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## REVIEW ARTICLE

# 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/Cul1/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)

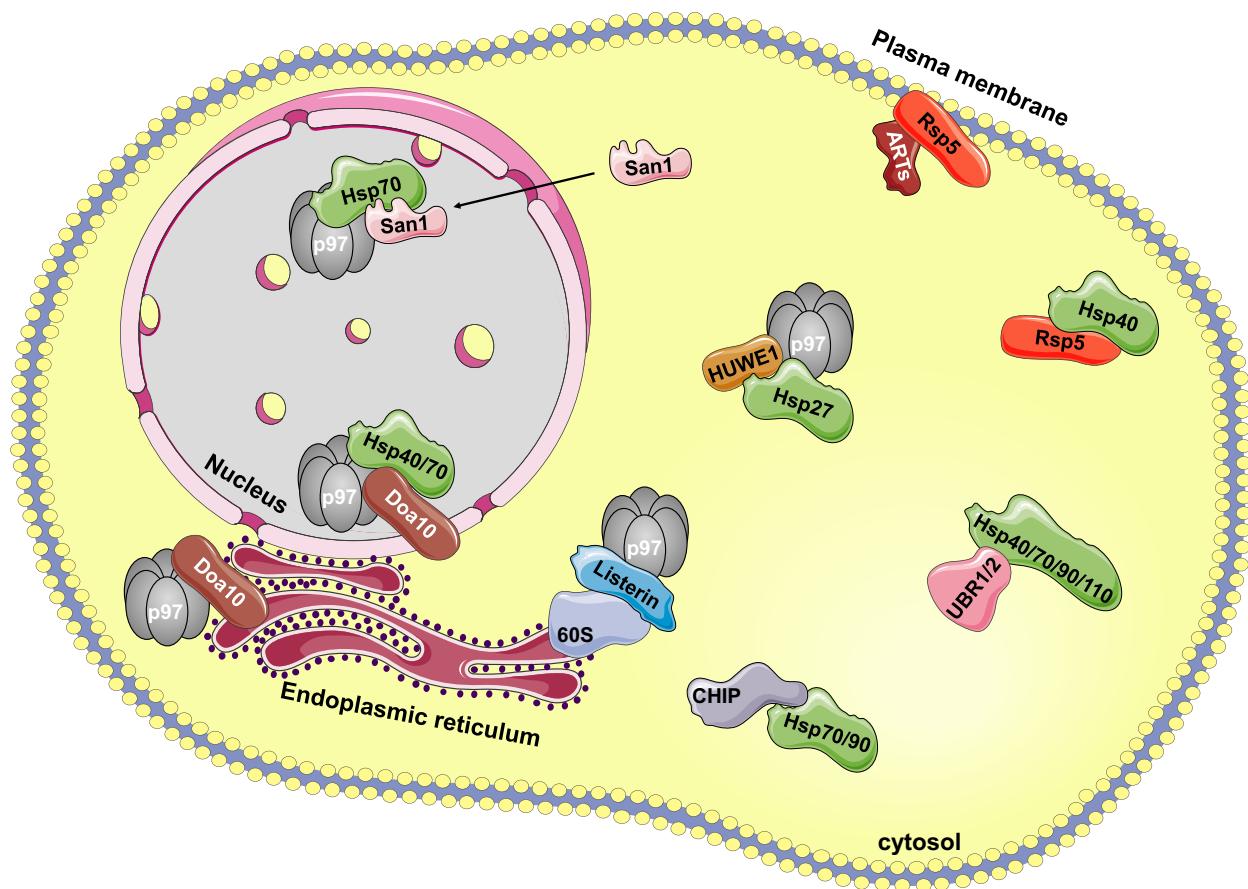
Selective degradation of misfolded or aggregated proteins is crucial to maintain functionality of the cell. A fundamental proteolytic module of the cellular proteostasis network is the UPS [9]. Substrates of the UPS are earmarked by covalent attachment of Ub to

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 coevolution 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

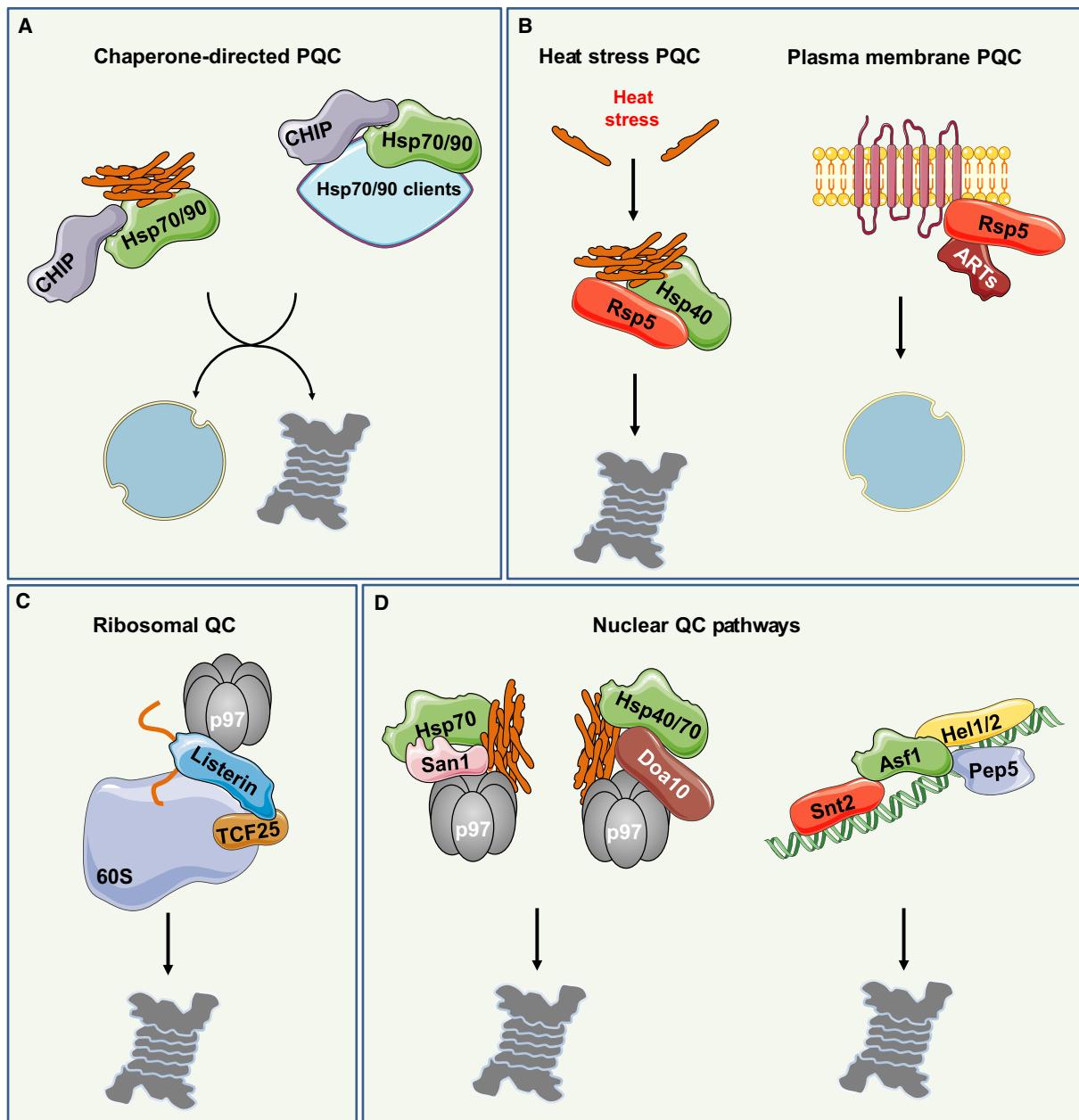
CHIP has been characterized as the first QC E3 Ub ligase important for the cellular proteostasis network. CHIP binds the molecular chaperones Hsp70/Hsp90 to coordinate the cellular balance between protein folding and degradation [71–75]. It was first identified as a chaperone binding protein based on 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);<br>Hsp70 (SSa1/2);<br>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;<br>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<br>Hsp70-Sgt1                                | LRR domain proteins; kinetochore                             | [125–131,220]             |
| Nuclei                            |   |  |                           |
| Pep5, Snt2, Hel1, Hel2            | Asf1  | Surplus histones   | [186]                     |
| Rtt101/Mms1/                      | Asf1  | H3   | [192]                     |
| Cul4A <sup>DDB1</sup>             | Asf1a/Asf1b   |  |                           |
| Psh1                              | FACT  | CENP-A (H3)  | [191]                     |
| Doa10/MARCH6                      | Hsp70 (Ssa1/Ssa2),<br>Hsp40 (Ydj1, Sis1)                | ER and INM   | [109,177]                 |
| Asi ligase complex<br>(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 HIF1 $\alpha$ ) [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 San1-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-recognition 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–Rqc1–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 Khmelnitskii *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<sup>DDB1</sup> and its yeast homolog Rtt101<sup>Mms1</sup> [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<sup>DDB1</sup> (Rtt101<sup>Mms1</sup>) E3 ligase preferentially binds to and ubiquitylates newly synthesized Lys<sup>56</sup>-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 Parkinson'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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## References

