Molecular Cell
Review

The UPR^{ER}: Sensor and Coordinator of Organismal Homeostasis

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http://dx.doi.org/10.1016/j.molcel.2017.05.031

Life is stressful. Organisms are repeatedly exposed to stressors that disrupt protein homeostasis (proteostasis), resulting in protein misfolding and aggregation. To sense and respond to proteotoxic perturbations, cells have evolved compartment-specific stress responses, such as the unfolded protein response of the endoplasmic reticulum (UPR^{ER}). However, UPR^{ER} function is impaired with age, which, we propose, creates a permissive environment for protein aggregation, unresolved ER stress, and chronic inflammation. Understanding age-related changes to the UPR^{ER} will provide new avenues for therapeutic intervention in metabolic disease, neurodegeneration, and aging.

Introduction

Aging is broadly defined as the post-reproductive, physiological decline of an organism that occurs over time. The mechanisms leading to the gradual accumulation of cellular and tissue damage during aging are complex and are influenced by many environmental and genetic factors. Age is considered the primary risk factor for many diseases, including diabetes, neurodegeneration, cancer, and cardiovascular diseases (Kennedy et al., 2014). Low-grade chronic inflammation in the absence of overt infection is one pervasive feature of human aging that increases susceptibility to disease. Thus, the mechanisms underlying chronic inflammation and the aging process are under intense investigation, as these likely will hold therapeutic promise for delaying onset or progression of many age-onset diseases (López-Otín et al., 2013).

The loss of proteostasis and subsequent accumulation of unfolded and misfolded proteins is a central molecular hallmark of aging and many degenerative diseases (López-Otín et al., 2013; Taylor and Dillin, 2011). Proteostasis is essential for cell health and viability and is ensured by the coordinated regulation of protein translation, folding, trafficking, and degradation. The endoplasmic reticulum (ER) plays a major role in maintaining protein homeostasis and is responsible for folding and processing nearly all polypeptides destined for secretion. Throughout their lifetime, cells are repeatedly faced with a variety of stressors and physiological demands that can potentially disrupt ER proteostasis. This is especially true for immune and metabolic cells that need to dynamically adjust production of secreted proteins in response to environmental insult and nutrient availability. In times of ER stress, when the load of newly synthesized, unfolded, or misfolded proteins overwhelms the capacity of the ER machinery, the unfolded protein response of the endoplasmic reticulum (UPR^{ER}) is initiated to restore proteostasis (Wang and Kaufman, 2016).

We will first discuss how UPR^{ER} coordinates immunity and metabolism at all stages of life and then examine how these physiological roles are disrupted with age. We propose that the age-related collapse of UPR^{ER} causes protein aggregation, persistent ER stress, and chronic inflammation, which directly contribute to the accumulation of tissue damage and an increase in disease susceptibility (Figure 1). As a case study, we discuss evidence that ER stress dictates motor neuron vulnerability and may be an underlying cause of neuroinflammation in the adult-onset neurodegenerative disease amyotrophic lateral sclerosis (ALS).

The ER and UPR^{ER}

The ER coordinates many diverse cellular processes, such as maintaining intracellular calcium levels, synthesizing lipids, and folding and processing most polypeptides destined for secretion. The vast network of ER membrane physically and functionally interacts with every other membranous structure in the cell (Rutkowski and Hegde, 2010). Thus, the ER is well positioned to sense cellular perturbations, integrate this information, and adjust signaling pathways to restore homeostasis. In times of ER stress, the demand for ER function outweighs its capacity, and the UPR^{ER} is initiated to restore balance.

In multicellular eukaryotes, the UPR^{ER} consists of three distinct arms defined by ER transmembrane sensors: inositol-requiring protein 1 (IRE1), activating transcription factor 6 (ATF6), and protein kinase RNA (PKR)-like ER kinase (PERK). These sensors have luminal domains that sense the protein-folding environment and cytosolic regions that interact with transcriptional and translational machinery. Under basal conditions, the luminal domains of IRE1, ATF6, and PERK are bound by a chaperone binding immunoglobulin protein (BiP) and rendered inactive. In times of ER stress, BiP is recruited to unfolded or misfolded proteins and titrated away from the UPR sensors, resulting in UPR^{ER} activation (Bertolotti et al., 2000; Shen et al., 2005). Unfolded proteins can also bind directly to IRE1 and PERK, resulting in dimerization, oligomerization, and ultimately activation

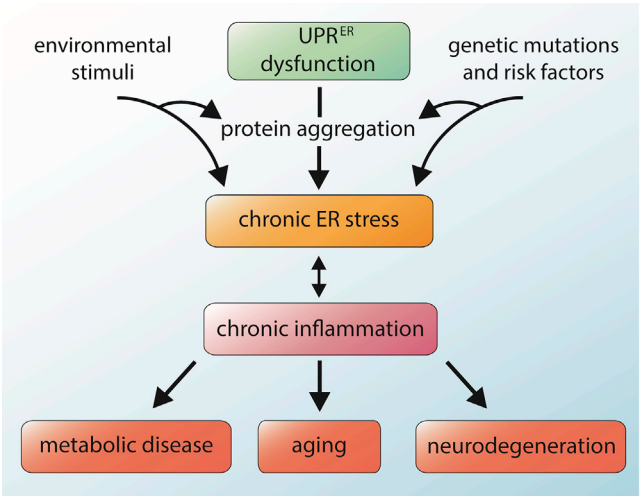

Figure 1. Impact of UPR^{ER} Dysfunction with Age
As an organism ages, the ability to induce a protective UPR^{ER} response declines. We propose that this, in combination with other factors (such as environmental stimuli and genetic risk factors), leads to protein aggregation, unresolved ER stress, and chronic inflammation. Ultimately, chronic inflammation increases susceptibility to disease and accelerates aging.

of UPR^{ER} (Gardner and Walter, 2011; Korennykh et al., 2009; Li et al., 2010; Walter and Ron, 2011). Once activated, these responses reduce the protein load entering the ER, lower protein synthesis, and induce a transcriptional program to increase the capacity of the ER and resolve the stress (Figure 2) (Ron and Walter, 2007).

