Protein Homeostasis at the Plasma Membrane

The plasma membrane (PM) and endocytic protein quality control (QC) in conjunction with the endosomal sorting machinery either repairs or targets conformationally damaged membrane proteins for lysosomal/vacuolar degradation. Here, we provide an overview of emerging aspects of the underlying mechanisms of PM QC that fulfill a critical role in preserving cellular protein homeostasis in health and diseases.

Protein quality control (QC) is essential to preserve the functional and structural integrity of polypeptides during their lifespan and offset potentially deleterious consequences of misfolding. Multiple QC systems have evolved at the cellular and transcellular level to compensate for the limited fidelity of protein translation, maturation, transport, and conformational maintenance at various cellular and extracellular destinations (109). The QC surveillance of DNA replication (107), transcription (126, 150), and maturation/transport of mRNAs (134) and tRNAs (16, 145) also contribute indirectly to cellular protein homeostasis or proteostasis (109). Conformational surveillance mechanisms of mature polypeptides involve the repair (or refolding) of unfolded molecules by chaperone networks and/or the proteolytic degradation of irreversibly damaged molecules via the ubiquitin-proteasome system (UPS), autophagosomes and/or lysosomal/vacuolar proteolysis. Partially overlapping proteostasis networks have evolved to meet the distinct requirements of polypeptides’ intrinsic designs at distinct locations, such as the nucleus, mitochondria, ER, cytosol, plasma membrane (PM), as well as the extracellular space (15, 40, 42, 60, 94, 98, 143) (FIGURE 1). Here, we survey the cellular and molecular basis of PM QC mechanisms that contribute to the refolding and/or elimination of nonnative polypeptides that either escaped the biosynthetic QC (3, 16) or were generated during aging and proteotoxic stress in situ.

Conformational Surveillance of PM Proteins is Necessary for Cellular and Organismal Homeostasis

In addition to serving to maintain intracellular homeostasis and communication with the extracellular environment of unicellular organisms, integral PM proteins also constitute signaling networks with pivotal roles in the proliferation, development, polarity, migration, and maintenance of the extracellular milieu in multicellular organisms. To regulate the myriad of activities and protein-protein interactions, it is imperative to maintain the native conformation of PM proteins, despite their modest folding free energy at physiological temperature (27). The limited thermal stability in concert with unavoidable cellular and environmental stresses (e.g., thermal, mechanical, oxidative, osmotic, and metabolic) and/or mutations that escaped the recognition by the ER or Golgi QC can contribute to the appearance of unfolded, misfolded, and/or aggregation-prone cytotoxic polypeptides at the PM (FIGURE 2). Failure to repair or eliminate these damaged PM proteins could lead to the loss- or gain-of-function phenotype, with severe consequences for cellular and organismal proteostasis (102). Conversely, the PM QC may allow the disposal of modestly unfolded and partially functional proteins, which could exacerbate the phenotypic manifestation of conformational diseases and pose additional hurdles on pharmacological therapies of folding defects (2, 96, 118).

The first evidence for PM QC operation came from studies demonstrating that the stressed uracil transporter (Fur4p), as well as the mutant multidrug resistance (Pdr5) and pheromone (Ste6p) transporter are rapidly degraded from the yeast PM (33, 54, 76). The downregulation of these proteins via endocytosis and vacuolar proteolysis required ubiquitination, a posttranslational modification by the cellular ubiquitination machinery, consisting of an Ub-activating E1 enzyme, E2 Ub-conjugating enzyme (e.g., Ubc4/Ubc5) (116), and E3 Ub-ligase (e.g., Rsp5, the yeast homologue of the Nedd-4) (32). Subsequent work has confirmed this paradigm and extended its counterpart to mammalian cells (118).

