

From the cradle to the grave: molecular chaperones that may choose between folding and degradation

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Molecular chaperones are known to facilitate cellular protein folding. They bind non-native proteins and orchestrate the folding process in conjunction with regulatory cofactors that modulate the affinity of the chaperone for its substrate. However, not every attempt to fold a protein is successful and chaperones can direct misfolded proteins to the cellular degradation machinery for destruction. Protein quality control thus appears to involve close cooperation between molecular chaperones and energy-dependent proteases. Molecular mechanisms underlying this interplay have been largely enigmatic so far. Here we present a novel concept for the regulation of the eukaryotic Hsp70 and Hsp90 chaperone systems during protein folding and protein degradation.

Introduction

The function of a protein is determined by the three-dimensional structure of its amino acid chain. The native structure is attained following translation or translocation and is under constant threat of unfolding as a consequence of chemical equilibrium and cellular stress. Systems that actively maintain and control protein structure are thus a prerequisite for cell survival, and involve both molecular chaperones and energy-dependent proteases (Wickner *et al.*, 1999). Molecular chaperones bind non-native proteins, inhibit protein aggregation and promote folding to the native state (Hartl, 1996; Bukau and Horwich, 1998). Energy-dependent proteases, on the other hand, eliminate proteins that fail to attain their native conformation (Wickner *et al.*, 1999). Thus, chaperones and proteases appear to form a cellular surveillance system that monitors protein quality. Recent findings provide insight into molecular mechanisms that underlie the interplay of chaperones and proteases.

The Hsp70 and Hsp90 chaperones

Major chaperones in the mammalian cytosol and nucleus are the 70 and 90 kDa heat shock proteins (Hsp70 and Hsp90). Hsp70 participates in the folding of newly synthesized proteins, the protection of proteins during cellular stress and intracellular protein trafficking (Hartl, 1996; Frydman and Höhfeld, 1997; Bukau and Horwich, 1998). Hsp90 function appears to be more restricted but, again, a role in stress protection has been demonstrated (Buchner, 1999; Caplan, 1999). Both classes of chaperones seem to associate with non-native protein substrates through recognition of hydrophobic patches ultimately buried in the native structure. In addition, they mediate the conformational regulation of a wide range of client proteins involved in signal transduction, cell proliferation and apoptosis (Frydman and Höhfeld, 1997; Caplan, 1999; Pandey *et al.*, 2000; Beere and Green, 2001). Interaction with non-native protein substrates and client proteins is highly dynamic and coupled to cycles of ATP binding and ATP hydrolysis by the chaperones (Frydman and Höhfeld, 1997; Prodromou *et al.*, 1999; Young and Hartl, 2000). Upon release, further protein folding or biogenesis can occur. This may involve rebinding to the same or another chaperone, or transfer to other cellular machines. Hsp70 and Hsp90 have been shown to cooperate with the degradation machinery (Schneider *et al.*, 1996; Bercovich *et al.*, 1997; Dul *et al.*, 2001). Of particular interest is the pharmacological shifting of Hsp90 function from protein folding to protein degradation, induced by anti-tumor agents like geldanamycin (Schneider *et al.*, 1996; Whitesell and Cook, 1996). However, the underlying molecular mechanisms remain largely elusive.

It is now widely recognized that Hsp70 and Hsp90 do not act on their own, but co-operate with several ancillary proteins, so-called chaperone cofactors or co-chaperones (Frydman and

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Höhfeld, 1997; Buchner, 1999; Caplan, 1999). In principle, chaperone cofactors have two options for modulating chaperone function. They can either regulate the ATPase cycle of the chaperone to influence its affinity for protein substrates, or recruit the chaperones to specific proteins, protein complexes and subcellular compartments. Many cofactors combine both activities and, consequently, exhibit a modular structure comprising chaperone-binding/chaperone-regulating motifs plus other functional domains. A chaperone-binding motif found in several Hsp70 and Hsp90 cofactors is characterized by a tandem arrangement of three degenerate 34 amino acid repeats (tetra- or pentapeptide repeats, TPRs; Frydman and Höhfeld, 1997). The Hsp70/Hsp90-organizing protein Hop possesses multiple TPR domains, enabling it to simultaneously bind Hsp70 and Hsp90, and to promote chaperone cooperation during the regulation of signal transduction pathways (Frydman and Höhfeld, 1997; Buchner, 1999; Caplan, 1999). In each domain, three tandem TPRs align with an adjacent α -helix to form a groove that accommodates conserved peptide motifs present at the C-terminus of Hsp70 and Hsp90 (Scheufler *et al.*, 2000). Although the presence of TPRs is not restricted to chaperone cofactors, they appear to form a stably folded adaptor well suited to mediate chaperone/cofactor contacts, even under conditions of cellular stress.

