ANNUAL REVIEWS

Annual Review of Cell and Developmental Biology

Coping with Protein Quality Control Failure

Esther Pilla,* Kim Schneider,* and Anne Bertolotti

MRC Laboratory of Molecular Biology, Cambridge CB2 0QH, United Kingdom;
email: aberto@mrc-lmb.cam.ac.uk

Annu. Rev. Cell Dev. Biol. 2017. 33:439–65

The *Annual Review of Cell and Developmental Biology* is online at cellbio.annualreviews.org

https://doi.org/10.1146/annurev-cellbio-111315-125334

*Both of these authors contributed equally to this review.

Keywords

protein quality control, stress responses, translation, protein misfolding, neurodegenerative diseases

Abstract

Cells and organisms have evolved numerous mechanisms to cope with and to adapt to unexpected challenges and harsh conditions. Proteins are essential to perform the vast majority of cellular and organismal functions. To maintain a healthy proteome, cells rely on a network of factors and pathways collectively known as protein quality control (PQC) systems, which not only ensure that newly synthesized proteins reach a functional conformation but also are essential for surveillance, prevention, and rescue of protein defects. The main players of PQC systems are chaperones and protein degradation systems: the ubiquitin-proteasome system and autophagy. Here we provide an integrated overview of the diverse PQC systems in eukaryotic cells in health and diseases, with an emphasis on the key regulatory aspects and their cross talks. We also highlight how PQC regulation may be exploited for potential therapeutic benefit.

Contents

1. INTRODUCTION: FROM STRESS RESPONSES TO CELLULAR HOMEOSTASIS

Cells devote a significant fraction of their resources to surveying and maintaining a healthy proteome, even under optimal conditions, because the accumulation of misfolded proteins represents a threat for proper cell and organismal function. Chaperones ensure that newly synthesized proteins fold properly and prevent their aggregation. Degradation systems—the ubiquitin-proteasome system and autophagy—degrade no longer needed and faulty proteins or organelles (**Figure 1**). Because species have evolved by surviving and adapting to challenges, and because PQC is vital, organisms not only keep an abundant supply of PQC components to accommodate the basic needs but also have evolved an ability to increase their abundance with increasing needs (**Figure 1**).

Although many components of the PQC systems were discovered in models of experimentally induced stress, most are evolutionarily conserved. PQC is used not only under stress conditions but also at all times to maintain cellular homeostasis, and indeed many components of PQC systems are essential genes. The diverse components of PQC are introduced below.

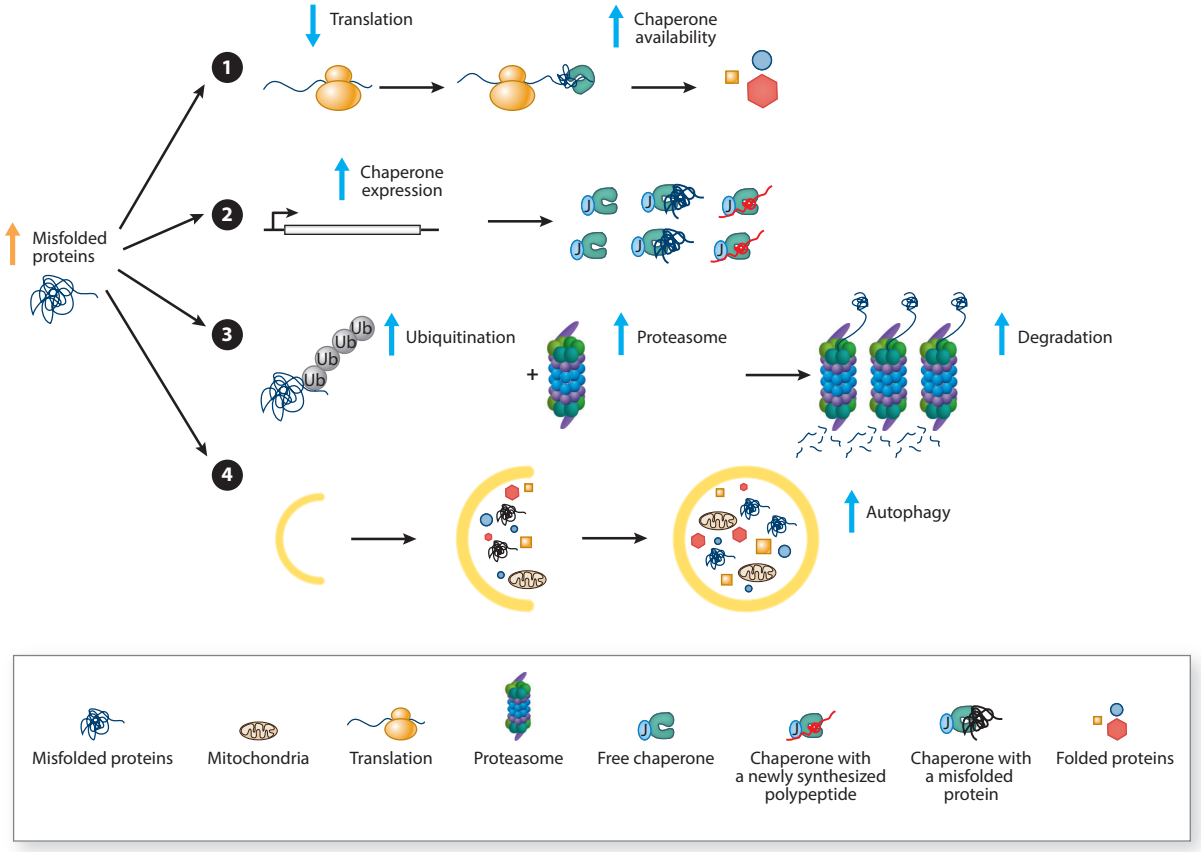

Figure 1

Cellular responses to protein misfolding: an integrated overview. Cells activate a variety of signaling pathways to neutralize misfolded proteins. (❶) An immediate response to protein misfolding consists of decreasing protein synthesis to lower the production of new clients and to increase chaperone availability. (❷) A transcriptional response to protein misfolding stress increases expression of chaperone-encoding genes. In this way, more chaperones can assist in the folding of newly synthesized polypeptides and misfolded proteins. (❸) Acute protein misfolding stresses lead to an increase in ubiquitination and proteasome abundance to increase proteasome degradation. (❹) Various stresses also increase autophagy to degrade large-molecular complexes and organelles.

2. OVERVIEW: PQC IN EUKARYOTIC CELLS

2.1. Molecular Chaperones in the Diverse Subcellular Compartments

Eukaryotic cells are subdivided into different compartments, many containing specific molecular chaperones, which bind unfolded regions of proteins, cotranslationally or posttranslationally. Chaperones thereby serve many functions such as assisting folding, preventing protein aggregation, or facilitating protein import in different subcellular compartments. The majority of the cytosolic chaperones are known to be heat shock proteins (HSPs) of diverse molecular weights (HSP40, HSP60, HSP70, HSP90, HSP100, HSP110, small HSPs). Indeed, chaperone-encoding genes are upregulated as a defense mechanism under conditions such as heat shock, which denatures proteins (Morimoto 1998, Parsell & Lindquist 1993). Protein folding in the cytosol is also

aided by chaperonins, cylindrical-shaped multisubunit complexes, which enclose the substrate proteins in their cavity, allowing them to fold in a shielded environment (Horwich et al. 2007). The endoplasmic reticulum (ER) has a dedicated set of chaperones, some of which are related to the cytosolic ones. The main ER chaperone is the HSP70 protein BiP (Kar2 in yeast), also known as GRP78. Specific also to the ER is HSP90 (GRP94) (Buchberger et al. 2010). Similarly to the ER and the cytosol, mitochondria have chaperones such as mtHSP70 and a mitochondrial chaperonin, HSP60 (Baker & Haynes 2011).

2.2. Degradation Systems

When proteins fail to fold, they must be recognized and degraded. Two main routes exist in the cell to degrade proteins: the ubiquitin proteasome system and autophagy.

2.2.1. Ubiquitin-proteasome system. The proteasome, the most complex protease known, is responsible for the degradation of a large number of proteins, usually following their marking by polyubiquitination (Hershko & Ciechanover 1998). The proteasome is a highly conserved multisubunit complex comprising 47 subunits that form a barrel-shaped structure. Proteasome degradation is vital, and most proteasome-encoding genes are essential (Finley 2009).

2.2.1.1. Regulation of proteasome degradation by ubiquitin. Proteasome degradation is highly regulated, and the marking of proteins by polyubiquitin chains, synthesized via a cascade of E1, E2, and E3 enzymes, represents the main signal for substrate recognition by the proteasome (Hershko & Ciechanover 1998). E3 ligases recognize substrates and thereby serve many functions in PQC in diverse cellular compartments/organelles. Just like chaperones, E3 ligases can act cotranslationally and posttranslationally and can respond to a number of different cellular stresses. A recent and comprehensive review describes these important enzymes (Metzger et al. 2012).

Proteins from the ER can also be degraded by the ubiquitin-proteasome system, a process that requires retrotranslocation of ER-resident proteins to the cytosol. This process, known as ER-associated degradation (ERAD), has been extensively reviewed elsewhere (Buchberger et al. 2010, Vembar & Brodsky 2008). Unlike the ER, which does not possess a luminal protein degradation system, mitochondria have, in their matrix, their own proteases: CLPXP and Lon, both barrel-shaped complexes distantly related to the proteasome (Bota & Davies 2002, Lupas et al. 1997).

2.2.1.2. The proteasome. The proteasome can be subdivided into two functional and structural subcomplexes: the regulatory particle (RP) and the core particle (CP), the latter of which harbors proteolytic subunits. The 19S RP is a cap-like structure essential for regulated and specific recognition of substrates. The RP comprises 19 subunits, 10 of which form the base and 9 of which form the lid. The motor of the RP base is a ring of 6 AAA+ ATPases, Rpt1–Rpt6, which unfold substrates to enable their entry through the narrow proteolytic cavity of the CP. In this way, only unfolded proteins can be degraded (Beckwith et al. 2013, Finley 2009). Rpn1 and Rpn2 are two large subunits of the base acting as a scaffold (He et al. 2012). Polyubiquitinated proteins are recognized by the RP via two ubiquitin receptors, Rpn10 and Rpn13 (Deveraux et al. 1994, Husnjak et al. 2008, Schreiner et al. 2008). Importantly, in addition to ubiquitin, the presence of an unstructured region in proteins is a second signal required to initiate efficient degradation (Prakash et al. 2004).

One function of the lid is deubiquitination of proteasome substrates prior to their degradation. This function is executed by Rpn11, a metalloprotease that sits near to the substrate entry port and usually removes the entire polyubiquitin chain by cleaving the substrate-proximal

ubiquitin (Matyskiela et al. 2013). Ubp6 is another proteasomal deubiquitinating enzyme that can inhibit proteasome degradation by premature removal of the polyubiquitin chain before the substrate has been correctly engaged to the proteasome (Finley 2009), thereby antagonizing the activity of Rpn11 (Bashore et al. 2015). The proteasome-associated E3 ligase Hul5 can further elongate polyubiquitin chains on proteasome substrates, in this way tilting the balance toward the degradation of some substrates (Crosas et al. 2006). Thus, editing polyubiquitin chains at the proteasome may represent a final PQC process before the substrates get irreversibly degraded.

The 20S CP is highly conserved throughout evolution, and it is composed of 28 subunits (products of 14 genes) that form a twofold-symmetric barrel-shaped structure. The core is formed by four hetero-heptameric rings. The α-rings, composed of α1–α7 subunits, are the two outer ends of the stack, whereas the β-rings (β1–β7) lie innermost. The catalytic activity of the CP lies within the β1, β2, and β5 subunits (Stock et al. 1996).

2.2.1.3. Proteasome assembly. The assembly of proteasome subunits follows a complex and orderly process that is facilitated by assembly chaperones (Murata et al. 2009, Schmidt & Finley 2014, Tomko & Hochstrasser 2013). Assembly of the CP starts with the formation of the α1–α7 ring, assisted by the chaperones Pba1/2 and Pba3/4 (mammalian PAC1/2 and PAC3/4, respectively) (Hirano et al. 2005, 2006; Kusmierczyk et al. 2011; Le Tallec et al. 2007; Yashiroda et al. 2008). Assembly of the β-ring starts upon completion of the α-ring (Frentzel et al. 1994, Nandi et al. 1997, Schmidtke et al. 1997, Yang et al. 1995), with the assistance of the assembly chaperone Ump1 (Ramos et al. 1998), which is degraded after completed β-rings assemble with the α-ring (Li et al. 2007, Marques et al. 2007).