- Douglas PM and Dillin A (2010) Protein homeostasis and aging in neurodegeneration. *J Cell Biol* **190**, 719–729.
- Morimoto RI and Cuervo A (2014) Proteostasis and the aging proteome in health and disease. *J Gerontol A Biol Sci Med Sci* **69**, S33–S38.
- Ellis RJ and Minton AP (2006) Protein aggregation in crowded environments. *Biol Chem* **387**, 485–497.
- Dunker AK, Silman I, Uversky VN and Sussman JL (2008) Function and structure of inherently disordered proteins. *Curr Opin Struct Biol* **18**, 756–764.
- Ciryam P, Kundra R, Morimoto RI, Dobson CM and Vendruscolo M (2015) Supersaturation is a major driving force for protein aggregation in neurodegenerative diseases. *Trends Pharmacol Sci* **36**, 72–77.
- Hartl FU, Bracher A and Hayer-Hartl M (2011) Molecular chaperones in protein folding and proteostasis. *Nature* **475**, 324–332.
- Labbadia J and Morimoto RI (2015) The biology of proteostasis in aging and disease. *Annu Rev Biochem* **84**, 435–464.
- Douglas PM and Cyr DM (2010) Interplay between protein homeostasis networks in protein aggregation and proteotoxicity. *Biopolymers* **93**, 229–236.
- Morimoto RI (2008) Proteotoxic stress and inducible chaperone networks in neurodegenerative disease and aging. *Genes Dev* **22**, 1427–1438.
- Powers ET, Morimoto RI, Dillin A, Kelly JW and Balch WE (2009) Biological and chemical approaches to diseases of proteostasis deficiency. *Annu Rev Biochem* **78**, 959–991.
- Saez I and Vilchez D (2014) The mechanistic links between proteasome activity, aging and age-related diseases. *Curr Genomics* **15**, 38–51.
- Lopez-Otin C, Blasco MA, Partridge L, Serrano M and Kroemer G (2013) The hallmarks of aging. *Cell* **153**, 1194–1217.
- Balch WE, Morimoto RI, Dillin A and Kelly JW (2008) Adapting proteostasis for disease intervention. *Science* **319**, 916–919.
- Kenyon CJ (2010) The genetics of ageing. *Nature* **464**, 504–512.
- Kuhlbrodt K, Mouysset J and Hoppe T (2005) Orchestra for assembly and fate of polyubiquitin chains. *Essays Biochem* **41**, 1–14.
- Kerscher O, Felberbaum R and Hochstrasser M (2006) Modification of proteins by ubiquitin and ubiquitin-like proteins. *Annu Rev Cell Dev Biol* **22**, 159–180.
- Komander D and Rape M (2012) The ubiquitin code. *Annu Rev Biochem* **81**, 203–229.
- Lee BH, Lee MJ, Park S, Oh DC, Elsasser S, Chen PC, Gartner C, Dimova N, Hanna J, Gygi SP *et al.* (2010) Enhancement of proteasome activity by a small-molecule inhibitor of USP14. *Nature* **467**, 179–184.
- Kuhlbrodt K, Janiesch PC, Kevei E, Segref A, Barikbin R and Hoppe T (2011) The Machado-Joseph disease deubiquitylase ATX-3 couples longevity and proteostasis. *Nat Cell Biol* **13**, 273–281.

20 Kim HT, Kim KP, Uchiki T, Gygi SP and Goldberg AL (2009) S5a promotes protein degradation by blocking synthesis of nondegradable forked ubiquitin chains. *EMBO J* **28**, 1867–1877.

21 Sowa ME, Bennett EJ, Gygi SP and Harper JW (2009) Defining the human deubiquitinating enzyme interaction landscape. *Cell* **138**, 389–403.

22 Amerik A, Swaminathan S, Krantz BA, Wilkinson KD and Hochstrasser M (1997) In vivo disassembly of free polyubiquitin chains by yeast Ubp14 modulates rates of protein degradation by the proteasome. *EMBO J* **16**, 4826–4838.

23 Finley D (2009) Recognition and processing of ubiquitin-protein conjugates by the proteasome. *Annu Rev Biochem* **78**, 477–513.

24 Xu P, Duong DM, Seyfried NT, Cheng D, Xie Y, Robert J, Rush J, Hochstrasser M, Finley D and Peng J (2009) Quantitative proteomics reveals the function of unconventional ubiquitin chains in proteasomal degradation. *Cell* **137**, 133–145.

25 Behrends C, Sowa ME, Gygi SP and Harper JW (2010) Network organization of the human autophagy system. *Nature* **466**, 68–76.

26 Xie Z and Klionsky DJ (2007) Autophagosome formation: core machinery and adaptations. *Nat Cell Biol* **9**, 1102–1109.

27 Jin M, Liu X and Klionsky DJ (2013) SnapShot: selective autophagy. *Cell* **152**, 368e1–368.e2.

28 Salminen A and Kaarniranta K (2009) Regulation of the aging process by autophagy. *Trends Mol Med* **15**, 217–224.

29 Levine B and Kroemer G (2008) Autophagy in the pathogenesis of disease. *Cell* **132**, 27–42.

30 Mizushima N, Levine B, Cuervo AM and Klionsky DJ (2008) Autophagy fights disease through cellular self-digestion. *Nature* **451**, 1069–1075.

31 Kraft C, Peter M and Hofmann K (2010) Selective autophagy: ubiquitin-mediated recognition and beyond. *Nat Cell Biol* **12**, 836–841.