CHIP links chaperones to the degradation machinery

CHIP was initially identified in a screen for human TPR-containing proteins (Ballinger *et al.*, 1999). At its N-terminus three tandem TPRs are located, which, together with an adjacent, highly charged α -helix, form a chaperone adaptor (Figure 1). CHIP utilizes this single adaptor to contact either Hsp70 or Hsp90 (Ballinger *et al.*, 1999; Connell *et al.*, 2001). Association with Hsp70 blocks the ATPase cycle of the chaperone and inhibits its ability to refold non-native proteins *in vitro* (Ballinger *et al.*, 1999). Similarly, binding of CHIP to Hsp90 prevents the cooperation of the chaperone with other cofactors required for productive chaperone function (Connell *et al.*, 2001). Apparently, CHIP abrogates the ability of both chaperones, Hsp70 and Hsp90, to assist cellular protein folding.

Interestingly, the C-terminus of CHIP displays structural similarities to components of the ubiquitin/proteasome system, a major protein degradation pathway in eukaryotic cells (Figure 1). Proteins destined for degradation are labelled with a multiubiquitin chain and then targeted to a large heterooligomeric protease, the 26S proteasome (Varshavsky, 1997; Baumeister *et al.*, 1998). Ubiquitylation is mediated by a complex cellular machinery comprising a ubiquitin activator (the E1 enzyme), a ubiquitin conjugating enzyme (E2), and a ubiquitin ligase (E3). E2 and E3 enzymes are recruited from large protein families and the broad repertoire of distinct E2/E3 pairs is likely to ensure specific recognition of diverse substrate proteins. Additional proteins, including the yeast ubiquitylation factor Ufd2, cooperate with the E2/E3 machinery (Koepl *et al.*, 1999). Interestingly, Ufd2 and CHIP both possess a U-box domain shown to participate in ubiquitin conjugation. The modular structure of CHIP may thus enable the cofactor to directly link molecular chaperones to the degradation machinery.

In support of this notion, CHIP was recently shown to promote the degradation of chaperone substrates by the ubiquitin/proteasome system (Connell *et al.*, 2001; Meacham *et al.*, 2001). Connell *et al.* (2001) investigated the effects of CHIP on the activation and degradation of the glucocorticoid hormone receptor (GR). Like several other steroid hormone receptors, GR undergoes transient interactions with the Hsp70 and Hsp90 chaperone systems during its activation (Frydman and Höhfeld, 1997). The high-affinity hormone binding state is finally reached in association with a Hsp90 heterocomplex that includes cofactors such as p23. *In vitro* experiments revealed that incorporation of CHIP into Hsp90 heterocomplexes leads to a dissociation of p23 and, consequently, Hsp90-associated GR no longer reaches its steroid-binding conformation (Connell *et al.*, 2001). In addition, remodelling of the Hsp90 chaperone complex by CHIP is accompanied by induced ubiquitylation of the hormone receptor. Apparently, the cofactor not only blocks GR activation but also diverts the receptor to the degradation machinery; increasing the cellular level of CHIP is indeed sufficient to induce degradation of GR by the proteasome.

Similar findings were obtained for the cystic fibrosis transmembrane conductance regulator (CFTR), a plasma membrane chloride-ion channel. Folding and maturation of CFTR is rather inefficient, and most nascent CFTR is retained in the endoplasmic reticulum (ER) and degraded by the ubiquitin/proteasome system (Kopito, 1999). Hsp70 and Hsp90 were shown to interact with cytoplasmic domains of CFTR and to facilitate the folding process (Loo *et al.*, 1998; Meacham *et al.*, 1999). Consequently, CFTR maturation is sensitive to expression of the CHIP cofactor. Elevation of CHIP induced the ubiquitylation and degradation of immature, ER-localized forms of CFTR (Meacham *et al.*, 2001). CHIP apparently targets diverse chaperone substrates to the ubiquitin/proteasome system and in this way modulates the balance of protein folding and protein degradation during protein quality control.

