Cell. Mol. Life Sci. 64 (2007) 2525 – 2541
1420-682X/07/192525-17
DOI 10.1007/s00018-007-7188-6

Cellular and Molecular Life Sciences

Review

To be, or not to be – molecular chaperones in protein degradation

V. Arndt, C. Rogon and J. Höhfeld*

Institut für Zellbiologie, Rheinische Friedrich Wilhelms-Universität Bonn, Ulrich-Haberland-Str. 61a, 53121 Bonn (Germany), Fax: +49 228 735302, e-mail: hoehfeld@uni-bonn.de

Received 16 April 2007; received after revision 10 May 2007; accepted 16 May 2007
Online First 14 June 2007

Abstract. To be, or not to be – that is the question not only for Hamlet in Shakespeare's drama but also for a protein associated with molecular chaperones. While long viewed exclusively as cellular folding factors, molecular chaperones recently emerged as active participants in protein degradation. This places chaperones at the center of a life or death decision during protein triage. Here we highlight molecular mechanisms that underlie chaperone action at the folding/degradation interface in mammalian cells. We discuss the importance of chaperone-assisted degradation for the regulation of cellular processes and its emerging role as a target for therapeutic intervention in cancer and amyloid diseases.

Keywords. CHIP, Parkin, Hsp70, Hsp90, ubiquitin, proteasome.

Introduction

Molecular chaperones are defined by their ability to associate with non-native proteins. They participate in the folding of newly translated and damaged polypeptides, in the transport of proteins across cellular membranes and in the assembly of protein complexes [1–3]. In addition, chaperones assist the conformational regulation of signaling proteins and apoptosis regulators [4–6]. Chaperones are therefore widely considered as cellular folding and assembly factors. In recent years, however, an active involvement of molecular chaperones in protein degradation became apparent [7–9]. Again, this activity relies on the ability of chaperones to selectively associate with non-native proteins (or inactive conformers of signaling proteins). However, instead of being directed onto a folding pathway (or getting activated), the chaperone-associated protein is presented to the degradation machinery in this situation. The emerging role as facilitators of protein degradation represents a significant extension of previous concepts regarding chaperone function [8,10]. Chaperone binding to a non-native protein initially prevents aggregation and subsequent triage decisions towards folding as well as degradation occur in association with the chaperone machinery (Fig. 1a). As we open a new chapter of cell biology many questions arise. How are folding and degradation activities regulated? What are the cellular processes that critically depend on chaperone-assisted degradation? Are alterations of the involved components related to human diseases and ageing? Answers to these questions are currently emerging and are discussed here.

* Corresponding author.

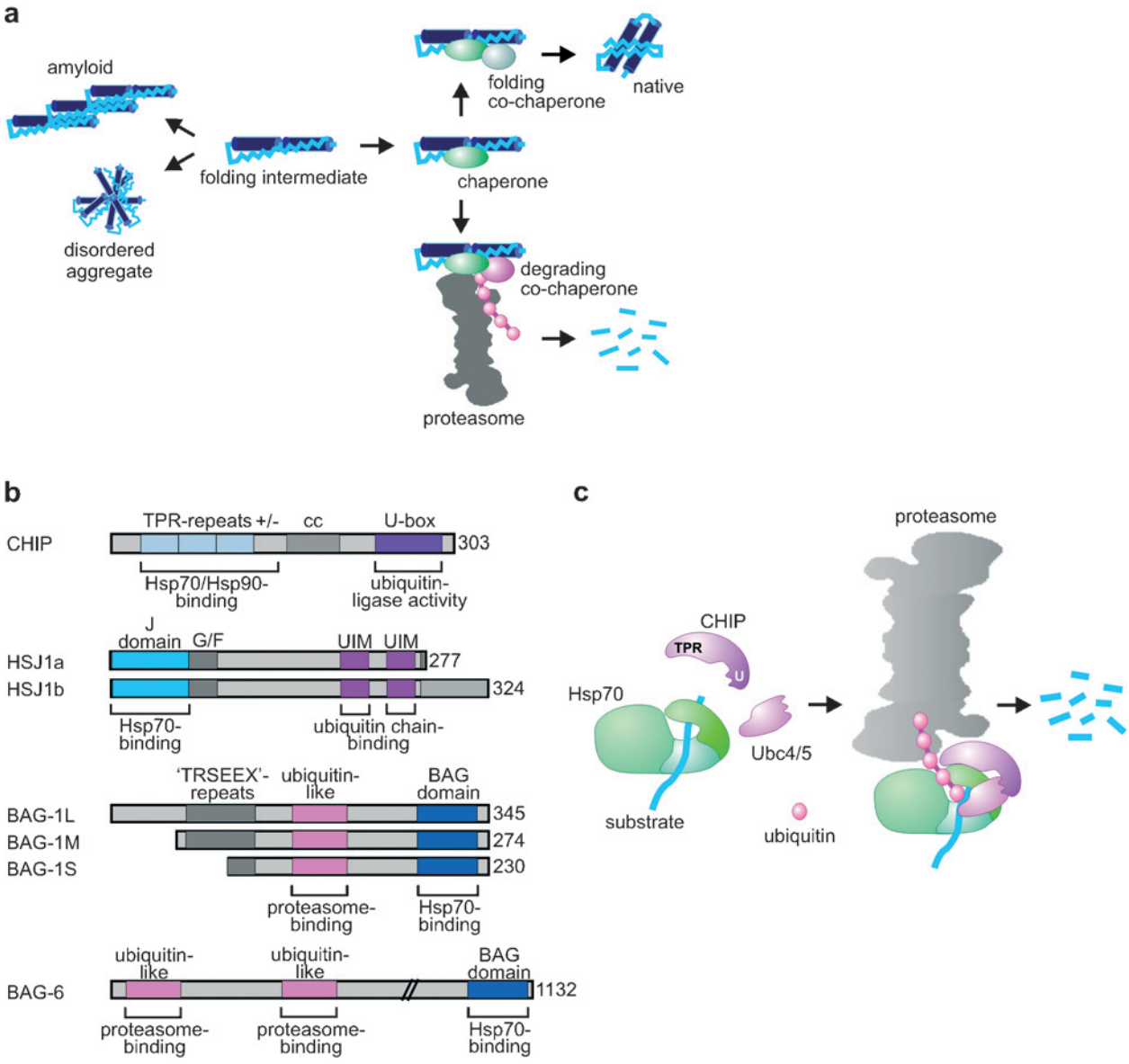

Figure 1. (*a*) Model of chaperone-mediated protein triage. Aggregation of a folding intermediate, leading to the formation of disordered aggregates or disease-associated amyloid fibers, is prevented by binding of the chaperone that subsequently mediates folding to the native state or targeting to the proteasome for degradation through a cooperation with distinct co-chaperones. (*b*) Co-chaperones that link chaperone function to the ubiquitin/proteasome system. CHIP utilizes a TPR domain and a subsequent highly charged region (+/–) for contacting Hsp70 and Hsp90. A coiled coil domain mediates dimerization (cc) and the U-box is essential for the ubiquitin ligase function. The HSJ1 escort factor is expressed as two alternatively spliced isoforms, which differ at their C termini. The ATP-hydrolysis stimulating J-domain is followed by a glycine/phenylalanine rich region (G/F) and two UIM domains. The latter are used for ubiquitin chain binding. At least three isoforms of BAG-1 are expressed in human cells. All contain an integrated ubiquitin-like domain for proteasome binding and a BAG domain for contacting Hsp70. BAG-6 may participate in chaperone-assisted degradation because it possesses two ubiquitin-like domains, which both contribute to proteasome binding of BAG-6, and a BAG domain. (*c*) Schematic presentation of CHIP-mediated degradation involving Hsp70. CHIP binds to the C terminus of Hsp70 using its TPR domain and recruits Ubc4/5 family members *via* its U-box. This leads to ubiquitin chain formation on the chaperone bound substrate, targeting to the proteasome and substrate degradation. During sorting ubiquitylation of Hsp70 may facilitate docking at the proteasome (not shown).

Background on molecular chaperones

Molecular chaperones comprise diverse groups of proteins. In this review we focus mainly on the Hsp70 and Hsp90 families, both of which are involved in chaperone-assisted degradation in mammalian cells (see Table 1 for abbreviations) [8]. The activity of Hsp70 and Hsp90 chaperones relies on similar functional principles. In both cases a cycle of ATP binding and hydrolysis gives rise to a dynamic cycle of substrate binding and release [2, 11]. Moreover, both chaperones cooperate with a plethora of co-

chaperones that regulate the ATPase cycle, assist substrate loading onto the chaperones, and recruit Hsp70 and Hsp90 to diverse protein complexes and subcellular locations. The engagement of Hsp70 or Hsp90 in a specific cellular process thus depends on its cooperation with a distinct set of co-chaperones. It is the assembled chaperone/co-chaperone complex rather than the chaperone alone that forms the functional entity in cells. Accordingly, our understanding of chaperone-assisted degradation is based on the characterization of certain co-chaperones.

Co-chaperones involved in chaperone-assisted degradation

In the last couple of years several co-chaperones were identified that link chaperone function to the ubiquitin/proteasome system [8, 10, 12, 13]. The latter provides the main degradation pathway for the removal of misfolded proteins and short-lived regulatory proteins in eukaryotes. A protein doomed for degradation is modified by attachment of a chain of ubiquitin moieties, targeted to the proteasome, and cleaved into peptide fragments [13–15]. Recognition of the substrate protein depends on the concerted action of a ubiquitin-conjugating enzyme (E2) and a ubiquitin ligase (E3). The two enzymes receive activated ubiquitin from the ubiquitin-activating enzyme (E1) and jointly transfer the ubiquitin moiety onto lysine residues of the substrate protein. In some instances, additional factors are involved to modulate the length of the formed ubiquitin chain [16–19]. Moreover, several ubiquitin binding proteins and ubiquitin domain-containing proteins were recently shown to act as escort factors during sorting to the proteasome and as adaptors to facilitate proteasomal binding [12, 13, 20–23]. Dedicated co-chaperones can operate at each step of chaperone-assisted sorting to the proteasome: initial recognition, substrate delivery and docking at the proteolytic complex.

Labeling chaperone substrates for degradation depends on the activity of chaperone-associated ubiquitin ligases. The key component is here certainly the C terminus of Hsp70-interacting protein CHIP. While initially identified as a binding partner of Hsp70, CHIP also binds to Hsp90 [24, 25]. Chaperone binding is mediated by an N-terminal tetratricopeptide repeat (TPR-) domain in conjunction with a highly charged region (Fig. 1b). Moreover, CHIP possesses a U-box that enables the co-chaperone to interact with ubiquitin-conjugating enzymes, such as human Ubc4/5 family members [26–29]. CHIP and its partner Ubc mediate the ubiquitylation of chaperone substrates that are presented by Hsp70 or Hsp90, thereby initiating sorting to the proteasome and degradation [24, 30]. Figure 1c illustrates the cooperation of Hsp70 and CHIP on such a degradation pathway. The chaperone/CHIP/Ubc complex may actually be viewed as a multi-subunit ubiquitin ligase complex, in which the chaperone acts as the main substrate recognition factor [8]. This does not exclude, however, that CHIP itself transiently associates with the substrate. Indeed, CHIP is able to modify some model substrates even in the absence of Hsp70 or Hsp90 with low efficiency [26]. Direct recognition by CHIP may contribute to the selection of substrates during chaperone-assisted degradation.

Chaperone substrates affected by CHIP can be broadly divided into two subfamilies: (i) proteins that undergo conformational changes in association with Hsp70 and/or Hsp90 as part of their cellular regulation such as the glucocorticoid hormone receptor and the oncogenic receptor tyrosine kinase ErbB2 [24, 31], and (ii) aggregation-prone proteins that are recognized by the chaperones during protein quality control (see Table 2) [30, 32]. Among the latter substrates are immature cystic fibrosis transmembrane conductance regulator (CFTR), mutations in which cause cystic fibrosis [30, 33, 34], and hyperphosphorylated tau that accumulates in patients with Alzheimer's disease [32, 35, 36]. With regard to protein quality control, the characterization of CHIP answers the long-standing question, as to how the ubiquitin/proteasome system recognizes non-native proteins for degradation [10]. Molecular chaperones that are anyway streamlined for the recognition of such proteins are directly employed by the ubiquitin conjugation machinery. Chaperone-associated ubiquitin ligases such as CHIP switch chaperone activity from protein folding to protein degradation.