32 Cuervo AM and Wong E (2014) Chaperone-mediated autophagy: roles in disease and aging. *Cell Res* **24**, 92–104.

33 Ferreira JV, Fôfo H, Bejarano E, Bento CF, Ramalho JS, Girão H and Pereira P (2013) STUB1/CHIP is required for HIF1A degradation by chaperone-mediated autophagy. *Autophagy* **9**, 1349–1366.

34 Kettern N, Dreiseidler M, Tawo R and Hohfeld J (2010) Chaperone-assisted degradation: multiple paths to destruction. *Biol Chem* **391**, 481–489.

35 Arndt V, Dick N, Tawo R, Dreiseidler M, Wenzel D, Hesse M, Fürst DO, Saftig P, Saint R, Fleischmann BK *et al.* (2010) Chaperone-assisted selective autophagy is essential for muscle maintenance. *Curr Biol* **20**, 143–148.

36 Gamerdinger M, Hajieva P, Kaya AM, Wolfrum U, Hartl FU and Behl C (2009) Protein quality control during aging involves recruitment of the macroautophagy pathway by BAG3. *EMBO J* **28**, 889–901.

37 Carra S, Seguin SJ, Lambert H and Landry J (2007) HspB8 chaperone activity toward poly(Q)-containing proteins depends on its association with Bag3, a stimulator of macroautophagy. *J Biol Chem* **283**, 1437–1444.

38 Crippa V, Sau D, Rusmini P, Boncoraglio A, Onesto E, Bolzoni E, Galbiati M, Fontana E, Marino M, Carra S *et al.* (2010) The small heat shock protein B8 (HspB8) promotes autophagic removal of misfolded proteins involved in amyotrophic lateral sclerosis (ALS). *Hum Mol Genet* **19**, 3440–3456.

39 Ulbricht A, Eppler Felix J, Tapia Victor E, van der Ven Peter FM, Hampe N, Hersch N, Vakeel P, Stadel D, Haas A, Saftig P *et al.* (2013) Cellular mechanotransduction relies on tension-induced and chaperone-assisted autophagy. *Curr Biol* **23**, 430–435.

40 Powers ET and Balch WE (2013) Diversity in the origins of proteostasis networks—a driver for protein function in evolution. *Nat Rev Mol Cell Biol* **14**, 237–248.

41 Brehme M, Voisine C, Rolland T, Wachi S, Soper JH, Zhu Y, Orton K, Villella A, Garza D, Vidal M *et al.* (2014) A chaperome subnetwork safeguards proteostasis in aging and neurodegenerative disease. *Cell Rep* **9**, 1135–1150.

42 Saibil H (2013) Chaperone machines for protein folding, unfolding and disaggregation. *Nat Rev Mol Cell Biol* **14**, 630–642.

43 Park C and Cuervo AM (2013) Selective autophagy: talking with the UPS. *Cell Biochem Biophys* **67**, 3–13.

44 Kaushik S and Cuervo AM (2012) Chaperones in autophagy. *Pharmacol Res* **66**, 484–493.

45 Buchner J (1996) Supervising the fold: functional principles of molecular chaperones. *FASEB J* **10**, 10–19.

46 Zuehlke A and Johnson JL (2010) Hsp90 and co-chaperones twist the functions of diverse client proteins. *Biopolymers* **93**, 211–217.

47 Tzankov S, Wong MJH, Shi K, Nassif C and Young JC (2008) Functional divergence between co-chaperones of Hsc70. *J Biol Chem* **283**, 27100–27109.

48 Nillegoda NB, Kirstein J, Szlachcic A, Berynskyy M, Stank A, Stengel F, Arnsburg K, Gao X, Scior A, Aebersold R *et al.* (2015) Crucial HSP70 co-chaperone complex unlocks metazoan protein disaggregation. *Nature* **524**, 247–251.

49 Nillegoda NB, Stank A, Malinvern D, Alberts N, Szlachcic A, Barducci A, De Rios Los P, Wade RC and Bukau B (2017) Evolution of an intricate J-protein network driving protein disaggregation in eukaryotes. *Elife* **6**, e24560.

50 Craig EA and Marszalek J (2017) How do J-proteins get Hsp70 to do so many different things? *Trends Biochem Sci* **42**, 355–368.

51 Zuiderweg ER, Hightower LE and Gestwicki JE (2017) The remarkable multivalency of the Hsp70 chaperones. *Cell Stress Chaperones* **22**, 173–189.

52 Schopf FH, Biebl MM and Buchner J (2017) The HSP90 chaperone machinery. *Nat Rev Mol Cell Biol* **18**, 345–360.

53 Zhao R, Davey M, Hsu YC, Kaplanek P, Tong A, Parsons AB, Krogan N, Cagney G, Mai D, Greenblatt J *et al.* (2005) Navigating the chaperone network: an integrative map of physical and genetic interactions mediated by the hsp90 chaperone. *Cell* **120**, 715–727.

54 Hemmingsen SM, Woolford C, van der Vies SM, Tilly K, Dennis DT, Georgopoulos CP, Hendrix RW and Ellis RJ (1988) Homologous plant and bacterial proteins chaperone oligomeric protein assembly. *Nature* **333**, 330–334.

55 Ranford JC, Coates AR and Henderson B (2000) Chaperonins are cell-signalling proteins: the unfolding biology of molecular chaperones. *Expert Rev Mol Med* **2**, 1–17.

56 Lopez T, Dalton K and Frydman J (2015) The mechanism and function of group II chaperonins. *J Mol Biol* **427**, 2919–2930.

57 Kampinga HH and Craig EA (2010) The HSP70 chaperone machinery: J proteins as drivers of functional specificity. *Nat Rev Mol Cell Biol* **11**, 579–592.

58 Bar-Lavan Y, Shemesh N and Ben-Zvi A (2016) Chaperone families and interactions in metazoa. *Essays Biochem* **60**, 237–253.

59 Takayama S and Reed JC (2001) Molecular chaperone targeting and regulation by BAG family proteins. *Nat Cell Biol* **3**, E237–E241.

60 Morano KA and Thiele D (1999) Heat shock factor function and regulation in response to cellular stress, growth, and differentiation signals. *Gene Expr* **7**, 271–282.

61 Anckar J and Sistonen L (2007) Heat shock factor 1 as a coordinator of stress and developmental pathways. In *Advances in Experimental Medicine and Biology* (Csermely P and Vigh L, eds.), pp. 78–88. Springer, New York.

62 Ciocca DR and Calderwood SK (2005) Heat shock proteins in cancer: diagnostic, prognostic, predictive, and treatment implications. *Cell Stress Chaperones* **10**, 86–103.

63 Calderwood SK and Gong J (2016) Heat shock proteins promote cancer: it's a protection racket. *Trends Biochem Sci* **41**, 311–323.

64 Rodina A, Wang T, Yan P, Gomes ED, Dunphy MP, Pillarsetty N, Koren J, Gerecitano JF, Taldone T, Zong H *et al.* (2016) The epichaperome is an integrated chaperome network that facilitates tumour survival. *Nature* **538**, 397–401.

65 Taipale M, Tucker G, Peng J, Krykbaeva I, Lin ZY, Larsen B, Choi H, Berger B, Gingras AC and Lindquist S (2014) A quantitative chaperone interaction network reveals the architecture of cellular protein homeostasis pathways. *Cell* **158**, 434–448.

