Review

Cooperation of molecular chaperones with the ubiquitin/proteasome system

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Abstract

Molecular chaperones and energy-dependent proteases have long been viewed as opposing forces that control protein biogenesis. Molecular chaperones are specialized in protein folding, whereas energy-dependent proteases such as the proteasome mediate efficient protein degradation. Recent data, however, suggest that molecular chaperones directly cooperate with the ubiquitin/proteasome system during protein quality control in eukaryotic cells. Modulating the intracellular balance of protein folding and protein degradation may open new strategies for the treatment of human diseases that involve chaperone pathways such as cancer and diverse amyloid diseases.

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1. Introduction

Protein folding is a delicate process. The linear amino acid chain must be converted to a unique three-dimensional structure that defines the biologically active state of the protein. Out of a broad repertoire of sterically available conformations, one specific structure must be selected. During the folding process the non-native polypeptide and folding intermediates often expose hydrophobic surfaces that are buried in the native conformation. This exposure can cause inappropriate intra- and intermolecular interactions and can lead to the adoption of alternative conformations with an increased tendency to aggregate (Fig. 1) [1]. Protein misfolding can be stimulated by amino acid misincorporation, as a consequence of genetic mutation or errors during transcription and translation, and by thermal, osmotic or oxidative stresses. In fact, a recent study suggests that up to 30% of all newly synthesized polypeptides never reach their native state [2]. The failure of polypeptides to adopt their correct structure poses a major threat to cell function and viability. This is best illustrated by human diseases that are pathologically defined by the abnormal deposition of misfolded polypeptides. Among these diseases are severe neurodegenerative diseases, such as prion diseases, Alzheimer’s and Huntington’s disease, in which misfolded polypeptides aggregate in fibrillar structures, known as amyloids [3]. Although it is still unclear whether the presence of protein aggregates in diseased neurons is a cause or consequence of the underlying cellular pathology, there is now ample evidence that the inherent tendency of a misfolded polypeptide to aggregate with itself or other proteins can severely affect cellular function and can cause cytotoxicity (reviewed in Refs. [3,4]). It is therefore not surprising that molecular mechanisms have evolved to prevent the accumulation of misfolded polypeptides and thus aggregate formation. Two different preventive strategies appear to be realized. Non-native proteins can be recognized by molecular chaperones that promote folding to the native state whenever possible or may be removed by proteolytic machineries such as the ubiquitin/proteasome system. In this review, we will discuss the role of molecular chaperones and the ubiquitin/proteasome system in protein quality control in the eukaryotic cytosol. We will focus in particular on the cytosolic Hsp70
chaperone machinery and its recently revealed cooperation with the ubiquitin/proteasome system.

2. Role of the ubiquitin/proteasome system in the removal of misfolded proteins

The ubiquitin/proteasome system has been known for a long time to be involved in the degradation of misfolded proteins. In the early 1980s it was shown that abnormal proteins, induced through feeding of amino acid analogs, are rapidly turned over in mammalian cells in a process involving ubiquitin conjugation [5–7]. When inhibitors of the proteasome became available about 10 years later a central role of the proteasome in this degradation process was established [8]. At the same time, genetic analysis of the yeast *Saccharomyces cerevisiae* provided additional evidence for a function of the proteasome in protein quality control. Mutations in genes encoding subunits of the yeast proteasome resulted in a hypersensitivity to amino acid analogs, and ubiquitinated proteins accumulated in the corresponding mutant strains under stress conditions [9]. Since then, numerous studies demonstrated that impairment of the proteasome leads to an accumulation of abnormal proteins and increases protein aggregation. A prominent example involves the cystic fibrosis transmembrane conductance regulator (CFTR), a chloride channel localized at the apical surface of polarized epithelial cells [10]. The folded state of CFTR is controlled during trafficking from the endoplasmic reticulum (ER) to the Golgi complex [11]. The protein is therefore affected by ER-associated quality control mechanisms (see review by Wolf and Sommer in this issue). Still, we would like to discuss the biogenesis of CFTR also in the context of cytosolic quality control because the polytopic CFTR protein exposes large domains into the cytosol and the misfolding of these domains is monitored by components of cytosolic control systems. This may eventually result in extraction from the ER membrane and degradation of CFTR in the cytosol [10,12–14]. Mutations in the CFTR gene cause the recessively inherited fatal disease cystic fibrosis. The majority of Caucasian cystic fibrosis patients have at least one copy of the ΔF508 mutation. The encoded temperature-sensitive molecule is unable to fold correctly under physiological conditions. However, also wild-type CFTR folds very inefficiently. As a consequence, all immature ΔF508 molecules and about 60–80% of wild-type CFTR are rapidly degraded (reviewed in Ref. [10]). Several lines of evidence suggest that misfolded CFTR molecules are substrates of the ubiquitin/proteasome system. For example, proteasome inhibition results in the accumulation of polyubiquitinated forms of CFTR and a dominant negative form of ubiquitin impairs degradation of CFTR [15,16]. The ΔF508 mutation maps to one of two cytosolically exposed nucleotide binding domains of CFTR (NBD1). The mutation seems to stabilize an intermediate state during the folding of NBD1 that is prone to self-aggregation [17]. This misfolded state is apparently recognized by cytosolic control systems resulting in membrane extraction of CFTR and sorting to the proteasome for degradation.

CFTR biogenesis illustrates how the activity of the ubiquitin/proteasome system and the formation of protein aggregates correlate. If CFTR cannot be efficiently degraded, because of inhibition of the proteasome, immature and ubiquitinated forms of the protein accumulate in a distinct pericentriolar structure, termed the aggresome [18]. Formation of the aggresome is an active process and requires dynein-dependent transport of aggregated molecules along microtubules. This finding challenges the widespread assumption that protein aggregation is simply...
driven by passive self-assembly of misfolded proteins. At least in some cases, active transport and sequestration take place. Interestingly, aggresome formation is not only caused by inhibition of the proteasome but also occurs upon massive overexpression of CFTR [18]. Aggresome formation may therefore be seen as a cellular response when the capacity of the proteasome is exceeded by the production of aggregation-prone misfolded proteins.