The degradative PM QC can be divided into four steps. 1) Recognition of nonnative clients and the recruitment of ubiquitination machinery that leads to 2) the internalization and 3) the endosomal sorting of ubiquitinated cargo into budding intraluminal vesicles (ILV) by the endosomal sorting complexes required for transport (ESCRT) and the formation of multivesicular endosome (MVE).
Multiple cellular organelles have folding sensor machineries to detect protein conformers; however, it is not clear how these systems discriminate terminally misfolded molecules from on-pathway folding intermediates. Aberrant or partially translated products are ubiquitinated by the ribosomal E3 ligases, Ltn1, and/or Not4 (98). Nonnative proteins in the cytosol are recognized by Hsp/Hsc70/Hsp40 and ubiquitinated by E3 ligases CHIP, Ubr1 and 2, and Hu5 (closest mammalian homologs UBE3B and UBE3C) (24, 38, 92) and directed to proteasomal degradation. In the yeast nucleus, the E3 San1 (42) can directly recognize misfolded proteins; however, it needs chaperone recruitment for transporting misfolded substrate into the nucleus (53). Ubr1–2 functions both in the cytosol and nucleus (53). In the ER-associated degradation (ERAD), the role of Hsp70/40/90 is conserved, but UDP-glucose:glycoprotein-glucosyltransferase (UGGT) can also directly bind nonnative proteins. Gp78 (mammalian autocrine motility factor receptor [AMFR]) functions in the ERAD with association of p97/VCP. The E3 Hrd1 and Hrd3 (mammalian Synoviolin and Sel1L, respectively) bind to misfolded transmembrane and luminal ER proteins (ERAD-M and ERAD-L pathways). Cytosolically exposed domains of membrane proteins (ERAD-C) as well as cytosolic polypeptides are ubiquitinated by CHIP and in yeast by Doa10 (mammalian MARCH VI) (15). In yeast, the Golgi QC prominently contributes to the vacuolar degradation pathways. Whether this mechanism exists in mammalian cells remains to be investigated, although both misfolded scrapie prions (Prsc) (45) and LDL-receptors use this route for lysosomal degradation or for ERAD (misfolded LDL-receptor) (25). At the PM, the Hsc70/Hsp90/CHIP complex ubiquinates nonnative MPs, a prerequisite for MVH/lysosomal targeting via the ESCRT multiprotein complex, which contains multiple Ub-receptors (2, 95). Hsp90/Cdc37 may play a role in recognition of misfolded extracellular domains (35). Clusterin and other soluble chaperones mediate the clearance of misfolded extracellular proteins by delivering them via receptor-mediated endocytosis for lysosomal proteolysis (143). Following mitofusion and PINK1-dependent recruitment to damaged mitochondria, Parkin ubiquitinates damaged mitochondrial outer membrane proteins, a prerequisite for mitophagy (40). Degradation of ubiquitinated protein aggregates can occur in multiple cellular locations via autophagy relying on fusion with endosomes (130).
The fusion of MVE with the lysosomal/vacuolar compartment ensures reutilization of amino acids from cleaved polypeptides (55, 81). Although the first stage of the PM QC displays unique features, subsequent steps appear to overlap mechanistically with the signal-dependent downregulation of ubiquitinated, native PM transporters, receptors, and structural proteins, topics covered in more depth by a number of excellent review articles (21, 100, 113).

**Recognition and Ubiquitination of Misfolded PM Protein**

**Cytoplasmic Domain Recognition**

Several yeast and mammalian integral PM proteins [e.g., mutant variants of the cystic fibrosis transmembrane conductance regulator (CFTR), α₂-receptor, transferrin receptor, bile salt export pump (BSEP), megalencephalic leukoencephalopathy with subcortical cyst 1 (MLC1), vasopressin V2 receptor (V2R), dopamine D4.4 receptor (DRD4), Na⁺/H⁺ exchanger 6 (NHE6), unliganded MHC I and human ether-a-go-go K⁺ channel] are prematurely eliminated from the cell surface as a result of documented or perceived structural defect (1–2, 10, 17, 39, 79 – 80, 95, 115, 117, 141, 151). The nature of the structural defect that signals substrate recognition by the PM QC remains poorly defined. Destabilizing mutations confined to cytoplasmic regions may disrupt the secondary, tertiary, or quaternary structure directly, whereas mutations that reside in the transmembrane (TM) or extracellular regions, similarly to various stressors, may be relayed to cytosolic domains and recognized by molecular chaperones as exemplified by a subset of misfolded ER membrane proteins (114) or recognized by specialized mechanisms (see following sections).

The highly conserved cytosolic chaperone system, Hsp70/Hsc70, transiently interacts with short extended peptide segments enriched in hydrophobic and basic amino acids (112, 154) in the cytoplasmic region of unfolded PM proteins. Similar to Hsp70/Hsc70, the chaperone activity of the Hsp90 family members is coupled to ATP binding and hydrolysis, and is regulated by co-chaperones (57, 127). Based on the interaction of Hsp90 with a range of metastable mature polypeptides (e.g., kinases, E3 ligases, receptors, and steroid receptors) and biogenic folding intermediates, the Hsp90 machinery can recognize both partially folded as well as extended polypeptide conformation, with preference toward thermally unstable domains (127). 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If structural perturbation by the PM protein unfolding cannot be counteracted by the profolding activity of chaperones, ubiquitination and lysosomal/vacuolar degradation prevail. The biochemical basis of this “triage” decision is only partly understood and probably relies on the extended residence time of Hsp70/Hsc70/CHIP complex on the misfolded polypeptide (22). The NH₂-terminal...
tetra-tricopeptide repeat (TPR) of CHIP binds to the COOH terminus of Hsc70/Hsp90 chaperones with low affinity. The COOH-terminal U-box, a RING-like domain, serves as scaffold for E2 enzymes UbcH5 or Ubc13/UEV1 to assemble either K48- or K63-linked Ub chains (4). In case of native-like proteins, it is postulated that transient association-dissociation cycles of Hsc70-Hop-Hsp90 and co-chaperones ensure that the client protein rapidly (re)folds and undergoes only limited ubiquitination that is reversed by the activity of deubiquitinating (DUB) enzyme(s). In contrast, extended association of Hsc70-CHIP with slowly folding clients, promoted by the CHIP-induced inhibition of the Hsc70/Hsp70 ATPase activity, leads to more efficient ubiquitination (22). This model is supported by in vitro biochemical studies (95), demonstrating complex formation between Hsc70/Hsp90/Hsp40, CHIP, and the temperature rescued ΔF508-CFTR or the CD4-ΔC chimeric model protein upon thermal unfolding of the nucleotide binding domains (NBDs) or the ΔC domain, respectively, at the PM (2, 95) (FIGURE 3).