CHIP is a chaperone-associated ubiquitin ligase

It was conceivable that CHIP-induced degradation was simply a consequence of the cofactor's ability to inhibit chaperone function. However, structure/function analysis of CHIP suggests an active role in the ubiquitylation of chaperone substrates. A deletion fragment that lacks the C-terminal U-box retains the ability to contact Hsp70 and to inhibit the ATPase cycle of the chaperone (Ballinger *et al.*, 1999; Meacham *et al.*, 2001). Moreover, it is sufficient to induce remodelling of Hsp90 heterocomplexes (Connell *et al.*, 2001). Nevertheless, the fragment does not induce the ubiquitylation and degradation of GR and CFTR in transient transfection assays (Connell *et al.*, 2001; Meacham *et al.*, 2001). In fact, it acts as a dominant-negative factor that blocks ubiquitylation and degradation. These findings point to a critical role for the U-box in CHIP-induced targeting of chaperone substrates for proteasomal degradation.

Another U-box-containing protein is Ufd2, which cooperates with certain E2 and E3 enzymes to promote the formation of multiubiquitin chains, and was therefore termed E4 (Koepl *et al.*, 1999). The enzymatic function of Ufd2 remains enigmatic. However, the U-box was shown to bind to ubiquitin moieties of the modified substrate protein. This may stabilize the association

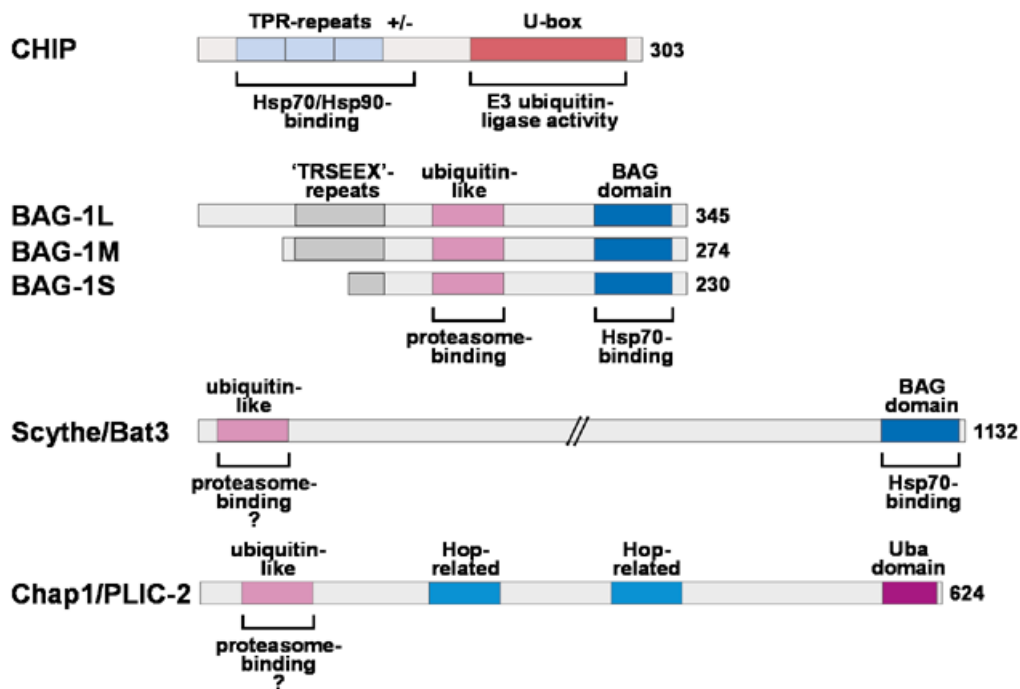


Fig. 1. Cofactors that appear to link molecular chaperones to the ubiquitin/proteasome system. CHIP possesses an N-terminal chaperone binding motif formed by three TPRs and an adjacent highly charged region. A U-box required for ubiquitin ligase activity is present at the C-terminus. The BAG-1 isoforms share a ubiquitin-like domain involved in proteasome binding and a BAG domain that mediates interaction with Hsp70. Like the BAG-1 proteins, Scythe/Bat3 possesses a ubiquitin-like domain that may be used for proteasome association and a BAG domain used for binding and regulation of Hsp70. Chap1/PLIC-2 combines a ubiquitin-like domain and a Uba domain, the latter of which is found in several proteins involved in ubiquitin conjugation. In addition, regions structurally related to the chaperone cofactor Hop are present in Chap1/PLIC-2.