The formation of intracellular protein inclusions is also a hallmark of polyglutamine repeat diseases such as Huntington’s disease, Kennedy’s disease and different forms of spinocerebellar ataxia (SCA) [19]. These diseases are all characterized by a progressive degeneration of the nervous system that is typically fatal. The responsible genes encode proteins that are functionally unrelated. However, in all cases a genetic alteration was found, which results in the expansion of CAG trinucleotide repeats in the disease genes. As a consequence, the encoded proteins possess an extended polyglutamine tract. Polyglutamine (polyQ) expansion confers dominant toxicity on the respective proteins, leading to progressive neuronal dysfunction and eventual neuronal loss. Exactly how polyQ expansion causes neuronal dysfunction is still obscure. However, extended polyQ tracts tend to form self-associating β-sheet structures that oligomerize into amyloid protein inclusions (reviewed in Refs. [4,20,21]). The cytotoxicity of polyQ tracts appears to depend on this oligomerization process, as its inhibition preserves normal cellular function [22]. Interestingly, certain transcription factors that possess polyQ segments of a nonpathological range, such as TATA-binding protein and CREB-binding protein (CBP), have been detected in protein inclusions of diseased neurons [23–25]. It was therefore proposed that sequestration of essential proteins in amyloid aggregates during polyQ oligomerization may cause neuronal toxicity. This sequestration may also include essential components of the ubiquitin/proteasome system. PolyQ inclusions stain positively for ubiquitin and proteasomal components of the ubiquitin/proteasome system. PolyQ pathogenesis has been suggested [26–28]. In fact, several reports show that formation of polyQ inclusions is enhanced upon proteasome inhibition in transfected cells [28–30]. However, in vitro a polyQ-expanded ataxin-1 protein was found to be more resistant to proteasome-mediated degradation than nonpathological forms of the protein [29]. Sequestration of components of the ubiquitin/proteasome system in polyQ inclusions may thus reflect rather futile attempts of the proteolytic machinery to degrade the disease protein. In any case, the resulting sequestration may be detrimental to the cell. An elegant study by Bence et al. [31] recently provided experimental evidence for this notion by showing that polyQ-dependent protein aggregation impairs the activity of the ubiquitin/proteasome system. In this study, the green fluorescent protein was fused to a degradation signal that induces ubiquitination and sorting to the proteasome (GFPu). The fusion protein made it possible to monitor the activity of the ubiquitin/proteasome system by fluorescence microscopy. Remarkably, GFPu was strongly stabilized in cells containing polyQ inclusions induced by co-expression of a pathological fragment of the huntingtin protein, the causative agent of Huntington’s disease. Similar findings were obtained upon co-expression of mutant ΔF508 CFTR [31]. The data provide strong evidence that protein aggregation impairs the activity of the ubiquitin/proteasome system. This finding may help to explain the progressive loss of cellular function observed in many misfolding diseases. Any impairment of the ubiquitin/proteasome system would cause the accumulation of misfolded proteins, which in turn would further impair the ubiquitin/proteasome system, resulting in a vicious circle.

An involvement of the ubiquitin/proteasome system in protein quality control is not restricted to a few disease-causing proteins. Using proteasome inhibitors, it was recently estimated that up to 30% of all newly synthesized proteins are rapidly degraded by the ubiquitin/proteasome system [2]. It was proposed that this astonishingly high proportion represents defective polypeptides, so-called DRiPs (defective ribosomal products), which are unable to attain their native conformation owing to genetic mutation or errors during transcription and translation. Recognition of DRiPs by the ubiquitin/proteasome system may already occur before translation is completed as the proteolytic system is able to degrade proteins cotranslationally [32]. Even under normal growth conditions, cells must apparently cope with a continuous flux of misfolded proteins. Their rapid and efficient degradation by the ubiquitin/proteasome system seems to ensure cellular survival.

How are misfolded proteins recognized by the ubiquitin/proteasome system? A recent report suggests that at least some aggregation-prone proteins are degraded by the proteasome in a manner that does not depend on ubiquitin conjugation [33]. Nonetheless, sorting to the proteasome usually involves attachment of a polyubiquitin chain to the protein substrate and also most misfolded proteins may follow such a sorting pathway. Ubiquitin conjugation is mediated by the concerted action of an E2 ubiquitin-conjugating enzyme and an E3 ubiquitin ligase. E2 and E3 enzymes are recruited from large protein families and team up to form functionally distinct E2/E3 pairs, which specifically recognize diverse substrate proteins [34]. Consequently, E2 and E3 enzymes should exist that are specialized in the recognition of misfolded proteins. These enzymes should be able to distinguish between non-native conformations and the native state of a substrate protein. This will most likely involve a recognition of hydrophobic regions that are exposed in non-native conformations while buried in the native structure. Studies in the yeast *S. cerevisiae* provided evidence for a central role of E2 ubiquitin-conjugating enzymes of the Ubc4/5 family in the removal of abnormal proteins [35]. Expression of their corresponding genes is induced under thermal stress, when protein unfolding and aggregation occurs. In addition, loss
of Ubc4/5 activity impairs cell growth, leads to inviability at elevated temperatures or in the presence of amino acid analogs, and induces the stress response. Ubc4/5 family members therefore appear to act as E2 ubiquitin-conjugating enzymes during the labeling of misfolded proteins for degradation by the proteasome. On the other hand, candidate proteins that may act as E3 ligases during this process remained elusive for a long time. Recently, however, such a candidate protein was identified [36–40]. The protein, termed CHIP (for carboxyl terminus of Hsp70 interacting protein), is intimately involved in the regulation of molecular chaperonins of the 70- and 90-kDa heat shock protein families (Hsp70s and Hsp90s). Its functional characterization therefore points to a close collaboration of molecular chaperones and the ubiquitin/proteasome system during protein quality control.

3. Molecular chaperones: mode of action and cellular functions

The term molecular chaperone describes members of diverse protein families that are all characterized by the ability to bind and stabilize non-native conformations of other proteins. Through controlled binding and release cycles, the chaperones facilitate the correct fate of the non-native polypeptide, be it folding, oligomeric assembly, transport to subcellular compartments or disposal by degradation [1,41]. Chaperones are ubiquitously expressed and are found in all cellular compartments of the eukaryotic cell (except for peroxisomes), which reflects their essential function under normal growth conditions. In addition, cells greatly increase chaperone concentration as a response to diverse stresses, when proteins become unfolded and require protection and stabilization. Accordingly, many chaperones are heat shock proteins (Hsps). Recent years have seen major advances in our understanding of how chaperones mediate protein folding in the cellular environment. The best understood chaperone proteins are certainly the so-called chaperonins that are defined by a barrel-shaped double ring structure [1,4,41,42]. Members include bacterial GroEL, Hsp60 of mitochondria and chloroplasts, and the TRiC/CCT complex localized in the eukaryotic cytosol. Based on their characteristic ring structure, a central cavity is formed, which binds non-native proteins via hydrophobic interactions. Conformational changes of the chaperonin subunits induced through ATP hydrolysis change the inner lining of the cavity from a hydrophobic to a hydrophilic character [43]. As a consequence, the unfolded polypeptide is released into the central chamber and can proceed on its folding pathway in a protected environment. Similar to the proteasome that forms a subcellular compartment for protein degradation owing to its unique oligomeric structure, the double ring chaperonins provide a compartment ideally suited for protein folding and assembly.

In many cases, non-native proteins are delivered to the chaperonins by other molecular chaperones, most notably by members of the Hsp70 family [1,41]. Hsp70 proteins comprise a highly conserved protein family with members present in eubacteria, eukarya and some archaea. They are among the first chaperones that bind newly synthesized polypeptides during de novo protein folding and are intimately involved in the translocation of unfolded polypeptides across intracellular membranes. Accordingly, Hsp70 family members are found associated with ribosomes and are present in eukaryotic organelles, such as mitochondria and the endoplasmic reticulum. In the mammalian cytosol two main Hsp70 proteins are present: the constitutively expressed Hsc70 (70 kDa heat shock cognate protein) and the heat-inducible Hsp70 (in the following we will refer to the two mammalian cytosolic Hsp70s with the term Hsc/Hsp70). In contrast to the double ring chaperonins, Hsp70 proteins act as monomers apparently dedicated to the initial recognition and stabilization of non-native polypeptides. The chaperones recognize short segments of the polypeptide chain, which are composed of clusters of hydrophobic amino acids flanked by basic residues [41]. Such binding motifs occur frequently within protein sequences, every 36 residues in average. They are likely to be found exposed on nascent polypeptides during translation, on polypeptides trafficking across intracellular membranes in an extended conformation, and on misfolded and damaged polypeptides. In fact, mammalian Hsc/Hsp70 bind to a wide range of nascent and newly synthesized polypeptides comprising about 15–20% of total protein [44]. This percentage is most likely further increased under stress conditions. By shielding hydrophobic regions, Hsp70 proteins apparently prevent protein aggregation and promote proper folding.

