional perturbation of protein folding, leading to more ROS and ROS-induced damage and ultimately, to the demise of the organelles (18, 57).

In this review, we summarize the current knowledge about the MQC mechanisms by which cells cope with biochemical stresses arising from the unique functional and organizational properties of mitochondria, and how these mechanisms sustain normal mitochondrial function and integrity. We will primarily focus on the proteolytic facet of MQC and outline recent advances and current concepts in the field.

Overview of Mitochondrial Protein Quality Control

Because of inherent susceptibility of mitochondria to biochemical stresses, elaborate multilayer quality control mechanisms have evolved that survey, repair, or eliminate damaged mitochondria. Depending on the extent of damage, MQC mechanisms can engage at several levels (Fig. 3). The molecular level of MQC (referred hereafter as protein MQC [PMQC]) includes a network of evolutionary conserved mitochondrial proteases and chaperones distributed across mitochondrial compartments (Fig. 3A), as well as cytosolic proteolytic systems like the ubiquitin–proteasome system (UPS), which can associate with the OM (79, 96, 116, 175) (Fig. 3B). Although the number of studies addressing PMQC in mammalian cells is steadily growing, most of our current knowledge stems from studies in bacteria and the yeast model. The key molecules involved in PMQC are summarized in Table 1. One layer of PMQC is represented by ATP-dependent chaperones of mtHsp70 and Hsp60 heat-shock protein families, responsible for the sorting, folding, and disaggregation of proteins in the matrix compartment (136, 187). Similarly, the Hsp70-type and Hsp90-type chaperones operate in the cytosol and prevent aggregation and facilitate transport of unfolded newly synthesized or nascent polypeptides into mitochondria (60, 75, 197). The proteolytic facet of PMQC includes multiple conserved proteases (7, 18, 21, 157). The proteases are distributed across mitochondrial subcompartments and generally can be divided into two

groups: (i) ATP-dependent, also known as AAA$^+$ (ATPase associated with diverse cellular activities) proteases and (ii) ATP-independent proteolytic enzymes. The former group includes ClpXP and Lon/Pim1 proteases that reside in the matrix and degrade oxidatively damaged or aggregated polypeptides in the compartment (24, 25, 26, 34, 94, 77, 171, 181). Another two AAA$^+$ proteases localize to the IM. The m-AAA (matrix AAA) protease has its active site exposed to the matrix side of the IM where it performs its quality control functions (11). The active site of i-AAA (intermembrane space AAA) peptidase faces the IMS (113, 190). These proteases appear to be important for removal of dysfunctional or orphaned proteins that are intrinsic to or associated with the IM (11, 105, 112, 113, 190). The AAA$^+$ proteases typically exist as homo-oligomeric (Lon/Pim1, i-AAA) or hetero-oligomeric (m-AAA, ClpXP) complexes, and their proteolytic activity is coupled to ATP hydrolysis (13, 22, 159, 181). Such molecular architecture also permits chaperone-like functions of AAA$^+$ proteases and appears to confer the ability to recognize misfolded or unassembled proteins and refold, and/or extract these polypeptides from phospholipid bilayers (159). The second group of proteases is more heterogeneous and includes the following: (i) processing peptidases (MPP, Oct1, Icp55, Cym1, and IMP) involved in sequential removal and/or degradation of mitochondrial targeting sequences (MTS) and thus proper biogenesis, sorting, and stabilization of matrix-and IM-targeted proteins (3, 73, 92, 131, 133, 176, 185); (ii) soluble peptidases (Prd1/Neurolysin, Atp23, and Ynm3/Omi) that seemingly contribute to MQC in the IMS (92, 143, 145, 180, 199); and (iii) IM-bound proteases (Pcp1/PARL, Oma1) implicated in the quality control of the IM proteome and regulation of mitochondrial dynamics (8, 19, 54, 58, 78, 83, 97, 127).

Growing evidence indicates that the cytosolic UPS also represents an important facet of PMQC. First, UPS has been shown to participate in quality control and removal of several mitochondria-targeted proteins before or during their import into the organelle (7, 35, 116). Moreover, UPS can access the OM subproteome and mediate retrotranslocation and degradation of OM resident proteins—a process termed mitochondria-associated degradation (MAD) (79, 96, 116). MAD apparently relies on p97/Cdc48 AAA$^+$ protein (79, 193), which is also involved in a well-described extraction of ubiquitylated proteins from the endoplasmic reticulum (ER) (37, 88).

Several MQC mechanisms are available on the organellar level (Fig. 3C–E). Fusion and fission events (Fig. 3C) mediate organellar dynamics and facilitate mitochondrial biogenesis, and even redistribution of mtDNA and proteome throughout the mitochondrial network (39, 41, 64, 196). Such redistribution due to fusion of several mitochondria permits the dilution of damaged molecules and/or replenishment of depleted components in malfunctioning organelles (41, 196). A phenomenon known as stress-induced mitochondrial hyperfusion (164, 177) represents an example of coupling between mitochondrial fusion and cellular stress response and highlights the significance of mitochondrial dynamics in stress management. Upon homeostatic insults like oxidative stress or starvation, mitochondria in the stressed cells form highly interconnected networks thereby increasing content mixing, ATP production, and protecting mitochondria from autophagic removal (68, 138, 155, 164, 177). When transient

stress protection *via* mitochondrial hyperfusion is not sufficient, damaged organelles are removed from the network through fragmentation (fission) events (178). Mitochondrial fission serves to increase the number of mitochondria in the cell before mitochondrial biogenesis or cellular division, as well as to segregate dysfunctional or depolarized mitochondria away from the healthy network (144, 178, 196). Once malfunctioning (*e.g.,* severely depolarized) mitochondria have been segregated, the components of their OM subproteome that are involved in establishing contact/tethering sites with other mitochondria are ubiquitylated and proteolyzed by UPS to prevent their rejoining with healthy mitochondria (56, 66, 172, 189). Then, damaged organelles are removed *via* another facet of organellar MQC (Fig. 3E)—a mitochondria-specific type of autophagy known as mitophagy (see Refs. 119, 195 for detailed review). An acute overwhelming stress such as treatment with oxidants causes massive fragmentation of the mitochondrial network followed by initiation of apoptosis (194). Conversely, genetic inhibition of mitochondrial fission increases apoptotic resistance and cell survival (109, 154).

