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Repair or destruction—an intimate liaison between ubiquitin ligases and molecular chaperones in proteostasis

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Cellular differentiation, developmental processes, and environmental factors challenge the integrity of the proteome in every eukaryotic cell. The maintenance of protein homeostasis, or proteostasis, involves folding and degradation of damaged proteins, and is essential for cellular function, organismal growth, and viability [1,2]. Misfolded proteins that cannot be refolded by chaperone machineries are degraded by specialized proteolytic systems. A major degradation pathway regulating cellular proteostasis is the ubiquitin (Ub)/proteasome system (UPS), which regulates turnover of damaged proteins that accumulate upon stress and during aging. Despite a large number of structurally unrelated substrates, Ub conjugation is remarkably selective. Substrate selectivity is mainly provided by the group of E3 enzymes. Several observations indicate that numerous E3 Ub ligases intimately collaborate with molecular chaperones to maintain the cellular proteome. In this review, we provide an overview of specialized quality control E3 ligases playing a critical role in the degradation of damaged proteins. The process of substrate recognition and turnover, the type of chaperones they team up with, and the potential pathogeneses associated with their malfunction will be further discussed.

Keywords: aging; chaperone; CHIP; E3 ligase; proteostasis; quality control; ubiquitin

Concept of proteostasis

The three-dimensional organization and conformation of a polypeptide chain is important for its cellular function. Maintaining the correct folding state of a protein is challenging particularly due to kinetic effects of a crowded cellular environment [3]. The high concentration of macromolecules within most intracellular compartments strongly increases the tendency of misfolding of non-native and structurally flexible proteins,

Abbreviation
AD, Alzheimer's disease; ALS, amyotrophic lateral sclerosis; ARTs, arrestin-related trafficking adaptors; BMP, bone morphogenetic proteins; CAP, chaperone-assisted proteasomal degradation; CASA, chaperone-assisted selective autophagy; CFTR, cystic fibrosis transmembrane conductance regulator; CHIP, C terminus of Hsc70-interacting protein; CMA, chaperone-mediated autophagy; CP, core particle; DUBs, deubiquitylating enzymes; ER, endoplasmic reticulum; ERBB2, erythroblastic leukemia viral oncogene homolog 2; GR, glucocorticoid receptor; H3, Histone3; HD, Huntington's disease; HSF1, heat shock factor 1; HSP, heat shock proteins; Hul5, HECT ubiquitin ligase 5; IIS, insulin/IGF signaling; INM, inner nuclear membrane; LRR, leucine-rich repeat; MLPs, mislocalized membrane proteins; NAC, nascent polypeptide-associated complex; ONM, outer nuclear membrane; PD, Parkinson's disease; PML, promyelocytic leukemia; polyQ, polyglutamine; PRMT5, protein arginine methyltransferase 5; Psh1, Pob3/Spt16/histone 1; RING, really interesting new gene; RP, regulatory particle; SCF, Skp1/Cullin/F-box; Smad1, Sma-mother against decapentaplegic 1; SOD1, superoxide dismutase1; TPR, tetratricopeptide repeat; Ub, ubiquitin; UPS, ubiquitin/proteasome system.

often causing their polymerization and aggregate formation [3]. Certain proteins only obtain an ordered, native structure and adopt folded conformations upon binding to the appropriate partner molecule or chaperone [4,5]. Cells are frequently exposed to proteotoxic conditions, including heat or oxidative stress, which makes it even more difficult to establish and preserve native protein structures [6]. Genetic mutations might also increase the tendency of protein aggregation resulting in significant pressure on the cellular protein quality control systems [7]. Intracellular pathways involved in the maintenance of the proteome build a complex proteostasis network. The term proteostasis refers to the preservation of the proper concentration, distribution, and function of proteins. The intricate balance is mainly achieved by vigilant control and safeguarding of protein synthesis, protein maturation, and folding, protein transport, as well as the timely disposal of unwanted and damaged proteins by the main proteolytic routes: the ubiquitin/proteasome system (UPS) or the lysosome-autophagy pathway [8–10].

With age, the ability of postmitotic cells to keep a balanced proteome is gradually compromised particularly by downregulation of molecular chaperones and reduced efficiency of protein degradation [7]. As such, impairment of proteostasis is seen as one major hallmark of aging, correlated with dementia and neurodegeneration, type 2 diabetes, cystic fibrosis, cancer, and cardiovascular diseases [11,12]. Notably, longevity-promoting pathways, such as dietary restriction, insulin/IGF signaling (IIS), mitochondrial respiration, or germ line immortality provide increased stability to the proteome, delaying the onset of age-related diseases [1,9,13,14]. One of the central nodes in the eukaryotic proteostasis network is the interaction between molecular chaperones and proteolytic machineries. To maintain the cellular proteome molecular chaperones and ubiquitin (Ub)-dependent degradation pathways coordinate protein refolding and removal of terminally damaged proteins. Irreversibly affected proteins are particularly recognized by chaperone-assisted E3 Ub ligases, which target them for degradation by the UPS or autophagy.

Protein degradation machineries

Ubiquitin/proteasome system (UPS)

Selective degradation of misfolded or aggregated proteins is crucial to maintain functionality of the cell. A fundamental proteolytic module of the cellular proteostasis network is the UPS [9]. Substrates of the UPS are earmarked by covalent attachment of Ub to internal lysine residues through the concerted action of E1 Ub-activating enzymes, E2 Ub-conjugating enzymes, and E3 Ub ligases [15–17]. Ubiquitylation is a dynamic and reversible process as deubiquitylating enzymes (DUBs) modulate the size and topology of poly Ub chains and thereby influence the fate of the conjugated substrate. DUBs that bind to the proteasome either remove proteolytic Ub tags [18] or edit the topology of Ub chains to generate efficient degradation signals [19,20]. Moreover, DUBs catalyze processing of inactive Ub precursors and recycling of inhibitory free Ub chains, which otherwise would inhibit polyubiquitin–substrate binding at the proteasome [21,22]. The 26S proteasome is a multicatalytic protease complex composed of a barrel-shaped 20S proteolytic core particle (CP) and a 19S regulatory particle (RP) translocating substrates into the 20S CP where they are degraded into short peptides [23]. Usually polyubiquitin attachment is sufficient for targeting substrate proteins for proteasomal turnover [17]. While chains connected through Lys48 of Ub promote proteasomal degradation, Lys63-linked chains provide regulatory or targeting functions [24].