The RP components, lid and base, assemble independently. The six ATPases of the RP, Rpt1–Rpt6, assemble in a unique order upon the formation of a trimer of specific pairs of ATPases (Förster et al. 2009, Tomko et al. 2010). Four evolutionarily conserved RP assembly chaperones (RACs) bind to the carboxy-terminal tails of Rpts to prevent their binding to the CP until the ring is complete (Tomko & Hochstrasser 2013). In addition to these evolutionarily conserved assembly chaperones, yeast have an additional RAC, Adc17 (Hanssum et al. 2014). Adc17 binds to the amino-terminal region of Rpt6 to facilitate its pairing with Rpt3 (Hanssum et al. 2014).

Assembly of the lid is poorly understood. Dedicated chaperones assisting the maturation of the lid have not yet been identified, although a recent study implicated SEM1 as a chaperone-like protein in lid biogenesis in yeast, despite its presence in the mature 19S particle (Tomko & Hochstrasser 2014).

2.2.2. Autophagy. Autophagy is a self-eating process that was initially identified as a response to nutrient starvation, in which cells engulf part of their cytosol that contains large protein complexes, protein aggregates, and/or organelles to target them for lysosomal degradation (Nakatogawa et al. 2009). The autophagosome fuses with the vacuole (in yeast) or the lysosome (in mammals), where its contents are degraded. Unlike the proteasome, which is essential, cells lacking autophagy genes are viable under optimal culture conditions but are sensitized to nutrient deprivation (Nakatogawa et al. 2009).

3. ADAPTING PQC TO RISING NEEDS

Cells have the ability to increase PQC in response to demand. This increase is achieved by multiple overlapping mechanisms, following two simple principles: decreasing protein synthesis

to increase the availability of existing PQC components or increasing the abundance of these PQC components.

3.1. Increasing PQC Capacity by Decreasing Translation

Protein folding is influenced by the availability of chaperones and by the rate of protein synthesis. Indeed, the synthesis of new proteins utilizes a large fraction of cellular resources, including PQC components such as chaperones. Thus, an immediate and powerful response to many forms of stresses consists of decreasing protein synthesis rates (**Figure 1**). This response is achieved through two main signaling pathways in the cell: phosphorylation of the alpha subunit of the eukaryotic translation initiation factor 2 (eIF2α) and inhibition of the mammalian target of rapamycin (mTOR). These two modes of translational control are presented below.

3.1.1. Translational control through eIF2α phosphorylation. Phosphorylation of eIF2α is a vital first line of defense against many stresses. This signaling pathway is therefore referred to as the integrated stress response (ISR) (Ron & Harding 2007). Four different eIF2α kinases, each responding to different stresses, have been identified in mammals. The eIF2α kinase GCN2 is induced by amino acid shortage (Sonenberg & Hinnebusch 2009); viral infections and heme deficiency activate the eIF2α kinases PKR and HRI, respectively (Ron & Harding 2007); and accumulation of misfolded proteins in the ER activates the kinase PKR-like ER kinase (PERK) (Harding et al. 2001a). These activated kinases phosphorylate eIF2α, which sequesters eIF2B, thereby preventing the exchange of eIF2-GDP to eIF2-GTP that is required for the recycling of eIF2 and for a new round of protein synthesis (Sonenberg & Hinnebusch 2009). Thus, phosphorylation of eIF2α reduces global translation. Because protein synthesis consumes a large fraction of cellular resources, a subtle decrease in the rates of protein synthesis has a profound impact on cellular homeostasis, allowing cells to spare their existing resources to effectively manage stress conditions. In this way, eIF2α phosphorylation has a broad range of benefits (Ron & Harding 2007). Under physiological conditions, this signaling pathway is most likely used as a rheostat to fine-tune the rates of protein synthesis to the conditions.

Although eIF2α phosphorylation leads to a general decrease in translation, a few transcripts escape this general repression. This is the case of the bZIP (basic leucine zipper) transcriptional activator ATF4, which is translated through the bypass of two otherwise inhibitory upstream open reading frames (uORFs) located in its *Atf4* 5′ untranslated region (5′UTR) (Harding et al. 2000). ATF4 controls transcription of other transcription factors, such as ATF3 and CHOP [also known as DNA damage inducible transcript 3 (DDIT3)], which together reprogram gene expression to restore homeostasis or, if the stress is prolonged, activate apoptosis (Oyadomari & Mori 2004). Importantly, ATF3 and CHOP also enhance expression of the regulatory subunit of the dedicated stress-induced eIF2α phosphatase subunit, PPP1R15A (GADD34). Once translated through ribosomal bypass of an inhibitory uORF located in the *Ppp1r15a* 5′UTR, PPP1R15A recruits its catalytic subunit, protein phosphatase 1 (PP1), to dephosphorylate eIF2α. This represents a negative feedback loop, terminating stress signaling (Novoa et al. 2001).

3.1.2. Translational control through mTOR. mTOR is the catalytic subunit of two macromolecular kinase complexes, mTORC1 and mTORC2, that regulate cellular growth and proliferation and are often misregulated in disease (Zoncu et al. 2011). mTOR senses the availability of nutrients and transduces this signal to many effector molecules to control growth and stress responses (Efeyan et al. 2015, Loewith & Hall 2011, Zoncu et al. 2011). In favorable environmental

conditions, a major function of mTORC1 is to regulate protein synthesis through phosphorylation of several substrates, including the S6 kinases (S6Ks), the inhibitory eIF4E-binding proteins (4E-BPs), and the eIF4G initiation factors. mTORC1 activity also promotes ribosome biogenesis, a highly energy demanding process that is therefore coupled to the energetic supply of the cell (Albert et al. 2015).

As a consequence of mTOR activation, phosphorylated S6K promotes mRNA translation by phosphorylating or binding multiple proteins such as eEF2K (eukaryotic elongation factor 2 kinase) (Wang et al. 2001), sKAR (s6K1 Aly/REF-like target) (Ma et al. 2008), CBP80 (80-kDa nuclear cap–binding protein) (Wilson et al. 2000), and eIF4B (Holz et al. 2005). Phosphorylation of S6K targets in turn facilitates translation initiation and hence enhances translation of newly synthesized mRNAs. Phosphorylation of 4E-BP by mTORC1 derepresses translation because phosphorylated 4E-BP dissociates from eIF4E, allowing translation initiation to occur through the recruitment of eIF4E to the 5′ end of mRNAs containing a 5′ terminal oligopyrimidine (TOP) or TOP-like motif (Haghighat et al. 1995, Hara et al. 1997, Thoreen 2013).

mTOR is activated when nutrients and energy supplies are high, whereas stresses or perturbations, such as in the presence of the mTORC1 inhibitor rapamycin, negatively regulate mTOR. Because mTOR controls translation and ribosome biogenesis, inhibition of mTOR leads to a decrease in protein synthesis (Gingras et al. 2001).

3.2. Increasing the PQC Capacity by Increasing Protein Degradation

When the PQC systems are overburdened or when the growth conditions/nutrient supply is unfavorable, the cells can respond by increasing protein degradation via autophagy or via the proteasome.

3.2.1. Increasing autophagy. Autophagy was initially discovered as a response to nutrient starvation. Autophagy degrades intracellular components and recycles their constituents, thereby providing nutrients to cells (Nakatogawa et al. 2009). Under basal circumstances, in a normally fed animal, autophagy levels are low (Mizushima et al. 2004). Autophagy genes are essential for surviving amino acid starvation in yeast, as well as the neonatal starvation period in mammals (Kuma et al. 2004). Autophagy is a tightly regulated process, and the necessary autophagy genes, known as *Atg* genes, were identified through a heroic phenotypic screen in yeast that isolated mutants defective in the formation of autophagic vesicles (Matsuura et al. 1997, Mizushima et al. 1998). Autophagy is an evolutionarily conserved process, and many of the lessons learned in yeast hold true in mammals. In yeast grown in favorable nutrient conditions, TORC1 phosphorylates Atg13 and in this way represses autophagy. Phosphorylation of Atg13 inhibits its interaction with Atg1 and consequently the recruitment of a complex also including Atg17, Atg29, and Atg31; this recruitment/complex is a crucial early step in autophagosome formation (Kamada et al. 2000) (**Figure 2**). Similarly, in mammals, autophagosome formation is inhibited when mTORC1 phosphorylates ATG13 (an Atg13 homolog) and ULK1 (an Atg1 homolog), which disrupts the complex of FIP200 and ATG101 (Hosokawa et al. 2009a,b; Mercer et al. 2009). In response to nutrient starvation or inhibition of mTOR with rapamycin, Atg13 in yeast (or ATG13 and ULK1 in mammals) is rapidly dephosphorylated and can associate with Atg1 (in yeast) or FIP200 and ATG101 (in mammals). The so-formed Atg1 or ULK1 complexes can trigger the formation of the autophagosome (Kamada et al. 2000, Mizushima 2010). In this way, mTOR represses autophagy, and autophagy is rapidly induced when mTOR is inhibited under stress or nutrient limitations (**Figure 2**).

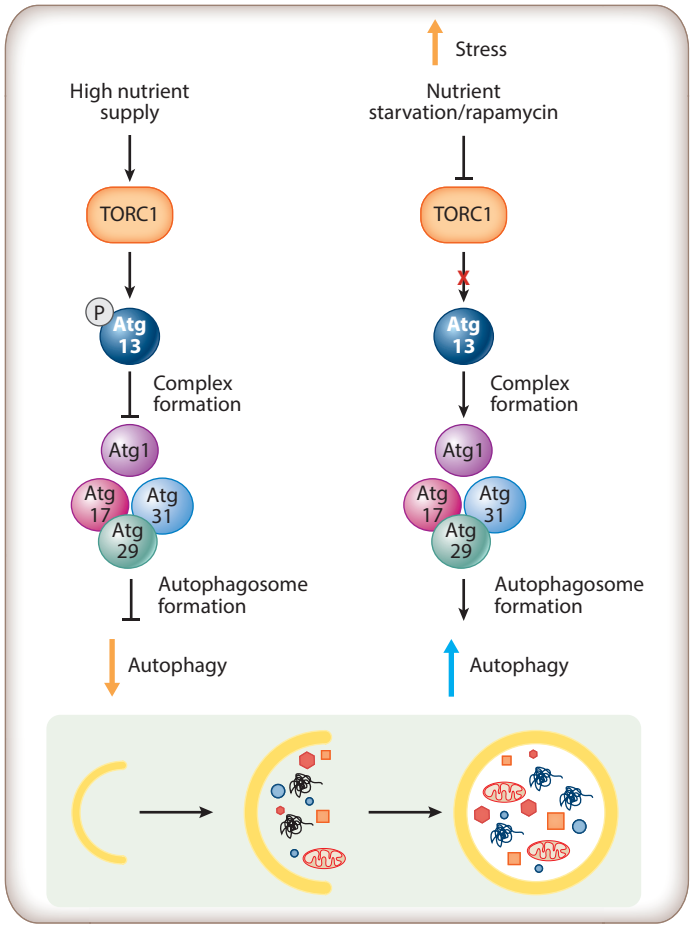

Figure 2

Induction of autophagy. Autophagy is regulated by nutrient availability or stress. In cells growing with a high nutrient supply, target of rapamycin complex 1 (TORC1) is active and phosphorylates Atg13. Phosphorylation of Atg13 inhibits its interaction with Atg1 and consequently the recruitment of a complex also including Atg17, Atg29, and Atg31, which is a crucial early step in autophagosome formation. Under stress conditions such as limited nutrient availability or upon treatment with rapamycin, TORC1 is inactive. Unphosphorylated Atg13 associates with the Atg1 complex to initiate the formation of the autophagosome, leading to an increase in autophagy.

3.2.2. Increasing proteasome abundance. Cells can adjust the abundance of the proteasome on the basis of cellular needs. They do so by coordinating the expression of proteasome subunits and proteasome assembly factors.

3.2.2.1. Regulation of proteasome subunit expression. In contrast to autophagy, which is low under optimal growth conditions, the proteasome is abundant because proteasome degradation serves a broad range of housekeeping functions. In addition, recent studies have shown that proteasome abundance is also upregulated when the demands on proteasome degradation are overwhelming. Increasing proteasome abundance when needed is important for sustaining protein degradation and cell viability.