Finally, a novel MQC mechanism has recently been described by the McBride laboratory (Fig. 3D). Mitochondria-derived vesicles (MDVs), which carry selected oxidized cargo and deliver this cargo to lysosomes, have been reported to facilitate MQC (137, 170). The MDV route appears to function at both normal and oxidative stress conditions and is independent of mitochondrial dynamics and mitophagy (166, 170). Although the identity of the vesicle cargo has not been fully characterized, this mechanism offers a potential strategy to remove segments of mitochondrial membranes containing damaged, hard to dissociate protein complexes and/or reactive prosthetic groups such as heme, which cannot be catabolized within the mitochondrion.

Mitochondrial Subproteomes and Their Regulation by PMQC

Matrix subproteome

Virtually all proteins of the dense matrix subproteome are synthesized in the cytosol and imported into the compartment as precursors in an unfolded state (136). Proper maturation and folding of these proteins in the matrix are facilitated by

several PMQC factors (Fig. 4). First, mtHsp70 and its J-type cochaperones participate in the import of precursor proteins through the TIM23 IM translocase complex and later, in conjunction with the Hsp60-Hsp10 chaperone system, they promote folding of the imported polypeptides (136, 187). Second, proteolytic removal of the N-terminal MTS is mediated through the action of the two-subunit MPP processing metallopeptidase complex (73, 131, 176). Some proteins undergo an additional processing by the mitochondrial intermediate peptidase (MIP/Oct1), which removes additional residues following the MTS (131, 176). Recent studies identified an additional intermediate cleaving aminopeptidase Icp55 that stabilizes multiple matrix proteins *via* the removal of a single, potentially destabilizing N-terminal amino acid residue following the MTS (131, 133, 185). Interestingly, Oct1 processing also appears to contribute to such stabilization (184). This situation resembles the N-end rule protein stabilization pathway described for the cytoplasm (173); however, the downstream protease(s) degrading destabilized polypeptides remain(s) to be identified.

The mitochondrial presequence peptidase, Cym1/PreP, also appears to contribute to the matrix MQC. Originally misidentified as an IMS-localized enzyme, this conserved metallopeptidase has been implicated in the clearance of free targeting peptides generated by MPP and MIP, as well as small (up to 65 amino acid residues), unstructured oligopeptides (92, 176), which upon accumulation may impair mitochondrial integrity (85, 139). Also, studies on mammalian PreP showed that the peptidase is required to prevent mitochondrial accumulation of the amyloid-β (Aβ) peptide, which upon accumulation can cause mitochondrial dysfunction (59).

Finally, two conserved AAA$^+$ serine proteases, Lon/Pim1 and ClpXP, are found in the mitochondrial matrix (94, 181) (Fig. 4). The Lon/Pim1 protease exists as a large homooligomeric complex with each subunit containing both ATPase and serine protease motifs (167, 181). It preferentially targets heat-damaged or oxidatively damaged proteins (24, 25, 26, 34). While protein misfolding and/or loss of prosthetic groups appear to contribute to substrate recognition by mitochondrial Lon (72, 121, 186), the exact recognition determinants remain to be identified. Studies in yeast indicate that Lon/Pim1 cooperates with ClpB-type AAA$^+$ chaperone

FIG. 3. Branches of the mitochondrial quality control (MQC) system. Multiple interdependent mechanisms exist at both molecular and organellar/cellular levels to sustain mitochondrial health. Conserved mitochondrial proteases and chaperones distributed across mitochondrial compartments represent one layer of MQC **(A)**. Removal of the proteins localized to the outer mitochondrial membrane (OM) and potentially other mitochondrial subproteomes, termed mitochondria-associated degradation (MAD), is mediated by the cytosolic ubiquitin–proteasome system (UPS) and assisted by several E3 ubiquitin ligases **(B)**. At the organellar level, MQC is provided through mitochondrial fusion (*left panel* **C**) and fission (*right panel* **C**) events, necessary for exchange and mixing of mitochondrial content and thus damage dilution, and segregation of damaged mitochondria from the network, respectively. Mitochondrial fusion is mediated by conserved GTPases in the OM (Mitofusins/Fzo1) and the IM (long and short isoforms of OPA1/Mgm1). Another OM-associated GTPase–Dynamin-related protein/Dnm1 is a key mediator of mitochondrial fission. Mitochondria-derived vesicles (MDVs), destined for lysosome, appear to represent yet another facet of organellar MQC **(D)**. This mechanism allows selective removal of fragments of mitochondria without affecting the entire organelle. Reportedly, the MDVs contain oxidized cargo and lipids and their formation in mammalian cells depends on the function of PINK1 kinase and E3 ubiquitin ligase Parkin (see text for details). When mitochondrial damage overwhelms the aforementioned mechanisms, failing organelles are segregated and targeted to autophagosomes, and subsequently to lysosomes where their content is degraded. The PINK1-Parkin functional tandem and UPS play important roles in the initiation of this process known as mitophagy **(E)**. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars

Hsp78 (24, 186) and mtHsp70 (160) to accelerate disaggregation/degradation of aggregated protein. The significance of this cooperation in higher eukaryotes remains unclear, as metazoans lack an apparent Hsp78 ortholog. In addition, Lon plays an important role in regulation of stability and ex-

pression of mtDNA *via* proteolytic control of the abundance of mitochondrial transcription factor TFAM (117, 124).

