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Review

Selective destruction of abnormal proteins by ubiquitin-mediated protein quality control degradation

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ABSTRACT

Misfolded proteins are continuously produced in the cell and present an escalating detriment to cellular physiology if not managed effectively. As such, all organisms have evolved mechanisms to address misfolded proteins. One primary way eukaryotic cells handle the complication of misfolded proteins is by destroying them through the ubiquitin-proteasome system. To do this, eukaryotes possess specialized ubiquitin-protein ligases that have the capacity to recognize misfolded proteins over normally folded proteins. The strategies used by these Protein Quality Control (PQC) ligases to target the wide variety of misfolded proteins in the cell will likely be different than those used by ubiquitin-protein ligases that function in regulated degradation to target normally folded proteins. In this review, we highlight what is known about how misfolded proteins are recognized by PQC ubiquitin-protein ligases.

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1. Introduction

To function properly, cells rely on proteins successfully accomplishing specific actions. Fundamental to protein action is the acquisition of a protein's 3-dimensional structure, and thus the proper folding of a cell's protein cohort is critical for cells. Due to the central importance of protein folding, cells have evolved a collection of protein quality control (PQC) mechanisms that maintain overall cellular protein homeostasis, or proteostasis [1]. PQC systems can be divided into those that function as either primary or secondary PQC defenses. The cell's primary PQC defenses are directly involved in repairing or removing misfolded proteins. Repair systems are chiefly composed of protein chaperones, whereas removal systems are principally involved in proteolytic destruction either by the proteasome or via autophagy. In many cases, the PQC repair and removal machinery function together in a triage hierarchy that has the potential to determine if a misfolded protein is salvageable and then direct the PQC action towards either repair or removal [2]. In contrast, the cell's secondary PQC defenses are adaptive transcriptional responses that balance the primary PQC capacities with the extent of the cellular burden caused by misfolded proteins. They can also reduce global translation as a way to attenuate the production of misfolded proteins. In eukaryotes, PQC systems typically mitigate protein misfolding in a compartment-specific way, with each subcellular compartment housing a distinct set of PQC repair, removal, and adaptive capabilities.

There is now a considerable wealth of information on the different types of cellular PQC, which we cannot cover in its entirety here. We refer the readers to excellent reviews for chaperone-mediated folding and repair and for PQC adaptive stress responses [3–7]. Herein, we will discuss the ubiquitin-dependent PQC removal systems that operate in each eukaryotic cellular compartment (Fig. 1).

1.1. Ubiquitin-mediated proteasomal degradation: regulation versus quality control

Protein degradation by the ubiquitin-proteasome system has two primary purposes in the cell: (1) the temporal or spatial regulation of normal proteins, and (2) the removal of misfolded proteins. For each type of degradation, a specific subset of proteins must first be uniquely distinguished from the global pool of cellular proteins and subsequently ubiquitylated. Ubiquitylation is canonically achieved via an enzymatic cascade wherein a ubiquitin-protein ligase (E3) partners with a specific ubiquitin-conjugating enzyme (E2) that has been charged with ubiquitin by a ubiquitin-activating enzyme (E1) [8]. The ubiquitin-protein ligase typically confers substrate specificity within each ubiquitylation cascade, either by possessing intrinsic substrate-binding domains or by recruiting auxiliary proteins that impart substrate specificity.

One critical aspect of ubiquitylation is the ability of a ubiquitin-protein ligase to distinguish its substrates from other proteins. In regulated degradation of normal proteins, ubiquitin-protein ligases typically recognize degrons, which are small specific linear amino acid sequences located within substrates [9]. The degron recognized by the ligase often varies based on the auxiliary proteins that are bound to the ligase. For example, cullin RING ubiquitin-protein ligases (CRLs) are able to recognize many different types of degrons through the directed binding of distinct adapter F-box proteins [10]. The interaction of a particular ubiquitin-protein ligase with different substrate-binding auxiliary proteins allows it to target a larger number of protein substrates. Importantly, these ubiquitin-protein ligases are still only able to target a limited number of substrates due to this sequence specificity requirement.