Recognition of hydrophobic segments is mediated by the central peptide-binding domain of Hsp70 proteins (Fig. 2). The structure of this domain complexed with a peptide substrate was revealed in crystallographic studies on bacterial Hsp70 [45]. The domain is composed of two sheets of β-strands that, together with connecting loops, form a cleft which can accommodate extended peptides of about seven amino acids in length. The adjacent carboxyl-terminal domain of Hsp70 is largely α-helical in structure and folds back over the β-sandwich in the obtained crystal structure. It was therefore speculated that the carboxyl-terminal domain of Hsp70 functions as a lid in permitting entry and release of polypeptide substrates [41,45] (Fig. 2). Lid opening and closure is regulated by cycles of ATP binding and hydrolysis by the amino-terminal ATPase domain of Hsp70, although the mechanism underlying interdomain communication is not yet understood. In the ATP-bound conformation of Hsp70 the peptide binding pocket is open, resulting in rapid binding and release of the polypeptide substrate and consequently in a low binding affinity (Fig. 2). Stable holding of the polypeptide requires closing of the binding pocket, which is induced upon ATP
Hsp70 proteins display a characteristic domain structure comprising an amino-terminal ATPase domain, a peptide-binding domain and a carboxyl-terminal lid domain. The latter ends with the tetrapeptide EEVD, which is recognized by the Hsp70 binding sites of the co-chaperones CHIP and Hop. Several other co-chaperones interact with the amino-terminal ATPase domain. Listed are co-chaperones that bind to Hsc/Hsp70 in the mammalian cytosol. Interaction of Hsp70 with co-chaperones is required to establish a functional chaperone cycle. In the ATP-bound state, Hsp70 does not stably associate with a non-native substrate protein because the peptide binding pocket is open. Upon Hsp40-stimulated transfer into the ADP state, the lid domain is closed and the substrate is locked in the peptide binding pocket. Nucleotide exchange and substrate release are stimulated by BAG proteins and possibly by HspBP1 in the mammalian cytosol, whereas Hip blocks ADP release.

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Fig. 2. The domain structure and reaction cycle of Hsp70. Hsp70 proteins display a characteristic domain structure comprising an amino-terminal ATPase domain, a peptide-binding domain and a carboxyl-terminal lid domain. The latter ends with the tetrapeptide EEVD, which is recognized by the Hsp70 binding sites of the co-chaperones CHIP and Hop. Several other co-chaperones interact with the amino-terminal ATPase domain. Listed are co-chaperones that bind to Hsc/Hsp70 in the mammalian cytosol. Interaction of Hsp70 with co-chaperones is required to establish a functional chaperone cycle. In the ATP-bound state, Hsp70 does not stably associate with a non-native substrate protein because the peptide binding pocket is open. Upon Hsp40-stimulated transfer into the ADP state, the lid domain is closed and the substrate is locked in the peptide binding pocket. Nucleotide exchange and substrate release are stimulated by BAG proteins and possibly by HspBP1 in the mammalian cytosol, whereas Hip blocks ADP release.

Cycling of Hsp70 in bacteria requires a second regulatory co-chaperone, the GrpE protein, which acts as a nucleotide exchange factor [1,41]. By stimulating the dissociation of ADP and rebinding of ATP, GrpE promotes substrate release and chaperone recycling. However, a GrpE-like nucleotide exchange factor seems to be dispensable in the eukaryotic cytosol, as dissociation of bound ADP is normally not the rate-limiting step in the chaperone cycle of eukaryotic Hsp70s, but rather ATP hydrolysis itself [51,52]. Nonetheless, Hsp70-mediated processes in the eukaryotic cytosol may be subjected to regulation through accelerated nucleotide exchange. In fact, the BAG-1 protein was recently shown to act as a nucleotide exchange factor in the eukaryotic cytosol [53,55]. BAG-1 binds to the ATPase domain of Hsc/Hsp70 via its carboxyl-terminal BAG domain and stimulates ADP release and substrate unloading from the chaperone (Table 1; Fig. 2). Although BAG-1 thus fulfills a GrpE-like function in the eukaryotic cytosol, it is structurally unrelated to the bacterial co-chaperone [54]. A role in stimulating nucleotide exchange was also discussed for the Hsc/Hsp70 co-chaperone HspBP1, which lacks BAG-1- and GrpE-related domains [56,57]. During evolution, functional convergence has apparently allowed proteins with different architectures to act as nucleotide exchange factors of Hsp70 family members. Interestingly, human cells contain several BAG-1-related proteins: BAG-2, BAG-3 (CAIR-1; Bis), BAG-4 (SODD), BAG-5 and BAG-6 (Scythe, BAT3) [58,59]. In addition to the conserved BAG domain required for binding and regulation of Hsc/Hsp70, the BAG family members possess additional functional domains that seem to mediate targeting to diverse partner proteins and subcellular compartments [58–61]. It appears that BAG proteins act as nucleotide exchange factors to induce substrate unloading from Hsc/Hsp70 on diverse protein folding, assembly and degradation pathways.

Besides regulating the ATPase and peptide binding cycle of Hsp70, co-chaperones play a key role in establishing distinct chaperone pathways. This is best illustrated for the cooperation of Hsp70 with Hsp90 during the regulation of signal transduction pathways. Signaling proteins such as steroid hormone receptors and certain protein kinases associate with Hsp70 and Hsp90 when inactive and the chaperones jointly mediate conformational changes necessary for activation (Fig. 3). The sequential and coordinated interaction of the two chaperones with a client protein critically depends on the activity of several chaperone cofactors (reviewed in Refs. [46,62]). An initial recognition of the signaling protein appears to be mediated by Hsp40, which subsequently delivers the substrate protein to Hsp70 [63]. Hsp40-induced conversion of Hsp70 to the ADP-bound state induces substrate transfer to the chaperone and stimulates an association of Hsp70 with the co-chaperone Hip (Hsc70-interacting protein). Hip binds to the ATPase
In contrast to BAG-1, Hip stabilizes the ADP-bound state of the chaperone and promotes chaperone/substrate interaction. At the same time, the cofactor Hop (Hsp70/Hsp90-organizing protein) associates with Hsp70 through binding to the carboxyl terminus of the chaperone. Importantly, Hop possesses nonoverlapping binding sites for Hsp70 and Hsp90 and therefore acts as coupling factor between the two chaperones. Hop-mediated tethering of the chaperones seems to be necessary for an efficient transfer of the signaling protein from Hsp70 to Hsp90. The components of the Hsp70 chaperone system are subsequently released, and further conformational changes of the client protein are mediated by Hsp90 in conjunction with another set of cofactors, most notably p23 and different immunophilins. Eventually, the signaling protein reaches an activatable conformation, for example a high affinity state for hormone binding in the case of steroid receptors. If the signaling protein is not activated at this stage, it again adopts its inactive conformation upon release from Hsp90 and enters a new cycle of chaperone interaction.