Soluble client binding to and refolding activity of Hsc70/Hsp70 and Hsp90 are regulated by co-chaperones both in vitro and in vivo (49, 119, 140). A subset of co-chaperones, DNAJA1 (Hdj2), DNAJB2 (Hsj1), Aha1, and HOP have been invoked to influence the peripheral triage decision by facilitating the temperature-rescued ΔF508-CFTR ubiquitination in post-Golgi compartment (2, 95). Ablation of DNAJA1 by siRNA decreased the ubiquitination and concomitantly stabilized the ΔF508-CFTR at the PM, probably via impaired client recognition and transfer to Hsc70 similar to that observed at the ER QC (148–149). Although HOP and Aha1 can facilitate Hsp90 refolding activity in vitro (83, 138), depletion of these co-chaperones enhanced the biosynthetic maturation of ΔF508-CFTR and induced the rescued ΔF508-CFTR stabilization by a poorly understood mechanism (95). Interestingly, DNAJB2 harboring an Ub-interacting motif (UIM) and J-domain regulates rescued ΔF508-CFTR ubiquitination only at the post-endocytic stage (95) (Table 1).

Contrary to the chaperone-dependent PM QC in higher eukaryotes, conformationally damaged yeast nutrient transporters are subjected to chaperone-independent ubiquitination upon exposure to cellular stress or excess substrate (44, 73, 76). Both heat and oxidative stress, similar to high concentration of substrates, lead to conformational perturbations that may destabilize the hydrogen bonded NH2-terminal “loop interaction domain” (or LID) association with multiple cytosolic loops in Fur4 and Mup1, the yeast uracil and methionine transporter, respectively (69). This structural alteration serves as a degron by exposing two Lys ubiquitin acceptor sites for Rsp5-mediated ubiquitination (69).

Both arrestin-related protein (ART)-dependent and -independent mechanisms have been implicated in degron recognition by Rsp5 (69, 77, 91, 152): however, the precise biochemical basis remains to be determined. Intriguingly, the Rsp5-dependent ubiquitination and elimination of unfolded Fur4p could not be substituted by Ubr1 or San1 E3 ligases that can recognize unfolded cytosolic and nuclear client protein (152). The possible redundancy of PM QC ligases is supported by the observation that CHIP ablation only partially inhibits the proteolytic downregulation of unfolded ΔF508-CFTR, DRD4, V2R, and hERG, whereas depletion of other E3 Ub-ligases (e.g., Hrd1 and Gp78) also impeded CFTR clearance from the PM (1, 2, 95). Similarly, at least two E3 ligases (Cullin5 and CHIP) are responsible for the geldanamycin-induced ubiquitination and lysosomal downregulation of ErbB2 (HER2/neu) (34, 153). These observations with the modest phenotypic manifestation of the CHIP knockout mouse (87) suggest that a complex network of QC E3 ligases has evolved to maintain PM proteostasis with overlapping substrate specificity.

**Transmembrane Segment Recognition**

Conformational defects caused by mutations in the TM segments may be recognized directly in the membrane plane and/or via allosteric perturbations of the cytosolic region due to rearrangement of exposed cytosolic loops and domains, as suggested by the phenotype of missense TM mutations in the V2R (W164S), DRD4 (M345T) (1, 2). These mutations introduce polar residues and induce the CHIP recruitment and polyubiquitination probably in a chaperone-dependent manner at the cytosolic surface of the G-protein-coupled receptors (GPCRs) (1, 2). It is plausible that intracellular and/or extracellular K+ depletion results in the global unfolding of the hERG channel, including its major cytosolic domains, leading to the CHIP recruitment (1). Unfolding is likely initiated by depletion of one or more K+ ions from the ion-conducting pore of the channel (1). In addition to mutations, reduction of the membrane sphingolipid content can also provoke conformational destabilization, followed by the ubiquitination-dependent vacuolar degradation of the yeast PM Pma-1 (H-ATPase) and Gap1p (general amino acid permease) transporters, conceivably by altering the TM domain packing (39, 73, 136, 151).