The co-chaperone HSJ1 recently emerged as an escort factor for chaperone substrates during proteasomal sorting in neuronal cells [12]. HSJ1 belongs to the J-domain co-chaperone family, members of which stimulate ATP hydrolysis of Hsp70, driving the chaperone into a high-affinity state for substrate binding [2]. J-domain co-chaperones therefore play pivotal roles in the loading of substrates onto Hsp70 family members. This often involves a direct interaction of the J-domain protein with the substrate based on an intrinsic ability to recognize non-native proteins [2]. The particular feature of the HSJ1 protein is the combination of a J-domain with two ubiquitin interaction motifs (UIMs) (Fig. 1b) [12]. The latter are utilized for binding to polyubiquitin chains on chaperone substrates after their initial encounter with the CHIP/Ubc5 conjugation machinery. The combined activity as a substrate loading factor of Hsp70 and ubiquitin chain binding protein

Table 1. Abbreviations and glossary.

Abbreviation	Explanation
BAG-1, Bcl-2-associated athanogene 1	BAG-1 was initially identified as a binding partner of the apoptosis inhibitor Bcl-2. Subsequently, its role as a co-chaperone of Hsp70 was established. Binding to Hsp70 is mediated by a conserved BAG domain that is also present in five other proteins in humans (BAG-2 to 6). The domain enables the co-chaperones to stimulate nucleotide exchange during the ATPase cycle of Hsp70. BAG-1 binds to the proteasome *via* an integrated ubiquitin-like domain and stimulates CHIP mediated degradation.
BAG-2, Bcl-2-associated athanogene 2	BAG-2 inhibits the CHIP ubiquitin ligase upon binding to the Hsp70/CHIP complex.
BAG-5, Bcl-2-associated athanogene 5	BAG-5 interacts with Parkin and inhibits its ubiquitin ligase activity.
CFTR, cystic fibrosis transmembrane conductance regulator	CFTR is an ion channel of central importance in lung and pancreas. Mutations in CFTR cause cystic fibrosis. During maturation in the endoplasmic reticulum the folded state of large cytoplasmic domains of the ion channel is controlled by the Hsp70 and Hsp90 chaperone systems.
CHIP, C terminus of Hsp70 interacting protein	CHIP is a ubiquitin ligase that interacts with Hsp70 and Hsp90 through binding to the C termini of the two chaperones. It switches chaperone activity from protein folding to protein degradation.
DALIS, dendritic cell aggresome-like induced structures	DALIS are protein aggregates that transiently form in dendritic cells after induced maturation. DALIS contain ubiquitylated polypeptides and are considered as processing sites for antigenic peptides.
Hip, Hsp70 interacting protein	Hip utilizes a TPR domain for binding to the ATPase domain of Hsp70. It stabilizes the substrate-binding ADP state of the chaperone and stimulates Hsp70-mediated protein folding.
Hop, Hsp70/Hsp90 organizing protein	Hop possesses separate TPR domains for binding to Hsp70 and Hsp90. This enables the co-chaperone to couple both chaperones.
HSJ1, Homo sapiens J domain protein	HSJ1 belongs to the family of J domain co-chaperones that stimulate ATP hydrolysis of Hsp70 family members. HSJ1 is primarily expressed in neuronal cells and binds ubiquitylated chaperone substrates through two UIM domains.
Hsp70, 70-kDa heat shock protein	Two main Hsp70 family members exist in the mammalian cytoplasm and nucleus: the constitutively expressed heat shock cognate protein Hsc70 (also termed Hsp73) and the stress inducible Hsp70 (also termed Hsp72). For sake of simplicity we use the term Hsp70 here to refer to both family members.
Hsp90, 90-kDa heat shock protein	Hsp90 acts subsequent to Hsp70 on many folding pathways. It has a prominent role in the regulation of cell signaling and is currently explored as a potential drug target in cancer and tauopathies.
HspBP1, Hsp70 binding protein 1	HspBP1 is a nucleotide exchange factor of Hsp70, which binds to the ATPase domain of the chaperone in a manner competitive to BAG-domain co-chaperones and Hip. It inhibits the ubiquitin ligase activity of CHIP upon binding to an Hsp70/CHIP complex.
TPR, tetratricopeptide repeat domain	The TPR repeat comprises an alpha helical 34-amino acid motif that forms a knob and hole structure, ideally suited for mediating protein/protein interactions. TPR domains comprising three consecutively arranged repeat motifs are often found in co-chaperones as chaperone-binding sites.
U-box, UFD2 homology domain	The U-box is a cysteine-containing protein domain that resembles RING finger domains, but lacks a coordinated Zn ion. U-boxes are found in E3 ubiquitin ligases and E4 ubiquitin chain assembly factors.
Ubc4/5, ubiquitin conjugating enzymes of the Ubc4/5 family	Ubc4/5 family members cooperate with CHIP in the ubiquitylation of chaperone substrates.
UFD2, ubiquitin-fusion degradation 2	UFD2 is a U-box containing protein, which was initially identified in yeast, and mediates ubiquitin chain assembly in conjunction with certain ubiquitin conjugating enzymes and ubiquitin ligases.
UIM, ubiquitin interaction motif	UIM domains are used for ubiquitin and ubiquitin chain binding in diverse proteins, including receptors of the proteasome and escort factors.
UNC-45, uncoordinated – 45	UNC-45 is a myosin-specific co-chaperone of Hsp90, which was identified in a genetic screen in *C. elegans* for gene products necessary for coordinated movement.

makes HSJ1 ideally suited to accompany chaperone substrates during sorting to the proteasome. The chaperone/substrate complex is a very dynamic assembly that is likely to undergo ATP-induced dissociation during the sorting process. HSJ1 could stabilize the ubiquitylated substrate upon release from Hsp70 because of its intrinsic chaperone activity and due to UIM-mediated interactions, and could facilitate reloading onto Hsp70 through its J-domain. In this way, HSJ1 would ensure Hsp70 engagement until docking

at the proteasome is achieved. The provided escort function might be of particular importance in post-mitotic neuronal cells to prevent cytotoxic protein aggregation. In fact, HSJ1 cooperates with CHIP in the proteasomal degradation of pathological forms of the Huntingtin protein, which cause neurodegeneration upon accumulation [12, 37].

With regard to the docking step the co-chaperone BAG-1 seems to exert important functions that, again, rely on a unique combination of functional protein domains. BAG-1 possesses a BAG-domain for binding to Hsp70 and an integrated ubiquitin-like domain for contacting the proteasome (Fig. 1b) [38–40]. The domain arrangement allows BAG-1 to facilitate chaperone/proteasome interactions and to stimulate the CHIP-mediated degradation of certain chaperone substrates, such as the glucocorticoid hormone receptor [26]. The degradation-stimulating activity may also in part rely on the Hsp70-regulating activity of BAG-1. The co-chaperone acts as a nucleotide exchange factor and triggers the release of substrates from Hsp70 [38, 40, 41]. This might be of critical importance for the insertion of chaperone substrates into the catalytic core of the proteasome.

The characterization of CHIP, HSJ1, and BAG-1 illustrates mechanistic principles of how co-chaperones can exert chaperone-regulating activities at different stages during sorting to the proteasome. A cooperation of CHIP with HSJ1 and BAG-1, respectively, has been demonstrated and relies on the usage of different docking sites on Hsp70 by the co-chaperones [12, 26]. While CHIP binds to the C-terminal EEVD motif of Hsp70, BAG-1 associates with the N-terminal ATPase domain (Fig. 2). The binding region of HSJ1 on Hsp70 has not yet been defined, but most likely involves the interface of the ATPase and peptide binding domains of Hsp70 in agreement with findings for other J proteins [2]. It remains to be established, however, whether CHIP, BAG-1 and HSJ1 cooperate on one sorting pathway. In this regard a specificity of HSJ1 and BAG-1 for certain chaperone substrates has to be considered [12, 26]. Moreover, additional proteins, whose functions seem to overlap with those of CHIP and BAG-1, appear to assist molecular chaperones during proteasomal sorting. The fact that chaperone-assisted degradation proceeds normally in cells that lack CHIP, indeed, points to the existence of other ubiquitin ligases that can cooperate with Hsp70 and Hsp90 [31]. A possible candidate in this regard is the ubiquitin ligase Parkin, mutations of which cause early onset Parkinson's disease [42, 43]. Parkin was found to associate with Hsp70 and CHIP and may therefore represent another entry point for the targeting of certain chaperone substrates to the proteasome [44].

At the proteasome itself, components related to BAG-1 may assist substrate delivery and transfer. BAG-1 actually belongs to a heterogeneous family of multi-domain co-chaperones that all carry an Hsp70-binding BAG domain in conjunction with diverse other protein interaction motifs [45]. Notably, BAG-6 (also known as BAT-3 or Scythe) possesses two integrated ubiquitin-like domains utilized for docking at the proteasome (Fig. 1b) [46]. BAG-6 may therefore facilitate Hsp70/proteasome cooperation on certain chaperone-assisted degradation pathways.

Apparently, chaperone-assisted degradation does not rely on just one well-defined pathway, but rather comprises a multitude of pathways involving diverse sets of Hsp70 and Hsp90 regulators that are recruited from a large co-chaperone network. Pathways may vary depending on the chaperone-bound substrate, the cell type or the physiological situation of the cell. We probably still underestimate the diversity of these degradation pathways. Additional degradation regulating co-chaperones are likely to be identified in the near future because our knowledge about protein domains involved in chaperone binding and in the regulation of ubiquitin-mediated protein sorting is increasing very rapidly at the moment.

Inhibitors of chaperone-assisted degradation

Co-chaperones not only facilitate chaperone-assisted degradation, but also control and restrict degradation functions of molecular chaperones. The Hsp70 co-chaperones HspBP1, BAG-2 and BAG-5 exert such control functions. BAG-5 was found to interact directly with Parkin and to abrogate its ubiquitin ligase activity [47]. HspBP1 and BAG-2 are able to bind to the Hsp70/CHIP complex through recognition of the ATPase domain of Hsp70, and inhibit CHIP-mediated ubiquitylation upon binding [48–50]. In the case of BAG-2, inhibition involves an abrogation of the interaction between CHIP and its partner ubiquitin conjugating enzyme (Fig. 2b). As a consequence a chaperone/co-chaperone complex is formed, which still contains CHIP, but the ubiquitin ligase is no longer able to exert its destructive activity. In such a complex, CHIP may participate in the regulation of the ATPase cycle of Hsp70 and engage in transient interactions with the chaperone substrate, consistent with an observed stimulating activity of CHIP in the refolding of certain substrates [51].

Remarkably, HspBP1 and BAG-2 were both found to inhibit the CHIP-mediated degradation of the CFTR ion channel [48, 50]. The folding and assembly of the ion channel in the endoplasmic reticulum membrane

Table 2. Known substrate proteins of the CHIP ubiquitin ligase. Substrates include proteins that undergo conformational regulation in association with Hsp70 and/or Hsp90, and aggregation-prone proteins that are subjected to Hsp70/Hsp90-mediated protein quality control.

