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Proteasomal and Autophagic Degradation Systems

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Abstract

Autophagy and the ubiquitin–proteasome system are the two major quality control pathways responsible for cellular homeostasis. As such, they provide protection against age-associated changes and a plethora of human diseases. Ubiquitination is utilized as a degradation signal by both systems, albeit in different ways, to mark cargoes for proteasomal and lysosomal degradation. Both systems intersect and communicate at multiple points to coordinate their actions in proteostasis and organelle homeostasis. This review summarizes molecular details of how proteasome and autophagy pathways are functionally interconnected in cells and indicates common principles and nodes of communication that can be therapeutically exploited.

INTRODUCTION

Proteostasis, the maintenance of a healthy proteome, is a key requirement for cell metabolism, organelle biogenesis, stress adaptation, and consequently the long-term viability and well-being of any cell type and organ (1). A major challenge in proteostasis concerns protection against the detrimental consequences of unfolded, misfolded, or damaged proteins that severely disturb cellular functions and are associated with aging and age-related diseases, including neurodegeneration, cancer, and immunological and metabolic diseases, most of which are currently incurable (1–3).

Because of the fundamental importance of proteostasis, cells have evolved a sophisticated protein quality control (PQC) system consisting of interconnected processes that constantly survey the cellular proteome throughout the complete life cycle of a protein. During protein translation, mRNA surveillance processes recognize aberrant mRNA molecules and mediate the degradation of their nascent protein products (4–6). The folding of properly synthesized polypeptide chains on ribosomes is guided by molecular chaperones to minimize misfolding. Yet, chaperones not only assist protein folding but also facilitate protein degradation when proper folding fails (7). Further, as cells are permanently subjected to external and internal changes as well as stressful insults, a functional proteome requires constant remodeling and repair or elimination of damaged proteins. Disused and defective proteins are removed by regulated degradation or, in the case of extensive damage that exceeds the intracellular refolding and degradative capacity, are sequestered into dedicated compartments (so-called inclusion bodies, or aggresomes, e.g., juxtanuclear quality control compartments and insoluble protein deposits) where they await clearance through different mechanisms (8–12).

Two major degradation systems have evolved to handle these tasks, the ubiquitin–proteasome system (UPS) and the autophagy-lysosome pathway (ALP). The fundamental importance of both degradation systems has been acknowledged with two Nobel Prizes: In 2004, the Nobel Prize in Chemistry was awarded jointly to Aaron Ciechanover, Avram Hershko, and Irwin Rose for the discovery of ubiquitin (Ub)-mediated protein degradation, and in 2016, Yoshinori Ohsumi received the Nobel Prize in Medicine and Physiology for his groundbreaking studies on autophagy. The UPS is the primary proteolytic route for short-lived, misfolded, and damaged proteins (see the sidebar titled Structure and Function of the Proteasome). It has important functions in the regulation of cell signaling and transcription and is involved in a variety of cellular functions, including cell cycle progression, cell survival, proliferation, apoptosis, and other critical cellular pathways (reviewed in 13). ALP recognizes and removes large and potentially dangerous cellular components such as protein aggregates and dysfunctional or superfluous organelles and has emerged as a crucial adaptive mechanism to cope with various cellular stresses (nutrient deprivation, hypoxia, oxidative stress, etc.) (14). Importantly, all of the systems contributing to PQC are interconnected and communicate with each other via different mechanisms.

REGULATION OF PROTEASOMAL CAPACITY AND FUNCTION

It has long been assumed that the rate of protein degradation by the UPS is determined by the rate of substrate ubiquitination. Instead, it turns out that abundance and degradative capacity of proteasomes are rate limiting and crucial to sustain viability under challenging conditions. Moreover, the composition of proteasomes is not static but can (and needs to) be dynamically adjusted to changing demands as highlighted by the existence of specialized immunoproteasomes and aggresome-associated proteasomes or the employment of 20S proteasomes for direct protein hydrolysis and 26S proteasomes for degradation of ubiquitinated proteins (15, 16). Moreover, cells depleted of free Ub, a condition known as Ub stress, actively change the composition of their

STRUCTURE AND FUNCTION OF THE PROTEASOME

The 26S proteasome is a multicatalytic, ATP-dependent protease of approximately 2.5 MDa, which is made up of two subcomplexes—a barrel-shaped, twofold-symmetric core particle (CP, or 20S proteasome) and a regulatory particle (RP, or 19S particle; the most abundant form in mammals is called PA700)—attached to one or both ends of the CP. Three β-type subunits, β1, β2, and β5, possess catalytic activities that face the interior space. The subunits are associated with caspase-like, trypsin-like, and chymotrypsin-like activities, respectively, that digest the substrate into peptides of 2–24 amino acids, ensuring that no protein will remain intact after entry into the CP and providing an essential source for amino acids. In fact, increased cell lethality due to severe shortage of amino acids is a major consequence of proteasome inhibition (132).

The RP is composed of base and lid subassemblies that serve to recognize ubiquitinated substrate proteins and prepare them for degradation in the CP (219). The central part of the RP base consists of six AAA ATPases (Rpt1–Rpt6) that form a hexameric Rpt ring and are endowed with several crucial functions: (*a*) They hook the RP via their C-terminal tails to the heptameric α-ring of the CP and (*b*) cause the gated substrate-entry channel of the CP to open. (*c*) They use the energy of ATP hydrolysis to unfold the substrates and (*d*) translocate them through the narrow pore into the 20S chamber (220). Of note, the six ATPases are not functionally redundant and have to work coordinately. The base of the RP also contains non-ATPase proteins: Rpn13 and Rpn10 possess ubiquitin-binding domains and function as receptors for ubiquitinated substrates (13). In addition, shuttling factors (Rad23, Dsk2, and Ddi1), referred to as the UBL/UBA proteins, deliver ubiquitinated proteins to the proteasome for degradation (221). Three deubiquitinases, the RP subunit Rpn11 (Poh1), and the RP-associated Usp14 and Uch37, release the ubiquitin chain from the substrate and disassemble it. The binding of ubiquitinated substrate to Usp14 and Uch37 was shown to stimulate ATP hydrolysis by Rpt ATPases as well as 20S gate opening (222).

proteasomes by enhancing their loading with Ubp6, a deubiquitinase (DUB) that spares Ub from proteasomal degradation (17).

Proteasomal abundance, function, and composition are tightly controlled by (*a*) factors that induce the transcription of genes encoding proteasomal subunits and assembly factors, (*b*) factors triggering degradation of proteasomes, and (*c*) posttranslational modifications that modulate the degradative capacity of proteasomes. Another important issue is that proteasomal capacity can be compromised by several external and internal insults, such as chemical inhibitors (bortezomib, carfilzomib, and others), oxidative stress, and aberrant proteins or over the course of aging as a result of decreased expression and oxidative modification of proteasomal proteins (18–20). Given the vital importance of proteasome function, there are several ways for the cells to react to reduced proteasomal activity and altered needs. These responses include not only efforts to adjust the UPS activity itself but also compensatory and regulatory actions of other PQC network components.

TRANSCRIPTIONAL REGULATION OF PROTEASOME BIOGENESIS

Transcriptional Regulation Under Normal Conditions

In yeast, the main transcriptional regulator of proteasome biogenesis is the transcription factor Rpn4, which binds to the promoters of proteasome subunit (PSM) genes via the conserved proteasome associated control element (PACE) motif (5′-GGTGGCAAA-3′) (21). Rpn4 functions in a negative feedback circuit. It is itself rapidly degraded in the proteasome, when proteolytic demand is low but becomes stabilized during proteotoxic stress, for example, when proteasomal function is inhibited or overloaded (22). Such increased de novo formation of proteasomes upon proteasome inhibition also occurs in higher eukaryotes and is mediated by members of the cap'n'collar basic

leucine zipper (CnC-bZip) family of transcription factors, Nrf1 and Nrf2, and others, depending on the cellular context.

Nrf1 is a glycoprotein bound to endoplasmic reticulum (ER) that upon activation enters the nucleus and binds to antioxidant response elements (ARE) in the promoter regions of proteasome subunit genes to upregulate their expression (23–25). Under normal conditions, Nrf1 is constitutively retrotranslocated from the ER into the cytosol by the p97 ATPase, ubiquitinated, and rapidly degraded in the proteasome. The active form of Nrf1 is released from the ER and enters the nucleus only when proteasomal capacity needs to be enhanced or proteasomal activity is inhibited (23, 26–30). This "bounce back" mechanism has a significant medical relevance because therapeutic proteasome inhibition (e.g., by bortezomib) promptly triggers the upregulation of proteasome genes in malignant cells (31). This represents an important compensatory response that promotes tumor cell survival and likely hampers therapeutic efficacy, for example, in the case of multiple myeloma, for which proteasome inhibitors are an important component of combination chemotherapy. Recently, DNA-damage inducible 1 homolog 2 (DDI2) was identified as the protease required to cleave and activate Nrf1 upon proteasome inhibition in mammalian cells (32) and in *Caenorhabditis elegans* (33). It remains to be investigated whether DDI2 and DDI-1 (*C. elegans*) can sense proteasomal activity and are specifically activated by inhibited or overloaded proteasomes or whether they are constitutively active and cytosolic accumulation of their substrate Nrf1, upon proteasome impairment, is sufficient to trigger cleavage.

In the presence of proper amounts of intracellular nutrients and energy, Nrf1 is also activated by mammalian target of rapamycin complex 1 (mTORC1), which promotes anabolic processes, such as protein synthesis and lipogenesis, in response to growth signals. mTORC1 induces Nrf1 through the posttranslational activation of SREBP1, which in turn switches on the transcription of the Nrf1 gene along with genes involved in lipid synthesis (34). Importantly, mTORC1 also functions as a master regulator of autophagy, the second cellular degradation pathway. Although active mTORC1 induces the production of new proteasomes, it simultaneously suppresses ALP that is normally active in situations that require catabolic activity (**Figure 1*a***).

