Edited by R. J. Mayer, A. Ciechanover,
M. Rechsteiner

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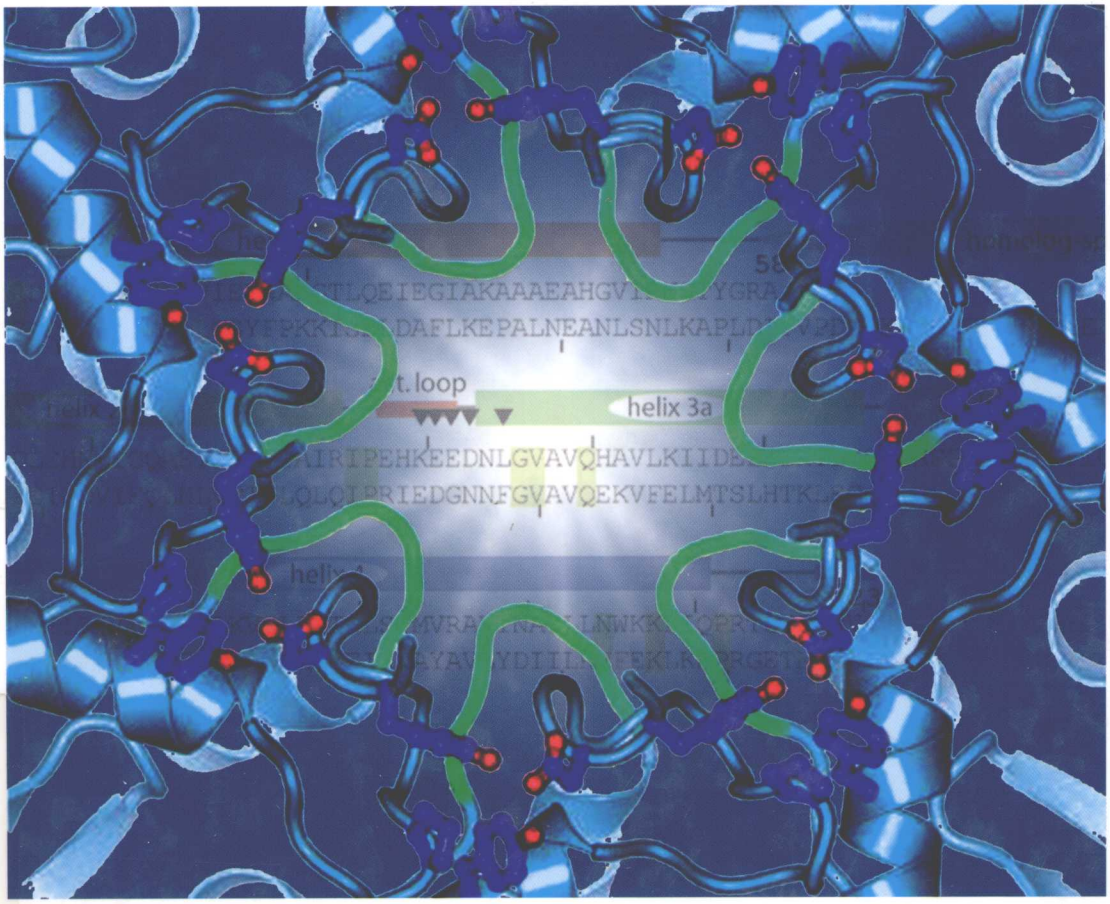
Protein Degradation

The Ubiquitin-Proteasome System

Volume 2



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

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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

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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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Contents

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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–

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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,

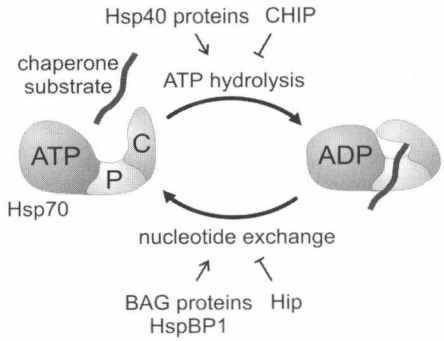

Fig. 1.1. Schematic presentation of the domain architecture and chaperone cycle of Hsp70. Hsp70 proteins display a characteristic domain structure comprising an amino-terminal ATPase domain (ATP), a peptide-binding domain (P), and a carboxyl-terminal domain (C) that is supposed to form a lid over the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