Substrates of CHIP	Function	Disease-related	Ref.
Conformational regulation			
AhR (aryl hydrocarbon receptor)	Metabolism	Cancer, cleft palate, primordial follicles	[138]
AR (androgen receptor)	Differentiation	Cancer	[139]
ASK-1 (apoptosis signal-regulating kinase 1)	Apoptosis	Cancer	[140]
DAPK (death associated protein kinase)	Apoptosis	Cancer	[141]
DLK (dual leucine zipper-bearing kinase)	Differentiation, wound healing		[142]
DR (dioxine receptor)	Xenobiotic metabolism	Cancer	[143]
E2A (elegantin isoform 2a)	Lymphocyte development	Cancer	[144]
Epsin (epidermal growth factor pathway substrate interacting)	Endocytosis	Schizophrenia	[145]
ERα (estrogen receptor α)	Differentiation, growth factor signalling	Breast Cancer	[146,147]
ERß	Differentiation, growth factor signalling	Breast Cancer	[148]
ErbB2 (avian erythroblastosis oncogene B like receptor 2)	Differentiation, proliferation	Cancer	[31]
GR (glucocorticoid receptor)	Metabolism	Lateral sclerosis	[24]
hEST1B (human ever-shorter telomeres 1B)	Ageing	Cancer	[149]
nNos (neuronal nitric-oxide synthase)	Oxidative stress response	Myocardial disease, asthma	[150]
NPM-ALK (nucleophosmin-anaplastic lymphoma kinase)	Differentiation, proliferation, survival	Human anaplastic large cell lymphomas	[151]
p53	Tumour suppressor	Cancer	[82]
Proto Dbl (diffuse B-cell lymphoma)	Signalling pathway	Cancer	[152]
Ron (recepteur d'origine nantais)	Migration, proliferation	Cancer	[153]
SGK-1 (serum- and glucocorticoid-regulated kinase-1)	Cell survival	Diabetes mellitus, cancer	[154]
Quality control			
α-synuclein	Synaptic function	Parkinson, DLB, multiple system atrophy	[155]
ATX1 (ataxin-1)		Spinocerebellar ataxia type 1	[156]
ATX3 (ataxin-3)		Spinocerebellar ataxia type 3	[157]
ßAPP (ß-amyloid-precursor-protein)	Cell adhesion, synaptogenesis	Alzheimer	[114]
CFTR (cystic fibrosis transmembrane conductance regulator)	Ion transport	Cystic Fibrosis	[30]
Huntingtin	Development	Huntington	[157]
NR2A (N-methyl-D-aspartate receptor subunit 2A)	Neuronal survival	Stroke	[158]
Pael-R (Parkin associated endothelin-receptor like receptor)		Parkinson	[159]
SOD1 (Cu/Zn super oxide dismutase1) (mutant form)	Oxidative stress response	Amyotrophic lateral sclerosis	[160]
Tau	Cytoskeletal organization	Alzheimer, tauopathies	[32,35]

is slow, taking about 10 min in most cell types tested, and very inefficient with only about 30 % of CFTR reaching its final destination in the plasma membrane [52–54]. The rate-limiting step in CFTR biogenesis appears to be the folding of the second of two cytoplasmic nucleotide binding domains (NBD2) [55], which is subjected to Hsp70/CHIP-mediated quality control [53]. Employing CHIP inhibitors at this stage apparently ensures that inefficient folding proceeds to completion at least for a fraction of CFTR

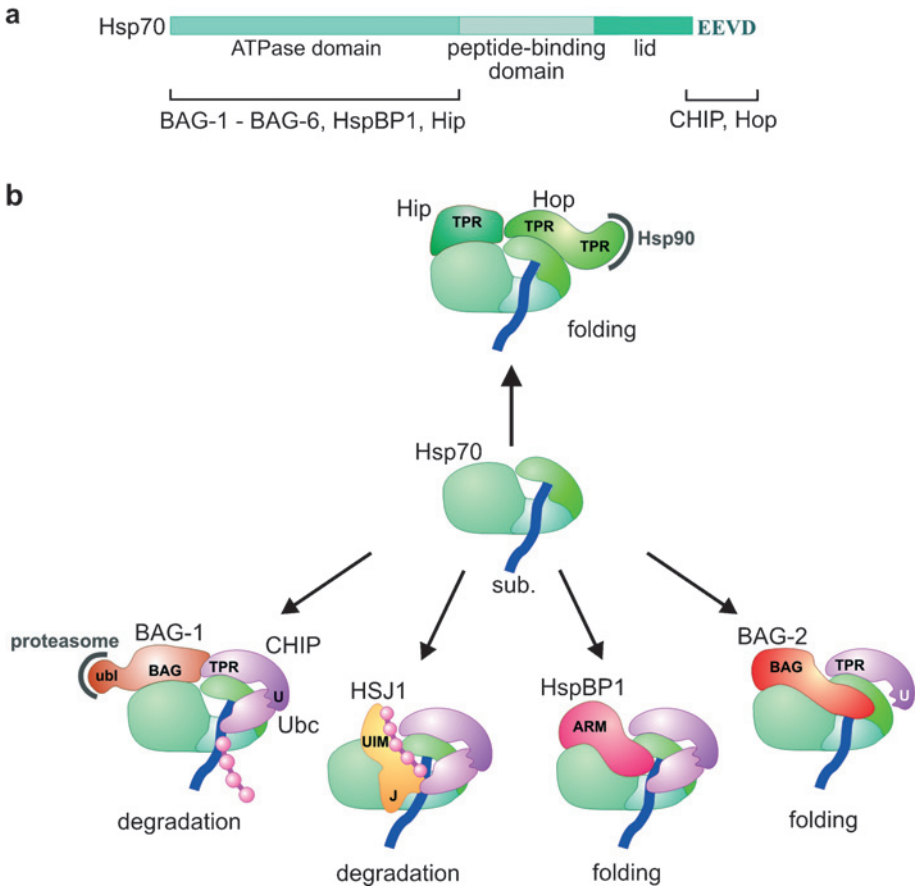

Figure 2. (*a*) Domain arrangement of Hsp70 and co-chaperone binding sites. The co-chaperones BAG-1 to BAG-6, HspBP1 and Hip all bind to the N-terminal ATPase domain of Hsp70 in a competitive manner. CHIP and Hop utilize the C-terminal peptide EEVD for binding. (*b*) Schematic presentation of the co-chaperone network that determines folding and degradation activities of Hsp70. Competitive and cooperative binding of co-chaperones gives rise to functionally distinct Hsp70 complexes. BAG-1 and Hop provide links to the proteasome and Hsp90, respectively. HSJ1 utilizes its UIM domains to bind to the ubiquitin chain assembled on the chaperone substrate, and facilitates substrate reloading onto Hsp70 through its J domain. HspBP1 inhibits the ubiquitin ligase activity of CHIP, most likely by shielding ubiquitin attachment sites within the assembled chaperone complex. BAG-2 acts as an CHIP inhibitor by abrogating the CHIP/Ubc interaction (TPR, tetratricopeptide repeat domain; sub., substrate protein; ubl, ubiquitin-like domain; BAG, BAG domain; U, U-box; UIM, ubiquitin interactions motif; J, J domain; ARM, armadillo repeat).

molecules, despite the presence of the chaperone-associated ubiquitin ligase.

HspBP1 and BAG-2 both utilize the ATPase domain of Hsp70 as the docking site and thus compete with the degradation stimulating co-chaperone BAG-1 in the regulation of the Hsp70/CHIP complex (Fig. 2) [48, 50]. Moreover, the ATPase domain can also be occupied by the Hsp70-interacting protein Hip, which assists Hsp70 in folding reactions [56]. Although about five to ten times more abundant than BAG-1 or BAG-2, Hip is not detectable in CHIP complexes isolated from human cells, suggesting steric interference between the binding of Hip to the ATPase domain and CHIP binding to the C terminus of Hsp70 [48]. The access of CHIP to its partner chaperones is further restricted by the presence of other TPR-domain containing co-chaperones that, like CHIP, bind to the C terminus of either Hsp70 or Hsp90. Among these CHIP 'competitors' is the Hsp70/Hsp90 organizing protein Hop, which facilitates the cooperation of both chaperones during protein folding and during the conformational regulation of signaling proteins [24, 57–59]. Preventing CHIP binding to Hsp70 or Hsp90 is apparently a second strategy, besides inhibition within the assembled chaperone/co-chaperone complex, to control the degradation-inducing activity of the ubiquitin ligase. This emphasizes again the importance of the co-chaperone network for determining folding and degradation functions. The concerted action of competing and cooperating co-chaperones gives rise to functionally distinct chaperone machines. In Figure 2b we try to illustrate this for Hsp70/co-chaperone complexes. It is an intriguing possibility that some of the shown complexes even give rise to higher order assemblies. Several of the involved co-chaperones, including Hip, CHIP and BAG-2, are known to

dimerize and may thus contact two Hsp70 s at a time [48, 60, 61].

The balance between chaperone-assisted folding and degradation seems to depend to a significant extent on the intracellular concentration of the different co-chaperones. Under normal growth conditions folding stimulating factors are more abundant than degradation inducing factors [8], suggesting that chaperone-assisted folding is usually favored over chaperone-assisted degradation. However, the binding affinity of each co-chaperone for its partner chaperone also has to be considered in this regard as it influences the abundance of certain chaperone/co-chaperone complexes. Accordingly, alterations in protein homeostasis could be achieved by changes of co-chaperone expression or by posttranslational modifications of co-chaperones, which affect chaperone binding. Physiological stimuli that alter co-chaperone expression largely remain to be elucidated. Indeed, none of the co-chaperones described above is strongly up-regulated under heat shock conditions [25, 38, 56, 62]. Posttranslational modification, *i.e.*, phosphorylation, has been observed for the CHIP inhibitor BAG-2 and the Parkin inhibitor BAG-5, but consequences for ubiquitin ligase inhibition and chaperone binding have not been established [63, 64]. Apparently, much remains to be learned about the regulation of chaperone-assisted degradation.

The utilized ubiquitin code

Sorting to the proteasome is typically initiated by the attachment of lysine 48 (K48) linked ubiquitin chains onto substrate proteins [65, 66]. In these canonical chains ubiquitin moieties are connected with each other *via* K48. However, other lysine residues of ubiquitin are also used for chain formation, leading to chains with reduced binding to the proteasome or even with degradation independent functions [65, 67–69]. The CHIP/Ubc5 conjugation machinery was shown to assemble non-canonical as well as canonical chains [28, 70, 71]. Chaperone-assisted degradation may therefore often involve sub-optimal targeting information. This information might be sufficient because the substrate protein is delivered in a partially unfolded state (the prerequisite for chaperone recognition), and might therefore be rapidly inserted into the proteolytic core of the proteasome [72]. However, chaperones and co-chaperones that cooperate with CHIP during proteasomal sorting may contribute targeting information. Indeed, CHIP was shown to ubiquitylate Hsp70, BAG-1 and HSJ1 *in vitro* and *in vivo* [12, 28, 70, 71]. In an assembled BAG-1/Hsp70/CHIP complex BAG-1 is modified by CHIP through attachment of mixed chains with a preference for K27 linkages [70]. Ubiquitylation of BAG-1 facilitates proteasome binding without inducing the degradation of the co-chaperone. Also, CHIP-mediated ubiquitylation of the constitutively expressed form of Hsp70 in the mammalian cytoplasm does not trigger the degradation of the chaperone but seems to stimulate binding to the proteasome [28,71]. The chaperone complex involved in substrate delivery may thus display multiple sorting signals that could be recognized by the multiple receptors for ubiquitin and ubiquitin chains present in the regulatory complex of the proteasome [8, 73–75]. Occupation of multiple docking sites might enable the chaperone to participate in substrate processing at the proteasome and to coordinate its own chaperone activity with that of the regulatory particle [72]. At the same time the folded state of the chaperone and associated co-chaperones may prevent their insertion into the proteolytic core, while the non-native substrate protein is processed and inserted.

Although CHIP does not trigger the degradation of constitutively expressed Hsp70 (usually termed Hsc70), it targets the stress inducible form of the chaperone for proteasomal degradation [71]. This activity was shown to be essential for the recovery of cells after heat stress, when the elevated concentration of stress-inducible Hsp70 must be reduced to the low basal level. The observed differences between the two Hsp70 s might arise from an increased formation of K48-linked chains on the stress inducible form in the presence of the CHIP conjugation machinery [71]. Attachment of the canonical sorting signal may result in prolonged binding to the proteasome and thus in sufficient processing time to initiate degradation of the chaperone. The two Hsp70 s are highly homologous but vary in their C termini, which is the region that is occupied by CHIP [25]. Subtle differences in the positioning of ubiquitin attachment sites or of the ubiquitin ligase on the chaperones seem to give rise to different chain architectures and to result in different physiological consequences of CHIP binding. Intriguingly, stress inducible Hsp70 is degraded by CHIP in heat-stressed cells only after the removal of non-native protein substrates [71]. It seems that CHIP preferentially modifies the substrate protein in a substrate-occupied chaperone complex. In stressed cells that are flooded with non-native protein substrates, this might be sufficient to prevent chaperone ubiquitylation. Under non-stressed conditions, however, ubiquitylation activity apparently extends towards chaperones and co-chaperones that cooperate with CHIP in proteasomal sorting (see above).