A distinct substrate-binding mode is envisioned for the membrane-associated RING-CH (MARCH) ligases, representing a small family of 11 RING-CH E3 Ub ligases with incompletely understood physiological functions in PM remodeling (37, 90).
MARCH ligases are orthologs of the Kaposi sarcoma-associated herpes virus (KSHV) immunoevasion ligase K3 and K5 (137) and contain two transmembrane segments and a COOH-terminal cytosolic RING-CH domain. Endogenous or overexpressed MARCHs play a key role in the ubiquitination-dependent lysosomal downregulation of native cell surface receptors (e.g., MHC-I: K3, K5, MARCH-9 and -4; MHC-II: MARCH-2 and -8, CD4, CD44 and CD98, MARCH-4 and -8) (6, 7, 23, 26, 41, 46, 62, 65, 66, 90). MARCH-8 and -1 can recognize a transmembrane hydrophobic segment in concert with charged residues and results in the Ub-conjugation to a Lys residue at the membrane-cytosol interface in the case of the HLA-DR and transferrin receptor (41, 46). Although the consensus signal for TM recognition by most MARCHes remains to be identified, it is reasonable to assume that a similar recognition mechanism may come into play upon unfolding and/or disassembly of polytopic PM polypeptides. It is also possible that otherwise buried hydrophilic residues become exposed and are recognized by QC E3 ligases. Although the latter scenario has not been documented at the PM yet, a cluster of intramembrane hydrophilic residues was found in both ER and Golgi E3 ligases (Hrd1, gp78, Doa10, and Tul1) that can facilitate binding to charged TM residues of nonnative client proteins (8, 106).

**QC at the Exofacial Surface of the PM**

The QC machinery that directly recognizes the extracellular surface of nonnative PM proteins and renders them susceptible for rapid elimination has not been described. Conformational defects emanating from the exofacial surface of PM proteins, however, may be relayed allosterically to cytoplas-
Impaired N-glycosylation of H+^-K^+^-ATPase, K- and δ-opioid receptor, Kv1.4 potassium channel, glucose transporter 1 (GLUT1), and CFTR has been associated with metabolic destabilization at the PM (43, 75, 82, 133, 139) and was sufficient to compromise the conformational stability of CFTR cytosolic domains, with consequential polyubiquitination in post-Golgi compartments (43). Unfolding of the extracellular domain of the LDL receptor provokes Derlin-1-/syntaxin-dependent retrograde transport of the internalized receptor from early endosomes to the Golgi complex and ultimately to the ER, where their degradation occurs (25). The signal that preferentially targets unfolded PM proteins toward ER-associated degradation remains to be elucidated.

Molecular chaperones secreted into the extracellular space may impact the PM homeostasis (89, 101, 144, 147). Hsp90 and its co-chaperone, the cell division cycle Cdc37, are involved in cell migration and are expressed in various tissues (89, 101, 144, 147). They contribute to the stability of a variety of proteins, including signaling molecules and structural proteins, by maintaining their correct conformation and preventing their aggregation and degradation (89, 101, 144, 147).

Table 1. Endocytic components capable of binding Ub

<table>
<thead>
<tr>
<th>Component</th>
<th>Adaptor</th>
<th>Binding Domains</th>
<th>Binding Partners</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>ESCRT-0</td>
<td>Hrs (Vps27)</td>
<td>DUIM</td>
<td>STAM, Ub</td>
<td>Ub-cargo binding and concentration</td>
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<td></td>
<td></td>
<td>VHS</td>
<td>ESCRT-I, Ub</td>
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<td></td>
<td>FYVE</td>
<td>Phosphodiyl-inositol-3 phosphate, Ub</td>
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<td></td>
<td></td>
<td>GAT</td>
<td>Clathrin</td>
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<td>PTAP</td>
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<tr>
<td></td>
<td>Stam1-2 (Hse1)</td>
<td>VHS</td>
<td>Hrs, Ub</td>
<td>Ub-cargo binding and concentration</td>
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<tr>
<td></td>
<td></td>
<td>UIM</td>
<td>Ub</td>
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<td></td>
<td></td>
<td>SH3</td>
<td>STAMBP and USP8</td>
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<td></td>
<td></td>
<td>GAT</td>
<td>Ub</td>
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<tr>
<td>ESCRT-I</td>
<td>Tsg101 (Vps23)</td>
<td>UEV</td>
<td>Ub, ESCRT-0, HDPTP/PTPN23</td>
<td>Ub-cargo binding and concentration</td>
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<tr>
<td>ESCRT-II</td>
<td>EAP45 (Vps36)</td>
<td>GLUE</td>
<td>Ub</td>
<td>Ub-cargo binding and concentration</td>
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<tr>
<td></td>
<td></td>
<td>WH2</td>
<td>Phosphoinositide lipids ESCRT-I</td>
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<tr>
<td>ESCRT associated</td>
<td>Alix (Bro1)</td>
<td>Bro1 V-domain</td>
<td>K63 polyUb, CHMP4 (ESCRT-III)</td>
<td>Viral budding and cytokinesis</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>In yeast, Bro1 binds Doa4 (USP8) and ESCRT-0</td>
<td>Ubiquitinated cargo, sorting to MVB</td>
</tr>
<tr>
<td>ARF associated</td>
<td>GGA1-3</td>
<td>VHS, GAE</td>
<td>Accessory proteins with DFGX0</td>
<td>Trans Golgi-to-endosome transport</td>
</tr>
<tr>
<td>Adaptor</td>
<td>Tom1 and Tom1L1 and L2</td>
<td>VHS, GAT</td>
<td>Ub, Myosin VI, Endofin, Clathrin, ZFYVE16, Tollip</td>
<td>Endosomal trafficking</td>
</tr>
<tr>
<td>Adaptor</td>
<td>Tollip</td>
<td>CUE, C2, TBD</td>
<td>Ub or phosphodiyl-inositol-3 phosphate, Tom1 Tom1L2</td>
<td>Component of the signaling pathway for Toll-like receptor complex and interleukin receptor-1</td>
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<tr>
<td>GTP exchange factor</td>
<td>Rabex-5 (Vps9)</td>
<td>A20 Zn-finger (CUE domain in yeast)</td>
<td>Ub, RGS14, Rabaptin-5 Rab4/5/21/22, GGAs</td>
<td>Endosomal fusion</td>
</tr>
<tr>
<td>Molecular chaperone</td>
<td>DNAJB2 (Hsp40 homolog)</td>
<td>J-domain UIM</td>
<td>Hsp70/Hsc70 binding Polyubiquitin</td>
<td>Regulates activity of Hsp70, Negative regulator of Hsp70 chaperone activity</td>
</tr>
<tr>
<td>Molecular co-chaperone</td>
<td>BAG1</td>
<td>BAG-domain</td>
<td>Hsp70 binding</td>
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</table>