The most evolutionarily conserved branch of the UPR^{ER} is mediated through the transmembrane kinase and ribonuclease IRE1. Upon sensing unfolded proteins, IRE1 is activated, leading to the regulated splicing of an unconventional 26-nt intron from X-box binding protein 1 (*Xbp1*) by the IRE1. This splicing event results in a frameshift, generating the spliced *Xbp1* (XBP1s) isoform. Once translated, XBP1s functions as a transcription factor regulating a range of targets, including chaperones necessary for ER-assisted polypeptide folding and genes required for lipid synthesis and membrane expansion (Calfon et al., 2002). In mammals, the remaining, unspliced *Xbp1* (*Xbp1u*) is also translated and can function as a negative regulator of XBP1s by binding XBP1s, sequestering it from the nucleus, and enhancing its degradation via the proteasome (Yoshida et al., 2006).

Analyzing promoter elements of ER stress-induced genes led to the identification of the ER transmembrane sensor ATF6. Under basal conditions, the luminal domain of ATF6 is bound to BiP. Upon ER stress, BiP binding to ATF6 is disrupted, and ATF6 is transported to the Golgi apparatus, where it is cleaved and transported into the nucleus. It then induces expression of *Xbp1* and genes required for ER-associated protein degradation (ERAD) (Haze et al., 1999; Lee et al., 2002; Yamamoto et al., 2007).

PERK is a protein kinase that contains a luminal domain similar to IRE1. PERK responds to ER stress by autophosphorylation and homomultimerization. The cytosolic kinase domain of PERK subsequently phosphorylates the α subunit of eukaryotic translation initiation factor 2 (eIF2α). This phosphorylation inhibits guanine nucleotide exchange factor (eIF2B), thus lowering levels of translation initiation (Harding et al., 1999). Despite decreased global translation, certain transcripts are preferentially translated, including activating transcription factor 4 (ATF4), which regulates various genes, including pro-apoptotic C/EBP homologous protein (*Chop*) (Harding et al., 2003; Marciniak et al., 2004). If homeostasis cannot be restored, the PERK branch initiates apoptosis in an effort to protect the organism from dysfunctional cells with unresolved ER stress and misfolded proteins (Wang and Kaufman, 2016).

UPR^{ER} and Immunity

Certain cell types require a higher ER stress-induced apoptosis threshold in order to perform their normal physiological functions. For example, immune cells need to rapidly increase protein production in response to pathogens while avoiding ER stress-induced apoptosis. In macrophages, sensing pathogens via Toll-like receptors (TLRs) induces the IRE1-XBP1 branch of UPR^{ER}. XBP1 in macrophages is required for sustained production of pro-inflammatory cytokines and efficient clearance of pathogenic bacteria (Martinon et al., 2010). TLR signaling also suppresses *Chop* expression to prevent apoptosis in macrophages subjected to chronic ER stress. This mechanism to raise the apoptosis threshold by suppressing *Chop* is thought to have evolved to promote macrophage survival in times of prolonged ER stress during persistent pathogen infection (Woo et al., 2009).

The UPR^{ER} may also be induced in an anticipatory fashion to increase a cell's capacity to cope with ER stress prior to overburdening the ER with a surge in secretory proteins. B lymphocytes induce the UPR^{ER} during differentiation into plasma cells to preemptively expand the ER to prepare for antibody production and secretion (van Anken et al., 2003). Mouse chimeras with XBP1-deficient lymphocytes fail to develop plasma cells, suggesting that XBP1 is essential for terminal B cell differentiation into plasma cells (Iwakoshi et al., 2003; Reimold et al., 2001). Similarly, plasmacytoid dendritic cells (pDCs) proactively constitutively splice *Xbp1* to allow for immediate and optimal pDC function and survival upon pathogen exposure (Iwakoshi et al., 2007; Osorio et al., 2014). Plasmacytoid DCs with defective XBP1 signaling secrete less IFN-α and are more susceptible to ER stress-induced apoptosis (Iwakoshi et al., 2007; Osorio et al., 2014). Thus, XBP1 signaling can operate preemptively to protect cells from an anticipated ER stress.

In addition to optimizing secretory function in immune cells, XBP1 can protect host cells from a potentially cytotoxic immune response. During development in *Caenorhabditis elegans*, XBP-1 has a protective role in response to robust immune activation induced upon exposure to the pathogenic bacteria *Pseudomonas aeruginosa*. Loss-of-function *xbp-1* mutant worms exhibit disturbed ER morphology and larval lethality when exposed to *P. aeruginosa*. Surprisingly, larval lethality of *xbp-1* mutant animals is rescued by dampening p38 mitogen-activating protein kinase (PMK-1)-mediated innate immune function. Because XBP-1 does not play a bactericidal role in controlling *P. aeruginosa* infection in *C. elegans*, it presumably protects the host's intestine from detrimental ER stress induced by an overwhelming immune response (Richardson et al., 2010).