In contrast to the regulated degradation of normal proteins, substrate recognition in PQC degradation is unlikely to be achieved via the recognition of linear sequence-specific degrons in misfolded substrates for two key reasons. First, any protein has the capacity to misfold and most proteins in each cellular compartment share little, if any sequence homology. Thus, two different misfolded proteins will not likely possess the same sequence-specific degron. Second, a key purpose of PQC degradation is to destroy structurally abnormal proteins that share the same linear sequence with their normal counterparts. Thus, the features of misfolding recognized by PQC ubiquitin-protein ligases must transcend primary structure. One prevalent hypothesis is that PQC ubiquitin-protein ligases distinguish abnormal proteins by recognizing the exposure of hydrophobic residues typically buried in the core of a normal protein. In the subsequent sections, we will introduce the PQC ubiquitin-protein ligases in each cellular compartment, highlighting what is known about substrate recognition mechanisms and the features of structural abnormality recognized within the substrates.

2. Endoplasmic reticulum

The unique properties of the endoplasmic reticulum (ER) present numerous challenges for PQC ubiquitin-protein ligases in substrate recognition. First, *de novo* protein folding occurs in the ER and ER PQC ubiquitin-protein ligases must be capable of differentiating between nascent polypeptides that are in the process of folding and proteins that have become misfolded. In addition, the ER is a membrane-bound organelle where structural lesions may be present in transmembrane segments or in regions located on the luminal or cytoplasmic side of the membrane. Thus, ER PQC ubiquitin-protein ligases must have location-specific recognition mechanisms that can sense where the lesions are relative to the ER membrane. Another complication in ER PQC substrate recognition is the presence of disulfide bonds and glycosylation moieties in resident proteins. These posttranslational modifications must be queried for defects by PQC machinery. Lastly, ubiquitin and proteasomes are not present in the ER lumen, so ubiquitylation and proteasome degradation can occur only on the cytoplasmic side of the ER membrane. Accordingly, the ER PQC ubiquitin-protein ligases must have a means to recognize substrates on the luminal side of the ER membrane while directing substrate ubiquitylation on the cytoplasmic side of the ER membrane.

2.1. The Hrd1 pathway

The first ubiquitin-protein ligase found to play a role in ER PQC degradation is the yeast protein Hrd1/Der3 [11,12]. Hrd1 is an integral ER-membrane protein containing a transmembrane domain that traverses the ER membrane six times and a cytoplasmic RING domain that mediates the transfer of ubiquitin to its substrates [12–14]. Several mammalian homologs of Hrd1 have been identified–gp78, hHrd1 (synovilin), and Rfp2 [15–19], with their roles in ER PQC degradation explored to varying degrees. Here, we will focus on yeast Hrd1 as it has been the best characterized.

All Hrd1 substrates require a core group of proteins to mediate substrate ubiquitylation. In addition to Hrd1, the core complex contains Hrd1's cognate ubiquitin-conjugating enzyme Ubc7 [11,20–22], which is localized to the Hrd1 core complex by the protein Cue1 [23]. Cue1 possesses a single transmembrane span and a cytoplasmic domain that is important for its interaction with Ubc7. There is no Cue1 mammalian homolog, but gp78 contains a cytoplasmic CUE domain that is similar to Cue1 [24]. Also in the core Hrd1 complex is Hrd3 (SEL1L in mammals [25,26]), which is predominantly luminal with a single transmembrane span and a small cytoplasmic region [12,13,27]. Hrd3 directly binds misfolded proteins in the ER lumen [28]. Additionally, Hrd3 regulates Hrd1 autoubiquitylation and stability [13]. Hrd1 autoubiquitylation, as well as Hrd1 oligomerization, also depends on the protein Usa1