ARF, ADP ribosylation factor; BAG, apoptosis regulator Bcl-2; CUE, coupling of ubiquitin conjugation to endoplasmic reticulum degradation; DUIM, double-sided ubiquitin-interacting motif (UIM); FYVE, Fab1, YetB, Vac1p, and EEA1; GAE, γ-adaptin ear domain; GAT, GGA and Tom1; GGA, Golgi-localizing/γ-adaptin ear homology domain/ADP-ribosylation factor-binding; GLUE, GRAM-like ubiquitin binding in EAP45; PTAP, Pro-Thr-Ala-Pro; Rab, Ras superfamily of monomeric G proteins; RGS, regulator of G protein signaling; SH3, Src homology 3; SOUBA, solenoid of overlapping ubiquitin-associated domain; UEV, Ubiquitin E2 variant; VHS, Vps27p, Hrs, and STAM domain; WH2, WASP-homology 2 or Wiskott-Aldrich homology 2; UBL, Ubiquitin-like domains.
and invasion in normal and cancer cells (36, 123). The extracellular Hsp90-Cdc37 complex interacts with kinase receptors (HER2, EGFR, and ErbB2) and metalloproteinases (35). In analogy to the stabilization of cytosolic kinase domains (127), Hsp90-Cdc37 in concert with extracellular Hsp70, Hop, and p23 maintains the active conformation of the extracellular domains of selected receptors (35).

Clusterin (apolipoprotein J), haptoglobin, and \( \alpha_2 \)-macroglobulin accumulate extracellularly after stress induction and participate in conformational protection of PM proteins by their ATP-independent holdase activity (101). The scavenger capacity of these chaperones can prevent further aggregation by sequestering misfolded conformers of extracellular proteins (89, 101, 144, 147). Extended association of clusterin with client proteins culminates in receptor-mediated endocytosis and lysosomal degradation to circumvent the PM permeabilization, triggered by extracellular oligomers (89) (see FIGURE 1). It was demonstrated 20 years ago that expression of clusterin is increased in Alzheimer’s disease (85), which since has been attributed to the neuroprotective response of the brain (135). By binding to \( \beta \)-amyloid peptides, clusterin prevents fibril formation (146) and may have a similar function in several sub-classes of high-density lipoprotein (HDL) particles. Accordingly, loss-of-function mutations and haploinsufficiency of clusterin, as well as elevated cholesterol levels, are implicated as a risk factor for Alzheimer’s disease (78, 122, 142).