Transcriptional Regulation Under Stress Conditions

This situation changes fundamentally under stress conditions, such as starvation, oxidative stress, or growth factor deprivation, that often require the (transient) stoppage of anabolic processes and cell growth. In this situation, mTORC1 is inhibited (**Figure 1*a***). As a consequence, (*a*) biosynthetic processes are halted; (*b*) autophagy, as an important stress response pathway and source of free amino acids, is induced; and (*c*) the transcription factor Nrf2 (35) and probably other currently unknown factors are activated and induce the expression of factors that maintain proteasome assembly and abundance. Proteasome assembly is a highly complex process, which is even more challenging under stressful conditions, that is, when cells need to deal with additional obstacles such as misfolded or damaged proteins. In yeast, Adc17, a newly identified ATPase-dedicated chaperone, helps to cope with these situations by supporting the function of the four known regulatory particle (RP) assembly chaperones Nas2, Nas6, Rpn14, and Hsm3. Adc17 interacts with the amino terminus of Rpt6 to assist formation of the critical Rpt6–Rpt3 ATPase pair during assembly of the RP of the 26S proteasome (36). This function of Adc17 is mechanistically distinct from the other RP assembly factors. The dramatic increase of Adc17 levels (along with levels of the other RP assembly chaperones) upon cell stress is mediated by the inhibition of mTORC1, which leads to Mpk1 activation, whereas this increase in Adc17 is independent from the transcriptional regulator Rpn4 (37). The mammalian homolog of Adc17 has not been identified yet, and it is currently unclear which protein(s) fulfills its critical task in mammalian cells. Nevertheless, this

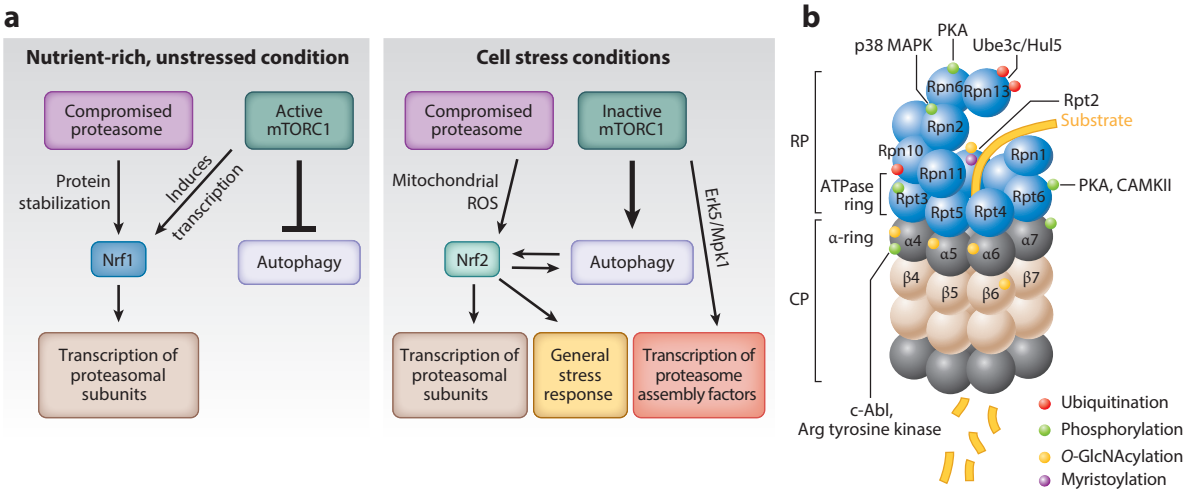


Figure 1

Transcriptional regulation and posttranslational modifications of the proteasome. (*a*) In mammalian cells, the transcriptional regulation of proteasome biogenesis is dependent on the cellular context. In the nutrient-rich, unstressed condition (*left*), compromised proteasome activity due to chemical inhibition or capacity overload leads to the stabilization of the transcription factor Nrf1, which would be otherwise degraded in the proteasome. In addition, Nrf1 transcription is upregulated by active mTORC1 (via SREBP1). At the same time, mTORC1 inhibits autophagy. In cell stress conditions (*right*) of starvation, oxidative stress, protein misfolding, etc., Nrf2 becomes stabilized. Also, proteasome inhibition contributes to Nrf2 stabilization through mitochondrial reactive oxygen species (ROS) generation. Nrf2 not only regulates the transcription of proteasomal subunits but also activates a general stress response that includes oxidative and heat stress response pathways and an increase in cellular protein folding capacity. Importantly, under cell stress conditions, mTORC1 is inactive leading to the activation of autophagy. mTORC1 inhibition also promotes proteasome biogenesis by enhancing the expression of several proteasome assembly factors, such as the four regulatory particle (RP) chaperones and Adc17 (yeast). (*b*) Posttranslational modifications of the proteasome: a simplified illustration of the proteasome holoenzyme composed of the RP and the core particle (CP). Of the more than 110 posttranslational modifications that have been detected thus far, only those modifications that have been functionally characterized are shown. Monoubiquitination of the Ub-receptors Rpn10 and Rpn13 impairs substrate binding and recruitment of shuttling factors. *O*-GlcNAcylation of the APTase Rpt2 and the α-ring of the CP can be induced by high glucose, is reversible, and results in proteasome inactivation. Myristoylation of Rpt2 affects the localization of the proteasome. Phosphorylation seems to be the most versatile modification. It can have an activating or inactivating function and affect stability/composition and localization of the holoenzyme. In addition, *N*(α)-acetylation, *N*(α)-methylation, and N-terminal truncation have been described to occur at several proteasomal subunits (primarily of the RP).

adaptive yeast pathway is generally conserved in mammals, in which mTOR and Erk5 control proteasome abundance by stimulating the expression of all four mammalian RP assembly chaperones in stressful situations. Importantly, these studies reveal that the two major degradative systems are under the control of one central regulator, mTORC1 (**Figure 1*a***).

In contrast to Nrf1, Nrf2 is not tethered to the ER but localized in the cytosol as well as on the mitochondrial surface (38) and plays a well-established role in the defense against oxidative stress by upregulating detoxifying and antioxidant factors (see details below). Yet, emerging evidence indicates that the same Nrf2 pathway also mediates a response to proteasome inhibition. Proteasome dysfunction in both *Drosophila* and mammalian cells induces a generalized stress response involving induction of autophagy-related genes as well as higher expression levels and assembly rates of proteasomal subunits (39, 40). This latter process is mediated by a burst of mitochondrially generated ROS triggered by mitochondrial proteome damage that in turn leads to a Nrf2-dependent upregulation of the proteasome subunits (41). Interestingly, this feedback circuit seems to be functional mainly in young organisms, as proteasome inhibition in aged flies

resulted only in minimal PSM gene expression changes (39). In addition, Nrf2 associates with mitochondria upon proteasome inhibition via interaction with PGAM5 (38). This interaction is required for the expression of Hsp40 and Hsp70 that were shown to target protein aggregates accumulating following proteasome inhibition. Thus, cells not only increase de novo synthesis of proteasomes to handle the accumulation of proteasomal substrates but also enhance refolding capacity to alleviate the burden of accumulating misfolded/aggregated proteins.

Recently, Nrf2 has also been shown to cooperate with p53 in the regulation of proteasome abundance (42). p53 is an important tumor suppressor that is frequently mutated in human cancers. New evidence now suggests that mutant p53 and Nrf2 together promote PSM gene transcription in cancer cells, thereby affecting the cancer cell proteome, including several tumor-suppressive pathways.

The stress-responsive role of Nrf1/2 appears to be largely conserved in *C. elegans*, with SKN-1 being orthologous to Nrf1 and 2. Loss-of-function mutants of the proteasome subunit Rpn10, which lead to a moderate proteasome dysfunction, increase longevity and resilience to multiple cellular stresses in *C. elegans*, including heat, oxidative stress, and the presence of aggregation-prone proteins (43). These effects are mediated through the activation of the SKN-1/NRF and ELT-2/GATA transcription factors that promote expression of PSM genes as well as the oxidative and heat stress response pathways. Yet, SKN1/NRF also upregulates multiple autophagy genes that are required for the enhanced resistance of the *rpn-10* mutant to aggregation-prone proteins. Interestingly, in mammalian cells, Rpn10 has been shown to be subjected to posttranslational regulation by ubiquitination leading to reduced proteasome activity (44) (see following section); however, a potential link to the induction of proteasomal subunits or autophagy genes has not been identified yet.

POSTTRANSLATIONAL MODIFICATIONS REGULATE ASSEMBLY, LOCALIZATION, AND DEGRADATION CAPACITY OF 26S PROTEASOMES

Recent findings corroborate the notion that proteasomal activity is subjected to extensive posttranslational regulation by phosphorylation, *N*-acetylation, ubiquitination, myristoylation, proteolytic cleavage, *O*-GlcNAc modification, and ribosylation (44–53) (**Figure 1*b***). In fact, first reports describing phosphorylation and ubiquitination of the proteasome date back to the 1990s. With the help of modern proteomic methodologies, as many as 110 co- and posttranslational modifications of the yeast proteasome have been reported, but as yet only a minor fraction of them have been functionally characterized (54, 55). Number, site, and type of the modifications differ considerably among the otherwise highly conserved yeast, human, and mouse proteasomes (55). There are basically three types of modifications: (*a*) those that affect stability/assembly of the holoenzyme and thus the abundance of functional proteasomes, (*b*) those that impact directly on the degradative capacity of existing proteasomes, and (*c*) those that control the localization of the proteasomes. For example, ADP-ribosylation of proteasome-associated factor PI31 has been reported to promote proteasome assembly (56). *O*-GlcNAcylation of Rpt2, one of the AAA ATPases in the 19S RP, inhibits the proteasome through inhibition of ATPase activity, thereby coupling proteasome function to glucose metabolism (47). In yeast, *N*-myristoylation of the Rpt2 subunit regulates the intracellular localization of the 26S proteasome (57).

Phosphorylation of Core and Regulatory Particle Subunits Modulates Proteasomal Stability and Activity

Almost all subunits of the 26S proteasome are phosphorylated (55). Phosphorylation of the CP α-ring primarily affects proteasome stability and composition. Phosphorylation of the core particle

(CP) α-subunit α7 stabilizes the association of the RP with the CP to form the 26S proteasome but does not affect peptidase activities (58, 59). γ-Interferon treatment decreases α7 phosphorylation and results in unstable 26S proteasome complexes facilitating the exchange of the RP into PA28-capped proteasomes (59). PA28 is an activator present only in organisms with an adaptive immune system, and PA28-containing proteasome complexes have been implicated in antigen processing.