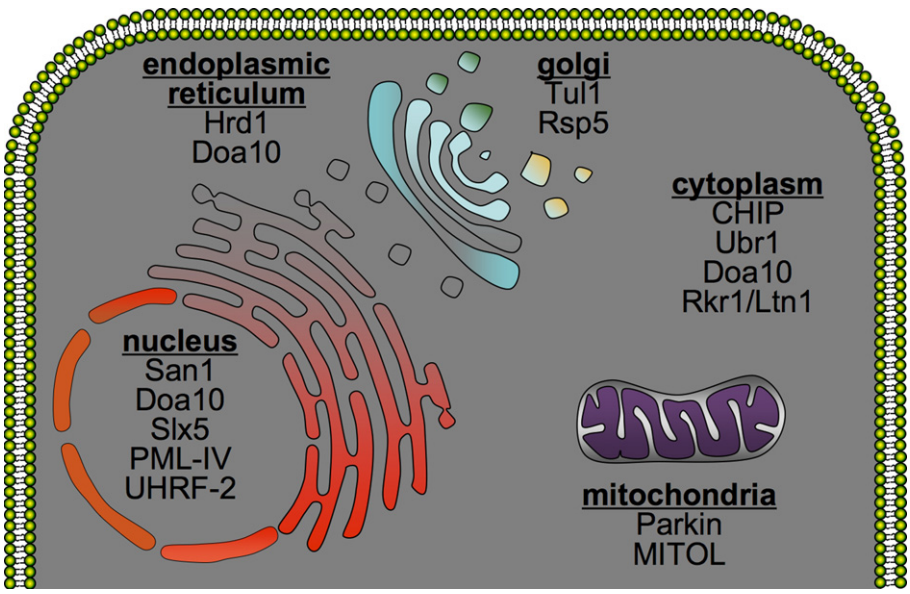

Fig. 1. PQC ubiquitin-protein ligases in the cell. Schematic representation of the cell with PQC ubiquitin-protein ligases listed in the appropriate cellular compartment. Yeast PQC ligases are listed as the main example, except in the cases where there is no yeast homolog.

(Herp in mammals [29,30]), which contains two transmembrane spans and interacts directly with Hrd1 [31–34]. For many Hrd1 substrates, this core complex of Hrd1, Ubc7, Cue1, Hrd3, and Usa1 is sufficient for substrate ubiquitylation [33,34].

One class of PQC substrates degraded by the core Hrd1 complex comprises proteins with lesions in their ER-membrane spanning segments and are thus referred to as ERAD-M substrates (ER-Associated Degradation-Membrane) [34]. ERAD-M substrate recognition appears to be performed directly by Hrd1 [35]. It has been proposed that the transmembrane domain of Hrd1 recognizes improperly exposed hydrophilic residues in the hydrophobic environment of the ER membrane [35]. Mutational analysis of Hrd1's transmembrane domain demonstrated that the degradation of Hrd1's ERAD-M substrates could be disrupted without affecting degradation of other Hrd1 substrates [35], suggesting separate substrate recognition mechanisms.

Other Hrd1 substrates contain lesions in their ER luminal domains (ERAD-L substrates [34]), and their degradation typically requires the core Hrd1 complex and additional ancillary factors. One such factor is Der1 [36], a transmembrane protein localized to the Hrd1 complex via Usa1 [32,34]. Der1 function in substrate recognition is unclear, though its mammalian homologue Derlin-1 is thought to be involved in postubiquitylation processes that deliver ER luminal proteins to the cytoplasm [37–39]. Another cofactor required in the degradation of ERAD-L substrates is the Hsp70 chaperone, Kar2 (also known as BiP) [40]. Kar2 likely couples to the core Hrd1 complex via interaction with a tetratricopeptide repeat (TPR) domain in Hrd3. Because Kar2 is an Hsp70 chaperone, its involvement in substrate degradation suggests that initial recognition of a misfolded protein could be mediated by the chaperone, which typically involves binding regions of hydrophobicity surrounded by basic residues [41]. However, exposed hydrophobicity in ERAD-L substrates has not yet been demonstrated as the abnormal structural feature recognized by the Hrd1 complex.

