1
Molecular Chaperones and the Ubiquitin–Proteasome System

Cam Patterson and Jörg Höhfeld

Abstract

A role for the ubiquitin–proteasome system in the removal of misfolded and abnormal proteins is well established. Nevertheless, very little is known about how abnormal proteins are recognized for degradation by the proteasome. Recent advances suggest that substrate recognition and processing require a close cooperation of the ubiquitin–proteasome system with molecular chaperones. Chaperones are defined by their ability to recognize nonnative conformations of other proteins and are therefore ideally suited to distinguish between native and abnormal proteins during substrate selection. Here we discuss molecular mechanisms that underlie the cooperation of molecular chaperones with the ubiquitin–proteasome system. Advancing our knowledge about such mechanisms may open up opportunities to modulate chaperone–proteasome cooperation in human diseases.

1.1
Introduction

The biological activity of a protein is defined by its unique three-dimensional structure. Attaining this structure, however, is a delicate process. A recent study suggests that up to 30% of all newly synthesized proteins never reach their native state [1]. As protein misfolding poses a major threat to cell function and viability, molecular mechanisms must have evolved to prevent the accumulation of misfolded proteins and thus aggregate formation. Two protective strategies appear to be followed. Molecular chaperones are employed to stabilize nonnative protein conformations and to promote folding to the native state whenever possible. Alternatively, misfolded proteins are removed by degradation, involving, for example, the ubiquitin–proteasome system. For a long time molecular chaperones and cellular degradation systems were therefore viewed as opposing forces. However, recent evidence suggests that certain chaperones (in particular members of the 70- and 90-kDa heat shock protein families) are able to cooperate with the ubiquitin–

Protein Degradation, Vol. 2: The Ubiquitin-Proteasome System.
Edited by R. J. Mayer, A. Ciechanover, M. Rechsteiner

ISBN: 3-527-31130-0

proteasome system. Protein fate thus appears to be determined by a tight interplay of cellular protein-folding and protein-degradation systems.

1.2 A Biomedical Perspective

The aggregation and accumulation of misfolded proteins is now recognized as a common characteristic of a number of degenerative disorders, many of which have neurological manifestations [2, 3]. These diseases include prionopathies, Alzheimer's and Parkinson's diseases, and polyglutamine expansion diseases such as Huntington's disease and spinocerebellar ataxia. At the cellular level, these diseases are characterized by the accumulation of aberrant proteins either intracellularly or extracellularly in specific groups of cells that subsequently undergo death. The precise association between protein accumulation and cell death remains incompletely understood and may vary from disease to disease. In some cases, misfolded protein accumulations may themselves be toxic or exert spatial constraints on cells that affect their ability to function normally. In other cases, the sequestering of proteins in aggregates may itself be a protective mechanism, and it is the overwhelming of pathways that consolidate aberrant proteins that is the toxic event. In either case, lessons learned from genetically determined neurodegenerative diseases have helped us to understand the inciting events of protein aggregation that ultimately lead to degenerative diseases.

Mutations resulting in neurodegenerative diseases fall into two broad classes. The first class comprises mutations that affect proteins, irrespective of their native function, and cause them to misfold. The classic example of this is Huntington's disease [4, 5]. The protein encoded by the huntingtin gene contains a stretch of glutamine residues (or polyglutamine repeat), and the genomic DNA sequence that codes for this polyglutamine repeat is subject to misreading and expansion. When the length of the polyglutamine repeat in huntingtin reaches a critical threshold of approximately 35 residues, the protein becomes prone to misfolding and aggregation [6]. This appears to be the proximate cause of neurotoxicity in this invariably fatal disease [7, 8]. A number of other neurodegenerative diseases are caused by polyglutamine expansions [9, 10]. For example, spinocerebellar ataxia is caused by polyglutamine expansions in the protein ataxin-1 [11]. In other diseases, protein misfolding occurs due to other mutations that induce misfolding and aggregation; for example, mutations in superoxide dismutase-1 lead to aggregation and neurotoxicity in amyotrophic lateral sclerosis [12, 13].

Other mutations that result in neurodegenerative diseases are instructive in that they directly implicate the ubiquitin–proteasome system in the pathogenesis of these diseases [14]. For example, mutations in the gene encoding the protein parkin are associated with juvenile-onset Parkinson's disease [15, 16]. Parkin is a RING finger–containing ubiquitin ligase, and mutations in this ubiquitin ligase cause accumulation of target proteins that ultimately result in the neurotoxicity and motor dysfunction associated with Parkinson's disease [17–20].

Repressor screens of neurodegeneration phenotypes in animal models have also linked the molecular chaperone machinery to neurodegeneration [21–24]. Taken together, the pathophysiology of neurodegenerative diseases provides a compelling demonstration of the importance of the regulated metabolism of misfolded proteins and provides direct evidence of the role of both molecular chaperones and the ubiquitin–proteasome system in guarding against protein misfolding and its consequent toxicity.

1.3 Molecular Chaperones: Mode of Action and Cellular Functions

Molecular chaperones are defined by their ability to bind and stabilize nonnative conformations of other proteins [25, 26]. Although they are an amazingly diverse group of conserved and ubiquitous proteins, they are also among the most abundant intracellular proteins. The classical function of chaperones is to facilitate protein folding, inhibit misfolding, and prevent aggregation. These folding events are regulated by interactions between chaperones and ancillary proteins, the co-chaperones, which in general assist in cycling unfolded substrate proteins on and off the active chaperone complex [25, 27, 28]. In agreement with their essential function under normal growth conditions, chaperones are ubiquitously expressed and are found in all cellular compartments of the eukaryotic cell (except for peroxisomes). In addition, cells greatly increase chaperone concentration as a response to diverse stresses, when proteins become unfolded and require protection and stabilization [29]. Accordingly, many chaperones are heat shock proteins (Hsps). Four main families of cytoplasmic chaperones can be distinguished: the Hsp70 family, the Hsp90 family, the small heat shock proteins, and the chaperonins.

1.3.1 The Hsp70 Family

The Hsp70 proteins bind to misfolded proteins promiscuously during translation or after stress-mediated protein damage [26, 30]. Members of this family are highly conserved throughout evolution and are found throughout the prokaryotic and eukaryotic phylogeny. It is common for a single cell to contain multiple homologues, even within a single cellular compartment; for example, mammalian cells express two inducible homologues (Hsp70.1 and Hsp70.3) and a constitutive homologue (Hsc70) in the cytoplasm. These homologues have overlapping but not totally redundant cellular functions. Members of this family are typically in the range of 70 kDa in size and contain three functional domains: an amino-terminal ATPase domain, a central peptide-binding cleft, and a carboxyl terminus that seems to form a lid over the peptide-binding cleft [28] (Figure 1.1). The chaperones recognize short segments of the protein substrate, which are composed of clusters of hydrophobic amino acids flanked by basic residues [31]. Such binding motifs occur frequently within protein sequences and are found exposed on nonnative proteins. In fact,

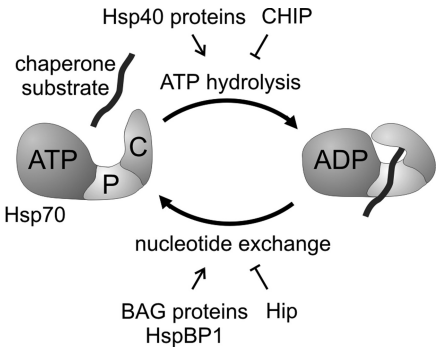

Fig. 1.1. Schematic presentation of the domain architecture and chaperone cycle of Hsp70. Hsp70 proteins display a characteristic domain structure comprising an amino-terminal ATPase domain (ATP), a peptide-binding domain (P), and a carboxyl-terminal domain (C) that is supposed to form a lid over the peptide-binding domain. In the ATP-bound conformation, the binding pocket is open, resulting in a low affinity for the binding of a chaperone substrate. ATP hydrolysis induces stable substrate binding through a closure of the peptide-binding pocket. Substrate release is induced upon nucleotide exchange. ATP hydrolysis and nucleotide exchange are regulated by diverse co-chaperones.

mammalian Hsp70 binds to a wide range of nascent and newly synthesized proteins, comprising about 15–20% of total protein [32]. This percentage is most likely further increased under stress conditions. Hsp70 proteins apparently prevent protein aggregation and promote proper folding by shielding hydrophobic segments of the protein substrate. The hydrophobic segments are recognized by the central peptide-binding domain of Hsp70 proteins (Figure 1.1). The domain is composed of two sheets of β strands that together with connecting loops form a cleft to accommodate extended peptides of about seven amino acids in length, as revealed in crystallographic studies of bacterial Hsp70 [33]. In the obtained crystal structure, the adjacent carboxyl-terminal domain of Hsp70 folds back over the β sandwich, suggesting that the domain may function as a lid in permitting entry and release of protein substrates (Figure 1.1). According to this model, ATP binding and hydrolysis by the amino-terminal ATPase domain of Hsp70 induce conformational changes of the carboxyl terminus, which lead to lid opening and closure [28]. In the ATP-bound conformation of Hsp70, the peptide-binding pocket is open, resulting in rapid binding and release of the substrate and consequently in a low binding affinity (Figure 1.1). Stable holding of the protein substrate requires closing of the binding pocket, which is induced upon ATP hydrolysis and conversion of Hsp70 to the ADP-bound conformation. The dynamic association of Hsp70 with nonnative polypeptide substrates thus depends on ongoing cycles of ATP binding, hydrolysis, and nucleotide exchange. Importantly, ancillary co-chaperones are employed to regulate the ATPase cycle [27, 30]. Co-chaperones of the Hsp40 family (also termed J proteins due to their founding member bacterial DnaJ) stimulate the ATP hydrolysis step within the Hsp70 reaction cycle and in this way promote substrate binding [34] (Figure 1.1). In contrast, the carboxyl terminus of Hsp70-interacting protein CHIP attenuates ATP hydrolysis [35]. Similarly, nucleo-

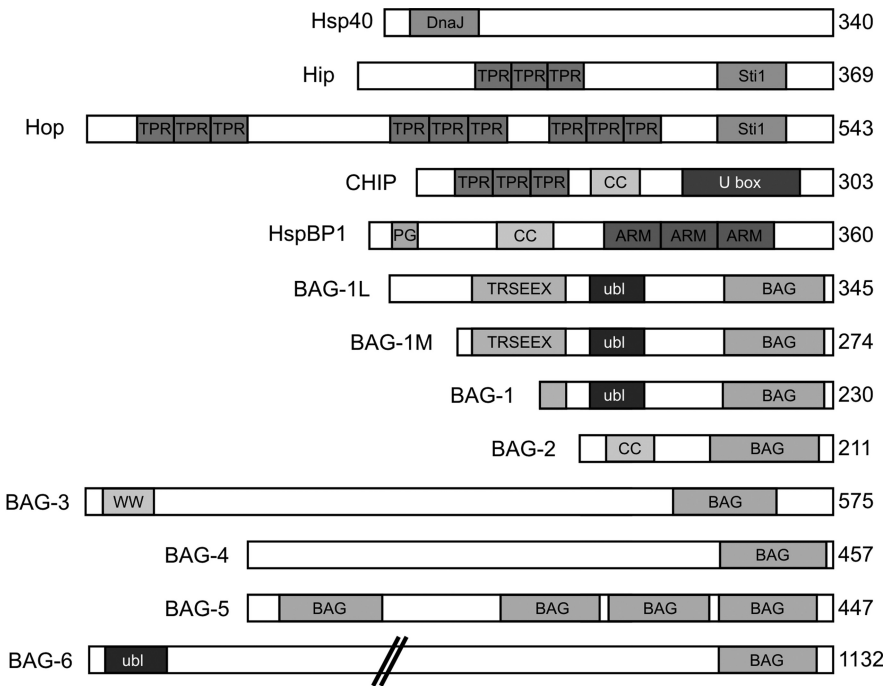

Fig. 1.2. Domain architecture of diverse co-chaperones of Hsp70. DnaJ: domain related to the bacterial co-chaperone DnaJ; TPR: tetratricopeptide repeat; Sti1: domain related to the yeast co-chaperone Sti1; CC: coiled-coil domain; U box: E2-interacting domain present in certain ubiquitin ligases; PG: polyglycine region; ARM: armadillo repeat; TRSEEX: repeat motif found at the amino terminus of BAG-1 isoforms; ubl: ubiquitin-like domain; BAG: Hsp70-binding domain present in BAG proteins; WW: protein interaction domain.

tide exchange on Hsp70 is under the control of stimulating and inhibiting co-chaperones. The Hsp70-interacting protein Hip slows down nucleotide exchange by stabilizing the ADP-bound conformation of the chaperone [36], whereas nucleotide exchange is stimulated by the co-chaperone BAG-1 (Bcl-2-associated athanogene 1), which assists substrate unloading from Hsp70 [37–39]. By altering the ATPase cycle, the co-chaperones directly modulate the folding activity of Hsp70. In addition to chaperone-recognition motifs, co-chaperones often possess other functional domains and therefore link chaperone activity to distinct cellular processes [27, 40] (Figure 1.2). Indeed, as discussed below, the co-chaperones BAG-1 and CHIP apparently modulate Hsp70 function during protein degradation.