Despite the large number of structurally unrelated substrates, Ub conjugation is remarkably selective. E3 Ub ligases represent the largest group of enzymes within the UPS, which is linked to their key role in substrate selection. Most E3 enzymes are not essential for cell growth and exhibit only mild loss-of-function phenotypes, suggesting the existence of similar functional redundancy and adaptation mechanisms even between degradation pathways of the UPS. The detailed analysis of several classes of E3 ligases led to identification of specific substrates and molecular pathways that they regulate [15,16]. Interestingly, a subgroup of specialized quality control E3 ligases have been identified to team up with a variety of molecular chaperones in order to recognize and target particularly damaged proteins for proteolysis (Fig. 1).

Selective autophagy

The other central component of the proteolytic system is the autophagy-lysosome pathway, which supports proteostasis by turnover of defective and aggregated proteins, multimeric complexes, and even whole organelles that cannot be handled by the proteasome [25–27]. A characteristic hallmark of macroautophagy (hereafter autophagy) is the formation of double-membrane autophagosomes, which engulf their particular cargo substrate and deliver it to the lysosome for degradation. Although nonselective autophagy is mainly induced to recycle nutrients upon starvation,

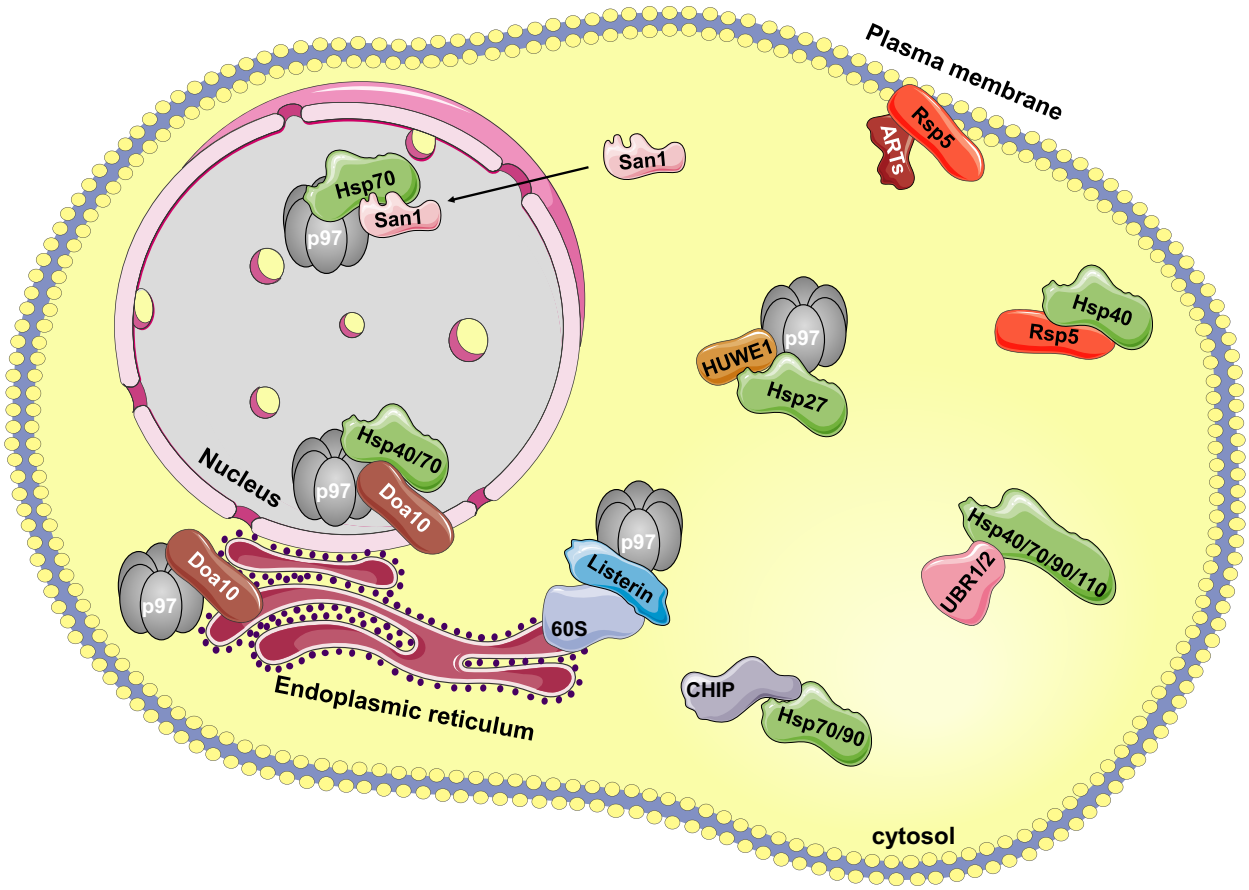

Fig. 1. Illustrative representation of subcellular localization of various PQC Ub ligases. E3 enzymes operating in quality control pathways (e.g., CHIP, Doa10, HUWE1, Listerin, Rsp5, and San1) and associated chaperones (in green and gray) are widely distributed in most subcellular compartments, including nucleus, cytoplasm, ER, and plasma membrane.

selective autophagy degrades specific cargos in response to environmental stress conditions and physiological changes including aging [28–30]. Substrate selectivity is ensured by a cargo–ligand–receptor–scaffold interaction [27]. As an initial step, the interaction between receptor and scaffold recruits a specific cargo to the phagophore assembly site for subsequent autophagosome formation. Commonly, the receptor proteins bind ATG8/LC3, which couples the cargo directly with the macroautophagy apparatus. Interestingly, some branches of selective autophagy also use Ub as recognition signal on target proteins, which involves Ub-selective autophagy receptors and subsequent degradation of targets in the lysosome [31].

In higher organisms, selective degradation of single proteins is also arranged via chaperone-mediated autophagy (CMA) and chaperone-assisted selective autophagy (CASA), initiating cargo uptake directly at the lysosomal or endosomal membrane through a specific protein translocation complex [32]. In both variants of selective autophagy, chaperones, and quality control E3 ligases play a key role. For instance, CMA-based degradation of the transcription factor HIF1A requires the concerted action of HspA8/Hsc70 (heat shock 70 kDa protein 8) and the E3 ligase C terminus of Hsc70-interacting protein (CHIP) [33].

Upon impaired capacity of the UPS and CMA, substrates are degraded by CASA [34]. The CASA complex contains the molecular chaperones Hsc70 and HspB8, the cochaperone BAG3, and the E3 ligase CHIP [35]. Under normal growth conditions, CASA is the main route for chaperone-mediated lysosomal degradation, whereas CMA is induced by proteotoxic stress. CASA (like CMA) is also necessary for protein quality control in aged cells, which is reflected by elevated BAG3 levels and increased targeting of oxidized and ubiquitylated proteins to the lysosome in aged neurons [36]. Substrates to be degraded by this

complex include polyglutamine (polyQ)-expanded huntingtin [37] and superoxide dismutase (SOD1) [38]. Furthermore, CASA is essential for muscle maintenance especially during aging [39].