Members of the Ubc4/5 family are not the only ubiquitin-conjugating enzymes that cooperate with

CHIP. The uncharacterized E2Q and the heterodimeric Ubc13/Uev1a were shown to interact with the chaperone-associated ubiquitin ligase [76, 77]. The relevance of the interaction with E2Q is completely enigmatic because there are no functional data on the ubiquitin-conjugating enzyme. In contrast, Ubc13/Uev1a are well known to mediate the formation of K63-linked ubiquitin chains that fulfill degradation independent functions during endocytosis, DNA repair, ribosome stability and signaling *via* the Toll-like receptor system [65, 78]. The heterodimeric E2 can cooperate with CHIP in K63 chain formation *in vitro* [76], raising the interesting possibility that CHIP besides its degradation-inducing function also employs regulatory K63 ubiquitylation to alter the biogenesis of chaperone substrates.

Role in signaling and apoptosis regulation

Many chaperone substrates that are amenable to CHIP-mediated degradation were previously found to undergo regulatory changes of their protein conformation in association with Hsp70 and Hsp90, the classical examples being steroid hormone receptors and certain signaling kinases (Table 2) [4, 24, 26, 59, 79]. In their unactivated state these proteins are structurally labile and expose binding sites for Hsp70 and Hsp90. The molecular mechanisms underlying conformational regulation may therefore largely resemble those involved in chaperone action during protein quality control. Nevertheless, we would like to make a conceptual distinction here to illustrate that chaperone-mediated protein triage does not only represent a form of cellular waste management but also contributes to the posttranslational regulation of protein activity. Signaling proteins are initially recognized by Hsp70 in conjunction with its J-domain co-chaperones, and subsequently transferred onto Hsp90 in a manner assisted by Hop (Fig. 3). Hsp90 then teams up with its own cohort of co-chaperones (*i.e.*, p23 and diverse immunophilins) to assist final conformational changes [4, 79, 80]. In the case of the steroid receptors, this sequential interaction is required to attain a conformation with high affinity for hormone binding. Kinases such as v-src are recognized *via* their exposed catalytic domains before assembly into the final signaling complex [59]. However, the pathway should not be viewed as a one-way street. Rather, in the absence of an activation signal, the substrate is released from Hsp90, returns to its labile conformation and enters a new cycle of sequential Hsp70 and Hsp90 interaction (Fig. 3) [81]. CHIP can interfere with the activation cycle at multiple stages because of its ability to bind to Hsp70 and Hsp90, respectively. This may explain why many signaling proteins are highly sensitive to alterations of the cellular CHIP concentration (Table 2 and references therein). The chaperone-assisted degradation pathway seems to play a critical role in the degradation of signal transducers in the absence of physiological stimuli and may thus determine cellular responsiveness to many intra- and extracellular signals.

In a similar manner, CHIP might control apoptotic processes. CHIP was shown to participate in the degradation of the tumor suppressor p53 under normal growth conditions [82]. Upon stress an increased fraction of Hsp70 and CHIP will handle non-native proteins, which could in turn result in p53 accumulation, leading to an inhibition of the cell cycle and possibly entry into the apoptotic program.

There is also multiple evidence that links BAG-1 to the regulation of apoptosis. The co-chaperone was initially identified as a binding partner of the death suppressor Bcl-2 and shown to display anti-apoptotic activity on its own [83]. Targeted ablation of BAG-1 in mice results in embryonic lethality due to increased apoptosis of neuronal and hematopoietic cells, supporting a central function as an apoptosis regulator [84]. Moreover, BAG-1 is frequently overexpressed in human cancers, in particular breast cancer, and seems to contribute to oncogenic transformation [85–89]. Indeed, elevated levels of BAG-1 protect breast cancer cell lines against apoptosis and stress-induced growth inhibition [86, 88]. Protection requires functional Hsp70- and proteasome-binding sites on BAG-1, suggesting that apoptosis regulation relies on induced chaperone/proteasome coupling and probably chaperone-assisted degradation. Although the critical chaperone substrates in this context remain to be identified, BAG-1 emerges as a potential molecular target for cancer treatment.

Intriguingly, drugs that alter the balance between chaperone-assisted folding and chaperone-assisted degradation have already entered clinical trials as anti-cancer agents. They are directed against Hsp90 and include derivatives of the ansamycin antibiotic geldanamycin, the antifungal antibiotic radicicol and coumarin antibiotics such as novobiocin [90–92]. All these compounds inhibit the ATP-driven peptide binding and release cycle of Hsp90. Most notably, chaperone inhibition results in the rapid proteasomal degradation of Hsp90 substrates, including key regulators of cell proliferation and apoptosis such as Raf-1, mutant p53 and ErbB2 [93]. It is the induced degradation of these proteins that apparently explains the anti-tumor activity of the Hsp90 inhibitors. In the presence of the inhibitors the Hsp70/Hsp90-mediated activation pathway appears to be blocked at an intermediate stage when

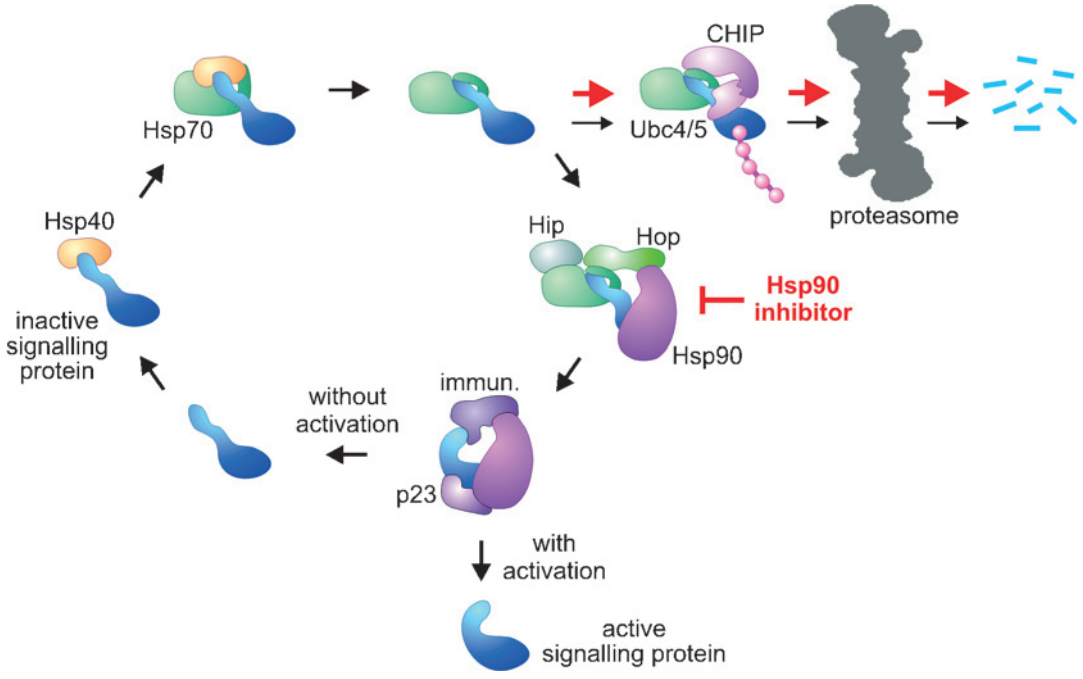

Figure 3. Cooperation of Hsp70 and Hsp90 during the conformational regulation of signal transducers and apoptosis regulators. The structurally labile, inactive protein undergoes transient interactions with Hsp70 and Hsp90 facilitated by their co-chaperones. This leads to an activatable conformation. In the absence of an activation signal the substrate protein is released, returns to its initial labile conformation and enters a new cycle of chaperone binding. CHIP is able to direct the substrate onto a degradation pathway, thus influencing cellular responsiveness (small arrows). In the presence of Hsp90 inhibitors the activation cycle is interrupted, leading to increased degradation (red arrows). A similar chaperone pathway seems to influence the biogenesis of the tau protein.

the substrate protein is primarily associated with Hsp70 (Fig. 3) [31]. This is followed by substrate sorting to the proteasome in a manner involving CHIP and probably other chaperone-associated ubiquitin ligases. Other ligases have to be invoked because the degradation of chaperone substrates proceeds normally in response to Hsp90 inhibition in cells that lack CHIP [31]. Although these ligases remain to be identified, the findings illustrate how important it is also from a clinical perspective to elucidate molecular mechanisms that operate at the folding/degradation interface.

Despite the high concentration of Hsp90 in all cell types, tumor cells are particularly prone to Hsp90 inhibition [94]. In those cells the chaperone displays a 100-fold higher affinity for drugs, such as geldanamycin, which target the ATPase domain. Indeed, tumor Hsp90 displays increased ATPase activity and is present entirely in multi-protein complexes comprising co-chaperones and chaperone substrates [94]. Because malignant progression results in the accumulation of mutant and overexpressed signaling proteins, Hsp90 seems to become increasingly engaged in the chaperoning of oncoproteins. This may explain the observed alterations of the Hsp90 machinery in tumor cells and the increased sensitivity towards drug-mediated inhibition.

Impact on neurodegeneration

The biomedical relevance of research into chaperone-assisted degradation becomes even more apparent when one looks at neurodegenerative diseases that are caused by the accumulation of abnormal proteins, including prion and polyglutamine diseases, and Parkinson's and Alzheimer's disease [95–98]. These diseases often involve an age-dependent or mutation-induced impairment of protein quality control, leading to the formation of amyloid protein aggregates and neuronal cell death. Contrary to decades of speculation, accumulating evidence suggests that visible aggregates are not the cause of toxicity; rather soluble intermediates that form during the aggregation process seem to be the major culprits [99–101]. Chaperone-based quality control systems can deal with such toxic intermediates in multiple ways and, accordingly, chaperones and co-chaperones are among the most potent suppressors of neurodegeneration known for animal models of human diseases [96, 102–104]. Binding of chaperones to toxic intermediates was shown to prevent detrimental interactions with other proteins essential for cell viability and is often accompanied by conformational changes that interfere with amyloid formation [99, 105]. Targeting of intermediates for proteasomal degradation represents

another rescue strategy, as is illustrated by the function of the neuronal co-chaperone HSJ1, which cooperates with CHIP in the proteasomal sorting of polyglutamine expanded Huntingtin in cellular models of Huntington's disease [12]. Furthermore, CHIP has a major impact on the aggregation and degradation of the microtubule- and actin-binding protein tau [32, 35, 106, 107]. Hyperphosphorylated forms of tau accumulate as intracellular tangles in brains of Alzheimer patients, and tau abnormalities are the primary cause of another neurodegenerative disease, frontotemporal dementia with parkinsonism linked to chromosome 17 (FTDP-17) [36, 108–110]. CHIP cooperates with Hsp70 and Hsp90 in the ubiquitylation and proteasomal degradation of tau, whereby hyperphosphorylated, pathological forms are preferentially recognized [32, 35, 111, 112]. Accordingly, both homo- and heterozygous CHIP knockout mice were shown to have increased levels of insoluble tau in the brain [113]. It is interesting to note, however, that an independently generated CHIP-deficient mouse displayed elevated levels of hyperphosphorylated tau without aggregate formation [107]. This may indicate that CHIP-mediated ubiquitylation of tau facilitates not only degradation but also aggregation. Varying concentrations of escort factors, such as HSJ1, which act after ubiquitylation to promote sorting to the proteasome might be invoked to explain the observed differences. In any case, both, triggered aggregation as well as degradation, would prevent neuronal cell death, because soluble hyperphosphorylated tau is apparently the toxic entity [35].

