Protein quality control: chaperones culling corrupt conformations

Amie J. McClellan, Stephen Tam, Daniel Kaganovich and Judith Frydman

Achieving the correct balance between folding and degradation of misfolded proteins is critical for cell viability. The importance of defining the mechanisms and factors that mediate cytoplasmic quality control is underscored by the growing list of diseases associated with protein misfolding and aggregation. Molecular chaperones assist protein folding and also facilitate degradation of misfolded polypeptides by the ubiquitin–proteasome system. Here we discuss emerging links between folding and degradation machineries and highlight challenges for future research.

Cell function and viability are dependent upon efficient protein folding. Accordingly, cells contain an elaborate enzymatic machinery of so-called molecular chaperones that bind non-native polypeptides and promote their folding to the native state in an ATP-dependent manner[1,2]. However, many cellular events such as genetic mutation, biosynthetic errors, or the absence of a necessary post-translational binding partner result in protein misfolding. Cellular stresses such as chemical or temperature perturbation can also unfold proteins (Fig. 1A). Growing evidence indicates that failure to eliminate misfolded proteins can lead to the formation of potentially toxic aggregates, inactivation of functional proteins, and ultimately cell death[3]. The number of disease states linked to aberrant protein conformations underscores the importance of effective quality control for cell survival[3].

In eukaryotic cells, the ubiquitin–proteasome system (UPS) is the main pathway for eliminating misfolded proteins[4,5]. As a result, blocking its function pharmacologically or genetically inhibits the clearance of misfolded proteins and eventually leads to the formation of intracellular aggregates. Proteins are earmarked for UPS-mediated degradation by the covalent attachment of a polyubiquitin chain(s), which is recognized by the 26S proteasome[4,5]. With few exceptions, only substrates targeted to the proteasome by polyubiquitination are able to gain access to its proteolytic core. Ubiquitination is a multi-step process involving an E1 ubiquitin-activating enzyme, E2 ubiquitin-conjugating enzymes, and E3 ubiquitin ligases, which select the substrate and facilitate ubiquitination. Polyubiquitination of some proteins also requires so-called E4 enzymes that cooperate with E3 ligases to extend the polyubiquitin chain[6]. Whereas it has long been known that the yeast E2s Ubc4/5 and Ubc6/7 are involved in the ubiquitination of misfolded proteins[7], specific E3s for these proteins have been more elusive. However, a number of candidate E3 ligases for quality control of cytosolic proteins have been described in recent years.

Amie J. McClellan, Stephen Tam, Daniel Kaganovich and Judith Frydman are in the Department of Biological Sciences and BioX Program, E200 Clark Center, Stanford University, Stanford, CA 94305, USA.
e-mail: jfrydman@stanford.edu

Aberrant protein conformation as the molecular basis of disease

Protein misfolding leading to either loss of function of the affected protein or gain of function due to toxicity of the misfolded species has been linked to human disease. For instance, loss-of-function mutations that impair the correct folding of the tumour suppressors p53 and von Hippel–Lindau (VHL) lead to their enhanced degradation and concomitant tumour development[8]. Similarly, mutations that affect the folding of the cystic fibrosis transmembrane conductance regulator (CFTR) affect the transport of mature CFTR to the plasma membrane, thus resulting in cystic fibrosis[9].