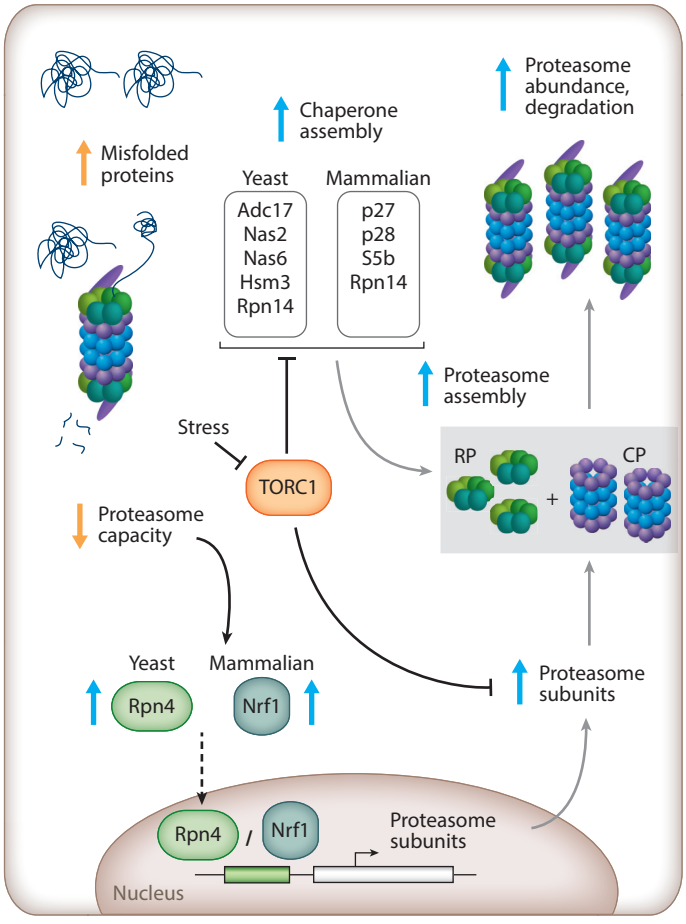

Figure 3

Increasing proteasome abundance. When proteasome capacity is reduced in yeast, the otherwise short-lived transcription factor Rpn4 is stabilized. Mammalian cells respond to proteasome dysfunction by accumulating nuclear factor erythroid-derived 2–related factor 1 (Nrf1) in the endoplasmic reticulum. Nrf1 is then cleaved into an active transcription factor that translocates into the nucleus. Rpn4 and Nrf1 increase the expression of proteasome subunits. Various stresses also increase the abundance of the regulatory particle (RP) assembly chaperones to facilitate the assembly of RP subunits. The increase in RP assembly in turn results in an increase in proteasome abundance and degradation. Mammalian target of rapamycin complex 1 (mTORC1) is a central component of an evolutionarily conserved signaling pathway controlling the coordinated upregulation of proteasome subunits and RP assembly chaperones to maintain proteasome homeostasis. Black lines denote forward signaling; dashed lines, translocation of factors to the nucleus; gray lines, feedback loop; CP, core particle.

The proteasome is a large, multisubunit complex. Proteasome production requires the coordinated expression and assembly of its subunits. In yeast, the expression of all proteasome subunits is coordinated by the transcription factor Rpn4 (Xie & Varshavsky 2001) (**Figure 3**). The steady-state levels of Rpn4 are kept low because Rpn4 is rapidly degraded by the proteasome under normal conditions (Xie & Varshavsky 2001). Thus, proteasome dysfunction results in the stabilization of Rpn4, which drives an increased expression of proteasome subunits. In mammals, the regulated expression of proteasome subunits is under the control of nuclear factor erythroid-derived

2–related factor 2 and 1 (Nrf2 and Nrf1) (Ishii et al. 2000, Kwak et al. 2003, Radhakrishnan et al. 2010). Nrf1, a transcription factor expressed in the ER, is continually degraded by ERAD and the proteasome (**Figure 3**). Upon proteasome inhibition, Nrf1 accumulates and is cleaved by the aspartyl protease DNA damage–inducible 1 homolog 2 (DDI2), releasing an active transcription factor that can translocate to the nucleus and activate target genes (Koizumi et al. 2016, Lehrbach & Ruvkun 2016).

3.2.2.2. Regulation of proteasome assembly. Proteasome assembly was initially thought to be a constitutive process, but recent studies have found that proteasome assembly is in fact highly regulated. An initial hint came from the discovery of a stress-inducible assembly chaperone, Adc17, that facilitates an early step in proteasome biogenesis: the pairing of Rpt6 and Rpt3 (Hanssum et al. 2014) (**Figure 3**). A search for the regulation of Adc17 identified an evolutionarily conserved signaling pathway regulating proteasome homeostasis. A genetic investigation led to TORC1, which negatively regulates Adc17 as well as the other four RACs (Rousseau & Bertolotti 2016). TORC1 negatively regulates proteasome abundance because TORC1 restricts the expression of RACs as well as proteasome subunits in a coordinated manner (Rousseau & Bertolotti 2016). This new level of regulation of proteasome abundance occurs at the translational level and has therefore been missed in gene expression studies. Upon TORC1 inhibition, proteasome abundance increases, an adaptive response required to sustain viability under conditions that would otherwise overwhelm the proteasome (Rousseau & Bertolotti 2016). A different study reported that, when the cells lacking Tsc2 are cultured in absence of serum, proteasome degradation is increased; Tsc2 is a negative regulator of TORC1 (Zhang et al. 2014), suggesting an additional level of regulation under conditions of starvation combined with TORC1 inhibition. Downstream of TORC1 is the MAP kinase MPK1 (also known as Erk5), which is crucial to maintaining the adequate levels of proteasome required for sustaining protein degradation and cell viability (Rousseau & Bertolotti 2016). These findings demonstrate that protein degradation is regulated not only at the level of ubiquitination of the substrates but also by the abundance of the proteasome itself. In line with the general principle of adaptive responses, the increase in proteasome abundance through the coordinated increase in proteasome subunits and RACs is a transient and rapidly reversible response (Rousseau & Bertolotti 2016). Proteasome production is highly energy demanding, and it is therefore not surprising that cells adjust proteasome abundance to their needs: Too much would be an unnecessary energy cost, but too little would put the cells at risk of accumulating protein waste. In fact, cells under standard conditions apparently maintain optimum proteasome levels, with only 20% of proteasomes engaged with substrates and 80% free to rapidly buffer a proteasome stress (Asano et al. 2015). Thus, TORC1 is a rheostat adjusting proteasome homeostasis to metabolism.

3.3. Increasing PQC Capacity by Increasing Chaperone Abundance

Chaperones are abundant proteins that help proteins to fold and prevent protein aggregation. Some chaperones are constitutively expressed, and some are inducible: Their abundance increases when needs increase under conditions, such as heat stress, that cause the accumulation of misfolded proteins.

3.3.1. In the cytosol. As a response to the detrimental accumulation of misfolded proteins, cells increase transcription of genes encoding chaperones to neutralize the potentially toxic species. In eukaryotes, the transcriptional control of HSPs is mediated by the heat shock transcription factors (HSFs); HSF1 is conserved from yeast to humans through invertebrates; and in addition to HSF1, mammals also have HSF2–4 (Vihervaara & Sistonen 2014) (**Figure 4**).

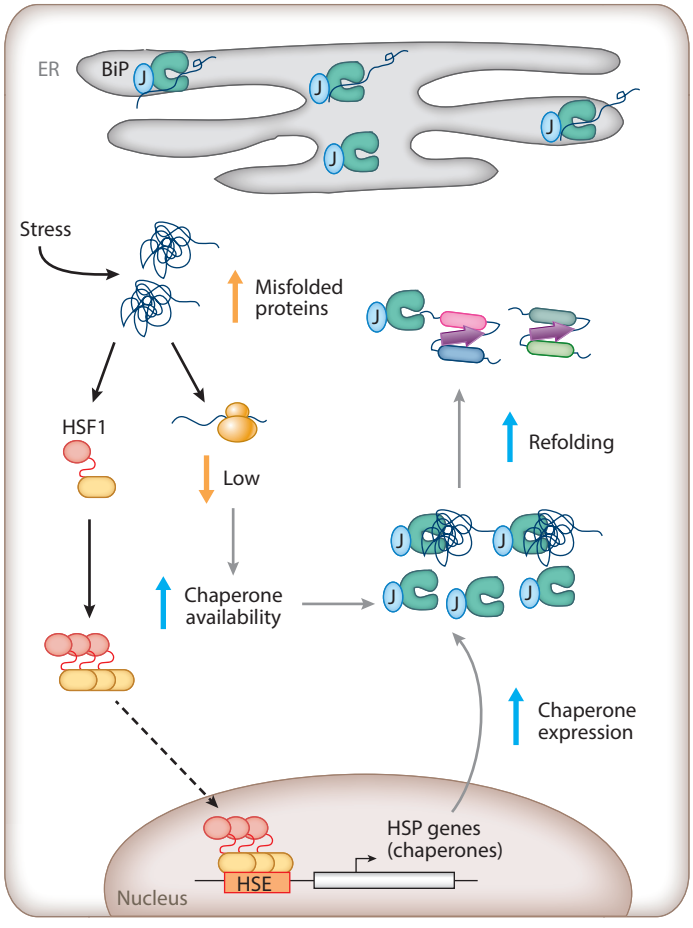

Figure 4

Cytosolic chaperones and the heat shock response. In unstressed cells, cytosolic chaperones assist the folding of cellular proteins. In the absence of stress, chaperones sequester the transcription factor heat shock factor 1 (HSF1) in its monomeric form and keep it inactive in the cytosol. When misfolded proteins accumulate, the cytosolic chaperones are overwhelmed with clients, releasing HSF1, which trimerizes and translocates to the nucleus to increase the transcription of heat shock protein (HSP) genes (chaperones). This increases chaperone capacity to neutralize misfolded proteins. HSE denotes heat shock element.

HSF1 is normally present in the cytosol in a monomeric form that is kept repressed by binding with the chaperones HSP70 and HSP90. An increase in abundance of misfolded polypeptides activates HSF1, which trimerizes and translocates to the nucleus to activate transcription of HSPs (Åkerfelt et al. 2010, Verghese et al. 2012). When stress is neutralized, chaperone availability increases, and as a consequence, HSP70s and HSP90s can sequester HSF1 in the cytosol. Diverse posttranslational modifications also regulate HSF1 activity (Åkerfelt et al. 2010).

Chaperones neutralize misfolded proteins in different ways. For example, aggregated proteins can be solubilized by the ATPase HSP100 (Hsp104 in yeast) (Glover & Lindquist 1998). HSP70s and HSP40s act together with HSP100 to solubilize aggregates and to refold misfolded proteins. Importantly, chaperones and cochaperones function as a network to prevent protein aggregation and to solubilize protein aggregates (Nillegoda & Bukau 2015). Chaperones also play a role in

the spatial sequestration of misfolded proteins to neutralize their deleterious effects (Sontag et al. 2014).

3.3.2. In the endoplasmic reticulum. The ER has unique demands on PQC because the load of protein production within this organelle varies considerably between cells and conditions. To cope with the high demand in protein folding in the ER required at times of high rates of protein synthesis, cells have evolved the unfolded protein response (UPR). The UPR consists of a coordinated translational and transcriptional program aimed at adjusting PQC capacity in the ER as needed (**Figure 5**).

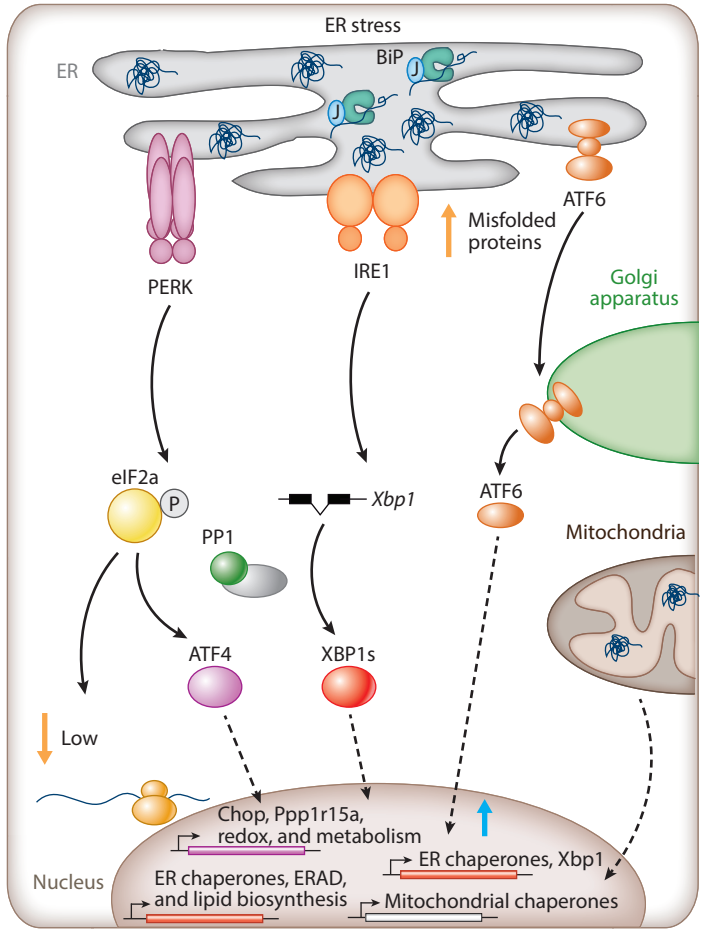

Figure 5

The unfolded protein response (UPR). Protein misfolding stress in the mammalian ER activates the three branches of the UPR. PERK phosphorylates eIF2α, leading to general translational inhibition. eIF2α phosphorylation also facilitates ATF4 translation, leading to transcriptional activation of target genes (e.g., those encoding PPP1R15A and CHOP). IRE1 mediates *Xbp1* splicing. XBP1 activates transcription of chaperones, ER-associated degradation (ERAD), and lipid biosynthesis genes. ATF6 translocates to the Golgi apparatus, where it is cleaved by S1P and S2P proteases, releasing an active transcription factor that can translocate to the nucleus to activate UPR genes. Protein misfolding in the mitochondria also induces a UPR, enhancing transcription of mitochondrial chaperones. Abbreviation: PP1, protein phosphatase 1c.