### Table 1

<table>
<thead>
<tr>
<th>Cofactor family</th>
<th>Cofactor</th>
<th>Functional domains</th>
<th>Binding site on Hsc/Hsp 70</th>
<th>Cellular function of the cofactor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hsp40-family</td>
<td>Hdj-1 (Hsp40)</td>
<td>J domain, (G/F)-rich region</td>
<td>probably formed by ATPase and peptide binding domain</td>
<td>stimulates Hsc/Hsp70 ATPase activity, has weak affinity for unfolded polypeptides</td>
</tr>
<tr>
<td></td>
<td>Hdj-2</td>
<td>J domain, (G/F)-rich region, cysteine-rich zinc finger</td>
<td>–</td>
<td>stimulates Hsc/Hsp70 ATPase activity, possesses chaperone activity on its own and recruits Hsc/Hsp70 to bound unfolded polypeptides</td>
</tr>
<tr>
<td></td>
<td>Hsj1</td>
<td>J domain, UIM domain</td>
<td>–</td>
<td>neuronal Hsp40 family member</td>
</tr>
<tr>
<td></td>
<td>Mtj (Hsj2)</td>
<td>J domain, (G/F)-rich region</td>
<td>–</td>
<td>is involved in inhibition of huntingtin aggregation and of cellular toxicity</td>
</tr>
<tr>
<td></td>
<td>auxilin</td>
<td>J domain, clathrin-binding domain</td>
<td>–</td>
<td>involved in clathrin uncoating</td>
</tr>
<tr>
<td>CSP (cysteine string protein)</td>
<td>J domain, cysteine-rich string</td>
<td>–</td>
<td>role in neurotransmitter release, binds CFTR</td>
<td></td>
</tr>
<tr>
<td>TPR2</td>
<td>J domain, TPR domains</td>
<td>possibly additional contacts via TPR-repeats</td>
<td>–</td>
<td>regulates Rad9 during the cell cycle</td>
</tr>
<tr>
<td>p58 IPK (inhibitor of protein kinase)</td>
<td>J domain, TPR domains</td>
<td>–</td>
<td>inhibitor of the interferon-induced protein kinase PKR</td>
<td></td>
</tr>
<tr>
<td>BAG-family</td>
<td>BAG-1</td>
<td>BAG domain, Ubiquitin-like domain</td>
<td>ATPase domain</td>
<td>stimulates the ADP dissociation rate of Hsc/Hsp70, accelerates ATP-triggered substrate release by Hsc/Hsp70, recruits the proteasome</td>
</tr>
<tr>
<td></td>
<td>BAG-2</td>
<td>BAG domain</td>
<td>ATPase domain</td>
<td>most likely regulates the Hsc/Hsp70 ATPase in a manner similar to BAG-1</td>
</tr>
<tr>
<td></td>
<td>BAG-3 (CAIR-1; Bis)</td>
<td>BAG domain, WW domain, proline rich-region with several PXXP-motifs</td>
<td>ATPase domain</td>
<td>forms complex with Hsc/Hsp70 and inactive phospholipase C-γ regulated by the epidermal growth factor, binds the SH3 domain of PLC-γ</td>
</tr>
<tr>
<td></td>
<td>BAG-4 (SODD)</td>
<td>BAG domain</td>
<td>ATPase domain</td>
<td>most likely regulates the Hsc/Hsp70 ATPase in a manner similar to BAG-1</td>
</tr>
<tr>
<td></td>
<td>BAG-5</td>
<td>four putative BAG domains</td>
<td>ATPase domain</td>
<td>most likely regulates the Hsc/Hsp70 ATPase in a manner similar to BAG-1</td>
</tr>
<tr>
<td></td>
<td>BAG-6 (scythe, BAT3)</td>
<td>BAG domain, Ubiquitin-like domain</td>
<td>ATPase domain</td>
<td>nuclear regulator of apoptosis, target of the Drosophila killer protein reaper</td>
</tr>
<tr>
<td></td>
<td>Hip</td>
<td>TPR domain</td>
<td>ATPase domain</td>
<td>promotes folding, prevents dissociation of ADP from Hsc/Hsp70, stabilizes chaperone-substrate complex</td>
</tr>
<tr>
<td></td>
<td>HspBP1</td>
<td></td>
<td>ATPase domain</td>
<td>inhibits the Hsp40-stimulated ATPase activity of Hsc/Hsp70, inhibits Hsc/Hsp70-mediated protein refolding</td>
</tr>
<tr>
<td></td>
<td>Hop</td>
<td>TPR domains</td>
<td>C-terminal EEVD motif</td>
<td>binds also to Hsp90, represents a coupling factor between Hsc/Hsp70 and Hsp90</td>
</tr>
<tr>
<td></td>
<td>CHIP</td>
<td>TPR domain, U-box</td>
<td>C-terminal EEVD motif</td>
<td>inhibits Hsp40-stimulated ATPase activity of Hsc/Hsp70, functions as E3-ubiquitin ligase promoting ubiquitination of Hsc/Hsp70/Hsp90 substrates</td>
</tr>
</tbody>
</table>

[51, 53, 58, 64] In contrast to BAG-1, Hip stabilizes the ADP-bound state of the chaperone and seems to promote chaperone/substrate interaction. At the same time, the cofactor Hop (Hsp70/Hsp90-organizing protein) associates with Hsp70 through binding to the carboxyl terminus of the chaperone. Importantly, Hop possesses nonoverlapping binding sites for Hsp70 and Hsp90 and therefore acts as coupling factor between the two chaperones [65]. Hop-mediated tethering of the chaperones seems to be necessary for an efficient transfer of the signaling protein from Hsp70 to Hsp90. The components of the Hsp70 chaperone system are subsequently released, and further conformational changes of the client protein are mediated by Hsp90 in conjunction with another set of cofactors, most notably p23 and different immunophilins. Eventually, the signaling protein reaches an activatable conformation, for example a high affinity state for hormone binding in the case of steroid receptors. If the signaling protein is not activated at this stage, it again adopts its inactive conformation upon release from Hsp90 and enters a new cycle of chaperone interaction (Fig. 3). The analysis of chaperone cooperation during signal transduction reveals the central role of regulatory cofactors in establishing a distinct folding pathway. Indeed, the functions of Hsp40, Hop and p23 were shown to be essential on this folding pathways [63, 66]. The role of Hip in Hsp70/Hsp90 cooperation is still a matter of debate, however. Dominant-negative mutant forms of Hip were shown to cause a dose-dependent inhibition of receptor assembly with Hsp90 in vitro [67]. Yet, Hip is not essential...
for the formation of the multi-chaperone complex on purified hormone receptor [68,69]. Notably, the in vitro data do not exclude that Hip contributes to the formation of Hsp70/Hsp90 complexes in cells by preventing an interaction of Hsp70 with cofactors such as BAG-1 that disturb the cooperation of the chaperones [69]. Indeed, evidence for a stimulating role of Hip in the functional maturation of the glucocorticoid hormone receptor was recently provided [70].

The identification and characterization of Hsp70 cofactors illustrate that the chaperone does not act on its own in the cellular environment. A functional chaperone system is formed only when Hsp70 tightly cooperates with regulatory cofactors that modulate the ATPase cycle of the chaperone or mediate targeting to other proteins and protein complexes. In particular, the large diversity of co-chaperones present in the eukaryotic cytosol seems to enable Hsp70 to fulfill its multiple functions in this compartment.