Diseases in which non-native polypeptides gain a toxic function also result from misfolding[3,10]. These diseases are characterized by the accumulation of intracellular aggregates or inclusion bodies, often consisting of insoluble heat-stable β-sheet amyloid deposits. Aggregation-based diseases disproportionately affect post-mitotic cells such as neurons, presumably because they cannot dilute the toxic species during cell division, and underlie various neurodegenerative disorders, including Alzheimer's disease, Parkinson's disease and amyotrophic lateral sclerosis (ALS)[10]. A number of amyloid diseases are caused by the expansion of a polyglutamine (polyQ) tract, usually beyond a critical threshold of approximately 40 repeats. These include Huntington's disease, spinocerebellar ataxia and spinal bulbar muscular atrophy (SBMA)[10]. Despite a lack of amino-acid sequence similarity, these aberrant proteins appear to adopt a common toxic conformation that affects cell viability (Fig. 1A). Amyloidogenic oligomers of proteins that are associated with Alzheimer's, Parkinson's and polyQ diseases share a common structural signature that can be recognized by the same antibody[11]. Notably, whereas soluble early intermediates in the aggregation pathway seem to be toxic[12], the larger amyloid deposits themselves are not pathogenic[13]. Ultimately, aggregation-based diseases reflect a failure of the quality control system, either in surveillance or in elimination, and an imbalance between protein synthesis, folding and degradation.