Unlike Lon, the ClpXP protease is a hetero-oligomeric complex consisting of two stacked ClpP serine protease heptamers topped with two hexameric rings formed by
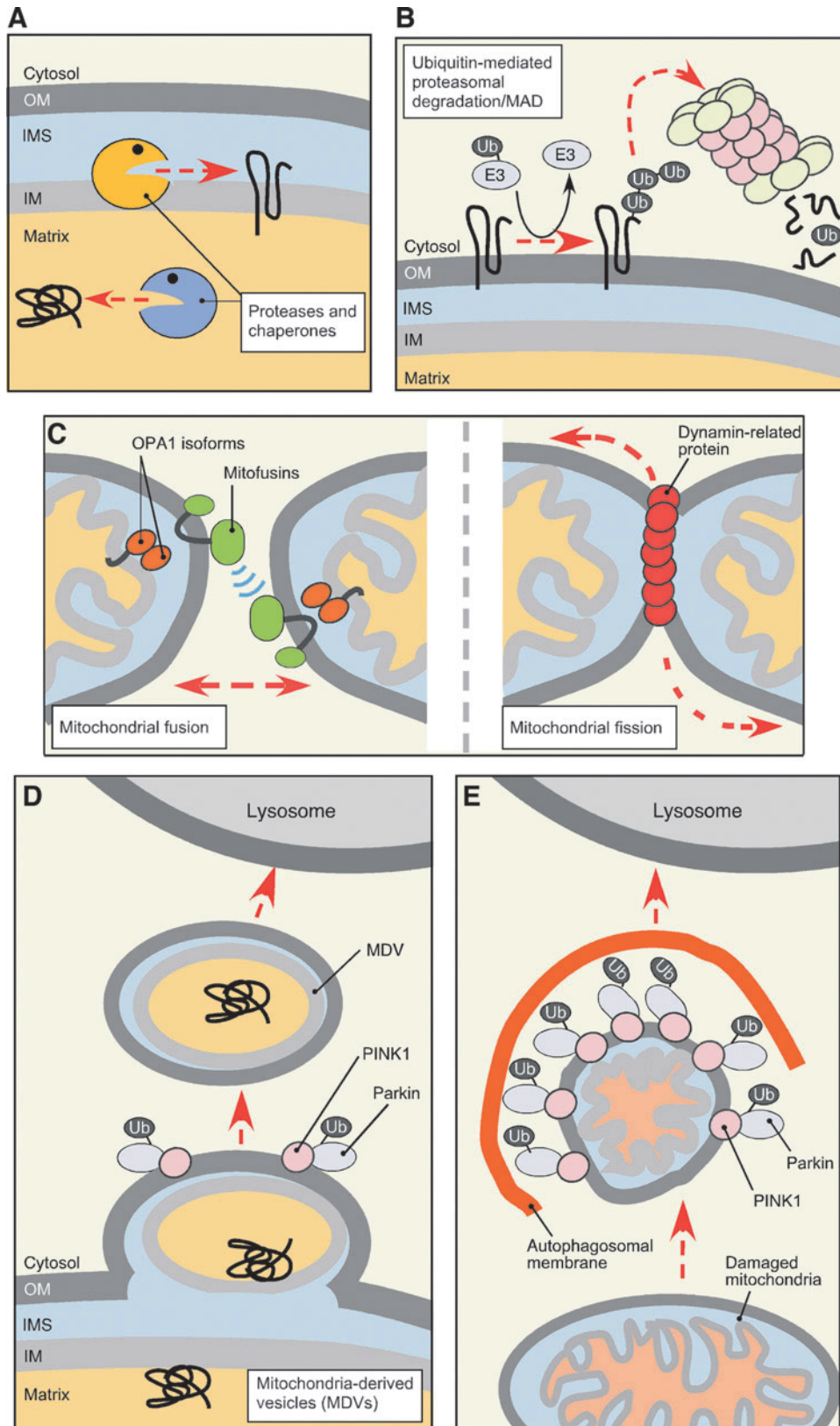
TABLE 1. Proteins Involved in Protein Mitochondrial Quality Control

Protein or component	Role	Localization	Yeast	Mammals
ClpX	ATP-dependent specificity component of ClpXP complex	Matrix	Mcx1	CLPX
ClpP	ATP-dependent protease component of ClpXP complex	Matrix	—	CLPP
Lon	ATP-dependent AAA+ serine protease	Matrix	Pim1	LONP1
MPP	Mitochondrial processing peptidase	Matrix	Mas1; Mas2	βMPP; αMPP
MIP	Mitochondrial intermediate peptidase	Matrix	Oct1	MIPEP
Icp55	Intermediate cleaving peptidase	Matrix	Icp55	XPNPEP3
PreP	Presequence metallopeptidase	Matrix	Cym1 (Mop112)	PreP
mtHsp70	Mitochondrial heat shock 70 kDa protein; molecular chaperone	Matrix	Ssc1	HSPA9 (Grp75)
Hsp78	Heat shock 78 kDa protein; ClpB-type AAA+ chaperone	Matrix	Hsp78	—
Hsp60	Heat shock 60 kDa protein; ATP-dependent chaperonin	Matrix	Hsp60 (Mna2)	HSPD1
Hsp10	Heat shock 10 kDa protein; Hsp60 cochaperonin	Matrix	Hsp10 (Cpn2)	HSPE1
Afg1	ATPase family gene 1; AAA+ protein	Matrix	Afg1	LACE1
m-AAA	Matrix-facing ATP-dependent AAA+ metalloprotease	IM	Yta10 (Afg3); Yta12 (Rca1)	AFG3L2; AFG3L1; SPG7/Paraplegin
i-AAA	IMS-facing ATP-dependent AAA+ metalloprotease	IM	Yme1 (Osd1)	YME1L
IMP	Intermediate mitochondrial protease	IM	Imp1; Imp2	IMMP1L; IMMP2L
PARL	Presenilin-associated rhomboid-like serine protease	IM	Pcp1 (Rbd1)	PARL
Oma1	Overlapping with m-AAA 1; ATP-independent metalloprotease	IM	Oma1	OMA1
Mgr1	Mitochondrial genome required 1; likely adaptor for i-AAA protease	IM	Mgr1	—
Mgr3	Mitochondrial genome required 3; likely adaptor for i-AAA protease	IM	Mgr3	C10orf118
OPA1	Optic atrophy 1; dynamin-related GTPase	IM	Mgm1	OPA1
Prd1	Proteinase yscD 1; zinc metallopeptidase	IMS	Prd1	Neurolysin
Atp23	ATP synthase 23; ATP-independent metalloprotease	IMS	Atp23	XRCC6BP1
HtrA2	High-temperature required A2; serine protease	IMS	Ynm3	HtrA2 (Omi)
PINK1	Phosphate and tensin homolog-induced putative kinase 1	IM/OM	—	PINK1
Msp1	Mitochondrial sorting of proteins 1; AAA+ protein	OM	Msp1	ATAD1
MFN	Mitofusin; transmembrane GTPase	OM	Fzo1	Mfn1; Mfn2
Mdm30	Mitochondrial distribution and morphology 30; ubiquitin ligase	OM	Mdm30	—
MARCH-V	Membrane-associated ring finger (C3HC4) 5; ubiquitin ligase	Cyto./OM	—	MARCH5 (MITOL)
Parkin	Autosomal recessive Parkinson's disease 2; ubiquitin ligase	Cyto./OM	—	Parkin (PARK2)
MULAN	Mitochondrial ubiquitin ligase activator of NFKB1; ubiquitin ligase	Mult.	—	MUL1
Vms1	VCP/Cdc48-associated mitochondrial stress responsive 1	Mult.	Vms1	VMS1
VCP	Valosin-containing peptide; AAA+ protein	Mult.	Cdc48	VCP (p97)
Ubiquitin	Small modifying protein; component of ubiquitin–proteasome system	Mult.	Ubi4	UBC; UBD
DRP	Dynamin-related protein; GTPase	Mult.	Dnm1	DRP1
Hsp70	Heat shock 70 kDa protein; molecular chaperone	Cyto.	Ssb1; Ssb2	HSPA1A
Hsp90	Heat shock 90 kDa protein; molecular chaperone	Cyto.	Hsc82; Hsp82	HSP90AA1; AB1