The decision to fold or degrade ER proteins can depend on posttranslational modifications. For instance, specific N-glycan moieties on ERAD-L substrates lead to degradation after their recognition by additional Hrd1 complex factors, such as the lectin Yos9 [42–45] (OS-9 in mammals [46]). Yos9 is coupled to the Hrd1 complex via interactions with Hrd3 and Kar2 [46–48]. N-glycan interaction with Yos9 depends on Yos9's mannose-6-phosphate receptor function and is required for degradation of glycolsylated ERAD-L substrates [42,49,50]. The particular location of the glycan modification within the misfolded protein, including the peptide sequences surrounding the glycosylation site, is important for efficient recognition [51–53]. Interestingly, Yos9 is involved in the degradation of certain non-glycosylated substrates [54,55], suggesting it might have additional substrate recognition roles.

2.2. The Doa10 pathway

Another PQC ubiquitin-protein ligase in the ER is Doa10, which was initially identified as the ubiquitin-protein ligase involved in degradation of the MATα2 transcription factor [56]. Doa10 has been subsequently shown to target misfolded proteins in the ER [57]. Doa10 contains a transmembrane domain that traverses the ER membrane fourteen times and a cytoplasmic RING domain [58]. In mammalian cells, TEB4 is described as a homolog of Doa10 due to its similar membrane topology [58]. TEB4 has recently been shown to be involved in the degradation of the ER-resident type 2 deiodinase [59].

Doa10, like Hrd1, functions as part of a larger protein complex in substrate recognition and ubiquitylation. There are four proteins required for the Doa10 complex to ubiquitylate substrates: Doa10, the ubiquitin-conjugating enzymes Ubc6 and Ubc7, and Cue1 [60]. Select substrates also require the ubiquitin chain elongator Ufd2 for degradation [61]. The role of Ufd2 is likely postubiquitylation and not in substrate recognition. Also required for degradation of certain Doa10 substrates are Hsp70 (of the Ssa class) and Hsp40 chaperones [57].

The Doa10 complex primarily functions in the degradation of ER PQC substrates with lesions on the cytoplasmic side of the ER [56,57] (ERAD-C substrates [34]). Although it's not clear how the Doa10 complex recognizes its substrates, the requirement of Hsp70 and Hsp40 chaperones suggests that substrate recognition could be mediated by the chaperones binding to exposed hydrophobicity. Some evidence suggests the Doa10 pathway likely targets hydrophobicity in its substrates. This was revealed from mutational studies of Doa10's substrate MATα2, which possesses an amphipathic helix in its N-terminus that is both necessary and sufficient for Doa10-dependent degradation [62]. The hydrophobic portion of the amphipathic helix is the critical determinant

for Doa10-dependent degradation of MATα2 [62]. Furthermore, studies examining Doa10-dependent degradation of small peptides fused to a reporter protein also revealed a hydrophobic requirement for Doa10-targeting [63–65]. Further work will be required to determine if the Doa10 complex recognizes hydrophobicity in ERAD-C substrates.

2.3. Late secretory PQC degradation pathways

Golgi-localized ubiquitin-protein ligases have also been implicated as having potential roles in PQC degradation. The yeast RING-domain ubiquitin-protein ligase Tul1 was found to be involved in selectively sorting transmembrane proteins with exposed polar residues in their transmembrane spans into multivesicular bodies for delivery to the vacuole for degradation [66]. In addition to Tul1, two studies found that the yeast transmembrane protein Bsd2 recruits the HECT-domain ubiquitin-protein ligase Rsp5 to target membrane proteins with exposed polar residues in their transmembrane spans for delivery to the vacuole [67,68]. It is important to note that Tul1 and Bsd2-Rsp5 also assist in the correct trafficking of normal proteins to the vacuole [66–68]. Thus, it is unclear if these ubiquitin-protein ligases serve a PQC function, or if the mutant proteins identified mimic physiological substrates.