Hsp70 and at least some Hsp70-regulating cofactors are involved in protein quality control. This was nicely revealed in studies on the influence of the chaperone system on polyglutamine diseases using the fruitfly Drosophila melanogaster as a model organism (reviewed in Refs. [21,71]). Hallmarks of the polyglutamine disease spinocerebellar ataxia type 3 (SCA3), for example, were recapitulated in transgenic flies that expressed a pathological polyQ tract of the ataxin-3 protein in the eye disc [72]. Transgene expression caused a characteristic formation of abnormal protein inclusions and progressive neuronal degeneration. Co-expression of human cytosolic Hsp70, however, suppressed polyQ-induced neurotoxicity [72]. In a similar experimental approach, Hsp40 family members, too, were shown to protect neuronal cells against toxic polyQ expression [73]. Enhancing the activity of the Hsp70/Hsp40 chaperone system apparently mitigates cytotoxicity caused by the accumulation of aggregation-prone proteins. These findings obtained in Drosophila were confirmed in a mouse model of spinocerebellar ataxia type 1 (SCA1). Crossbreeding of SCA1 mice with mice overexpressing Hsp70 resulted in protection against neurodegeneration [74]. It should be pointed out, however, that the Hsp70 chaperone system was unable to prevent the formation of protein aggregates in these models of polyglutamine diseases and upon polyQ expression in yeast and mammalian cells [26,72,75–77]. Elevated cellular levels of Hsp70 and of some Hsp40 family members affected the number of protein aggregates and their biochemical properties, but did not inhibit the formation of polyQ aggregates. The question arises: how can the chaperone system reduce cytotoxicity without inhibiting protein aggregation? Experiments by Muchowski et al. [76] may provide an answer. They observed that Hsp70 and Hsp40, when present at sufficient levels, profoundly modulate the aggregation process of polyQ tracts. While a polyQ-expanded pathological fragment of huntingtin usually forms ordered, SDS-insoluble amyloid fibrils in vitro, the formation of amorphous, SDS-soluble aggregates was observed in the presence of Hsp70 and Hsp40 [76]. This effect of the chaperone system was reproduced in yeast and mammalian cells when the polyQ tract was co-expressed with Hsp70 and Hsp40 homologs [76,78]. Transient binding of the Hsp70 chaperone system to polyQ tracts is apparently insufficient to prevent the formation of amorphous protein aggregates, but seems to block ordered oligomerization and fibril growth. In this way, Hsp70 and Hsp40 could inhibit a sequestration of other polyQ-containing proteins such as CBP and TATA-binding protein into polyQ oligomers and therefore reduce cytotoxicity. In an alternate but not mutually exclusive model, the chaperones may cover potentially dangerous surfaces exposed by the polyQ tracts during the oligomerization

Fig. 3. Co-chaperone-induced cooperation of Hsp70 and Hsp90 during the regulation of signal transduction pathways. An inactive signaling protein, for example a steroid hormone receptor, is initially recognized by Hsp40 and delivered to Hsp70. Subsequently, the co-chaperones Hip and Hop associate with the chaperone/substrate complex. Hop stimulates recruitment of an Hsp90 dimer and in this way promotes a transfer of the client protein from Hsp70 to Hsp90. At the final stage of the chaperone pathway, Hsp90 associates with p23 and diverse immunophilins (immun.) to mediate conformational changes of the signaling protein necessary to reach an activatable state. Upon activation, i.e., hormone binding in the case of the steroid receptor, the signaling protein is released from Hsp90. In the absence of an activating stimulus, the signaling protein folds back to the inactive state when released and enters a new cycle of chaperone binding.
process or by the final oligomers. Intriguingly, elevated expression of Hsp70 also suppresses the toxicity of the non-polyQ-containing protein a-synuclein in a Drosophila model of Parkinson’s disease without inhibiting aggregate formation [79]. The finding suggests a rather general role of Hsp70 in protecting cells against toxic protein aggregation. Conceivably, a treatment of diverse forms of human neurodegenerative diseases may be achieved through up-regulation of Hsp70 activity.

The examples mentioned above show that the protective activity of Hsp70 in protein quality control does not necessarily include refolding of the misfolded polypeptide to the native state. Modulating the aggregation process or shielding of interaction surfaces of the misfolded polypeptide may be sufficient to decrease cytotoxic effects. Another option may involve presentation of the misfolded polypeptide to the ubiquitin/proteasome system for degradation. Mammalian Hsc70 was shown to be required for the ubiquitination of a subset of cellular proteins in vitro [80]. Furthermore, a role of the Hsp70 chaperone system in the degradation of an aggregation-prone protein was established by investigating the maturation of CFTR. Cytosolic Hsp70, the Hsp40 family members Hdj-2 and CSP, and also Hsp90 transiently associate with CFTR at the ER membrane and facilitate early steps during CFTR maturation [12,81–83]. The ΔF508 misfolding mutant of CFTR shows a prolonged interaction with the chaperone systems followed by proteasomal degradation [12,13,81,82]. That cytosolic Hsp70 is actually essential for CFTR degradation was revealed in studies using S. cerevisiae. Inhibiting the activity of a yeast cytosolic Hsp70 homolog significantly blocked the degradation of recombinant CFTR [14]. Capturing of immature forms of the aggregation-prone protein by the cytosolic Hsp70 chaperone system is apparently required for efficient degradation by the proteasome. In a similar manner Hsp70 seems to monitor the folded state of apolipoprotein B (apoB) at the cytosolic face of the ER membrane. ApoB is essential for the assembly and secretion of hepatic lipoproteins. In the absence of lipid ligands apoB is sorted to the proteasome and is rapidly degraded [84]. Degradation is enhanced upon Hsp70 overexpression and attenuated when chaperone levels are reduced [85,86]. Yet another example that illustrates a participation of cytosolic Hsp70 in protein degradation involves immunoglobulin light chains (LCs). Mutations in LCs can cause a dislocation from the ER to the cytosol, where they can accumulate in an aggresome-like structure or can be degraded by the proteasome [87]. Overexpression of cytosolic Hsp70 decreases aggresome formation and facilitates proteasomal degradation of LC mutant forms. Taken together, the data reveal a tight cooperation of Hsp70 with the ubiquitin/proteasome system in the eukaryotic cytosol.

What is the molecular basis of this cooperation? In the simplest model the chaperone would just prevent the aggregation of the misfolded polypeptide. If the polypeptide is unable to proceed to its native state, multiple cycles of binding and release from the chaperone would occur. After each cycle, the polypeptide could rebind to the chaperone system or could be recognized by the ubiquitin conjugation machinery followed by sorting to the proteasome. A defining feature of such a model is thus a kinetic partitioning of a non-native polypeptide between the chaperone system and the degradation machinery. On the other hand, an active participation of the chaperone in the degradation process is also conceivable. Through an association of the chaperone with components of the ubiquitin conjugation machinery and possibly with the proteasome itself, the chaperone may act as a substrate recognition factor during degradation and actively deliver the bound polypeptide to the proteasome. The characterization of the chaperone cofactor CHIP now provides evidence for the latter model.

4. The CHIP cofactor—a chaperone-associated ubiquitin ligase

The CHIP protein was initially identified in a screen for human proteins that possess a tetratricopeptide repeat (TPR) domain [36]. The domain comprises multiple repeats of a degenerate 34-amino-acid motif and is found in several cofactors of Hsp70 and Hsp90 [46,65,88] (Table 1). In fact, CHIP can associate with both Hsps and modulate their chaperone activity [13,36,37]. CHIP blocks the ATPase cycle of cytosolic Hsp70 and inhibits Hsp70-mediated protein folding [36]. Similarly, the interaction of the cofactor with Hsp90 abrogates the function of the chaperone in the conformational regulation of signaling proteins [37]. Clearly, the cofactor is not involved in the productive folding of chaperone substrates. With regard to a possible cellular role of CHIP, a closer look at its domain structure is revealing. In addition to the amino-terminal TPR domain, CHIP possesses a U-box at its carboxyl terminus (Fig. 4). The U-box is structurally related to RING-finger domains found in many ubiquitin ligases, which suggested a function of CHIP in ubiquitin conjugation [48,89,90]. Indeed, CHIP utilizes its U-box for binding to E2 ubiquitin-conjugating enzymes of the Ubc4/5 family and acts as an E3 ubiquitin ligase during the ubiquitination of known chaperone substrates [38–40,91,92]. As a consequence, elevating the cellular concentration of CHIP results in an increased degradation of chaperone substrates by the proteasome [13,37]. This was shown, for example, for the glucocorticoid hormone receptor (GR), whose activation depends on a sequential interaction with Hsp70 and Hsp90 (see above). When the receptor was co-expressed in mammalian cells with CHIP, the co-chaperone stimulated the ubiquitination and proteasomal degradation of the hormone receptor [37]. In addition, CHIP had a profound effect on the composition of Hsp90 heterocomplexes. By occupying a docking site on Hsp90 for TPR domain-containing co-chaperones, CHIP reduced the amount of
Hop associated with Hsp90 and completely blocked the interaction of Hsp90 with p23. As Hop and p23 are necessary for the Hsp70/Hsp90-mediated maturation of GR, CHIP mitigated receptor activation [37]. The co-chaperone apparently shifts the mode of action of Hsp70 and Hsp90 from protein folding to protein degradation.