Protein names in brackets indicate aliases. "/" denotes alternative subcellular localization.
Cyto., cytosol; IM, inner mitochondrial membrane; IMS, intermembrane space; OM, outer mitochondrial membrane; Mult., multiple cellular locations.
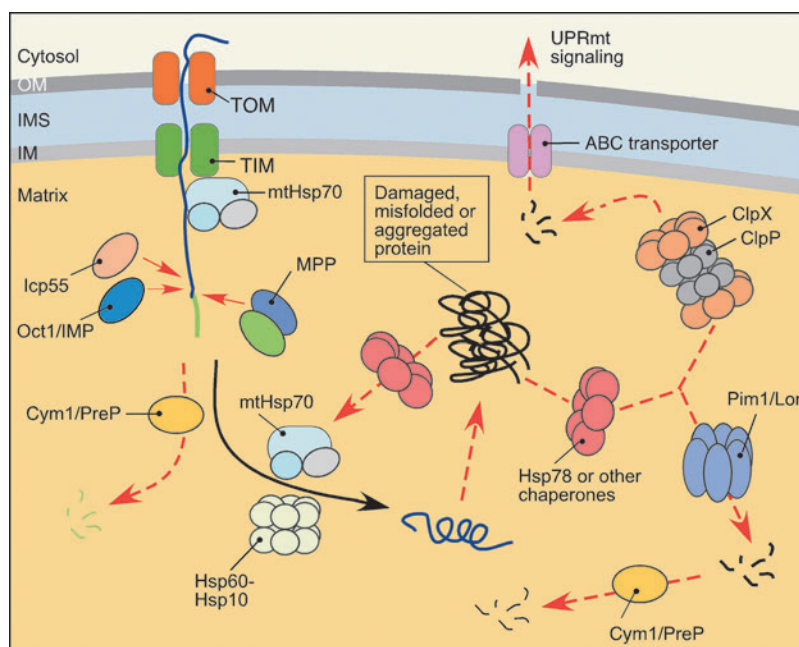
FIG. 4. PMQC in the matrix. Multiple proteases and molecular chaperones regulate the matrix subproteome. The regulation involves control of protein maturation and accumulation and degradation of poly- and oligopeptides. Proper maturation of the precursor proteins transported *via* the TIM23 translocase complex requires removal of mitochondrial targeting sequence by MPP processing metallopeptidase complex and, in certain cases, additional stabilizing processing by intermediate peptidases MIP/Oct1 and Icp55. Resulting free targeting peptides, as well as other small oligopeptides, are removed by mitochondrial presequence peptidase Cym1/PreP. Subsequent protein folding is facilitated by Hsp family chaperones. Stress-damaged, misfolded, and/or aggregated proteins are recognized and cleaved by AAA$^+$ proteases Lon/Pim1 and ClpXP. Peptides produced by these proteolytic events are either subjected to additional processing by oligopeptidases or extruded through ATP-binding cassette (ABC)-type transporters into the cytosol where they activate mitochondrial unfolded protein response (UPRmt). To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars

AAA$^+$ ClpX subunits (22, 94). Reportedly, the ClpX component participates in recognition of misfolded polypeptides and channels them into a proteolytic chamber formed by the ClpP subunits (12, 22). Although multiple studies implicate bacterial ClpXP as a protein quality control protease (22), its exact role within the mitochondrion remains elusive. Of particular interest is the recently postulated function of ClpXP in the mitochondrial unfolded protein response (UPRmt) in nematodes (76, 77). Peptides generated *via* ClpXP-mediated proteolysis of unfolded proteins in the matrix are extruded from mitochondria into the cytosol, whereby they trigger a specific transcriptional response that promotes expression and synthesis of nuclear-borne mitochondrial chaperones and proteases to restore mitochondrial proteostasis (76). This mechanism serves to sense and correct imbalances between the proteins of nuclear and mitochondrial origin, particularly subunits of the OXPHOS complexes. Recently, a Lon-regulated facet of UPRmt was reported in a roundworm model. The bZip transcriptional factor ATFS-1 required for UPRmt signaling is destined to the mitochondrial matrix where Lon degrades it; however, under stress conditions, ATFS-1 is stabilized and trafficked to the nucleus where it initiates the transcriptional response (76, 135). Although UPRmt is conserved among worms, mice, and humans (191), it remains to be determined if stress-sensing and signaling mechanisms in mammals are identical to the ones described for nematodes.