3. Cytoplasm

The cytoplasmic environment presents challenges to PQC ubiquitin-protein ligases that are both similar to and distinct from the ER. Similar to the ER, *de novo* protein folding occurs in the cytoplasm and cytoplasmic PQC ubiquitin-protein ligases must have the ability to differentiate between misfolded proteins and nascent unfolded polypeptides in the process of folding. Unlike the ER, cytoplasmic PQC does not have to contend with multiple classes of substrate lesions that present themselves differently in relation to a membrane. It is possible, however, that there might be specific distinct regions within the cytoplasm in which misfolded proteins behave differently [69], and these regions could require different ubiquitin-protein ligases.

3.1. The CHIP pathway

The mammalian protein CHIP is involved in cytoplasmic PQC degradation. CHIP contains a U-box domain necessary for its ubiquitin-protein ligase activity via interaction with the ubiquitin-conjugating enzyme UbcH5 [70–72], and a tetratricopeptide repeat (TPR) domain that is essential for CHIP's interactions with Hsp70 and Hsp90 chaperones [71,72]. Through direct interaction with chaperones, CHIP ubiquitylates client proteins that are bound by the chaperones [73]. While CHIP interacts with both Hsp70 and Hsp90 chaperones, data suggests that CHIP has a preference for ubiquitylating Hsp70-bound proteins [74]. Although substrate recognition by CHIP is heavily dependent on chaperone recognition, CHIP itself also appears capable of binding misfolded proteins directly [75].

CHIP's interactions with chaperones place it at a central decision-making hub that balances productive folding and PQC degradation of a chaperone-bound client protein. However, an open question is how CHIP determines if a chaperone-bound protein should be ubiquitylated and degraded or allowed to continue with productive folding. One possibility is that Hsp70 and Hsp90 accessory proteins mediate this decision. For example, CHIP ubiquitylation of Hsp70 client proteins is influenced by the BAG class of Hsp70 cochaperones, which contain a BCL2-associated athanogene (BAG) domain that mediates the interaction with Hsp70 [76]. BAG cochaperones vary in their CHIP-related function from negatively regulating CHIP-dependent ubiquitylation of Hsp70 client proteins (BAG-2 and BAG-5) [77,78], to facilitating the interaction of CHIP-chaperone complexes with the proteasome (BAG-1) [79], or in helping recruit the protein p62 to the CHIP-chaperone complex for substrate delivery to the lysosome (BAG-3) [80]. The Hsp70 cochaperone HspBP1 is also known to negatively regulate CHIP's substrate-ubiquitylating activity [81].

CHIP's interaction with chaperones suggests that the feature of structural abnormality recognized by CHIP is likely the same feature that the Hsp70 or Hsp90 chaperones recognize. CHIP has also been shown to have a chaperone function itself and is capable of binding thermally denatured proteins in an Hsp70-independent manner to prevent their aggregation [75]. However, the structurally abnormal feature that CHIP directly binds in its thermally denatured substrates is not known.

3.2. The Ubr1 pathway

In *S. cerevisiae* there is no identified homolog to CHIP. Rather, the RING-domain ubiquitin-protein ligase Ubr1 appears to mediate cytoplasmic PQC degradation [82–85]. Originally, Ubr1 was characterized for its role in N-end rule degradation, in which certain residues at the N-terminus of proteins serve as degrons [86]. Two specific regions in Ubr1 were identified that direct the ubiquitylation of substrates containing N-terminal residues of either type 1 (Arg, Lys, or His) or type 2 (Leu, Ile, Phe, Trp, or Tyr) [86]. It was recently found that Ubr1 also mediates the PQC degradation of misfolded cytoplasmic proteins [82–85], and this is independent of its role in the N-end rule pathway [83,84]. Additionally, yeast Ubr2 has been shown to mediate cytoplasmic PQC degradation, [83] as well as human Ubr1 [87].