Intriguingly, CHIP-induced degradation of GR requires both the TPR-domain that mediates chaperone binding and the U-box which is involved in ubiquitin conjugation [37]. This indicates that Hsp70 and Hsp90 are intimately involved in the conjugation process. Reconstitution of CHIP-mediated ubiquitination in vitro confirmed this conclusion. Efficient ubiquitination of heat-denatured firefly luciferase by the CHIP/Ubc5 conjugation machinery was dependent on the presence of either Hsp70 or Hsp90 and did not occur with native luciferase [40]. The chaperones apparently select substrates for CHIP-mediated ubiquitination. However, a direct interaction between CHIP and the protein substrate may contribute to the selection process, as CHIP is able to ubiquitinate at least some protein substrates even in the absence of chaperones [38] by directly recognizing certain regions of the substrate [93]. In any case, the chaperone/CHIP complex may be viewed as a multi-subunit ubiquitin ligase that contains either Hsp70 or Hsp90 as the main substrate recognition factor (Fig. 4). The complex thus resembles other multi-subunit ligases that are characterized by the association of certain ubiquitin ligases and ubiquitin-conjugating enzymes with substrate recognition factors, for example the Skp1/cullin/F-box protein (SCF) complex [89,94] (Fig. 4).

CHIP-mediated degradation of signaling proteins may be viewed as a special case of protein quality control. If the signaling protein becomes activated, for example by hormone binding in the case of steroid hormone receptors, it is released from the chaperones and escapes the destructive action of CHIP. In the inactivated state, however, the signaling protein undergoes multiple cycles of chaperone binding and release, and remains accessible for targeting to the proteasome through CHIP-mediated ubiquitination. In a similar manner, CHIP seems to cooperate with molecular chaperones to prevent the accumulation of misfolded polypeptides. Meacham et al. [13] demonstrated that CHIP is part of an Hsp70 chaperone complex that associates with immature forms of CFTR on the cytosolic face of the ER membrane. Consequently, elevated levels of CHIP induced the proteasomal degradation of CFTR and its folding incompetent ΔF508 mutant form [13]. The activity of CHIP in CFTR biogenesis was dependent on the presence of the U-box and the TPR domain of the co-chaperone, and involved enhanced ubiquitination of the membrane protein. Apparently, CHIP controls the fate of folding intermediates during CFTR maturation based on its function as a chaperone-associated ubiquitin ligase.

Further support for a role of CHIP in protein quality control stems from studies that link CHIP to Parkinson’s disease [95]. The disease is characterized by a progressive loss of dopaminergic neurons in the substantia nigra pars compacta, which is often accompanied by the accumulation of protein deposits, so-called Lewy bodies (reviewed in Ref. [3]). The disease usually affects elderly people that appear to develop symptoms spontaneously. However, recent data identified genetic mutations in early onset familial cases of Parkinson’s disease. So far, three genes have been associated with the disease, encoding α-synuclein, the ubiquitin ligase parkin and the ubiquitin hydrolase UCHL1 that removes ubiquitin moieties from modified protein substrates (reviewed in Ref. [3]). The

Fig. 4. Domain structure of CHIP and schematic presentation of the CHIP multi-subunit ubiquitin ligase. CHIP possesses a triple TPR domain that together with an adjacent charged region forms a binding site for Hsp70 and Hsp90. A central coiled coil domain (cc) may be involved in protein/protein interactions. The carboxyl-terminal U-box is required for the E3 ubiquitin ligase activity of CHIP and seems to mediate binding to E2 ubiquitin-conjugating enzymes of the Ubc4/5 family. Association of CHIP with the carboxyl-terminal domain of Hsp70 and possibly with Hsp90 gives rise to a multi-subunit ubiquitin ligase complex, in which the chaperones act as substrate recognition factors. Subunit and domain arrangement in the complex remains to be determined experimentally. For reasons of comparison, the multi-subunit ligase complex SCF is shown, which possesses an F-box protein for substrate recognition. The structure of the SCF complex was recently determined and is schematically presented.
genes may define a pathogenic biochemical pathway that seems to involve protein degradation by the ubiquitin/proteasome system. This notion has gained ground with the observation that α-synuclein, a major constituent of Lewy bodies, is a substrate of the parkin ubiquitin ligase [96]. Because pathogenic mutations in parkin are associated with a loss of function of the ubiquitin ligase [97], impaired removal of aggregation-prone conformers of α-synuclein may contribute to the development of the disease. Other pathologically relevant substrate proteins of parkin are the α-synuclein binding partner synphilin-1 and the G protein-coupled plasma membrane receptor Pael that accumulates in the brains of Parkinson patients in an insoluble form [98,99]. Similar to CFTR, Pael is an aggregation-prone membrane protein that is affected by protein quality control mechanisms at the ER membrane. The cytosolically localized parkin participates in these mechanisms by mediating the ubiquitination of unfolded Pael receptor in cooperation with ER-associated ubiquitin-conjugating enzymes, e.g., Ubc6 and Ubc7 [99]. Interestingly, parkin and Pael are present in a multi-protein complex that also includes Hsp70, Hdj-2 and the CHIP ubiquitin ligase [95]. The chaperone system appears to be involved in the recognition of the unfolded receptor. Moreover, CHIP together with its partner Ubc4 promoted ubiquitination of the Pael receptor. The data thus link CHIP to neurodegenerative diseases and further support a role of the cochaperone in protein quality control. In this regard, it is also noteworthy that CHIP participates in the degradation of the microtubule-binding protein tau, which forms protein aggregates in Alzheimer patients [100].

During sorting of chaperone substrates to the proteasome, CHIP may cooperate with the BAG-1 nucleotide exchange factor of Hsp70 (see above). In addition to the BAG domain that mediates Hsp70 binding and regulation, BAG-1 possesses a ubiquitin-like domain within its primary structure (Fig. 5). The presence of such a domain is the defining feature of a family of diverse proteins, termed ubiquitin domain proteins (UDPs) [101]. Similar to other UDps, BAG-1 utilizes the integrated ubiquitin domain for an association with the proteasome [60,102]. Because the domain does not overlap with the chaperone-binding site of the cofactor, BAG-1 can act as a coupling factor between Hsp70 and the proteasome. As a consequence, elevating BAG-1 levels induced a proteasomal association of Hsp70 in cell culture experiments [60]. BAG-1 may thus exert its nucleotide exchange and substrate release activity on Hsp70 in the vicinity of the proteasome to facilitate the transfer of chaperone substrates to the proteasome. In agreement with this hypothesis, BAG-1 was shown to stimulate CHIP-induced degradation of the glucocorticoid hormone receptor [38]. The cooperation of BAG-1 and CHIP in sorting to the proteasome seems to reflect the ability of the two cofactors to simultaneously associate with Hsp70. BAG-1 interacts with the amino-terminal ATPase domain of Hsp70 [53,54], whereas CHIP binds to the carboxyl terminus of the chaperone in a manner that appears to involve recognition of Hsp70’s terminal EEVD motif [36,65,103] (Figs. 2 and 5). Ternary complexes of BAG-1, Hsp70 and CHIP have been isolated from mammalian cells [38]. In addition, a direct interaction between BAG-1 and CHIP was observed in vitro, which may contribute to the formation of the ternary chaperone/cofactor complex [38]. It remains to be seen, however, whether BAG-1 is an essential component of the CHIP ubiquitin ligase complex. At least in vitro, CHIP-mediated degradation of certain protein substrates can be reconstituted without BAG-1 [104].