Inner membrane subproteome

The mitochondrial IM is among the most proteinaceous biological membranes and houses a significant portion of mitochondrial proteome, including the OXPHOS complexes. Assembly and function of the reactive respiratory complexes create major challenges for mitochondrial protein homeostasis (Fig. 2). Several mechanisms are in place to assure normal biogenesis and maintenance of the proteins residing in the IM. First, a large number of dedicated chaperones and chaperone-like assembly factors assist and regulate biogenesis and maintenance of the respiratory complexes (63, 125, 128). The second set of mechanisms controlling IM proteostasis involves proteolytic enzymes (Fig. 5). The two-subunit, IM-bound IMP proteolytic complex—similar to Oct1 and Icp55 peptidases—was shown to stabilize its substrate proteins (131). For instance, processing of the Mgr2 subunit of TIM23 translocase by IMP stabilizes Mgr2 and promotes TIM23 assembly (86).

The membrane-bound m-AAA and i-AAA metalloprotease complexes—surveying the matrix and the IMS sides of IM, respectively—are two major factors that provide quality control of the IM subproteome (7, 18, 21, 112, 113). The importance of coordinated actions between the IM AAA proteases is highlighted by the observation that simultaneous loss of these molecules in yeast is lethal (111, 112). The m-AAA protease is a large hetero-hexameric complex typically
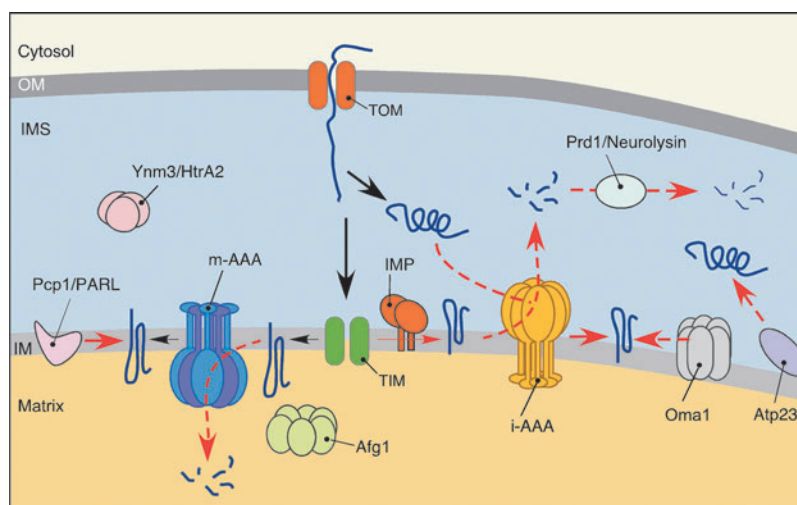
FIG. 5. PMQC in the IM and intermembrane space (IMS). Complexity of mitochondrial IM anticipates vastly efficient systems to maintain protein homeostasis. These include two tightly coordinated proteases matrix-facing AAA metalloprotease (m-AAA) and intermembrane space-facing AAA metalloprotease (i-AAA), which along with other regulatory functions recognize excessive, misassembled, and damaged subunits of OXPHOS complexes associated with the IM. Another IM protease complex Oma1, with m-AAA-overlapping functions, is also proposed to play a major role in mitochondrial dynamics and homeostasis upon stress conditions. Rhomboid-like Pcp1/PARL protease is implicated in the intramembrane proteolysis of several IM proteins in yeast, whereas in mammalian cells, it also contributes to regulation of mitochondrial turnover. The IMS PMQC is less studied. In addition to the i-AAA, which exerts both proteolytic and chaperone functions toward IMS-localized proteins, the IMS subproteome appears to be regulated by oligopeptidase Prd1/Neurolysin and serine protease Ymn3/HtrA2. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars

formed by Yta10/AFG3L2 and Yta12/SPG7/Paraplegin subunits in yeast and humans (11, 103). An additional rodent-specific subunit AFG3L1 that can substitute for AFG3L2 has been described (107). Also, studies on mammalian m-AAAs showed that other forms of the enzyme consisting of AFG3L2 subunits only (in humans) or AFGL32 and AFG3L1 (in rodents) do exist (103). The cryo-EM reconstruction of the yeast m-AAA revealed that the protease complex is ring shaped and contains a central pore, which may be restricted to unfolded polypeptide segments (108). This finding provides insights into the substrate recognition by mitochondrial AAA metallopeptidases, however, the detailed mechanism is yet to be determined. The molecular architecture of the i-AAA protease is similar to one of the m-AAA complex, except that the former proteolytic machine is always a homo-oligomer formed by six copies of the Yme1/YME1L peptidase (69, 70). Known substrates of the AAA metalloproteases include surplus, misassembled, and/or damaged subunits of the OXPHOS complexes that are intrinsic or peripheral to the IM (10, 45, 84, 105, 112, 113, 134, 148, 168, 190). In addition, *via* its involvement in maturation of the nuclear-borne constituent of the large mitoribosomal subunit MrpL32, the m-AAA is critical for synthesis of mtDNA-coded polypeptides (33, 140). The i-AAA complex is an important regulator of the IM's functional integrity and dynamics *via* several proteolytic events. One such event is constituent regulatory proteolysis of the PRELI protein family members Ups1 and Ups2 involved in transport, synthesis, and accumulation of mitochondrial phospholipids (46, 152). Second, the i-AAA is involved in biogenesis of the short isoform of the optic atrophy 1 (OPA1) dynamin-related GTPase, which is central to mitochondrial dynamics and mtDNA maintenance (8, 71).