of the substrate with the conjugation machinery and, as a consequence, may increase the efficiency of the conjugation reaction. The presence of the U-box within CHIP suggested an E4-like activity, but it was also conceivable that CHIP fulfils an E3-like function, as the U-box is structurally related to RING-finger domains found in several E3 ubiquitin ligases (Aravind and Koonin, 2000). In the E3s, the RING-finger mediates the binding and activation of E2 enzymes (Jackson *et al.*, 2000). In fact, CHIP utilizes its U-box for cooperation with E2 enzymes of the Ubc4/5 family, and thus displays E3 ubiquitin ligase activity (Demand *et al.*, 2001; Hatakeyama *et al.*, 2001; Jiang *et al.*, 2001). E3 and E4 enzymes appear to promote ubiquitylation in a similar fashion, by facilitating the interaction between the conjugation machinery and the substrate protein.

CHIP is the first ubiquitin ligase known to directly associate with molecular chaperones. Indeed, the chaperone itself can be seen as an integral part of the ubiquitin ligase complex (Figure 2). Through its ability to recognize non-native proteins and specific client proteins, the chaperone apparently selects substrates for CHIP-mediated ubiquitylation. The chaperone-CHIP complex thus resembles other multisubunit ubiquitin ligases, such as the Skp1-cullin-F-box protein (SCF) complex. In SCF, an E3 teams up with diverse F-box proteins that act as adaptors to specifically recognize a broad range of substrate proteins (Jackson *et al.*, 2000). Similarly, CHIP association with Hsp70 and Hsp90 appears to promote the ubiquitylation of diverse chaperone substrates.

Chaperone machines: folding versus degradation

Previously, kinetic partitioning of non-native proteins between molecular chaperones and proteases has been proposed to underlie protein quality control (Wickner *et al.*, 1999). This model infers a competition between chaperones and components of the degradation machinery. The characterization of the CHIP cofactor now points to a different concept. Through association with CHIP, the molecular chaperones Hsp70 and Hsp90 are directly turned into protein degradation factors (Figure 2). This suggests an elegant solution to the problem of how the degradation machinery recognizes aberrant proteins: the factors that bind non-native proteins in the course of protein folding also appear to be employed to select substrates for protein degradation. According to this model, a balance of protein folding and protein degradation would be achieved through regulation of chaperone function. As CHIP occupies commonly used cofactor binding sites on Hsp70 and Hsp90, competition with other chaperone cofactors may provide the means to modulate quality control decisions. The cofactor Hop most likely fulfils a role as a CHIP antagonist. Like CHIP, Hop associates with the C-terminus of Hsp70 via a TPR chaperone adaptor. Similarly, the two cofactors appear to compete for a common TPR acceptor site on Hsp90 (Demand *et al.*, 1998; Ballinger *et al.*, 1999; Connell *et al.*, 2001). In contrast to CHIP, Hop has been shown to assist chaperone-mediated protein folding (Buchner *et al.*, 1999; Caplan *et al.*, 1999). Competing cofactors may thus determine