66 Mehnert M, Sommer T and Jarosch E (2010) ERAD ubiquitin ligases: multifunctional tools for protein quality control and waste disposal in the endoplasmic reticulum. *BioEssays* **32**, 905–913.

67 Christianson JC and Ye Y (2014) Cleaning up in the endoplasmic reticulum: ubiquitin in charge. *Nat Struct Mol Biol* **21**, 325–335.

68 Ruggiano A, Foresti O and Carvalho P (2014) Quality control: ER-associated degradation: protein quality control and beyond. *J Cell Biol* **204**, 869–879.

69 Roberts RF, Tang MY, Fon EA and Durcan TM (2016) Defending the mitochondria: the pathways of mitophagy and mitochondrial-derived vesicles. *Int J Biochem Cell Biol* **79**, 427–436.

70 Rüb C, Wilkening A and Voos W (2016) Mitochondrial quality control by the Parkin system. *Cell Tissue Res* **367**, 111–123.

71 Arndt V, Rogon C and Höhfeld J (2007) To be, or not to be — molecular chaperones in protein degradation. *Cell Mol Life Sci* **64**, 2525–2541.

72 Connell P, Ballinger CA, Jiang J, Wu Y, Thompson LJ, Hohfeld J and Patterson C (2001) The co-chaperone CHIP regulates protein triage decisions mediated by heat-shock proteins. *Nat Cell Biol* **3**, 93–96.

73 Cyr DM, Hohfeld J and Patterson C (2002) Protein quality control: U-box-containing E3 ubiquitin ligases join the fold. *Trends Biochem Sci* **27**, 368–375.

74 Murata S, Chiba T and Tanaka K (2003) CHIP: a quality-control E3 ligase collaborating with molecular chaperones. *Int J Biochem Cell Biol* **35**, 572–578.

75 Petrucelli L (2004) CHIP and Hsp70 regulate tau ubiquitination, degradation and aggregation. *Hum Mol Genet* **13**, 703–714.

76 Ballinger CA, Connell P, Wu Y, Hu Z, Thompson LJ, Yin L-Y and Patterson C (1999) Identification of CHIP, a novel tetratricopeptide repeat-containing protein that interacts with heat shock proteins and negatively regulates chaperone functions. *Mol Cell Biol* **19**, 4535–4545.

77 Hatakeyama S, Yada M, Matsumoto M, Ishida N and Nakayama KI (2001) U box proteins as a new family of ubiquitin-protein ligases. *J Biol Chem* **276**, 33111–33120.

78 Jiang J (2001) CHIP Is a U-box-dependent E3 Ubiquitin Ligase. Identification of Hsc70 as a target for ubiquitylation. *J Biol Chem* **276**, 42938–42944.

79 Matsumura Y, Sakai J and Skach WR (2013) Endoplasmic reticulum protein quality control is determined by cooperative interactions between Hsp/c70 protein and the CHIP E3 ligase. *J Biol Chem* **288**, 31069–31079.

80 VanPelt J and Page RC (2017) Unraveling the CHIP: Hsp70 complex as an information processor for protein quality control. *Biochim Biophys Acta* **1865**, 133–141.

81 Dai Q (2003) CHIP activates HSF1 and confers protection against apoptosis and cellular stress. *EMBO J* **22**, 5446–5458.

82 Yu Y, Liu M, Zhang L, Cao Q, Zhang P, Jiang H, Zou Y and Ge J (2012) Heat shock transcription factor 1 inhibits H2O2-induced cardiomyocyte death through suppression of high-mobility group box 1. *Mol Cell Biochem* **364**, 263–269.

83 Murata S, Minami Y, Minami M, Chiba T and Tanaka K (2001) CHIP is a chaperone-dependent E3 ligase that ubiquitylates unfolded protein. *EMBO Rep* **2**, 1133–1138.

84 Soss SE, Rose KL, Hill S, Jouan S and Chazin WJ (2015) Biochemical and proteomic analysis of ubiquitination of Hsc70 and Hsp70 by the E3 ligase CHIP. *PLoS ONE* **10**, e0128240.

85 McDonough H and Patterson C (2003) CHIP: a link between the chaperone and proteasome systems. *Cell Stress Chaperones* **8**, 303.

86 Meacham GC, Patterson C, Zhang W, Younger JM and Cyr DM (2001) The Hsc70 co-chaperone CHIP targets immature CFTR for proteasomal degradation. *Nat Cell Biol* **3**, 100–105.

87 Rees I, Lee S, Kim H and Tsai FTF (2006) The E3 ubiquitin ligase CHIP binds the androgen receptor in a phosphorylation-dependent manner. *Biochim Biophys Acta* **1764**, 1073–1079.

88 Fan M, Park A and Nephew KP (2005) CHIP (carboxyl terminus of Hsc70-interacting protein) promotes basal and geldanamycin-induced degradation of estrogen receptor- $\alpha$ . *Mol Endocrinol* **19**, 2901–2914.

89 Zhang H-T, Zeng L-F, He Q-Y, Tao WA, Zha Z-G and Hu C-D (2016) The E3 ubiquitin ligase CHIP mediates ubiquitination and proteasomal degradation of PRMT5. *Biochim Biophys Acta* **1863**, 335–346.

90 Zhou P, Fernandes N, Dodge IL, Reddi AL, Rao N, Safran H, DiPetrillo TA, Wazer DE, Band V and Band H (2003) ErbB2 degradation mediated by the co-chaperone protein CHIP. *J Biol Chem* **278**, 13829–13837.

91 Li R-F, Shang Y, Liu D, Ren Z-S, Chang Z and Sui S-F (2007) Differential ubiquitination of smad1 mediated by CHIP: implications in the regulation of the bone morphogenetic protein signaling pathway. *J Mol Biol* **374**, 777–790.

92 Paul I and Ghosh MK (2015) A CHIPotle in physiology and disease. *Int J Biochem Cell Biol* **58**, 37–52.

93 Joshi V, Amanullah A, Upadhyay A, Mishra R, Kumar A and Mishra A (2016) A decade of boon or burden: what has the chip ever done for cellular protein quality control mechanism implicated in neurodegeneration and aging? *Front Mol Neurosci* **9**, 93.

94 Li X, Huang M, Zheng H, Wang Y, Ren F, Shang Y, Zhai Y, Irwin DM, Shi Y, Chen D *et al.* (2008) CHIP promotes Runx2 degradation and negatively regulates osteoblast differentiation. *J Cell Biol* **181**, 959–972.

95 Min JN, Whaley RA, Sharpless NE, Lockyer P, Portbury AL and Patterson C (2008) CHIP deficiency decreases longevity, with accelerated aging phenotypes accompanied by altered protein quality control. *Mol Cell Biol* **28**, 4018–4025.

96 Morishima Y, Wang AM, Yu Z, Pratt WB, Osawa Y and Lieberman AP (2008) CHIP deletion reveals functional redundancy of E3 ligases in promoting degradation of both signaling proteins and expanded glutamine proteins. *Hum Mol Genet* **17**, 3942–3952.

97 Tawo R, Pokrzywa W, Kevei É, Akyuz ME, Balaji V, Adrian S, Höhfeld J and Hoppe T (2017) The ubiquitin ligase CHIP integrates proteostasis and aging by regulation of insulin receptor turnover. *Cell* **169**, 470–482.e13.