The versatility of the IM AAA proteases is impressive and remains to be understood. A possible explanation is provided by cooperation of these proteolytic machines with other proteases and adaptor-like proteins. For instance, the conserved IM-associated peptidase Atp23 involved in maturation of the respiratory complex V (143, 199) reportedly cooperates with the Yme1 proteolytic complex to degrade Ups1 (152). Likewise, two adaptor-like proteins Mgr1 and Mgr3 have been shown to facilitate degradation of model substrates by the i-AAA in yeast (51, 52). The evolutionary conservation of Mgr3 suggests that the YME1L complex may also operate in a similar manner. Similarly, overexpression of the matrix AAA^+ protein Afg1/LACE1 (1, 110) has been shown to facilitate degradation of several m-AAA protease substrates in a series of respiratory-deficient yeast mutants (99). However, it remains to be determined if Afg1 is a *bona fide* adaptor/cooperating partner of the m-AAA complex. In addition, the m-AAA protease exerts overlapping activity with the ATP-independent membrane-bound metallopeptidase Oma1 complex (97, 100). Studies by our group established that Oma1 *per se* is also an important stress-activated protease required for cell survival (32). In mammalian cells, Oma1 mediates rapid proteolytic processing of the long membrane-anchored form of OPA1 (L-OPA1), thereby promoting IM fragmentation and subsequent cellular MQC actions in response to homeostatic insults (8, 19, 200). Conversely, the mitochondrial network remains intact or even hyperfused in stressed Oma1-deficient mouse embryonic fibroblasts (19, 152). While the exact mechanism of stress sensing by Oma1 only begins to emerge (19, 32, 120, 200), it is clear that Oma1 activation is central to the regulation of IM proteostasis and dynamics upon various stresses and/or pathological states.

Pcp1/PARL is yet another proteolytic module intrinsic to the IM. It belongs to the rhomboid family of serine proteases and mediates intramembrane cleavage of several IM polypeptides in yeast, including cytochrome *c* peroxidase Ccp1 (processed in conjunction with m-AAA protease) (174) and yeast OPA1 ortholog Mgm1 (81). Interestingly, the latter polypeptide does not appear to be an Oma1 substrate either under normal or under stress conditions (53, 114), thereby suggesting partial evolutionary repurposing of Oma1 function. Reciprocally, PARL does not seem to cleave OPA1 *in vivo* (53). Instead, recent studies have implicated mammalian PARL in constituent proteolytic removal of the phosphate and tensin homolog-induced putative kinase 1 (PINK1) —a crucial regulator of mitophagic (whole mitochondria) and nonmitophagic (respiratory chain complexes) mitochondrial turnover (48, 89, 182, 189). PINK1 is translated in the cytosol and imported into the mitochondria. Under normal conditions, the protein is sorted to the IM where it is degraded in a PARL-dependent manner (48). This pathway is blocked in severely depolarized or ATP-depleted mitochondria, whereby PINK1 is stabilized and accumulates on the OM subsequently recruiting and activating E3 ubiquitin ligase Parkin/PARK2, which in turn triggers segregation and mitophagic removal of malfunctioning mitochondria (87, 93, 106, 119, 195). Interestingly, a recent study demonstrated the important role of the PINK1-Parkin pathway in the formation of MDVs under mitochondrial stress conditions (126).

IMS subproteome

Relatively little is known about the IMS MQC (Fig. 5). Recent studies identified the i-AAA protease as an important regulator of the IMS subproteome through its involvement in the folding and degradation of unassembled and/or misfolded small TIM proteins (20, 162). Of note, small TIM proteins themselves have been postulated to chaperone several folding reactions in the IMS (82). Similarly to Cym1/PreP, the IMS-localized oligopeptidase Prd1/Neurolysin appears to degrade cleaved presequences and small oligopeptides (92, 131, 176), thereby preventing their accumulation in the IMS. The high-temperature requirement A2 (HtrA2/Omi/Ynm3) serine

protease is a highly conserved enzyme, whose functions and mechanism of action are understood primarily *via* studies on its bacterial orthologs—stress-inducible quality control peptidases DegP and DegS (44). HtrA2/Omi is a homotrimer and appears to be the only mitochondrial protease containing a PDZ-domain required for recognition of exposed hydrophobic stretches of misfolded proteins. The postulated modulation of Htr2A *via* PARL-assisted processing (40) and PINK1-mediated phosphorylation suggests its involvement in regulation of mitophagy (151). However, while studies in yeast and mammalian models indicated the role of Htr2A in thermotolerance (145), prevention of accumulation of aberrant respiratory chain subunits (123, 129), and even identified several substrates of the protease (91), its precise role in the IMS MQC awaits clarification.

Outer membrane subproteome

Recent findings provide several important insights into the PMQC mechanisms controlling OM proteostasis (Fig. 6). In addition to the aforementioned cytosolic Hsp70 and Hsp90 chaperones assuring proper delivery and likely the removal of nascent and/or newly synthesized unfolded polypeptides to be inserted into the OM (60, 122, 197), additional MQC modules have been characterized recently. The role of UPS in OM proteostasis becomes increasingly evident. Although it has been initially thought that the UPS may only intercept misfolded or damaged proteins *en route* to the mitochondrion (7, 35), numerous studies have suggested that mitochondria-localized polypeptides can be ubiquitylated and subsequently removed by the UPS in the process known as MAD (96, 116, 175). Consistently, several E3 ubiquitin ligases such as Mdm30, MITOL/MARCH-V, and MULAN (56) were found to associate with the cytosolic side of the OM. Likewise, the ubiquitin ligase Parkin is recruited to depolarized mitochondria (56, 66, 119, 172, 189, 195). Additional mitochondria-associated modifiers like SUMO ligases have also been described (56). Subsequently, multiple OM proteins have been identified as targets of these ligases and/or UPS (56, 66, 158). The discovery of VCP/p97/Cdc48-associated mitochondria stress responsive 1 (Vms1) protein in yeast that