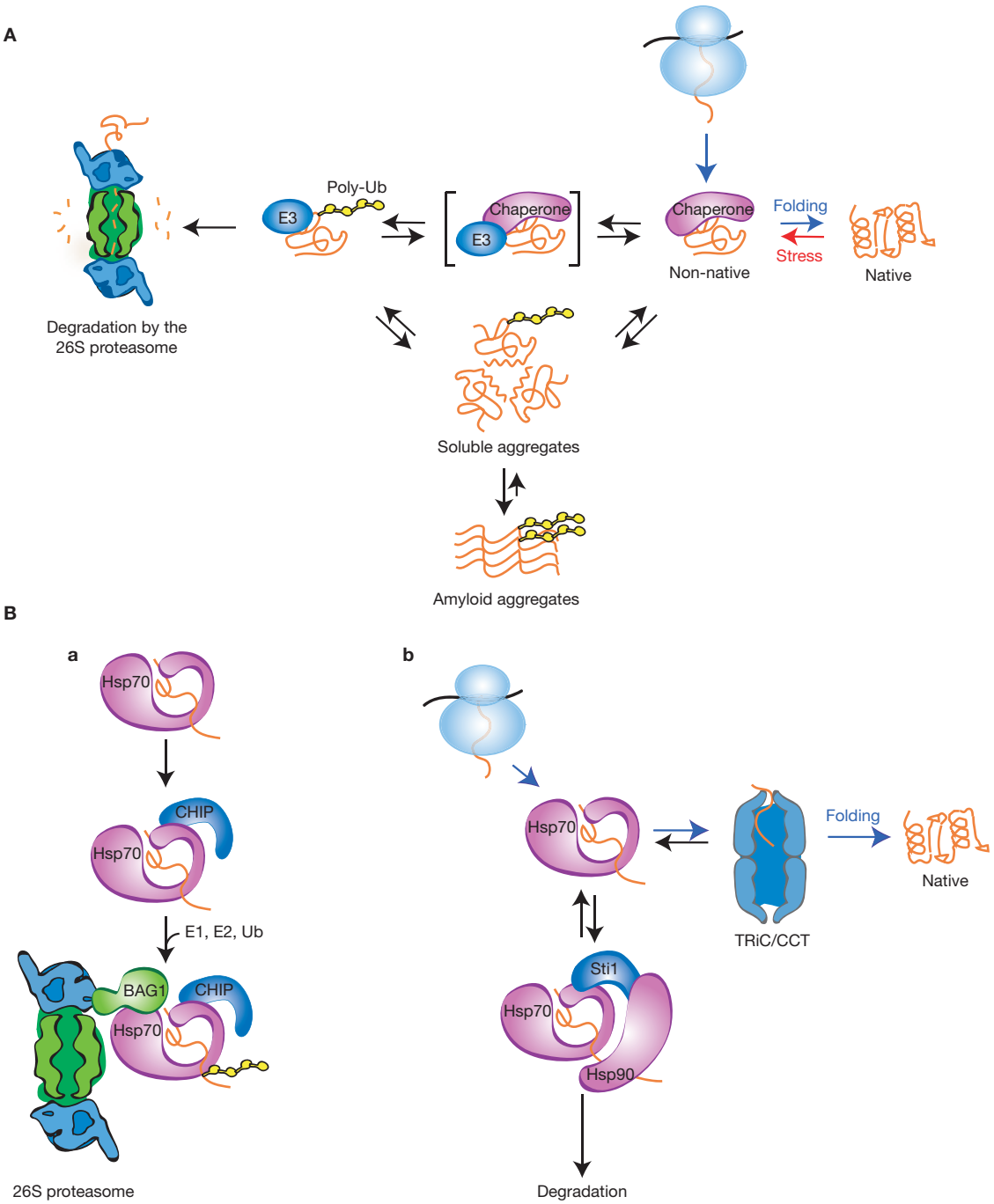

Figure 1 Role of molecular chaperones in the balance of folding, degradation and aggregation. (**A**) Molecular chaperones facilitate folding of newly synthesized polypeptides to the native state (blue arrows). Chaperones also bind to non-native intermediates that are generated when native proteins are denatured, for example by stress (red arrow). Cellular surveillance results in either refolding (blue 'folding' arrow) or elimination by the ubiquitin–proteasome system (arrows pointing to the left, which ultimately end in 'degradation'). Degradation generally requires tagging by ubiquitination via a quality control E3 ligase. In some cases, ubiquitination of the chaperone-bound substrate is directed by specific interaction of certain chaperones, for example Hsp70 or Hsp90, with E3s containing chaperone-interaction domains, such as CHIP (complex in brackets; see also part **B**). Under some circumstances, such as stress or aging, quality control efforts may fail, either before or after adding a polyubiquitin chain. In these cases, misfolded proteins may form small soluble aggregates, which, if unresolved by refolding or degradation, go on to form heat- and detergent-resistant amyloidogenic aggregates. (**B**) Examples of models for the transition from chaperone-mediated folding to chaperone-mediated degradation. (**a**) Action of CHIP and BAG1 on an Hsp70-bound polypeptide: CHIP recruitment to Hsp70 via its TPR domains promotes polyubiquitination of the bound polypeptide substrate. Hsp70 interaction with proteasome-associated BAG1 may promote substrate degradation. (**b**) A hierarchy of chaperone interactions determines the quality control of the VHL tumour suppressor. An initial association of newly translated VHL with TRiC would favour folding over degradation. However, failure to fold leads to Hsp90 and Sti1 binding and subsequent degradation.

Molecular chaperones: the first line of defense

Selective recognition of non-native proteins is the first step towards their elimination. Based on their ability to interact with non-native folding intermediates, molecular chaperones are prime candidates to aid in the triage of misfolded proteins. Once potentially damaging conformers have been identified, the cell can respond to their presence in three ways (Fig. 1A). First, cellular factors may attempt to rescue the misfolded conformations by refolding them to a functional native state. Second, the cell can sequester misfolded proteins in an attempt to prevent toxic interactions. Accordingly, chaperones alleviate the toxicity associated with aberrant protein conformations in neurodegenerative disease models[10]. For instance, overexpressing Hsp70 suppresses the toxicity associated with various proteins including amyloid-β (Aβ) and tau in Alzheimer's disease, α-synuclein in Parkinson's, superoxide dismutase (SOD1) in ALS, and polyQ-expanded proteins in Huntington's, SBMA and ataxias[10]. It seems that chaperones alter the conformation of these pathogenic proteins, because Hsp70, together with its cofactor Hsp40, induces a conformational rearrangement in mutant Huntingtin[14] and disfavours the accumulation of specific soluble polyQ fibril intermediates[15]. An intriguing trend emerging from these studies is that chaperone-mediated neuroprotection is not a consequence of reduced inclusion body formation. Instead, chaperones seem to alleviate toxicity by sequestering the soluble toxic oligomeric species or by modulating their conformation[10,15].