The chaperone network

With increasing genome size, the amount of proteostasis guarding factors expressed in eukaryotic cells has been adjusted to the growing complexity of the proteome during evolution [40]. The coevolvement of proteostasis networks helps to cope with the higher burden of protein folding allocated to different subcellular organelles, more complex developmental reorganization of cell type-specific metabolism, and unique stresses faced by multicellular, complex organisms. Thus, it is not surprising that the human chaperome consisting of chaperones, cochaperones, and adaptors possess more than 300 different members [41]. Molecular chaperones are folding machines that assist client proteins in acquiring and keeping their active conformation, directing their folding, unfolding, and refolding. In case of irreversible damage chaperones also direct misfolded proteins toward specialized proteolytic systems of the cell [42]. Chaperones coordinate lysosome-dependent degradation pathways like CASA and CMA, and also target misfolded proteins to the 26S proteasome in chaperone-assisted proteasomal degradation (CAP) [34,43,44]. Chaperones usually recognize exposed hydrophobic protein surface and help client proteins to acquire and keep their active conformation by directing (re)-folding [45]. The biggest classes of chaperones are named according to their molecular weight [Hsp100, Hsp90, Hsp70, Hsp60, Hsp40, and small heat shock proteins (HSPs)] [6] and form different subgroups based on their mechanistic function.

The major *de novo* protein folding chaperones in eukaryotes are Hsp90 and Hsp70. These ATP-dependent chaperones appear as constitutively expressed and stress-induced forms and team up with various cochaperones for substrate recognition, binding, and activation [46–49]. Hsp70 and Hsp90 chaperone systems play a role in different organelles of the eukaryotic cell and regulate a wide range of events including folding of *de novo* synthesized polypeptides, refolding, or degradation of misfolded proteins. Furthermore, they also show disaggregation activity, facilitate protein translocation through membranes and they are involved in remodeling of multimeric protein complexes [50,51]. Hsp90 is also involved in regulation of receptor-ligand binding or assembly of protein complexes, and has been implicated in regulatory pathways such as DNA repair or immune response [52]. The broader role of this chaperone is well reflected by its various client proteins. In fact, it has been shown that Hsp90 associates with more than 10% of the total proteome [53].

The HSP60 chaperones are also known as chaperonins [54]. The mitochondria localized HSP60–HSP10 and the cytosolic TriC/CCT complex chaperonins act as multimeric ring-shaped folding chambers that encapsulate client proteins for folding [55,56]. Cochaperones, like HSP40/J-proteins, and BAG family cochaperones regulate the activity of their cognate chaperones through the modulation of their ATPase cycle or via binding substrate proteins or other cochaperones [50,57–59].

Age-dependent and disease-linked changes of the chaperome

Molecular chaperones are vital for protein quality assurance recognizing non-native proteins and diminishing their toxicity. In the presence of proteotoxic conditions including heat stress, oxidative changes, and aging, all cells induce a highly conserved gene expression program, the heat stress response. This stress response is tightly regulated in eukaryotes, inducing expression of Hsp genes by the evolutionarily conserved transcription factor heat shock factor 1 (HSF1) [9,60,61].

Studying the *Caenorhabditis elegans* and human chaperome, Brehme *et al*. has shown that a conserved subnetwork of chaperones safeguards the aging process [41]. The expression of the major cytosolic chaperones, including Hsc70, Hsp90s, CCT/TRiC complex, Hsp40, and tetratricopeptide motif repeat (TPR)-domain cochaperones, is repressed in old worms and the adult human brain. This age-related chaperome repression leads to accumulation of misfolded proteins and increases the risk of proteotoxic diseases, such as Parkinson's disease (PD), Alzheimer's disease (AD), and Huntington's disease (HD). On the contrary, increased HSP expression have been attributed to many human cancer types, providing the fast dividing cells with ultimate protein folding capacity, thus promoting tumor cell survival, proliferation, and invasion [62,63]. A recent study sheds light on the underlying chaperome reorganization event in the cell, which could trigger the survival of tumors. Examining the chaperone complexes of different tumor species, Rodina and coworkers identified the formation of tumor-specific chaperone subnetworks that are distinct to physiological chaperone interactions [64]. This so-called cancer epi-chaperome is based on enhanced physical integration of the two major cellular

chaperone networks of Hsc70 and Hsp90. The epi-chaperome of these cancer cells is nucleated by stable, high molecular weight complexes of Hsp90 and Hsc70, and supports the survival of fast dividing cells [64].

E3–chaperone interaction in proteostasis maintenance

As a result of cellular and environmental stresses, the eukaryotic cell is continuously confronted with handling defective proteins. Compartmentalized degradation pathways are specifically involved in the removal of misfolded proteins of the endoplasmic reticulum (ER), mitochondrion, cytoplasm, and nucleus. In order to target damaged proteins for degradation, specialized E3 Ub ligases are recruited by targeting chaperones. So far only a few E3–chaperone complexes have been described mechanistically (Fig. 2 and Table 1). Quantitative protein interaction analysis suggests that more than 30% of all human E3 ligases interact with Hsp90 [65]. This observation indicates a highly complex and intricate PQC network guided by diverse E3–chaperone teams. In the following section, we will discuss PQC systems based on the coordination of protein folding and degradation. We will focus on the recent discoveries on cytosolic, nuclear, and membrane-directed PQC; ER- and mitochondrion-specific PQC regulation was previously described [66–70].

CHIP

CHIP has been characterized as the first QC E3 Ub ligase important for the cellular proteostasis network. CHIP binds the molecular chaperones Hsp70/Hsp90 to coordinate the cellular balance between protein folding and degradation [71–75]. It was first identified as a chaperone binding protein based on its N-terminal tandem TPR [76]. The evolutionarily conserved U-box domain of CHIP, responsible for its E3 ligase activity, is a modified form of the more frequently found really interesting new gene (RING) domain [77,78]. Notably, the chaperone-directed recruitment of CHIP involves a mutual allosteric interaction between the TPR and U-box domains [79]. Structural diversity and dynamics within CHIP are well described in the recent review by VanPelt and Page [80].