Ubr1-mediated PQC degradation requires the use of the ubiquitin-conjugating enzymes Rad6 and Ubc4 [83,84,88], as well as Hsp70 and Hsp110 chaperones [82–84,88]. Hsp70 functions to keep substrates soluble [88], but it is not yet clear if these chaperones also direct substrates to Ubr1 similar to CHIP or if Ubr1 directly binds misfolded proteins. In support of direct interaction with substrates, Ubr1 is able to ubiquitylate a thermally denatured substrate *in vitro* without the aid of a chaperone [83]. Addition of Hsp70 increases the Ubr1-dependent ubiquitylation of the denatured substrate and robust ubiquitylation only occurs when Ubr1 is added during thermal denaturation [83]. Additional work will be necessary to clarify the role of chaperones in Ubr1-mediated PQC ubiquitylation.

3.3. The cytoplasmic Doa10 pathway

In addition to its role in ER PQC degradation, Doa10 is also involved in the degradation of cytoplasmic proteins that are misfolded [63] or contain an acetylated N-terminus [89]. Doa10-dependent degradation of cytoplasmic misfolded proteins requires cytoplasmic Hsp70 and Hsp40 chaperones [63]. It is not known if Doa10 recognizes the same features in cytoplasmic misfolded proteins as it does in ER misfolded proteins.

3.4. The Hul5 pathway

Recently, the HECT-domain ubiquitin-protein ligase Hul5 was shown to function in the PQC degradation of misfolded cytoplasmic proteins generated after heat shock [90]. Hul5 was previously found to be a component of the 19S regulatory subunit of the proteasome where it functions to extend polyubiquitin chains on substrates [91], likely to facilitate their processive degradation [91,92]. It is not clear if Hul5's function in cytoplasmic PQC degradation is to extend polyubiquitin chains initially added by other cytoplasmic PQC ubiquitin-protein ligases, or if it functions independently as a primary PQC ubiquitin-protein ligase that directly

targets misfolded proteins itself. Because of this, how Hul5 targets its substrates is unknown.

3.5. The Rkr1/Ltn1 pathway

Translation of nonstop mRNAs is one way in which abnormal proteins are generated. Nonstop mRNAs can be created through DNA mutation or transcriptional mistakes that alter the stop codon, or by premature polyadenylation due to errors in processing [93]. Nonstop mRNAs can result in an aberrant sequence appended to a normal protein by read-through of the poly(A) tail, which would typically add a poly-Lys residue tract. Polybasic tracts have been shown to cause translational pausing and arrest [94]. *RKR1/LTN1/YMR247C* was initially identified as a gene that suppressed the phenotype of certain translated nonstop mRNAs [95]. Subsequently, it was demonstrated that Rkr1/Ltn1 is a ubiquitin-protein ligase [96,97], and is involved in the degradation of abnormal proteins with a polybasic tract of Lys resulting from nonstop translation [97]. Furthermore, it was found that Rkr1/Ltn1 associates with ribosomes and is involved in the degradation of newly synthesized proteins that have stalled on the ribosome by virtue of a nonstop transcript [97]. However, It is not yet clear what Rkr1/Ltn1 recognizes: the polybasic tract of a target substrate in the context of a stalled ribosome or some feature of a stalled ribosome itself.

4. Nucleus

Unlike the cytoplasm, protein biosynthesis does not occur in the nucleus. Instead, nuclear proteins are typically translated and folded in the cytoplasm and imported into the nucleus. Thus, PQC degradation systems designed to detect errors in nascent protein folding will likely be absent in the nucleus. The nucleus does have different subdomains such as chromatin, the nucleoplasm, the nuclear membrane, and the nucleolus. Thus, different PQC ubiquitin-protein ligases might be required to manage misfolded proteins that arise in each of these different subnuclear regions.

4.1. The San1 pathway

In *S. cerevisiae*, the nuclear RING ubiquitin-protein ligase San1 mediates the PQC degradation of mutant or misfolded nuclear proteins [98–102]. San1 does not target normal versions of the same proteins [99–102], establishing a specific role for San1 in nuclear PQC degradation. While no mammalian homologs of San1 have yet been identified, a *S. pombe* homolog was recently identified and its function in PQC degradation established [103].