Chaperone-Independent Ubiquitination of Nonnative PM Proteins

As an alternative route of ubiquitination, several adaptors have been invoked in the recruitment of E3 ligases to nonnative PM proteins. Ten arrestin-related trafficking adaptors or ARTs are implicated in client selection for Rsp5-mediated ubiquitination that invariably causes substrate and/or stress-induced downregulation of yeast PM proteins by accelerated internalization and ESCRT-dependent vacuolar degradation. Based on computer modeling and phylogenetic analysis, two subclasses of arrestins are distinguished (9). The more ancient alpha-arrestins (or ARTs) contain an NH\(_2\)-terminal arrestin domain similar to beta-arrestins and a COOH-terminal PPxY motif(s) (9), which is responsible for the Rsp5 recruitment via the WW domains. Although multiple ARTs have overlapping substrate specificity (e.g., ART1, 2, 4, and 8 are involved in Hxt6, Fur4, and Tat2 down-regulation), only ART1 is required to render Can1 and Mup1 metabolically unstable (77). Besides ARTs, arrestin-like adaptors also participate in the downregulation of PM transporters. Both Bul1/Bul2 and ART1–9 ablation are required to impede the downregulation of destabilized Fur4 from the PM (69). Mammalian cells have five members of the arrestin domain-containing proteins (ARRDC) in the alpha-arrestin subfamily, but their contribution to sensing unfolded PM proteins is not known (48). In contrast to alpha-arrestins, beta-arrestins have clathrin and AP-2 interacting scaffolding function to facilitate internalization and signaling of activated GPCRs upon phosphorylation in higher eukaryotes (120). Bsd2 is a transmembrane adaptor protein that harbors PY motifs, recruits Rsp5, and can recognize polar TM domains of unfolded or activated PM proteins (e.g., Smf1 metal transporter and mutant PM ATPase) at the endosomal and Golgi compartment (58). This mechanism complements the ART-dependent ubiquitination at the PM and facilitates the transporter vacuolar delivery along the endocytic pathway where deubiquitination may occur (19). Likewise, ubiquitination and lysosomal delivery of Smf1 was blocked if nine ARTs in concert with Bsd2 were deleted (91). ARTs can also recruit constituents of the ESCRT-I machinery (Vps28 and Vps23) to modulate cargo sorting efficiency via ESCRT-I ubiquitination (56, 104). Intriguingly, the human ortholog (NDFIP1) of Bsd2 is essential for the Nedd4–2-dependent polyubiquitination and downregulation of the divergent metal transporter (DMT1) to protect mammalian cells from toxic iron accumulation, suggesting a similar paradigm for the regulation of DMT1 PM density (63). In addition to being an adaptor, NDFIP1 also functions as an activator of multiple HECT domain E3 ligases of the Nedd4 family (88).

Configuration of Ubiquitin as Sorting Signal at the PM and Endosome

Ubiquitination of unfolded PM protein is one of the key sorting signals that ensures cargo recognition by the internalization and endocytic sorting apparatus to facilitate lysosomal/vacuolar targeting and degradation (47). The cellular aspects of this paradigm could be exemplified by several PM proteins, including a direct correlation between ubiquitination, internalization, and lysosomal delivery of the cell surface resident CD4T-\( \lambda_c \) on thermal unfolding (2, 44, 51, 95, 110, 118). Although a single Ub appears to be sufficient to direct internalization in yeast, MVE sorting requires poly- or multiple-mono-Ub for recognition by ESCRT0-II (2, 95, 105, 124). Notably, polyUb chain or multiple monoUb of numerous native and misfolded PM proteins, as well as model proteins in

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higher eukaryotes, significantly augments the internalization rate of cargo (5, 31, 50). In addition of K63-linked Ub chains, lysosomal sorting signals may contain K48-, K11-, and K29-linked chains to enhance the avidity for Ub-binding protein recognition (13). In misfolded CD4T-λC, the K63-linked Ub chain was more abundant than the K48-linked chain at the PM (2), and the Rsp5 synthesized K63-linked Ub-chain is susceptible to deubiquitination by the Ubp2 DUB (67, 68).

The Ub-chain configuration is dynamically determined by both the E2/E3 and the deubiquitinating enzyme (DUB) activities. Although some DUBs associate with specific E3 ligases (e.g., Ubp2-Rsp5), others [e.g., STAMBP (AMSH), STAMBP-LP (AMSH-LP), and USP8 (UBPY)] are confined to endosomes (19, 132), allowing regulated remodeling of cargo Ub configuration that can either facilitate lysosomal/vacuolar degradation or recycling, depending on the extent and configuration of ubiquitination. Interaction of USP20 and USP33 DUB with beta-arrestins ensures the agonist-stimulated beta2-adrenergic receptor deubiquitination (11, 121), whereas the USP2 variant 45 deubiquitinates ENaC (14). USP10 regulates CFTR recycling in airway epithelia (12), and USP46 prohibits lysosomal degradation of glutamate receptor in C. elegans (71). No specific DUB activity has been identified in association with the PM QC machinery.

Decoding Ubiquitin Modification as Sorting Signal Along the Endocytic Pathway

A large number of Ub-receptors containing a variety of Ub binding domains (UBD) are involved in the translation of Ub signals as internalization and lysosomal sorting motifs of PM cargo molecules (28). The UBDs may also protect against premature deubiquitination and proteasome-mediated cleavage of PM proteins, a prerequisite for MVE/lysosomal delivery. Although only a subset of the endocytic Ub receptors has been functionally identified in PM QC, it is reasonable to assume that the sorting machinery of native and nonnative ubiquitinated cargos partially overlaps.

Depending on their primary confinement, ubiquitinated PM cargo can be internalized via clathrin- or caveolin-dependent internalization route. Ubiquitinated cargo concentration into clathrin-coated pits is facilitated by the endocytic adaptors (Epsin 1–2 and Eps15/15R/15B), harnessing multiple UBDs with preferentially recognition of polyUb (5, 50, 111, 128). In the caveolin-dependent pathway, UBBD1 and the p97-UBBD1 ATPase complex are required for targeting of ubiquitinated caveolin-1 to early endosomes (70).