98 Mishra A, Godavarthi SK, Maheshwari M, Goswami A and Jana NR (2009) The ubiquitin ligase E6-AP is induced and recruited to aggresomes in response to proteasome inhibition and may be involved in the ubiquitination of Hsp70-bound misfolded proteins. *J Biol Chem* **284**, 10537–10545.

99 Ehrlich ES, Wang T, Luo K, Xiao Z, Niewiadomska AM, Martinez T, Xu W, Neckers L and Yu XF (2009) Regulation of Hsp90 client proteins by a Cullin5-RING E3 ubiquitin ligase. *Proc Natl Acad Sci USA* **106**, 20330–20335.

100 Samant RS, Clarke PA and Workman P (2014) E3 ubiquitin ligase Cullin-5 modulates multiple molecular and cellular responses to heat shock protein 90 inhibition in human cancer cells. *Proc Natl Acad Sci USA* **111**, 6834–6839.

101 Heck JW, Cheung SK and Hampton RY (2010) Cytoplasmic protein quality control degradation mediated by parallel actions of the E3 ubiquitin ligases Ubr1 and San1. *Proc Natl Acad Sci USA* **107**, 1106–1111.

102 Eisele F and Wolf DH (2008) Degradation of misfolded protein in the cytoplasm is mediated by the ubiquitin ligase Ubr1. *FEBS Lett* **582**, 4143–4146.

103 Nillegoda NB, Theodoraki MA, Mandal AK, Mayo KJ, Ren HY, Sultana R, Wu K, Johnson J, Cyr DM

and Caplan AJ (2010) Ubr1 and Ubr2 function in a quality control pathway for degradation of unfolded cytosolic proteins. *Mol Biol Cell* **21**, 2102–2116.

104 Gardner RG, Nelson ZW and Gottschling DE (2005) Degradation-mediated protein quality control in the nucleus. *Cell* **120**, 803–815.

105 Rosenbaum JC, Fredrickson EK, Oeser ML, Garrett-Engele CM, Locke MN, Richardson LA, Nelson ZW, Hetrick ED, Milac TI, Gottschling DE *et al.* (2011) Disorder targets disorder in nuclear quality control degradation: a disordered ubiquitin ligase directly recognizes its misfolded substrates. *Mol Cell* **41**, 93–106.

106 Russell SJ, Steger KA and Johnston SA (1999) Subcellular localization, stoichiometry, and protein levels of 26 S proteasome subunits in yeast. *J Biol Chem* **274**, 21943–21952.

107 Guerriero CJ, Weiberth KF and Brodsky JL (2013) Hsp70 targets a cytoplasmic quality control substrate to the San1p ubiquitin ligase. *J Biol Chem* **288**, 18506–18520.

108 Prasad R, Kawaguchi S and Ng DT (2010) A nucleus-based quality control mechanism for cytosolic proteins. *Mol Biol Cell* **21**, 2117–2127.

109 Boomsma W, Nielsen SV, Lindorff-Larsen K, Hartmann-Petersen R and Ellgaard L (2016) Bioinformatics analysis identifies several intrinsically disordered human E3 ubiquitin-protein ligases. *PeerJ* **4**, e1725.

110 Bartel B, Wunning I and Varshavsky A (1990) The recognition component of the N-end rule pathway. *EMBO J* **9**, 3179–3189.

111 Kwon YT, Xia Z, An JY, Tasaki T, Davydov IV, Seo JW, Sheng J, Xie Y and Varshavsky A (2003) Female lethality and apoptosis of spermatocytes in mice lacking the UBR2 ubiquitin ligase of the N-end rule pathway. *Mol Cell Biol* **23**, 8255–8271.

112 Tasaki T, Mulder LCF, Iwamatsu A, Lee MJ, Davydov IV, Varshavsky A, Muesing M and Kwon YT (2005) A family of mammalian E3 ubiquitin ligases that contain the UBR box motif and recognize N-degrons. *Mol Cell Biol* **25**, 7120–7136.

113 Tasaki T and Kwon YT (2007) The mammalian N-end rule pathway: new insights into its components and physiological roles. *Trends Biochem Sci* **32**, 520–528.

114 Summers DW, Wolfe KJ, Ren HY and Cyr DM (2013) The type II Hsp40 Sis1 cooperates with Hsp70 and the E3 ligase Ubr1 to promote degradation of terminally misfolded cytosolic protein. *PLoS ONE* **8**, e52099.

115 Sultana R, Theodoraki MA and Caplan AJ (2012) UBR1 promotes protein kinase quality control and sensitizes cells to Hsp90 inhibition. *Exp Cell Res* **318**, 53–60.

116 Hessa T, Sharma A, Mariappan M, Eshleman HD, Gutierrez E and Hegde RS (2011) Protein targeting and degradation are coupled for elimination of mislocalized proteins. *Nature* **475**, 394–397.

117 Rodrigo-Brenni MC, Gutierrez E and Hegde RS (2014) Cytosolic quality control of mislocalized proteins requires RNF126 recruitment to Bag6. *Mol Cell* **55**, 227–237.

118 Fourie AM, Sambrook JF and Gething MJ (1994) Common and divergent peptide binding specificities of hsp70 molecular chaperones. *J Biol Chem* **269**, 30470–30478.

119 Rüdiger S, Buchberger A and Bukau B (1997) Interaction of Hsp70 chaperones with substrates. *Nat Struct Biol* **4**, 342–349.

120 Higy M, Junne T and Spiess M (2004) Topogenesis of membrane proteins at the endoplasmic reticulum. *Biochemistry* **43**, 12716–12722.

121 Lerch-Bader M, Lundin C, Kim H, Nilsson I and von Heijne G (2008) Contribution of positively charged flanking residues to the insertion of transmembrane helices into the endoplasmic reticulum. *Proc Natl Acad Sci USA* **105**, 4127–4132.

122 Xu Y, Anderson DE and Ye Y (2016) The HECT domain ubiquitin ligase HUWE1 targets unassembled soluble proteins for degradation. *Cell Discov* **2**, 16040.

123 Sung MK, Porras-Yakushi TR, Reitsma JM, Huber FM, Sweredoski MJ, Hoelz A, Hess S and Deshaies RJ (2016) A conserved quality-control pathway that mediates degradation of unassembled ribosomal proteins. *Elife* **5**, e19105.

124 Katsogiannou M, Andrieu C, Baylot V, Baudot A, Dusetti NJ, Gayet O, Finetti P, Garrido C, Birnbaum D, Bertucci F *et al.* (2014) The functional landscape of Hsp27 reveals new cellular processes such as DNA repair and alternative splicing and proposes novel anticancer targets. *Mol Cell Proteomics* **13**, 3585–3601.

125 Davies AE and Kaplan KB (2010) Hsp90-Sgt1 and Skp1 target human Mis12 complexes to ensure efficient formation of kinetochore-microtubule binding sites. *J Cell Biol* **189**, 261–274.

126 Spiechowicz M, Zylicz A, Bieganowski P, Kuznicki J and Filipek A (2007) Hsp70 is a new target of Sgt1—an interaction modulated by S100A6. *Biochem Biophys Res Comm* **357**, 1148–1153.