Phosphorylation of RP subunits seems to predominantly influence the access of the substrate to the proteolytic chamber. For example, phosphorylation of Rpt6 (ATPase subunit of the RP base) by either PKA or CaMKII (48, 49, 60) results in increased degradation rates. cAMP-dependent PKA also phosphorylates Rpn6, a non-ATPase component of the RP lid, accelerating substrate degradation in vitro (61). Importantly, this also concerns aggregation-prone proteins such as mutant FUS, SOD1, TDP43, and tau, whose aggregation is a hallmark of neurodegenerative diseases. Indeed, in a mouse model of tauopathy, cAMP treatment ameliorated proteasome activity, clearance of tau aggregates, and cognitive function (62).

Evidently, the activity of kinases might control the subcellular localization of proteasomes as a phosphomimetic mutant of Rpt6 (S120D) is more resistant to detergent extraction, probably due to local tethering of proteasomes to scaffolds (48). In this context, CaMKII has been proposed to act as a scaffold for the proteasome in postsynaptic spines (50).

Ubiquitination of the Proteasome Reduces Its Degradative Capacity

Proteasome inhibitors or proteotoxic conditions induce a rapid ubiquitination of the proteasome at multiple sites (63, 64). In these conditions, five E3 ligases have been found to accumulate on proteasomes (Ube3a/E6AP, Ube3c/Hul5, Rnf181, Huwe1, and Ubr4), which mediate the ubiquitination of approximately 14 subunits. Although an extensive ubiquitination will result in the elimination of inactive proteasomes by autophagy (see the sidebar titled Proteaphagy: Autophagic Turnover of the Proteasome), the proteasomal Ub-receptor Rpn13 has been identified as a specific substrate of Ube3c/Hul5 unrelated to proteaphagy. The targeted lysines, K21 and K34, are located at the N-terminal end of the Ub-binding Pru domain, and their ubiquitination reduced the ability of Rpn13 to interact with ubiquitinated proteins, thereby impeding substrate degradation (63). Interestingly, partial proteasome inhibition already induces specific Rpn13 ubiquitination

PROTEAPHAGY: AUTOPHAGIC TURNOVER OF THE PROTEASOME

The term proteaphagy was coined by studies in *Arabidopsis* demonstrating that the turnover of proteasomes is mediated by autophagy (223). Proteaphagy in *Arabidopsis* can be stimulated by nitrogen starvation, which promotes bulk autophagy, or by chemical or genetic inhibition of the proteasome, which causes the selective removal of nonfunctional 26S proteasomes. Only the latter route involves extensive ubiquitination of inactive particles, enabling their recognition by (extraproteasomal) Rpn10, which binds the attached ubiquitin moieties through a ubiquitin-interacting motif (UIM) and simultaneously interacts with ATG8 exposed on the autophagic membranes through an Atg8-interacting motif (AIM), thus acting as a classical selective autophagy receptor (223).

In yeast, the majority of proteasomes reside in the nucleus, whereas autophagy is restricted to the cytosol. Moreover, yeast Rpn10 does not contain an AIM. Analogous to *Arabidopsis*, there are two proteaphagy routes in yeast that respond to either nitrogen starvation or particle inactivation (224, 225). Yet, instead of Rpn10, Cue5 was identified as the responsible selective proteaphagy receptor and before encapsulation, the oligomeric chaperone Hsp42 is required to export ubiquitinated nuclear proteasomes to insoluble protein deposit (IPOD)-type structures (224).

both in vivo and in vitro, suggesting that this is a sensitive autoregulatory response that prevents binding of ubiquitinated substrates in the case of impaired degradation capacity. The physiological advantage of such a mechanism remains to be determined.

The second proteasomal Ub-receptor, Rpn10, was also shown to be monoubiquitinated. Monoubiquitination of Ub-receptors very often blocks the protein's ability to bind its ubiquitinated targets in trans because of intramolecular Ub-binding domain (UBD)–Ub interactions (65). This also seems to be the case for Rpn10 (44) whose ability to recruit proteasomal substrates is markedly reduced upon ubiquitination. Moreover, Rpn10 is found in proteasome-bound and -unbound pools, and yeast proteasomes have the capacity to dynamically associate with Rpn10. Yet, Rpn10 monoubiquitination impairs the Rpn10–proteasome association and facilitates the interaction of the ubiquilin-type DSK2 receptor with the proteasome (66). In contrast to Rpn13 ubiquitination, which is increased by proteotoxic stress, Rpn10 monoubiquitination is decreased in stress conditions, suggesting that it may function as a mechanism to dynamically and quickly adjust not only the loading capacity but also the composition of the proteasome to changing cellular demands.

CONTROLLING PROTEASOME FUNCTION AND ACTIVITY

How the function and integrity of assembled proteasomes are surveyed and maintained is so far poorly understood. Ecm29, a large protein containing 29 HEAT repeats, has been proposed to act as a quality control factor that recognizes and inhibits aberrant proteasomes and helps to remodel proteasomes in stress conditions. On one hand, perturbations of the RP–CP interface or ATP depletion increase the loading of Ecm29 onto 26S particles inducing the closure of the substrate entry channel (67, 68) and reducing the ATPase activity of the RP so that substrates cannot be unfolded (69). On the other hand, Ecm29 promotes the dissociation process of the 26S proteasome under oxidative stress to generate 20S particles capable of degrading oxidized proteins (70, 71). Although its role as a proteasome quality control factor seems to be evolutionarily highly conserved, the mechanistic and structural basis of Ecm29's activities, for example, how it recognizes structurally compromised proteasomes, is still poorly defined but might involve distinct posttranslational modifications of the proteasome (72).

DISTURBED PROTEASOME ACTIVITY AFFECTS VARIOUS ASPECTS OF CELLULAR FUNCTION

On one hand, several decades of intensive research have revealed the fundamental importance of the proteasome for virtually every cellular process, and a decline in proteasome activity has been proposed as a central factor during aging and in the pathology of multiple diseases, especially neurodegenerative disorders (73, 74). On the other hand, targeting the proteasome in cancer has emerged as a powerful strategy to fight expansion of malignant cells (75). Thus, understanding the consequences of impaired proteasome activity is of broad interest.

Impaired proteasome activity disturbs cell signaling networks that control cell growth, apoptosis, immunity, and other factors, because several signal transduction pathways, such as the NF-κB pathway, depend on the ability of the proteasome to regulate quality, amplitude, and duration of their signal. The proteolytic activity of the proteasome has also been implicated in the generation of peptides for antigen presentation and thus its impairment will affect the performance of the immune system (76–79). The best-studied consequences of reduced proteasome activity probably concern the accumulation of potentially toxic un/misfolded proteins as well as protein aggregates (80). Besides threatening vital functions of cytosolic processes and organelles, the accumulating waste will also impede the recycling of amino acids and Ub that are both required for cell survival.

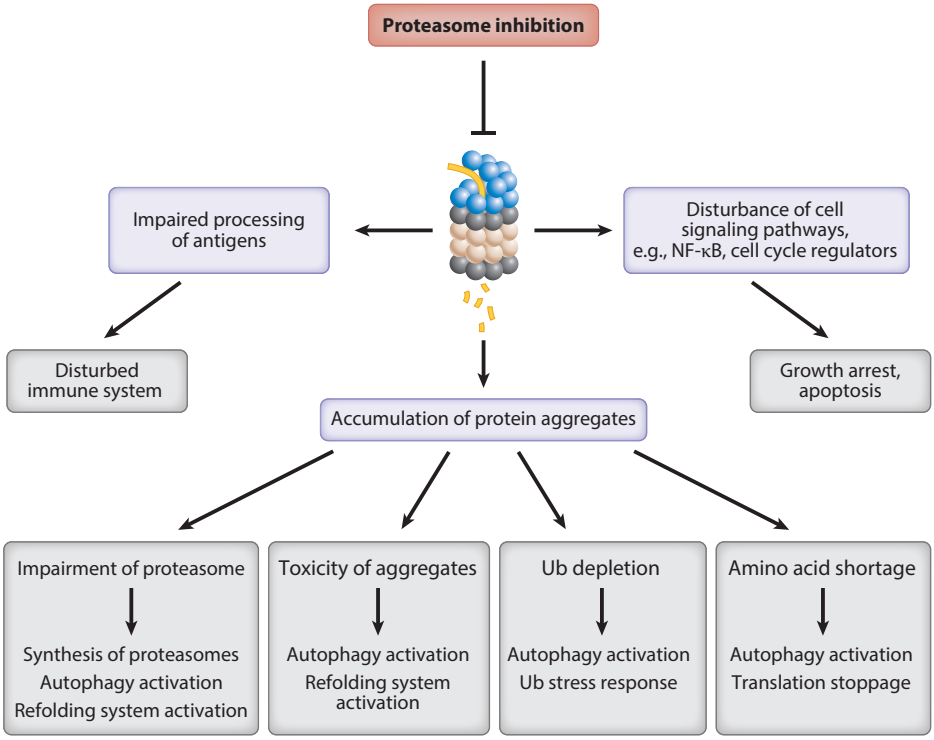


Figure 2

Cellular consequences of proteasome inhibition. Inhibiting the proteolytic activity of the proteasome affects almost every cellular process. Signaling networks regulating, for example, cell cycle progression, cell growth, or survival, depend on the proteasomal degradation of effector/inhibitor proteins or require proteasomal processing of precursor proteins for signal propagation. Also, a major task of proteasomes (in particular, of specialized immunoproteasomes) is the generation of major histocompatibility complex class I antigens through proteolytic cleavage of endogenous proteins. Proteasome inhibition can therefore contribute to immunopathology. The most prominent consequence of impaired proteasomal activity is the accumulation of misfolded and aggregated proteins that causes a depletion of essential amino acids and ubiquitin (Ub), is toxic to multiple organelles, and impairs the function of the proteasome itself. Cells react to these threats by upregulating autophagy, stalling protein translation, and activating protein refolding capacity to eliminate aggregates.

Some but not all of these consequences can be ameliorated by compensatory actions of other PQC pathways, with ALP being at the forefront (**Figure 2**).