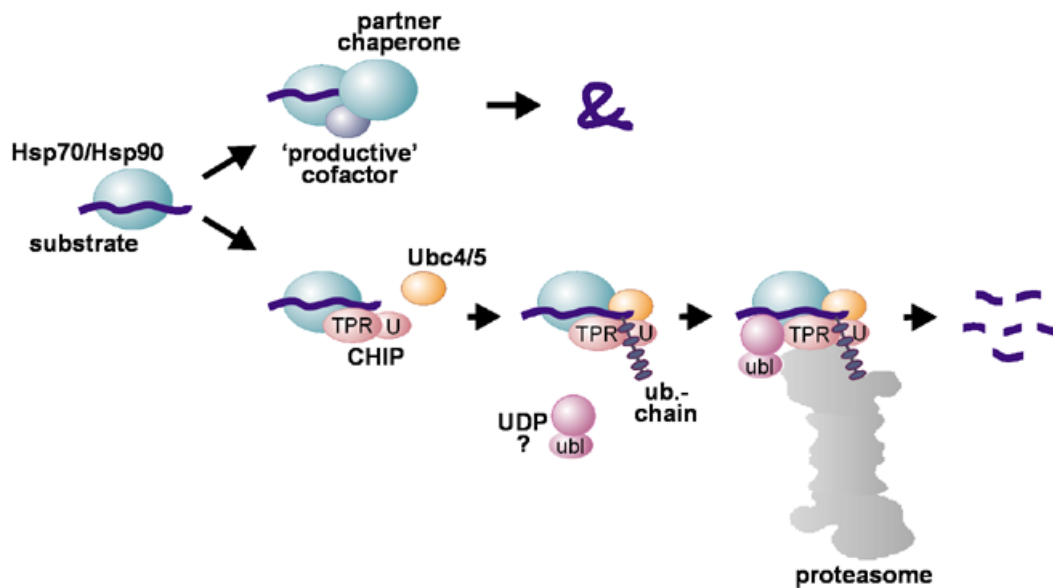


Fig. 2. Hsp70 and Hsp90 in protein folding and degradation. An initial decision to fold or degrade a Hsp70- or Hsp90-associated substrate protein may be reached through competition between a 'productive' cofactor such as Hop, and the ubiquitin ligase CHIP. During the folding process, Hsp70 and Hsp90 may co-operate with other chaperone proteins (termed partner chaperones). On the degradation pathway, CHIP associates with Hsp70 or Hsp90 via its TPR chaperone adaptor (TPR), and at the same time recruits E2 ubiquitin conjugating enzymes of the Ubc4/5 family to the chaperone complex. This may involve binding of the E2 to the U-box of the cofactor (U). In conjunction with E2, CHIP mediates ubiquitin attachment to the chaperone substrate and induces its targeting to the proteasome for degradation. The targeting process may be facilitated by a ubiquitin domain protein (UDP), such as BAG-1, which binds to Hsp70 and utilizes its ubiquitin-like domain (ubl) for proteasomal association.

whether a given chaperone machine is involved in protein folding or protein degradation (Figures 2 and 3).

The Hsp70 cofactor BAG-1 has also been proposed to act as a link between molecular chaperones and the ubiquitin/proteasome system (Lüders *et al.*, 2000). BAG-1 exists in multiple isoforms in mammalian cells. These share a C-terminal BAG domain required for Hsp70 interaction, and an N-terminal ubiquitin-like domain (Figure 1). BAG-1 thus belongs to a family of ubiquitin domain proteins (UDPs) (Jentsch and Pyrowolakis, 2000). The chaperone cofactor utilizes its ubiquitin domain at least in part for binding to the proteasome (Lüders *et al.*, 2000). In this way, BAG-1 promotes an association between Hsp70 and the proteolytic complex. As BAG-1 also acts as a nucleotide exchange factor that induces the release of substrate from Hsp70, a role in assisting substrate transfer to the proteasome is conceivable (Höhfeld and Jentsch, 1997; Lüders *et al.*, 1998; Sondermann *et al.*, 2001). However, experimental evidence in support of this notion is still missing. In fact, overexpression of BAG-1 was shown to promote the activation of the raf-1 protein kinase and to stimulate the anti-apoptotic function of Bcl-2 (Takayama *et al.*, 1995; Song *et al.*, 2001). These observations are difficult to reconcile with an exclusive role of BAG-1 in chaperone-proteasome coupling. Only a subpopulation of cellular BAG-1 may thus fulfil such a coupling function, or coupling may affect only a subset of BAG-1 and Hsp70 substrate proteins. Alternatively, BAG-1 may closely cooperate with other, possibly rate-limiting cofactors during the sorting of chaperone substrates to the proteasome. As a consequence, elevation of BAG-1 levels alone may be insufficient to promote substrate degradation.