Following the delivery of ubiquitinated cargoes from clathrin- and caveolin-coated vesicles into tubular early endosomes (52), cargo segregation from recycling toward lysosomal delivery is mediated by components of the ESCRT0-III. The four multimeric ESCRT (0–III) complexes consecutively assemble and disassemble with each other and numerous accessory proteins to ultimately ensure the sorting and budding of most ubiquitinated PM proteins into intraluminal vesicles (ILVs). ESCRT-0 consists of Hrs and Stam1/2, encompassing five UBDs, and has modestly higher affinity to K63- over K48-linked polyUb chain with ~50-fold increased binding preference to K63-linked tetra-Ub over mono-Ub (59, 108) (Table 1). Ubiquitination of constituents of the PM QC machinery (e.g., Hsc70, CHIP, and BAG1) may serve as a signal amplification mechanism to enhance the fidelity of the lysosomal targeting of nonnative membrane proteins. In concert, the Ub-like domain of BAG1, an Hsc70 cochaperone, could further facilitate nonnative cargo recognition by the Ub-receptor network both at the PM and endosomes, as observed for the misfolded CFTR mutant (95).

Subsequent interaction of ubiquitinated PM proteins with ESCRT I–III machinery is required for their delivery into MVE and the formation of ILV (55, 64). Depletion of Hrs, Stam1, or Tsg101 (ESCRT-I) attenuated the endocytosis and lysosomal delivery of misfolded ΔF508-CFTR, CD4T-λC, V2R-W164S, and DRD4-M345T from the cell surface. The polyubiquitinated nonnative rΔF508 and Δ70 CFTR (70 residues COOH-terminal truncation), but not the native channel, physically interact with ESCRT components (e.g., Hrs, Stam1/2, TSG101) (118). This functional and biochemical evidence supports the indispensable role of ESCRT complexes in the lysosomal degradation of nonnative PM proteins (2, 43, 95).

Several other Ub-receptors have been identified along the endocytic pathways that contribute to ubiquitinated PM protein recognition and clearance. TOM1 or TOM1 like–1–2 [TOML1–2] clathrin interacting ESCRT-0–like proteins (103) can form a complex with Tollip and Endofin and are capable of interfacing with Hrs and Tsg101 (131) (Table 1). The ESCRT-I proteins, Tsg101 and UBAPl, and the ESCRT-II EAP45 also incorporate UBDs. In addition, the Golgi protein gamma adaptin-ear-containing ARF-binding protein 3 (GGA3) has Hrs-like UBBDs and binds both ubiquitinated cargo and Tsg101 (64, 103). The early endosomal Rab5 GTP/ GDP exchange factor (Rabex-5) also contains two UBDs (74, 99); one of them (A20 Zn-finger) displays E3 Ub ligase activity (84) and can associate with ubiquitinated cargo both at PM and endosomes, as shown for the EGF receptor (99).
The ILV budding process is controlled by the assembly of ESCRT-III, lacking known UBDs, but has the capacity to recruit two DUBs, USP8 and STAMBP (20). Bro1 protein His domain protein tyrosine phosphatase (HD-PTP/PTPN23), which is an adaptor protein for ESCRTs, has been shown to compete with STAM2 for USP8 interaction, driving the ubiquitinated EGF receptor to ESCRT-III (125). In yeast, Bro1 interacts with ESCRT-0, recruits Doa4 (USP8), and selectively sorts ubiquitinated cargo to MVE (97). Bro1 is a distant relative to mammalian Alix, which captures K63-linked polyUb (30). HD-PTP may have inherited the yeast Bro1 role, representing the degradative Ub-adaptor link from sorting endosomes to MVE.

Following the removal of Ubs and targeting client proteins into the budding ILV from the limiting membrane of MVE, the cargo is considered to be at the point of no return, whereas MVE matures into lysosome. Endolysosomal trafficking pathway, however, may not be the exclusive degradation route of misfolded PM protein elimination. A possible link between clathrin-mediated endocytosis and autophagy has been proposed via the actin motor, myosin VI, and its adaptor proteins (Dab2, GIPC,

Table 2. Possible adaptor proteins for directing cargo to autophagosomes at the endocytic pathway