Notably, another BAG family protein, namely BAG-6/Scythe, displays a domain arrangement similar to BAG-1 by combining an integrated ubiquitin-like domain together with an Hsp70 binding site [61]. BAG-6/Scythe may thus replace BAG-1 on certain degradation pathways.

5. Chaperone machines: protein folding versus protein degradation

The characterization of the CHIP cofactor challenges previous concepts regarding the role of molecular chaperones in protein quality control. So far, a kinetic partitioning of non-native polypeptides between chaperones and proteases was proposed to underlie quality control [105]. Chaperone binding would direct the non-native polypeptide towards the folded state, whereas an association with components of the degradation machinery would result in polypeptide destruction. This model infers a competition
between chaperones and the degradation machinery in substrate binding. The identification of CHIP now suggests a different concept. Through association with the cofactor, molecular chaperones are directly turned into protein degradation factors [47,48]. Accordingly, folding as well as degradation of a non-native polypeptide would involve an initial recognition by molecular chaperones, which would prevent aggregation (Fig. 6). The fate of the chaperone-bound polypeptide would be determined to a significant extent by the cofactors that associate with the chaperone/substrate complex. Binding of CHIP and BAG-1 would lead to degradation, whereas attempts to fold the bound polypeptide would occur upon binding of folding cofactors, such as Hip and Hop (see above). The diverse cofactors apparently define functionally distinct Hsp70 chaperone machines (Fig. 7). Intriguingly, CHIP and Hop compete in chaperone binding [37]. Both cofactors seem to utilize the same docking site at the carboxyl terminus of Hsp70 and Hsp90, respectively [65,103]. A similar competition of cofactors is observed at the amino-terminal ATPase domain of Hsp70, where the proteasome recruitment factor BAG-1 competes with the folding-stimulating cofactor Hip [53,58]. The intracellular balance of the competing and cooperating cofactors may therefore set the threshold between folding and degradation. Concentrations of BAG-1 and CHIP are low compared to the folding-stimulating cofactors Hip and Hop, so the cell usually appears to set conditions that favor folding. However, very little is known about how the expression levels of these cofactors are regulated [106]. In addition, chaperone pathways may be controlled by modulating the binding affinity of distinct cofactors for its partner chaperone. Again, this is an area of research that has not been explored intensively so far. Another level of regulation may involve a direct inhibition of the destructive activity of CHIP. Indeed, the Hsp70-binding protein 1 (HspBP1) was recently identified by us as an inhibitor of the CHIP ubiquitin ligase [107]. Like BAG-1, HspBP1 binds to the ATPase domain of Hsp70 [56]. This binding promotes an association of CHIP with the carboxyl terminus of the chaperone. Intriguingly, in the formed ternary HspBP1/Hsp70/CHIP complex the ubiquitin ligase activity of CHIP is inhibited [107] (Fig. 7). As a consequence, HspBP1 attenuated the CHIP-induced degradation of CFTR in cell culture experiments and stimulated the maturation of the ion channel. HspBP1 thus emerges as a key regulator of chaperone-assisted degradation that involves the CHIP ubiquitin ligase.

It is tempting to speculate that the HspBP1-mediated inhibition of the ubiquitin ligase activity may enable CHIP to regulate the Hsp70 ATPase cycle without inducing degradation. In fact, degradation-independent functions of CHIP recently emerged [110–113]. CHIP participates in the chaperone-mediated folding and trafficking of the androgen receptor, endothelial nitric oxide synthase and the heat shock transcription factor without inducing degradation. Cooperation with HspBP1 may provide a molecular basis for exerting such degradation-independent functions. The data illustrate that CHIP is tightly embedded in the co-

![Fig. 6. Model for the action of molecular chaperones in protein folding and protein degradation. The non-native folding intermediate is initially recognized by a chaperone protein that prevents aggregation. Subsequently, the chaperone would associate with either a folding cofactor or a degrading cofactor, which would determine the fate of the substrate protein.](image-url)
chaperone network that determines the cellular functions of Hsp70 and Hsp90.

6. Pharmacological modulation of protein folding and degradation activities

An exciting example of chaperone/proteasome cooperation involves the drug-induced degradation of chaperone substrates. Antibiotics of the benzoquinone ansamycin class, such as geldanamycin (GA), the antifungal antibiotic radicicol and coumarin antibiotics (e.g., novobiocin) stimulate the degradation of diverse chaperone substrates by the ubiquitin/proteasome system [82,114–117]. Among the affected proteins are known regulators of cell proliferation and apoptosis, for example raf-1, mutant p53 and ErbB-2 [118–120]. This seems to provide the basis for the potent anti-tumor activity of the mentioned antibiotics. They were shown to reverse the malignant phenotype of transformed fibroblasts and displayed anti-tumor activity in vitro and in vivo (reviewed in Refs. [116,117,121]). Importantly, the antibiotics do not interact directly with the affected proteins. Rather, they all bind with high specificity to Hsp90 and occupy ATPase binding sites of the chaperone [122–124]. By inhibiting the ATP-dependent peptide binding and release cycle of Hsp90, the antibiotics apparently affect the biogenesis of chaperone clients that require an interaction with the chaperone during maturation and activation (see above) [125,126]. In the presence of Hsp90 inhibitors, chaperone-mediated maturation pathways appear to be blocked at an intermediate stage when the client proteins are primarily associated with Hsp70, followed by sorting of the client proteins to the ubiquitin/proteasome system for degradation [104] (Fig. 8). Accordingly, the drug-induced degradation of chaperone substrates may rely on the activity of co-chaperones such as CHIP. In fact, overexpression of CHIP in cell cultures results in a remodeling of chaperone complexes associated with client proteins, similar to that observed upon GA treatment [37,104,119]. In addition, a dominant-negative mutant form of CHIP inhibited GA-

![Fig. 7. Schematic presentation of functionally distinct Hsp70 chaperone complexes. The degrading complex is characterized by an association of BAG-1 with the ATPase domain of Hsp70 while CHIP is bound to the carboxyl terminus. BAG-1 mediates an association of Hsp70 with the proteasome via its ubiquitin-like domain (ubl), whereas CHIP acts in conjunction with Ubc4/5 as a chaperone-associated ubiquitin ligase to target chaperone substrates for degradation by the proteasome. The Hsp70/Hsp90 coupling factor Hop competes with CHIP in binding to the carboxyl terminus of Hsp70. Hop cooperates with the ATPase domain binding co-chaperone Hip, which blocks binding of BAG-1 to Hsp70 and stimulates the folding activity of the chaperone. When HspBP1 occupies the ATPase domain of Hsp70, the co-chaperone stimulates binding of CHIP to the carboxyl terminus and inhibits the ubiquitin ligase activity of CHIP, resulting in a chaperone complex that mediates protein folding.