ROLE OF AUTOPHAGY IN CLEANING THE CELL INTERIOR

Whereas the proteasome is in charge of small, short-lived proteins, autophagy is the preferred degradative route for large, heterogeneous cytoplasmic materials such as protein aggregates, organelles, lipid droplets, or invading bacteria whose sizes exceed the spatial capacity of proteasomes. There are three different types of autophagy pathways: macroautophagy, microautophagy, and chaperone-mediated autophagy (CMA; only in mammals). These pathways have in common the capacity to deliver cytosolic substrates to the lysosomes for degradation, yet they differ in the way the cargo is directed to the lytic compartment. In CMA, cytosolic proteins that expose a

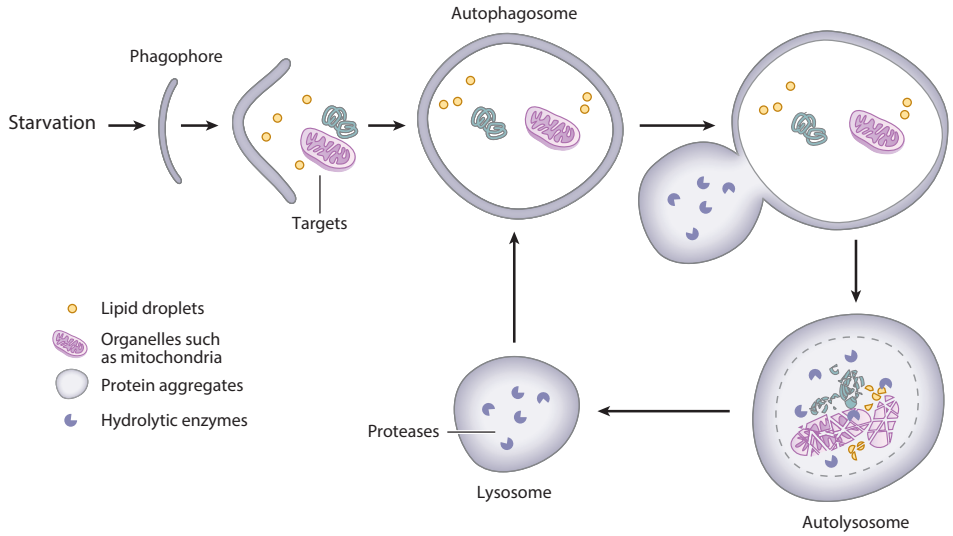


Figure 3

Bulk autophagy. Bulk autophagy is induced by starvation and starts with the formation of a double-layered isolation membrane that elongates and nonselectively envelops cytosolic material such as proteins and organelles. Various organelles, including the endoplasmic reticulum, mitochondria, mitochondria-associated membranes, the Golgi apparatus, the plasma membrane, and recycling endosomes, have been suggested to contribute to formation and growth of the membrane. Closure of the membrane gives rise to an organelle known as the autophagosome, the outer membrane of which fuses with lysosomes to create the autolysosome. The autophagosomal cargo is then degraded by lysosomal hydrolases and released into the cytoplasm for protein biosynthesis and energy production.

pentapeptide signature motif (KFERQ) are recognized by heat shock 70 kDa protein 8 (HSPA8/HSC70), which in turn binds to lysosomal-associated membrane protein 2A (LAMP2A). The target proteins are then unfolded and translocated into the lysosomal lumen where they are degraded (2). During microautophagy, cytosolic content is sequestered by a small invagination of the lysosomal membrane that pinches off into the lumen (81). The best-characterized form of autophagy is macroautophagy (hereafter called autophagy). Its hallmark is the engulfment of cellular material by a double-membrane structure called the phagophore, which closes to form the autophagosome (14) (**Figure 3**). Mature autophagosomes are transported along microtubules toward their minus-ends and ultimately fuse with lysosomes resulting in the degradation of the contents by lysosomal hydrolases. In this way, autophagy provides molecular building blocks during periods of nutrient deprivation but also eliminates unwanted cellular contents.

Under normal conditions, signals that regulate autophagy are connected to metabolism and growth (82). The threonine-serine kinase ULK1 is the most upstream kinase within the autophagy core machinery and its phosphorylation status is the main determinant of autophagy activation (see the sidebar titled The Autophagy Core Machinery). Both AMP-activated protein kinase (AMPK) and mTORC1 are able to directly phosphorylate ULK1 and are thus key regulators that couple low energy and nutrient depletion to autophagy activation (83, 84). AMPK activity positively regulates autophagy, whereas mTORC1 is the key repressor of autophagy under nutrient-rich conditions and its inhibition (e.g., by rapamycin or starvation) is sufficient to rapidly induce autophagy (85, 86). Moreover, mTORC1 has been reported to target other proteins known to modulate autophagy, such as the proautophagic factor AMBRA1. Under nonautophagic conditions, mTORC1 inhibits

THE AUTOPHAGY CORE MACHINERY

The core machinery that is essential and sufficient to drive this highly complex process consists of 17 conserved autophagy-related (ATG) proteins (autophagy-related genes). The ATG proteins are recruited to the site of autophagosome formation in a hierarchical manner and function in several protein complexes: (*a*) a multiprotein complex organized around unc-51-like autophagy activating kinase 1 (ULK1) as well as FIP200 (also known as RB1-inducible coiled-coil 1, or RB1CC1), autophagy-related 13 (ATG13), and ATG101; (*b*) a second multiprotein complex, involving vacuolar protein sorting 34 (VPS34; also known as phosphatidylinositol 3-kinase, catalytic subunit type 3, or PIK3C3), Beclin 1 (BECN1), and autophagy/beclin-1 regulator 1 (AMBRA1), whose activity is dependent on phosphorylation by ULK1 and can be blocked by inhibitory signals from antiapoptotic members of the Bcl-2 protein family; (*c*) two transmembrane proteins, ATG9 and vacuole membrane protein 1 (VMP1), that circulate between the Golgi apparatus, endosomes, and autophagosomes; (*d*) the ubiquitin-like (UBL) Atg12–Atg5–Atg16 system that covalently couples an Ubl of the Atg8/LC3/GABARAP family to autophagic membranes, which is required for autophagosome biogenesis; (*e*) the Atg8/LC3/GABARAP family of Ubls that are exposed on autophagic membranes and have a dual function in that they contribute to the maturation of the phagophore and interact with selective autophagy adaptors to facilitate substrate sequestration; and (*f*) several SNARE-like protein complexes, which promote the fusion between mature autophagosomes and lysosomes.

AMBRA1 by phosphorylation. Upon autophagy induction, that is, mTORC1 inhibition, AMBRA1 is dephosphorylated and regulates ULK1 stability and kinase activity by promoting the assembly of K63-linked Ub chains on ULK1 by the E3 ligase TRAF6. As ULK1 is known to activate AMBRA1 by phosphorylation, this pathway acts as a positive regulation loop (87).

Under nutrient or growth factor deprivation, autophagy is thought to be nonselective and dedicated to bulk degradation of any cytosolic protein and other macromolecules to deliver essential nutrients. However, autophagy can also selectively target cellular structures such as organelles, nuclear components, the proteasome, protein aggregates, stress granules, or invading bacteria (**Figure 3**). In this context, selective autophagy functions as an important cytoprotective mechanism that can be activated in nutrient-rich conditions by various stress signals (88).

Autophagy receptors are the master players in selective autophagy. They specifically recognize cargo and tether it tightly to the nascent autophagosomal membrane (**Figure 4**). To this end, they are equipped with the LC3-interacting region (LIR) that binds to members of the LC3/GABARAP-family of Ub-like modifiers exposed on the autophagosomal membrane. Moreover, emerging evidence suggests that cargo-bound autophagy receptors are able to locally trigger autophagy by recruiting and activating crucial components of the autophagy machinery (such as ULK1) (89, 90). This is mechanistically different from starvation-induced autophagy where the initiation of autophagy and the generation of the isolation membrane is independent of cargo recognition but is regulated by mTORC1 and/or AMPK (14). The crucial role of autophagy receptors is underlined by the fact that a growing number of them as well as their regulator Tank-binding kinase 1 (TBK1) are found to be mutated in neurodegenerative diseases (91–94).

In addition to binding to autophagosomal membranes, autophagy receptors have to recognize their cognate cargo, that is, distinguish healthy organelles or cellular structures from damaged or superfluous ones. This topic has been comprehensively covered by a recent review (95). Briefly, in yeast and mammals, autophagy receptors are often integral components of the autophagic cargo and expose their LIR or AIM (in yeast) upon internal or external stress signals. Alternatively, the binding affinity of the LIR/AIM is modulated by phosphorylation. In addition to

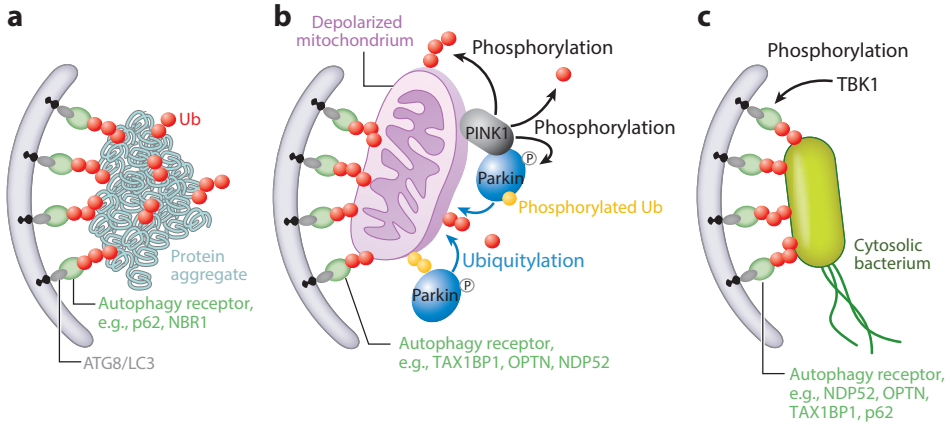


Figure 4

Selective autophagy targets specific cellular structures. In mammals, autophagic cargo is often labeled with ubiquitin (Ub), which is recognized by selective autophagy receptors. By simultaneously binding to LC3 exposed on autophagic membranes, these receptors sequester the cargo into the autophagosome. (*a*) Aggrephagy: Protein aggregates are targeted in this form of selective autophagy. The major autophagy receptor implicated in this process is p62. (*b*) Mitophagy: Depolarized mitochondria are labeled with phosphorylated Ub through the PINK1/Parkin1 pathway. PINK1 becomes stabilized on damaged mitochondria and phosphorylates free Ub, the Ubl domain of Parkin and Ub chains attached to the mitochondrial surface. Parkin is initially recruited to mitochondria by PINK1 and activated through PINK1-mediated phosphorylation and noncovalent binding to phosphorylated free Ub. Subsequently, Parkin ubiquitinates several mitochondrial proteins. Approximately 10–20% of the attached Ub chains are in turn phosphorylated by PINK1. Parkin exhibits a high binding affinity for phosphorylated Ub chains, resulting in prolonged residence time and enhanced K63-linked ubiquitination. K63-linked chains serve as a recognition signal for multiple autophagy receptors. (*c*) Xenophagy: Cytosolic bacteria, such as *Salmonella*, become covered by a dense Ub coat that is recognized by OPTN, NDP52, and TAXBP1. Tank-binding kinase 1 (TBK1) phosphorylates all four receptors on several sites, including the Ub- and LC3-binding domains, thereby altering their preference for polyUb linkages and enhancing their Ub-binding capacity. TBK1-mediated phosphorylation of OPTN also enables binding to phosphorylated Ub chains and has been implicated in PINK1/Parkin–driven mitophagy as well.