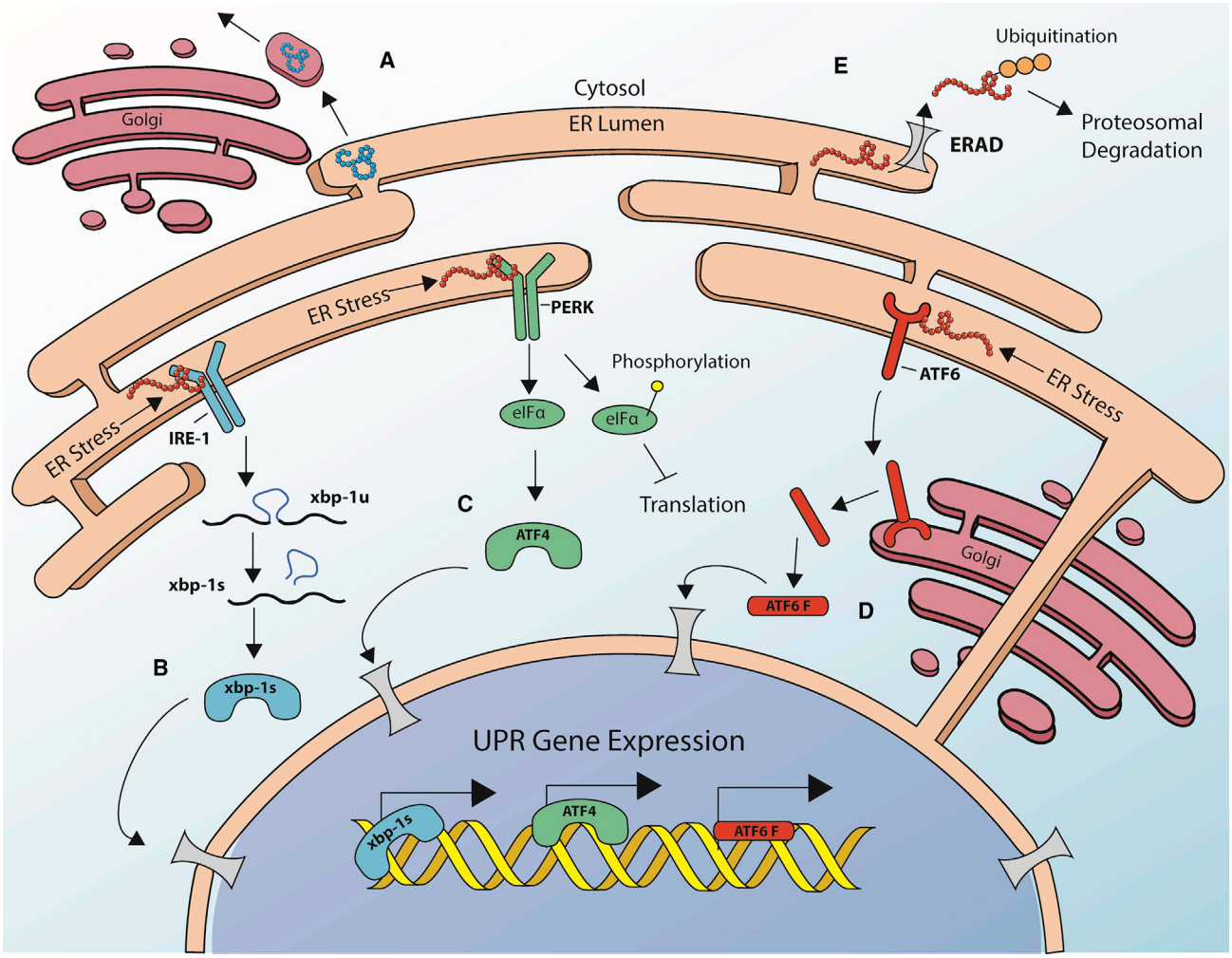

Figure 2. The Unfolded Protein Response of the Endoplasmic Reticulum

(A) The ER is responsible for folding and processing nearly all polypeptides destined for secretion. The UPR^{ER} is initiated when the capacity of the basal machinery of the ER is overwhelmed by unfolded or misfolded proteins.

(B) Upon activation, IRE1 splices *xbp-1u* to generate *xbp-1s*, which is translated and functions as a transcription factor regulating expression genes such as chaperones.

(C) Activated PERK phosphorylates eIF2α, which leads to translation inhibition. However, phospho-eIF2α preferentially increases translation of ATF4, which activates transcription of UPR^{ER} and apoptotic genes such as CHOP.

(D) During ER stress, ATF6 is transported to the Golgi apparatus, where it is cleaved, transported to the nucleus, and induces expression of *Xbp-1* and genes required for ER-associated protein degradation (ERAD).

(E) ERAD functions to remove terminally unfolded or misfolded proteins from the ER for proteasomal degradation.

The cytoprotective role of intestinal XBP1 during a robust inflammatory response is conserved in mammals. Independent of pathogen or chemical insult, mice with XBP1-deficient intestinal intraepithelial cells (IECs) develop spontaneous enteritis (intestinal inflammation), resembling human inflammatory bowel disease, with features such as intestinal crypt abscesses, leukocyte infiltration, ulcers, and Paneth cell death (Kaser et al., 2008). Paneth cells are highly secretory specialized IECs that produce antimicrobial peptides to mediate the balance of colonizing microbiota and enteric pathogen host defense at the gastrointestinal barrier. Paneth cell depletion alone does not induce spontaneous enteritis, which highlights the important role of XBP1 in maintaining ER homeostasis in the gut (Garabedian et al., 1997). Not surprisingly, mice with XBP1-deficient IECs are more sensitive to inflammation caused by chemical-induced colitis (Kaser et al., 2008). Deep sequencing of 1,200 patients with inflammatory bowel disease revealed rare XBP1 hypomorphic variants not present in control subjects. Reconstituting these XBP1 mutations in vitro demonstrated that the variants exhibited reduced UPR^{ER} in response to chemically induced ER stress (Kaser et al., 2008). Together, these studies highlight that the IRE1-XBP1 branch of UPR^{ER} has an evolutionarily conserved role to protect the intestine from ER stress induced by robust immune activation. Compromise of this system likely leads to chronic inflammation and substantial tissue damage.

The UPR^{ER} in Metabolism of Development

In addition to the immune system, the UPR^{ER} plays an integral role in metabolic function, as evidenced by loss-of-function studies in both mice and humans. For example, both IRE1 and XBP1 are required for mouse embryonic development, since global knockout of either results in embryonic lethality due to liver hypoplasia and apoptosis (Reimold et al., 2000; Zhang et al., 2005). Rescuing liver-specific expression of *Xbp-1* in *Xbp-1*-deficient mice prevents death in utero. However, these mice die a few days after birth from growth retardation and hypoglycemia due to malformed salivary glands and apoptosis of pancreatic acinar cells. In the absence of XBP1, acinar cells succumb to apoptosis due to ER stress from an imbalance of ER capacity and demand for secretory proteins, such as pancreatic digestive enzymes (Lee et al., 2005).