FIG. 6. PMQC in the OM. In addition to interception of mitochondria-destined proteins *en route*, the UPS provides an additional level of OM PMQC. It removes misfolded, damaged, or surplus proteins in the OM *via* the MAD process. MAD involves ubiquitylation by E3 ubiquitin ligases that tag proteins to be degraded and extraction of the peptides by the AAA$^+$ protein VCP/Cdc48/p97 complex, which is, in turn, recruited to the OM through several mechanisms, including targeting by stress-responsive factor Vms1 or PINK1-Parkin functional tandem. Several reports (80, 123, 158) suggest that some IM proteins might also be subject of MAD. Another AAA$^+$ protein Msp1/ATAD1 targets and removes tail-anchored proteins mislocalized to the OM. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars
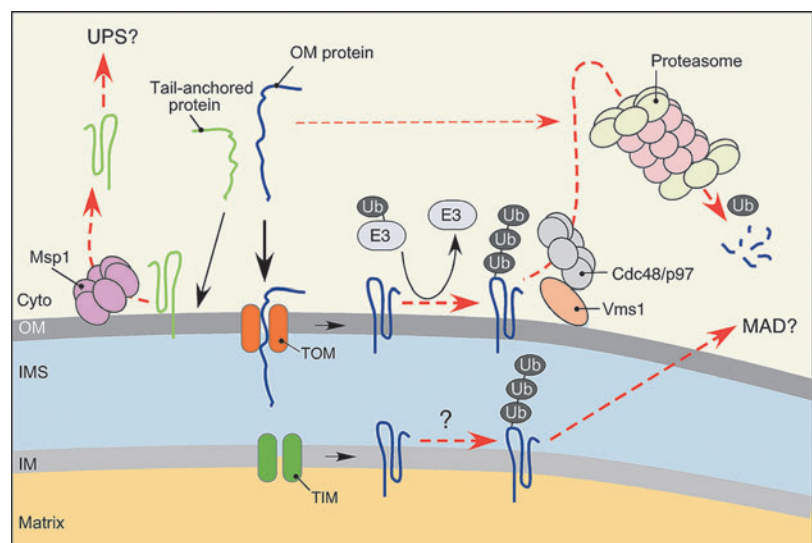
translocates from the cytosol to the mitochondrial OM upon stress and recruits the Cdc48-Npl4 complex, provided further mechanistic understanding of MAD (79, 80). The AAA$^+$ protein VCP/p97/Cdc48 complex is involved in multiple cellular processes but plays a key role in the extraction of ubiquitylated ER- and OM-anchored proteins for proteasomal degradation (37, 88, 193). Reciprocally, loss of Vms1 results in accumulation of ubiquitylated OM proteins and mitochondrial damage (79). Vms1 apparently is not the only VCP/p97/Cdc48-Npl4 recruiting factor, as a fraction of the complex can still associate with dysfunctional mitochondria in the absence of Vms1 (55, 79). Therefore, additional UPS recruitment factors are likely to exist and remain to be identified. Another intriguing line of findings suggests the involvement of MAD in retrotranslocation and removal of several IM- and matrix-localized proteins (14, 15, 122). However, more work is needed to corroborate this postulate.

Recent studies identified yet another OM-associated AAA$^+$ protein Msp1/ATAD1 required to prevent accumulation of mislocalized ER-destined tail-anchored proteins in the OM, thereby maintaining proper mitochondrial function and morphology (43, 142). It has been proposed that Msp1 represents a novel PMQC component involved in the extraction of mistargeted tail-anchored polypeptides. It remains to be determined if Msp1 constitutes an additional branch of MAD or is an independent facet of the OM MQC.

PMQC in Disease and Aging

Over time, a combination of increasing oxidative damage, failing protein homeostasis, and mitochondrial and organellar QC capacity can contribute to cellular aging and instability of mtDNA and mitochondrial proteome (27, 41). The resulting decline in mitochondrial health can primarily affect organs and tissues with high energetic demands and contribute to the onset of cardiovascular, neurodegenerative, and complex metabolic diseases like cancer.

Reduced activity/abundance of Lon/Pim1 and ClpP proteases has been demonstrated in cells from a patient with late-onset autosomal dominant hereditary spastic paraplegia (type SPG13) and aged rat hepatocytes (17, 74). In line with these findings, overexpression of Lon in the *Podospora anserina* fungal model of aging significantly extended the lifespan of the organism (118). Interestingly, the same effect is achieved through the depletion of *P. anserina* ClpP (62). The prospective role of Lon in cancer stems from its regulation by hypoxia-inducible factor HIF1α and Lon's role in hypoxia-induced remodeling of the respiratory chain (65, 153). Indeed, a recent study identified Lon as a prospective anticancer target (28).

The ability of the oligopeptidase PreP to prevent accumulation of mitochondria-targeted Aβ peptides (59) indicates its protective role against Alzheimer's disease (AD). Consistently, the activity but not abundance of PreP is attenuated in AD patients' brain mitochondria and in AD transgenic mouse models (4). The exact role of PreP in the onset of AD is yet to be determined.

Mutations in genes encoding the subunits of m-AAA protease are known with both familial and sporadic forms of autosomal recessive hereditary spastic paraplegia (Paraplegin) (38, 140), spinocerebellar ataxia type 28 (AFG3L2) (49), and spastic ataxia-neuropathy syndrome (150) in hu-

mans. Studies in mice also indicate that the AFG3L2 subunit of m-AAA is important for the survival of Purkinje cells (5) and anterograde mitochondrial transport in murine cortical neurons (102), thereby highlighting the role of failing m-AAA function in late-onset neurodegeneration. In addition, a recent report identified a variant of Paraplegin, which is linked to enhanced ROS generation and several clinical phenotypes, including type 2 diabetes mellitus and coronary artery disease (6).