San1-mediated degradation uses the ubiquitin-conjugating enzymes Cdc34 and Ubc1 [99]. Substrate recognition involves San1 directly binding its substrates through N- and C-terminal regions that possess multiple substrate-binding sites embedded within highly disordered sequences [104]. It has been proposed that San1's unique topology of high intrinsic disorder interspersed with substrate-binding modules allows San1 to use conformational plasticity to accommodate the binding of San1 to the diverse array of misfolded substrate conformations that it is likely to encounter [104].

The abnormal structural feature that San1 recognizes appears to be exposed hydrophobic residues in substrates. Through the use of a two-hybrid assay, it was found that San1 mediates the degradation of reporter proteins fused to hydrophobic peptides [104,105]. Further exploration revealed that as few as five contiguous hydrophobic residues in the peptides defined the minimal recognition motif for San1-mediated degradation [105]. San1 can also target exposed hydrophobicity in larger misfolded proteins [105].

Surprisingly, it was recently found that some presumably cytoplasmic misfolded proteins become nuclear-localized and are degraded in a San1-dependent manner [82,84]. It was found that cytoplasmic Hsp70 and Hsp110 chaperones are required for the nuclear localization of these substrates [82,84], suggesting that chaperones might be involved in misfolded protein trafficking to the nucleus. This could be through a direct action of the chaperones in the nuclear import process, or an indirect involvement of the chaperones in maintaining the solubility of the misfolded proteins prior to nuclear import. Due to their potential role in the nuclear trafficking of substrates and the fact that San1 can directly bind its substrates, the role of these chaperones in substrate recognition in the San1 pathway is not clear.

4.2. The nuclear Doa10 pathway

In addition to its PQC roles in the ER and cytoplasm, Doa10 is also involved in nuclear PQC degradation. The ER membrane and nuclear envelope are contiguous, and a portion of Doa10 localizes to the inner nuclear envelope [106]. Doa10's nuclear localization is required for the Doa10-dependent regulated degradation of the MATα2 transcription factor [106]. Doa10 also selectively recognizes a temperature-sensitive mutant of the nuclear protein Ndc10 [60]. Mutant Ndc10 is targeted for degradation by exposure of the hydrophobic side of an amphipathic helix and a hydrophobic C-terminal tail [107], indicating that Doa10 recognizes exposed hydrophobicity in misfolded nuclear proteins.

4.3. The Slx5 pathway

Another potential nuclear PQC ubiquitin-protein ligase is the yeast RING-domain protein Slx5, which was previously characterized for its role in ubiquitylating sumoylated proteins [108,109]. The Slx5 pathway is required for the ubiquitylation and degradation of the SUMOylated transcription factor Mot1 in its normal form, as well as its mutant form Mot1-301 [110]. Because Mot1-301 is degraded more rapidly than normal Mot1, Slx5 was suggested to be involved in nuclear PQC [110]. Enhanced degradation of a mutant protein compared to its normal form is seen in the PQC degradation of certain abnormal proteins. For example, 95% of mutant cystic fibrosis transmembrane conductance regulator (CFTR) is degraded by the ubiquitin-proteasome system, whereas 75% of normal CFTR is degraded due to slow folding kinetics [111,112]. Additional PQC substrates will need to be identified to solidify Slx5's role in nuclear PQC degradation.

4.4. Potential mammalian nuclear PQC ubiquitin-protein ligases

Two mammalian ubiquitin-protein ligases that are posited to function in nuclear PQC degradation are UHRF-2 and PML IV [113–115]. UHRF-2 is a RING-domain ubiquitin-protein ligase that has been shown to be involved in the degradation of a truncated form of the huntingtin (htt) protein [113]. Both the non-toxic, normal polyglutamine tract version of truncated htt and a toxic, expanded polyglutamine tract version are degraded in a UHRF-2 dependent manner [113]. Similar to UHRF-2, PML IV has been shown to be involved in the degradation of a nuclear protein with an expanded polyglutamine tract [114]. PML IV also associates with the nuclear aggregates formed by expressing polyglutamine-expanded proteins [114,115]. It has not yet been shown if UHRF-2 and PML IV can distinguish between normal and abnormal versions of a protein. Thus, additional studies will be needed to clarify their roles in nuclear PQC degradation.