The PERK branch of UPR^{ER} is also implicated in metabolic function, since loss-of-function mutations in the gene encoding PERK cause Walcott-Rallison syndrome, a rare, autosomal recessive disorder affecting neonates and infants. These patients develop diabetes, epiphyseal dysplasia, and, in some cases, exocrine pancreatic dysfunction, mental retardation, hepatic and kidney dysfunction, and cardiac abnormalities (Delépine et al., 2000; Senée et al., 2004). This pathology is recapitulated in PERK-deficient mice, which develop diabetes mellitus and exocrine pancreatic dysfunction early in adulthood despite normal pancreatic development (Harding et al., 2001). Together, these studies highlight the fundamental role the UPR^{ER} plays in metabolic tissue homeostasis, considering that loss of IRE1/XBP1 or PERK causes severe metabolic defects early in life.

ER Stress in Adult-Onset Metabolic Disease

In states of chronic overnutrition, the UPR^{ER} machinery in metabolically active cells can be overwhelmed, leading to unresolved ER stress and deterioration of metabolic tissue. In fact, ER stress is emerging as a major contributor to the pathogenicity of age-onset metabolic disease, such as obesity, insulin resistance, and type 2 diabetes. Type 2 diabetes is characterized by increased levels of blood glucose due to decreased insulin secretion from pancreatic β cells and insulin resistance in adipose tissue, muscle, and liver (Hotamisligil, 2006). Liver and adipose tissue from mice fed a high-fat diet or mice with a genetic mutation that causes obesity (*ob*/*ob*) exhibit increased phosphorylation of eIF2α and PERK as well as increased levels of BiP. Mice heterozygous for a null *Xbp1* mutation develop glucose intolerance, severe insulin resistance, and diabetes (Ozcan et al., 2004).

In *ob*/*ob* mice, translocation of XBP1s to the nucleus is disrupted, further exacerbating ER stress and insulin resistance. Nuclear migration of XBP1s is enhanced by phosphorylation by MAP kinase kinase 6 (MKK6Glu). Overexpressing constitutively active MKK6Glu continuously phosphorylates XBP1s, restoring UPR^{ER} and glucose tolerance in severely obese and diabetic mice (Lee et al., 2011). Similarly, treating *ob*/*ob* mice or mice fed a high-fat diet with the chemical chaperones 4-phenyl butyric acid and tauroursodeoxycholic acid (TUDCA) to relieve ER stress normalizes hyperglycemia and restores insulin sensitivity (Ozcan et al., 2006).

In addition to peripheral tissues, ER stress is induced in the central nervous system (CNS) of mice fed a high-fat diet and plays a direct role in the development of insulin and leptin resistance. Leptin is a satiety hormone that is released by adipocytes and binds leptin receptors in the hypothalamus. The hypothalamus is a brain region responsible for regulating neuroendocrine communication between the brain and peripheral tissues to orchestrate homeostatic processes, such as hunger, thirst, and body temperature. ER stress is prevalent in the hypothalamus when mice are fed a high-fat diet, and induction of ER stress specifically in the brain is sufficient to induce leptin resistance in mice fed a normal diet (Ozcan et al., 2009; Schneeberger et al., 2013). ER stress suppresses leptin and insulin signaling by induction of suppressor of cytokine signaling-3 (SOCS3) and protein tyrosine phosphatase 1b (Ptp1b) (Howard and Flier, 2006; White et al., 2009).

Mice with compromised UPR^{ER} via conditional deletion of *Xbp-1* in neurons and glia are more susceptible to diet-induced obesity and are severely leptin resistant (Ozcan et al., 2009). Conversely, overexpression of *Xbp-1s* in hypothalamic pro-opiomelanocortin (POMC) neurons protects mice from diet-induced obesity and cell-non-autonomously induced UPR^{ER} in the liver (Williams et al., 2014). This transcellular response is similar to cell-autonomous UPR^{ER} triggered by hepatocytes after normal feeding as they switch from glucose production to glucose storage. This postprandial state can be induced in the absence of food by ectopic expression of *Xbp-1s* in the liver and is mediated by UDP-galactose-4-epimerase (GalE) (Deng et al., 2013). Interestingly, *Xbp-1s* expression in POMC neurons is sufficient to mimic a fed state in the liver independent of caloric intake, shown by induction of *Xbp-1s* and *GalE*. This postprandial transcriptional program initiated by POMC neurons expressing *Xbp-1s* improves insulin and leptin sensitivity, promotes browning of adipose tissue, and decreases production of endogenous liver glucose. These mice exhibit decreased levels of *Ptp1b* and *Socs3*, suggesting a mechanism by which insulin and leptin resistance are abrogated (Williams et al., 2014) (Figure 3). This study suggests that XBP1 can function as a regulatory nexus between the brain and the periphery to coordinate metabolic homeostasis. Since the nervous system coordinates organism-wide energy homeostasis, it is tempting to hypothesize that neuronal XBP1s can function as a pre-emptive signal to the liver in an effort to anticipate physiological changes in glucose.

UPR^{ER} Decline Contributes to Protein Aggregation and Aging

Despite its fundamental role in maintaining immune and metabolic function, the UPR^{ER} becomes dysfunctional with age, causing massive problems for organismal protein homeostasis, including protein aggregation and widespread tissue failure. Studies in *C. elegans* have begun to shed light on how protein aggregation contributes to tissue decline in normal aging. In *C. elegans*, protein aggregates accumulate with age in multiple tissues (David et al., 2010; Reis-Rodrigues et al., 2012). This organism-wide failure of protein homeostasis occurs rapidly in early adulthood and coincides with tissue deterioration, such as the development of sarcopenia (Ben-Zvi et al., 2009).