Finally, proteins that cannot be refolded must be eliminated by the UPS. A function for chaperones in targeting misfolded proteins for degradation has been established in various ways. Hsp70 is required for the *in vitro* degradation of some misfolded proteins[16], whereas *in vivo* experiments implicate the yeast Hsp40 Ydj1p[17]. Hsp70 and Hsp90 are required for degrading CFTR[18,19] and misfolded VHL variants[20]. In addition, overexpressing Hsp70 and Hsp40 increases the proteasome-mediated degradation of α-synuclein and polyQ-expanded proteins[10].

The precise role of chaperones in eliminating misfolded proteins is still unclear. In the simplest model, chaperones would be primarily dedicated to stabilizing and refolding non-native polypeptides. In this case, their role in quality control could be an extension of their primary role in folding; that is, to maintain the solubility of misfolded intermediates and facilitate sampling by the ubiquitination machinery. On the other hand, recent analysis of the quality control mechanisms of misfolded variants of the VHL tumour suppressor suggests that chaperones have an active role selecting proteins for degradation[20] (see below). The observation that some chaperones specifically interact with E3s (discussed below) raises the possibility that, at least in some cases, chaperones could recognize misfolded proteins and subsequently mediate their polyubiquitination by directly recruiting an E3 ligase (for example, the complex in brackets in Fig. 1A; see also Fig. 1B)[7,21,22]. In addition, a post-ubiquitination function for chaperones has been proposed[22]. For instance, the neuronal Hsp70 cofactor HSJ1 stimulates the ubiquitination of Hsp70-bound proteins via its ubiquitin-interaction motif (UIM) domains (Fig. 2) and their subsequent sorting to the proteasome[23]. Furthermore, Hsp90 associates with the proteasome in an ATP-dependent manner[21]. Thus, Hsp70- or Hsp90-bound substrates may be directed to proteasomes by virtue of direct or indirect chaperone–UPS interactions.