127 Kaplan KB, Hyman AA and Sorger PK (1997) Regulating the yeast kinetochore by ubiquitin-dependent degradation and Skp1p-mediated phosphorylation. *Cell* **91**, 491–500.

128 Catlett MG and Kaplan KB (2006) Sgt1p is a unique co-chaperone that acts as a client adaptor to link Hsp90 to Skp1p. *J Biol Chem* **281**, 33739–33748.

129 Kitagawa K, Skowyra D, Elledge SJ, Harper JW and Hieter P (1999) SGT1 encodes an essential component of the yeast kinetochore assembly pathway and a

novel subunit of the SCF ubiquitin ligase complex. *Mol Cell* **4**, 21–33.

130 Stuttmann J, Parker JE and Noel LD (2008) Staying in the fold: the SGT1/chaperone machinery in maintenance and evolution of leucine-rich repeat proteins. *Plant Signal Behav* **3**, 283–285.

131 Willhoft O, Kerr R, Patel D, Zhang W, Al-Jassar C, Daviter T, Millson SH, Thalassinos K and Vaughan CK (2017) The crystal structure of the Sgt1-Skp1 complex: the link between Hsp90 and both SCF E3 ubiquitin ligases and kinetochores. *Sci Rep* **7**, 41626.

132 Fang NN, Ng AH, Measday V and Mayor T (2011) Hul5 HECT ubiquitin ligase plays a major role in the ubiquitylation and turnover of cytosolic misfolded proteins. *Nat Cell Biol* **13**, 1344–1352.

133 Fang NN, Chan GT, Zhu M, Comyn SA, Persaud A, Deshaies RJ, Rotin D, Gsponer J and Mayor T (2014) Rsp5/Nedd4 is the main ubiquitin ligase that targets cytosolic misfolded proteins following heat stress. *Nat Cell Biol* **16**, 1227–1237.

134 Crosas B, Hanna J, Kirkpatrick DS, Zhang DP, Tone Y, Hathaway NA, Buecker C, Leggett DS, Schmidt M, King RW *et al.* (2006) Ubiquitin chains are remodeled at the proteasome by opposing ubiquitin ligase and deubiquitinating activities. *Cell* **127**, 1401–1413.

135 Aviram S and Kornitzer D (2010) The ubiquitin ligase Hul5 promotes proteasomal processivity. *Mol Cell Biol* **30**, 985–994.

136 Fang NN and Mayor T (2012) Hul5 ubiquitin ligase: good riddance to bad proteins. *Prion* **6**, 240–244.

137 Koegl M, Hoppe T, Schlenker S, Ulrich HD, Mayer TU and Jentsch S (1999) A novel ubiquitination factor, E4, is involved in multiubiquitin chain assembly. *Cell* **96**, 635–644.

138 Hoppe T (2010) Life and destruction: ubiquitin-mediated proteolysis in aging and longevity. *FI1000 Biol Rep*, **2**.

139 Chu BW, Kovary KM, Guillaume J, Chen LC, Teruel MN and Wandless TJ (2013) The E3 ubiquitin ligase UBE3C enhances proteasome processivity by ubiquitinating partially proteolyzed substrates. *J Biol Chem* **288**, 34575–34587.

140 Kuo C-L and Goldberg AL (2017) Ubiquitinated proteins promote the association of proteasomes with the deubiquitinating enzyme Usp14 and the ubiquitin ligase Ube3c. *Proc Natl Acad Sci* **114**, E3404–E3413.

141 Shahsavaran H, Sugiyama M, Kaneko Y, Chuenchit B and Harashima S (2012) Superior thermotolerance of *Saccharomyces cerevisiae* for efficient bioethanol fermentation can be achieved by overexpression of RSP5 ubiquitin ligase. *Biotechnol Adv* **30**, 1289–1300.

142 Apaja PM, Xu H and Lukacs GL (2010) Quality control for unfolded proteins at the plasma membrane. *J Cell Biol* **191**, 553–570.

143 Hettema EH, Valdez-Taubas J and Pelham HR (2004) Bsd2 binds the ubiquitin ligase Rsp5 and mediates the ubiquitination of transmembrane proteins. *EMBO J* **23**, 1279–1288.

144 Zhao Y, Macgurn JA, Liu M and Emr S (2013) The ART-Rsp5 ubiquitin ligase network comprises a plasma membrane quality control system that protects yeast cells from proteotoxic stress. *Elife* **2**, e00459.

145 Shiga T, Yoshida N, Shimizu Y, Suzuki E, Sasaki T, Watanabe D and Takagi H (2014) Quality control of plasma membrane proteins by *Saccharomyces cerevisiae* Nedd4-like ubiquitin ligase Rsp5p under environmental stress conditions. *Eukaryot Cell* **13**, 1191–1199.

146 Becuwe M, Herrador A, Haguenauer-Tsapis R, Vincent O and Leon S (2012) Ubiquitin-mediated regulation of endocytosis by proteins of the arrestin family. *Biochem Res Int* **2012**, 242764.

147 MacGurn JA, Hsu PC and Emr SD (2012) Ubiquitin and membrane protein turnover: from cradle to grave. *Annu Rev Biochem* **81**, 231–259.

148 Duttler S, Pechmann S and Frydman J (2013) Principles of cotranslational ubiquitination and quality control at the ribosome. *Mol Cell* **50**, 379–393.

149 Maier T, Ferbitz L, Deuerling E and Ban N (2005) A cradle for new proteins: trigger factor at the ribosome. *Curr Opin Struct Biol* **15**, 204–212.

150 Merz F, Boehringer D, Schaffitzel C, Preissler S, Hoffmann A, Maier T, Rutkowska A, Lozza J, Ban N, Bukau B *et al.* (2008) Molecular mechanism and structure of Trigger Factor bound to the translating ribosome. *EMBO J* **27**, 1622–1632.

151 Lakshminpathy SK, Gupta R, Pinkert S, Etchells SA and Hartl FU (2010) Versatility of trigger factor interactions with ribosome-nascent chain complexes. *J Biol Chem* **285**, 27911–27923.

152 Bengtson MH and Joazeiro CA (2010) Role of a ribosome-associated E3 ubiquitin ligase in protein quality control. *Nature* **467**, 470–473.

153 Wang F, Canadeo LA and Huijbregts JM (2015) Ubiquitination of newly synthesized proteins at the ribosome. *Biochimie* **114**, 127–133.

154 Shao S, Brown A, Santhanam B and Hegde RS (2015) Structure and assembly pathway of the ribosome quality control complex. *Mol Cell* **57**, 433–444.

155 Yonashiro R, Tahara EB, Bengtson MH, Khokhrina M, Lorenz H, Chen KC, Kigoshi-Tansho Y, Savas JN, Yates JR, Kay SA *et al.* (2016) The Rqc2/Tae2 subunit of the ribosome-associated quality control (RQC) complex marks ribosome-stalled nascent polypeptide chains for aggregation. *Elife* **5**, e11794.

156 Verma R, Oania RS, Kolawa NJ and Deshaies RJ (2013) Cdc48/p97 promotes degradation of aberrant nascent polypeptides bound to the ribosome. *Elife* **2**, e00308.

157 Rape M, Hoppe T, Gorr I, Kalocay M, Richly H and Jentsch S (2001) Mobilization of processed, membrane-tethered SPT23 transcription factor by CDC48 UFD1/NPL4, a ubiquitin-selective chaperone. *Cell* **107**, 667–677.