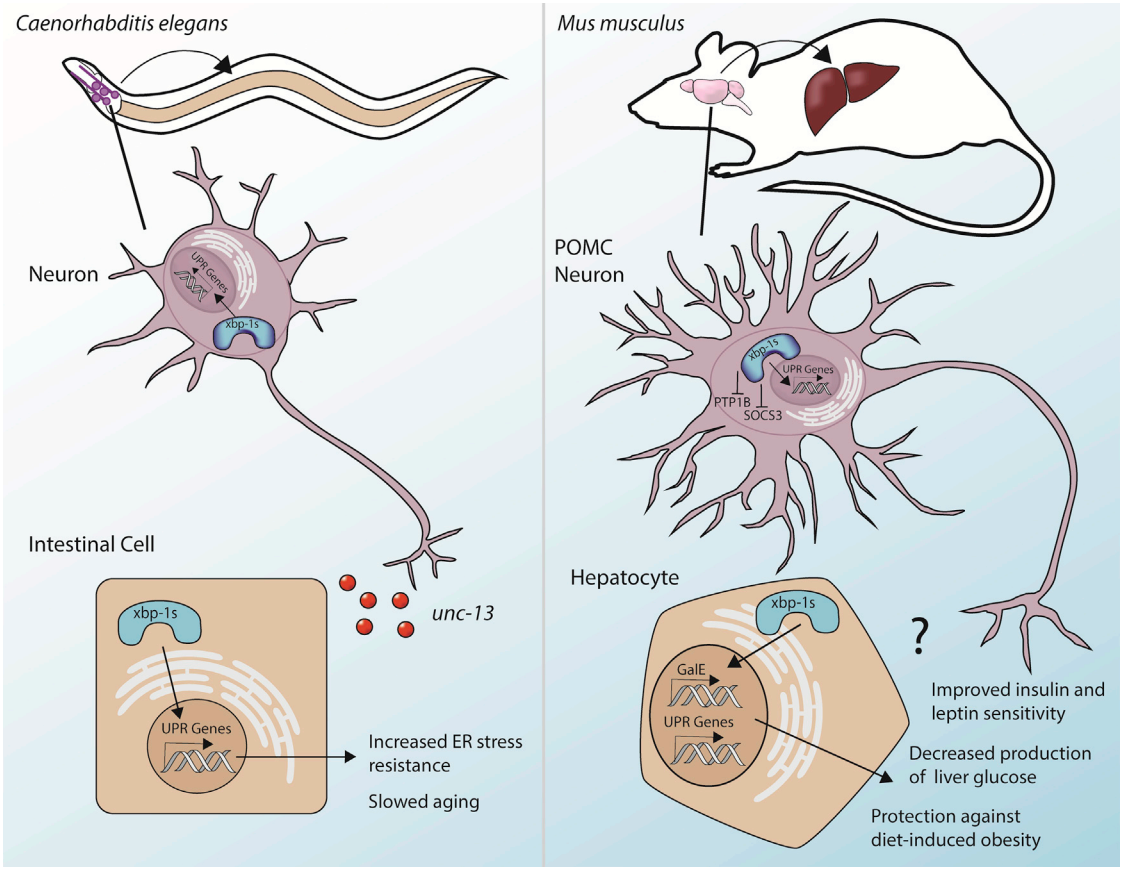

Figure 3. Cell-Non-autonomous Communication of UPR^{ER}

In *C. elegans*, ectopic expression of *xbp-1s* under a pan-neuronal promoter induces UPR^{ER} in distal intestinal tissue, which is sufficient to restore age-onset loss of UPR^{ER} and prolong lifespan. This transcellular signaling mechanism is dependent on small clear vesicle release, suggesting that the secreted cell-non-autonomous mediator is a neurotransmitter. Similarly, expression of Xbp1s in POMC neurons in the mouse hypothalamus results in activation of the UPR^{ER} in the liver, mimicking a postprandial transcriptional program (GalE and UPR genes). These mice exhibit improved insulin and leptin sensitivity and are protected against diet-induced obesity. The signaling molecules required for this response remain elusive.

Pharmacologically slowing protein aggregation in *C. elegans* markedly extends lifespan, providing a direct link between protein aggregation and aging (Alavez et al., 2011).

During normal aging, the accumulation of misfolded and damaged proteins is exacerbated by a seemingly organism-wide loss in the ability to mount cellular stress responses, such as UPR^{ER} (Taylor and Dillin, 2011, 2013). In mammals, chaperones required for ER protein homeostasis, such as BiP, calnexin, and protein disulfide isomerase (*Pdi*), decline both in the CNS and in peripheral tissues with age (Naidoo et al., 2008; Nuss et al., 2008; Paz Gavilán et al., 2006). This coincides with decreased eIF2α phosphorylation and increased levels of the pro-apoptotic factors CHOP, caspase-12, and GADD34, a subunit of a eIF2α phosphatase, all indicative of chronic, maladaptive ER stress (Hussain and Ramaiah, 2007). Moreover, many ER chaperones accumulate oxidative damage with age, limiting their function in UPR^{ER} (Rabek et al., 2003; Uehara et al., 2006; Yang et al., 2015).

Enhancing protein homeostasis pathways in *C. elegans* protects the organism against protein aggregation and extends lifespan, highlighting the crucial link between maintenance of a healthy proteome and longevity. For example, increasing the synthesis of N-glycan precursors, which are required for the proper folding and N-glycosylation of membrane-bound and secreted proteins, extends lifespan and alleviates protein misfolding in *C. elegans*. IRE-1 and XBP-1 are required for these benefits (Denzel et al., 2014).

Furthermore, ectopic expression of *xbp-1s* in neurons is sufficient to restore age-onset loss of UPR^{ER}, confer stress resistance, and prolong lifespan in *C. elegans*. Surprisingly, neuronal *xbp-1s* activates the UPR^{ER} in distal intestinal cells. Thus, *C. elegans* have an analogous cell-non-autonomous signaling circuit as described in mice (Williams et al., 2014). Loss of UPR^{ER} components in worm intestinal cells abrogates the cell-non-autonomous induction of UPR^{ER}, preventing lifespan extension. Transcellular UPR^{ER} induction is dependent on release of small clear vesicles, suggesting that the cell-non-autonomous signal in *C. elegans* may be a neurotransmitter (Taylor and Dillin, 2013) (Figure 3). Therefore, neuronal control of organismal ER stress is a conserved, fundamental mechanism in metazoans to integrate stress signals into physiological changes to maintain homeostasis. It is tempting to speculate whether ectopic

expression *Xbp1s* in neurons may increase longevity in mammals. Identifying downstream signaling mediators of neuronal XBP1s may give rise to novel therapeutic strategies to combat age-onset decline in UPR^{ER} or chronic ER stress.