Various studies provide evidence that CHIP tightly regulates chaperone function. Upon acute stress, CHIP facilitates nuclear translocation and activation of HSF1 to protect against stress-induced apoptosis [81,82]. CHIP also modulates the proteotoxic stress response by reducing the level of Hsc70/Hsp70 chaperone after heat shock [72,80,83,84]. Furthermore, CHIP modulates the activity of certain chaperones. For instance, this E3 ligase stimulates the release of the Hsp90 ATPase activity modulating cofactor p23 from the Hsp90 complex, thereby suppressing the affinity and folding activity of Hsp90, which results in Ub-dependent degradation of substrate proteins [72,85]. On the other hand, CHIP competes for Hsp70 binding with Hsp40, which attenuates Hsp40 ATPase activity and suppresses protein folding by Hsp70 [76].

Importantly, CHIP orchestrates regulation of cellular proteins from folding to degradation, including a coordinated degradation of substrates, which are beyond the refolding range. In addition to misfolded proteins of the cytoplasm, CHIP promotes degradation of a broad array of substrates when bound to Hsps, like cystic fibrosis transmembrane conductance regulator (CFTR), glucocorticoid receptor (GR), androgen receptor, estrogen receptor, erythroblastic leukemia viral oncogene homolog 2 (ERBB2), or protein arginine methyltransferase 5 (PRMT5) [86–90] (Fig. 2A). Hypothetically, all clients of HSP70 or HSP90 are potential targets of CHIP. However, not all CHIP substrates are recruited via interaction with Hsps. For example, Sma-mother against decapentaplegic 1 (Smad1) level is regulated by CHIP, which subsequently influences bone morphogenetic proteins (BMP) signal transduction [91]. Identified substrates of the CHIP/Hsp complex are detailed in the recent reviews by Paul and Gosh, and Joshi *et al.* [92,93].

Aside from ameliorating proteotoxicity, CHIP plays a role in developmental regulation and aging. For instance, CHIP is involved in osteoblast differentiation by regulating the protein level of Runx2 [94]. In agreement with its role in protein quality control, CHIP knockout mice show reduced lifespan associated with age-related pathophysiological defects [95]. However, CHIP deletion mice exhibit normal embryonic development and unaffected turnover of many known CHIP substrates, suggesting functional redundancy among quality control Ub ligases [95,96]. In contrast, CHIP deficiency induces accelerated aging, which suggests the existence of at least one critical CHIP-specific substrate that controls longevity. We have recently revealed an important function of CHIP-mediated proteolysis in insulin/IGF-like signaling (IIS). CHIP triggers degradation of the insulin receptor (INSR), which regulates metabolic changes and determines lifespan in metazoan organisms. Upon proteotoxic stress and during aging, CHIP preferentially functions in PQC, causing a stabilization of the INSR. Accordingly, proteotoxic accumulation of damaged proteins or aberrant CHIP function attenuates INSR degradation and affects metabolism and longevity through increased IIS [97].