In remarkable analogy to the pharmacological modulation of cancer-relevant chaperone pathways, Dickey and coworkers [112] recently validated the use of Hsp90 inhibitors for alleviating tau pathology. They identified a low molecular weight Hsp90 inhibitor that can cross the blood-brain barrier and causes the proteasomal degradation of hyperphosphorylated tau in a mouse disease model. The chaperone/co-chaperone network that handles hyperphosphorylated tau was found to resemble the machinery involved in the conformational regulation of signaling proteins, including the Hsp70/Hsp90 organizing co-chaperone Hop, Hsp90 itself and its co-chaperone p23 [112]. The Hsp90 heterocomplex may provide a platform for tau dephosphorylation and refolding. Interfering with this activity causes CHIP-mediated degradation of tau (Fig. 3). Again, altering the balance of chaperone-assisted folding and degradation emerges as a promising therapeutic strategy.

The biogenesis of tau is interconnected with the trafficking and processing of the β-amyloid precursor protein (βAPP), which gives rise to β-amyloid (Aβ)-positive extracellular plaques in Alzheimer patients [36, 108, 109]. Intriguingly, recent data reveal that CHIP associates with βAPP in brain and cultured cells, and triggers the proteasomal degradation of Aβ, thereby reducing neuronal cytotoxicity [114]. The findings emphasize the neuroprotective function of chaperone-assisted degradation.

Role in muscle cells

CHIP is highly expressed in skeletal muscle and heart [25]. Muscle cells seem to represent a particularly hostile environment for protein homeostasis, involving contraction-induced heat stress and increased oxidative stress because of high energy consumption and mitochondrial activity. Chaperones provide a major line of defense against these insults [115, 116]. Indeed, expression of Hsp70 is elevated during physical exercise and expression levels correlate with exercise intensity in human beings and animal models [117]. In addition, the chaperone machinery exerts protective functions through a modulation of apoptotic responses under ischemic conditions when oxygen supply is insufficient. Notably, BAG-1 expression is up-regulated in cardiomyocytes under such conditions, and BAG-1 and CHIP were both found to protect cardiomyocytes against apoptosis in cellular models of ischemia and reperfusion injury [118, 119]. Furthermore, CHIP-deficient mice display increased mortality following heart injury, related to an increased incidence of arrhythmias during reperfusion and increased infarct size [118]. CHIP deficiency also led to a strong elevation of BAG-1 levels in cardiomyocytes, possibly as a compensatory response. These data strongly suggest a cooperation of CHIP and BAG-1 during cardioprotection. Whether it involves an induced proteasomal degradation of chaperone substrates remains to be explored, however, because CHIP fulfils a degradation-independent function in the regulation of the heat-shock transcription factor HSF1 and is therefore generally required for stress protection [120]. Nevertheless, direct damage to proteins is likely to occur upon myocardial injury due to mitochondrial dysfunction and generation of reactive oxygen species, resulting in an increased requirement for protein quality control [121].

Studies in *Caenorhabditis elegans* also point to an important role of CHIP in muscle development in conjunction with the myosin-associated protein UNC-45 [122]. Organization of the motor protein myosin into motile structures requires a precise temporal and spatial regulation. UNC-45 participates in this regulation by functioning as a myosin-specific co-chaperone of Hsp90 during muscle thick filament assembly

[123, 124]. Hoppe and coworkers identified *C. elegans* CHIP (known as CHN-1) as a binding partner of UNC-45 and showed that the chaperone-associated ubiquitin ligase mediates ubiquitylation of the myosin co-chaperone in conjunction with UFD2 that stimulates ubiquitin chain formation [122]. The phenotype of *unc-45* temperature-sensitive mutant worms is partially suppressed by loss of CHIP function, while UNC-45 overexpression in CHIP-deficient worms results in severe disorganization of muscle cells. The findings are consistent with a model, in which CHIP controls the function of UNC-45, and thus myosin assembly, through regulated degradation. It is likely that this activity of CHIP is not restricted to worm development but is conserved in humans and mice and may even contribute to muscle maintenance and regeneration [122]. A recent publication supports this notion by providing evidence that a human isoform of UNC-45 expressed in striated muscles, SM UNC-45, is targeted for degradation by a heterocomplex containing CHIP, UFD2, and the AAA-type chaperone CDC48/p97 [125]. Remarkably, mutations in human p97, known to cause hereditary inclusion-body myopathy, abrogate UNC-45 degradation and result in severely disorganized myofibrils. Apparently, chaperone-assisted degradation is essential for maintaining muscle cell function.

Antigen presentation

Chaperone-assisted degradation even seems to be involved in the generation of antigenic peptides that are translocated across the endoplasmic reticulum membrane and loaded onto MHC class I molecules for presentation on the cell surface [126]. In fact, siRNA-mediated depletion of CHIP in cultured cells impaired peptide loading onto MHC class I molecules [127]. Moreover, also Hsp90 has been shown to participate in antigen presentation prior to proteasomal degradation [128]. CHIP may thus cooperate with Hsp90 and possibly Hsp70 to mediate the initial targeting of polypeptides onto an antigen-presentation pathway. As a source of such polypeptides, defective ribosomal products might be considered [129, 130]. In professional immune cells, *i.e.* dendritic cells, ribosomal products transiently accumulate in protein aggregates, so-called dendritic cell aggresome-like induced structures (DALIS), after the maturation of the cells is induced [131, 132]. The aggregates resemble aggresomes that have been observed in diverse cell types upon impairment of the proteasome system [133], and contain polyubiquitylated proteins [131]. DALIS seem to define specific functional sites in dendritic cells where antigen processing occurs [132, 134]. Intriguingly, CHIP and BAG-1 are located in DALIS [134] (Schild and Höhfeld, unpublished data), pointing to the importance of the chaperone machinery for regulated antigen processing.

Concluding remarks

Chaperone-assisted degradation is not a rare curiosity but a vital and essential aspect of chaperone activity, indispensable for the posttranslational regulation of protein function and for coping with protein damage and misfolding. This new perspective has to be incorporated into current functional concepts. In evolutionary biology, for example, chaperones are widely recognized as potent and general agents of genetic robustness [135, 136]. They reduce phenotypic expression of a wide spectrum of genetic alterations. This was attributed to their ability to uphold the function of proteins that are destabilized by mutation [137]. It is tempting to speculate, however, that chaperone-assisted degradation contributes to phenotypic buffering.

Many questions remain to be answered. A particular challenging question deals with the initial recognition of chaperone substrates for degradation. Is there an active distinction between a substrate that has to be folded and one that will be degraded? Despite the high degree of organization of chaperone pathways, induced by the co-chaperone network, it is still conceivable that kinetic partitioning underlies substrate selection. A damaged protein substrate that has to be removed may undergo repeated and prolonged interactions with the chaperone machinery because of its inability to adopt a folded confirmation. In turn, the likelihood increases of encountering a chaperone complex that contains degradation inducing co-chaperones. On the other hand, many co-chaperones, including CHIP, BAG-1 and HSJ1, possess an Hsp70-independent intrinsic ability to recognize chaperone substrates. Multiple low-affinity interactions of a substrate with chaperones, dedicated co-chaperones and additional ancillary factors may thus divert a substrate from a folding onto a degradation pathway. To be or not to be that is the question.

1 Ellis, R. J. (2006) Molecular chaperones: assisting assembly in addition to folding. Trends Biochem. Sci. 31, 395 – 401.
2 Mayer, M. P. and Bukau, B. (2005) Hsp70 chaperones: cellular functions and molecular mechanism. Cell. Mol. Life Sci. 62, 670 – 684.
3 Young, J. C., Agashe, V. R., Siegers, K. and Hartl, F. U. (2004) Pathways of chaperone-mediated protein folding in the cytosol. Nat. Rev. Mol. Cell. Biol. 5, 781 – 791.
4 Picard, D. (2006) Chaperoning steroid hormone action. Trends Endocrinol. Metab. 17, 229 – 235.