the direct mode of cargo-coupling to autophagosomal membranes, higher eukaryotes have evolved a Ub-dependent mechanism, which in fact became the major form of cargo recognition in mammals (96). The affinity of autophagy receptors for both Ub (on the substrate) and LC3/GABARAP (on the autophagosomal membrane) can be additionally modified by phosphorylation of the UBD and LIR/AIM, respectively (97) (**Figure 4**).

COMPENSATORY ACTIONS BETWEEN PROTEASOME AND AUTOPHAGY

The observation that proteasome inhibition or overload induces autophagy in the majority of cell types provided a first indication for a functional connection between the two systems. The proteasome-to-autophagy direction of regulation is well documented by now. It primarily compensates the reduced degradative capacity of the proteasome and eliminates the threat resulting from damaged organelles or the accumulation of potentially toxic protein aggregates. In contrast, evidence for an opposite autophagy-to-proteasome shift, activated upon impairment of autophagy, is currently weak. In starved colon cancer cells, inhibition of autophagy was shown

to induce proteasomal capacity by increasing both the proteolytic activity and the expression of proteasomal subunits (70). However, other reports did not detect an upregulation of proteasomal activity upon lysosomal inhibition (98). Moreover, inhibition of autophagy in HeLa cells was shown to rather impair flux through the UPS due to the stabilization of p62 that is normally degraded along with the autophagic cargo. Accumulated p62 sequesters ubiquitinated proteins and thereby delays their shuttling toward the proteasome, but proteasomal activity itself was not affected (99). Notably, increased p62 levels were also shown to activate the Nrf2-dependent stress response (100), which includes the transcriptional activation of several Ub-associated genes, such as E3 ligases and DUBs (for details, see the section on the p62–Keap1–Nrf2 axis in cellular stress response). Yet, a direct compensatory function of the proteasome in the case of impaired autophagy might be precluded by the fact that most of the autophagosomal substrates are simply too large and complex to be handled by the proteasome.

Several recent reports strongly corroborate the notion that both AMPK and mTORC1 can "sense" impaired proteasome activity in several ways (**Figure 5**). In fact, inhibition of the 26S proteasome results in rapid activation of AMPK in macrophages and epithelial and endothelial cells (101) as well as in several cancer lines (102, 103). In some types of cancer cells, this is controlled via a Ca^{2+}-CaMKKβ–dependent pathway, whereas in human breast and prostate cancer cells, proteasome inhibition was shown to decrease glycogen synthase kinase-3β (GSK-3β) activity, which in turn stimulates autophagy via AMPK activation (104–106). Notably, such a GSK-3β-dependent cross talk seems to also be active in stressed hippocampal neurons but appears to decline during aging (105).

In addition, proteasome impairment will eventually affect the function of all other organelles due to the accumulation of unfolded and damaged proteins. Several reports indicate that one of the first detectable consequences of proteasome dysfunction concerns mitochondria, which develop deleterious alterations in their proteome (39, 101). This in turn leads to a burst of mitochondria-originating ROS that can directly activate AMPK through oxidative modification (glutathionylation) of specific cysteine thiols (107). As an inactive proteasome also compromises the ER-associated degradation (ERAD) pathway, severe ER stress is another unavoidable consequence of sustained proteasome inhibition. Consequently, several mechanisms that increase autophagy are triggered (reviewed in 108). A Perk-mediated pathway activates the transcription factor ATF4, which induces the expression of Sestrin2, which in turn induces autophagy by inhibiting mTORC1 (109). Moreover, a CamKK/AMPK–dependent pathway has been described that also targets mTORC1 (110). Another arm of the ER-stress response pathway causes the dissociation of the Nrf2–Keap1 complex and upregulation of Nrf2 target genes, including several autophagy-related genes (111).

COORDINATED ACTION OF THE UPS AND AUTOPHAGY IN PROTEOSTASIS

Apart from the compensatory function of autophagy upon proteasome impairment, both systems intersect and communicate at multiple points to coordinate and balance their actions in proteostasis and homeostasis of organelles. The UPS and autophagy influence each other through a powerful mechanism of mutual control of the levels of their key components. This is the case, for example, in the oxidative stress response in which the autophagy receptor p62 mediates the autophagic degradation of the E3 ligase Keap1 (for details, see the section on the p62–Keap1–Nrf2 axis in cellular stress response) or the control of proteasome abundance by proteaphagy (see the sidebar titled Proteaphagy: Autophagic Turnover of the Proteasome). The termination of autophagy that is mediated by proteasomal degradation of ULK1 and other autophagy regulators is yet another

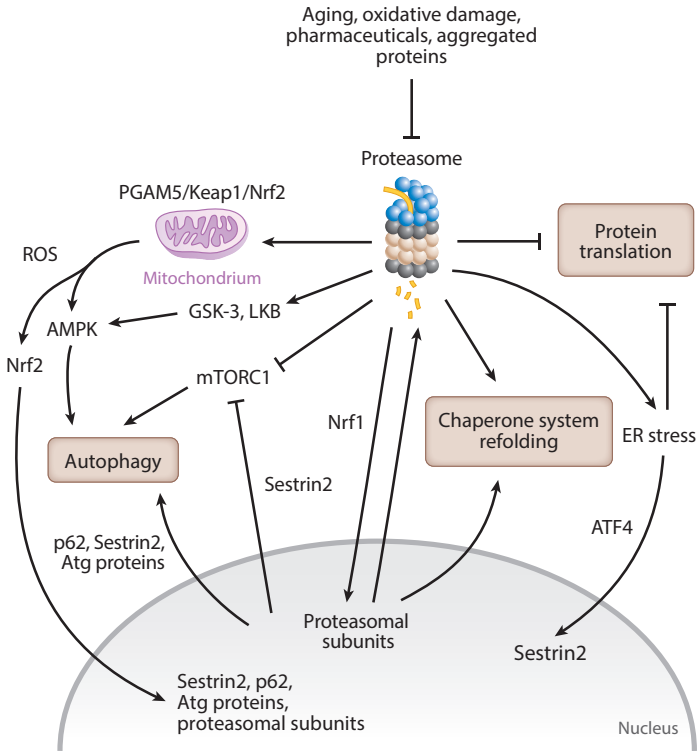


Figure 5

Cross talk between the ubiquitin–proteasome system and autophagy. A reduced proteasome activity affects several other cellular compartments. Therefore, multiple pathways are activated to communicate compromised proteasomal capacity to different cellular systems to avoid or reduce deleterious disturbances. Nrf1 translocates to the nucleus to upregulate de novo synthesis of the proteasome. CAMKKβ- or GSK-3β-dependent pathways as well as ROS production in the mitochondria cause the activation of autophagy via AMPK. In addition, mitochondrial ROS stimulate Nrf2 stabilization through inactivation of Keap1. Nrf2 in turn induces expression of proteasomal subunits, several autophagy-related genes, p62, and Sestrin2. Sestrin2 is also upregulated by ATF4 in response to ER stress due to disturbed ER function. On one hand, Sestrin2 directly activates autophagy by inhibiting mTORC1. On the other hand, it supports p62-dependent autophagic degradation of Keap1, thereby further stabilizing Nrf2 and establishing a positive feedback loop. Thus, autophagy is upregulated at multiple levels and primarily serves to clear accumulating protein aggregates. Finally, protein translation is halted and chaperone activity increased to minimize the load of unprocessed proteasomal substrates. Abbreviations: ER, endoplasmic reticulum; ROS, reactive oxygen species.

example (112, 113). LC3 can also undergo proteasomal degradation, which is mediated by the 20S proteasome. Intriguingly, LC3 can be rescued from proteolysis by the autophagy receptor p62 (114). Further examples of E3 ligases that control the proteasomal degradation of various autophagy effectors are described in a recent review (115).

In addition, depending on the context, autophagy and the proteasome share common substrates as well as regulatory factors. Several key players of either pathway were shown to physically interact with each other, thereby allowing mutual control and concerted actions. The following sections illustrate how the degradation routes are molecularly adjusted to the specific needs of the cell.