Chronic ER Stress and Inflammation

Despite ample evidence that the UPR^{ER} is essential for immunity, it is not understood how UPR^{ER} deterioration with age impacts the immune system. During aging, adaptive immunity declines substantially (termed immunosenescence), whereas innate immunity increases to a state of chronic inflammation (termed inflamm-aging) (Franceschi and Campisi, 2014; Franceschi et al., 2000). We propose that dysfunction of the UPR^{ER} leads to persistent ER stress with age, which is likely pivotal for the development of chronic inflammation.

Upon transient ER stress, all three branches of the UPR^{ER} can interface with inflammatory and stress signaling pathways, including nuclear factor kappa B (NF-κB) (Zhang and Kaufman, 2008). NF-κB is a master transcriptional regulator of innate immunity, driving gene expression of pro-inflammatory cytokines, chemokines, enzymes, adhesion molecules, and anti-apoptotic factors. Under basal conditions NF-κB is inactive, bound by inhibitory kappa B (IκB), which sequesters NF-κB in cytoplasm by blocking its nuclear localization signal (Ghosh and Karin, 2002; Jacobs and Harrison, 1998). Upon ER stress, the PERK branch can activate NF-κB by repressing translation of IκB via eIF2α (Deng et al., 2004; Jiang et al., 2003). The cytoplasmic domain of IRE1 can bind TNF receptor-associated factor 2 (TRAF2) and form a complex with IκB kinase (IKK), which initiates a kinase-signaling cascade to phosphorylate and activate NF-κB (Hu et al., 2006). Transient induction of NF-κB is likely beneficial to prevent apoptosis during acute ER stress (Karin and Lin, 2002). However, unresolved ER stress leads to sustained NF-κB signaling. The resulting chronic inflammation driven by NF-κB accelerates aging and increases susceptibility to disease.

NF-κB is chronically activated in many tissues with age and has been implicated as a culprit in inflamm-aging (Helenius et al., 2001; Korhonen et al., 1997; Salminen et al., 2008). Recent studies provide direct evidence that hypothalamic inflammation induced by microglial NF-κB activity increases with age and mediates systemic aging. Inhibiting NF-κB in the hypothalamus significantly extends lifespan and confers beneficial effects in systemic aging. These mice exhibit increased neurogenesis, thicker dermis, and enhanced muscle endurance compared to mice with intact NF-κB activity. Conversely, constitutively activating the NF-κB pathway in the hypothalamus shortens lifespan and accelerates peripheral aging, shown by marked bone loss, and muscle and skin atrophy (Zhang et al., 2013).

NF-κB in Metabolic Disease

In the context of overnutrition, chronic ER stress can lead to NF-κB activation in both the CNS and metabolically active peripheral tissues (Carlsen et al., 2009; Zhang et al., 2008). Globally reducing NF-κB activation by heterozygous deletion of IKKβ, a subunit of the IKK complex that is required for NF-κB activation, protects mice from developing insulin resistance in response to diet-induced or genetically induced obesity (Yuan et al., 2001). To identify the cell types responsible for IKKβ-induced insulin resistance, IKKβ was selectively deleted in muscle, liver, or myeloid cells. Surprisingly, mice with IKKβ-deficient myeloid cells exhibited global insulin sensitivity (Arkan et al., 2005). Muscle ablation of IKKβ conferred no benefit to insulin resistance induced by high-fat diet (Röhl et al., 2004). Mice with IKKβ-deficient hepatocytes retained insulin sensitivity in the liver; however, muscle and fat tissue remained insulin resistant (Arkan et al., 2005).

In contradiction with these studies, a recent report demonstrates that activation of liver IKKβ in obese mice significantly improves insulin sensitivity and glucose homeostasis by its interaction with XBP1s. During normal feeding, IKKβ binds and phosphorylates XBP1s, and this interaction is lost in obese *ob/ob* mice. Ectopic expression of constitutively active IKKβ selectively in the liver of diet-induced obese or *ob/ob* mice restores XBP1s activity, reduces ER stress, re-establishes insulin sensitivity, and improves glucose homeostasis. Decreasing XBP1s levels diminishes the beneficial effect of IKKβ in the liver (Liu et al., 2016). It remains unknown how both deletion and constitutive activation of IKKβ in the liver leads to improved glucose homeostasis. However, it may be that embryonic deletion of IKKβ results in compensatory protective mechanisms in the liver. It is also possible that these studies highlight complex, pleiotropic effects of IKKβ in the liver.

A recent study demonstrates that the inflammatory milieu in obese mice compromises IRE1α-mediated XBP-1 splicing. Inducible nitric oxide synthase (iNOS) activity is increased in obese mice and causes S-nitrosylation of IRE1α, impairing ribonuclease activity. Reconstituting a variant of IRE1α that is resistant to nitrosylation in mice with IRE1α-deficient livers restores XBP-1 splicing and improves glucose homeostasis (Yang et al., 2015). Thus, the NF-κB-regulated inflammatory mediator iNOS can contribute to the reduced function of the IRE1-XBP-1 branch of UPR^{ER} in diet-induced obesity.

When mice are fed a high-fat diet, phosphorylation of PERK is induced concurrently with the classical NF-κB pathway in the hypothalamus. Alleviating high-fat-diet-induced ER stress with TUDCA decreases NF-κB activation. Conversely, chemically induced ER stress in the hypothalamus induces NF-κB activation, independent of caloric intake. Thus, ER stress is at least one of the underlying mechanisms that activate NF-κB in the hypothalamus after a high-fat diet. However, these two signaling pathways do not always function unilaterally. Hyperactivation of NF-κB via overexpression of constitutively active IKKβ in the hypothalamus is sufficient to induce phosphorylation of PERK and eIF2α and disrupt leptin signaling (Zhang et al., 2008).