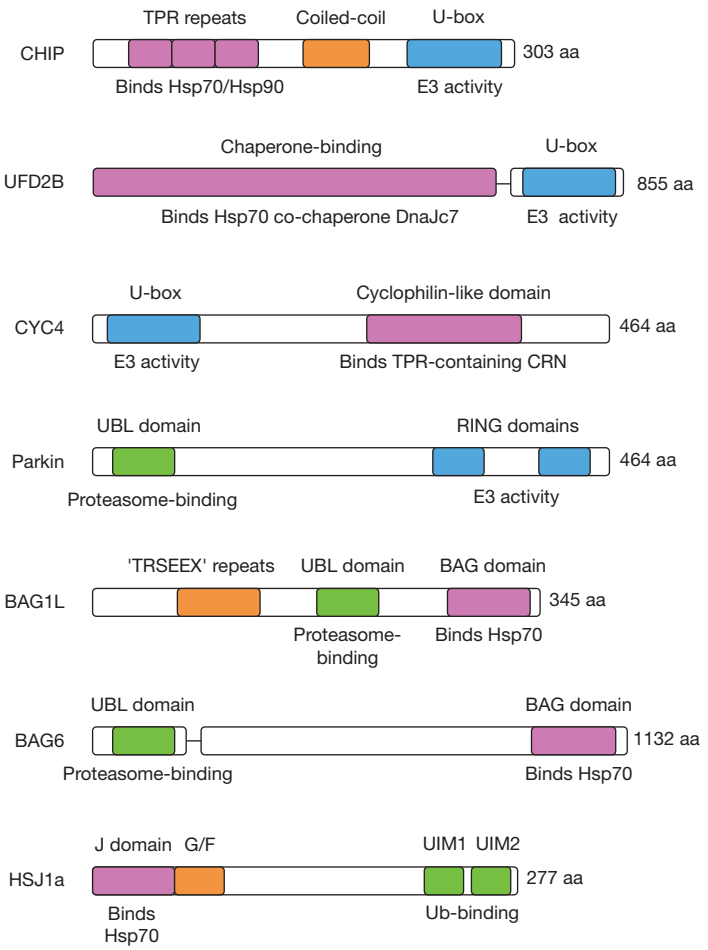

Figure 2 Domain organization of selected proteins linking chaperones and the UPS. E3 ubiquitin-ligase domains (blue; U-box and RING) and domains mediating interaction with the proteasome (green; UBL, ubiquitin-like; UIM, ubiquitin-interacting motif) or chaperones (pink), are highlighted in the selected proteins. UFD2B and CYC4 do not bind directly to Hsp70; UFD2B binds to an Hsp70 co-chaperone containing both a DnaJ domain and TPR repeats (DnaJc7), whereas CYC4 binds to the TPR-containing CRN (crooked neck). These TPR-containing proteins then mediate interaction with Hsp70. Defined domains with other functions are shown in orange. Proteins are not drawn to scale; the amino-acid length is indicated to the right of each protein schematic.

Modular proteins link chaperones to degradation

The identification of E3 ligases and chaperone cofactors that physically link chaperones to the UPS further supports the idea of direct communication between the folding and degradation machineries[7,22]. A trademark of these emerging families of modular proteins is the presence of a

combination of chaperone-interaction domains with domains that function in the UPS (Fig. 2).

Two proteins with these structural features, CHIP and BAG1, were initially identified in chaperone interaction screens and have since emerged as linkers between chaperones and the proteasome[24] (Fig. 1B and Fig. 2). CHIP contains three chaperone-interacting tetratricopeptide (TPR) domains at the amino terminus, which confer binding to Hsp70 and Hsp90, and a U-box with E3 ligase activity at the carboxyl terminus (Fig. 2). BAG1, a member of a larger family of proteins containing a BAG domain with Hsp70 nucleotide-exchange activity, also contains a ubiquitin-like (UBL) domain that binds the 26S proteasome[22]. Whereas CHIP has been established as an E3 capable of associating with chaperones and polyubiquitinating their bound substrates[7,22], BAG1 has been shown to link Hsp70 to the 26S proteasome, presumably to facilitate delivery of Hsp70-bound targets to the proteasome for degradation[22]. Because BAG1 and CHIP also regulate the ATPase activity of Hsp70, they may not only physically link chaperones and UPS enzymes but may also modulate the transition between the two pathways[22,24].

Quality control E3 ligases

Several groups have shown that CHIP overexpression in cultured cells promotes clearance of Hsp70/Hsp90 substrates such as CFTR, the glucocorticoid receptor, and the ErbB2 receptor[7,25]. Purified CHIP, along with recombinant Hsp40 and Hsp70 can ubiquitinate CFTR *in vitro*[26]; chaperone association is a prerequisite for CHIP-mediated CFTR ubiquitination because CFTR polyubiquitination by CHIP requires both its TPR and U-box domains[18]. A similar *in vitro* approach demonstrated that a CHIP–Hsp70 complex, together with E1, E2, ATP and ubiquitin, can ubiquitinate heat-denatured luciferase bound to either Hsp70/Hsp40 or Hsp90 (ref. 27). These experiments indicate that CHIP can ubiquitinate chaperone clients *in vitro* and, when overexpressed, can shift the quality control equilibrium from folding to ubiquitination and degradation[6]. In some cases, CHIP itself exhibits E4 activity such as in facilitating polyubiquitination of unfolded Pael-R receptor in collaboration with the RING-finger E3 ligase Parkin. CHIP can also associate with aggregation-prone proteins, including tau and polyQ-expanded proteins[22,28], and in some cases facilitates their ubiquitination and clearance from the cell. Thus, CHIP may alleviate the toxicity of aggregation-prone proteins in disease states. Whereas the cumulative data indicate that CHIP is a strong candidate for a quality control ligase, the physiologically relevant substrates of this pathway remain to be found[25].