The most ancient and evolutionarily conserved branch of the UPR is composed of the ER-resident transmembrane protein kinase IRE1, which detects stress in the ER lumen and dimerizes to transduce this information on the other side of the ER membrane (Cox et al. 1993, Mori et al. 1993). The effector domain of IRE1 contains a kinase and an RNase domain that cleaves the mRNA encoding the transcription factor Hac1 in yeast (XBP1 in mammals) (Mori 2000). Only the spliced *HAC1/Xbp1* mRNA can be productively translated into a transcription factor that in turn activates UPR target genes (**Figure 5**). Hac1/XBP1 binds to the unfolded protein response element in the promoter of its target genes, which include ER chaperone–encoding genes, to increase their transcription (Cox et al. 1993, Mori et al. 1993).

In addition to the IRE1 branch, the mammalian UPR has two other branches, which are initiated by the two ER-resident transmembrane proteins PERK and ATF6 (Mori 2000). Activated PERK phosphorylates eIF2α to reduce global rates of protein synthesis, decreasing the biosynthetic burden and increasing PQC capacity (**Figure 5**). ATF6 is an ER-resident transmembrane transcription factor that upon ER stress translocates to the Golgi apparatus, where it is cleaved by site-1 and -2 proteases (Ye et al. 2000). This cleavage releases the active form of the transcription factor from the Golgi membrane. Activated ATF6 increases expression of ER-resident chaperones, which neutralize the misfolded proteins (Mori 2000) (**Figure 5**). The different branches of the UPR act in a coordinated manner. Translation attenuation is an immediate response to stress, followed by changes in gene expression. In the third phase of the response, expression of UPR target genes requires translation recovery (Novoa et al. 2003).

The three mammalian ER stress sensors IRE1, ATF6, and PERK are regulated by a similar mechanism. Under optimal conditions, the ER chaperone BiP (Kar2 in yeast) binds to their luminal domains to keep them inactive (Bertolotti et al. 2000, Liu et al. 2000, Okamura et al. 2000, Shen et al. 2002). Accumulation of misfolded proteins leads to BiP dissociation, promoting activation of IRE1, PERK, and ATF6 (Bertolotti et al. 2000, Shen et al. 2002). The BiP-mediated UPR-sensing mechanism was recently reconstituted in vitro with purified components (Carrara et al. 2015). BiP binds to the luminal domains of IRE1 and PERK through its ATPase domain, leaving the substrate-binding domain of BiP free to bind misfolded proteins (Carrara et al. 2015). Binding of unfolded proteins to BiP then induces a conformational change that is transduced from BiP's substrate-binding site to its ATPase domain, causing BiP dissociation and activation of IRE1 and PERK (Carrara et al. 2015). Thus, UPR sensors exploit the chaperone BiP as a sentinel to detect folding perturbation in the ER. Yeast Ire1 also binds Kar2, which also dissociates upon stress (Okamura et al. 2000), but the importance of Kar2 dissociation in the sensing of the UPR in yeast is debated (Kimata 2004, Kimata et al. 2007, Pincus et al. 2010). Unlike the case for mammalian IRE1 (Oikawa et al. 2009), the luminal domain of yeast Ire1 directly binds to unfolded proteins (Gardner & Walter 2011, Kimata et al. 2007).

3.3.3. In the mitochondria. Mitochondria respond to folding perturbation in their matrix by mounting a mitochondrial UPR (UPR^{mt}), which consists of inducing the expression of mitochondrial chaperones (**Figure 5**). The UPR^{mt} is best characterized in *Caenorhabditis elegans*, in which a mitochondria-to-nucleus signaling pathway senses protein misfolding stress in the mitochondrial matrix and responds by adjusting expression of mitochondrial chaperones and proteases that are encoded in the nucleus (Pellegrino et al. 2013). In addition to this transcriptional response, translation of mitochondrial proteins also decreases upon stress (Münch & Harper 2016).

4. LINKS BETWEEN PROTEIN AND AMINO ACID HOMEOSTASIS

As proteins are made of amino acids, it is not surprising that close links exist between protein homeostasis and amino acid homeostasis. To produce the thousands of proteins required for

cellular and organismal function, cells need to keep an adequate supply of amino acids. There is a dynamic equilibrium between protein synthesis and protein degradation: Protein synthesis consumes amino acids, whereas protein degradation resupplies them. The mTOR and the eIF2α pathways are the two cellular pathways that sense amino acid abundance and in turn adapt cellular metabolism to fine-tune the rates of protein synthesis according to the supply of amino acids.

4.1. The mTOR Pathway

The nature of amino acid sensing was recently elucidated with the discovery of two families of amino acid sensors, Sestrin2 and CASTOR proteins (Chantranupong et al. 2016, Wolfson et al. 2016). Amino acids signal to mTORC1 through the Rag GTPases (Efeyan et al. 2015). Activated mTORC1 is recruited by Rag GTPases to the outer surface of lysosomes, a prime location because this is a site of amino acid recycling through lysosomal degradation. When amino acid supply is low, mTORC1 activity is diminished, leading to a reduction of protein synthesis and an increase in protein degradation. The decrease in protein synthesis reduces amino acid consumption, a rapid adaptive response to restore amino acid homeostasis. A parallel response to TORC1 inhibition is increased protein degradation, which results in the recycling of amino acids to restore amino acid homeostasis.

4.2. The eIF2α Pathway

Phosphorylation of eIF2α is a central modulator of protein and amino acid homeostasis. The kinase GCN2 is activated by uncharged tRNAs. Unlike in the mTORC1 pathway, which is activated by a high abundance of amino acids, in the eIF2α pathway a minute decrease in amino acid abundance may result in elevated uncharged tRNAs, which triggers GCN2 activation to phosphorylate eIF2α, decreasing overall protein synthesis (Wek et al. 1995). It is easy to rationalize why this response has evolved as a consequence of amino acid starvation. By decreasing protein synthesis, cells spare amino acids, and if this first response is not sufficient to restore amino acid homeostasis, cells will mount a transcriptional response to increase production and import amino acids through the Gcn4 and ATF4 pathways. The fact that many forms of stresses increase eIF2α phosphorylation and thus ATF4 expression indicates that this response has evolved because diverse stresses also impact amino acid homeostasis.

4.3. Amino Acid Homeostasis Is Central in Coordinating Protein Synthesis and Degradation

Autophagy is inducible upon starvation, and nitrogen-starved yeast cells require functional autophagy to maintain the levels of amino acids needed to sustain protein synthesis (Onodera & Ohsumi 2005). Proteasome inhibition also impairs protein synthesis under conditions of amino acid deprivation (Vabulas & Hartl 2005).

Autophagy inhibition is deleterious to nutrient-starved cells and organisms, whereas proteasome degradation is essential from unicellular organisms to metazoans, even under conditions in which the nutrient supply is high. Proteasome inhibition is lethal, and this property has been exploited to develop proteasome inhibitors for the treatment of multiple myeloma (Schwartz & Ciechanover 2009). Proteasome inhibition leads to the accumulation of proteins that should be degraded, but it also causes a lethal depletion in the pool of amino acids (Suraweera et al. 2012). In diverse experimental systems, amino acid supplementation rescues the viability of yeast, mammalian cells, and flies when the proteasome is inhibited, without affecting the load of

undegraded proteins, demonstrating that proteasome inhibition causes a lethal depletion of amino acids (Suraweera et al. 2012). This amino acid shortage activates GCN2 to induce eIF2α phosphorylation, as well as autophagy (through the Rag GTPases), and is thus an adaptive response aimed at replenishing the pool of intracellular amino acids. This highlights the central role of the proteasome in the vital dynamic equilibrium between protein synthesis and degradation: proteasome degradation is vital to maintaining amino acid homeostasis. Incidentally, this work reveals that amino acid deprivation is the signal that induces autophagy when the proteasome is inhibited, providing the molecular basis for the cross talk between these two pathways (Suraweera et al. 2012).

The ATPase valosin-containing protein (VCP; also known as p97) is involved in multiple pathways regulating proteasome degradation and is currently being evaluated as a target for cancer therapy. VCP inhibitors have been developed and are toxic to cells, causing accumulation of misfolded proteins (Chou et al. 2011). VCP inhibitors also trigger a deleterious shortage of amino acids, to which cells try to adapt by inhibiting mTOR and inducing eIF2α phosphorylation (Parzych et al. 2015).

Although amino acid scarcity is deleterious, an excess of amino acids may also be undesirable. Cells harboring an editing-defective alanyl-tRNA synthetase accumulate ubiquitinated (presumably misfolded) proteins that associate with HSP72 in response to treatment with an excess of a single amino acid (Lee et al. 2006). This finding suggests that excessive amino acid levels can result in their misincorporation and in protein misfolding (Lee et al. 2006).

5. WHEN THINGS GO WRONG: PQC FAILURE IN DISEASES

Age-related diseases such as neurodegenerative diseases and type 2 diabetes (T2D) are, after cancer, among the most frequent causes of human death. In this section, we discuss how these diverse diseases may result from a gradual decline in PQC with age. Because PQC failure is a common feature of diverse human diseases, rescuing PQC functionality could lead to common therapeutic strategies.

5.1. Neurodegenerative Diseases

Neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis (ALS), and prion diseases are caused by the deposition and aggregation of one or two misfolding-prone proteins (Bertolotti 2008, Soto et al. 2003). The disease-causing proteins are normally soluble; what causes their assembly and aggregation in diseases is unclear. Neurodegenerative diseases have a late onset, suggesting that accumulation of misfolding-prone proteins may be due to a failure of PQC systems. This model predicts that PQC are normally very efficient in young cells but that their efficacy gradually declines with age (Morimoto & Cuervo 2014, Vilchez et al. 2014).

The diverse protein aggregates in neurodegenerative diseases are ubiquitinated, suggesting that proteasome failure may lead to neurodegeneration (Bertolotti 2011). Likewise, genetic inactivation of autophagy in mouse neurons is sufficient to trigger neurodegeneration (Hara et al. 2006). Misfolded proteins and aggregates in disease-specific deposits have been found to be associated with chaperones (Chai et al. 1999, Goswami et al. 2006), and chaperones are well known for their ability to neutralize aggregation of a variety of disease-associated proteins (Kobayashi et al. 2000). Failure to mount adequate stress responses has also been reported in aging *C. elegans* (Morimoto & Cuervo 2014, Vilchez et al. 2014). Thus, multiple lines of evidence support the idea that defective PQC underlies diverse age-related diseases.

5.2. Diabetes

Pancreatic β-cells have to secrete very large amounts of insulin in a controlled manner, and maintenance of β-cell homeostasis is crucial. With time, overwhelming demands on β-cells to secrete insulin may cause persistent and unresolved ER stress (Back & Kaufman 2012). The association between β-cell failure/death and chronic ER stress was highlighted soon after the discovery of the mammalian UPR, with the finding that an intact UPR is essential for β-cell function and glucose homeostasis (Harding et al. 2001b, Scheuner et al. 2001). Moreover, several mouse models for T2D display increased expression of genes associated with the UPR and oxidative stress in the pancreatic islets (Song et al. 2008). In addition, *Perk*-deficient β-cells show retention of misfolded proinsulin in the ER; such retention is accompanied by increased cell death, leading to progressive diabetes (Gupta et al. 2010, Oyadomari & Mori 2004).