1.3.2 The Hsp90 Family

The 90-kDa cytoplasmic chaperones are members of the Hsp90 family, and in mammals two isoforms exist: Hsp90α and Hsp90β. The Hsp70 and Hsp90 families exhibit several common features: both possess ATPase activity and are regulated

by ATP binding and hydrolysis, and both are further regulated by ancillary co-chaperones [41–48]. Unlike Hsp70, however, cytoplasmic Hsp90 is not generally involved in the folding of newly synthesized polypeptide chains. Instead it plays a key role in the regulation of signal transduction networks, as most of the known substrates of Hsp90 are signaling proteins, the classical examples being steroid hormone receptors and signaling kinases. On a molecular level, Hsp90 binds to substrates at a late stage of the folding pathway, when the substrate is poised for activation by ligand binding or associations with other factors. Consequently, Hsp90 accepts partially folded conformations from Hsp70 for further processing. In the case of the chaperone-assisted activation of the glucocorticoid hormone receptor and also of the progesterone receptor, the sequence of events leading to attaining an active conformation is fairly well understood [49–53]. It appears that the receptors are initially recognized by Hsp40 and are then delivered to Hsp70 [54] (Figure 1.3). Subsequent transfer onto Hsp90 requires the Hsp70/Hsp90-organizing protein Hop, which possesses non-overlapping binding sites for Hsp70 and Hsp90 and therefore acts as a coupling factor between the two chaperones [55]. In conjunction with p23 and different cyclophilins, Hsp90 eventually medi-

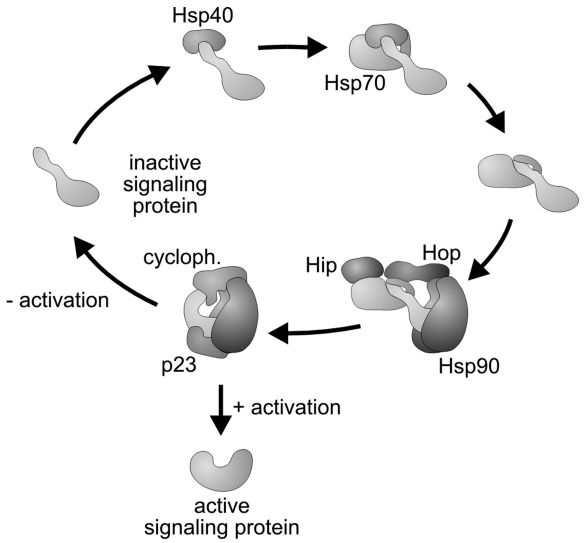

Fig. 1.3. Cooperation of Hsp70 and Hsp90 during the regulation of signal transduction pathways. The inactive signaling protein, e.g., a steroid hormone receptor, is initially recognized by Hsp40 and delivered to Hsp70. Subsequently, a multi-chaperone complex assembles that contains the Hsp70 co-chaperone Hip and the Hsp70/Hsp90-organizing protein Hop. Hop stimulates recruitment of an Hsp90 dimer that accepts the substrate from Hsp70. At the final stage of the chaperone pathway, Hsp90 associates with p23 and diverse cyclophilins (cycloph.) 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.

ates conformational changes that enable the receptor to reach a high-affinity state for ligand binding. On other signaling pathways Hsp90 serves as a scaffolding factor to permit interactions between kinases and their substrates, as is the case for Akt kinase and endothelial nitric oxide synthase [56]. Since many of the Hsp90 substrate proteins are involved in regulating cell proliferation and cell death, it is not surprising that the chaperone recently emerged as a drug target in tumor therapy [57–59]. The antibiotics geldanamycin and radicicol specifically bind to Hsp90 in mammalian cells and inhibit the function of the chaperone by occupying its ATP-binding pocket [60–63]. Drugs based on these compounds are now being developed as anticancer agents, as they potentially inactivate multiple signaling pathways that drive carcinogenesis. Remarkably, drug-induced inhibition of Hsp90 blocks the chaperone-assisted activation of signaling proteins and leads to their rapid degradation via the ubiquitin–proteasome pathway [64–69] (Figure 1.4). Hsp90 inhibitors therefore have emerged as helpful tools to study chaperone–proteasome cooperation.

1.3.3 The Small Heat Shock Proteins

The precise functions of small heat shock proteins (sHsps) including Hsp27 and the eye-lens protein αB-crystallin are incompletely understood. However, they

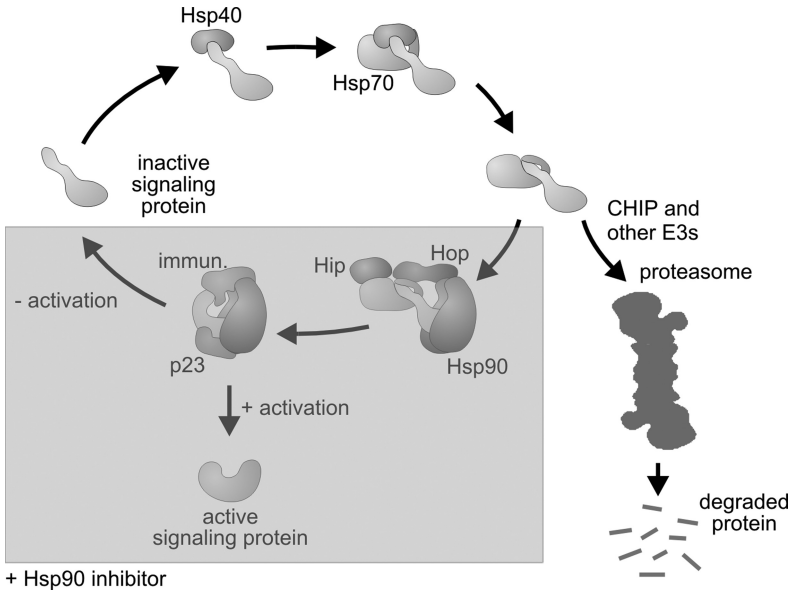

Fig. 1.4. Alteration of chaperone action during signal transduction induced by Hsp90 inhibitors such as geldanamycin and radicicol. In the presence of the inhibitors the activation pathway is blocked, and signaling proteins are targeted to the proteasome for degradation in a process that involves the co-chaperone CHIP and other E3 ubiquitin ligases that remain to be identified.

seem to play a major role in preventing protein aggregation under conditions of cellular stress [70–73]. All members investigated so far form large oligomeric complexes of spherical or cylindrical appearance [74, 75]. Complex formation is independent of ATP binding and hydrolysis, but appears to be regulated by temperature and phosphorylation. The structural analysis of wheat Hsp16.9 suggested that the oligomeric complex acts as a storage form rather than an enclosure for substrates, as the active chaperone appears to be a dimer [75]. In agreement with this notion, dissociation of the oligomeric complex formed by yeast Hsp26 was found to be a prerequisite for efficient chaperone activity [76]. Subsequent refolding may occur spontaneously or may involve cooperation with other chaperones such as Hsp70 [77].

1.3.4 Chaperonins

The chaperone proteins best understood with regard to their mode of action are certainly the so-called chaperonins, which are defined by a barrel-shaped, double-ring structure [25, 28]. Members include bacterial GroEL, Hsp60 of mitochondria and chloroplasts, and the TriC–CCT complex localized in the eukaryotic cytoplasm. Based on their characteristic ring structure, a central cavity is formed, which accommodates nonnative 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 [78–80]. As a consequence the unfolded polypeptide is released into the central chamber and can proceed on its folding pathway in a protected environment [81]. The chaperonins are therefore capable of folding proteins such as actin that cannot be properly folded via other mechanisms [82].

1.4 Chaperones: Central Players During Protein Quality Control

Due to their ability to recognize nonnative conformations of other proteins, molecular chaperones are of central importance during protein quality control. This was elegantly revealed in studies on the influence of the Hsp70 chaperone system on polyglutamine diseases using the fruit fly *Drosophila melanogaster* as a model organism (reviewed in Refs. [23] and [83]). 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 [84]. Transgene expression caused formation of abnormal protein inclusions and progressive neuronal degeneration. Intriguingly, co-expression of human cytoplasmic Hsp70 suppressed polyQ-induced neurotoxicity. In a similar experimental approach, Hsp40 family members protected neuronal cells against toxic polyQ expression [22]. Enhancing the activity of the Hsp70/Hsp40 chaperone system apparently mitigates cytotoxicity caused by the accumulation of aggregation-prone pro-

teins. These findings obtained in *Drosophila* were confirmed in a mouse model of spinocerebellar ataxia type 1 (SCA1) [85, 86]. Unexpectedly, however, 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 [84, 85, 87–89]. Elevating the 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. Notably, Hsp70 and Hsp40 profoundly modulated the aggregation process of polyQ tracts in biochemical experiments; this led to the formation of amorphous, SDS-soluble aggregates, instead of the ordered, SDS-insoluble amyloid fibrils that form in the absence of the chaperone system [88]. These biochemical data were confirmed in yeast and mammalian cells [88, 90]. Although unable to prevent the formation of protein aggregates, the Hsp70 chaperone system apparently prevents the ordered oligomerization and fibril growth that is characteristic of the disease process. In an alternate but not mutually exclusive model to explain their protective role, the chaperones may cover potentially dangerous surfaces exposed by polyQ-containing proteins during the oligomerization process or by the final oligomers. Intriguingly, elevated expression of Hsp70 also suppresses the toxicity of the non-polyQ-containing protein α-synuclein in a *Drosophila* model of Parkinson's disease without inhibiting aggregate formation [24]. Hsp70 may thus exert a rather general function in protecting cells against toxic protein aggregation. This raises the exciting possibility that treatment of diverse forms of human neurodegenerative diseases may be achieved through upregulation of Hsp70 activity.

The mentioned examples illustrate that one does not have to evoke the refolding of an aberrant protein to the native state in order to explain the protective activity of Hsp70 observed in models of amyloid diseases. In some cases it might be sufficient for Hsp70 to modulate the aggregation process or to shield interaction surfaces of the misfolded protein to decrease cytotoxic effects. Another option may involve presentation of the misfolded protein to the ubiquitin–proteasome system for degradation.

1.5 Chaperones and Protein Degradation

Hsp70 and Hsp90 family members as well as small heat shock proteins have all been implicated to participate in protein degradation. For example, the small heat shock protein Hsp27 was recently shown to stimulate the degradation of phosphorylated IκBα via the ubiquitin–proteasome pathway, which may account for the antiapoptotic function of Hsp27 [91]. Similarly, Hsp27 facilitates the proteasomal degradation of phosphorylated tau, a microtubule-binding protein and component of protein deposits in Alzheimer's disease [92]. Hsp70 participates in the degradation of apolipoprotein B100 (apoB), which is essential for the assembly and secretion of very low-density lipoproteins from the liver [93]. Under conditions of limited availability of core lipids, apoB translocation across the ER membrane is

attenuated, resulting in the exposure of some domains of the protein into the cytoplasm and their recognition by Hsp70. This is followed by the degradation of apoB via the ubiquitin–proteasome pathway. Elevating cellular Hsp70 levels stimulated the degradation of the membrane protein, suggesting that the chaperone facilitates sorting to the proteasome. Genetic studies in yeast indicate that cytoplasmic Hsp70 may fulfill a rather general role in the degradation of ER-membrane proteins that display large domains into the cytoplasm [94]. In agreement with this notion, Hsp70 also takes part in the degradation of immaturely glycosylated and aberrantly folded forms of the cystic fibrosis transmembrane conductance regulator (CFTR) [95–98]. CFTR is an ion channel localized at the apical surface of epithelial cells. Its functional absence causes cystic fibrosis, the most common fatal genetic disease in Caucasians [99, 100]. The disease-causing allele, ΔF508, which is expressed in more than 70% of all patients, drastically interferes with the protein's ability to fold, essentially barring it from functional expression in the plasma membrane. However, wild-type CFTR also folds very inefficiently, and less than 30% of the protein reaches the plasma membrane [99]. While trafficking from the endoplasmic reticulum (ER) to the Golgi apparatus, immature forms of CFTR are recognized by quality-control systems and are eventually directed to the proteasome for degradation [101–104]. A critical step during CFTR biogenesis is the inefficient folding of the first of two cytoplasmically exposed nucleotide-binding domains (NBD1) of the membrane protein [105, 106]. The disease-causing ΔF508 mutation localizes to NBD1 and further decreases the folding propensity of this domain. During the co-translational insertion of CFTR into the ER membrane, cytoplasmic Hsp70 and its co-chaperone Hdj-2 bind to NBD1 and facilitate intramolecular interactions between the domain and another cytoplasmic region of CFTR, the regulatory R-domain [96, 107]. However, Hsp70 is also able to present CFTR to the ubiquitin–proteasome system [97], and heterologous expression of CFTR in yeast revealed an essential role of cytoplasmic Hsp70 in CFTR turnover [98]. Hsp70 is thus a key player in the cellular surveillance system that monitors the folded state of CFTR at the ER membrane.