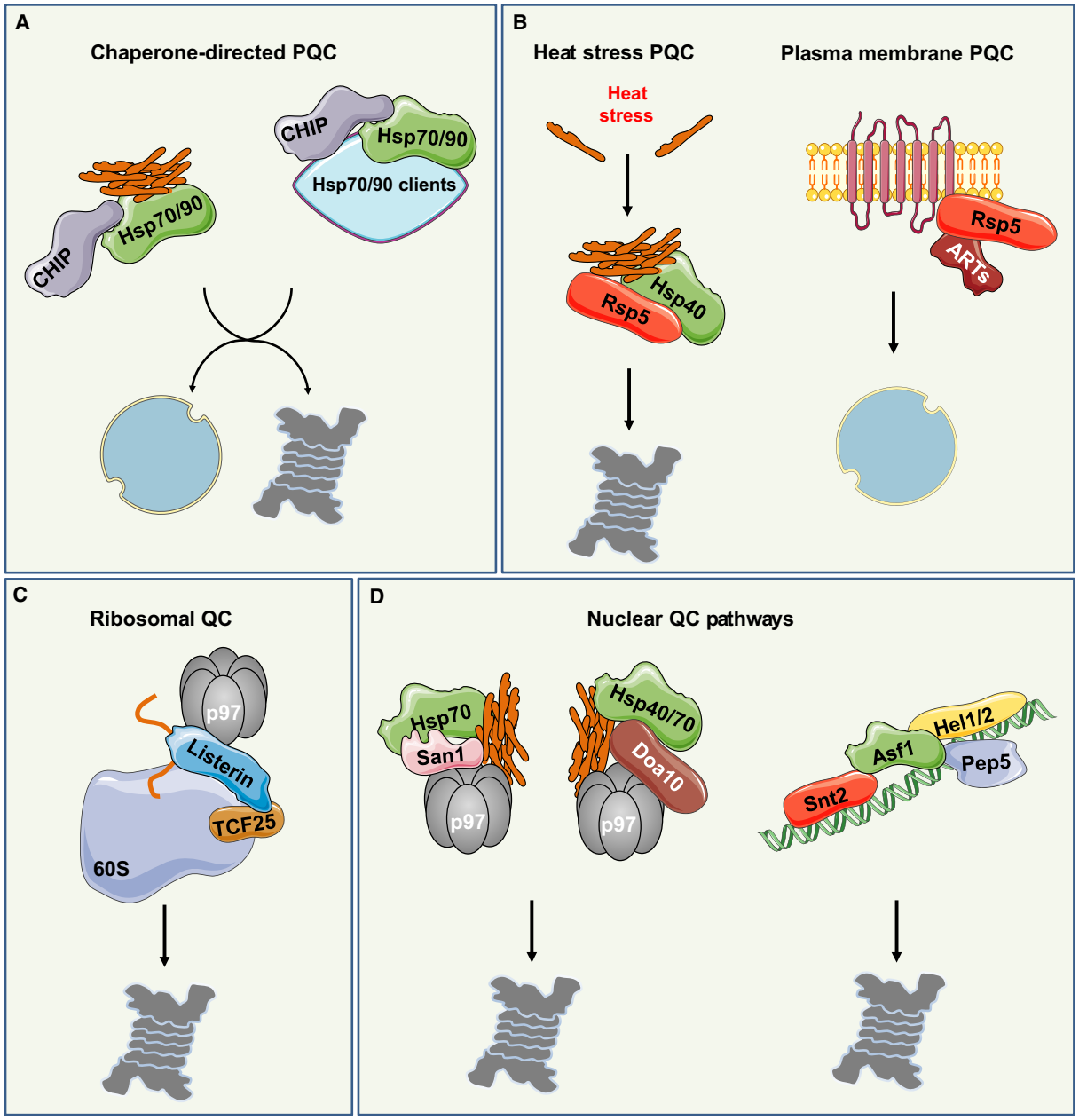

Fig. 2. E3 Ub ligases with different roles in PQC. (A) CHIP is a major PQC ligase of the cytosol. (left panel) In cooperation with Hsp70/90, CHIP ameliorates proteotoxicity in various proteinopathies by referring aggregates of beta-amyloid, mutant SOD1, polyQ protein, or alpha-synuclein for degradation. (right panel) In addition to misfolded proteins of the cytoplasm, CHIP promotes degradation of a broad array of substrates when bound to Hsps, such as CFTR, GR, ERBB2, or HIF1A. (B) (left panel) Rsp5/Nedd4 participates in the removal of cytosolic misfolded proteins. Upon heat stress Rsp5/Nedd4 associates with cochaperone Hsp40 (Ydj1), which supports recognition and degradation of misfolded proteins. (right panel) Beyond its role in the removal of cytosolic substrates, Rsp5/Nedd4 also targets misfolded proteins at the plasma membrane. ARTs enable Rsp5 to selectively target a wide range of plasma membrane proteins and initiate their endocytosis and lysosomal degradation. (C) The E3 ligase Listerin directly associates with the 60s ribosomal subunit to specifically target newly synthesized aberrant polypeptides expressing a translated polyA tail. Listerin collaborates with three cofactors for ribosomal binding and substrate processing: NEMF, TCF25, and the Ub-selective chaperone p97. (D) The role of chaperone-directed E3 ligases in nuclear PQC. (left panel) San1 cooperates with Hsp70 chaperones to recruit misfolded proteins from the cytosol for proteasomal degradation in the nucleus, whereas Doa10 targets substrates independent of Hsp70/Hsp40. In addition, both Doa10 and San1 interact with Cdc48/p97 to facilitate proteasomal degradation of a subset of their substrates. (right panel) The yeast E3 ligases Hel1, Hel2, Snt2 together with the histone chaperones Pep5 and Asf1 trigger ubiquitylation and subsequent proteasomal turnover of surplus histones.

Table 1. List of quality control E3 ligases and their chaperone partners reviewed in this paper.

E3 ligase	Chaperone	Target	References
Cytosol			
CHIP	Hsp70; Hsp90	Misfolded proteins; Hsp90 clients	[72,73]
Ubr1/UBR1	Hsp110 (Sse1); Hsp70 (SSa1/2); Hsp40 (Ydj1, Sis1)	Misfolded proteins	[101–103,108,114]
Ubr2	Hsp110; Hsp70	Misfolded proteins	[101,103]
Tom1/HUWE1	Hsp27; CDC48	Unassembled proteins	[122–124]
E6-AP	Hsp70/Hsc70	Misfolded, aggregated proteins	[98]
RNF126	BAG6 complex	Mislocalized ER proteins	[116,117]
Rsp5/NEDD4	Hsp40	Heat-induced misfolded proteins; plasma membrane proteins	[133,143–147]
Cullin5	Hsp90	Hsp90 clients	[99,100]
Hul5/UBE3C		Heat shock-induced misfolded proteins	[132,134–136,139,140,219]
SCF	Hsp90-Sgt1 Hsp70-Sgt1	LRR domain proteins; kinetochore	[125–131,220]
Nuclei			
Pep5, Snt2, Hel1, Hel2	Asf1	Surplus histones	[186]
$Rtt101^{Mms1/}$ $Cul4A^{DDB1}$	Asf1 Asf1a/Asf1b	H3	[192]
Psh1	FACT	CENP-A (H3)	[191]
Doa10/MARCH6	Hsp70 (Ssa1/Ssa2), Hsp40 (Ydj1, Sis1)	ER and INM	[109,177]
Asi ligase complex (Asi1-Asi3)		Mislocalized proteins at INM	[175,176]
Cytosol/Nuclei			
San1	Hsp70; CDC-48/p97	Nuclear misfolded proteins	[101,105,107,108,221]
Ribosomes			
Ltn1p/Listerin	Cdc48/p97	Aberrant nonstop polypeptides	[148,152,161]

Our observation suggests an evolutionarily conserved coordination of proteostasis and aging regulated by CHIP-assisted protein degradation.

Different strategies for targeting misfolded proteins in the cytosol

The cytosol of the eukaryotic cell melds protein synthesis, folding, and transport, all of which are continuously defining cellular proteostasis. The key insights into how E3 ligases and chaperones work together have been uncovered by research directed towards understanding the role of CHIP in degradation of misfolded proteins. As described above CHIP is the most extensively studied E3 Ub ligase associated with molecular chaperones [83], but not the only one maintaining proteostasis. PQC pathways deploy a variety of E3 ligases linked to various degradation routes to cope with the constant protein folding stress applied by physiological or stress-related processes. Similarly to CHIP, E6-AP—a HECT-domain Ub ligase found in higher eukaryotes—interacts with Hsp70/Hsc70 chaperones and ubiquitylates their client proteins, such as aggregated proteins [98]. Multisubunit Cullin-based E3 ligases have also been implicated in PQC within the cytoplasm. The mammalian Cullin5–RING E3 Ub ligase interacts with the Hsp90 chaperone and mediates Ub-dependent degradation of Hsp90 client proteins, including protein kinases (such as ERBB2) or transcription factors (like HIF1α) [99,100].

Although CHIP is thought to be the major PQC E3 ligase in the cytosol of higher eukaryotes, budding yeast lacks this enzyme. Instead, Ubr1, Ubr2, and San1 are major PQC E3 ligases in *Saccharomyces cerevisiae* which ubiquitylate misfolded proteins to maintain proteostasis [101–103]. Interestingly, these enzymes evolved two different strategies to safeguard the proteome. San1 has been first described as nuclear PQC E3 ligase with intrinsic capacity to bind aberrant proteins in the nucleus [104]. As Rosenbaum and coworkers have shown, San1 can directly bind to its substrates through its disordered N-terminal and C-terminal domains, which provide conformational flexibility and serve as substrate recognition sites for misfolded proteins [105]. In yeast, where proteasomal degradation capacity of the cell is highly concentrated in the nucleus [106],

numerous cytosolic PQC substrates are degraded on San1-dependent clearance mechanisms. Misfolded proteins in the cytosol are delivered to the nucleus by Hsp70 where San-1-driven ubiquitylation initiates proteasomal degradation [101,107,108]. This suggests a major role of San1 in proteostasis by removing a wide range of cytoplasmic and nuclear misfolded proteins. So far no mammalian San1 homolog has been identified, but recent bioinformatic analysis suggests the existence of several mammalian E3 ligases that bear related, disordered regions and thus might function in a similar way [109].