Autophagy is important for maintaining normal islet architecture and function. Indeed, generation of β-cell-specific autophagy–deficient mice (Atg7) resulted in degeneration of islets and in impaired glucose tolerance with reduced insulin secretion (Ebato et al. 2008, Jung et al. 2008). Also, autophagosomes are upregulated in the pancreatic β-cell in diabetic *db/db* and nondiabetic high fat–fed mice (Ebato et al. 2008).

Thus, PQC mechanisms are crucial to maintaining protein homeostasis in healthy cells and organisms, and the failure to do so has dramatic consequences. If PQC failures are a common cause of diverse human diseases, then identifying strategies to survive such failures could lead to the development of therapeutics against diverse protein misfolding diseases.

6. EXPLOITING PQC SYSTEMS TO DEVELOP RATIONAL THERAPEUTICS

Targeting PQC could have, in principle, two major outcomes of potential therapeutic value. Proliferating cells, such as cancer cells, have a high metabolic demand and a higher reliance on functional PQC systems. Thus, inhibition of these vital pathways could promote apoptosis and could specifically kill cancer cells. Alternatively, boosting quality control systems could enhance the cellular defenses against misfolded proteins to alleviate protein misfolding, which could represent a common therapeutic approach against neurodegenerative diseases.

6.1. Weakening PQC Systems to Destroy the Bad Cells: An Anticancer Strategy

Conditions of tumor microenvironments such as hypoxia, nutrient limitation, and low pH activate the UPR, and hyperactivation of the three branches of the UPR (PERK, IRE1α, and ATF6) has been reported in a variety of human tumors (Maly & Papa 2014). Mutations in the *Ire1a* gene have been reported in rare cases of human solid tumors (Greenman et al. 2007). Thus, small-molecule inhibitors of the UPR have been investigated as potential cancer drugs.

6.1.1. IRE1 inhibitors. Compounds inhibiting IRE1α could target either the catalytic core of the RNase domain or the ATP-binding pocket of the kinase domain. To date, only small molecules targeting the RNase domain have been shown to display solid antioncogenic functions in vivo. The inhibitor STF-083010 significantly inhibits the growth of tumorigenic cells in mice bearing human multiple myeloma xenografts (Papandreou et al. 2011, Suh et al. 2012). Also, MKC-3946 inhibits the formation of tumors in vivo in a xenograft model of myeloma in a bortezomib-synergistic

manner (Mimura et al. 2012; for a comprehensive review of IRE1α inhibitors, see Hetz et al. 2013).

6.1.2. Inhibiting PERK signaling. GSK2606414, a potent inhibitor of PERK activity, binds near the active site and leads to decreased tumor growth in a xenograft model of pancreatic cancer (Axten et al. 2012). A related yet optimized PERK inhibitor, GSK2656157, also inhibits tumor growth in several mouse xenograft models of pancreatic cancer by blocking PERK autophosphorylation (Atkins et al. 2013). Although these findings show the anticipated promises, the inhibitors exhibit on-target pancreatic toxicity, as expected from the phenotype of the PERK knockout mice (Harding et al. 2001b).

Similarly, a potent inhibitor of eIF2α phosphorylation, ISRIB, was discovered in a small-molecule screen using an ATF4 luciferase reporter (Sidrauski et al. 2013). ISRIB blocks signaling downstream of PERK, and like PERK inhibitors, it kills cells subjected to chronic ER stress (Sidrauski et al. 2013). Although PERK inhibitors and ISRIB were originally developed to kill cancer cells, the use of these molecules in vivo has revealed some surprises. In vivo, ISRIB improves cognitive functions (Sidrauski et al. 2013), and PERK inhibitors are protective against prion diseases (Moreno et al. 2013). It is important to note that the PERK inhibitor GSK2606414 was used in the prion study at a concentration that is >40-fold higher than the IC_{50} (Moreno et al. 2013), questioning whether the observed effects are mediated by PERK inhibition. Indeed, the GSK2606414 inhibitor has also not been optimized to cross the blood-brain barrier and has many other targets (**http://www.kinase-screen.mrc.ac.uk/screening-compounds/600854**). Another paper demonstrates that GSK2606414 is a potent inhibitor of RIPK1. The inhibitor completely represses TNF-mediated RIPK1 kinase–dependent death, an effect independent of PERK inactivation but mediated by direct inhibition of RIPK1 (Rojas-Rivera et al. 2017).

6.1.3. ATF6 inhibitors. Recent efforts have identified inhibitors of the ATF6 branch of the UPR, which could be promising to exploit as agents to increase the death of stressed cells. The small molecules, termed Ceapins, specifically reduce the activity of the ATF6 branch of the UPR during ER stress by preventing its translocation to the Golgi apparatus (Gallagher & Walter 2016, Gallagher et al. 2016).

6.1.4. Targeting protein chaperones. Inhibition of protein chaperones is also thought to be a promising approach to kill cancer cells. For example, inhibition of the chaperone Hsp90 by geldanamycin, or 17-AAG, a less toxic and more potent derivative, induced apoptosis of B chronic lymphocytic leukemia cells (Jones et al. 2004). In addition, the small compound honokiol, a BiP inhibitor, induced apoptosis in brain tumors (Martin et al. 2013).

Taken together, these approaches reveal that inhibiting protective signaling of PQC systems has potential efficacy.

6.2. Boosting PQC Systems to Protect the Good Cells: A Strategy Against Degenerative Diseases

Degenerative diseases in which protein misfolding causes the accumulation of detrimental aggregates can potentially be addressed with strategies aimed at boosting PQC systems to rescue cells from the burden of aggregates. This goal could be obtained by increasing chaperone availability and activity, decreasing the biosynthetic burden, or increasing protein degradation, as we discuss in this section.

6.2.1. Enhancing chaperone expression. The main efforts to increase chaperone expression have focused on either modulating the activity of HSF1, a key transcription factor involved in chaperone expression, or inhibiting HSP90, a negative regulator of HSF1. Several compounds, such as geldanamycin or its analogs 17-AAG and 17-DMAG, inhibit HSP90 and protect *Drosophila* and mouse models from neurodegeneration (Fujikake et al. 2008, Waza et al. 2005) and muscular atrophy (Waza et al. 2005). Essentially, increasing chaperone availability decreases aggregate formation, thus reducing motor neuron loss and degeneration (Bose & Cho 2016). However, the use of these compounds in vivo has not progressed beyond experimental models, because of their toxicity as well as their poor pharmacokinetics properties. A recently discovered molecule, arimoclomol, believed to be a coinducer of HSPs, is progressing to the clinic (Kieran et al. 2004) to treat inclusion body myositis (Ahmed et al. 2016). It will be interesting to elucidate the mechanism of action of arimoclomol.

6.2.2. Chemical chaperones. Chemical chaperones are a group of low-molecular-mass molecules that improve protein folding. One such chemical chaperone, tafamidis, is now an FDA-approved drug that slows the progression of a rare disease, transthyretin familial amyloid polyneuropathy, by stabilizing the misfolding-prone transthyretin mutant (Eisele et al. 2015).

Some chemical chaperones enhancing folding in the ER have been reported as research tools; for example, the chaperone *N*-(*n*-nonyl)deoxynojirimycin helps cells to fold the enzyme β-glucosidase, the faulty enzyme in Gaucher disease (Sawkar et al. 2002). The chemical chaperones 4-PBA and TUDCA have also been shown to reduce ER stress and to improve insulin sensitivity and glucose homeostasis in a mouse model of diabetes and in human patients (Kars et al. 2010, Ozcan et al. 2006, Xiao et al. 2011). Likewise, administration of chemical chaperones alleviates ER stress and reduces pathologies in mouse models of brain and spinal cord ischemia and liver steatosis (Ben Mosbah et al. 2010, Mizukami et al. 2010, Qi et al. 2004). Thus, chemical chaperones are worth exploring further, following the therapeutic success of tafamidis.

6.2.3. ATF6 activators. Activators of the ATF6 branch have also been identified and have been shown to reduce aggregation of extracellular proteins (Chen et al. 2014, Cooley et al. 2014, Plate et al. 2016). Therefore, modulating ATF6 signaling could be a promising strategy to attenuate aggregation of secreted amyloidogenic proteins.

6.2.4. Boosting protein degradation. Activation of autophagy using mTOR inhibitors was also proposed many years ago as a possible strategy to alleviate accumulation of disease-associated protein aggregates (Rubinsztein et al. 2012). However, mTOR controls many cellular processes, so it is not straightforward to rationalize the beneficial effects of mTOR inhibition. Pharmacological inhibition of mTOR prevents translation initiation (Gingras et al. 2001); hence, not surprisingly, treatment with rapamycin has also been shown to ameliorate the aggregation of toxic polyQ in a cellular model of Huntington's disease independently of autophagy by reducing protein synthesis (King et al. 2008, Wyttenbach et al. 2008).

The idea of boosting proteasome degradation to prevent neurodegenerative disease models has recently gained increasing attention. Inhibitors of USP14, the mammalian homolog of Ubp6, have been shown to increase proteasome degradation in cells (Lee et al. 2010). However, the validity of USP14 as a therapeutic target has been questioned (Ortuno et al. 2016). Proteasome impairment has been suggested as a possible cause of diverse neurodegenerative diseases. Tau aggregates cause proteasome impairment and cognitive deficits in mice, and these defects can be alleviated by activating cAMP-PKA signaling, perhaps by enhancing proteasome phosphorylation and function (Myeku et al. 2015).

6.2.5. Increasing PQC capacity by slowing down translation. All the above-described approaches were rationally designed. Our lab has opted for unbiased approaches to identify strategies helping cells to survive PQC failures. The small molecule guanabenz rescues cells from otherwise lethal protein misfolding stress in the ER by selective inhibition of the stress-induced eIF2α phosphatase (Tsaytler et al. 2011). The stress-induced eIF2α phosphatase is composed of a catalytic subunit, PP1, which is shared by approximately 200 phosphatases in mammals (Bollen et al. 2010), and a regulatory subunit, PPP1R15A, which provides substrate selectivity (Connor et al. 2001). Guanabenz is selective because it binds to PPP1R15A without affecting the constitutive and related PPP1R15B-PP1 phosphatase. The selectivity is important because inhibiting both eIF2α phosphatases is lethal (Harding et al. 2009). Salubrinal is a compound that increases eIF2α phosphorylation and protects cells from lethal ER stress (Boyce et al. 2005). It has been used in diverse mouse models and has shown some benefits but also some toxic effects. Salubrinal increases eIF2α phosphorylation by a mechanism proposed to inhibit the two eIF2α phosphatases (Boyce et al. 2005). However, because the target of salubrinal has not been identified, the molecular basis of salubrinal effects is unclear. A possible mechanism came with a recent study showing that salubrinal causes aggregation of diverse purified proteins (Carrara et al. 2017).

Guanabenz inhibits PPP1R15A but is also a centrally active hypotensive drug with nanomolar affinity for the α2-adrenergic receptor (Das et al. 2015). The adrenergic activity of guanabenz becomes a liability for in vivo studies. A structure-activity relationship effort identified Sephin1, which is an inhibitor of PPP1R15A and is devoid of adrenergic activity (Das et al. 2015). Sephin1 is safe in vivo because it selectively inhibits PPP1R15A, but not the related PPP1R15B. Sephin1 prolongs the translation attenuation resulting from stress (Das et al. 2015), thereby extending the benefit of this evolutionarily optimized signaling pathway. As a consequence, the availability of chaperones to target misfolded protein increases so as to rescue cells from a PQC failure (Tsaytler & Bertolotti 2013). Recently, an in vitro system with purified proteins recapitulated the function and selectivity of the human eIF2α holophosphatases, enabling the functional characterization of these enzymes and revealing that Guanabenz and Sephin1 induce a selective conformational change in the amino-terminal region of R15A. This altered the recruitment of eIF2α, preventing its dephosphorylation (Carrara et al. 2017). Because Sephin1 is closely related to guanabenz, it has similar pharmacokinetic properties, rapidly crossing the blood-brain barrier to concentrate in the brain (Das et al. 2015). In mice, Sephin1 treatment prevents the motor, morphological, and molecular defects of two otherwise unrelated protein misfolding diseases, Charcot-Marie-Tooth 1B and SOD1-ALS (Das et al. 2015). This work demonstrated that PPP1R15A inhibition can safely prevent protein misfolding diseases associated with maladaptive ISR signaling. These results are promising, and the next step will consist of assessing whether PPP1R15A is also a target in human diseases. The discovery of selective PPP1R15A inhibitors and their benefits in vivo demonstrates that regulatory subunits of protein phosphatases can be selectively inhibited in vivo. Approximately 200 PP1 phosphatases in cells were previously thought to be undruggable but could in principle be inhibited in the same way.