<table>
<thead>
<tr>
<th>Component</th>
<th>Adaptor</th>
<th>Binding Domains</th>
<th>Binding Partners</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endocytic vesicles</td>
<td>Vps34</td>
<td>Phosphoinositide-3-kinase FYVE, PX</td>
<td>Phosphoinositide-3-kinase regulatory subunit 4, Beclin, either UVRAG or rubicon, or ATG14, Rab7A</td>
<td>Phosphoinositol to phosphatidylinositol (3)-phosphate formation, transport from early to late endosomes, lysosomal enzyme transport, autophagosome formation</td>
</tr>
<tr>
<td>Clathrin-dependent endocytosis complex</td>
<td>Dab2</td>
<td>Clathrin binding AP2 binding Phosphotyrosine binding motif</td>
<td>Clathrin EH domain-containing proteins (EPS15, EPS15L1 and ITS1) Myosin VI</td>
<td>Clathrin-associated sorting protein, through myosin VI dimerisation to autophagy</td>
</tr>
<tr>
<td>Endocytic vesicles</td>
<td>Tom1 and/or Tom1L2</td>
<td>VHS, GAT</td>
<td>Ub</td>
<td>Myosin VI, Endophilin, Clathrin, ZFYVE16</td>
</tr>
<tr>
<td>Endocytic vesicles</td>
<td>GIPC</td>
<td>PDZ</td>
<td>PDZ-binding proteins Myosin VI, APPL1</td>
<td>Endocytosis</td>
</tr>
<tr>
<td>Endocytic vesicles</td>
<td>LMTK2</td>
<td>Transmembrane serine/threonine kinase</td>
<td>Myosin VI</td>
<td>Early endocytic trafficking and recycling</td>
</tr>
<tr>
<td>NDP52 (CALCOCO2)</td>
<td>UBD/Zn-finger</td>
<td>LIR, SKICH</td>
<td>Ub</td>
<td>Myosin VI, LC3</td>
</tr>
<tr>
<td>T6BP (TIFA)</td>
<td>UBD/Zn-finger</td>
<td>LIR, SKICH, FHA</td>
<td>Ub</td>
<td>Myosin VI, ITCH (AIP4), PtdIns(4,5)P2 binding</td>
</tr>
<tr>
<td>Optineurin</td>
<td>UBD</td>
<td>LIR, Zn-finger</td>
<td>Ub</td>
<td>Myosin VI, CYLD, TBK1, TBC1D17</td>
</tr>
<tr>
<td>ATG16L1</td>
<td>7 WD40 repeats</td>
<td>Clathrin/AP2</td>
<td>Clathrin/AP2</td>
<td>Early autophagosome formation, probably in the dynamin-dependent scission process</td>
</tr>
</tbody>
</table>

AP, adaptin subunit of the adaptor protein; APPL1, adaptor protein, phosphotyrosine interaction; ATG, autophagy related gene; CYLD, cylindromatosis; Dab2, disabled-2; EH, Eps15 homology; FHA, fork head associated domain; FYVE, Fab1, YotB, Vac1p, and EEA1; GAT, GGA and Tom1; ITS1N1, intersectin 1; LC3, microtubule-associated protein 1 light chain 3; LIR, LC3-interaction motif; LMTK2, lemur tyrosine kinase-2; NDP52/CALCOCO2, calcium binding and coiled-coil domain 2, also known as nuclear domain 10 protein 52; PDZ, postsynaptic density protein (PSD95), Drosophila disc large tumor suppressor (Dlg1), and zonula occludens-1 protein (zo-1) or Discs-large homologous regions; PX, phosphoinositide-binding domain; Rab, Ras-associated protein; SKICH, SKIP-carboxyl homology domain; TBC, Tre-2, Bub2p, and Cdc16p domain family (Rab GTPase-activating protein); TBK1, TANK-binding kinase 1; UVRAG, UV radiation-resistance-associated gene protein; VHS, Vps27p, Hrs, and STAM domain, PH domain, and leucine zipper containing 1; Vps, class III phosphoinositide 3-kinase vacuolar protein sorting; WD, Trp-Asp solenoid repeat containing domain; UBD, ubiquitin binding domain; ZF, zinc finger domain.
Tom1, and LMTK2), harboring Ub, clathrin/AP2, and phospholipid binding domains (Table 2). Myosin VI links Tom1 to the autophagy adaptor proteins (T6BP, NDP52, and optineurin), which can recruit ubiquitinated cargo and via LC3 association may facilitate autophagosome formation (130). It is also possible that unfolded proteins are targeted for degradation in the absence of covalent Ub modification, either via complex formation with an E3 ligase, via component(s) of the ESCRT machinery, or by exposing a sorting signal that can be recognized, e.g., by ALIX, as exemplified by various transmembrane cargoes (e.g., Sna3, delta opioid receptor, and PAR1) (29, 61, 86, 93).

Conclusions

Although significant progress has been made to uncover the peripheral or PM QC machinery, many questions remain unanswered regarding the inner working of this homeostatic mechanism in cellular and organismal physiology. Little is known about the conformational sensitivity of the peripheral QC toward metastable or partially unfolded membrane proteins and the redundancy of the recognition, ubiquitination, and sorting machinery involved. We still do not have a comprehensive inventory of adaptors and chaperones that account for the recognition of conformationally damaged PM proteins, and we lack knowledge of possible contribution of alternative disposal pathways, such as autophagosome, proteasome, and retrograde ER delivery. Finally, the regulation of molecular chaperones that may suppress conformational defects of PM proteins by their foldase activity and/or delay ubiquitination will have to be elucidated. Better understanding of the cellular proteostasis networks at the molecular level may lead to novel opportunities for influencing inherited or acquired diseases affecting the conformational integrity of PM proteins.

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