5 Caplan, A. J., Mandal, A. K. and Theodoraki, M. A. (2007) Molecular chaperones and protein kinase quality control. Trends Cell Biol. 17, 87 – 92.
6 Mosser, D. D. and Morimoto, R. I. (2004) Molecular chaperones and the stress of oncogenesis. Oncogene 23, 2907 – 2918.
7 Cyr, D. M., Höhfeld, J. and Patterson, C. (2002) Protein quality control: U-box-containing E3 ubiquitin ligases join the fold. Trends Biochem. Sci. 27, 368 – 375.
8 Esser, C., Alberti, S. and Höhfeld, J. (2004) Cooperation of molecular chaperones with the ubiquitin/proteasome system. Biochim. Biophys. Acta 1695, 171 – 188.
9 Wiederkehr, T., Bukau, B. and Buchberger, A. (2002) Protein turnover: a CHIP programmed for proteolysis. Curr. Biol. 12, R26 – 28.
10 McClellan, A. J., Tam, S., Kaganovich, D. and Frydman, J. (2005) Protein quality control: chaperones culling corrupt conformations. Nat. Cell Biol. 7, 736 – 741.
11 Pearl, L. H. and Prodromou, C. (2006) Structure and mechanism of the Hsp90 molecular chaperone machinery. Annu. Rev. Biochem. 75, 271 – 294.
12 Westhoff, B., Chapple, J. P., van der Spuy, J., Höhfeld, J. and Cheetham, M. E. (2005) HSJ1 is a neuronal shuttling factor for the sorting of chaperone clients to the proteasome. Curr. Biol. 15, 1058 – 1064.
13 Hartmann-Petersen, R., Seeger, M. and Gordon, C. (2003) Transferring substrates to the 26S proteasome. Trends Biochem. Sci. 28, 26 – 31.
14 Finley, D., Ciechanover, A. and Varshavsky, A. (2004) Ubiquitin as a central cellular regulator. Cell 116, S29 – 32, 2 p following S32.
15 Varshavsky, A. (2005) Regulated protein degradation. Trends Biochem. Sci. 30, 283 – 286.
16 Koegl, M., Hoppe, T., Schlenker, S., Ulrich, H. D., Mayer, T. U. and Jentsch, S. (1999) A novel ubiquitination factor, E4, is involved in multiubiquitin chain assembly. Cell 96, 635 – 644.
17 Wilkinson, K. D. (2000) Ubiquitination and deubiquitination: targeting of proteins for degradation by the proteasome. Semin. Cell Dev. Biol. 11, 141 – 148.
18 Richly, H., Rape, M., Braun, S., Rumpf, S., Hoege, C. and Jentsch, S. (2005) A series of ubiquitin binding factors connects CDC48/p97 to substrate multiubiquitylation and proteasomal targeting. Cell 120, 73 – 84.
19 Amerik, A. Y. and Hochstrasser, M. (2004) Mechanism and function of deubiquitinating enzymes. Biochim. Biophys. Acta 1695, 189 – 207.
20 Rumpf, S. and Jentsch, S. (2006) Functional division of substrate processing cofactors of the ubiquitin-selective Cdc48 chaperone. Mol. Cell 21, 261 – 269.
21 Schmidt, M., Hanna, J., Elsasser, S. and Finley, D. (2005) Proteasome-associated proteins: regulation of a proteolytic machine. Biol. Chem. 386, 725 – 737.
22 Elsasser, S. and Finley, D. (2005) Delivery of ubiquitinated substrates to protein-unfolding machines. Nat. Cell Biol. 7, 742 – 749.
23 Raasi, S., Varadan, R., Fushman, D. and Pickart, C. M. (2005) Diverse polyubiquitin interaction properties of ubiquitin-associated domains. Nat. Struct. Mol. Biol. 12, 708 – 714.
24 Connell, P., Ballinger, C. A., Jiang, J., Wu, Y., Thompson, L. J., Höhfeld, J. and Patterson, C. (2001) The co-chaperone CHIP regulates protein triage decisions mediated by heat-shock proteins. Nat. Cell Biol. 3, 93 – 96.
25 Ballinger, C. A., Connell, P., Wu, Y., Hu, Z., Thompson, L. J., Yin, L. Y. and Patterson, C. (1999) Identification of CHIP, a novel tetratricopeptide repeat-containing protein that interacts with heat shock proteins and negatively regulates chaperone functions. Mol. Cell. Biol. 19, 4535 – 4545.
26 Demand, J., Alberti, S., Patterson, C. and Höhfeld, J. (2001) Cooperation of a ubiquitin domain protein and an E3 ubiquitin ligase during chaperone/proteasome coupling. Curr. Biol. 11, 1569 – 1577.
27 Murata, S., Minami, Y., Minami, M., Chiba, T. and Tanaka, K. (2001) CHIP is a chaperone-dependent E3 ligase that ubiquitylates unfolded protein. EMBO Rep. 2, 1133 – 1138.
28 Jiang, J., Ballinger, C. A., Wu, Y., Dai, Q., Cyr, D. M., Höhfeld, J. and Patterson, C. (2001) CHIP is a U-box-dependent E3 ubiquitin ligase: identification of Hsc70 as a target for ubiquitylation. J. Biol. Chem. 276, 42938 – 42944.
29 Xu, Z., Devlin, K. I., Ford, M. G., Nix, J. C., Qin, J. and Misra, S. (2006) Structure and interactions of the helical and U-box domains of CHIP, the C terminus of HSP70 interacting protein. Biochemistry 45, 4749 – 4759.
30 Meacham, G. C., Patterson, C., Zhang, W., Younger, J. M. and Cyr, D. M. (2001) The Hsc70 co-chaperone CHIP targets immature CFTR for proteasomal degradation. Nat. Cell Biol. 3, 100 – 105.
31 Xu, W., Marcu, M., Yuan, X., Mimnaugh, E., Patterson, C. and Neckers, L. (2002) Chaperone-dependent E3 ubiquitin ligase CHIP mediates a degradative pathway for c-ErbB2/Neu. Proc. Natl. Acad. Sci. USA 99, 12847 – 12852.
32 Petrucelli, L., Dickson, D., Kehoe, K., Taylor, J., Snyder, H., Grover, A., De Lucia, M., McGowan, E., Lewis, J., Prihar, G., Kim, J., Dillmann, W. H., Browne, S. E., Hall, A., Voellmy, R., Tsuboi, Y., Dawson, T. M., Wolozin, B., Hardy, J. and Hutton, M. (2004) CHIP and Hsp70 regulate tau ubiquitination, degradation and aggregation. Hum. Mol. Genet. 13, 703 – 714.
33 Younger, J. M., Ren, H. Y., Chen, L., Fan, C. Y., Fields, A., Patterson, C. and Cyr, D. M. (2004) A foldable CFTR(Delta)F508 biogenic intermediate accumulates upon inhibition of the Hsc70-CHIP E3 ubiquitin ligase. J. Cell Biol. 167, 1075 – 1085.
34 Knowles, M. R. (2006) Gene modifiers of lung disease. Curr. Opin. Pulm. Med. 12, 416 – 421.
35 Shimura, H., Schwartz, D., Gygi, S. P. and Kosik, K. S. (2004) CHIP-Hsc70 complex ubiquitinates phosphorylated tau and enhances cell survival. J. Biol. Chem. 279, 4869 – 4876.
36 Goedert, M. and Spillantini, M. G. (2006) A century of Alzheimer's disease. Science 314, 777 – 781.
37 Howarth, J., Kelly, S., Keasey, M., Glover, C., Lee, Y. B., Mitrophanous, K., Chapple, J., Gallo, J., Cheetham, M. and Uney, J. (2007) Hsp40 molecules that target to the ubiquitin-proteasome system decrease inclusion formation in models of polyglutamine disease. Mol Ther. 15, 1100 – 1105.
38 Höhfeld, J. and Jentsch, S. (1997) GrpE-like regulation of the hsc70 chaperone by the anti-apoptotic protein BAG-1. EMBO J. 16, 6209 – 6216.
39 Lüders, J., Demand, J. and Höhfeld, J. (2000) The ubiquitin-related BAG-1 provides a link between the molecular chaperones Hsc70/Hsp70 and the proteasome. J. Biol. Chem. 275, 4613 – 4617.
40 Sondermann, H., Scheufler, C., Schneider, C., Höhfeld, J., Hartl, F. U. and Moarefi, I. (2001) Structure of a Bag/Hsc70 complex: convergent functional evolution of Hsp70 nucleotide exchange factors. Science 291, 1553 – 1557.
41 Brehmer, D., Rudiger, S., Gassler, C. S., Klostermeier, D., Packschies, L., Reinstein, J., Mayer, M. P. and Bukau, B. (2001) Tuning of chaperone activity of Hsp70 proteins by modulation of nucleotide exchange. Nat. Struct. Biol. 8, 427 – 432.
42 Dawson, T. M. (2006) Parkin and defective ubiquitination in Parkinson's disease. J. Neural Transm. Suppl. (70), 209 – 213.
43 Kitada, T., Asakawa, S., Hattori, N., Matsumine, H., Yamamura, Y., Minoshima, S., Yokochi, M., Mizuno, Y. and Shimizu, N. (1998) Mutations in the parkin gene cause autosomal recessive juvenile parkinsonism. Nature 392, 605 – 608.
44 Imai, J., Yashiroda, H., Maruya, M., Yahara, I. and Tanaka, K. (2003) Proteasomes and molecular chaperones: cellular machinery responsible for folding and destruction of unfolded proteins. Cell Cycle 2, 585 – 590.

45 Takayama, S. and Reed, J. C. (2001) Molecular chaperone targeting and regulation by BAG family proteins. Nat. Cell Biol. 3, E237 – 241.
46 Kikukawa, Y., Minami, R., Shimada, M., Kobayashi, M., Tanaka, K., Yokosawa, H. and Kawahara, H. (2005) Unique proteasome subunit Xrpn10c is a specific receptor for the antiapoptotic ubiquitin-like protein Scythe. FEBS J. 272, 6373 – 6386.
47 Kalia, S. K., Lee, S., Smith, P. D., Liu, L., Crocker, S. J., Thorarinsdottir, T. E., Glover, J. R., Fon, E. A., Park, D. S. and Lozano, A. M. (2004) BAG5 inhibits parkin and enhances dopaminergic neuron degeneration. Neuron 44, 931 – 945.
48 Arndt, V., Daniel, C., Nastainczyk, W., Alberti, S. and Höhfeld, J. (2005) BAG-2 acts as an inhibitor of the chaperone-associated ubiquitin ligase CHIP. Mol. Biol. Cell 16, 5891 – 5900.
49 Dai, Q., Qian, S. B., Li, H. H., McDonough, H., Borchers, C., Huang, D., Takayama, S., Younger, J. M., Ren, H. Y., Cyr, D. M. and Patterson, C. (2005) Regulation of the cytoplasmic quality control protein degradation pathway by BAG2. J. Biol. Chem. 280, 38673 – 38681.
50 Alberti, S., Böhse, K., Arndt, V., Schmitz, A. and Höhfeld, J. (2004) The cochaperone HspBP1 inhibits the CHIP ubiquitin ligase and stimulates the maturation of the cystic fibrosis transmembrane conductance regulator. Mol. Biol. Cell 15, 4003 – 4010.
51 Kampinga, H. H., Kanon, B., Salomons, F. A., Kabakov, A. E. and Patterson, C. (2003) Overexpression of the cochaperone CHIP enhances Hsp70-dependent folding activity in mammalian cells. Mol. Cell. Biol. 23, 4948 – 4958.
52 Farinha, C. M. and Amaral, M. D. (2005) Most F508del-CFTR is targeted to degradation at an early folding checkpoint and independently of calnexin. Mol. Cell. Biol. 25, 5242 – 5252.
53 Younger, J. M., Chen, L., Ren, H. Y., Rosser, M. F., Turnbull, E. L., Fan, C. Y., Patterson, C. and Cyr, D. M. (2006) Sequential quality-control checkpoints triage misfolded cystic fibrosis transmembrane conductance regulator. Cell 126, 571 – 582.
54 Ward, C. L., Omura, S. and Kopito, R. R. (1995) Degradation of CFTR by the ubiquitin-proteasome pathway. Cell 83, 121 – 127.
55 Du, K., Sharma, M. and Lukacs, G. L. (2005) The DeltaF508 cystic fibrosis mutation impairs domain-domain interactions and arrests post-translational folding of CFTR. Nat. Struct. Mol. Biol. 12, 17 – 25.
56 Höhfeld, J., Minami, Y. and Hartl, F. U. (1995) Hip, a novel cochaperone involved in the eukaryotic Hsc70/Hsp40 reaction cycle. Cell 83, 589 – 598.
57 Demand, J., Lüders, J. and Höhfeld, J. (1998) The carboxy-terminal domain of Hsc70 provides binding sites for a distinct set of chaperone cofactors. Mol. Cell. Biol. 18, 2023 – 2028.
58 Brinker, A., Scheufler, C., Von Der Mulbe, F., Fleckenstein, B., Herrmann, C., Jung, G., Moarefi, I. and Hartl, F. U. (2002) Ligand discrimination by TPR domains. Relevance and selectivity of EEVD-recognition in Hsp70 × Hop × Hsp90 complexes. J. Biol. Chem. 277, 19265 – 19275.
59 Young, J. C., Moarefi, I. and Hartl, F. U. (2001) Hsp90: a specialized but essential protein-folding tool. J. Cell Biol. 154, 267 – 273.
60 Nikolay, R., Wiederkehr, T., Rist, W., Kramer, G., Mayer, M. P. and Bukau, B. (2004) Dimerization of the human E3 ligase CHIP *via* a coiled-coil domain is essential for its activity. J. Biol. Chem. 279, 2673 – 2678.
61 Velten, M., Villoutreix, B. O. and Ladjimi, M. M. (2000) Quaternary structure of the HSC70 cochaperone HIP. Biochemistry 39, 307 – 315.
62 Takayama, S., Bimston, D. N., Matsuzawa, S., Freeman, B. C., Aime-Sempe, C., Xie, Z., Morimoto, R. I. and Reed, J. C. (1997) BAG-1 modulates the chaperone activity of Hsp70/Hsc70. EMBO J. 16, 4887 – 4896.
63 Ueda, K., Kosako, H., Fukui, Y. and Hattori, S. (2004) Proteomic identification of Bcl2-associated athanogene 2 as a novel MAPK-activated protein kinase 2 substrate. J. Biol. Chem. 279, 41815 – 41821.
64 Fu, Z., Larson, K. A., Chitta, R. K., Parker, S. A., Turk, B. E., Lawrence, M. W., Kaldis, P., Galaktionov, K., Cohn, S. M., Shabanowitz, J., Hunt, D. F. and Sturgill, T. W. (2006) Identification of yin-yang regulators and a phosphorylation consensus for male germ cell-associated kinase (MAK)-related kinase. Mol. Cell. Biol. 26, 8639 – 8654.
65 Pickart, C. M. and Fushman, D. (2004) Polyubiquitin chains: polymeric protein signals. Curr. Opin. Chem. Biol. 8, 610 – 616.
66 Dubiel, W. and Gordon, C. (1999) Ubiquitin pathway: another link in the polyubiquitin chain? Curr. Biol. 9, R554 – 557.
67 Thrower, J. S., Hoffman, L., Rechsteiner, M. and Pickart, C. M. (2000) Recognition of the polyubiquitin proteolytic signal. EMBO J. 19, 94 – 102.
68 Fang, S. and Weissman, A. M. (2004) A field guide to ubiquitylation. Cell. Mol. Life Sci. 61, 1546 – 1561.
69 Peng, J., Schwartz, D., Elias, J. E., Thoreen, C. C., Cheng, D., Marsischky, G., Roelofs, J., Finley, D. and Gygi, S. P. (2003) A proteomics approach to understanding protein ubiquitination. Nat. Biotechnol. 21, 921 – 926.
70 Alberti, S., Demand, J., Esser, C., Emmerich, N., Schild, H. and Höhfeld, J. (2002) Ubiquitylation of BAG-1 suggests a novel regulatory mechanism during the sorting of chaperone substrates to the proteasome. J. Biol. Chem. 277, 45920 – 45927.
71 Qian, S. B., McDonough, H., Boellmann, F., Cyr, D. M. and Patterson, C. (2006) CHIP-mediated stress recovery by sequential ubiquitination of substrates and Hsp70. Nature 440, 551 – 555.
72 Pickart, C. M. and Cohen, R. E. (2004) Proteasomes and their kin: proteases in the machine age. Nat. Rev. Mol. Cell. Biol. 5, 177 – 187.
73 Young, P., Deveraux, Q., Beal, R. E., Pickart, C. M. and Rechsteiner, M. (1998) Characterization of two polyubiquitin binding sites in the 26 S protease subunit 5a. J. Biol. Chem. 273, 5461 – 5467.
74 Lam, Y. A., Lawson, T. G., Velayutham, M., Zweier, J. L. and Pickart, C. M. (2002) A proteasomal ATPase subunit recognizes the polyubiquitin degradation signal. Nature 416, 763 – 767.
75 Elsasser, S., Gali, R. R., Schwickart, M., Larsen, C. N., Leggett, D. S., Muller, B., Feng, M. T., Tubing, F., Dittmar, G. A. and Finley, D. (2002) Proteasome subunit Rpn1 binds ubiquitin-like protein domains. Nat. Cell Biol. 4, 725 – 730.
76 Zhang, M., Windheim, M., Roe, S. M., Peggie, M., Cohen, P., Prodromou, C. and Pearl, L. H. (2005) Chaperoned ubiquitylation – crystal structures of the CHIP U box E3 ubiquitin ligase and a CHIP-Ubc13-Uev1a complex. Mol. Cell 20, 525 – 538.
77 Grelle, G., Kostka, S., Otto, A., Kersten, B., Genser, K. F., Muller, E. C., Walter, S., Boddrich, A., Stelzl, U., Hanig, C., Volkmer-Engert, R., Landgraf, C., Alberti, S., Höhfeld, J., Strodicke, M. and Wanker, E. E. (2006) Identification of VCP/p97, carboxyl terminus of Hsp70-interacting protein (CHIP), and amphiphysin II interaction partners using membrane-based human proteome arrays. Mol. Cell. Proteomics 5, 234 – 244.
78 Mukhopadhyay, D. and Riezman, H. (2007) Proteasome-independent functions of ubiquitin in endocytosis and signaling. Science 315, 201 – 205.
79 Pratt, W. B. and Toft, D. O. (2003) Regulation of signaling protein function and trafficking by the hsp90/hsp70-based chaperone machinery. Exp Biol Med (Maywood) 228, 111 – 133.
80 Zhao, R. and Houry, W. A. (2007) Molecular interaction network of the Hsp90 chaperone system. Adv. Exp. Med. Biol. 594, 27 – 36.
81 Smith, D. F. (1993) Dynamics of heat shock protein 90-progesterone receptor binding and the disactivation loop