158 Bodnar NO and Rapoport TA (2017) Molecular mechanism of substrate processing by the Cdc48 ATPase complex. *Cell* **169**, 722–735.e9.

159 Brandman O, Stewart-Ornstein J, Wong D, Larson A, Williams CC, Li GW, Zhou S, King D, Shen PS, Weibezaahn J *et al.* (2012) A ribosome-bound quality control complex triggers degradation of nascent peptides and signals translation stress. *Cell* **151**, 1042–1054.

160 Defenouillere Q, Zhang E, Namane A, Mouaikel J, Jacquier A and Fromont-Racine M (2016) Rqc1 and Ltn1 prevent c-terminal alanine-threonine tail (CAT-tail)-induced protein aggregation by efficient recruitment of Cdc48 on stalled 60S subunits. *J Biol Chem* **291**, 12245–12253.

161 Choe YJ, Park SH, Hassemer T, Korner R, Vincenz-Donnelly L, Hayer-Hartl M and Hartl FU (2016) Failure of RQC machinery causes protein aggregation and proteotoxic stress. *Nature* **531**, 191–195.

162 Maurer MJ, Spear ED, Yu AT, Lee EJ, Shahzad S and Michaelis S (2016) Degradation signals for ubiquitin-proteasome dependent cytosolic protein quality control (CytoQC) in yeast. *G3* **6**, 1853–1866.

163 Boban M and Foisner R (2016) Degradation-mediated protein quality control at the inner nuclear membrane. *Nucleus* **7**, 41–49.

164 Meng F, Na I, Kurgan L and Uversky V (2015) Compartmentalization and functionality of nuclear disorder: intrinsic disorder and protein-protein interactions in intra-nuclear compartments. *Int J Mol Sci* **17**, 24.

165 Roberts P (2002) Piecemeal microautophagy of nucleus in *Saccharomyces cerevisiae*. *Mol Biol Cell* **14**, 129–141.

166 Park Y-E, Hayashi YK, Bonne G, Arimura T, Noguchi S, Nonaka I and Nishino I (2009) Autophagic degradation of nuclear components in mammalian cells. *Autophagy* **5**, 795–804.

167 Fredrickson EK, Rosenbaum JC, Locke MN, Milac TI and Gardner RG (2011) Exposed hydrophobicity is a key determinant of nuclear quality control degradation. *Mol Biol Cell* **22**, 2384–2395.

168 Iwata A, Nagashima Y, Matsumoto L, Suzuki T, Yamanaka T, Date H, Deoka K, Nukina N and Tsuji S (2009) Intranuclear degradation of polyglutamine aggregates by the ubiquitin-proteasome system. *J Biol Chem* **284**, 9796–9803.

169 Swanson R (2001) A conserved ubiquitin ligase of the nuclear envelope/endoplasmic reticulum that functions in both ER-associated and Matalpha 2 repressor degradation. *Genes Dev* **15**, 2660–2674.

170 Bordallo J, Plemper RK, Finger A and Wolf DH (1998) Der3p/Hrd1p is required for endoplasmic reticulum-associated degradation of misfolded luminal and integral membrane proteins. *Mol Biol Cell* **9**, 209–222.

171 Bays NW, Gardner RG, Seelig LP, Joazeiro CA and Hampton RY (2001) Hrd1p/Der3p is a membrane-anchored ubiquitin ligase required for ER-associated degradation. *Nat Cell Biol* **3**, 24–29.

172 Laba J, Steen A, Popken P, Chernova A, Poolman B and Veenhoff L (2015) Active nuclear import of membrane proteins revisited. *Cells* **4**, 653–673.

173 Boban M, Zargari A, Andreasson C, Heessen S, Thyberg J and Ljungdahl PO (2006) Asil is an inner nuclear membrane protein that restricts promoter access of two latent transcription factors. *J Cell Biol* **173**, 695–707.

174 Zargari A, Boban M, Heessen S, Andreasson C, Thyberg J and Ljungdahl PO (2007) Inner nuclear membrane proteins Asi1, Asi2, and Asi3 function in concert to maintain the latent properties of transcription factors Stp1 and Stp2. *J Biol Chem* **282**, 594–605.

175 Foresti O, Rodriguez-Vaello V, Funaya C and Carvalho P (2014) Quality control of inner nuclear membrane proteins by the Asi complex. *Science* **346**, 751–755.

176 Khmelinskii A, Blaszcak E, Pantazopoulou M, Fischer B, Omnis DJ, Le Dez G, Brossard A, Gunnarsson A, Barry JD, Meurer M *et al.* (2014) Protein quality control at the inner nuclear membrane. *Nature* **516**, 410–413.

177 Deng M and Hochstrasser M (2006) Spatially regulated ubiquitin ligation by an ER/nuclear membrane ligase. *Nature* **443**, 827–831.

178 Shiber A, Breuer W, Brandeis M and Ravid T (2013) Ubiquitin conjugation triggers misfolded protein sequestration into quality control foci when Hsp70 chaperone levels are limiting. *Mol Biol Cell* **24**, 2076–2087.

179 Ravid T, Kreft SG and Hochstrasser M (2006) Membrane and soluble substrates of the Doa10 ubiquitin ligase are degraded by distinct pathways. *EMBO J* **25**, 533–543.

180 Metzger MB, Maurer MJ, Dancy BM and Michaelis S (2008) Degradation of a cytosolic protein requires endoplasmic reticulum-associated degradation machinery. *J Biol Chem* **283**, 32302–32316.

181 Guo L, Giasson Benoit I, Glavis-Bloom A, Brewer Michael D, Shorter J, Gitler Aaron D and Yang X (2014) A cellular system that degrades misfolded proteins and protects against neurodegeneration. *Mol Cell* **55**, 15–30.

182 Venkatesh S and Workman JL (2015) Histone exchange, chromatin structure and the regulation of transcription. *Nat Rev Mol Cell Biol* **16**, 178–189.

183 Commerford S, Carsten A and Cronkite E (1982) Histone turnover within nonproliferating cells. *Proc Natl Acad Sci* **79**, 1163–1165.

184 Singh RK, Kabbaj M-HM, Paik J and Gunjan A (2009) Histone levels are regulated by phosphorylation and ubiquitylation-dependent proteolysis. *Nat Cell Biol* **11**, 925–933.

185 Singh RK, Liang D, Gajjalaiahvari UR, Kabbaj M-HM, Paik J and Gunjan A (2010) Excess histone levels mediate cytotoxicity via multiple mechanisms. *Cell Cycle* **9**, 4236–4244.

186 Singh RK, Gonzalez M, Kabbaj M-HM and Gunjan A (2012) Novel E3 ubiquitin ligases that regulate histone protein levels in the budding yeast *Saccharomyces cerevisiae*. *PLoS ONE* **7**, e36295.

187 Goldberg AD, Banaszynski LA, Noh K-M, Lewis PW, Elsaesser SJ, Stadler S, Dewell S, Law M, Guo X, Li X *et al.* (2010) Distinct factors control histone variant H3.3 localization at specific genomic regions. *Cell* **140**, 678–691.

188 Hondele M and Ladurner AG (2011) The chaperone–histone partnership: for the greater good of histone traffic and chromatin plasticity. *Curr Opin Struct Biol* **21**, 698–708.