These studies demonstrate how persistent ER stress and NF-κB activity lead to metabolic disease, chronic inflammation, and accelerated aging. However, the upstream mechanisms triggering unresolved ER stress and chronic inflammation are still unknown. Is the collapse of UPR^{ER} with age simply a loss of function, exacerbating protein misfolding and causing unresolved ER stress? Is the aged ER not able to properly sense and coordinate organismal homeostasis in response to metabolic and immune stimuli? Are misfolded or aggregated proteins themselves inducing an inflammatory response? Insight into the mechanisms by which ER stress and protein aggregation with age

influence inflammation has emerged from studies of neurodegenerative disease.

ALS: Protein Aggregation Meets Chronic Inflammation

Here, we discuss evidence that susceptibility to ER stress dictates motor neuron vulnerability and may be an underlying cause of neuroinflammation in amyotrophic lateral sclerosis (ALS). ALS is a late-onset, relentlessly progressive neurodegenerative disease characterized by motor neuron death in the spinal cord, motor cortex, and brainstem, resulting in severe muscle atrophy, paralysis, and death within an average of 3–5 years after diagnosis (Taylor et al., 2016). Dysfunctional proteostasis and neuroinflammation are common features of both inherited and sporadic ALS, exemplified by ER stress, inclusions of abnormal oligomerized or aggregated proteins, and reactive gliosis (Ferraiuolo et al., 2011).

ER stress is one of the earliest pathological processes observed in ALS, detectable prior to denervation of the neuromuscular junction. PDI, phospho-PERK, CHOP, and caspase-12 are elevated in spinal cords of ALS mice at pre-symptomatic ages and at disease onset (Atkin et al., 2006; Nagata et al., 2007). ER stress is also evident in ALS patients with *C9orf72* hexanucleotide repeat expansions, the most common genetic variant (Prudencio et al., 2015). Non-AUG translation of *C9orf72* expansions generates repeat proteins (DPRs) that are likely the source of ER stress in *C9orf72* patients (Ash et al., 2013). DPRs form soluble and insoluble high-molecular-weight species and intracellular inclusions in patients and in primary neurons in culture (Mori et al., 2013a, 2013b; Zhang et al., 2014). DPRs can undergo cell-to-cell transmission in vitro and are detectible in cerebrospinal fluid of *C9orf72* ALS patients, suggesting that DPRs may be secreted (Su et al., 2014; Westergard et al., 2016).

In addition to serving as pathological hallmark of ALS, ER stress dictates motor neuron vulnerability to disease in mouse models of ALS that ubiquitously express mutant superoxide dismutase 1 (SOD1). Specific subtypes of motor neurons are differentially affected during the course of ALS. Fast-fatigable motor neurons display pathological changes long before noticeable symptoms present (termed VUL-MNs), whereas slow-fatigable motor neurons are more resistant to disease and only denervate from muscle at late to end stages of disease (RES-MNs) (Pun et al., 2006). Long before clinical onset of disease BiP, *Atf4* and phospho-eIF2α levels are increased in VUL-MNs, but not RES-MNs. RES-MNs eventually induce a UPR^{ER}; however, the response is substantially delayed (Saxena et al., 2009).

It is not fully understood how mutant SOD1 induces ER stress in VUL-MNs months prior to disease onset. Biochemical and microscopic studies show that mutant SOD1 can localize to the ER and bind BiP, leading to UPR^{ER} activation (Kikuchi et al., 2006). At clinical onset, insoluble mutant SOD1 aggregates are detectable in motor neurons and glia of ALS mice, and the abundance of aggregates markedly increases as disease progresses (Bruijn et al., 1997, 1998). Ribosome profiling of motor neurons at disease onset revealed increased levels of *Pdi*, sequestosome-1 (*p62*), *Atf4*, *Chop*, and activated PERK. However, *BiP* and *Xbp1s* are not elevated, suggesting that motor neurons fail to initiate the ATF6 and IRE1α branches of UPR^{ER} at disease onset (Sun et al., 2015). Interestingly, microglia undergo rapid morphological changes concomitantly with VUL-MN UPR^{ER} induction, suggesting that these myeloid-derived sentinels of the CNS may sense very early disturbances in neuronal protein homeostasis (Saxena et al., 2009).

Independent of ALS-causing mutations, vulnerable motor neuron pools are intrinsically less able to cope with prolonged ER stress. For example, mice expressing a knockin mutant *BiP* allele acquire age-related motor dysfunction attributed to selective motor neuron loss. Mutant BiP causes ER stress, ubiquitin-positive aggregates, and aggregates of wild-type SOD1 in motor neurons (Jin et al., 2014). In wild-type mice, *BiP* and a co-chaperone of *BiP*, *Sil1*, are highly expressed in RES-MNs compared to VUL-MNs (Filézac de L'Etang et al., 2015). Genetic ablation of *Sil1* in mutant SOD1 mice enhances ER stress and accelerates ALS pathology. Conversely, overexpression of *Sil1* ameliorates disease manifestation and improves ER homeostasis (Filézac de L'Etang et al., 2015). Together, these studies provide evidence that intrinsic susceptibility to ER stress renders distinct motor neuron subtypes more vulnerable to mutant SOD1-induced ALS.