Interestingly, CFTR and the disease form ΔF508 are deposited in distinct pericentriolar structures, termed aggresomes, upon overexpression or proteasome inhibition [108]. Subsequent studies established that aggresomes are induced upon ectopic expression of many different aggregation-prone proteins (reviewed in Refs. [109] and [110]). Aggresomes form near the microtubule-organizing center in a manner dependent on the microtubule-associated motor protein dynein, and are surrounded by a ''cage'' of filamentous vimentin [108, 111]. Aggresome formation is apparently a specific and active cellular response when production of misfolded proteins exceeds the capacity of the ubiquitin–proteasome system to tag and remove these proteins. They likely serve to protect the cell from toxic ''gain-of-function'' activities acquired by misfolded proteins. Aggresomes are also of clinical relevance as they share remarkable biochemical and structural features, for example, with Lewy bodies, the cytoplasmic inclusion bodies found in neurons affected by Parkinson's disease [112]. The pathways that regulate aggresome assembly are only now being explicated. Histone deacetylase 6 (HDAC6) appears to be a key reg-

ulator of aggresome assembly [113]. HDAC6 is a microtubule-associated deacetylase that has the capacity to bind both multi-ubiquitinated proteins and dynein motors and is believed to recruit misfolded proteins to the pericentriolar region for aggresome assembly. Deletion of HDAC6 prevents aggresome formation and sensitizes cells to the toxic effects of misfolded proteins, which supports the hypothesis that aggresomes sequester misfolded proteins to protect against their toxic activities. Components of the ubiquitin–proteasome system and chaperones such as Hsp70 are abundantly present in and are actively recruited to aggresomes [114–116]. Furthermore, elevating cellular Hsp70 levels can reduce aggresome formation by stimulating proteasomal degradation [117]. It appears that these subcellular structures are major sites of chaperone–proteasome cooperation to mediate the metabolism of misfolded proteins.

The formation of aggresome-like structures is also observed in dendritic cells that present foreign antigens to other immune cells [118]. Immature dendritic cells are located in tissues throughout the body, including skin and gut. When they encounter invading microbes, the pathogens are endocytosed and processed in a manner that involves the generation of antigenic peptides by the ubiquitin–proteasome system. Upon induction of dendritic cell maturation, ubiquitinated proteins transiently accumulate in large cytosolic structures that resemble aggresomes and were therefore termed DALIS (dendritic cell aggresome-like induced structures). It was speculated that DALIS formation may enable dendritic cells to regulate antigen processing and presentation. DALIS contain components of the ubiquitin–proteasome machinery as well as Hsp70 and the co-chaperone CHIP [118, 119]. Again, an interplay of molecular chaperones and the ubiquitin–proteasome system during regulated protein turnover is suggested.

The cellular function of molecular chaperones is apparently not restricted to mediating protein folding; instead, chaperones emerge also as vital components on protein-degradation pathways. Remarkably, the balance between folding and degradation activities of chaperones can be manipulated. In cells treated with Hsp90 inhibitors, for example, with geldanamycin (see above), the chaperone-assisted activation of signaling proteins is abrogated and chaperone substrates such as the protein kinases Raf-1 and ErbB2 are rapidly degraded by the ubiquitin–proteasome system [64–69, 120]. This appears to be due, in part, to transfer of the substrates back to Hsp70 and progression toward the ubiquitin-dependent degradation pathway.

Substrate interactions with chaperones – and consequently their commitment either toward the folding pathway or to their degradation via the ubiquitin–proteasome machinery – apparently serve as an essential post-translational protein quality-control mechanism within eukaryotic cells. The partitioning of proteins to either one of these mutually exclusive pathways is referred to as ''protein triage'' [121]. Although some misfolded proteins may be directly recognized by the proteasome [122], specific pathways within the ubiquitin–proteasome system are probably relied on for the degradation of most misfolded and damaged proteins. For example, E2 enzymes of the Ubc4/5 family selectively mediate the ubiquitylation of abnormal proteins as revealed in genetic studies in *Saccharomyces cerevisiae* [123].

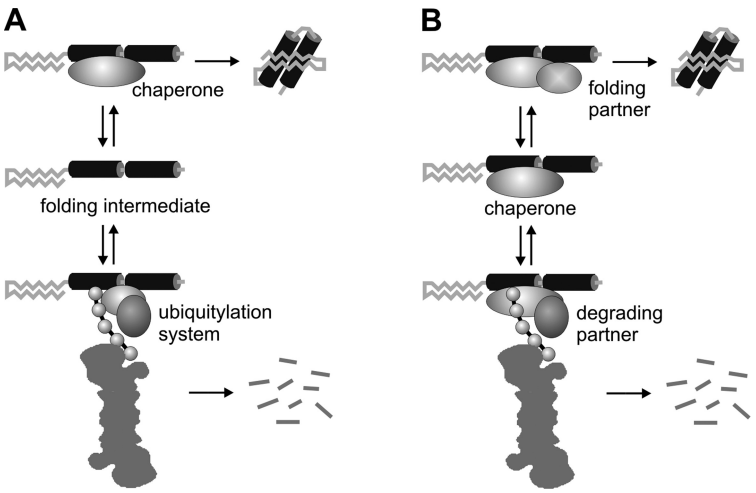

Fig. 1.5. Interplay of molecular chaperones with the ubiquitin–proteasome system. (A) Chaperones and the degradation machinery (i.e., ubiquitylation systems) compete with each other in the recognition of folding intermediates. Interaction with the chaperones directs the substrate towards folding. However, when the protein substrate is unable to attain a folded conformation, the chaperones maintain the folding intermediate in a soluble state that can be recognized by the degradation machinery. (B) The chaperones are actively involved in protein degradation. Through an association with certain components of the ubiquitin conjugation machinery (degrading partner), the chaperones participate in the targeting of protein substrates to the proteasome. A competition between degrading partners and folding partners determines chaperone action and the fate of the protein substrate.

It is well accepted that chaperones play a central role in the triage decision; however, less well understood are the events that lead to the cessation of efforts to fold a substrate, and the diversion of the substrate to the terminal degradative pathway. It is possible that chaperones and components of the ubiquitin–proteasome pathway exist in a state of competition for these substrates and that repeated cycling of a substrate on and off a chaperone maintains the substrate in a soluble state and increases, in a stochastic fashion, its likelihood of interactions with the ubiquitin machinery (Figure 1.5A). However, some data argue for a more direct role of the chaperones in the degradation process. Hsp70 plays an active and necessary role in the ubiquitylation of some substrates [124]; this activity of Hsp70 requires its chaperone function, indicating that conformational changes within substrates may facilitate recognition by the ubiquitylation machinery. Plausible hypotheses to explain these observations include direct associations between the chaperone and ubiquitin–proteasome machinery to facilitate transfer of a substrate from one pathway to the other, or conversion of the chaperone itself to a ubiquitylation complex (Figure 1.5B). It is also entirely possible that several quality-control pathways may exist and that the endogenous triage decision may involve aspects of each of these hypotheses.

1.6
The CHIP Ubiquitin Ligase: A Link Between Folding and Degradation Systems

Major insights into molecular mechanisms that underlie the cooperation of molecular chaperones with the ubiquitin–proteasome system were obtained through the functional characterization of the co-chaperone CHIP (reviewed in Ref. [40]). CHIP was initially identified in a screen for proteins containing tetratricopeptide repeat (TPR) domains, which are found in several co-chaperones – including Hip, Hop, and the cyclophilins – as chaperone-binding domains [27, 55] (Figure 1.2). CHIP contains three TPR domains at its amino terminus, which are used for binding to Hsp70 and Hsp90 [35, 125]. Besides the TPR domains, CHIP possesses a U-box domain at its carboxyl terminus [35] (Figure 1.2). U-box domains are similar to RING finger domains, but they lack the metal-chelating residues and instead are structured by intramolecular interactions [126]. The predicted structural similarity suggests that U boxes, like RING fingers, may also play a role in targeting proteins for ubiquitylation and subsequent proteasome-dependent degradation, and this possibility is borne out in functional analyses of U box–containing proteins [127, 128]. The TPR and U-box domains in CHIP are separated by a central domain rich in charged residues. The charged domain of CHIP is necessary for TPR-dependent interactions with Hsp70 [35] and is also required for homodimerization of CHIP [129].

The tissue distribution of CHIP supports the notion that it participates in protein folding and degradation decisions, as it is most highly expressed in tissues with high metabolic activity and protein turnover: skeletal muscle, heart, and brain. Although it is also present in all other organs, including pancreas, lung, liver, placenta, and kidney, the expression levels are much lower. CHIP is also detectable in most cultured cells, and is particularly abundant in muscle and neuronal cells and in tumor-derived cell lines [35]. Intracellularly, CHIP is primarily localized to the cytoplasm under quiescent conditions [35], although a fraction of CHIP is present in the nucleus [97]. In addition, cytoplasmic CHIP traffics into the nucleus in response to environmental challenge in cultured cells, which may serve as a protective mechanism or to regulate transcriptional responses in the setting of stress [130].

CHIP is distinguished among co-chaperones in that it is a bona fide interaction partner with both of the major cytoplasmic chaperones Hsp90 and Hsp70, based on their interactions with CHIP in the yeast two-hybrid system and *in vivo* binding assays [35, 125]. CHIP interacts with the terminal-terminal EEVD motifs of Hsp70 and Hsp90, similar to other TPR domain–containing co-chaperones such as Hop [55, 131, 132]. When bound to Hsp70, CHIP inhibits ATP hydrolysis and therefore attenuates substrate binding and refolding, resulting in inhibition of the ''forward'' Hsp70 substrate folding/refolding pathway, at least in *in vitro* assays [35]. The cellular consequences of this ''anti-chaperone'' function are not yet clear, and in fact CHIP may actually facilitate protein folding under conditions of stress, perhaps by slowing the Hsc70 reaction cycle [130, 133]. CHIP interacts with Hsp90 with approximately equivalent affinity to its interaction with Hsp70 [125]. This interaction

results in remodeling of Hsp90 chaperone complexes, such that the co-chaperone p23, which is required for the appropriate activation of many, if not all, Hsp90 client proteins, is excluded. The mechanism for this activity is unclear – p23 and CHIP bind Hsp90 through different sites – yet the consequence of this action is predictable: CHIP should inhibit the function of proteins that require Hsp90 for conformational activation. The glucocorticoid receptor is an Hsp90 client that undergoes activation through a well-described sequence of events that depend on interactions of the glucocorticoid receptor with Hsp90 and various Hsp90 co-chaperones, including p23, making it an excellent model to test this prediction. Indeed, CHIP inhibits glucocorticoid receptor substrate binding and steroid-dependent transactivation ability [125]. Surprisingly, this effect of CHIP is accompanied by decreased steady-state levels of glucocorticoid receptor, and CHIP induces ubiquitylation of the glucocorticoid receptor *in vivo* and *in vitro*, as well as subsequent proteasome-dependent degradation. This effect is both U-box- and TPR-domain-dependent, suggesting that CHIP's effects on GR require direct interaction with Hsp90 and direct ubiquitylation of GR and delivery to the proteasome.