A likely candidate for a rate-limiting cofactor is CHIP. In fact, the ubiquitin ligase was recently shown to team up with the BAG-1 ubiquitin domain protein to regulate proteasomal sorting of chaperone substrates (Demand *et al.*, 2001; Figure 2). This apparently involves the formation of a chaperone complex that comprises both cofactors (Demand *et al.*, 2001; Figure 3). Notably, BAG-1 binds to the amino-terminal ATPase domain of Hsp70, whereas CHIP occupies the carboxyl terminus of the chaperone (Ballinger *et al.*, 1999; Sondermann *et al.*, 2001). Intriguingly, BAG-1 competes with the folding-stimulating cofactor Hip in binding to the ATPase domain of Hsp70 (Höhfeld and Jentsch, 1997). This further points to a role of cooperating and competing cofactors in determining the function of molecular chaperones in protein folding and protein degradation (Figure 3).

BAG-1 was previously shown to associate with another RING-finger type ubiquitin ligase, the Siah protein (Matsuzawa *et al.*, 1998). The functional consequences of this association remain unclear. However, it could provide a means for Siah to gain access to chaperone substrates and to promote their ubiquitylation and degradation. BAG-1/Siah cooperation would provide an additional pathway for protein sorting between Hsp70 and the proteasome. The existence of additional pathways is also implied by the fact that BAG-1 is not the only ubiquitin domain protein that cooperates with molecular chaperones (Figure 1). The human UDPs Chap1 and Chap2 were recently identified in a screen for proteins which, like BAG-1, bind to the ATPase domain of a Hsp70 family member (Kaye *et al.*, 2000). Chap1 (also termed PLIC-2) and its relative PLIC-1 represent human homologues of the yeast Dsk2 protein which is required for

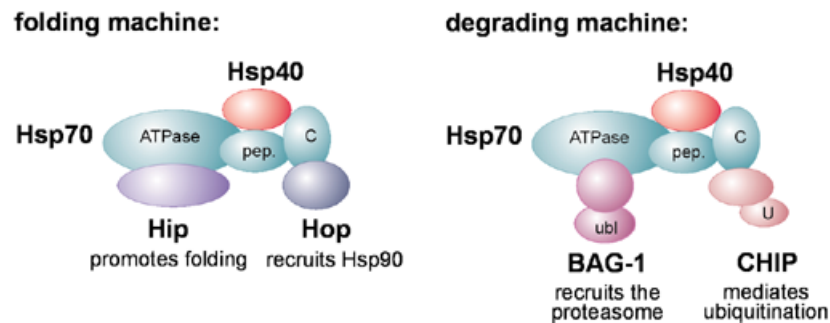


Fig. 3. Model for Hsp70 chaperone machines. Binding of distinct chaperone cofactors to the N-terminal ATPase domain of Hsp70 (ATPase) and to its C-terminus (C) may give rise to chaperone machines involved in protein folding and protein degradation, respectively. The cofactors Hip and BAG-1 compete for binding to the ATPase domain, while Hop and CHIP associate with the C-terminus in a competitive manner. During folding and degradation, Hsp70 appears to co-operate with cofactors of the Hsp40 protein family. pep., peptide binding domain of Hsp70; ubl, ubiquitin-like domain of BAG-1; U, U-box of CHIP.

cell-cycle regulation. Both PLIC proteins were shown to associate with the proteasome and with ubiquitin ligases, and may thus coordinate the activities of the ubiquitin conjugation machinery and the proteolytic complex (Kleijnen *et al.*, 2000). The observed association with molecular chaperones suggests that this coordinating function may involve chaperone regulation. Affected chaperone substrates, however, remain to be identified. Similarly, a role for Chap2 in the degradation of chaperone substrates awaits experimental confirmation. Chap2 is also known as Bat3 and human Scythe. Like BAG-1, Scythe combines an N-terminal ubiquitin domain with a BAG domain that is used for binding to Hsp70 and for inhibition of Hsp70 chaperone function (Thress *et al.*, 2001; Figure 1). Thus, Chap2/Scythe most likely acts as an additional coupling factor between the molecular chaperone and the proteasome.

Perspectives

Interactions of Hsp70 and Hsp90 with a variety of cofactors enable these chaperones to accompany substrate proteins from the cradle to the grave. The emergence and characterization of multiple cofactor families now paves the way to elucidate the roles of chaperones in protein folding and degradation. The modulation of quality control decisions during signal transduction and apoptosis becomes feasible. Nevertheless, our knowledge of cellular targets that are affected by diverse chaperone cofactors is still limited, and further work is required to understand the mechanisms underlying substrate selection. In any case, molecular chaperones and their ancillary cofactors have taken centre stage in protein quality control.

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