As ALS progresses, the accumulation of inclusions and chronic ER stress is accompanied by extensive neuroinflammation characterized by marked astrogliosis and microglial activation (Glass et al., 2010). As observed in the hypothalamus during aging and metabolic disease, NF-κB is upregulated in spinal cords of ALS patients and in mutant SOD1 mice. Selective inhibition of NF-κB signaling by conditional IKKβ-deletion in microglia rescues motor neurons from microglial-mediated death in vitro and extends survival in ALS mice by dampening pro-inflammatory microglial activation. Conversely, constitutively activating IKKβ in microglia recapitulated several hallmarks of ALS, such as gliosis and motor neuron death (Frakes et al., 2014). It is not known whether ER stress plays a causative role in microglial NF-κB activity in mutant SOD1 mice. However, genetic deletion of mutant SOD1 (i.e., the proteotoxic stress) in microglia rescues motor neurons from microglial-mediated motor neuron death in vitro and extends survival of mutant SOD1 mice (Boillée et al., 2006; Frakes et al., 2014). Furthermore, combining microglial NF-κB inhibition with SOD1 reduction in motor neurons and astrocytes additively extends lifespan. Thus, co-targeting inflammation and proteotoxic stress leads to additive benefits in the SOD1 ALS mouse model (Frakes et al., 2017).

Concluding Remarks

In light of accumulating evidence, the UPR^{ER} has emerged as a major sensor and coordinator of organismal homeostasis. We propose that the collapse of UPR^{ER} in aged organisms directly contributes to the accumulation of tissue damage and the increase in disease susceptibility (Figure 4). Identifying the affected cell types and delineating the molecular mechanisms by which a decrepit UPR^{ER} contributes to unresolved ER stress and chronic inflammation will be critical to our understanding of age-onset metabolic and neurodegenerative disease.

Recent studies reviewed here highlight the role of cell-non-autonomous communication of UPR^{ER} between the brain and peripheral tissues to coordinate organism-wide homeostasis. It is tempting to hypothesize that neuronal XBP1s could be functioning as a pre-emptive signal to peripheral tissues in an effort

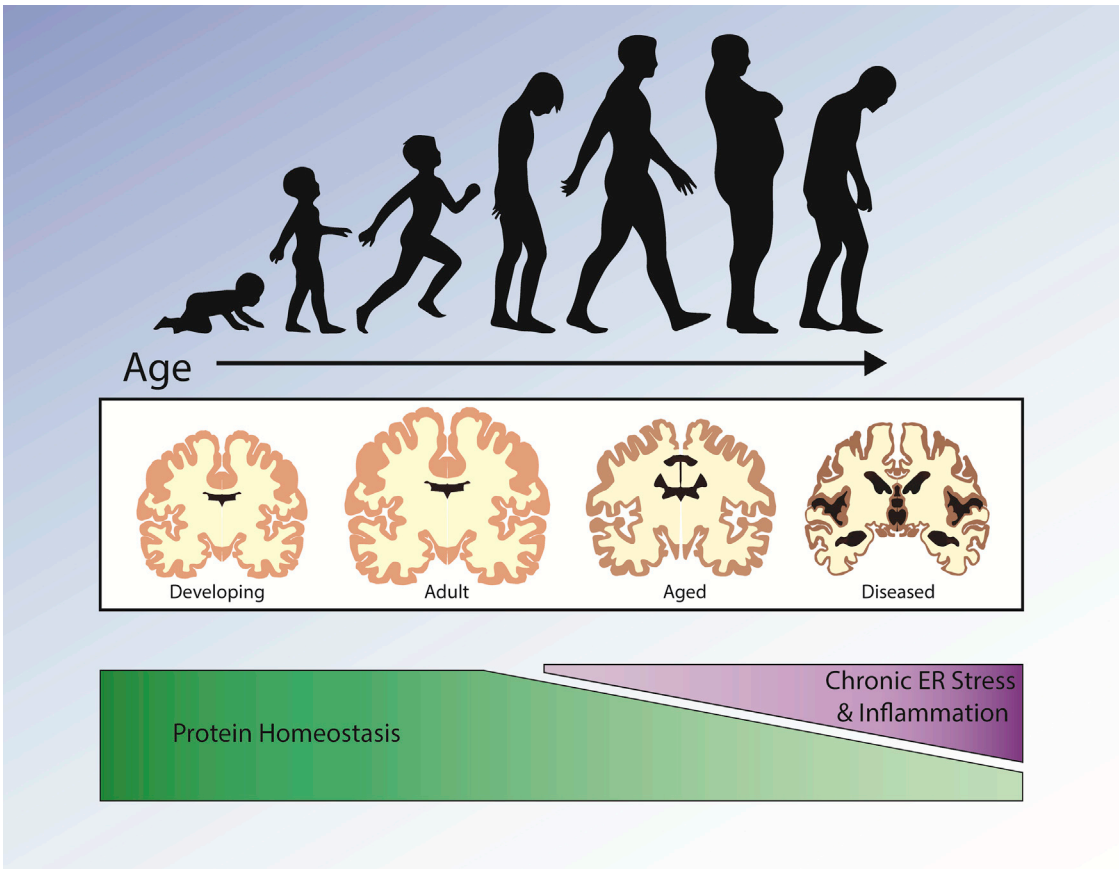

Figure 4. Decline of Protein Homeostasis with Age
Cell stress response pathways maintain proteostasis throughout the lifetime of an organism. Recent studies suggest that these protective mechanisms are diminished with age. Also, UPR^{ER} components are reduced in brain tissue of rodents with age. Impaired UPR^{ER} likely contributes to unresolved ER stress and chronic inflammation, which leads to tissue decline with age and exacerbates pathology in diseases of protein aggregation.

to prepare for anticipated physiological stressors. Identifying the genetic requirements for trans-cellular UPR^{ER} may have enormous therapeutic implications for metabolic disease and aging. Due to the complex, multi-factorial mechanisms involved in aging, metabolic disease, and neurodegeneration, the most effective therapeutic strategies will likely be cell specific and combinatorial in nature.

ACKNOWLEDGMENTS

We are grateful to Sarah Uhlein Tronnes for the artwork and Holly Gildea, Melissa Metcalf, Rebekah Zahedi, Ryo Higuchi-Sanabria, and Brant Webster for helpful comments and discussion. A.E.F. is supported by the NIH (F32 AG051355). A.D. is supported by the Thomas and Stacey Siebel Foundation, the Howard Hughes Medical Institute, and NIH grant AG042679.

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