These observations are not limited to the glucocorticoid receptor. ErbB2, another Hsp90 client, is also degraded by CHIP in a proteasome-dependent fashion [120]. Nor are they limited to Hsp90 clients. For example, CHIP cooperates with Hsp70 during the degradation of immature forms of the CFTR protein at the ER membrane and during the ubiquitylation of phosphorylated forms of the microtubule-binding protein tau, which is of clinical importance due to its role in the pathology of Alzheimer's disease [97, 134]. The effects of CHIP are dependent on both the TPR domain, indicating a necessity for interactions with molecular chaperones, and the U box, which suggests that the U box is most likely the "business end" with respect to ubiquitylation. The means by which CHIP-dependent ubiquitylation occurs is not clear. In the case of ErbB2, ubiquitylation depends on a transfer of the client protein from Hsp90 to Hsp70 [120], indicating that the final ubiquitylation complex consists of CHIP, Hsp70 (but not Hsp90), and the client protein. In any event, the studies are consistent in supporting a role for CHIP as a key component of the chaperone-dependent quality-control mechanism. CHIP efficiently targets client proteins, particularly when they are partially unfolded (as is the case for most Hsp90 clients when bound to the chaperone) or frankly misfolded (as is the case for most proteins binding to Hsp70 through exposed hydrophobic residues).

Once the ubiquitylation activity of CHIP was recognized, it was logical to speculate that its U box might function in a manner analogous to that of RING fingers, which have recently been appreciated as key components of the largest family of ubiquitin ligases. If CHIP is a ubiquitin ligase, then its ability to ubiquitylate a substrate should be reconstituted *in vitro* when a substrate is added in the presence of CHIP, E1, an E2, and ubiquitin. Indeed, this is the case [135–137] (Figure 1.6). CHIP is thus the first described chaperone-associated E3 ligase. The ubiquitin ligase activity of CHIP depends on functional and physical interactions with a specific family of E2 enzymes, the Ubc4/Ubc5 family, which in humans comprises the E2s UbcH5a, UbcH5b, and UbcH5c. Of interest is the fact that the Ubc4/Ubc5 E2s

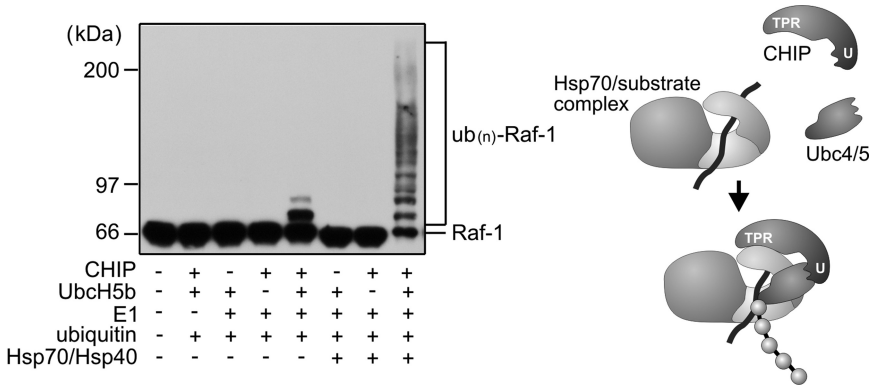

Fig. 1.6. Characterization of CHIP as a chaperone-associated ubiquitin ligase. Purified CHIP, UbcH5b, the ubiquitin-activating enzyme E1, ubiquitin, and the Hsp70–Hsp40 chaperone system were incubated with the bacterially expressed protein kinase Raf-1 (for details, see Ref. [137]). Raf-1 and ubiquitylated forms of the kinase ($ub_{(n)}$-Raf-1) were detected by immunoblotting using a specific anti-Raf-1 antibody. Efficient ubiquitylation of Raf-1 through the CHIP conjugation machinery depends on the recognition of the chaperone substrate by Hsp70, which presents the kinase to the conjugation machinery.

are stress-activated, ubiquitin-conjugating enzymes [123]. CHIP can therefore be seen as a co-chaperone that, in addition to inhibiting traditional chaperone activity, converts chaperone complexes into chaperone-dependent ubiquitin ligases. Indeed, the chaperones themselves seem to act as the main substrate-recognition components of these ubiquitin ligase complexes, as efficient ubiquitylation of chaperone substrates by CHIP depends on the presence of Hsp70 or Hsp90 in reconstituted systems [136, 137] (Figure 1.6). The chaperones apparently function in a manner analogous to F-box proteins, which are required as substrate recognition modules in many RING finger–containing ubiquitin ligase complexes [138–140].

Recently, another surprising function for CHIP has been identified, that of activation of the stress-responsive transcription factor heat shock factor-1 (HSF1) [130]. Through this association, CHIP regulates the expression of chaperones such as Hsp70 independently of its ability to modify their function through direct interactions. The mechanisms through which CHIP activates HSF1 are not entirely clear, but they are dependent in part on the induction of HSF1 trimerization, which is required for nuclear import and DNA binding. In addition, activation of HSF1 by CHIP seems to be independent of CHIP's ubiquitin ligase activity. The consequences of this activation are important for the response to stress, in that cells lacking CHIP are prone to stress-dependent apoptosis and mice deficient in CHIP (through homologous recombination) succumb rapidly to thermal challenge. These data indicate that CHIP plays a heretofore unsuspected role in coordinating the response to stress, not only by serving as a rate-limiting step in the degradation of damaged proteins but also by increasing the buffering capacity of the chaperone system to guard against stress-dependent proteotoxicity.

1.7
Other Proteins That May Influence the Balance Between Chaperone-assisted Folding and Degradation

CHIP is ideally suited to mediate chaperone–proteasome cooperation, as it combines a chaperone-binding motif and a domain that functions in ubiquitin-dependent degradation within its protein structure (Figure 1.2). Some other co-chaperones display a similar structural arrangement [40]. For example, BAG-1 contacts Hsp70 through a BAG-domain located at its carboxyl terminus and, in addition, possesses a central ubiquitin-like domain that is used for binding to the proteasome [141] (Figure 1.2). The co-chaperone thus belongs to a family of ubiquitin domain proteins (UDPs), many of which were shown to be associated with the proteasome [142]. This domain architecture enables BAG-1 to provide a physical link between Hsp70 and the proteasome, and elevating the cellular levels of BAG-1 results in a recruitment of the chaperone to the proteolytic complex. Notably, BAG-1 and CHIP occupy distinct domains on Hsp70 (Figure 1.7). Whereas BAG-1 associates with the amino-terminal ATPase domain, CHIP binds to the carboxyl-terminal EEVD motif of Hsp70 [35, 37]. Ternary complexes that comprise both co-chaperones associated with Hsp70 can be isolated from mammalian cells, suggesting a cooperation of BAG-1 and CHIP in the regulation of Hsp70 activity on certain degradation pathways. In fact, BAG-1 is able to stimulate the CHIP-induced degradation of the glucocorticoid hormone receptor [137]. A cooperation of diverse co-chaperones apparently provides additional levels of regulation to alter chaperone-assisted folding and degradation pathways.

Interestingly, BAG-1 and also Hsp70 and Hsp90 are themselves substrates of the CHIP ubiquitin ligase [135, 143] (J.H. unpublished). Yet, CHIP-mediated ubiquitylation of the chaperones and the co-chaperone does not induce their proteasomal degradation. Instead, it seems to provide additional means to regulate the association of the chaperone systems with the proteasome. In the case of BAG-1, ubiquitylation mediated by CHIP indeed stimulates the binding of the co-chaperone to the proteasome [143]. It remains to be elucidated, however, why Hsp70 and BAG-1 are not degraded when sorted to the proteasome through CHIP-induced ubiqui-

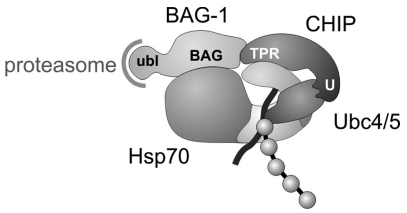

Fig. 1.7. Schematic presentation of the BAG-1–Hsp70–CHIP complex. BAG-1 associates with the ATPase domain of Hsp70, while CHIP is bound to the carboxyl terminus. BAG-1 mediates an association of Hsp70 with the proteasome via its ubiquitin-like domain (ubl), whereas CHIP acts in conjunction with Ubc4/5 as a chaperone-associated ubiquitin ligase to mediate the attachment of a polyubiquitin chain to the chaperone substrate.

tylation, in contrast to chaperone substrates such as the glucocorticoid hormone receptor. Possibly, the folded state of the proteins may serve to distinguish targeting factors and substrates doomed for degradation.

Efficient ubiquitylation of BAG-1 mediated by CHIP is dependent on the formation of the ternary BAG-1–Hsp70–CHIP complex [143]. The formed chaperone complex would thus expose multiple signals for sorting to the proteasome, e.g., the integrated ubiquitin-like domain of BAG-1 and polyubiquitin chains attached to BAG-1, Hsp70, and the bound protein substrate. Such a redundancy of sorting information might be considered unnecessary. Intriguingly, however, several subunits of the regulatory 19S particle of the proteasome are currently thought to act as receptors for polyubiquitin chains and integrated ubiquitin-like domains, including Rpn1, Rpn2, Rpt5, and Rpn10. 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 [144–146]. Rpn10 possesses two distinct ubiquitin-binding domains, of which only one is used for UDP recognition [145–147]. However, conflicting data exist as to whether the subunit acts as a ubiquitin receptor in the context of the assembled 19S complex [148, 149]. More recently, Rpn1 was identified as a receptor for integrated ubiquitin-like domains [149], and a similar function may be fulfilled by the Rpn1-related subunit Rpn2 [150]. Polyubiquitin chains seem to be recognized by the Rpt5 subunit, one of the AAA ATPases present in the ring-like base of the regulatory 19S complex [151]. Its receptor function was revealed when tetraubiquitin was cross-linked to intact proteasomes [148]. Multiple docking sites for ubiquitin-like domains and polyubiquitin chains are apparently displayed by the regulatory particle of the proteasome. This may provide a structural basis for the recognition of multiple sorting signals exposed by the CHIP–chaperone complex (Figure 1.8). 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 [152]. In these cases substrate delivery involves interactions of proteasomal subunits with the substrate-bound ubiquitin ligase, with the polyubiquitin chain attached to the substrate, and with the substrate itself. 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.

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) [153] (Figure 1.2). It appears that BAG proteins act as nucleotide-exchange factors to induce substrate unloading from Hsp70 on diverse protein folding, assembly, and degradation pathways. Notably, BAG-6 is another likely candidate for a co-chaperone that regulates protein degradation via the ubiquitin–proteasome pathway. Similar to BAG-1, BAG-6 contains a ubiquitin-like domain that is possibly used for proteasome binding [154]. However, experimental data verifying a role of BAG-6 in protein degradation remain elusive so far.

The cooperation of diverse co-chaperones not only may allow promotion of chaperone-associated degradation but also may provide the means to confine the

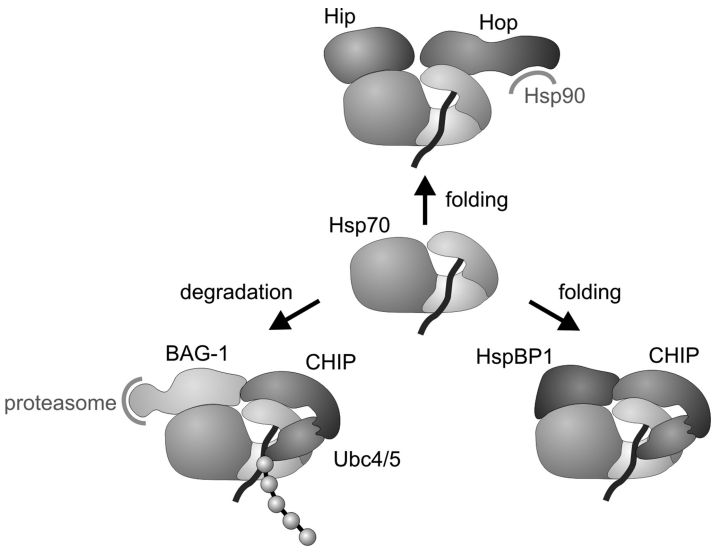

Fig. 1.8. The co-chaperone network that determines folding and degradation activities of Hsp70. BAG-1 and CHIP associate with Hsp70 to induce the proteasomal degradation of a Hsp70-bound protein substrate. When BAG-1 is displaced by binding of HspBP1 to the ATPase domain of Hsp70, the ubiquitin ligase activity of CHIP is attenuated in the formed complex, enabling CHIP to modulate Hsp70 activity without inducing degradation. The ATPase domain can also be occupied by Hip, which stimulates the chaperone activity of Hsp70 and participates in the Hsp70/Hsp90-mediated regulation of signal transduction pathways. At the same time, Hop displaces CHIP from the carboxyl terminus of Hsp70 and recruits Hsp90 to the chaperone complex.

destructive activity of CHIP. The Hsp70-binding protein 1 (HspBP1) seems to fulfill such a regulatory function [155]. HspBP1 was initially identified in a screen for proteins that associate with the ATPase domain of Hsp70 and was shown to stimulate nucleotide release from the chaperone [156, 157]. Notably, association of HspBP1 with the ATPase domain blocks binding of BAG-1 to Hsp70 and at the same time promotes an interaction of CHIP with Hsp70's carboxyl terminus. In the formed ternary HspBP1–Hsp70–CHIP complex, the ubiquitin ligase activity of CHIP is attenuated and Hsp70 as well as a chaperone substrate are no longer efficiently ubiquitylated [155]. By interfering with CHIP-mediated ubiquitylation, HspBP1 stimulates the maturation of CFTR and promotes the sorting of the membrane protein to the cell surface. HspBP1 apparently functions as an antagonist of the CHIP ubiquitin ligase to regulate Hsp70-assisted folding and degradation pathways (Figure 1.8).