7. CONCLUSION

In the past 20 years, we have learned a great deal about PQC systems. Many components of PQC systems are evolutionarily conserved, and it is fascinating to see that fundamental cell biological studies, many of which started in simple unicellular organisms, have paved the way to our current understanding of mammalian biology in health and diseases. The fundamental knowledge gained regarding the PQC systems is now being harnessed with small molecules, with the hope that

some manipulations may translate into therapeutic benefits. Exploiting PQC systems for potential therapeutics will be a very active and exciting area of research in the years to come.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

The authors are grateful to members of the Bertolotti lab for discussions, especially Marta Carrara and Joseph Watson for their comments on this manuscript. Research in the Bertolotti lab is funded by the UK Medical Research Council and by the European Research Council (ERC) under the European Union's Seventh Framework Program (FP7/2007–2013) and ERC grant 309516. A.B. is an honorary fellow of the Department of Clinical Neurosciences of Cambridge University. K.S. is supported by the Swiss National Science Foundation.

LITERATURE CITED

Ahmed M, Machado PM, Miller A, Spicer C, Herbelin L, et al. 2016. Targeting protein homeostasis in sporadic inclusion body myositis. *Sci. Transl. Med.* 8(331):331ra41

Albert V, Hall MN. 2015. mTOR signaling in cellular and organismal energetics. *Curr. Opin. Cell Biol.* 33:55–66

Åkerfelt M, Morimoto RI, Sistonen L. 2010. Heat shock factors: integrators of cell stress, development and lifespan. *Nat. Rev. Mol. Cell Biol.* 11(8):545–55

Asano S, Fukuda Y, Beck F, Aufderheide A, Förster F, et al. 2015. A molecular census of 26S proteasomes in intact neurons. *Science* 347(6220):439–42

Atkins C, Liu Q, Minthorn E, Zhang S-Y, Figueroa DJ, et al. 2013. Characterization of a novel PERK kinase inhibitor with antitumor and antiangiogenic activity. *Cancer Res.* 73(6):1993–2002

Axten JM, Medina JR, Feng Y, Shu A, Romeril SP, et al. 2012. Discovery of 7-methyl-5-(1-{[3-(trifluoromethyl)phenyl]acetyl}-2,3-dihydro-1*H*-indol-5-yl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine (GSK2606414), a potent and selective first-in-class inhibitor of protein kinase R (PKR)-like endoplasmic reticulum kinase (PERK). *J. Med. Chem.* 55(16):7193–207

Back S-H, Kaufman RJ. 2012. Endoplasmic reticulum stress and type 2 diabetes. *Annu. Rev. Biochem.* 81:767–93

Baker BM, Haynes CM. 2011. Mitochondrial protein quality control during biogenesis and aging. *Trends Biochem. Sci.* 36(5):254–61

Bashore C, Dambacher CM, Goodall EA, Matyskiela ME, Lander GC, Martin A. 2015. Ubp6 deubiquitinase controls conformational dynamics and substrate degradation of the 26S proteasome. *Nat. Struct. Biol.* 22(9):712–19

Beckwith R, Estrin E, Worden EJ, Martin A. 2013. Reconstitution of the 26S proteasome reveals functional asymmetries in its AAA+ unfoldase. *Nat. Struct. Mol. Biol.* 20(10):1164–72

Ben Mosbah I, Alfany-Fernández I, Martel C, Zaouali MA, Bintanel-Morcillo M, et al. 2010. Endoplasmic reticulum stress inhibition protects steatotic and non-steatotic livers in partial hepatectomy under ischemia-reperfusion. *Cell Death Dis.* 1:e52

Bertolotti A. 2008. Protein misfolding in neurodegenerative diseases. *Dementia Forum* 5:17–20

Bertolotti A. 2011. *The Ubiquitin–Proteasome System in Neurodegenerative Diseases: More than the Usual Suspects*. Hoboken, NJ: John Wiley & Sons

Bertolotti A, Zhang Y, Hendershot LM, Harding HP, Ron D. 2000. Dynamic interaction of BiP and ER stress transducers in the unfolded-protein response. *Nat. Cell Biol.* 2:326–32

Bollen M, Peti W, Ragusa MJ, Beullens M. 2010. The extended PP1 toolkit: designed to create specificity. *Trends Biochem. Sci.* 35(8):450–58

Bose S, Cho J. 2016. Targeting chaperones, heat shock factor-1, and unfolded protein response: promising therapeutic approaches for neurodegenerative disorders. *Ageing Res. Rev.* 35:155–75

Bota DA, Davies KJA. 2002. Lon protease preferentially degrades oxidized mitochondrial aconitase by an ATP-stimulated mechanism. *Nat. Cell Biol.* 4(9):674–80

Boyce M, Bryant KF, Jousse C, Long K, Harding HP, et al. 2005. A selective inhibitor of eIF2alpha dephosphorylation protects cells from ER stress. *Science* 307(5711):935–39

Buchberger A, Bukau B, Sommer T. 2010. Protein quality control in the cytosol and the endoplasmic reticulum: brothers in arms. *Mol. Cell* 40(2):238–52

Carrara M, Prischi F, Nowak PR, Kopp MC, Ali MM. 2015. Noncanonical binding of BiP ATPase domain to Ire1 and Perk is dissociated by unfolded protein C_H1 to initiate ER stress signaling. *eLife* 4:e03522

Carrara M, Sigurdardottir A, Bertolotti A. 2017. Decoding the selectivity of eIF2α holophosphatases and PPP1R15A inhibitors. *Nat. Struct. Mol. Biol.* In press. **http://doi.org/10.1038/nsmb.3443**

Chai Y, Koppenhafer SL, Bonini NM, Paulson HL. 1999. Analysis of the role of heat shock protein (Hsp) molecular chaperones in polyglutamine disease. *J. Neurosci.* 19(23):10338–47

Chantranupong L, Scaria SM, Saxton RA, Gygi MP, Shen K, et al. 2016. The CASTOR proteins are arginine sensors for the mTORC1 pathway. *Cell* 165(1):153–64

Chen JJ, Genereux JC, Qu S, Hulleman JD, Shoulders MD, Wiseman RL. 2014. ATF6 activation reduces the secretion and extracellular aggregation of destabilized variants of an amyloidogenic protein. *Chem. Biol.* 21(11):1564–74

Chou T-F, Brown SJ, Minond D, Nordin BE, Li K, et al. 2011. Reversible inhibitor of p97, DBeQ, impairs both ubiquitin-dependent and autophagic protein clearance pathways. *PNAS* 108(12):4834–39

Connor JH, Weiser DC, Li S, Hallenbeck JM, Shenolikar S. 2001. Growth arrest and DNA damage–inducible protein GADD34 assembles a novel signaling complex containing protein phosphatase 1 and inhibitor 1. *Mol. Cell. Biol.* 21(20):6841–50

Cooley CB, Ryno LM, Plate L, Morgan GJ, Hulleman JD, et al. 2014. Unfolded protein response activation reduces secretion and extracellular aggregation of amyloidogenic immunoglobulin light chain. *PNAS* 111(36):13046–51

Cox JS, Shamu CE, Walter P. 1993. Transcriptional induction of genes encoding endoplasmic reticulum resident proteins requires a transmembrane protein kinase. *Cell* 73(6):1197–206

Crosas B, Hanna J, Kirkpatrick DS, Zhang DP, Tone Y, et al. 2006. Ubiquitin chains are remodeled at the proteasome by opposing ubiquitin ligase and deubiquitinating activities. *Cell* 127:1401–13

Das I, Krzyzosiak A, Schneider K, Wrabetz L, D'Antonio M, et al. 2015. Preventing proteostasis diseases by selective inhibition of a phosphatase regulatory subunit. *Science* 348(6231):239–42

Deveraux Q, Ustrell V, Pickart C, Rechsteiner M. 1994. A 26 S protease subunit that binds ubiquitin conjugates. *J. Biol. Chem.* 269(10):7059–61

Ebato C, Uchida T, Arakawa M, Komatsu M, Ueno T, et al. 2008. Autophagy is important in islet homeostasis and compensatory increase of beta cell mass in response to high-fat diet. *Cell Metab.* 8(4):325–32

Efeyan A, Comb WC, Sabatini DM. 2015. Nutrient-sensing mechanisms and pathways. *Nature* 517(7534):302–10

Eisele YS, Monteiro C, Fearns C, Encalada SE, Wiseman RL, et al. 2015. Targeting protein aggregation for the treatment of degenerative diseases. *Nat. Rev. Drug Discov.* 14(11):759–80

Finley D. 2009. Recognition and processing of ubiquitin-protein conjugates by the proteasome. *Annu. Rev. Biochem.* 78:477–513

Förster F, Lasker K, Beck F, Nickell S, Sali A, Baumeister W. 2009. An atomic model AAA-ATPase/20S core particle sub-complex of the 26S proteasome. *Biochem. Biophys. Res. Commun.* 388(2):228–33

Frentzel S, Pesold-Hurt B, Seelig A, Kloetzel PM. 1994. 20 S proteasomes are assembled via distinct precursor complexes. Processing of LMP2 and LMP7 proproteins takes place in 13–16 S preproteasome complexes. *J. Mol. Biol.* 236(4):975–81

Fujikake N, Nagai Y, Popiel HA, Okamoto Y, Yamaguchi M, Toda T. 2008. Heat shock transcription factor 1–activating compounds suppress polyglutamine-induced neurodegeneration through induction of multiple molecular chaperones. *J. Biol. Chem.* 283(38):26188–97

Gallagher CM, Garri C, Cain EL, Ang KK-H, Wilson CG, et al. 2016. Ceapins are a new class of unfolded protein response inhibitors, selectively targeting the ATF6α branch. *eLife* 5:e11878

Gallagher CM, Walter P. 2016. Ceapins inhibit ATF6α signaling by selectively preventing transport of ATF6α to the Golgi apparatus during ER stress. *eLife* 5:e11880

Gardner BM, Walter P. 2011. Unfolded proteins are Ire1-activating ligands that directly induce the unfolded protein response. *Science* 333(6051):1891–94

Gingras AC, Raught B, Sonenberg N. 2001. Regulation of translation initiation by FRAP/mTOR. *Genes Dev.* 15(7):807–26

Glover JR, Lindquist S. 1998. Hsp104, Hsp70, and Hsp40: a novel chaperone system that rescues previously aggregated proteins. *Cell* 94(1):73–82

Goswami A, Dikshit P, Mishra A, Mulherkar S, Nukina N, Jana NR. 2006. Oxidative stress promotes mutant huntingtin aggregation and mutant huntingtin-dependent cell death by mimicking proteasomal malfunction. *Biochem. Biophys. Res. Commun.* 342(1):184–90

Greenman C, Stephens P, Smith R, Dalgliesh GL, Hunter C, et al. 2007. Patterns of somatic mutation in human cancer genomes. *Nature* 446(7132):153–58

Gupta S, McGrath B, Cavener DR. 2010. PERK (EIF2AK3) regulates proinsulin trafficking and quality control in the secretory pathway. *Diabetes* 59(8):1937–47

Haghighat A, Mader S, Pause A, Sonenberg N. 1995. Repression of cap-dependent translation by 4E-binding protein 1: competition with p220 for binding to eukaryotic initiation factor-4E. *EMBO J.* 14:5701–9

Hanssum A, Zhong Z, Rousseau A, Krzyzosiak A, Sigurdardottir A, Bertolotti A. 2014. An inducible chaperone adapts proteasome assembly to stress. *Mol. Cell* 55(4):566–77

Hara K, Yonezawa K, Kozlowski MT, Sugimoto T, Andrabi K, et al. 1997. Regulation of eIF-4E BP1 phosphorylation by mTOR. *J. Biol. Chem.* 272:26457–63

Hara T, Nakamura K, Matsui M, Yamamoto A, Nakahara Y, et al. 2006. Suppression of basal autophagy in neural cells causes neurodegenerative disease in mice. *Nature* 441(7095):885–89

Harding HP, Novoa I, Bertolotti A, Zeng H, Zhang Y, et al. 2001a. Translational regulation in the cellular response to biosynthetic load on the endoplasmic reticulum. *Cold Spring Harb. Symp. Quant. Biol.* 66(5521):499–508

Harding HP, Novoa I, Zhang Y, Zeng H, Wek R, et al. 2000. Regulated translation initiation controls stress-induced gene expression in mammalian cells. *Mol. Cell* 6(5):1099–108

Harding HP, Zeng H, Zhang Y, Jungries R, Chung P, et al. 2001b. Diabetes mellitus and exocrine pancreatic dysfunction in *Perk*$^{-/-}$ mice reveals a role for translational control in secretory cell survival. *Mol. Cell* 7(6):1153–63