![Fig. 8. Model to explain the drug-induced degradation of signaling proteins by the ubiquitin/proteasome system. Hsp90 inhibitors, such as geldanamycin, radicicol and novobiocin, block the chaperone-mediated activation pathway at an intermediate stage when the client proteins are primarily associated with Hsp70. From this intermediate complex the signaling proteins are sorted to the proteasome in a manner that appears to involve the activity of CHIP and possibly other yet to be identified ubiquitin ligases.](image-url)
induced ubiquitination [104]. Although these data suggest an involvement of CHIP in the degradation of chaperone substrates in the presence of Hsp90 inhibitors, fibroblasts that lacked genomic copies of the chip gene remained sensitive to GA [104]. At least one additional as yet unidentified ubiquitin ligase may therefore participate in the sorting of chaperone clients to the proteasome. In any case, using Hsp90 inhibitors to alter the balance between protein folding and protein degradation seems to open new strategies for the treatment of cancer. Hsp90 inhibitors have now entered clinical trials and second generation inhibitors are currently developed to improve therapeutic benefit (reviewed in Refs. [116,117,127]). Moreover, this strategy may be applicable to other medical conditions involving chaperone pathways.

7. New insights into sorting to the proteasome

Remarkably, CHIP not only mediates ubiquitin attachment to chaperone clients but also to certain chaperones and co-chaperones itself. Mammalian Hsc/Hsp70 has been identified as a main target of the CHIP ubiquitin ligase [39], and also the co-chaperone BAG-1 was shown to be a substrate of CHIP in vitro and in vivo [102]. However, in contrast to the destructive action of CHIP on chaperone clients, Hsc/Hsp70 and BAG-1 are not targeted for degradation by the CHIP ubiquitin ligase [39,102]. In the case of BAG-1, CHIP was shown to stimulate a degradation-independent association of the co-chaperone with the proteasome [102]. CHIP-mediated attachment of polyubiquitin chains to BAG-1 and probably also to Hsc/Hsp70 may thus provide the means to regulate the sorting of chaperone clients to the proteasome. It is still unclear, however, why Hsc/Hsp70 and BAG-1 are not degraded when targeted to the proteasome by CHIP, whereas chaperone clients are rapidly destroyed. Interestingly, CHIP mediates the attachment of a noncanonical polyubiquitin chain to Hsc/Hsp70 and BAG-1 [39,102]. While ubiquitin moieties are linked via lysine 48 in the typical degradation signal, CHIP utilizes other lysine residues of ubiquitin (in particular lysine 27) for chain assembly. The unusual polyubiquitin chain together with structural features of Hsc/Hsp70 and BAG-1 may render the chaperone and co-chaperone rather resistant to proteasomal degradation.

Efficient ubiquitination of BAG-1 mediated by CHIP was dependent on the formation of the ternary BAG-1/Hsp70/CHIP complex [102]. It seems that the assembly of the functional multi-subunit ubiquitin ligase is accompanied by polyubiquitin chain attachment to BAG-1 and Hsc/Hsp70. The resultant complex would thus expose multiple signals for sorting to the proteasome, e.g., the integrated ubiquitin-like domain of BAG-1 (see above) and polyubiquitin chains attached to BAG-1, Hsc/Hsp70 and the bound protein substrate. Such a redundancy of sorting information may be considered unnecessary. However, it mirrors the identification of multiple receptors for polyubiquitin chains and integrated ubiquitin-like domains within the regulatory 19S complex of the proteasome. Currently, the proteasomal subunits Rpn1, Rpn2, Rpt5 and Rpn10 are discussed to fulfill receptor functions (Fig. 9). The Rpn10 subunit was initially identified as a polyubiquitin chain receptor and was later shown to also bind integrated ubiquitin-like domains presented by UDPs [128–131]. In addition, two distinct ubiquitin binding domains were identified in Rpn10, of which only one is used for UDP recognition [129,130]. However, conflicting data exist as to whether the subunit acts as a ubiquitin receptor in the context of the assembled 19S complex [132,133]. More recently, Rpn1 was identified as a receptor for integrated ubiquitin-like domains [133]. Rpn1 associates with different UDPs through a leucine-rich-repeat-like domain. A similar function in the recognition of integrated ubiquitin-like domains may be fulfilled by the Rpn1-related subunit Rpn2 [134]. On the other hand, polyubiquitin chains may be primarily recognized by the Rpt5 subunit, one of the AAA-ATPases present in the ring-like base of the regulatory 19S complex [135]. The subunit was identified as a receptor when tetraubiquitin was cross-linked to intact proteasomes [132]. The presence of several distinct docking sites within the regulatory complex of the proteasome may provide the structural basis for the recognition of multiple sorting signals displayed by the CHIP/chaperone complex (Fig. 9). Interestingly, a similar mechanism involving multiple-site binding at the proteasome was recently proposed based on the observation that two unrelated yeast ubiquitin ligases associate with specific subunits of the 19S regulatory complex [136]. Removal of the proteasome binding site of one of the ligases did not impair substrate ubiquitination, but abrogated efficient

![Fig. 9. Multiple-site binding of sorting signals at the 19S regulatory cap of the proteasome. The subunits Rpn1, Rpn2, Rpt5 and Rpn10 were shown to be involved in the recognition of polyubiquitin chains and integrated ubiquitin-like domains (ubl). Multiple docking sites at the regulatory complex may ensure efficient transfer of protein substrates into the proteolytic core of the proteasome through ATP-dependent movements of the ring-like base of the 19S cap.](Image)
degradation. In this case, docking at the proteasome therefore seems to involve contacts of proteasomal subunits with the substrate-bound ubiquitin ligase, with the poly-ubiquitin chain attached to the substrate and with the substrate itself [136]. Multiple-site binding may function to slow down dissociation of the substrate from the proteasome and to facilitate transfer into the central proteolytic chamber through ATP-dependent movements of the subunits of the 19S particle.

Another way to increase degradation efficiency may involve the delivery of protein substrates in a non-native conformation, which would allow direct insertion into the proteasomal core. The BAG-1/Hsp70/CHIP complex is apparently ideally suited for this task. In addition, a similar function seems to be provided by the hexameric AAA-ATPase Cdc48 (reviewed in Ref. [137]). Through association with two regulatory proteins, Ufd1 and Npl4, Cdc48 participates in ER-associated degradation and in the ubiquitin-dependent activation of membrane-bound transcription factors (see also the review by Sommer and Wolf in this issue; [138]). During these processes the AAA-ATPase appears to act in a chaperone-like manner to mediate the disassembly of protein complexes and possibly substrate unfolding [139]. As the latter is a rate limiting step in proteasomal degradation [140], chaperone-mediated delivery of non-native polypeptides to the proteasome could facilitate degradation. Notably, Cdc48 has been found associated with the proteasome and with components involved in ubiquitin conjugation [141–143]. Cdc48 and its binding partners may therefore form a multi-functional protein complex which, similar to the BAG-1/Hsp70/CHIP machinery, mediates substrate processing in the vicinity of the proteasome. Direct physical contacts between molecular chaperones and chaperone-like proteins, ubiquitin ligases and subunits of the 19S regulatory complex appear to ensure a tight coordination of ubiquitin conjugation, substrate unfolding and insertion into the proteasomal core.

8. Concluding remarks

The elucidation of the cooperation of molecular chaperones with the ubiquitin/proteasome system has significantly advanced our understanding of how protein substrates are sorted to the proteasome for degradation. In the future, we need to learn more about the molecular mechanisms that regulate chaperone-mediated degradation pathways. The possibility to modulate such pathways may provide the basis for the treatment of cancer and of a broad range of protein misfolding diseases.

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