The HspBP1-mediated inhibition of the ubiquitin ligase activity may enable CHIP to modulate the Hsp70 ATPase cycle without inducing degradation. In fact, degradation-independent functions of CHIP have recently emerged [130, 133, 158, 159]. CHIP was shown to regulate the chaperone-assisted folding and sorting of

the androgen receptor and of endothelial nitric oxide synthase without inducing degradation [158, 159]. Moreover, CHIP fulfills an essential role in the chaperone-mediated regulation of the heat shock transcription factor, independent of its degradation-inducing activity [130]. It remains to be seen, however, whether HspBP1 cooperates with CHIP in these instances, as HspBP1 displayed a certain specificity with regard to chaperone substrates. The co-chaperone interfered with the degradation of CFTR, but did not influence the CHIP-mediated turnover of the glucocorticoid hormone receptor. Such a client specificity may arise in part from the fact that HspBP1 inhibits the ubiquitin ligase activity of CHIP in a complex with Hsc70, but leaves Hsp90-associated ubiquitylation unaffected [155]. In addition, direct interactions between HspBP1 and a subset of chaperone substrates may contribute to substrate selection. In any case, the cooperation of CHIP with other co-chaperones apparently provides a means to regulate chaperone-assisted protein degradation.

It is likely that there are multiple degradation pathways for misfolded proteins in the eukaryotic cytoplasm. Although CHIP participates in the degradation of chaperone substrates induced by applying Hsp90 inhibitors to cell cultures (see above), drug-induced degradation is not abrogated in cells that lack the CHIP ubiquitin ligase [120]. Furthermore, CHIP cooperates with Hsp70 in the ER-associated degradation of CFTR, but the Hsp70-assisted degradation of apoB at the cytoplasmic face of the ER membrane does not involve CHIP [97]. Taken together, these data strongly argue for the existence of other, yet to be identified, ubiquitin ligases that are able to target chaperone substrates to the proteasome. A likely candidate in this regard is Parkin, a RING finger ubiquitin ligase, whose activity is impaired in juvenile forms of Parkinson's disease [17]. Hsp70 and CHIP were found to be associated with Parkin in neuronal cells, suggesting an involvement of Parkin in the proteasomal degradation of chaperone substrates [160]. Interestingly, α-synuclein, the main component of protein deposits observed in dopaminergic neurons of Parkinson patients, and synphilin, a protein that binds α-synuclein and induces deposit formation, both associate with yet other ubiquitin ligases: Siah-1 and Siah-2 [161, 162]. In the case of Siah-1, a link to cytoplasmic chaperone systems is suggested by the finding that the Hsp70 co-chaperone BAG-1 is a binding partner of the ubiquitin ligase and suppresses some of the cellular activities of Siah-1 [163]. Taken together, it is tempting to speculate about a role of Parkin and Siah on chaperone-assisted degradation pathways; yet, this remains to be explored in detail.

1.8 Further Considerations

Although the appreciation of interplay between molecular chaperones and ubiquitin-dependent proteolysis has greatly expanded over the past decade, a number of critical issues remain to be resolved. It is not entirely clear what determines whether a misfolded protein will undergo repeated attempts at misfolding versus

diversion to the ubiquitin–proteasome pathway. Recruitment of CHIP into chaperone complexes appears to be a critical component of this reaction, which therefore begs the question as to what regulates this step. Since this step in protein quality control must be both rapidly activated and easily reversible, it is likely that regulation occurs at the post-translational level rather than through changes in steady-state protein levels. The precise sorting mechanisms for ubiquitinated proteins are also unclear. BAG-1 is a player, and it is also likely that overlap exists to some extent for sorting of the cytoplasmic and endoplasmic reticulum quality-control pathways. Nevertheless, much remains to be learned about these steps.

From a broader perspective, it is now also imperative to understand the pathophysiological roles of cytoplasmic quality-control mechanisms regulated by chaperone–proteasome interactions. As mentioned previously, there is a strong association between chaperone dysfunction and accumulations of misfolded proteins that characterizes genetic neurodegenerative diseases. An imbalance between protein folding and degradation may also contribute to some features of senescence and organismal aging. The link between chaperone systems and aging is based on increasing appreciation that modified, misfolded, and aggregated proteins accumulate with age [164]. Dysregulation of chaperone expression has been observed with aging and is therefore implicated in aging-related changes [165]; in general, it is accepted that induction of the major chaperones is impaired with aging, a fact confirmed by recent gene-profiling experiments *in vivo* [166], although given the diversity of chaperones it is probably not surprising that age-related changes in expression are fairly complicated [167]. The mechanism underlying this dysregulation is not entirely clear, but seems to be due in part to impaired activation of the stress-responsive transcription factor HSF1. Overexpression of heat shock proteins in yeast, *C. elegans*, and *Drosophila* leads to increased longevity [168–170]. More recently, conclusive genetic evidence from *C. elegans* indicates that mutation of HSF1 causes a dramatic and significant reduction in lifespan [170, 171], further implicating the accumulation of misfolded proteins in age-related phenotypes.

1.9 Conclusions

The associations between molecular chaperones and the ubiquitin–proteasome system represent a critical step in the response to proteotoxic damage. Whether attempts should be made to refold damaged proteins (thus conserving cellular resources) or degrade them instead (to prevent the possibility of protein aggregation and concomitant toxicity) requires a consideration of cellular economy. Defects in the quality-control mechanisms may have enormous consequences even if only slight imbalances occur between protein folding and degradation, as these imbalances can cause accumulated toxicity over time. The relationship between chaperone–proteasome interactions and pathophysiological events is only now being unraveled. Modulation of this system may provide a unique therapeutic target for degenerative diseases and pathologies associated with aging.

References

1 U. Schubert, L.C. Anton, J. Gibbs, C.C. Norbury, J.W. Yewdell, J.R. Bennink, Rapid degradation of a large fraction of newly synthesized proteins by proteasomes, Nature 404 (2000) 770–774.

2 J.P. Taylor, J. Hardy, K.H. Fischbeck, Toxic proteins in neurodegenerative disease, Science 296 (2002) 1991–1995.

3 A. Horwich, Protein aggregation in disease: a role for folding intermediates forming specific multimeric interactions, J. Clin. Invest. 110 (2002) 1221–1232.

4 E. Scherzinger, R. Lurz, M. Turmaine, L. Mangiarini, B. Hollenbach, R. Hasenbank, G.P. Bates, S.W. Davies, H. Lehrach, E.E. Wanker, Huntingtin-encoded polyglutamine expansions form amyloid-like protein aggregates *in vitro* and *in vivo*, Cell 90 (1997) 549–558.

5 E. Scherzinger, A. Sittler, K. Schweiger, V. Heiser, R. Lurz, R. Hasenbank, G.P. Bates, H. Lehrach, E.E. Wanker, Self-assembly of polyglutamine-containing huntingtin fragments into amyloid-like fibrils: implications for Huntington's disease pathology, Proc. Natl. Acad. Sci. USA 96 (1999) 4604–4609.

6 A. Kazantsev, E. Preisinger, A. Dranovsky, D. Goldgaber, D. Housman, Insoluble detergent-resistant aggregates form between pathological and nonpathological lengths of polyglutamine in mammalian cells, Proc. Natl. Acad. Sci. U S A 96 (1999) 11404–11409.

7 D. Martindale, A. Hackam, A. Wieczorek, L. Ellerby, C. Wellington, K. McCutcheon, R. Singaraja, P. Kazemi-Esfarjani, R. Devon, S.U. Kim, D.E. Bredesen, F. Tufaro, M.R. Hayden, Length of huntingtin and its polyglutamine tract influences localization and frequency of intracellular aggregates. Nat. Genet. 18 (1998) 150–154.

8 I. Sanchez, C. Mahlke, J. Yuan, Pivotal role of oligomerization in expanded polyglutamine neurodegenerative disorders, Nature 421 (2003) 373–379.

9 M.F. Perutz, Glutamine repeats and neurodegenerative diseases: molecular aspects, Trends Biochem. Sci. 24 (1999) 58–63.

10 H.Y. Zoghbi, H.T. Orr, Glutamine repeats and neurodegeneration, Annu. Rev. Neurosci. 23 (2000) 217–247.

11 H.T. Orr, M.Y. Chung, S. Banfi, T.J. Jr. Kwiatkowski, A. Servadio, A.L. Beaudet, A.E. McCall, L.A. Duvick, L.P. Ranum, H.Y. Zoghbi, Expansion of an unstable trinucleotide CAG repeat in spinocerebellar ataxia type 1, Nat. Genet. 4 (1993) 221–226.

12 D.R. Rosen, T. Siddique, D. Patterson, D.A. Figlewicz, P. Sapp, A. Hentati, D. Donaldson, J. Goto, J.P. O'Regan, H.X. Deng, et al. Mutations in Cu/Zn superoxide dismutase gene are associated with familial amyotrophic lateral sclerosis. Nature 362 (1993) 59–62.

13 J.A. Johnston, M.J. Dalton, M.E. Gurney, R.R. Kopito, Formation of high molecular weight complexes of mutant Cu, Zn-superoxide dismutase in a mouse model for familial amyotrophic lateral sclerosis, Proc. Natl. Acad. Sci. USA 97 (2000) 12571–12576.

14 B.I. Giasson, V.M. Lee, Are ubiquitination pathways central to Parkinson's disease? Cell 114 (2003) 1–8.

15 T. Kitada, S. Asakawa, N. Hattori, H. Matsumine, Y. Yamamura, S. Minoshima, M. Yokochi, Y. Mizuno, N. Shimizu, Mutations in the parkin gene cause autosomal recessive juvenile parkinsonism, Nature 392 (1998) 605–608.

16 C.B. Lücking, A. Durr, V. Bonifati, J. Vaughan, G. De Michele, T. Gasser, B.S. Harhangi, G. Meco, P. Denefle, N.W. Wood, Y. Agid, A. Brice, Association between early-onset Parkinson's disease and mutations in the parkin gene, N. Engl. J. Med. 342 (2000) 1560–1567.

17 H. Shimura, N. Hattori, S. Kubo, Y. Mizuno, S. Asakawa, S. Minoshima, N. Shimizu, K. Iwai, T. Chiba, K. Tanaka, T. Suzuki, Familial Parkinson disease gene product, parkin, is a ubiquitin-protein ligase, Nat. Genet. 25 (2000) 302–305.

18 K.K. Chung, Y. Zhang, K.L. Lim, Y. Tanaka, H. Huang, J. Gao, C.A. Ross, V.L. Dawson, T.M. Dawson, Parkin ubiquitinates the α-synuclein-interacting protein, synphilin-1: implications for Lewy-body formation in Parkinson disease, Nat. Med. 7 (2001) 1144–1150.

19 H. Shimura, M.G. Schlossmacher, N. Hattori, M.P. Frosch, A. Trockenbacher, R. Schneider, Y. Mizuno, K.S. Kosik, D.J. Selkoe, Ubiquitination of a new form of α-synuclein by parkin from human brain: implications for Parkinson's disease, Science 293 (2001) 263–269.

20 Y. Imai, M. Soda, H. Inoue, N. Hattori, Y. Mizuno, R. Takahashi, An unfolded putative transmembrane polypeptide, which can lead to endoplasmic reticulum stress, is a substrate of Parkin, Cell 105 (2001) 891–902.

21 P. Fernandez-Funez, M.L. Nino-Rosales, B. de Gouyon, W.C. She, J.M. Luchak, P. Martinez, E. Turiegano, J. Benito, M. Capovilla, P.J. Skinner, A. McCall, I. Canal, H.T. Orr, H.Y. Zoghbi, J. Botas, Identification of genes that modify ataxin-1-induced neurodegeneration, Nature 408 (2000) 101–106.