189 Hondele M, Stuwe T, Hassler M, Halbach F, Bowman A, Zhang ET, Nijmeijer B, Kotthoff C, Rybin V, Amlacher S *et al.* (2013) Structural basis of histone H2A–H2B recognition by the essential chaperone FACT. *Nature* **499**, 111–114.

190 Mattiroli F, D’Arcy S and Luger K (2015) The right place at the right time: chaperoning core histone variants. *EMBO Rep* **16**, 1454–1466.

191 Deyter GMR and Biggins S (2014) The FACT complex interacts with the E3 ubiquitin ligase Psh1 to prevent ectopic localization of CENP-A. *Genes Dev* **28**, 1815–1826.

192 Han J, Zhang H, Zhang H, Wang Z, Zhou H and Zhang Z (2013) A Cul4 E3 ubiquitin ligase regulates histone hand-off during nucleosome assembly. *Cell* **155**, 817–829.

193 Keller JN, Huang FF and Markesberry WR (2000) Decreased levels of proteasome activity and proteasome expression in aging spinal cord. *Neuroscience* **98**, 149–156.

194 Cuervo AM (2004) Impaired degradation of mutant  $\alpha$ -synuclein by chaperone-mediated autophagy. *Science* **305**, 1292–1295.

195 Vilchez D, Morantte I, Liu Z, Douglas PM, Merkworth C, Rodrigues AP, Manning G and Dillin A (2012) RPN-6 determines *C. elegans* longevity under proteotoxic stress conditions. *Nature* **489**, 263.

196 Kahn NW, Rea SL, Moyle S, Kell A and Johnson TE (2008) Proteasomal dysfunction activates the transcription factor SKN-1 and produces a selective oxidative-stress response in *Caenorhabditis elegans*. *Biochem J* **409**, 205–213.

197 Ciechanover A and Kwon YT (2017) Protein quality control by molecular chaperones in neurodegeneration. *Front Neurosci* **11**, 185.

198 Terman A (1995) The effect of age on formation and elimination of autophagic vacuoles in mouse hepatocytes. *Gerontology* **41**, 319–326.

199 Donati A, Cavallini G, Paradiso C, Vittorini S, Pollera M, Gori Z and Bergamini E (2001) Age-related changes in the regulation of autophagic proteolysis in rat isolated hepatocytes. *J Gerontol A Biol Sci Med Sci* **56**, B288–B293.

200 Walther Dirk M, Kasturi P, Zheng M, Pinkert S, Vecchi G, Ciryam P, Morimoto Richard I, Dobson Christopher M, Vendruscolo M, Mann M *et al.* (2015) Widespread proteome remodeling and aggregation in aging *C. elegans*. *Cell* **161**, 919–932.

201 Kaushik S and Cuervo AM (2015) Proteostasis and aging. *Nat Med* **21**, 1406–1415.

202 Morimoto RI and Santoro MG (1998) Stress-inducible responses and heat shock proteins: new pharmacologic targets for cytoprotection. *Nat Biotechnol* **16**, 833–838.

203 Taylor JP, Hardy J and Fischbeck KH (2002) Toxic proteins in neurodegenerative disease. *Science* **296**, 1991–1995.

204 Broersen K, Rousseau F and Schymkowitz J (2010) The culprit behind amyloid beta peptide related neurotoxicity in Alzheimer’s disease: oligomer size or conformation? *Alzheimer’s Res Ther* **2**, 12.

205 Sulistio YA and Heese K (2016) The ubiquitin-proteasome system and molecular chaperone deregulation in Alzheimer’s disease. *Mol Neurobiol* **53**, 905–931.

206 Shin Y, Klucken J, Patterson C, Hyman BT and McLean PJ (2005) The co-chaperone carboxyl terminus of Hsp70-interacting protein (CHIP) mediates  $\alpha$ -synuclein degradation decisions between proteasomal and lysosomal pathways. *J Biol Chem* **280**, 23727–23734.

207 Kumar P, Pradhan K, Karunya R, Ambasta RK and Querfurth HW (2011) Cross-functional E3 ligases Parkin and C-terminus Hsp70-interacting protein in neurodegenerative disorders. *J Neurochem* **120**, 350–370.

208 Qian S-B, McDonough H, Boellmann F, Cyr DM and Patterson C (2006) CHIP-mediated stress recovery by sequential ubiquitination of substrates and Hsp70. *Nature* **440**, 551–555.

209 Koren J 3rd, Jinwal UK, Lee DC, Jones JR, Shultz CL, Johnson AG, Anderson LJ and Dickey CA (2009) Chaperone signalling complexes in Alzheimer’s disease. *J Cell Mol Med* **13**, 619–630.

210 Urushitani M, Kurisu J, Tateno M, Hatakeyama S, Nakayama K-I, Kato S and Takahashi R (2004)

CHIP promotes proteasomal degradation of familial ALS-linked mutant SOD1 by ubiquitinating Hsp/Hsc70. *J Neurochem* **90**, 231–244.

211 Jana NR, Dikshit P, Goswami A, Kotliarova S, Murata S, Tanaka K and Nukina N (2005) Co-chaperone CHIP associates with expanded polyglutamine protein and promotes their degradation by proteasomes. *J Biol Chem* **280**, 11635–11640.

212 Williams AJ, Knutson TM, Colomer Gould VF and Paulson HL (2009) In vivo suppression of polyglutamine neurotoxicity by C-terminus of Hsp70-interacting protein (CHIP) supports an aggregation model of pathogenesis. *Neurobiol Dis* **33**, 342–353.

213 Garrido C, Brunet M, Didelot C, Zermati Y, Schmitt E and Kroemer G (2006) Heat shock proteins 27 and 70: anti-apoptotic proteins with tumorigenic properties. *Cell Cycle* **5**, 2592–2601.

214 Jego G, Hazoumé A, Seigneuric R and Garrido C (2013) Targeting heat shock proteins in cancer. *Cancer Lett* **332**, 275–285.

215 Barrott JJ and Haystead TA (2013) Hsp90, an unlikely ally in the war on cancer. *FEBS J* **280**, 1381–1396.

216 Nahleh Z, Tfayli A, Najm A, El Sayed A and Nahle Z (2012) Heat shock proteins in cancer: targeting the ‘chaperones’. *Future Med Chem* **4**, 927–935.

217 Wang X, Chen M, Zhou J and Zhang X (2014) HSP27, 70 and 90, anti-apoptotic proteins, in clinical cancer therapy (Review). *Int J Oncol* **45**, 18–30.

218 Fang NN, Zhu M, Rose A, Wu KP and Mayor T (2016) Deubiquitinase activity is required for the proteasomal degradation of misfolded cytosolic proteins upon heat-stress. *Nat Commun* **7**, 12907.

219 Finley D (2011) Misfolded proteins driven to destruction by Hul5. *Nat Cell Biol* **13**, 1290–1292.

220 Zhang M, Boter M, Li K, Kadota Y, Panaretou B, Prodromou C, Shirasu K and Pearl LH (2008) Structural and functional coupling of Hsp90- and Sgt1-centred multi-protein complexes. *EMBO J* **27**, 2789–2798.

221 Gallagher PS, Clowes Candadai SV and Gardner RG (2014) The requirement for Cdc48/p97 in nuclear protein quality control degradation depends on the substrate and correlates with substrate insolubility. *J Cell Sci* **127**, 1980–1991.