5. Mitochondria

Mitochondria present a specific set of challenges for PQC degradation. One challenge is that mitochondria are bound by both inner and outer membranes that divide the mitochondria into distinct subcompartments. This means that separate PQC degradation pathways will be required to recognize misfolded proteins in each subcompartment. Similar to the ER, ubiquitin and proteasomes are not located inside the mitochondria. Because of this, inner mitochondria PQC degradation appears to be independent of the proteasome, instead relying on AAA-ATPase proteases similar to bacterial systems [116]. Another challenge for PQC degradation in the mitochondria is that there is a continual production of reactive oxygen species (ROS) as a byproduct of ATP production. An environment with a high level of ROS can lead to increased protein oxidation and misfolding, which the mitochondrial PQC degradation machinery will need to mange robustly.

5.1. The Parkin pathway

Parkin is a mammalian PQC ubiquitin-protein ligase associated with the outer mitochondrial membrane. Inactivation of Parkin is a major cause of juvenile Parkinson's disease [117]. Parkin contains a RING-in between-RING domain [118], which is required for its ubiquitin-protein ligase activity [119,120]. Parkin also associates with CHIP and Hsp70, and this association leads to increased Parkin-mediated ubiquitylation of unfolded Pael receptor [121]. Association with CHIP and Hsp70 suggests Parkin may have additional roles in cytoplasmic PQC degradation. To date, the abnormal structural feature in the substrates targeted by Parkin is unknown. Parkin mediates the degradation of specific proteins that are associated with Parkinson's disease [122], but it is not known how or why Parkin targets these substrates.

5.2. The MITOL pathway

Another mammalian ubiquitin-protein ligase associated with the outer mitochondrial membrane is MITOL (mitochondrial ubiquitin-protein ligase) [123]. MITOL contains a multi-spanning membrane domain that passes through the outer mitochondrial membrane four times [123]. In addition, MITOL possesses a PHD-variant of the RING domain that is responsible for its ligase activity [123]. Certain point mutants of superoxide dismutase 1 (Sod1), but not normal Sod1 are degraded in a MITOL-dependent manner [124]. Components required for MITOL-mediated ubiquitylation are unknown, and MITOL does not contain any obvious chaperone-binding motifs. The lack of multiple substrates and known partners means substrate recognition mechanisms are unclear at this time.

6. Conclusions

In this review, we have attempted to describe the current state of knowledge for how each ubiquitin-protein ligase implicated in PQC degradation operates in substrate recognition. In a few cases, the substrate-targeting mechanisms are becoming better understood. In most cases, however, there is still considerable work that is needed to discover the modes of substrate recognition for each PQC ligase. In particular, we currently have only a rudimentary knowledge about how the individual PQC ubiquitin-protein ligases actually bind their substrates and what they recognize as abnormal within their substrates.

After exploring the literature on the topic of substrate recognition in PQC degradation, we think there are a few main questions that need to be resolved moving forward. First, it is clear that the survey of PQC ubiquitin-protein ligases is incomplete, so what are the other ligases that participate in cellular PQC degradation? Second, what is the purpose for having multiple PQC ubiquitin ligases in a single compartment? In the ER, Hrd1 and Doa10 recognize structural lesions presented in distinct locations in relation to the ER membrane making the utility of two pathways obvious. But why, for example, do San1 and Doa10 both function in nuclear PQC degradation? Do they recognize different abnormal structural features in their substrates? If so, having two separate PQC degradations systems in the nucleus would broaden the cell's substrate recognition capabilities. Or, do they function in different subcompartments of the nucleus? Consistent with this hypothesis, Doa10 is membrane bound, while San1 is not. Determining the reasons for multiple PQC degradation pathways in a single compartment will require a larger pool of substrates for each pathway and a better understanding of the abnormal structural features recognized by each PQC ligase.

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