22 P. Kazemi-Esfarjani, S. Benzer, Genetic suppression of polyglutamine toxicity in Drosophila, Science 287 (2000) 1837–1840.

23 N.M. Bonini, Chaperoning brain degeneration, Proc. Natl. Acad. Sci. U S A 99 Suppl. 4 (2002) 16407–16411.

24 P.K. Auluck, H.Y. Chan, J.Q. Trojanowski, V.M. Lee, N.M. Bonini, Chaperone suppression of α-synuclein toxicity in a Drosophila model for Parkinson's disease, Science 295 (2002) 865–868.

25 F.U. Hartl, M. Hayer-Hartl, Molecular chaperones in the cytosol: from nascent chain to folded protein, Science 295 (2002) 1852–1858.

26 J. Frydman, Folding of newly translated proteins *in vivo*: the role of molecular chaperones, Annu. Rev. Biochem. 70 (2001) 603–647.

27 J. Frydman, J. Höhfeld, Chaperones get in touch: the Hip-Hop connection, Trends Biochem. Sci. 22 (1997) 87–92.

28 B. Bukau, A.L. Horwich, The Hsp70 and Hsp60 chaperone machines, Cell 92 (1998) 351–366.

29 R.I. Morimoto, Regulation of the heat shock transcriptional response: cross talk between a family of heat shock factors, molecular chaperones, and negative regulators. Genes Dev. 12 (1998) 3788–3796.

30 M.P. Mayer, D. Brehmer, C.S. Gässler, B. Bukau, Hsp70 chaperone machines, Adv. Protein Chem. 59 (2001) 1–44.

31 S. Rüdiger, L. Germeroth, J. Schneider-Mergener, B. Bukau, Substrate specificity of the DnaK chaperone determined by screening cellulose-bound peptide libraries, EMBO J. 16 (1997) 1501–1507.

32 V. Thulasiraman, C.F. Yang, J. Frydman, *In vivo* newly translated polypeptides are sequestered in a protected folding environment, EMBO J. 18 (1999) 85–95.

33 X. Zhu, X. Zhao, W.F. Burkholder, A. Gragerov, C.M. Ogata, M.E. Gottesman, W.A. Hendrickson, Structural analysis of substrate binding by the molecular chaperone DnaK, Science 272 (1996) 1606–1614.

34 Y. Minami, J. Höhfeld, K. Ohtsuka, F.U. Hartl, Regulation of the heat-shock protein 70 reaction cycle by the mammalian DnaJ homolog, Hsp40, J. Biol. Chem. 271 (1996) 19617–19624.

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

36 J. Höhfeld, Y. Minami, F.U. Hartl, Hip, a novel cochaperone involved in the eukaryotic Hsc70/Hsp40 reaction cycle, Cell 83 (1995) 589–598.

37 J. Höhfeld, S. Jentsch, GrpE-like regulation of the hsc70 chaperone by the anti-apoptotic protein BAG-1, Embo J. 16 (1997) 6209–6216.

38 H. Sondermann, C. Scheufler, C. Schneider, J. Höhfeld, F.U. Hartl, I. Moarefi, Structure of a Bag/Hsc70 complex: convergent functional evolution of Hsp70 nucleotide exchange factors, Science 291 (2001) 1553–1557.

39 C.S. Gässler, T. Wiederkehr, D. Brehmer, B. Bukau, M.P. Mayer, Bag-1M accelerates nucleotide release for human Hsc70 and Hsp70 and can act concentration-dependent as positive and negative cofactor, J. Biol. Chem. 276 (2001) 32538–32544.

40 J. Höhfeld, D.M. Cyr, C. Patterson, From the cradle to the grave: molecular chaperones that may choose between folding and degradation, EMBO Rep. 2 (2001) 885–890.

41 W.M. Obermann, H. Sondermann, A.A. Russo, N.P. Pavletich, F.U. Hartl, *In vivo* function of Hsp90 is dependent on ATP binding and ATP hydrolysis, J. Cell Biol. 143 (1998) 901–910.

42 J. Buchner, Hsp90 & Co. – a holding for folding, Trends Biochem. Sci. 24 (1999) 136–141.

43 J.C. Young, I. Moarefi, F.U. Hartl, Hsp90: a specialized but essential protein-folding tool, J. Cell Biol. 154 (2001) 267–273.

44 L.H. Pearl, C. Prodromou, Structur, function and mechanism of the Hsp90 molecular chaperone, Adv. Protein Chem. 59 (2001) 157–186.

45 B. Panaretou, G. Siligardi, P. Meyer, A. Maloney, J.K. Sullivan, S. Singh, S.H. Millson, P.A. Clarke, S. Naaby-Hansen, R. Stein, R. Cramer, M. Mooapour, P. Workman, P.W. Piper, L.H. Peal, C. Prodromou, Activation of the ATPase activity of hsp90 by the stress-regulated cochaperone aha1, Mol. Cell 10 (2002) 1307–1318.

46 J.C. Young, N.J. Hoogenraad, F.U. Hartl, Molecular chaperones Hsp90 and Hsp70 deliver preproteins to the mitochondrial import receptor Tom70, Cell 112 (2003) 41–50.

47 A. Brychzy, T. Rein, K.F. Winklhofer, F.U. Hartl, J.C. Young, W.M. Obermann, Cofactor Tpr2 combines two TPR domains and a J domain to regulate the Hsp70/Hsp90 chaperone system, EMBO J. 22 (2003) 3613–3623.

48 S.M. Roe, M.M. Ali, P. Meyer, C.K. Vaughan, B. Panaretou, P.W. Piper, C. Prodromou, L.H. Pearl, The mechanism of Hsp90 regulation by the protein kinase-specific cochaperone p50 (cdc37), Cell 116 (2004) 87–98.

49 K.D. Dittmar, W.B. Pratt, Folding of the glucocorticoid receptor be the reconstituted Hsp90-based chaperone machinery. The initial hsp90.p60.hsp70 dependent step is sufficient for creating the steroid binding conformation, J. Biol. Chem. 272 (1997) 13047–13054.

50 K.D. Dittmar, D.R. Demady, L.F. Stancato, P. Krishna, W.B. Pratt, Folding of the glucocorticoid receptor by the heat shock protein (hsp) 90-based chaperone machinery. The role of p23 is to stabilize receptor.hsp90 heterocomplexes formed by hsp90.p60.hsp70, J. Biol. Chem. 272 (1997) 21213–21220.

51 S. Chen, V. Prapapanich, R.A. Rimerman, B. Honore, D.F. Smith, Interactions of p60, a mediator of progesterone receptor assembly, with heat shock proteins hsp90 and hsp70. Mol. Endocrinol. 10 (1996) 682–693.

52 H. Kosano, B. Stensgard, M.C. Charlesworth, N. McMahon, D. Toft, The assembly of progesterone receptor-hsp90 complexes using purified proteins, J. Biol. Chem. 273 (1998) 32973–32979.

53 W.B. Pratt, D.O. Toft, Regulation of signaling protein function and trafficking by the hsp90/hsp70-based chaperone machinery, Exp. Biol. Med. 228 (2003) 111–133.

54 M.P. Hernandez, A. Chadli, D.O.

Toft, Hsp40 binding is the first step in the Hsp90 chaperoning pathway for the progesterone receptor, J. Biol. Chem. 277 (2002) 11873–11881.

55 C. Scheufler, A. Brinker, G. Bourenkov, S. Pegoraro, L. Moroder, H. Bartunik, F.U. Hartl, I. Moarefi, Structure of TPR domain-peptide complexes: critical elements in the assembly of the Hsp70-Hsp90 multichaperone machine, Cell 101 (2000) 199–210.

56 J. Fontana, D. Fulton, Y. Chen, T.A. Fairchild, T.J. McCabe, N. Fujita, T. Tsuruo, W.C. Sessa, Domain mapping studies reveal that the M domain of hsp90 serves as a molecular scaffold to regulate Akt-dependent phosphorylation of endothelioal nitric oxide synthase and NO release, Circ. Res. 90 (2002) 866–873.

57 D.B. Solit, H.I. Scher, N. Rosen, Hsp90 as a therapeutic target in prostate cancer, Semin. Oncol. 30 (2003) 709–716.

58 L. Neckers, S.P. Ivy, Heat shock protein 90, Curr. Opin. Oncol. 15 (2003) 419–424.

59 A. Kamal, L. Thao, J. Sensintaffar, L. Zhang, M.F. Boehm, L.C. Fritz, F.J. Burrows, A high-affinity conformation of Hsp90 confers tumour selectivity on Hsp90 inhibitors, Nature 425 (2003) 407–410.

60 C. Prodromou, S.M. Roe, R. O'Brien, J.E. Ladbury, P.W. Piper, L.H. Pearl, Identification and structural characterization of the ATP/ADP-binding site in the Hsp90 molecular chaperone, Cell 90 (1997) 65–75.

61 C.E. Stebbins, A.A. Russo, C. Schneider, N. Rosen, F.U. Hartl, N.P. Pavletich, Crystal structure of an Hsp90-geldanamycin complex: targeting of a protein chaperone by an antitumor agent, Cell 89 (1997) 239–250.

62 S.V. Sharma, T. Agatsuma, H. Nakano, Targeting of the protein chaperone, Hsp90, by the transformation suppressing agent, radicicol, Oncogene 16 (1998) 2639–2645.

63 T.W. Schulte, S. Akinaga, T. Murakata, T. Agatsuma, S. Sugimoto, H. Nakano, Y.S. Lee, B.B. Simen, Y. Argon, S. Felts, D.O. Toft, L.M. Neckers, S.V. Sharma, Interaction of radicicol with members of the heat shock protein 90 family of molecular chaperones, Mol. Endocrinol. 13 (1999) 1435–1448.

64 C. Schneider, L. Sepp-Lorenzino, E. Nimmesgern, O. Ouerfelli, S. Danishefsky, N. Rosen, F.U. Hartl, Pharmacologic shifting of a balance between protein refolding and degradation mediated by Hsp90, Proc. Natl. Acad. Sci. U S A 93 (1996) 14536–14541.

65 E.G. Mimnaugh, C. Chavany, L. Neckers, Polyubiquitination and proteasomal degradation of the p185c-erbB-2 receptor protein-tyrosine kinase induced by geldanamycin, J. Biol. Chem. 271 (1996) 22796–22801.

66 M.G. Marcu, T.W. Schulte, L. Neckers, Novobiocin and related coumarins and depletion of heat shock protein 90-dependent signaling proteins, J. Natl. Cancer Inst. 92 (2000) 242–248.

67 W. Xu, E. Mimnaugh, M.F. Rosser, C. Nicchitta, M. Marcu, Y. Yarden, L. Neckers, Sensitivity of mature Erbb2 to geldanamycin is conferred by its kinase domain and is mediated by the chaperone protein Hsp90, J. Biol. Chem. 276 (2001) 3702–3708.

68 D.B. Solit, F.F. Zheng, M. Drobnjak, P.N. Munster, B. Higgins, D. Verbel, G. Heller, W. Tong, C. Cordon-Cardo, D.B. Agus, H.I. Scher, N. Rosen, 17-Allylamino-17-demethoxygeldanamycin induces the degradation of androgen receptor and HER-2/neu and inhibits the growth of prostate cancer xenografts, Clin. Cancer Res. 8 (2002) 986–993.

69 A. Citri, I. Alroy, S. Lavi, C. Rubin, W. Xu, N. Grammatikakis, C. Patterson, L. Neckers, D.W. Fry, Y. Yarden, Drug-induced ubiquitylation and degradation of ErbB receptor tyrosine kinases: implications for cancer therapy, Embo J. 21 (2002) 2407–2417.

70 R. van Montfort, C. Slingsby, E. Vierling, Structure and function of the small heat shock protein/alpha-crystallin family of molecular chaperones, Adv. Protein Chem. 59 (2001) 105–156.

71 M. Haslbeck, N. Braun, T. Stromer, B. Richter, N. Model, S. Weinkauf, J. Buchner, Hsp42 is the general small heat shock protein in the cytosol of Saccharomyces cerevisiae, EMBO J. 23 (2004) 638–649.

72 T. Stromer, E. Fischer, K. Richter, M. Haslbeck, J. Buchner, Analysis of the regulation of the molecular chaperone Hsp26 by temperature-induced dissociation: the N-terminal domain is important for oligomer assembly and the binding of unfolding proteins. J. Biol. Chem. 279 (2004) 11222–11228.