Two additional E3 ligases, Parkin and Dorfin, have also been implicated in the clearance of disease-related misfolded proteins. The role of these E3s in quality control and their connection to the chaperone machinery is less well understood than for CHIP[29]. Parkin inactivation is a major cause of early onset Parkinson's disease[29]. Parkin contains both a RING-finger domain with E3 ligase activity and a UBL domain[29], and can promote the ubiquitination of three Parkinson's disease-associated proteins, α-synuclein, Pael-R and synphilin-1, both *in vitro* and *in vivo*[30]. However, it is unclear whether defective ubiquitination of these proteins underlies Parkinson's disease. Interestingly, the intracellular aggregates, called Lewy bodies, commonly associated with Parkinson's disease are absent in the Parkin-deficient form of the disease, indicating that their formation requires Parkin function[31].

Parkin can bind to and cooperate with CHIP and Hsp70, because CHIP overexpression enhances Parkin's ubiquitin ligase activity towards the Parkinson's disease-associated receptor Pael-R[32]. As with CHIP, Parkin also binds to polyQ-expanded Huntingtin *in vitro* and localizes to Huntingtin inclusions in the brains of humans with Huntington's disease[33]. Furthermore, overexpressing Parkin in cultured cells improves clearance of polyQ-expanded proteins and increases the survival rate of these cells[33]. Conversely, BAG5, an inhibitor of both Parkin and Hsp70, accelerates neuronal degeneration in rat brains[34]. Parkin may also shuttle certain aggregation-prone substrates to the proteasome, because it interacts with the 26S proteasome, presumably via its UBL domain[33]. Thus, similar to CHIP, Parkin may link Hsp70-bound substrates and the proteasome while also acting as an E3. However, many questions remain about the functions of CHIP and Parkin in quality control. For instance, although CHIP and Parkin are able to ubiquitinate misfolded or aggregating substrates *in vitro* and when overexpressed *in vivo*, neither has been shown to distinguish between the wild type and folding-defective mutants of its substrates, as would be expected for quality control components. Moreover, although CHIP has been suggested to act in heat-shock response[35], neither CHIP nor Parkin are stress inducible, unlike other quality control components including ubiquitin, the E2s Ubc4 and Ubc5, many chaperones, and proteasome subunits[36]. Because stress produces a massive accumulation of misfolded proteins that need to be cleared, it is likely that there are additional E3s that function in quality control. Indeed, deletion or knockdown of CHIP does not prevent the degradation of its known substrates *in vivo*[25], suggesting the existence of redundant pathways to ubiquitinate chaperone-bound proteins.

Dorfin, yet another mammalian E3 implicated in quality control, associates with and selectively ubiquitinates mutant but not wild-type SOD1 (ref. 37). Dorfin colocalizes with SOD1 inclusions in transgenic mice expressing an aggregation-prone SOD1 mutant[37] and with Lewy bodies in Parkinson's disease brains[38]. Dorfin overexpression increases the viability of cells that express aggregation-prone SOD1 (ref. 37); it also promotes ubiquitination of the Parkin substrate synphilin-1 in cultured cells[38]. Dorfin, like Parkin, contains two RING domains, but associates with its known substrates without an obvious link to Hsp70 or other chaperones.

While ongoing characterization of these E3s provides a glimpse into the mechanism of protein quality control and strengthens the idea that the chaperone machinery directly communicates with the UPS, more components of the quality control system remain to be described. For instance, CHIP, Parkin and Dorfin lack homologues in *Saccharomyces cerevisiae*, which nonetheless perform efficient quality control of misfolded proteins. Interestingly, a novel nuclear quality control E3, San1p, has recently been identified in yeast[39,40]. San1p selectively targets misfolded nuclear proteins for proteasome-mediated degradation but it does not recognize the native proteins[40]. How San1p recognizes and interacts with its misfolded substrates remains to be determined. It may bind non-native structures directly or through an intermediary recognition factor such as a chaperone, although San1p does not contain any canonical chaperone interaction domains. It will be interesting to see whether San1p homologues function in nuclear protein quality control in mammalian cells.