Depletion of OMA1 in the mouse model reduces energy expenditure and specifically leads to obesity, hepatic steatosis, and altered thermogenesis/cold stress resistance (154). These phenotypes are likely due to defective L-OPA1 processing and inability to initiate fragmentation of the mitochondrial networks in response to metabolic and/or oxidative insults (154, 192). Also, a recent high-throughput sequencing study of patients with familial and sporadic forms of amyotrophic lateral sclerosis (ALS) identified several mutations in the conserved residues of OMA1, thus implicating OMA1 as a prospective ALS-causing gene (47).

The IMS protease HtrA2/Omi plays an important role in protecting neurons from degeneration (90, 91, 123) and has been associated with Parkinson's disease (PD) (98, 169). Likewise, a PD-associated mutation in PARL has been recently identified (163). Although *in vitro* studies highlight the importance of MAD for prevention of neurodegenerative pathologies such as PD, its physiological role in human health awaits further investigation. Two lines of evidence implicate MAD in mitochondrial disease. First, a murine knockin model of p97 mutations (associated with inclusion body myopathy and Paget's bone disease) presents with mitochondriopathy-like phenotypes (16). Second, the PINK1-Parkin system, which likely represents a facet of the MAD that links molecular and organellar MQC—is clearly associated with neurodegenerative processes. Loss-of-function mutations in Parkin have been described in juvenile PD patients and account for $\sim$50% of the familial cases of PD (101).

Concluding Remarks and Perspectives

The role of functionally intertwined multilayer MQC mechanisms in assuring mitochondrial health becomes increasingly evident. It is important to note that humans sustain mitochondrial damage not only from age-related decline in mitochondrial function but also from causes that can affect younger populations. For instance, aggressive anticancer or antiviral therapies may enhance the formation of malfunctioning mitochondria in nontarget cells in patients undergoing such treatments (42). Modulation of the activity of certain MQC components can therefore be a promising approach to enhance cellular health and lifespan (62, 118). Reciprocally, proteases like Lon can be prospective targets in anticancer therapies to promote the death of drug-treated malignant cells (28, 67).

A number of important unresolved questions regarding MQC persist. For instance, the mechanisms by which MQC components promptly recognize mitochondrial stress signals remain obscure. Likewise, it remains to be determined how MQC modules in different mitochondrial subcompartments coordinate their actions in response to homeostatic insults. The regulation of nuclear output and physiological responses

via function/dysfunction of MQC modules is another exciting question. Answering these questions is likely to yield new insights into MQC in health and disease and may lead to novel or supplementary therapeutic and preventive approaches against mitochondria-related maladies.

Acknowledgments

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Abbreviations Used

AAA$^+$ = ATPase associated with diverse cellular activities
ABC = ATP-binding cassette
AD = Alzheimer's disease
AFG = ATPase family gene
ALS = amyotrophic lateral sclerosis
ATAD1 = ATPase family AAA domain containing 1
ATFS-1 = activating transcription factor associated with stress 1
ATPase = adenosine triphosphate hydrolyzing enzyme
Aβ = amyloid-β peptide
bZip = basic leucine zipper domain
Cdc48 = cell division cycle 48, AAA ATPase
cryo-EM = cryoelectron microscopy
Cym1 = cytosolic metalloprotease, metallopeptidase of the intermembrane space
Cyto. = cytosol
ER = endoplasmic reticulum
GTPase = guanosine triphosphate hydrolyzing enzyme
Hsp = heat shock protein
HtrA2 = high-temperature required A2 (also known as Omi)
i-AAA = intermembrane space-facing AAA metalloprotease
Icp55 = intermediate cleaving peptidase 55
IM = inner mitochondrial membrane
IMP = intermediate mitochondrial protease
IMS = intermembrane space
LACE1 = lactation elevated 1, AAA ATPase
Lon = long filaments forming, AAA protease
m-AAA = matrix-facing AAA metalloprotease

Abbreviations Used (Cont.)

MAD = mitochondria-associated degradation
MARCH-V = membrane-associated ring finger (C3HC4) 5 ubiquitin ligase
Mdm30 = mitochondrial distribution and morphology 30
MDVs = mitochondria-derived vesicles
Mgm1 = mitochondrial genome maintenance 1, yeast homolog of OPA1
Mgr = mitochondrial genome required
MIP = mitochondrial intermediate peptidase
MITOL = mitochondrial ubiquitin ligase
MPP = mitochondrial processing peptidase
MQC = mitochondrial quality control
Msp1 = mitochondrial sorting of proteins 1
mtDNA = mitochondrial DNA
mtHsp = mitochondrial heat shock protein
MTS = mitochondrial targeting sequence
MULAN = mitochondrial ubiquitin ligase activator of NFKB1
Mult. = multiple cellular locations
Npl4 = nuclear protein localization 4, substrate-recruiting cofactor of Cdc48 complex
Oct1 = octapeptidyl aminopeptidase 1
OM = outer mitochondrial membrane
Oma1 = overlapping with m-AAA 1
OPA1 = optic atrophy 1
OXPHOS = oxidative phosphorylation system
PARL = presenilin-associated rhomboid-like

Pcp1 = processing of cytochrome *c* peroxidase 1
PD = Parkinson's disease
PDZ = postsynaptic density protein domain
Pim1 = proteolysis in mitochondria 1, yeast homolog of Lon
PINK1 = phosphate and tensin homolog-induced putative kinase 1
PMQC = protein mitochondrial quality control
Prd1 = proteinase yscD homolog 1
PRELI = protein of relevant evolutionary and lymphoid interest
PreP = presequence peptidase
RNS = reactive nitrogen species
ROS = reactive oxygen species
SPG = spastic paraplegia
SUMO = small ubiquitin-like modifier
TFAM = mitochondrial transcription factor A
TIM = translocase of the inner membrane
TOM = translocase of the outer membrane
UPRmt = mitochondrial unfolded protein response
UPS = ubiquitin–proteasome system
Ups = unprocessed, mitochondrial phosphatidic acid transport protein
VCP = valosin-containing protein (or p97), mammalian homolog of Cdc48
Vms1 = VCP/Cdc48-associated mitochondrial stress responsive 1
Yme1 = yeast mitochondrial escape 1