R. John Mayer, Aaron Ciechanover, and Martin Rechsteiner (Eds.)

Protein Degradation

The Ubiquitin-Proteasome System

Volume 2

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Contents

Preface

There is an incredible amount of current global research activity devoted to understanding the chemistry of life. The genomic revolution means that we now have the basic genetic information in order to understand in full the molecular basis of the life process. However, we are still in the early stages of trying to understand the specific mechanisms and pathways that regulate cellular activities. Occasionally discoveries are made that radically change the way in which we view cellular activities. One of the best examples would be the finding that reversible phosphorylation of proteins is a key regulatory mechanism with a plethora of downstream consequences. Now the seminal discovery of another post-translational modification, protein ubiquitylation, is leading to a radical revision of our understanding of cell physiology. It is becoming ever more clear that protein ubiquitylation is as important as protein phosphorylation in regulating cellular activities. One consequence of protein ubiquitylation is protein degradation by the 26S proteasome. However, we are just beginning to understand the full physiological consequences of covalent modification of proteins, not only by ubiquitin, but also by ubiquitin-related proteins.

Because the Ubiquitin Proteasome System (UPS) is a relatively young field of study, there is ample room to speculate on possible future developments. Today a handful of diseases, particularly neurodegenerative ones, are known to be caused by malfunction of the UPS. With perhaps as many as 1000 human genes encoding components of ubiquitin and ubiquitin-related modification pathways, it is almost certain that many more diseases will be found to arise from genetic errors in the UPS or by pathogen subversion of the system. This opens several avenues for the development of new therapies. Already the proteasome inhibitor Velcade is producing clinical success in the fight against multiple myeloma. Other therapies based on the inhibition or activation of specific ubiquitin ligases, the substrate recognition components of the UPS, are likely to be forthcoming. At the fundamental research level there are a number of possible discoveries especially given the surprising range of biochemical reactions involving ubiquitin and its cousins. Who would have guessed that the small highly conserved protein would be involved in endocytosis or that its relative Atg8 would form covalent bonds to a phospholipid during autophagy? We suspect that few students of ubiquitin will be surprised if it or a

ubiquitin-like protein is one day found to be covalently attached to a nucleic acid for some biological purpose.

We are regularly informed by the ubiquitin community that the initiation of this series of books on the UPS is extremely timely. Even though the field is young, it has now reached the point at which the biomedical scientific community at large needs reference works in which contributing authors indicate the fundamental roles of the ubiquitin proteasome system in all cellular processes. We have attempted to draw together contributions from experts in the field to illustrate the comprehensive manner in which the ubiquitin proteasome system regulates cell physiology. There is no doubt then when the full implications of protein modification by ubiquitin and ubiquitin-like molecules are fully understood we will have gained fundamental new insights into the life process. We will also have come to understand those pathological processes resulting from UPS malfunction. The medical implications should have considerable impact on the pharmaceutical industry and should open new avenues for therapeutic intervention in human and animal diseases. The extensive physiological ramifications of the ubiquitin proteasome system warrant a series of books of which this is the first one.

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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–

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,

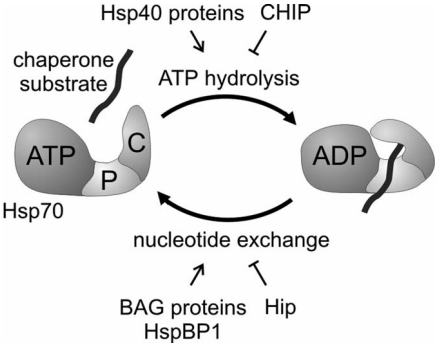

Fig. 1.1. Schematic presentation of the domain architecture and chaperone cycle of Hsp70. Hsp70 proteins display a characteristic domain structure comprising an amino-terminal ATPase domain (ATP), a peptide-binding domain (P), and a carboxyl-terminal domain (C) that is supposed to form a lid over the 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-

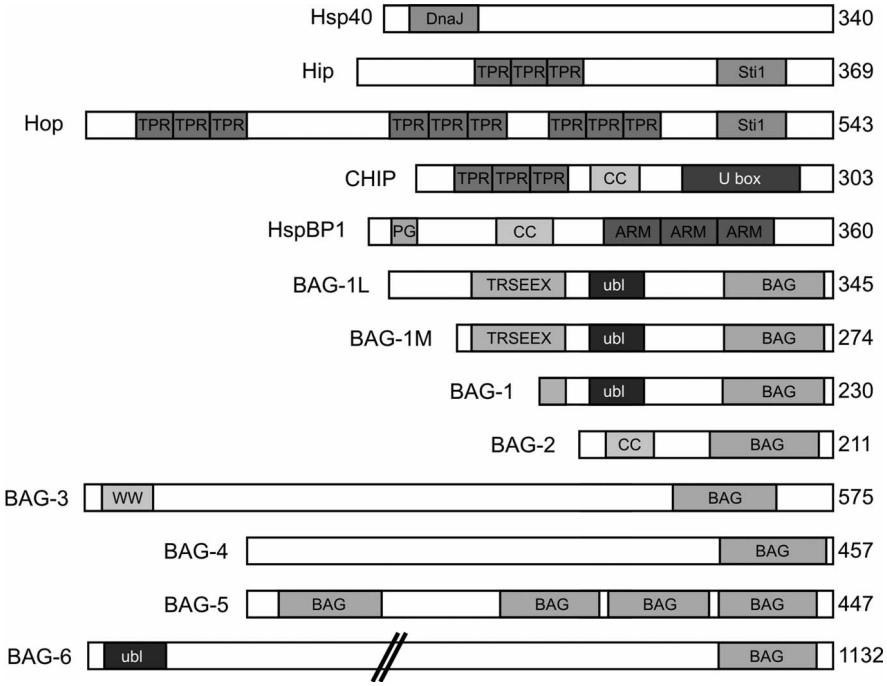

Fig. 1.2. Domain architecture of diverse co-chaperones of Hsp70. DnaJ: domain related to the bacterial co-chaperone DnaJ; TPR: tetratricopeptide repeat; Sti1: domain related to the yeast co-chaperone Sti1; CC: coiled-coil domain; U box: E2-interacting domain present in certain ubiquitin ligases; PG: 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-