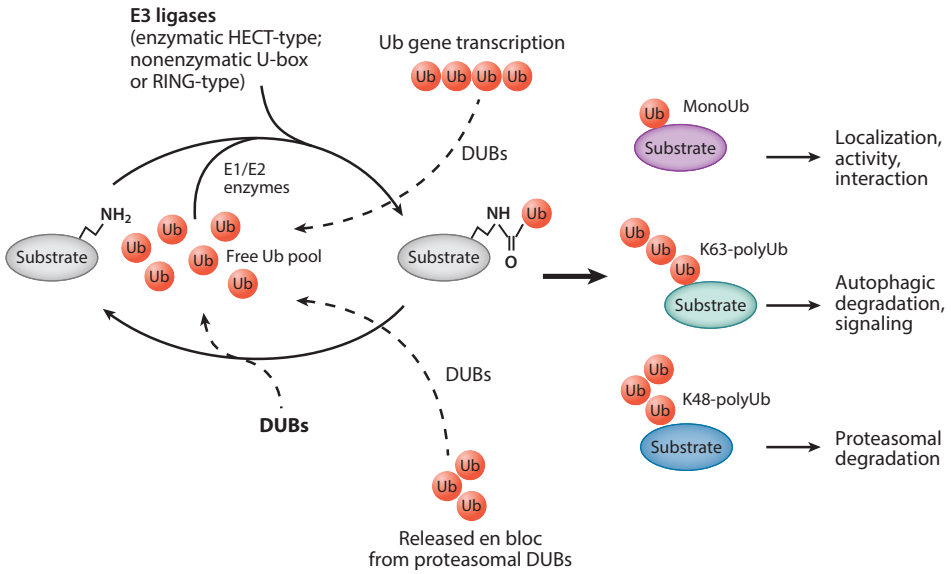


Figure 6

Ubiquitination. Ubiquitin (Ub) is a highly conserved, small protein composed of 76 amino acids, which can be covalently coupled to the ε-amino group of a lysine within a substrate protein. Ubiquitination is both inducible and reversible and requires a cascade of three enzymatic reactions: In the first step, the Ub-activating enzyme (E1) activates the C-terminal Gly of Ub and then transfers it to the Ub conjugating enzyme (E2), which cooperates with the Ub-ligating enzyme (E3) to attach it to the substrate protein. There are basically two types of E3s: The enzymatic homologous to E6-AP C terminus (HECT) domain E3s and non-HECT domain E3s contain a RING, variant RING, or a U-box domain and do not possess enzymatic activity. The RING E3s function as adaptors that bring the Ub-charged E2 in close proximity to the substrate protein to facilitate the transfer of Ub from the E2 to the substrate lysine. Ub itself contains seven lysine residues as well as Met1 that can be targeted in recurrent rounds of this cascade, giving rise to differently linked and branched Ub chains (polyUb). In this way, a complex Ub code can be generated. The action of E3 ligases can be reversed by specific deubiquitinases (DUBs) that can cleave Ub from substrates or process different types of Ub chains, thus turning ubiquitination into a highly dynamic modification. The pool of free Ub is maintained by the action of DUBs and transcription of two polyUb genes, *Ubb* and *Ubc*, composed of 3–4 and 9–10 tandem Ub-coding units, respectively.

UBIQUITINATION REGULATES BOTH PROTEASOMAL DEGRADATION AND SELECTIVE AUTOPHAGY

The most striking commonality of both degradation systems is the use of ubiquitination as a labeling system for their substrates (**Figure 6**). Thus, regulated protein degradation almost exclusively relies on ubiquitination, and it is not surprising that free Ub is a precious commodity. In fact, Ub depletion has been identified as a key mediator of toxicity by translational inhibitors (116). Polyubiquitin genes and DUB activity feed into the pool of free Ub and are essential for cellular function and stress tolerance as well as in development (117–121). Moreover, the degradation of multiple substrates (both proteasomal and autophagosomal) additionally requires the p97/VCP/Cdc48 ATPase complex, which functions as a segregase to extract ubiquitinated proteins from membranes and large protein complexes to facilitate their degradation by proteasomes (122) or their delivery to selective autophagy receptors (123, 124).

Interestingly, even E3 ligases that are endowed with strong substrate specificity cannot be sharply assigned to either the UPS or ALP. For example, the E3 ligase Parkin, which plays a central role in mitophagy, mediates proteasomal degradation of a subset of mitochondrial substrates, whereas another subset undergoes autophagic degradation (for details, see the section on regulation of mitochondrial dynamics). A major future challenge is to dissect which factors determine the degradation route of a given substrate and what the particular advantage is of taking one route but not the other if both are available.

TURNOVER OF COMMON SUBSTRATES: PROTEIN AGGREGATES

The accumulation of unfolded, misfolded, or damaged proteins severely impairs the function of organelles and cells and has been recognized as a crucial factor in aging and a wide variety of diseases, including neurodegenerative and cardiovascular diseases, type II diabetes, and cancer. Moreover, there are multiple examples of disease-relevant misfolded proteins, such as α-synuclein, Aβ, or huntingtin, that affect the function of the proteasome and the autophagy machinery (125–131). Recent studies further suggest that mammalian cells can in fact tolerate the accumulation of substantial amounts of protein waste but are more sensitive to the lack of amino acids and Ub molecules resulting from the sequestration of large amounts of ubiquitinated proteins into aggregates. Therefore, cells induce response pathways, including autophagy, to compensate for the amino acid shortage resulting from proteasome inhibition (132) (**Figure 2**). mTORC1 plays a key role in this process. In the presence of sufficient amino acids, mTORC1 localizes to the lysosomal surface and is subsequently activated (133–136) to promote anabolic processes. Upon depletion of free amino acids, mTORC1 activity seems to be actively shut down (137), triggering the switch to the autophagy-inducing catabolic state.

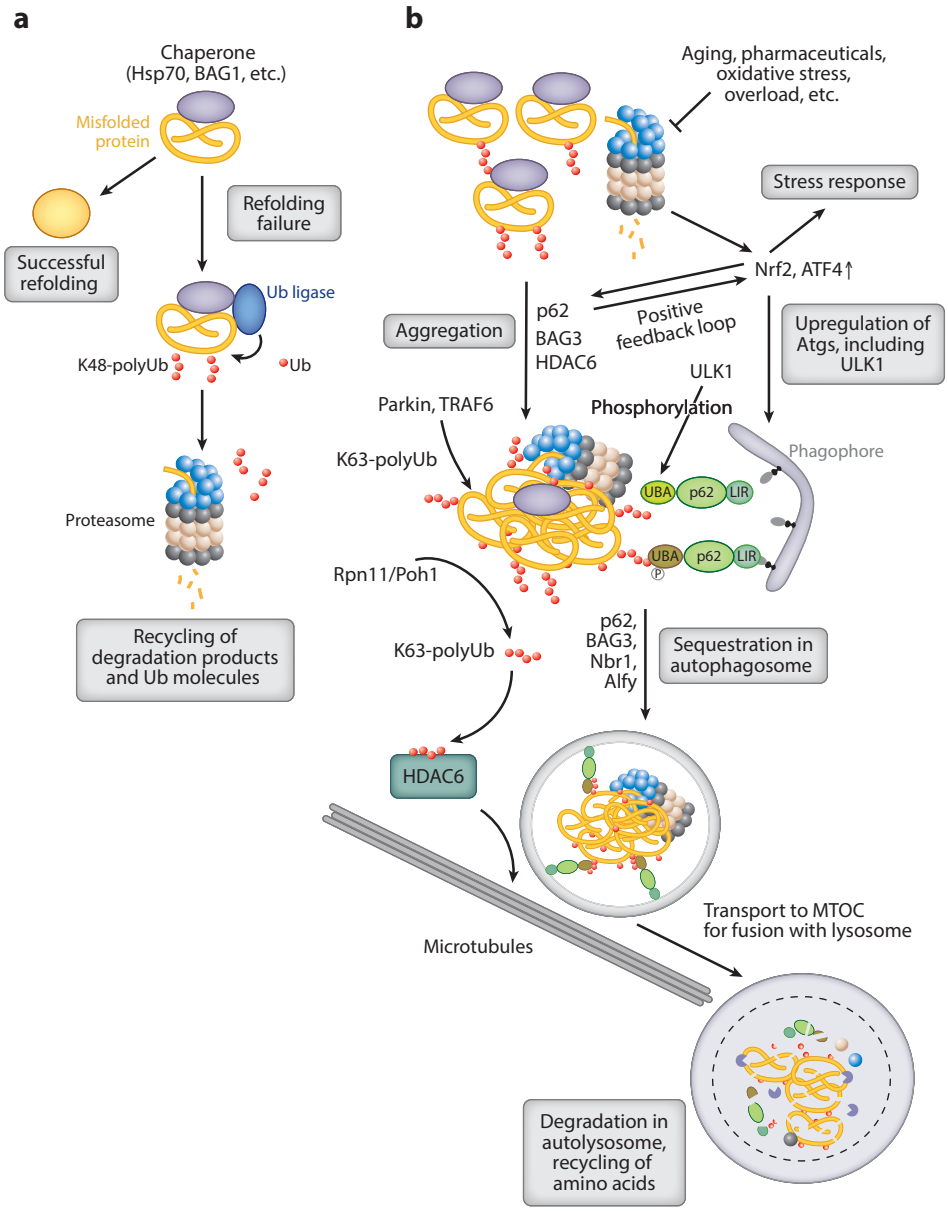
The destruction of most misfolded proteins occurs through the UPS (**Figure 7*a***). Their recognition by E3 ligases is often coupled to chaperone systems that try to rescue the proteins by refolding before handing them over to destruction. For example, the E3 ligase CHIP interacts with Hsp70 and Hsp90 to ubiquitinate chaperone-bound substrates whose refolding has failed. In

Figure 7

Handling of misfolded proteins and protein aggregates. (*a*) In normal conditions, misfolded or unfolded proteins are bound by molecular chaperones that prevent their aggregation and help the proteins to reach their native state and functionality in an ATP-dependent manner. Molecular chaperones are often classified according to their molecular weight, and members include Hsp90, Hsp70, Hsp60, Hsp40 (DnaJ), and the small Hsps. If refolding fails, chaperones recruit ubiquitin (Ub) E3 ligases and facilitate the delivery of the substrate to the proteasome for elimination. Both Ub and amino acids are recycled. (*b*) In the case of compromised proteasome activity (due to aging, overload, chemical inhibition, etc.), misfolded, ubiquitinated proteins accumulate in the cytosol, mitochondria, and endoplasmic reticulum, and the transcription factors Nrf2 and ATF4 induce the transcription of several autophagy-related genes, including ULK1 and p62. p62, HDAC6, and BAG3 mediate the aggregation of misfolded proteins at perinuclear regions, which is required for sequestration by autophagosomal membranes. Besides misfolded proteins, aggresomes also contain proteasomes and chaperones. Aggresome formation is also accompanied by the recruitment of the Ub E3 ligase Parkin, which decorates preaggresomal structures as well as the mature aggresome with K63-linked polyUb chains. ULK1 mediates phosphorylation of S406 within the ubiquitin-associated (UBA) domain of p62 to selectively enhance the affinity of p62 for K63-linked Ub chains, thereby enabling p62 to selectively couple mature aggresomes to the autophagosomal membrane. The proteasomal deubiquitinase Rpn11/Poh1 liberates part of the K63-polyUb chains that are found by HDAC6, thereby activating HDAC6 to mediate the transport of the autophagosome and the fusion of the autophagosome with the lysosome. Abbreviations: LIR, LC3-interacting region; MTOC, microtubule-organizing center.

line with this finding, mild proteotoxic stress was shown to stimulate proteasome activity (138); however, persistent stress eventually suppresses its proteolytic function. Autophagy steps in when the primary repair pathways and the proteasome become overwhelmed or are inhibited leading to the accumulation of misfolded proteins that eventually form large protein aggregates that cannot be recognized or efficiently cleared by the UPS (139) (**Figure 7*b***).