73 E. Basha, G.J. Lee, L.A. Breci, A.C. Hausrath, N.R. Buan, K.C. Giese, E. Vierling, The identity of proteins associated with a small heat shock protein during heat stress *in vivo* indicates that these chaperones protect a wide range of cellular functions, J. Biol. Chem. 279 (2004) 7566–7575.

74 K.K. Kim, R. Kim, S.H. Kim, Crystal structure of a small heat-shock protein, Nature 394 (1998) 595–599.

75 R.L. van Montfort, E. Basha, K.L. Friedrich, C. Slingsby, E. Vierling, Crystal structure and assembly of a eukaryotic small heat shock protein. Nat. Struct. Biol. 8 (2001) 1025–1030.

76 M. Haslbeck, S. Walke, T. Stromer, M. Ehrnsperger, H.E. White, S. Chen, H.R. Saibil, J. Buchner, Hsp26: a temperature-regulated chaperone, EMBO J. 18 (1999) 6744–6751.

77 G.J. Lee, A.M. Roseman, H.R. Saibil, E. Vierling, A small heat shock protein stably binds heat-denatured model substrates and can maintain a substrate in a folding-competent state, EMBO J. 16 (1997) 659–671.

78 Z. Xu, A.L. Horwich, P.B. Sigler, The crystal structure of the asymmetric GroEL-GroES-(ADP)7 chaperonin complex, Nature 388 (1997) 741–750.

79 H.R. Saibil, N.A. Ranson, The chaperonin folding machine, Trends Biochem. Sci. 27 (2002) 627–632.

80 A.S. Meyer, J.R. Gillespie, D. Walther, I.S. Millet, S. Doniach, J. Frydman, Closing the folding chamber of the eukaryotic chaperonin requires the transition state of ATP hydrolysis, Cell 113 (2003) 369–381.

81 V. Thulasiraman, C.F. Yang, J. Frydman, *In vivo* newly translated polypeptides are sequestered in a protected folding environment, EMBO J. 18 (1999) 85–95.

82 S.A. Lewis, G. Tian, I.E. Vainberg, N.J. Cowan, Chaperonin-mediated folding of actin and tubulin, J. Cell Biol. 132 (1996) 1–4.

83 H. Sakahira, P. Breuer, M.K. Hayer-Hartl, F.U. Hartl, Molecular chaperones as modulators of polyglutamine protein aggregation and toxicity, Proc. Natl. Acad. Sci. USA 99 Suppl. 4 (2002) 16412–16418.

84 J.M. Warrick, H.Y. Chan, G.L. Gray-Board, Y. Chai, H.L. Paulson, N.M. Bonini, Suppression of polyglutamine-mediated neurodegeneration in Drosophila by the molecular chaperone Hsp70. Nat. Genet. 23 (1999) 425–428.

85 C.J. Cummings, M.A. Mancini, B. Antalffy, D.B. DeFranco, H.T. Orr, H.Y. Zoghbi, Chaperone suppression of aggregation and altered subcellular proteasome localization imply protein misfolding in SCA1, Nat. Genet. 19 (1998) 148–154.

86 C.J. Cummings, Y. Sun, P. Opal, B. Antalffy, R. Mestril, H.T. Orr, W.H. Dillmann, H.Y. Zoghbi, Over-expression of inducible HSP70 chaperone suppresses neuropathology and improves motor function in SCA1 mice, Hum. Mol. Genet. 10 (2001) 1511–1518.

87 S. Krobitsch, S. Lindquist, Aggregation of huntingtin in yeast varies with the length of the polyglutamine expansion and the expression of chaperone proteins, Proc. Natl. Acad. Sci. U S A 97 (2000) 1589–1594.

88 P.J. Muchowski, G. Schaffar, A.

Sittler, E.E. Wanker, M.K. Hayer-Hartl, F.U. Hartl, Hsp70 and hsp40 chaperones can inhibit self-assembly of polyglutamine proteins into amyloid-like fibrils, Proc. Natl. Acad. Sci. U S A 97 (2000) 7841–7846.

89 J.Z. Chuang, H. Zhou, M. Zhu, S.H. Li, X.J. Li, C.H. Sung, Characterization of a brain-enriched chaperone, MRJ, that inhibits Huntingtin aggregation and toxicity independently, J. Biol. Chem. 277 (2002) 19831–19838.

90 A. Sittler, R. Lurz, G. Lueder, J. Priller, H. Lehrach, M.K. Hayer-Hartl, F.U. Hartl, E.E. Wanker, Geldanamycin activates a heat shock response and inhibits huntingtin aggregation in a cell culture model of Huntington's disease, Hum. Mol. Genet. 10 (2001) 1307–1315.

91 A. Parcellier, E. Schmitt, S. Gurbuxani, D. Seigneurin-Berny, A. Pance, A. Chantome, S. Plenchette, S. Khochbin, E. Solary, C. Garrido, Hsp27 is a ubiquitin-binding protein involved in IκBα proteasomal degradation, Mol. Cell Biol. 23 (2003) 5790–5802.

92 H. Shimura, Y. Miura-Shimura, K.S. Kosick, Binding of tau to heat shock protein 27 leads to decreased concentration of hyperphosphorylated tau and enhanced cell survival, J. Biol. Chem. 279 (2004) 17957–17962.

93 V. Gusarova, A.J. Caplan, J.L. Brodsky, E.A. Fisher, Apoprotein B degradation is promoted by the molecular chaperones hsp90 and hsp70, J. Biol. Chem. 276 (2001) 24891–24900.

94 C. Taxis, R. Hitt, S.H. Park, P.M. Deak, Z. Kostova, D.H. Wolf, Use of modular substrates demonstrates mechanistic diversity and reveals differences in chaperone requirement of ERAD, J. Biol. Chem. 278 (2003) 35903–35913.

95 Y. Yang, S. Janich, J.A. Cohn, J.M. Wilson, The common variant of cystic fibrosis transmembrane conductance regulator is recognized by hsp70 and degraded in a pre-Golgi nonlysosomal compartment, Proc. Natl. Acad. Sci. U S A 90 (1993) 9480–9484.

96 G.C. Meacham, Z. Lu, S. King, E. Sorscher, A. Tousson, D.M. Cyr, The Hdj-2/Hsc70 chaperone pair facilitates early steps in CFTR biogenesis, Embo J. 18 (1999) 1492–1505.

97 G.C. Meacham, C. Patterson, W. Zhang, J.M. Younger, D.M. Cyr, The Hsc70 co-chaperone CHIP targets immature CFTR for proteasomal degradation, Nat. Cell Biol. 3 (2001) 100–105.

98 Y. Zhang, G. Nijbroek, M.L. Sullivan, A.A. McCracken, S.C. Watkins, S. Michaelis, J.L. Brodsky, Hsp70 molecular chaperone facilitates endoplasmic reticulum-associated protein degradation of cystic fibrosis transmembrane conductance regulator in yeast, Mol. Biol. Cell 12 (2001) 1303–1314.

99 R.R. Kopito, Biosynthesis and degradation of CFTR, Physiol. Rev. 79 (1999) S167–173.

100 J.R. Riordan, Cystic fibrosis as a disease of misprocessing of the cystic fibrosis transmembrane conductance regulator glycoprotein, Am. J. Hum. Genet. 64 (1999) 1499–1504.

101 C.L. Ward, S. Omura, R.R. Kopito, Degradation of CFTR by the ubiquitin–proteasome pathway, Cell 83 (1995) 121–127.

102 T.J. Jensen, M.A. Loo, S. Pind, D.B. Williams, A.L. Goldberg, J.R. Riordan, Multiple proteolytic systems, including the proteasome, contribute to CFTR processing, Cell 83 (1995) 129–135.

103 M.S. Gelman, E.S. Kannegaard, R.R. Kopito, A principal role for the proteasome in endoplasmic reticulum-associated degradation of misfolded intracellular cystic fibrosis transmembrane conductance regulator, J. Biol. Chem. 277 (2002) 11709–11714.

104 Z. Kostova, D.H. Wolf, For whom the bell tolls: protein quality control of the endoplasmic reticulum and the ubiquitin–proteasome connection, EMBO J. 22 (2003) 2309–2317.

105 S. Sato, C.L. Ward, M.E. Krouse, J.J. Wine, R.R. Kopito, Glycerol reverses the misfolding phenotype of the most common cystic fibrosis mutation, J. Biol. Chem. 271 (1996) 635–638.

106 B.H. Qu, P.J. Thomas, Alteration of the cystic fibrosis transmembrane conductance regulator folding pathway, J. Biol. Chem. 271 (1996) 7261–7264.

107 E. Strickland, B.H. Qu, L. Millen, P.J. Thomas, The molecular chapeorne Hsc70 assists the *in vitro* folding of the N-terminal nucleotide-binding domain of the cystic fibrosis transmembrane conductance regulator, J. Biol. Chem. 272 (1997) 25421–25424.

108 J.A. Johnston, C.L. Ward, R.R. Kopito, Aggresomes: a cellular response to misfolded proteins, J. Cell Biol. 143 (1998) 1883–98.

109 R.R. Kopito, Aggresomes, inclusion bodies and protein aggregation, Trends Cell Biol. 10 (2000) 524–530.

110 S. Waelter, A. Boeddrich, R. Lurz, E. Scherzinger, G. Lueder, H. Lehrach, E.E. Wanker, Accumulation of mutant huntingtin fragments in aggresome-like inclusion bodies as a result of insufficient protein degradation, Mol. Biol. Cell 12 (2001) 1393–1407.

111 R. Garcia-Mata, Z. Bebok, E.J. Sorscher, E.S. Sztul, Characterization and dynamics of aggresome formation by a cytosolic GFP-chimera, J. Cell Biol. 146 (1999) 1239–1254.

112 K.S. McNaught, P. Shashidharan, D.P. Perl, P. Jenner, C.W. Olanow, Aggresome-related biogenesis of Lewy bodies, Eur. J. Neurosci. 16 (2002) 2136–2148.

113 Y. Kawaguchi, J.J. Kovacs, A. McLaurin, J.M. Vance, A. Ito, T.P. Yao, The deacetylase HDAC6 regulates aggresome formation and cell viability in response to misfolded protein stress, Cell 115 (2003) 727–738.

114 R. Garcia-Mata, Z. Bebok, E.J. Sorscher, E.S. Sztul, Characterization and dynamics of aggresome formation by a cytosolic GFP-chimera, J. Cell Biol. 146 (1999) 1239–1254.

115 W.C. Wigley, R.P. Fabunmi, M.G. Lee, C.R. Marino, S. Muallem, G.N. DeMartino, P.J. Thomas, Dynamic association of proteasomal machinery with the centrosome, J. Cell Biol. 145 (1999) 481–490.

116 A. Wyttenbach, J. Carmichael, J. Swartz, R.A. Furlong, Y. Narain, J. Rankin, D.C. Rubinsztein, Effects of heat shock, heat shock protein 40 (HDJ-2), and proteasome inhibition on protein aggregation in cellular models of Huntington's disease, Proc. Natl. Acad. Sci. USA 97 (2000) 2898–2903.

117 J.L. Dul, D.P. Davis, E.K. Williamson, F.J. Stevens, Y. Argon, Hsp70 and antifibrillogenic peptides promote degradation and inhibit intracellular aggregation of amyloidogenic light chains, J. Cell Biol. 152 (2001) 705–716.

118 H. Lelouard, E. Gatti, F. Cappello, O. Gresser, V. Camosseto, P. Pierre, Transient aggregation of ubiquitinated proteins during dendritic cell maturation, Nature 417 (2002) 177–182.

119 H. Lelouard, V. Ferrand, D. Marguet, J. Bania, V. Camosseto, A. David, E. Gatti, P. Pierre, Dendritic cell aggresome-like induced structures are dedicated areas for ubiquitination and storage of newly synthesized defective proteins, J. Cell Biol. 164 (2004) 667–675.

120 W. Xu, M. Marcu, X. Yuan, E. Mimnaugh, C. Patterson, L. Neckers, Chaperone-dependent E3 ubiquitin ligase CHIP mediates a degradative pathway for c-ErbB2/Neu, Proc. Natl. Acad. Sci. USA 99 (2002) 12847–12852.

121 S. Wickner, M.R. Maurizi, S. Gottesman, Posttranslational quality control: folding, refolding, and degrading proteins, Science 286 (1999) 1888–1893.

122 C.-W. Liu, M.J. Corboy, G.N. DeMartino, P.J. Thomas, Endoproteolytic activity of the proteasome, Science 299 (2003) 408–411.