The E3 ligases Ubr1 and Ubr2 have been first characterized in the N-end rule pathway regulating degradation of short-lived proteins presenting N-terminal destabilizing amino acids [110–113]. The Ubr1/Ubr2-dependent yeast PQC pathway operates in the cytosol, where Ubr1 employs the Hsp70 chaperones Ssa1/Ssa2, the Hsp40 cochaperone Ydj1 or Sis1, and the Hsp110 chaperone Sse1 for target recognition [101,103,114]. Similar quality control function has been recently attributed to the mammalian UBR1 (N-recognin 1) E3 ligase targeting HSP90 client proteins [115].

While the CHIP–Hsp70/Hsp90 complex directs the degradation of a multitude of different misfolded proteins, other ligase–chaperone complexes adopted more specialized strategies to bind target proteins, revealing a set of E3 ligases dedicated to distinct PQC pathways. RNF126 is an interesting example of a specialized PQC E3 ligase, which cooperates with the Bag6 chaperone [116]. Eukaryotic cells have extensive endomembrane systems, hosting a significant portion of the cellular proteome. Utilizing specific signal sequences, the newly synthesized membrane proteins are rapidly integrated into the ER membrane. However, those that fail to target to the ER must be removed from the cytosol to avoid protein aggregation. Rodrigo-Brenni *et al.* identified RNF126 as the key component of Bag6-dependent degradation of mislocalized membrane proteins (MLPs) in the cytosol [117]. The Bag6 chaperone preferentially binds to misfolded proteins with extensive hydrophobic domains [116], while the typical client proteins of Hsp70/Hsp90 characteristically expose shorter hydrophobic stretches [118,119]. This suggests that the Hsp70/Hsp90 chaperone system provides a different role in PQC compared to the Bag6-driven pathway. RNF126 specifically ubiquitylates lysine residues located directly next to the hydrophobic segment of the MLPs [117]. Interestingly, positively charged residues, such as lysines often flank chaperone-recognized hydrophobic regions in membrane proteins [120,121]. This observation supports the idea that the RNF126/Bag6 complex implements a degree of specialization in substrate recognition and binding.

Quality control of multiprotein complexes

Proteins destined to work in multimeric protein complexes often expose unshielded segments of hydrophobic residues on their surface that mediate their assembly into higher order molecular machineries. Excess of free subunits of unassembled protein complexes either cause protein aggregation or interfere with their normal function. Using a model substrate to study the degradation of unassembled soluble polypeptides of multisubunit complexes HUWE1 has been identified as novel PQC Ub ligase [122]. HUWE1 targets both cytosolic and nuclear subunits in human cells, providing a constant quality control and removal of incomplete protein assemblies. Similarly, the yeast HUWE1 homolog Tom1 facilitates ubiquitylation and proteasomal degradation of unassembled ribosomal proteins [123]. Although HUWE1-dependent degradation of unassembled proteins is linked to the Ub-selective chaperone p97, the possible involvement of other chaperones engaged in recognizing and presenting misfolded HUWE1 targets is not addressed yet. A recent proteomic study identified HUWE1–Hsp27 interaction, which might link HUWE1 to chaperone-dependent degradation of hydrophobic polypeptides [124].

As discussed above, chaperones are not only important for folding of proteins but also play vital roles in supporting accurate assembly of multiprotein complexes. The presence of available components in proper stoichiometric ratios is critical to facilitate the build-up of functional protein complexes. As such, the Hsp90–Sgt1 chaperone has a critical role in the assembly of kinetochores, the multivalent microtubule binding sites in the cells [125]. Sgt1 acts as an adaptor and cochaperone for Hsp90 and Hsp70 to connect to multiple client proteins during their folding and assembly into protein complexes [126]. Sgt1 also links Hsp90 to the Skp1/Cullin/F-box (SCF) E3 ligase via direct binding to Skp1, thereby regulating assembly and activity of the SCF complex [127–129]. The client proteins of Sgt1 and the Skp1 component of the SCF ligase share similar sequence feature, the leucine-rich repeat (LRR) domain that supports recognition and interaction with the cochaperone Sgt1 [130]. Recent studies also suggested that Sgt1 client proteins are often ubiquitylated by SCF to facilitate their removal, although this regulatory function needs further experimental evidence [125,131].

Heat stress E3 ligases

Heat stress increases protein unfolding and acutely overloads the cell with misfolded proteins. It has been shown that heat shock primarily increases the ubiquitylation of cytosolic proteins [132]. HECT ubiquitin ligase 5 (Hul5) and Rsp5 have been identified in yeast as major E3 ligases that regulate ubiquitylation and proteasomal degradation of heat-induced misfolded proteins [132,133]. Although degradation of many unfolded yeast proteins depends on the Hsp70 (SSA1-5) chaperones, Hul5 recognizes target proteins without the help of these chaperones. It is a critical challenge to discriminate between terminally or temporarily misfolded proteins and only target those for degradation that cannot be refolded and used anymore. Hul5-dependent ubiquitylation of terminally misfolded proteins occurs when mono-ubiquitylated proteins are not refolded for a longer time window. Hul5 directly associates with the 19S RP of the proteasome where it acts as an E4 enzyme elongating Ub chains on proteasome-bound substrates [134–138]. UBE3C, the human homolog of Hul5, is also a proteasome-associated E3 ligase which further ubiquitylates proteins that are difficult to degrade thereby assisting proteasomal degradation [135,139,140]. The potential involvement of the mammalian Hul5 homologs in heat stress-induced PQC is not verified yet. In addition to Hul5, the yeast Rsp5 and its mammalian homolog Nedd4 have major roles in the removal of cytosolic misfolded proteins upon heat stress [133] (Fig. 2B). Overexpression of Rsp5 increases thermotolerance in yeast [141], which is in agreement with its important role in response to heat-induced damage. In contrast to Hul5, Rsp5/Nedd4 uses a bipartite mechanism for recognition of its cytosolic misfolded substrates. Upon heat stress, Rsp5 associates with Hsp40 (Ydj1) cochaperone promoting ubiquitylation and degradation of misfolded proteins. On the other hand, Rsp5 can bind some of its targets directly. These substrates typically contain short stretches of amino acids, which are proline-rich motifs (called the PY or PY-like) that confer binding to the WW-domains of Rsp5 [133]. These motifs act as degrons promoting heat stress-induced substrate–Rsp5 interaction.