Making the triage decision: to fold, hold, or degrade

An important and poorly understood issue is how triage decisions maintain the balance between protecting non-native, newly

translated proteins until they reach their folded state, and preventing the accumulation of misfolded proteins that can lead to toxic aggregation-prone species. Are all folding intermediates, including those on a productive folding pathway, constantly sampled by the UPS or is there an ordered hierarchy of interactions that initially favours folding?

A potential model for the transition from chaperone-mediated folding to chaperone-mediated degradation comes from studies on CHIP[22] (Fig. 1B, a). Because CHIP can inhibit Hsp70 activity and also compete with Hsp70 co-chaperones that promote folding, such as the TPR-containing protein STI1/HOP, it is possible that unsuccessful folding attempts by Hsp70 lead to CHIP recruitment and the subsequent polyubiquitination of the offending polypeptide. CHIP and Hsp70 interaction with proteasome-associated BAG1, or its related homologue BAG6, may seal the fate of the misfolded protein by ensuring its proteasome-mediated degradation[22]. The question remains, however, as to what senses that folding has failed and that commitment to degradation is now required.

A recent study of the quality control of tumour-causing misfolded variants of VHL provides new insight into the logic of triage decisions[20] (Fig. 1B, b). The chaperone-mediated folding pathway of VHL is well characterized, allowing a comparison of the chaperone requirements for folding and degradation. Correct folding of VHL requires the cooperation of Hsp70 with the chaperonin TRiC/CCT[41,42]. Surprisingly, TRiC/CCT is dispensable for VHL degradation whereas Hsp90, which is not required for VHL folding, is specifically required for its degradation[20]. The identification of two distinct pathways of chaperone interactions for VHL, one leading to folding and the other to degradation, supports the idea that specific, rather than stochastic, chaperone pathways act in folding and degradation. In the case of VHL, co-translational coupling mechanisms may establish an initial association with TRiC, resulting in a chaperone hierarchy that favours folding over degradation. However, failure to fold may allow subsequent association with the Hsp90 complex, leading to degradation. Hsp70, uniquely required for both folding and degradation, may be key to the decision-making process. As discussed above for CHIP, the decision to fold or degrade might be regulated by cofactors that bind to and modulate Hsp70 function.

Perspectives and future questions

The cell must achieve and maintain a delicate equilibrium between protecting folding intermediates to ensure that they reach the native state and enforcing the rapid and efficient clearance of misfolded species. The late onset of most amyloid diseases in both humans and model organisms[43] suggests that throughout most of the life of the organism, misfolded and aggregation-prone species are kept in check by the quality control machinery. Disruption of this homeostatic balance by genetic mutation, stress, or the aging-induced decline in surveillance capacity by the folding or degradation machineries, has catastrophic consequences for cell viability. Paradoxically, once the aggregation process begins, it further disrupts the quality control machinery, presumably by overloading its capacity[10]. Although important advances in our understanding of this process have been made in recent years, many questions remain about the identity of quality control E3 ligases and the mechanisms that direct a polypeptide to either folding or degradation. The identification of chaperone-interacting E3/E4 ligase components[44], such as the CHORD domain-containing TPR repeat protein Sgt1 (ref. 45), or the UBL domain-containing UFD2 (ref. 44) (Fig. 2), suggests that cells contain several chaperone/E3 pathways that may ubiquitinate distinct classes of misfolded proteins. This might be expected given the critical importance of ensuring efficient quality control for cell viability. Future studies will reveal how quality control pathways recognize misfolded substrates and whether different structural classes of proteins require distinct cellular components for efficient recognition and elimination. The strong links between protein misfolding and disease call for a better understanding of the factors and mechanisms involved in protein quality control.

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