123 W. Seufert, S. Jentsch, Ubiquitin-conjugating enzymes UBC4 and UBC5 mediate selective degradation of

short-lived and abnormal proteins, EMBO J. 9 (1990) 543–550.
124 B. Bercovich, I. Stancovski, A. Mayer, N. Blumenfeld, A. Laszlo, A.L. Schwartz, A. Ciechanover, Ubiquitin-dependent degradation of certain protein substrates *in vitro* requires the molecular chaperone Hsc70, J. Biol. Chem. 272 (1997) 9002–9010.
125 P. Connell, C.A. Ballinger, J. Jiang, Y. Wu, L.J. Thompson, J. Höhfeld, C. Patterson, The co-chaperone CHIP regulates protein triage decisions mediated by heat-shock proteins, Nat. Cell Biol. 3 (2001) 93–96.
126 L. Aravind, E.V. Koonin, The U box is a modified RING finger – a common domain in ubiquitination, Curr. Biol. 10 (2000) R132–R134.
127 S. Hatakeyama, M. Yada, M. Matsumoto, N. Ishida, K.I. Nakayama, U box proteins as a new family of ubiquitin-protein ligases, J. Biol. Chem. 276 (2001) 33111–33120.
128 E. Pringa, G. Martinez-Noel, U. Müller, K. Harbers, Interaction of the ring finger-related U-box motif of a nuclear dot protein with ubiquitin-conjugating enzymes, J. Biol. Chem. 276 (2001) 19617–19623.
129 R. Nikolay, T. Wiederkehr, W. Rist, G. Kramer, M.P. Mayer, B. Bukau, Dimerization of the human E3 ligase CHIP via a coiled-coil domain is essential for its activity, J. Biol. Chem. 279 (2004) 2673–2678.
130 Q. Dai, C. Zhang, Y. Wu, H. McDonough, R.A. Whaley, V. Godfrey, H.H. Li, N. Madamanchi, W. Xu, L. Neckers, D. Cyr, C. Patterson, CHIP activates HSF1 and confers protection against apoptosis and cellular stress, EMBO J. 22 (2003) 5446–5458.
131 J. Demand, J. Lüders, J. Höhfeld, The carboxyl-terminal domain of Hsc70 provides binding sites for a distinct set of chaperone cofactors, Mol. Cell. Biol. 18 (1998) 2023–2028.
132 A. Brinker, C. Scheufler, F. Von Der Mulbe, B. Fleckenstein, C. Herrmann, G. Jung, I. Moarefi, F.U. Hartl, Ligand discrimination by TPR domains. Relevance and selectivity of EEVD-recognition in Hsp70 × Hop × Hsp90 complexes, J. Biol. Chem. 277 (2002) 19265–19275.
133 H.H. Kampinga, B. Kanon, F.A. Salomons, A.E. Kabakov, C. Patterson, Overexpression of the cochaperone CHIP enhances Hsp70-dependent folding activity in mammalian cells, Mol. Cell. Biol. 23 (2003) 4948–4958.
134 H. Shimura, D. Schwartz, S.P. Gygi, K.S. Kosik, CHIP-Hsc70 complex ubiquitinates phosphorylated tau and enhances cell survival, J. Biol. Chem. 279 (2004) 4869–4876.
135 J. Jiang, C.A. Ballinger, Y. Wu, Q. Dai, D.M. Cyr, J. Höhfeld, C. Patterson, CHIP is a U-box-dependent E3 ubiquitin ligase: identification of Hsc70 as a target for ubiquitylation, J. Biol. Chem 276 (2001) 42938–42944.
136 S. Murata, Y. Minami, M. Minami, T. Chiba, K. Tanaka, CHIP is a chaperone-dependent E3 ligase that ubiquitylates unfolded protein, EMBO Rep. 2 (2001) 1133–1138.
137 J. Demand, S. Alberti, C. Patterson, J. Höhfeld, Cooperation of a ubiquitin domain protein and an E3 ubiquitin ligase during chaperone/proteasome coupling, Curr. Biol. 11 (2001) 1569–1577.
138 P.K. Jackson, A.G. Eldridge, E. Freed, L. Furstenthal, J.Y. Hsu, B.K. Kaiser, J.D. Reimann, The lore of the RINGs: substrate recognition and catalysis by ubiquitin ligases, Trends Cell. Biol. 10 (2000) 429–439.
139 P.K. Jackson, A.G. Eldridge, The SCF ubiquitin ligase: an extended look, Mol. Cell 9 (2002) 923–925.
140 N. Zheng, B.A. Schulman, L. Song, J.J. Miller, P.D. Jeffrey, P. Wang, C. Chu, D.M. Koepp, S.J. Elledge, M. Pagano, R.C. Conaway, J.W. Conaway, J.W. Harper, N.P. Pavletich, Structure of the Cul1-Rbx1-Skp1-F boxSkp2 SCF ubiquitin ligase complex, Nature 416 (2002) 703–709.
141 J. Lüders, J. Demand, J. Höhfeld,

The ubiquitin-related BAG-1 provides a link between the molecular chaperones Hsc70/Hsp70 and the proteasome, J. Biol. Chem. 275 (2000) 4613–4617.

142 S. Jentsch, G. Pyrowolakis, Ubiquitin and its kin: how close are the family ties? Trends Cell. Biol. 10 (2000) 335–342.

143 S. Alberti, J. Demand, C. Esser, N. Emmerich, H. Schild, J. Höhfeld, Ubiquitylation of BAG-1 suggests a novel regulatory mechanism during the sorting of chaperone substrates to the proteasome, J. Biol. Chem. 277 (2002) 45920–45927; Erratum in J. Biol. Chem. 278 (2003) 15702–15703.

144 Q. Deveraux, V. Ustrell, C. Pickart, M. Rechsteiner, A 26 S protease subunit that binds ubiquitin conjugates, J. Biol. Chem. 269 (1994) 7059–7061.

145 P. Young, Q. Deveraux, R.E. Beal, C.M. Pickart, M. Rechsteiner, Characterization of two polyubiquitin binding sites in the 26 S protease subunit 5a, J. Biol. Chem. 273 (1998) 5461–5467.

146 H. Hiyama, M. Yokoi, C. Masutani, K. Sugasawa, T. Maekawa, K. Tanaka, J.H. Hoeijmakers, F. Hanaoka, Interaction of hHR23 with S5a. The ubiquitin-like domain of hHR23 mediates interaction with S5a subunit of 26 S proteasome, J. Biol. Chem. 274 (1999) 28019–28025.

147 K.J. Walters, M.F. Kleijnen, A.M. Goh, G. Wagner, P.M. Howley, Structural studies of the interaction between ubiquitin family proteins and proteasome subunit S5a, Biochemistry 41 (2002) 1767–1777.

148 Y.A. Lam, T.G. Lawson, M. Velayutham, J.L. Zweier, C.M. Pickart, A proteasomal ATPase subunit recognizes the polyubiquitin degradation signal, Nature 416 (2002) 763–767.

149 S. Elsasser, R.R. Gali, M. Schwickart, C.N. Larsen, D.S. Leggett, B. Muller, M.T. Feng, F. Tubing, G.A. Dittmar, D. Finley, Proteasome subunit Rpn1 binds ubiquitin-like protein domains, Nat. Cell Biol. 4 (2002) 725–730.

150 Y. Saeki, T. Sone, A. Toh-e, H. Yokosawa, Identification of ubiquitin-like protein-binding subunits of the 26S proteasome, Biochem. Biophys. Res. Commun. 296 (2002) 813–819.

151 M.H. Glickman, D.M. Rubin, O. Coux, I. Wefes, G. Pfeifer, Z. Cjeka, W. Baumeister, V.A. Fried, D. Finley, A subcomplex of the proteasome regulatory particle required for ubiquitin-conjugate degradation and related to the COP9-signalosome and eIF3, Cell 94 (1998) 615–623.

152 Y. Xie, A. Varshavsky, UFD4 lacking the proteasome-binding region catalyses ubiquitination but is impaired in proteolysis, Nat. Cell Biol. 4 (2002) 1003–1007.

153 S. Takayama, J.C. Reed, Molecular chaperone targeting and regulation by BAG family proteins, Nat. Cell Biol. 3 (2001) E237–241.

154 K. Thress, J. Song, R.I. Morimoto, S. Kornbluth, Reversible inhibition of Hsp70 chaperone function by Scythe and Reaper, EMBO J. 20 (2001) 1033–1041.

155 S. Alberti, K. Böhse, V. Arndt, A. Schmitz, J. Höhfeld, The co-chaperone HspBP1 inhibits the CHIP ubiquitin ligase and stimulates the maturation of the cystic fibrosis transmembrane conductance regulator, Mol. Biol. Cell (2004), in press.

156 D.A. Raynes, V. Guerriero, Inhibition of Hsp70 ATPase activity and protein renaturation by a novel Hsp70-binding protein, J. Biol. Chem. 273 (1998) 32883–32888.

157 M. Kabani, C. McLellan, D.A. Raynes, V. Guerriero, J.L. Brodsky, HspBP1, a homologue of the yeast Fes1 and Sls1 proteins, is an Hsc70 nucleotide exchange factor, FEBS Lett. 531 (2002) 339–342.

158 C.P. Cardozo, C. Michaud, M.C. Ost, A.E. Fliss, E. Yang, C. Patterson, S.J. Hall, A.J. Caplan, C-terminal Hsp-interacting protein slows androgen receptor synthesis and

reduces its rate of degradation, Arch. Biochem. Biophys. 410 (2003) 134–140.

159 J. Jiang, D. Cyr, R.W. Babbitt, W.C. Sessa, C. Patterson, Chaperone-dependent regulation of endothelial nitric-oxide synthase intracellular trafficking by the co-chaperone/ubiquitin ligase CHIP, J. Biol. Chem. 278 (2003) 49332–49341.

160 Y. Imai, M. Soda, S. Hatakeyama, T. Akagi, T. Hashikawa, K.I. Nakayama, R. Takahashi, CHIP is associated with Parkin, a gene responsible for familial Parkinson's disease, and enhances its ubiquitin ligase activity, Mol. Cell 10 (2002) 55–67.

161 Y. Nagano, H. Yamashita, T. Takahashi, S. Kishida, T. Nakamura, E. Iseki, N. Hattori, Y. Mizuno, A. Kikuchi, M. Matsumoto, Siah-1 facilitates ubiquitination and degradation of synphilin-1, J. Biol. Chem. 278 (2003) 51504–51514.

162 E. Liani, A. Eyal, E. Avraham, R. Shermer, R. Szargel, D. Berg, A. Bornemann, O. Riess, C.A. Ross, R. Rott, S. Engelender, Ubiquitylation of synphilin-1 and alpha-synuclein by SIAH and its presence in cellular inclusions and Lewy bodies imply a role in Parkinson's disease, Proc. Natl. Acad. Sci. USA 101 (2004) 5500–5505.

163 S. Matsuzawa, S. Takayama, B.A. Froesch, J.M. Zapata, J.C. Reed, p53-inducible homologue of Drosophila seven in absentia (Siah) inhibits cell growth: suppression by BAG-1, EMBO J. 17 (1998) 2736–2747.

164 C. Soti, P. Csermely, Aging and molecular chaperones, Exp. Gerontol. 38 (2003) 1037–1040.

165 J. Fargnoli, T. Kunisada, A.J. Jr. Fornace, E.L. Schneider, N.J. Holbrook, Decreased expression of heat shock protein 70 mRNA and protein after heat tretament in cells of aged rats, Proc. Natl. Acad. Sci. USA 87 (1990) 846–850.

166 C.K. Lee, R.G. Klopp, R. Weindruch, T.A. Prolla, Gene expression profile of aging and its retardation by caloric restriction, Science 285 (1999) 1390–1393.

167 S.X. Cao, J.M. Dhahbi, P.L. Mote, S.R. Spindler, Genomic profiling of short- and long-term caloric restriction effects in the liver of aging mice, Proc. Natl. Acad. Sci. USA 98 (2001) 10630–10635.

168 M. Tatar, A.A. Khazaeli, J.W. Curtsinger, Chaperoning extended life, Nature 390 (1997) 30.

169 G. Morrow, R.M. Tanguay, Heat shock proteins and aging in Drosophila melanogaster, Semin. Cell. Dev. Biol. 14 (2003) 291–299.

170 J.F. Morley, R.I. Morimoto, Regulation of longevity in Caenorhabditis elegans by heat shock factor and molecular chaperones, Mol. Biol. Cell 15 (2004) 657–664.

171 A.L. Hsu, C.T. Murphy, C. Kenyon, Regulation of aging and age-related disease by DAF-16 and heat-shock factor, Science 300 (2003) 1142–1145.