Harding HP, Zhang Y, Scheuner D, Chen J-J, Kaufman RJ, Ron D. 2009. Ppp1r15 gene knockout reveals an essential role for translation initiation factor 2 alpha (eIF2alpha) dephosphorylation in mammalian development. *PNAS* 106(6):1832–37

He J, Kulkarni K, da Fonseca PCA, Krutauz D, Glickman MH, et al. 2012. The structure of the 26S proteasome subunit Rpn2 reveals its PC repeat domain as a closed toroid of two concentric α-helical rings. *Structure* 20(3):513–21

Hershko A, Ciechanover A. 1998. The ubiquitin system. *Annu. Rev. Biochem.* 67:425–79

Hetz C, Chevet E, Harding HP. 2013. Targeting the unfolded protein response in disease. *Nat. Rev. Drug Discov.* 12(9):703–19

Hirano Y, Hayashi H, Iemura S-I, Hendil KB, Niwa S-I, et al. 2006. Cooperation of multiple chaperones required for the assembly of mammalian 20S proteasomes. *Mol. Cell* 24(6):977–84

Hirano Y, Hendil KB, Yashiroda H, Iemura S-I, Nagane R, et al. 2005. A heterodimeric complex that promotes the assembly of mammalian 20S proteasomes. *Nature* 437(7063):1381–85

Holz MK, Ballif BA, Gygi SP, Blenis J. 2005. mTOR and S6K1 mediate assembly of the translation preinitiation complex through dynamic protein interchange and ordered phosphorylation events. *Cell* 123(4):569–80

Horwich AL, Fenton WA, Chapman E, Farr GW. 2007. Two families of chaperonin: physiology and mechanism. *Annu. Rev. Cell Dev. Biol.* 23:115–45

Hosokawa N, Hara T, Kaizuka T, Kishi C, Takamura A, et al. 2009a. Nutrient-dependent mTORC1 association with the ULK1-Atg13-FIP200 complex required for autophagy. *Mol. Biol. Cell* 20(7):1981–91

Hosokawa N, Sasaki T, Iemura S-I, Natsume T, Hara T, Mizushima N. 2009b. Atg101, a novel mammalian autophagy protein interacting with Atg13. *Autophagy* 5(7):973–79

Husnjak K, Elsasser S, Zhang N, Chen X, Randles L, et al. 2008. Proteasome subunit Rpn13 is a novel ubiquitin receptor. *Nature* 453(7194):481–88

Ishii T, Itoh K, Takahashi S, Sato H, Yanagawa T, Katoh Y, et al. 2000. Transcription factor Nrf2 coordinately regulates a group of oxidative stress–inducible genes in macrophages. *J. Biol. Chem.* 275(21):16023–29

Jones DT, Addison E, North JM, Lowdell MW, Hoffbrand AV, et al. 2004. Geldanamycin and herbimycin A induce apoptotic killing of B chronic lymphocytic leukemia cells and augment the cells' sensitivity to cytotoxic drugs. *Blood* 103(5):1855–61

Jung HS, Chung KW, Won Kim J, Kim J, Komatsu M, et al. 2008. Loss of autophagy diminishes pancreatic beta cell mass and function with resultant hyperglycemia. *Cell Metab.* 8(4):318–24

Kamada Y, Funakoshi T, Shintani T, Nagano K, Ohsumi M, Ohsumi Y. 2000. Tor-mediated induction of autophagy via an Apg1 protein kinase complex. *J. Cell Biol.* 150(6):1507–13

Kars M, Yang L, Gregor MF, Mohammed BS, Pietka TA, et al. 2010. Tauroursodeoxycholic acid may improve liver and muscle but not adipose tissue insulin sensitivity in obese men and women. *Diabetes* 59(8):1899–905

Kieran D, Kalmar B, Dick JRT, Riddoch-Contreras J, Burnstock G, Greensmith L. 2004. Treatment with arimoclomol, a coinducer of heat shock proteins, delays disease progression in ALS mice. *Nat. Med.* 10(4):402–5

Kimata Y. 2004. A role for BiP as an adjustor for the endoplasmic reticulum stress-sensing protein Ire1. *J. Cell Biol.* 167(3):445–56

Kimata Y, Ishiwata-Kimata Y, Ito T, Hirata A, Suzuki T, et al. 2007. Two regulatory steps of ER-stress sensor Ire1 involving its cluster formation and interaction with unfolded proteins. *J. Cell Biol.* 179(1):75–86

King MA, Hands S, Hafiz F, Mizushima N, Tolkovsky AM, Wyttenbach A. 2008. Rapamycin inhibits polyglutamine aggregation independently of autophagy by reducing protein synthesis. *Mol. Pharmacol.* 73(4):1052–63

Kobayashi Y, Kume A, Li M, Doyu M, Hata M, et al. 2000. Chaperones Hsp70 and Hsp40 suppress aggregate formation and apoptosis in cultured neuronal cells expressing truncated androgen receptor protein with expanded polyglutamine tract. *J. Biol. Chem.* 275(12):8772–78

Koizumi S, Irie T, Hirayama S, Sakurai Y, Yashiroda H. 2016. The aspartyl protease DDI2 activates Nrf1 to compensate for proteasome dysfunction. *eLife* 5:e18357

Korolchuk VI, Menzies FM, Rubinsztein DC. 2010. Mechanisms of cross-talk between the ubiquitin-proteasome and autophagy-lysosome systems. *FEBS Lett.* 584(7):1393–98

Kuma A, Hatano M, Matsui M, Yamamoto A, Nakaya H, et al. 2004. The role of autophagy during the early neonatal starvation period. *Nature* 432(7020):1032–36

Kusmierczyk AR, Kunjappu MJ, Kim RY, Hochstrasser M. 2011. A conserved 20S proteasome assembly factor requires a C-terminal HbYX motif for proteasomal precursor binding. *Nat. Struct. Mol. Biol.* 18(5):622–29

Kwak MK, Wakabayashi N, Greenlaw JL, Yamamoto M, Kensler TW. 2003. Antioxidants enhance mammalian proteasome expression through the Keap1-Nrf2 signaling pathway. *Mol. Cell. Biol.* 23:8786–94

Le Tallec B, Barrault M-B, Courbeyrette R, Guérois R, Marsolier-Kergoat M-C, Peyroche A. 2007. 20S proteasome assembly is orchestrated by two distinct pairs of chaperones in yeast and in mammals. *Mol. Cell* 27(4):660–74

Lee B-H, Lee MJ, Park S, Oh D-C, Elsasser S, et al. 2010. Enhancement of proteasome activity by a small-molecule inhibitor of USP14. *Nature* 467(7312):179–84

Lee JW, Beebe K, Nangle LA, Jang J, Longo-Guess CM, et al. 2006. Editing-defective tRNA synthetase causes protein misfolding and neurodegeneration. *Nature* 443(7107):50–55

Lehrbach NJ, Ruvkun G. 2016. Proteasome dysfunction triggers activation of SKN-1A/Nrf1 by the aspartic protease DDI-1. *eLife* 5:e17721

Li X, Kusmierczyk AR, Wong P, Emili A, Hochstrasser M. 2007. β-Subunit appendages promote 20S proteasome assembly by overcoming an Ump1-dependent checkpoint. *EMBO J.* 26(9):2339–49

Liu CY, Schröder M, Kaufman RJ. 2000. Ligand-independent dimerization activates the stress-response kinases IRE1 and PERK in the lumen of the endoplasmic reticulum. *J. Biol. Chem.* 275:44881–85

Loewith R, Hall MN. 2011. Target of rapamycin (TOR) in nutrient signaling and growth control. *Genetics* 189(4):1177–201

Lupas A, Flanagan JM, Tamura T, Baumeister W. 1997. Self-compartmentalizing proteases. *Trends Biochem. Sci.* 22(10):399–404

Ma XM, Yoon S-O, Richardson CJ, Jülich K, Blenis J. 2008. SKAR links pre-mRNA splicing to mTOR/S6K1-mediated enhanced translation efficiency of spliced mRNAs. *Cell* 133(2):303–13

Maly DJ, Papa FR. 2014. Druggable sensors of the unfolded protein response. *Nat. Chem. Biol.* 10(11):892–901

Marques AJ, Glanemann C, Ramos PC, Dohmen RJ. 2007. The C-terminal extension of the beta7 subunit and activator complexes stabilize nascent 20 S proteasomes and promote their maturation. *J. Biol. Chem.* 282(48):34869–76

Martin S, Lamb HK, Brady C, Lefkove B, Bonner MY, et al. 2013. Inducing apoptosis of cancer cells using small-molecule plant compounds that bind to GRP78. *Br. J. Cancer* 109(2):433–43

Matsuura A, Tsukada M, Wada Y, Ohsumi Y. 1997. Apg1p, a novel protein kinase required for the autophagic process in *Saccharomyces cerevisiae*. *Gene* 192(2):245–50

Matyskiela ME, Lander GC, Martin A. 2013. Conformational switching of the 26S proteasome enables substrate degradation. *Nat. Struct. Mol. Biol.* 20(7):781–88

Mercer CA, Kaliappan A, Dennis PB. 2009. A novel, human Atg13 binding protein, Atg101, interacts with ULK1 and is essential for macroautophagy. *Autophagy* 5(5):649–62

Metzger MB, Hristova VA, Weissman AM. 2012. HECT and RING finger families of E3 ubiquitin ligases at a glance. *J. Cell. Sci.* 125(Pt 3):531–37

Mimura N, Fulciniti M, Gorgun G, Tai Y-T, Cirstea D, et al. 2012. Blockade of XBP1 splicing by inhibition of IRE1α is a promising therapeutic option in multiple myeloma. *Blood* 119(24):5772–81

Mizukami T, Orihashi K, Herlambang B, Takahashi S, Hamaishi M, et al. 2010. Sodium 4-phenylbutyrate protects against spinal cord ischemia by inhibition of endoplasmic reticulum stress. *J. Vasc. Surg.* 52(6):1580–86

Mizushima N. 2010. The role of the Atg1/ULK1 complex in autophagy regulation. *Curr. Opin. Cell Biol.* 22(2):132–39

Mizushima N, Noda T, Yoshimori T, Tanaka Y, Ishii T, et al. 1998. A protein conjugation system essential for autophagy. *Nature* 395(6700):395–98

Mizushima N, Yamamoto A, Matsui M, Yoshimori T, Ohsumi Y. 2004. In vivo analysis of autophagy in response to nutrient starvation using transgenic mice expressing a fluorescent autophagosome marker. *Mol. Biol. Cell* 15(3):1101–11

Moreno JA, Halliday M, Molloy C, Radford H, Verity N, et al. 2013. Oral treatment targeting the unfolded protein response prevents neurodegeneration and clinical disease in prion-infected mice. *Sci. Transl. Med.* 5(206):206ra138

Mori K. 2000. Tripartite management of unfolded proteins in the endoplasmic reticulum. *Cell* 101(5):451–54

Mori K, Ma W, Gething MJ, Sambrook J. 1993. A transmembrane protein with a cdc2+/CDC28− related kinase activity is required for signaling from the ER to the nucleus. *Cell* 74(4):743–56

Morimoto RI. 1998. Regulation of the heat shock transcriptional response: cross talk between a family of heat shock factors, molecular chaperones, and negative regulators. *Genes Dev.* 12(24):3788–96

Morimoto RI, Cuervo AM. 2014. Proteostasis and the aging proteome in health and disease. *J. Gerontol. A Biol. Sci. Med. Sci.* 69(Suppl. 1):33–38

Münch C, Harper JW. 2016. Mitochondrial unfolded protein response controls matrix pre-RNA processing and translation. *Nature* 534:710–13

Murata S, Yashiroda H, Tanaka K. 2009. Molecular mechanisms of proteasome assembly. *Nat. Rev. Mol. Cell Biol.* 10(2):104–15

Myeku N, Clelland CL, Emrani S, Kukushkin NV, Yu WH, et al. 2015. Tau-driven 26S proteasome impairment and cognitive dysfunction can be prevented early in disease by activating cAMP-PKA signaling. *Nat. Med.* 22:46–53

Nakatogawa H, Suzuki K, Kamada Y, Ohsumi Y. 2009. Dynamics and diversity in autophagy mechanisms: lessons from yeast. *Nat. Rev. Mol. Cell Biol.* 10(7):458–67