Degradation of plasma membrane proteins

In contrast to cytosolic or ER proteins, plasma membrane anchored or integral proteins are mainly degraded by the endolysosomal degradation pathway [142]. Proteotoxic stress dramatically changes the landscape of membrane proteins as a result of the endocytic removal of damaged proteins. Beyond its role in removal of cytosolic heat-induced misfolded proteins, Rsp5/Nedd4 has also been reported to target misfolded plasma membrane proteins for lysosomal degradation [143–145] (Fig. 2B). Furthermore, Rsp5 is also involved in a range of cargo-sorting events within the endosomal and Golgi transport pathway. In yeast, arrestin-related trafficking adaptors (ARTs) enable Rsp5 to specifically target a wide range of plasma membrane proteins and initiate their endocytosis and lysosomal degradation [144,146,147]. Defects of this PQC pathway result in severe loss of plasma membrane integrity.

Ribosome-associated quality control

Protein synthesis is a highly error-prone process. In eukaryotic cells, a fraction of the newly synthesized proteins is immediately degraded by the 26S proteasome, indicating the existence of a strictly cotranslational PQC to ensure elimination of aberrant proteins [148]. The ribosomal Ltn1/Rkr1 and Hel2 E3 ligases together with the nonribosomal Ub ligases Doa10, Hrd1 and Hul5 mediate ubiquitylation and proteasomal degradation of nascent proteins, which escaped cotranslational folding control of newly synthesized proteins directed by the ribosome-bound nascent polypeptide-associated complex (NAC) chaperone [148–151]. The recently described conserved PQC pathway requires the yeast Ltn1 Ub ligase, or its mammalian homolog Listerin, which directly associates with ribosomes to specifically target newly synthesized aberrant polypeptides expressing a translated polyA tail [152]. Ltn1-dependent polyubiquitylation and subsequent proteasomal degradation of nonstop proteins is triggered by stalling them at the translation machinery [153]. Ltn1/Listerin utilizes three cofactors for binding to the ribosome and for processing the targets: Tae2/NEMF, Rqc1/TCF25, and Cdc48/p97 (Fig. 2C). Tae2 (NEMF) recognizes the stalled ribosomes and recruits Ltn1 to the 60S–peptidyl–tRNA complex, which together with Rqc1 enables binding of the Cdc48/p97 Ub-selective chaperone [154,155]. Cdc48/p97 mediates segregation/unfolding of ubiquitylated substrates from the ribosomal complex and their proteasomal degradation [156–158]. Tae2 (Rqc2) also recruits an enzyme that generates chloramphenicol acetyltransferase tail on aberrant nascent peptides, which is crucial for induction of translational folding stress response [159]. When Cdc48 is not recruited, the Ltn1–Rcq1–Cdc48 PQC pathway fails to initiate the degradation of aberrant translation products arising from ribosomes. Consequently, the chloramphenicol

acetyltransferase-tailed ubiquitylated peptides localize to aggregates, which are specifically associated with Sis1, Sgt2, Ssa1/2, and Hsp82 chaperones [160]. Hence, Ltn1 Ub ligase-driven PQC of ribosomal translation is also essential in prevention of cytosolic aggregate formation [161]. A recent study using a large set of model substrates in yeast revealed that besides its role in ribosomal PQC, Ltn1 also mediates degradation of substrates bearing different degradation signals (degrons) fused to their C terminus, suggesting a broader role for Ltn1 in cellular PQC [162].

E3–chaperone complexes in the nuclear protein quality control

Although it is spared from folding burden of nascent polypeptides, when it comes to the nucleus, the PQC pathways face unique folding problems. PQC of the nucleus should have rigorous control over the identity and folding of nuclear membrane proteins as well as chromatin-associated proteins [163]. The nuclear PQC is exceptionally important because failure to repair or remove misfolded nuclear proteins can lead to a deterioration of the nuclear genome and mRNA integrity. In addition, the nucleus is especially enriched in proteins possessing low complexity and intrinsically disordered regions [164]. Compared to regulation in the cytosol, nuclear PQC is also governed by the cooperative action of HSPs, molecular chaperones, associated E3 ligases, and proteasomal degradation. In addition, increasing evidence suggests that nuclear envelope components are also degraded by autophagy [165,166].

Exposed hydrophobic protein stretches are key determinants of nuclear quality control degradation pathways. San1 is a central PQC E3 ligase of the yeast nucleus, involved in ubiquitylation and proteasomal degradation of a wide range of misfolded nuclear and imported cytosolic proteins [104] (Fig. 2D). Recognition of misfolded proteins by San1 is triggered via surface exposure of a few contiguous hydrophobic residues [167]. Lacking San1, mammalian cells employ other nuclear PQC E3 ligases, such as UHRF2, which associates with and ubiquitylates nuclear polyQ aggregates [168].

Asi protein ligase preserves the identity of the inner nuclear membrane

The double-membrane-based nuclear envelope has crucial function in providing compartmentalization for the genomic DNA. As the outer nuclear membrane (ONM) is contiguous with the ER, the quality control of proteins localized in this membrane layer is generally performed by the ER-associated PQC systems. As such, E3 ligase complexes based on Hrd1 and Doa10 drive ubiquitylation and proteasomal degradation of the majority of misfolded ER proteins through the yeast ERAD pathway [68,169–171]. The protein content of the inner nuclear membrane (INM) is distinct from that of the outer layer and it is thought that nuclear quality control mechanisms are in charge to maintain its integrity. The INM is connected to the outer membrane–ER system at the nuclear pores which, in addition to restricting protein exchange between the cytosol and nuclei, also regulates protein transport from the ER membrane to the INM [172]. In yeast, the Ub ligase Asi complex consisting of Asi1, Asi2, and As3 is involved in the process that controls promoter access of two transcription factors Spt1 and Spt2 [173,174]. Recently, Foresti *et al.* and Khmelinskii *et al.* found that the INM-localized Asi E3 ligases regulate degradation of mislocalized proteins that are not destined to INM, defining a novel PQC pathway of the eukaryotic cell that maintains and safeguards the identity of the INM [175,176]. Notably, ER membrane bound Doa10 has been linked to ubiquitylation of soluble and INM-associated nuclear proteins, by recognizing hydrophobic patches of proteins exposed to the nucleoplasm [177]. Doa10 targets proteins in an Hsp70/Hsp40-dependent manner [178], and teams up with Cdc48/p97 for proteasomal targeting of a subset of its substrates [179,180].

Aggregation-prone proteins, such as the pathogenic polyQ-exposing proteins, represent another major threat for the nucleus. Guo and coworkers recently described a dedicated nuclear team responsible for recognition and removal of polyQ aggregates [181]. This interesting mechanism is based on the promyelocytic leukemia protein (PML) that selectively recognizes and interacts with the nuclear, misfolded polyQ proteins and sumoylates them. In turn, the Ub ligase RNF4 attaches polyubiquitin chains to the aggregates, which targets them for proteasomal degradation. The role of E3 ligase-associated chaperones in nuclear protein quality control is not as well established as in the ER or cytoplasmic PQC. While San1 might use Hsp70 chaperones for delivering cytosolic misfolded proteins for nuclear degradation by the proteasome [107], chaperone partners of the Asi complex have not been described yet.