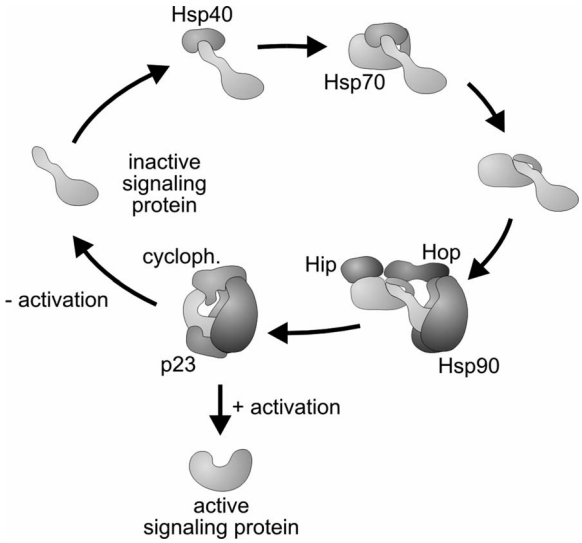

Fig. 1.3. Cooperation of Hsp70 and Hsp90 during the regulation of signal transduction pathways. The inactive signaling protein, e.g., a steroid hormone receptor, is initially recognized by Hsp40 and delivered to Hsp70. Subsequently, a multi-chaperone complex assembles that contains the Hsp70 co-chaperone 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

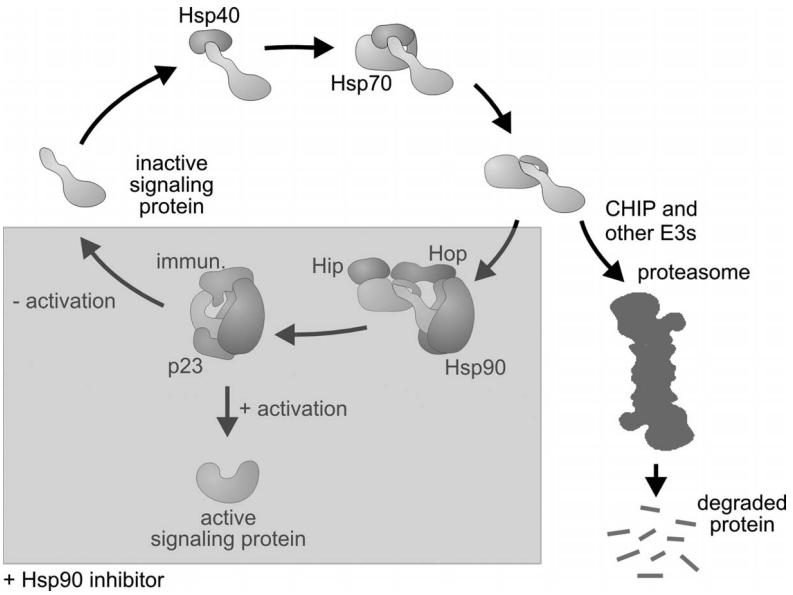

Fig. 1.4. Alteration of chaperone action during signal transduction induced by Hsp90 inhibitors such as geldanamycin and radicicol. In the presence of the inhibitors the activation pathway is blocked, and signaling proteins are targeted to the proteasome for degradation in a process that involves the 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].

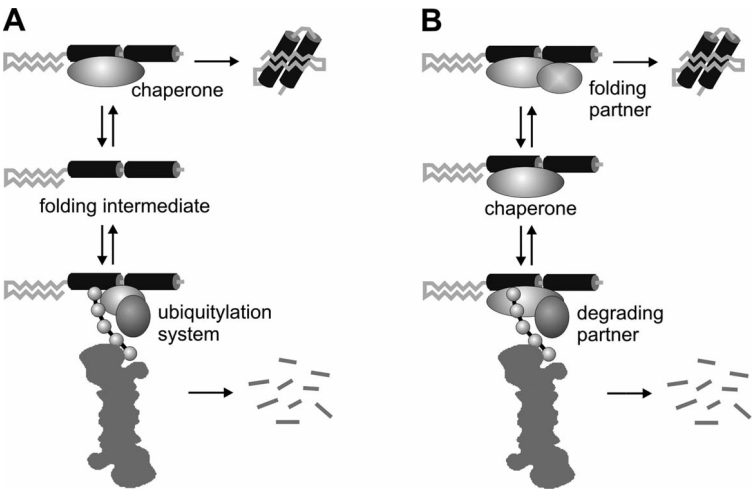

Fig. 1.5. Interplay of molecular chaperones with the ubiquitin–proteasome system. (A) Chaperones and the degradation machinery (i.e., ubiquitylation systems) compete with each other in the recognition of folding intermediates. Interaction with the chaperones directs the substrate towards folding. However, 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