Nandi D, Woodward E, Ginsburg DB, Monaco JJ. 1997. Intermediates in the formation of mouse 20S proteasomes: implications for the assembly of precursor beta subunits. *EMBO J.* 16(17):5363–75
Nillegoda NB, Bukau B. 2015. Metazoan Hsp70-based protein disaggregases: emergence and mechanisms. *Front. Mol. Biosci.* 2:57
Novoa I, Zeng H, Harding HP, Ron D. 2001. Feedback inhibition of the unfolded protein response by GADD34-mediated dephosphorylation of eIF2alpha. *J. Cell Biol.* 153(5):1011–22
Novoa I, Zhang Y, Zeng H, Jungreis R, Harding HP, Ron D. 2003. Stress-induced gene expression requires programmed recovery from translational repression. *EMBO J.* 22(5):1180–87
Oikawa D, Kimata Y, Kohno K, Iwawaki T. 2009. Activation of mammalian IRE1α upon ER stress depends on dissociation of BiP rather than on direct interaction with unfolded proteins. *Exp. Cell Res.* 315(15):2496–504
Okamura K, Kimata Y, Higashio H, Tsuru A, Kohno K. 2000. Dissociation of Kar2p/BiP from an ER sensory molecule, Ire1p, triggers the unfolded protein response in yeast. *Biochem. Biophys. Res. Commun.* 279(2):445–50
Onodera J, Ohsumi Y. 2005. Autophagy is required for maintenance of amino acid levels and protein synthesis under nitrogen starvation. *J. Biol. Chem.* 280(36):31582–86
Ortuno D, Carlisle HJ, Miller S. 2016. Does inactivation of USP14 enhance degradation of proteasomal substrates that are associated with neurodegenerative diseases? *F1000Res.* 5:137
Oyadomari S, Mori M. 2004. Roles of CHOP/GADD153 in endoplasmic reticulum stress. *Cell Death Differ.* 11(4):381–89
Ozcan U, Yilmaz E, Ozcan L, Furuhashi M, Vaillancourt E, et al. 2006. Chemical chaperones reduce ER stress and restore glucose homeostasis in a mouse model of type 2 diabetes. *Science* 313(5790):1137–40
Papandreou I, Denko NC, Olson M, Van Melckebeke H, Lust S, et al. 2011. Identification of an Ire1alpha endonuclease specific inhibitor with cytotoxic activity against human multiple myeloma. *Blood* 117(4):1311–14
Parsell DA, Lindquist S. 1993. The function of heat-shock proteins in stress tolerance: degradation and reactivation of damaged proteins. *Annu. Rev. Genet.* 27:437–96
Parzych K, Chinn TM, Chen Z, Loaiza S, Porsch F, et al. 2015. Inadequate fine-tuning of protein synthesis and failure of amino acid homeostasis following inhibition of the ATPase VCP/p97. *Cell Death Dis.* 6(12):e2031
Pellegrino MW, Nargund AM, Haynes CM. 2013. Signaling the mitochondrial unfolded protein response. *BBA Mol. Cell Res.* 1833(2):410–16
Pincus D, Chevalier MW, Aragón T, van Anken E, Vidal SE, et al. 2010. BiP binding to the ER-stress sensor Ire1 tunes the homeostatic behavior of the unfolded protein response. *PLOS Biol.* 8(7):e1000415
Plate L, Cooley CB, Chen JJ, Paxman RJ, Gallagher CM, et al. 2016. Small molecule proteostasis regulators that reprogram the ER to reduce extracellular protein aggregation. *eLife* 5:e15550
Prakash S, Tian L, Ratliff KS, Lehotzky RE, Matouschek A. 2004. An unstructured initiation site is required for efficient proteasome-mediated degradation. *Nat. Struct. Mol. Biol.* 11(9):830–37
Qi X, Hosoi T, Okuma Y, Kaneko M, Nomura Y. 2004. Sodium 4-phenylbutyrate protects against cerebral ischemic injury. *Mol. Pharmacol.* 66(4):899–908
Radhakrishnan SK, Lee CS, Young P, Beskow A, Chan JY, Deshaies RJ. 2010. Transcription factor Nrf1 mediates the proteasome recovery pathway after proteasome inhibition in mammalian cells. *Mol. Cell* 38(1):17–28
Ramos PC, Höckendorff J, Johnson ES, Varshavsky A, Dohmen RJ. 1998. Ump1p is required for proper maturation of the 20S proteasome and becomes its substrate upon completion of the assembly. *Cell* 92(4):489–99
Rojas-Rivera D, Delvaeye T, Roelandt R, Nerinckx W, Augustyns K, et al. 2017. When PERK inhibitors turn out to be new potent RIPK1 inhibitors: critical issues on the specificity and use of GSK2606414 and GSK2656157. *Cell Death Differ.* 24(6):1100–10
Ron D, Harding HP. 2007. eIF2α phosphorylation in cellular stress responses and disease. *Transl. Control Biol. Med.* 48:345–68
Rousseau A, Bertolotti A. 2016. An evolutionarily conserved pathway controls proteasome homeostasis. *Nature* 536(7615):184–89

Rubinsztein DC, Codogno P, Levine B. 2012. Autophagy modulation as a potential therapeutic target for diverse diseases. *Nat. Rev. Drug Discov.* 11(9):709–30

Sawkar AR, Cheng W-C, Beutler E, Wong C-H, Balch WE, Kelly JW. 2002. Chemical chaperones increase the cellular activity of N370S beta-glucosidase: a therapeutic strategy for Gaucher disease. *PNAS* 99(24):15428–33

Scheuner D, Song B, McEwen E, Liu C, Laybutt R, et al. 2001. Translational control is required for the unfolded protein response and in vivo glucose homeostasis. *Mol. Cell* 7(6):1165–76

Schmidt M, Finley D. 2014. Regulation of proteasome activity in health and disease. *Biochim. Biophys. Acta* 1843(1):13–25

Schmidtke G, Schmidt M, Kloetzel PM. 1997. Maturation of mammalian 20 S proteasome: purification and characterization of 13 S and 16 S proteasome precursor complexes. *J. Mol. Biol.* 268(1):95–106

Schreiner P, Chen X, Husnjak K, Randles L, Zhang N, et al. 2008. Ubiquitin docking at the proteasome through a novel pleckstrin-homology domain interaction. *Nature* 453(7194):548–52

Schwartz AL, Ciechanover A. 2009. Targeting proteins for destruction by the ubiquitin system: implications for human pathobiology. *Annu. Rev. Pharmacol. Toxicol.* 49:73–96

Shen J, Chen X, Hendershot L, Prywes R. 2002. ER stress regulation of ATF6 localization by dissociation of BiP/GRP78 binding and unmasking of Golgi localization signals. *Dev. Cell* 3:99–111

Sidrauski C, Acosta-Alvear D, Khoutorsky A, Vedantham P, Hearn BR, et al. 2013. Pharmacological brake-release of mRNA translation enhances cognitive memory. *eLife* 2:e00498

Sonenberg N, Hinnebusch AG. 2009. Regulation of translation initiation in eukaryotes: mechanisms and biological targets. *Cell* 136(4):731–45

Song B, Scheuner D, Ron D, Pennathur S, Kaufman RJ. 2008. Chop deletion reduces oxidative stress, improves beta cell function, and promotes cell survival in multiple mouse models of diabetes. *J. Clin. Investig.* 118(10):3378–89

Sontag EM, Vonk WIM, Frydman J. 2014. Sorting out the trash: the spatial nature of eukaryotic protein quality control. *Curr. Opin. Cell Biol.* 26:139–46

Soto C. 2003. Unfolding the role of protein misfolding in neurodegenerative diseases. *Nat. Rev. Neurosci.* 4(1):49–60

Stock D, Nederlof PM, Seemüller E, Baumeister W, Huber R, Löwe J. 1996. Proteasome: from structure to function. *Curr. Opin. Biotechnol.* 7(4):376–85

Suh DH, Kim M-K, Kim HS, Chung HH, Song YS. 2012. Unfolded protein response to autophagy as a promising druggable target for anticancer therapy. *Ann. N. Y. Acad. Sci.* 1271:20–32

Suraweera A, Münch C, Hanssum A, Bertolotti A. 2012. Failure of amino acid homeostasis causes cell death following proteasome inhibition. *Mol. Cell* 48(2):242–53

Thoreen CC. 2013. Many roads from mTOR to eIF4F. *Biochem. Soc. Trans.* 41:913–16

Tomko RJ Jr., Hochstrasser M. 2013. Molecular architecture and assembly of the eukaryotic proteasome. *Annu. Rev. Biochem.* 82:415–45

Tomko RJ Jr., Hochstrasser M. 2014. The intrinsically disordered Sem1 protein functions as a molecular tether during proteasome lid biogenesis. *Mol. Cell* 53(3):433–43

Tomko RJ, Funakoshi M, Schneider K, Wang J, Hochstrasser M. 2010. Heterohexameric ring arrangement of the eukaryotic proteasomal ATPases: implications for proteasome structure and assembly. *Mol. Cell* 38(3):393–403

Tsaytler P, Bertolotti A. 2013. Exploiting the selectivity of protein phosphatase 1 for pharmacological intervention. *FEBS J.* 280(2):766–70

Tsaytler P, Harding HP, Ron D, Bertolotti A. 2011. Selective inhibition of a regulatory subunit of protein phosphatase 1 restores proteostasis. *Science* 332(6025):91–94

Vabulas RM, Hartl FU. 2005. Protein synthesis upon acute nutrient restriction relies on proteasome function. *Science* 310(5756):1960–63

Vembar SS, Brodsky JL. 2008. One step at a time: endoplasmic reticulum–associated degradation. *Nat. Rev. Mol. Cell Biol.* 9(12):944–57

Verghese J, Abrams J, Wang Y, Morano KA. 2012. Biology of the heat shock response and protein chaperones: budding yeast (*Saccharomyces cerevisiae*) as a model system. *Microbiol. Mol. Biol. Rev.* 76(2):115–58

Vihervaara A, Sistonen L. 2014. HSF1 at a glance. *J. Cell. Sci.* 127(Pt 2):261–66
Vilchez D, Saez I, Dillin A. 2014. The role of protein clearance mechanisms in organismal ageing and age-related diseases. *Nat. Commun.* 5:5659
Wang X, Li W, Williams M, Terada N, Alessi DR, Proud CG. 2001. Regulation of elongation factor 2 kinase by $p90^{RSK1}$ and p70 S6 kinase. *EMBO J.* 20(16):4370–79
Waza M, Adachi H, Katsuno M, Minamiyama M, Sang C, et al. 2005. 17-AAG, an Hsp90 inhibitor, ameliorates polyglutamine-mediated motor neuron degeneration. *Nat. Med.* 11(10):1088–95
Wek SA, Zhu S, Wek RC. 1995. The histidyl-tRNA synthetase–related sequence in the eIF-2 alpha protein kinase GCN2 interacts with tRNA and is required for activation in response to starvation for different amino acids. *Mol. Cell. Biol.* 15(8):4497–506
Wilson KF, Wu WJ, Cerione RA. 2000. Cdc42 stimulates RNA splicing via the S6 kinase and a novel S6 kinase target, the nuclear cap-binding complex. *J. Biol. Chem.* 275(48):37307–10
Wolfson RL, Chantranupong L, Saxton RA, Shen K, Scaria SM, et al. 2016. Sestrin2 is a leucine sensor for the mTORC1 pathway. *Science* 351(6268):43–48
Wyttenbach A, Hands S, King MA, Lipkow K, Tolkovsky AM. 2008. Amelioration of protein misfolding disease by rapamycin: translation or autophagy? *Autophagy* 4(4):542–45
Xiao C, Giacca A, Lewis GF. 2011. Sodium phenylbutyrate, a drug with known capacity to reduce endoplasmic reticulum stress, partially alleviates lipid-induced insulin resistance and beta-cell dysfunction in humans. *Diabetes* 60(3):918–24
Xie Y, Varshavsky A. 2001. RPN4 is a ligand, substrate, and transcriptional regulator of the 26S proteasome: a negative feedback circuit. *PNAS* 98(6):3056–61
Yang Y, Früh K, Ahn K, Peterson PA. 1995. In vivo assembly of the proteasomal complexes, implications for antigen processing. *J. Biol. Chem.* 270(46):27687–94
Yashiroda H, Mizushima T, Okamoto K, Kameyama T, Hayashi H, et al. 2008. Crystal structure of a chaperone complex that contributes to the assembly of yeast 20S proteasomes. *Nat. Struct. Mol. Biol.* 15(3):228–36
Ye J, Rawson RB, Komuro R, Chen X, Davé UP, et al. 2000. ER stress induces cleavage of membrane-bound ATF6 by the same proteases that process SREBPs. *Mol. Cell* 6(6):1355–64
Zhang Y, Nicholatos J, Dreier JR, Ricoult SJH, Widenmaier SB, et al. 2014. Coordinated regulation of protein synthesis and degradation by mTORC1. *Nature* 513(7518):440–43
Zoncu R, Efeyan A, Sabatini DM. 2011. mTOR: from growth signal integration to cancer, diabetes and ageing. *Nat. Rev. Mol. Cell Biol.* 12(1):21–35