Histone chaperone–E3 complexes safeguard genome stability

In eukaryotic cells, the genomic DNA is packed by histones, building a compact chromatin structure. Histone complexes act as spools as DNA winds up

around them to form the basic structural elements of the chromatin, the nucleosomes. Histone proteins dynamically regulate chromatin structure to adapt its activity status to the cellular demand [182]. Chromatin-bound histones appear to be very stable [183]; however, nonchromatin-bound histones are rapidly degraded with a short half-life [184]. Degradation of excess histones is essential because they interfere with cellular viability and promote toxic effect leading to genomic instability [185]. The yeast E3 ligases Tom1, Pep5, Snt2, Hel1, and Hel2 are involved in ubiquitylation and subsequent proteasomal degradation of surplus histones, where Hel1, Hel2, Snt2, and Pep5 works together with the Asf1 histone chaperone [186] (Fig. 2D). Other histone chaperones such as FACT, NAP1, HIRA, and DAXX are involved in histone shuttling between the cytoplasm and the nuclei, as well as histone deposition into and extraction from chromatin [187–190]. Notably, the histone chaperone FACT cooperates with Pob3/Spt16/histone 1 (Psh1) E3 ligase that targets ectopically localized Histone3 (H3) variant CENP-A (Cse4) for degradation, to maintain centromere identity, and to support proper chromatin segregation, and genomic stability [191].

Recently, Han and coworkers described a novel player in nucleosome maintenance, the mammalian E3 Ub ligase $Cul4A^{DDB1}$ and its yeast homolog $Rtt101^{Mms1}$ [192]. Nucleosomes are dynamically formed and disassembled in order to allow the gene transcription and DNA replication machinery to access distinct regions of the genomic DNA on a temporally regulated fashion [182]. During nucleosome assembly, the $Cul4A^{DDB1}$ ($Rtt101^{Mms1}$) E3 ligase preferentially binds to and ubiquitylates newly synthesized Lys^{56}-acetylated H3. This promotes H3 dissociation from the Asf1a or Asf1b (Asf1) chaperone and facilitates its binding to downstream processing histone chaperones, such as the mammalian CAF-1 for H3.1 or Daxx and HIRA for H3.3, to support nucleosome formation. The Cul4 E3 ligase-driven histone hand-off between chaperones does not lead to histone degradation, but it stands as an interesting example of the nonproteolytic E3 ligase–chaperone role [192].

Conclusions and perspectives

Selective degradation of misfolded or aggregated proteins is crucial for maintaining functionality of the cell. PQC mechanisms are present at all steps of a protein's lifetime and specialized enzyme complexes safeguard distinct steps of proteostasis processes. Molecular chaperones are vital in the protein quality assurance pathways recognizing the non-native proteins and preventing their interference with the cellular functions. To deliver damaged proteins to the cellular degradation pathways, dedicated E3 ligases cooperate with a variety of chaperones (Table 1). Environmental threats, endogenous stress, and aging constantly challenge the cellular proteome, and ultimately affect organismal viability. As we described above, eukaryotic cells adopted various mechanisms to cope with proteotoxic insults, which put pressure on their limited protein folding capacity. The implications of the eukaryotic proteostasis pathways in human disease are far reaching, as failure of any components of the PQC pathways could lead to disease [7].

It is commonly thought that an age-related impairment of protein degradation affects general proteostasis networks, causing enhanced accumulation of damaged proteins that can be cytotoxic and shortens lifespan [28,193–199]. During aging, the cellular proteostasis network shows significant changes in expression, mainly causing an overall reduction in protein synthesis, which reflects age-dependent remodeling of an imbalanced proteome [200]. Progressive decline of proteostasis can lead to the development of various diseases [201]. An apparent consequence of PQC downregulation is the appearance of various forms of neurodegeneration. The formation of protein aggregates is universally observed in about 30 different human diseases [197,202–204]. Accordingly, the age-dependent deposition of protein aggregates linked to disturbed proteasomal degradation of misfolded proteins is a major hallmark of neurodegenerative proteinopathies such as AD, HD, or Parkinsons's disease [11,205]. Along with molecular chaperones, PQC E3 Ub ligases associate with dysfunctional proteins in different neurodegenerative disorders. CHIP for example marks alpha-synuclein in PD [206], beta-amyloid in AD [207], phosphorylated tau, mutant SOD1 aggregates in amyotrophic lateral sclerosis (ALS) [75,208–210], and polyQ aggregates in polyQ diseases [211,212], for their proteasomal degradation. Therefore, failure of chaperone-assisted degradation of these misfolded proteins might aggravate various neurodegenerative disorders. Chaperone upregulation is widely observed in different cancer types providing a cell with increased protein folding capacity [213–215]. Such deregulation in chaperone level may also lead to impaired or to excessive recruitment of E3 ligases that can significantly change the selectivity and pace of substrate degradation. Hsp90 client proteins, including various kinases, have been implicated in malignant transformation [52]. Therefore, Hsp inhibition has emerged as a central strategy in cancer treatment [216,217].

Although special E3 ligase–chaperone partners involved in different PQC pathways have been identified, it is still far from being understood how much these pathways overlap in target recognition and processing, and how substrate selectivity is driven by the chaperones or the E3 ligases involved. It is especially interesting regarding the diverse cytosolic PQC pathways, where numerous E3–chaperone complexes work in parallel. For instance, interaction of chaperones with cochaperones could provide another layer of regulating the activity of chaperone–E3 ligase complexes. While E3 ligases are continuously being identified, the role of DUBs counteracting the activity of PQC E3 ligases is fairly unknown. Although their role in fine tuning the ubiquitylation processes is well established, only few DUBs have been assigned to PQC pathways so far [218]. It would be also interesting to examine the potential consequences of disturbed proteostasis on the function of PQC E3 ligases and determine how the imbalance in protein folding alters substrate processing. Recent identification of the human chaperone network supports the idea that tight temporal and spatial regulation of the activity and abundance of chaperone groups, and potentially of their cofactors, armors the cell against specific challenges during aging. Therefore, future characterization of tissue-, age-, or disease-specific chaperomes might reveal important mechanistic insights into how E3 ligases team up with chaperones to safeguard the proteome especially in multicellular organisms. Future research on tissue-specific PQC pathways and their role in tissue functionality will enable therapeutic intervention strategies against age-related protein aggregation diseases.

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