model for steroid receptor complexes. Mol. Endocrinol. 7, 1418 – 1429.
82 Esser, C., Scheffner, M. and Höhfeld, J. (2005) The chaperone-associated ubiquitin ligase CHIP is able to target p53 for proteasomal degradation. J. Biol. Chem. 280, 27443 – 27448.
83 Takayama, S., Sato, T., Krajewski, S., Kochel, K., Irie, S., Millan, J. A. and Reed, J. C. (1995) Cloning and functional analysis of BAG-1: a novel Bcl-2-binding protein with anti-cell death activity. Cell 80, 279 – 284.
84 Gotz, R., Wiese, S., Takayama, S., Camarero, G. C., Rossoll, W., Schweizer, U., Troppmair, J., Jablonka, S., Holtmann, B., Reed, J. C., Rapp, U. R. and Sendtner, M. (2005) Bag1 is essential for differentiation and survival of hematopoietic and neuronal cells. Nat. Neurosci. 8, 1169 – 1178.
85 Gotz, R., Kramer, B. W., Camarero, G. and Rapp, U. R. (2004) BAG-1 haplo-insufficiency impairs lung tumorigenesis. BMC Cancer 4, 85.
86 Townsend, P. A., Cutress, R. I., Sharp, A., Brimmell, M. and Packham, G. (2003) BAG-1 prevents stress-induced long-term growth inhibition in breast cancer cells *via* a chaperone-dependent pathway. Cancer Res. 63, 4150 – 4157.
87 Townsend, P. A., Dublin, E., Hart, I. R., Kao, R. H., Hanby, A. M., Cutress, R. I., Poulsom, R., Ryder, K., Barnes, D. M. and Packham, G. (2002) BAG-i expression in human breast cancer: interrelationship between BAG-1 RNA, protein, HSC70 expression and clinico-pathological data. J. Pathol. 197, 51 – 59.
88 Kudoh, M., Knee, D. A., Takayama, S. and Reed, J. C. (2002) Bag1 proteins regulate growth and survival of ZR-75 – 1 human breast cancer cells. Cancer Res. 62, 1904 – 1909.
89 Turner, B. C., Krajewski, S., Krajewska, M., Takayama, S., Gumbs, A. A., Carter, D., Rebbeck, T. R., Haffty, B. G. and Reed, J. C. (2001) BAG-1: a novel biomarker predicting long-term survival in early-stage breast cancer. J. Clin. Oncol. 19, 992 – 1000.
90 Drysdale, M. J., Brough, P. A., Massey, A., Jensen, M. R. and Schoepfer, J. (2006) Targeting Hsp90 for the treatment of cancer. Curr. Opin. Drug Discov. Dev. 9, 483 – 495.
91 Neckers, L. (2006) Using natural product inhibitors to validate Hsp90 as a molecular target in cancer. Curr. Top. Med. Chem. 6, 1163 – 1171.
92 Cullinan, S. B. and Whitesell, L. (2006) Heat shock protein 90: a unique chemotherapeutic target. Semin. Oncol. 33, 457 – 465.
93 Powers, M. V. and Workman, P. (2006) Targeting of multiple signalling pathways by heat shock protein 90 molecular chaperone inhibitors. Endocr. Relat. Cancer 13 Suppl. 1, S125 – 135.
94 Kamal, A., Thao, L., Sensintaffar, J., Zhang, L., Boehm, M. F., Fritz, L. C. and Burrows, F. J. (2003) A high-affinity conformation of Hsp90 confers tumour selectivity on Hsp90 inhibitors. Nature 425, 407 – 410.
95 Muchowski, P. J. and Wacker, J. L. (2005) Modulation of neurodegeneration by molecular chaperones. Nat. Rev. Neurosci. 6, 11 – 22.
96 Bonini, N. M. (2002) Chaperoning brain degeneration. Proc. Natl. Acad. Sci. USA 99 Suppl 4, 16407 – 16411.
97 Ross, C. A. and Pickart, C. M. (2004) The ubiquitin-proteasome pathway in Parkinson's disease and other neurodegenerative diseases. Trends Cell Biol. 14, 703 – 711.
98 Sakahira, H., Breuer, P., Hayer-Hartl, M. K. and Hartl, F. U. (2002) Molecular chaperones as modulators of polyglutamine protein aggregation and toxicity. Proc. Natl. Acad. Sci. USA 99 Suppl. 4, 16412 – 16418.
99 Schaffar, G., Breuer, P., Boteva, R., Behrends, C., Tzvetkov, N., Strippel, N., Sakahira, H., Siegers, K., Hayer-Hartl, M. and Hartl, F. U. (2004) Cellular toxicity of polyglutamine expansion proteins: mechanism of transcription factor deactivation. Mol. Cell 15, 95 – 105.
100 Ross, C. A. and Poirier, M. A. (2004) Protein aggregation and neurodegenerative disease. Nat. Med. 10 Suppl., S10 – 17.
101 Nagai, Y., Inui, T., Popiel, H. A., Fujikake, N., Hasegawa, K., Urade, Y., Goto, Y., Naiki, H. and Toda, T. (2007) A toxic monomeric conformer of the polyglutamine protein. Nat. Struct. Mol. Biol. 14, 332 – 340.
102 Kazemi-Esfarjani, P. and Benzer, S. (2000) Genetic suppression of polyglutamine toxicity in *Drosophila.* Science 287, 1837 – 1840.
103 Finkbeiner, S., Cuervo, A. M., Morimoto, R. I. and Muchowski, P. J. (2006) Disease-modifying pathways in neurodegeneration. J. Neurosci. 26, 10349 – 10357.
104 Winklhofer, K. F. and Tatzelt, J. (2006) The role of chaperones in Parkinson's disease and prion diseases. Handb. Exp. Pharmacol. (172), 221 – 258.
105 Muchowski, P. J., Schaffar, G., Sittler, A., Wanker, E. E., Hayer-Hartl, M. K. and Hartl, F. U. (2000) Hsp70 and hsp40 chaperones can inhibit self-assembly of polyglutamine proteins into amyloid-like fibrils. Proc. Natl. Acad. Sci. USA 97, 7841 – 7846.
106 Kosik, K. S. and Shimura, H. (2005) Phosphorylated tau and the neurodegenerative foldopathies. Biochim. Biophys. Acta 1739, 298 – 310.
107 Dickey, C. A., Yue, M., Lin, W. L., Dickson, D. W., Dunmore, J. H., Lee, W. C., Zehr, C., West, G., Cao, S., Clark, A. M., Caldwell, G. A., Caldwell, K. A., Eckman, C., Patterson, C., Hutton, M. and Petrucelli, L. (2006) Deletion of the ubiquitin ligase CHIP leads to the accumulation, but not the aggregation, of both endogenous phospho- and caspase-3-cleaved tau species. J. Neurosci. 26, 6985 – 6996.
108 Fulga, T. A., Elson-Schwab, I., Khurana, V., Steinhilb, M. L., Spires, T. L., Hyman, B. T. and Feany, M. B. (2007) Abnormal bundling and accumulation of F-actin mediates tau-induced neuronal degeneration *in vivo.* Nat. Cell Biol. 9, 139 – 148.
109 Leschik, J., Welzel, A., Weissmann, C., Eckert, A. and Brandt, R. (2007) Inverse and distinct modulation of tau-dependent neurodegeneration by presenilin 1 and amyloid-beta in cultured cortical neurons: evidence that tau phosphorylation is the limiting factor in amyloid-beta-induced cell death. J. Neurochem. 101, 1303 – 1315.
110 Brandt, R. and Leschik, J. (2004) Functional interactions of tau and their relevance for Alzheimer's disease. Curr. Alzheimer Res. 1, 255 – 269.
111 Hatakeyama, S., Matsumoto, M., Kamura, T., Murayama, M., Chui, D. H., Planel, E., Takahashi, R., Nakayama, K. I. and Takashima, A. (2004) U-box protein carboxyl terminus of Hsc70-interacting protein (CHIP) mediates poly-ubiquitylation preferentially on four-repeat Tau and is involved in neurodegeneration of tauopathy. J. Neurochem. 91, 299 – 307.
112 Dickey, C. A., Kamal, A., Lundgren, K., Klosak, N., Bailey, R. M., Dunmore, J., Ash, P., Shoraka, S., Zlatkovic, J., Eckman, C. B., Patterson, C., Dickson, D. W., Nahman, N. S. Jr., Hutton, M., Burrows, F. and Petrucelli, L. (2007) The high-affinity HSP90-CHIP complex recognizes and selectively degrades phosphorylated tau client proteins. J. Clin. Invest. 117, 648 – 658.
113 Sahara, N., Murayama, M., Mizoroki, T., Urushitani, M., Imai, Y., Takahashi, R., Murata, S., Tanaka, K. and Takashima, A. (2005) *In vivo* evidence of CHIP up-regulation attenuating tau aggregation. J. Neurochem. 94, 1254 – 1263.
114 Kumar, P., Ambasta, R. K., Veereshwarayya, V., Rosen, K. M., Kosik, K. S., Band, H., Mestril, R., Patterson, C. and Querfurth, H. W. (2007) CHIP and HSPs interact with (beta)-APP in a proteasome-dependent manner and influence A(beta) metabolism. Hum. Mol. Genet. 16, 848 – 864.
115 Latchman, D. S. (2001) Heat shock proteins and cardiac protection. Cardiovasc. Res. 51, 637 – 646.
116 Patterson, C., Ike, C., Willis, P. W. t., Stouffer, G. A. and Willis, M. S. (2007) The bitter end: the ubiquitin-proteasome system and cardiac dysfunction. Circulation 115, 1456 – 1463.
117 Liu, Y., Gampert, L., Nething, K. and Steinacker, J. M. (2006) Response and function of skeletal muscle heat shock protein 70. Front. Biosci. 11, 2802 – 2827.
118 Zhang, C., Xu, Z., He, X. R., Michael, L. H. and Patterson, C. (2005) CHIP, a cochaperone/ubiquitin ligase that regulates protein quality control, is required for maximal cardiopro-