Multiple lines of evidence suggest that p62 might function as a central stress sensor that orchestrates the communication between the UPS and autophagy in the clearance of protein aggregates



(**Figure 7*b***). p62 is upregulated in response to proteasome impairment and consequent ER stress through the transcription factors ATF4 and Nrf2 that also stimulate expression of several other autophagy-related proteins (140). In fact, p62 was originally discovered as a Ub-binding protein involved in proteasomal degradation by binding to the RP subunits Rpn10 and Rpt1 via its Phox and Bem1p (PB1) domains (141, 142). p62 was suggested to redirect polyubiquitinated proteasomal substrates into the autophagosome upon proteasome inhibition, but how this destination change is achieved is currently elusive. In fact, p62 is involved both in aggresome formation, that is, targeting misfolded, aggregated proteins to the aggresome at perinuclear regions (143), and in the subsequent sequestration of aggresomes by the phagophore (11) (**Figure 7*b***). Both steps depend on K63-linked Ub chains that are attached to aggregated proteins as well as the mature aggresome by Parkin, TRAF6 and/or other E3 ligases (144–146). Proteotoxic stress causes the phosphorylation of p62 at S409 by ULK1 (147). S409 is located in the UBA domain of p62 and its phosphorylation further enhances the affinity to K63-polyubiquitinated proteins. Notably, phosphorylation of p62 by ULK1 does not occur upon nutrient starvation, highlighting the specificity of this process. Yet, the role of p62 goes beyond that of a simple autophagy receptor for ubiquitinated protein aggregates, because p62 also activates the Nrf2-dependent stress response (see details below in the section on the p62–Keap1–Nrf2 axis in cellular stress response).

HDAC6 represents another important factor in the communication between autophagy and the proteasome (139). It is equipped with a UBD and associates with both microtubules and the F-actin cytoskeleton (148, 149) enabling it to mediate the retrograde transport of ubiquitinated proteins along the microtubules to form aggresomes (150). HDAC6 is also involved in the subsequent autophagic clearance of the aggresomes, an activity that requires proteasomes (151): Although proteasomes cannot degrade protein aggregates, they accumulate at protein inclusion bodies where the proteasomal subunit Rpn11 (Poh1), a DUB, produces unanchored free Ub chains that bind and activate HDAC6. HDAC6 in turn induces an actinomyosin system that promotes autophagic clearance of the aggresome by supporting the transport of autophagosomes and their fusion with lysosomes (151, 152) (**Figure 7*b***). This activity is specific to aggrephagy as HDAC6 is completely dispensable for starvation-induced autophagy. Of note, in the context of proteasomal degradation, Rpn11 cleaves K48-linked Ub chains from proteasomal substrates prior to their degradation in the 26S proteasome. Yet, in the context of aggrephagy, Rpn11 liberates K63-linked Ub chains from protein aggregates. There is evidence that extensive Hsp90-mediated remodeling of the 26S proteasome facilitates this switch in Rpn11 specificity (153).

Finally, the Bcl2-associated athanogene (BAG) family of proteins has been implicated in the coordination of proteasomal and autophagic activities. BAG proteins interact with the heat shock protein HSP70/HSPA ATPase domain through a specific structural domain known as the BAG domain (154). Several lines of evidence suggest that BAG proteins are critical modulators of PQC systems and play a role in age-related degenerative diseases. BAG1 delivers chaperone-recognized mis-/unfolded substrates (such as huntingtin) to the proteasome and is essential for their effective proteasomal degradation (155, 156). BAG3, in contrast, promotes the degradation of proteins by autophagy (156–158). In fact, BAG1 and BAG3 seem to compete for HSP-bound (poly)ubiquitinated substrates and their expression levels are reciprocally regulated. The BAG3/BAG1 ratio thus determines the balance between proteasomal and autophagosomal degradation.

This balance is shifted from BAG1 to BAG3 upon oxidative/proteotoxic stress (156, 159), upon proteasomal inhibition (160, 161), and during aging (156). Upon chemical proteasome inhibition, HSF1 seems to be the key regulator of BAG3 induction. However, XBP1, HSR, the NF-κB pathway (162, 163), and the ERN1 branch of the unfolded protein response also seem to be able to regulate BAG3 expression. Mechanistically, BAG3 (but not BAG1) induces expression of p62

and physically interacts with it, thus itself participating in the redirection of (poly)ubiquitinated clients to autophagy for degradation (156). Whereas p62 and HDAC6 are selective for substrates modified with K63-linked polyubiquitin, BAG3 complements the repertoire of clients by handling proteins modified by K48-linked polyubiquitins originally destined for proteasomal degradation. Details of this process have been covered by a recent review (164).

REGULATION OF MITOCHONDRIAL DYNAMICS

Mitochondria are the cellular power plants that produce energy through oxidative phosphorylation. They also directly contact most (if not all) other cytosolic organelles (e.g., ER, plasma membrane, and peroxisomes) to generate specialized networks that control several cellular functions like Ca^{2+} homeostasis, lipid synthesis, and apoptosis. This requires that mitochondria be highly dynamic, undergo fission (fragmentation) and fusion (elongation), and undergo transport along microtubules to respond to changes in nutrient or oxygen supply or other kinds of stress (165–167). Importantly, dysfunctional mitochondria are also the major source for oxidative stress through aberrant production of ROS, which has profound implications for the pathogenesis of various diseases, in particular neurodegenerative disorders and cancer, as well as aging (167–171).

The UPS and autophagy preserve mitochondrial plasticity and quality by controlling several levels of mitochondrial dynamics. Depending on the degree of stress, they trigger adaptive responses (moderate stress) and removal of the damaged organelle by mitophagy (sustained stress) or, if these measures fail, induce cell death (irreparable damage). These activities and processes are tightly interconnected. During moderate stress, as a first line of defense against mitochondrial dysfunction, the UPS is in charge of outer membrane PQC and the degradation of damaged proteins in a process called outer mitochondrial membrane–associated degradation (OMMAD). Moreover, the UPS directly controls mitochondrial dynamics by ubiquitinating and degrading proteins involved in mitochondrial fusion and fission processes, such as Mfn-1/2, Drp1, and OPA1, as well as in mitochondrial movement, that is, Miro. Several E3 ligases localized on the mitochondrial outer membrane have been implicated in these events: MULAN (172), MARCH5/MITOL (173–175), Parkin (176, 177), and Mdm30 (178, 179). Through enhanced mitochondrial fission or decreased mitochondrial fusion, impaired mitochondria are separated from the mitochondrial network, thereby quarantining the damage. Unless dysfunctional mitochondria retain their membrane potential, they are unable to fuse with the mitochondrial network, thereby priming them for fragmentation and degradation by mitophagy (180). In contrast, mitochondrial elongation, which can, for example, be induced by amino acid starvation, was shown to protect functional mitochondria from autophagic turnover to maintain ATP production during starvation (181, 182). Mitochondrial elongation is achieved by inactivation of Drp1 as well as by preventing its localization to mitochondria, thus leaving Mfn-1/OPA1–mediated mitochondrial fusion events unopposed (182). MARCH5 mediates ubiquitination and proteasomal degradation of Drp1, Mfn-1, and MiD49, the mitochondrial receptor of Drp1, thus acting as a potent negative regulator of mitochondrial fission (173, 174, 183).

Irreparably damaged mitochondrial regions that have been separated from the mitochondrial network are specifically removed via mitophagy. This is achieved either via an Ub-dependent pathway mediated by the kinase PINK1 and the E3 ligase Parkin or via a receptor-mediated pathway, which is independent of Ub but requires integral mitochondrial autophagy receptors such as FUNDC1, BNIP3, or NIX. The mechanistic details have been covered by several recent reviews (184–186). Briefly, in PINK1/Parkin–mediated mitophagy PINK1 becomes specifically stabilized on depolarized mitochondria and mediates the recruitment and activation of Parkin. Parkin then polyubiquitinates multiple mitochondrial outer membrane proteins, which in turn

enables the recruitment of Ub-binding autophagy receptors (p62, OPTN, NPD52, TAX1BP1, and NBR1) (89, 187, 188). Notably, although all of these receptors can be specifically detected on damaged mitochondria, depletion of OPTN, NDP52, and TAX1BP1 is sufficient to completely inhibit mitophagy, at least in HeLa cells. The specific functions of p62 and NBR1 in the process are currently unclear. In addition, efficient mitophagy is critically dependent on a positive feedback loop that relies on PINK1-mediated phosphorylation of both Parkin and Ub and results in full E3 ligase activation of Parkin as well as sustained binding of Parkin to damaged mitochondria (189–192) (**Figure 4*b***).

However, the proteasome is also required to facilitate this process. Several targets of Parkin, in particular mitochondrial outer membrane (MOM) proteins such as Tom20, Tom40, Tom70, Omp25, and Mfn-1/2, are modified with K48-linked Ub chains and degraded by the proteasome (193–196). Parkin was also shown to directly interact with the proteasome via the proteasomal Ub-receptor Rpn13. Deletion of Rpn13 delayed the clearance of mitochondrial proteins (TIM23, TIM44, and TOM20) during mitophagy (197). By targeting selected MOM proteins for proteasomal degradation, Parkin seems to facilitate mitophagy through remodeling and rupture of the MOM of depolarized mitochondria prior to engulfment by the autophagosomal membrane.