tection after myocardial infarction in mice. Am. J. Physiol. Heart Circ. Physiol. 288, H2836 – 2842.
119 Townsend, P. A., Cutress, R. I., Carroll, C. J., Lawrence, K. M., Scarabelli, T. M., Packham, G., Stephanou, A. and Latchman, D. S. (2004) BAG-1 proteins protect cardiac myocytes from simulated ischemia/reperfusion-induced apoptosis *via* an alternate mechanism of cell survival independent of the proteasome. J. Biol. Chem. 279, 20723 – 20728.
120 Dai, Q., Zhang, C., Wu, Y., McDonough, H., Whaley, R. A., Godfrey, V., Li, H. H., Madamanchi, N., Xu, W., Neckers, L., Cyr, D. and Patterson, C. (2003) CHIP activates HSF1 and confers protection against apoptosis and cellular stress. EMBO J. 22, 5446 – 5458.
121 Willis, M. S. and Patterson, C. (2006) Into the heart: the emerging role of the ubiquitin-proteasome system. J. Mol. Cell. Cardiol. 41, 567 – 579.
122 Hoppe, T., Cassata, G., Barral, J. M., Springer, W., Hutagalung, A. H., Epstein, H. F. and Baumeister, R. (2004) Regulation of the myosin-directed chaperone UNC-45 by a novel E3/E4-multiubiquitylation complex in *C. elegans*. Cell 118, 337 – 349.
123 Barral, J. M., Hutagalung, A. H., Brinker, A., Hartl, F. U. and Epstein, H. F. (2002) Role of the myosin assembly protein UNC-45 as a molecular chaperone for myosin. Science 295, 669 – 671.
124 Landsverk, M. L., Li, S., Hutagalung, A. H., Najafov, A., Hoppe, T., Barral, J. M. and Epstein, H. F. (2007) The UNC-45 chaperone mediates sarcomere assembly through myosin degradation in *Caenorhabditis elegans*. J. Cell Biol. 177, 205 – 210.
125 Janiesch, P. C., Kim, J., Mouysset, J., Barikbin, R., Lochmuller, H., Cassata, G., Krause, S. and Hoppe, T. (2007) The ubiquitin-selective chaperone CDC-48/p97 links myosin assembly to human myopathy. Nat. Cell Biol. 9, 379 – 390.
126 Kloetzel, P. M. (2004) Generation of major histocompatibility complex class I antigens: functional interplay between proteasomes and TPPII. Nat. Immunol. 5, 661 – 669.
127 Kunisawa, J. and Shastri, N. (2006) Hsp90alpha chaperones large C-terminally extended proteolytic intermediates in the MHC class I antigen processing pathway. Immunity 24, 523 – 534.
128 Yamano, T., Murata, S., Shimbara, N., Tanaka, N., Chiba, T., Tanaka, K., Yui, K. and Udono, H. (2002) Two distinct pathways mediated by PA28 and hsp90 in major histocompatibility complex class I antigen processing. J. Exp. Med. 196, 185 – 196.
129 Qian, S. B., Princiotta, M. F., Bennink, J. R. and Yewdell, J. W. (2006) Characterization of rapidly degraded polypeptides in mammalian cells reveals a novel layer of nascent protein quality control. J. Biol. Chem. 281, 392 – 400.
130 Schubert, U., Anton, L. C., Gibbs, J., Norbury, C. C., Yewdell, J. W. and Bennink, J. R. (2000) Rapid degradation of a large fraction of newly synthesized proteins by proteasomes. Nature 404, 770 – 774.
131 Lelouard, H., Gatti, E., Cappello, F., Gresser, O., Camosseto, V. and Pierre, P. (2002) Transient aggregation of ubiquitinated proteins during dendritic cell maturation. Nature 417, 177 – 182.
132 Lelouard, H., Ferrand, V., Marguet, D., Bania, J., Camosseto, V., David, A., Gatti, E. and Pierre, P. (2004) Dendritic cell aggresome-like induced structures are dedicated areas for ubiquitination and storage of newly synthesized defective proteins. J. Cell Biol. 164, 667 – 675.
133 Kopito, R. R. (2000) Aggresomes, inclusion bodies and protein aggregation. Trends Cell Biol. 10, 524 – 530.
134 Herter, S., Osterloh, P., Hilf, N., Rechtsteiner, G., Höhfeld, J., Rammensee, H. G. and Schild, H. (2005) Dendritic cell aggresome-like-induced structure formation and delayed antigen presentation coincide in influenza virus-infected dendritic cells. J. Immunol. 175, 891 – 898.
135 Rutherford, S. L. (2003) Between genotype and phenotype: protein chaperones and evolvability. Nat. Rev. Genet. 4, 263 – 274.
136 Queitsch, C., Sangster, T. A. and Lindquist, S. (2002) Hsp90 as a capacitor of phenotypic variation. Nature 417, 618 – 624.
137 Sangster, T. A., Lindquist, S. and Queitsch, C. (2004) Under cover: causes, effects and implications of Hsp90-mediated genetic capacitance. Bioessays 26, 348 – 362.
138 Morales, J. L. and Perdew, G. H. (2007) Carboxyl terminus of hsc70-interacting protein (CHIP) can remodel mature aryl hydrocarbon receptor (AhR) complexes and mediate ubiquitination of both the AhR and the 90 kDa heat-shock protein (hsp90) *in vitro*. Biochemistry 46, 610 – 621.
139 Cardozo, C. P., Michaud, C., Ost, M. C., Fliss, A. E., Yang, E., Patterson, C., Hall, S. J. and Caplan, A. J. (2003) C-terminal Hsp-interacting protein slows androgen receptor synthesis and reduces its rate of degradation. Arch. Biochem. Biophys. 410, 134 – 140.
140 Hwang, J. R., Zhang, C. and Patterson, C. (2005) C-terminus of heat shock protein 70-interacting protein facilitates degradation of apoptosis signal-regulating kinase 1 and inhibits apoptosis signal-regulating kinase 1-dependent apoptosis. Cell Stress Chaperones 10, 147 – 156.
141 Zhang, L., Nephew, K. P. and Gallagher, P. J. (2007) Regulation of death associated protein kinase: Stablization by HSP90 heterocomplexes. J. Biol. Chem. 282, 11795 – 11804.
142 Daviau, A., Proulx, R., Robitaille, K., Di Fruscio, M., Tanguay, R. M., Landry, J., Patterson, C., Durocher, Y. and Blouin, R. (2006) Down-regulation of the mixed-lineage dual leucine zipper-bearing kinase by heat shock protein 70 and its co-chaperone CHIP. J. Biol. Chem. 281, 31467 – 31477.
143 Lees, M. J., Peet, D. J. and Whitelaw, M. L. (2003) Defining the role for XAP2 in stabilization of the dioxin receptor. J. Biol. Chem. 278, 35878 – 35888.
144 Huang, Z., Nie, L., Xu, M. and Sun, X. H. (2004) Notch-induced E2A degradation requires CHIP and Hsc70 as novel facilitators of ubiquitination. Mol. Cell. Biol. 24, 8951 – 8962.
145 Timsit, Y. E., Miller, S. L., Mohney, R. P. and O'Bryan, J. P. (2005) The U-box ligase carboxyl-terminus of Hsc 70-interacting protein ubiquitylates Epsin. Biochem. Biophys. Res. Commun. 328, 550 – 559.
146 Fan, M., Park, A. and Nephew, K. P. (2005) CHIP (carboxyl terminus of Hsc70-interacting protein) promotes basal and geldanamycin-induced degradation of estrogen receptor-alpha. Mol. Endocrinol. 19, 2901 – 2914.
147 Tateishi, Y., Kawabe, Y., Chiba, T., Murata, S., Ichikawa, K., Murayama, A., Tanaka, K., Baba, T., Kato, S. and Yanagisawa, J. (2004) Ligand-dependent switching of ubiquitin-proteasome pathways for estrogen receptor. EMBO J. 23, 4813 – 4823.
148 Tateishi, Y., Sonoo, R., Sekiya, Y., Sunahara, N., Kawano, M., Wayama, M., Hirota, R., Kawabe, Y., Murayama, A., Kato, S., Kimura, K. and Yanagisawa, J. (2006) Turning off estrogen receptor beta-mediated transcription requires estrogen-dependent receptor proteolysis. Mol. Cell. Biol. 26, 7966 – 7976.
149 Lee, H., Sengupta, N., Villagra, A., Rezai-Zadeh, N. and Seto, E. (2006) Histone deacetylase 8 safeguards the human ever-shorter telomeres 1B (hEST1B) protein from ubiquitin-mediated degradation. Mol. Cell. Biol. 26, 5259 – 5269.
150 Peng, H. M., Morishima, Y., Jenkins, G. J., Dunbar, A. Y., Lau, M., Patterson, C., Pratt, W. B. and Osawa, Y. (2004) Ubiquitylation of neuronal nitric-oxide synthase by CHIP, a chaperone-dependent E3 ligase. J. Biol. Chem. 279, 52970 – 52977.
151 Bonvini, P., Dalla Rosa, H., Vignes, N. and Rosolen, A. (2004) Ubiquitination and proteasomal degradation of nucleophosmin-anaplastic lymphoma kinase induced by 17-allylamino-demethoxygeldanamycin: role of the co-chaperone carboxyl heat shock protein 70-interacting protein. Cancer Res. 64, 3256 – 3264.
152 Kamynina, E., Kauppinen, K., Duan, F., Muakkassa, N. and Manor, D. (2007) Regulation of proto-oncogenic dbl by chaperone-controlled, ubiquitin-mediated degradation. Mol. Cell. Biol. 27, 1809 – 1822.

153 Germano, S., Barberis, D., Santoro, M. M., Penengo, L., Citri, A., Yarden, Y. and Gaudino, G. (2006) Geldanamycins trigger a novel Ron degradative pathway, hampering oncogenic signaling. J. Biol. Chem. 281, 21710 – 21719.
154 Belova, L., Sharma, S., Brickley, D. R., Nicolarsen, J. R., Patterson, C. and Conzen, S. D. (2006) Ubiquitin-proteasome degradation of serum- and glucocorticoid-regulated kinase-1 (SGK-1) is mediated by the chaperone-dependent E3 ligase CHIP. Biochem. J. 400, 235 – 244.
155 Shin, Y., Klucken, J., Patterson, C., Hyman, B. T. and McLean, P. J. (2005) The co-chaperone carboxyl terminus of Hsp70-interacting protein (CHIP) mediates alpha-synuclein degradation decisions between proteasomal and lysosomal pathways. J. Biol. Chem. 280, 23727 – 23734.
156 Al-Ramahi, I., Lam, Y. C., Chen, H. K., de Gouyon, B., Zhang, M., Perez, A. M., Branco, J., de Haro, M., Patterson, C., Zoghbi, H. Y. and Botas, J. (2006) CHIP protects from the neurotoxicity of expanded and wild-type ataxin-1 and promotes their ubiquitination and degradation. J. Biol. Chem. 281, 26714 – 26724.
157 Jana, N. R., Dikshit, P., Goswami, A., Kotliarova, S., Murata, S., Tanaka, K. and Nukina, N. (2005) Co-chaperone CHIP associates with expanded polyglutamine protein and promotes their degradation by proteasomes. J. Biol. Chem. 280, 11635 – 11640.
158 Nelson, R. F., Glenn, K. A., Miller, V. M., Wen, H. and Paulson, H. L. (2006) A novel route for F-box protein-mediated ubiquitination links CHIP to glycoprotein quality control. J. Biol. Chem. 281, 20242 – 20251.
159 Imai, Y., Soda, M., Hatakeyama, S., Akagi, T., Hashikawa, T., Nakayama, K. I. and Takahashi, R. (2002) CHIP is associated with Parkin, a gene responsible for familial Parkinson's disease, and enhances its ubiquitin ligase activity. Mol. Cell 10, 55 – 67.
160 Choi, J. S., Cho, S., Park, S. G., Park, B. C. and Lee, D. H. (2004) Co-chaperone CHIP associates with mutant Cu/Zn-superoxide dismutase proteins linked to familial amyotrophic lateral sclerosis and promotes their degradation by proteasomes. Biochem. Biophys. Res. Commun. 321, 574 – 583.