Mitochondrial phosphoglycerate mutase 5 (PGAM5) has emerged as a new key regulator of both PINK1/Parkin– and receptor-mediated mitophagy. On one hand, it functions as a protein serine/threonine phosphatase that stabilizes PINK1 in mitochondria, leading to enhanced recruitment of Parkin and mitophagy induction (198, 199). On the other hand, in response to mitochondrial damage or hypoxia, PGAM5 interacts with and dephosphorylates FUNDC1, thereby enhancing its interaction with LC3 (200). Intriguingly, PGAM5 by functioning as a binding partner of the Keap1–Nrf2 complex also integrates signals generated by proteasome inhibition and participates in oxidative stress response pathways. It remains to be investigated how these roles of PGAM5 are functionally and physiologically linked.

MANAGEMENT OF OXIDATIVE STRESS

ROS are produced as a by-product of aerobic metabolism, of exposure to various environmental stressors, during disease pathogenesis, and in the natural aging process. Mitochondria are the major source of cellular ROS. Whereas ROS at moderate levels function as signals to promote cell proliferation and survival, excessive ROS production (e.g., due to mitochondrial dysfunction) can cause serious oxidative damage to lipids, proteins, and DNA, severely affecting their molecular activities. The 20S proteasome plays a pivotal role in the selective recognition and degradation of oxidized proteins (18, 201), being responsible for the degradation of approximately 90% of all intracellular oxidation-damaged proteins. Notably, degradation by the 20S proteasome occurs in a Ub-independent manner. The major recognition motifs of the substrates seem to be hydrophobic patches exposed on the surface of the damaged protein. Studies indicate that the cell is able to reversibly disassemble 26S proteasomes to elevate the levels of free 20S particles under oxidative conditions or in response to mitochondrial dysfunction (15, 202). Upon prolonged oxidative stress, the proteasome itself becomes compromised (39, 203), leading to further accumulation of oxidized proteins and protein aggregates and requiring compensatory actions, the most prominent of which is the induction of autophagy for the elimination of protein aggregates.

Endogenous ROS production or exogenous H_2O_2 promote autophagy by several mechanisms, including ROS-mediated AMPK activation (107) and inactivation of Atg4 by thiol modifications (204). Atg4 is a cysteine protease that cleaves the precursor of Atg8/LC3 near the C terminus, thereby allowing the conjugation of Atg8/LC3 to phosphatidylethanolamine (PE), which decorates autophagosomal membranes and plays a critical role in autophagosome maturation as well as cargo

sequestration. However, as Atg8-PE is also a substrate of Atg4, it needs to become inactive to maintain the conjugation of Atg8 to the autophagosomal membrane. This (reversible) inactivation is achieved by ROS-mediated thiol modification.

The Keap1–Nrf2 pathway represents another line of defense against oxidative stress and provides an intriguing example of how well attuned the UPS and autophagy functions in the management of stress are.

THE p62–Keap1–Nrf2 AXIS IN CELLULAR STRESS RESPONSE

Under quiescent conditions, Keap1 serves as the adaptor component (for substrate recognition) of the Cullin 3 (Cul3)-based E3 ligase that promotes ubiquitination and proteasomal degradation of the transcription factor Nrf2, thus keeping its level low. Upon exposure to electrophilic or oxidative assaults, Keap1 is inactivated through modification of its reactive cysteine residues (205). Consequently, Nrf2 is stabilized and accumulates in the nucleus, where it activates the transcription of a cluster of genes encoding cytoprotective proteins, including antioxidant proteins, detoxifying enzymes, and multidrug transporters (206). Moreover, as described above, Nrf2 also induces expression of proteasomal genes to compensate for compromised activity of the proteasome (35, 39).

Intriguingly, the Nrf2–Keap1 pathway is engaged in a positive feedback loop with selective autophagy. The autophagy adaptor p62 directly interacts with Keap1, thereby dissociating it from Nrf2 and directing it toward autophagic degradation (207). The immediate consequence is similar to oxidative inactivation of Keap1: Nrf2 is stabilized and becomes transcriptionally active. However, in contrast to the reversible oxidative inactivation of Keap1, p62-mediated elimination of Keap1 is permanent. As Nrf2 also upregulates *p62* gene expression, a positive feedback loop is created that further enhances Keap1 inactivation and elimination (208). Importantly, p62's binding affinity for Keap1 can be markedly increased by phosphorylation of S351 within the Keap1-interacting region of p62 (209). Interestingly, p62 S351 phosphorylation is not only induced by oxidative stressors but also upon activation of various selective autophagy pathways including xenophagy, mitophagy and aggrephagy (209) as well as by proteasomal dysfunction (41).

Another important player in the game is Sestrin2. Sestrins are members of a stress-responsive protein family regulated by several transcription factors, including Nrf2, ATF4, and others, in response to different kinds of stress, such as oxidative stress (210, 211), ER stress (109), or proteasome inhibition (109). Sestrin2 has emerged as a potent regulator of autophagy as it both inhibits mTORC1 and positively regulates AMPK (210). In addition, Sestrin2 directly interacts with p62 and Keap1 to promote p62-dependent autophagic degradation of Keap1 (212). Taken together, the Nrf2–Keap1 pathway seems to act as a general regulator of the cellular stress response that not only induces detoxifying antioxidant factors and proteasome biogenesis (see above) but is also implicated in several selective autophagy pathways, including mitophagy and aggrephagy, that contribute to the elimination of toxic protein aggregates and damaged mitochondria as a major source of ROS.

CROSS TALK BETWEEN PROTEASOME AND AUTOPHAGY DURING ER STRESS

Perturbations in Ca^{2+} homeostasis, redox imbalance, altered protein glycosylation, or protein folding defects cause unfolded or misfolded proteins to accumulate in the ER lumen, which is known as ER stress. Because of the detrimental effects of ER stress, proteins that reside within the ER and transit along the secretory pathway are subject to the strict quality control of the unfolded protein response and ERAD. A first line of defense against the accumulation of misfolded

proteins in the ER is to decrease protein synthesis, thereby unburdening the ER protein-folding machinery (213, 214). This is achieved by phosphorylating the α-subunit of eukaryotic translation initiation factor 2 (eIF2α). This adaptive response can be pharmaceutically prolonged by the Guanabenz derivative Sephin-1 (selective inhibitor of a holophosphatase), which selectively inhibits the PPP1R15A–PP1c complex (but spares the related PPP1R15B–PP1c complex). As a result, Sephin-1 prolongs eIF2α phosphorylation after stress and delays translation recovery. Intriguingly, in mouse models, this effect of Sephin-1 was able to correct the two unrelated protein-misfolding diseases Charcot–Marie–Tooth 1B and amyotrophic lateral sclerosis without any obvious adverse side effects (215).

Although reducing protein synthesis upon ER stress is a rapid and vital response, it does not entirely prevent the occurrence of misfolded proteins. Incorrectly folded polypeptides are typically retrotranslocated to the cytosol, ubiquitinated, and sent to the proteasome for degradation. Moreover, as described above, it is well documented that impairment or overload of proteasome-dependent ERAD leads to induction of autophagy. This facilitates the disposal of polyubiquitinated protein aggregates, thus alleviating ER stress that would otherwise cause cell death (216–218).

NOVELTIES AND FUTURE DIRECTIONS IN THE FIELD

PQC is achieved by a complex network that surveys the quality and capacity of protein translation, folding, and degradation. Disturbances of either part of the system will be sensed and responded to in order to prevent the deleterious effects of a malfunctioning proteome. In particular, prolonged impairment of the degradative capacity of the proteasome constitutes a major threat for cell, organ, and organismal function as signaling networks are disbalanced and the function of other organelles is endangered by the accumulation and/or aggregation of unwanted, potentially toxic proteins. Therefore, cells react on multiple levels to an inappropriate decline in proteasome activity (**Figure 5**). Besides facilitating a direct communication between PQC components on a posttranslational level, cells activate transcriptional programs to upregulate proteasome biogenesis, protein (re)folding capacity, and autophagy to deal with the resulting damage (protein aggregates, damaged organelles) and to compensate at least partially for the reduced degradative capacity. Conversely, problems encountered during protein transcription, translation, or folding are signaled to the proteasome to engage its proteolytic activity. Although the proteasome, basal autophagy, and chaperone systems seem to primarily run the "daily business" of the cell, selective autophagy is called upon for reinforcement primarily in stressful conditions.

Genetic or chemical inhibition as well as an age-dependent activity decline of the PQC are associated with protein aggregation and particularly detrimental to neuronal cells, apparently contributing to various neurodegenerative proteinopathies, including Alzheimer's and Parkinson's diseases, tauopathies, and polyglutamine expansion diseases like Huntington's. Despite intensive research in the past decades, none of these diseases is currently curable. Cancer cells frequently exploit or depend on the cytoprotective power of proteasomes and autophagy to facilitate rapid growth even in suboptimal conditions. For these reasons, targeting the two major degradation systems is highly promising but also challenging. Given the complex nature of the cellular PQC network, a combination of genetic perturbation screens [e.g., using clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9 technology] and quantitative proteomics approaches is needed to further our understanding of the complex cross talk and compensatory actions among its components.

An activation of aggrephagy in the treatment of neurodegenerative disorders seems advantageous and has already yielded promising results in various mouse models. A beneficial side effect

of autophagy activation is likely its protective effect against proapoptotic insults and enhancement of the clearance of a range of infectious agents. A combination of autophagy activators with agents that stimulate proteasomal activity may maximize therapeutic efficiency.

In contrast to neurodegenerative disorders, strategies in cancer therapy aim at inhibiting the proteasome and/or autophagy. Initial approaches targeting the proteasome alone were promising, but success was limited most likely due to activation of one or more of the above-described response pathways resulting in de novo synthesis of proteasomes, autophagy activation, and several other cytoprotective pathways such as the Keap1–Nrf2 pathway. Efficient cancer therapy will therefore require a sophisticated targeting of at least both major degradative systems. Moreover, proteomics approaches are needed to reveal and understand global changes resulting from pharmacological manipulation of this complex system and to identify the most promising therapeutic targets in neurodegenerative diseases, aging and cancer.

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