Critical Review

Endoplasmic Reticulum Quality Control and Systemic Amyloid Disease: Impacting Protein Stability from the Inside Out

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Abstract

The endoplasmic reticulum (ER) is responsible for regulating proteome integrity throughout the secretory pathway. The ER protects downstream secretory environments such as the extracellular space by partitioning proteins between ER protein folding, trafficking, and degradation pathways in a process called ER quality control. In this process, ER quality control factors identify misfolded, aggregation-prone protein conformations and direct them toward ER protein folding or degradation, reducing their secretion to the extracellular space where they could further misfold or aggregate into proteotoxic conformations. Despite the general efficiency of ER quality control, many human diseases, such as the systemic amyloidoses, involve aggregation of destabilized, aggregation-prone proteins in the extracellular space. A common feature for all systemic amyloid diseases is the ability for amyloidogenic proteins to evade ER quality control and be efficiently secreted. The efficient secretion of these amyloidogenic proteins increases their serum concentrations available for the distal proteotoxic aggregation characteristic of these diseases. This indicates that ER quality control, and the regulation thereof, is a critical determinant in defining the onset and pathology of systemic amyloid diseases. Here, we discuss the pathologic and potential therapeutic relationship between ER quality control, protein secretion, and distal deposition of amyloidogenic proteins involved in systemic amyloid diseases. Furthermore, we present evidence that the unfolded protein response, the stress-responsive signaling pathway that regulates ER quality control, is involved in the pathogenesis of systemic amyloid diseases and represents a promising emerging therapeutic target to intervene in this class of human disease.

Keywords: *protein folding; stress-activated signaling; chaperones*

Systemic Amyloid Diseases are Dependent on Secretion of Amyloidogenic Proteins

The ability for proteins to attain their native three-dimensional conformation is critical for human health. The inability for proteins to maintain this folded conformation can lead to misfolding and subsequent proteotoxic aggregation associated with the onset and pathology of many diseases including the systemic amyloidoses. Systemic amyloid diseases are a class of human disorders characterized by the extracellular misfolding and proteotoxic aggregation of proteins that deposit as amyloid on tissues such as the heart, gut, and peripheral nerves (1,2). The deposition of amyloid fibrils is causatively associated with organ malfunction and eventual death in the pathogenesis of these diseases. Fourteen structurally diverse proteins (or

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TABLE 1 *List of amyloidogenic precursor proteins and their associated systemic amyloid disease*

Precursor protein	*Disease*	*Organ involvement*
α-Fibrinogen variants	Fibrinogen amyloidosis	Kidney
Apolipoprotein A–I variants	ApoAI amyloidosis	Kidney, heart, liver, peripheral nervous system, testis, larynx, skin
Apolipoprotein A–II variants	ApoAII amyloidosis	Kidney
Apolipoprotein A–IV wild type	ApoAIV amyloidosis	Kidney
β2-Microglobulin variants	β2-Microglobulin amyloidosis	Autonomic Nervous System
BriPP variants	Familial British dementia	Central nervous system
Cystatin C variants	Cystatin C amyloid angiopathy	Peripheral nervous system, skin
Gelsolin variants	Finnish hereditary amyloidosis	Eyes, peripheral nervous system
Immunoglobulin light chain	Light-chain amyloidosis	All organs (except central nervous system)
Immunoglobulin heavy chain	Heavy-chain amyloidosis	All organs (except central nervous system)
Leukocyte chemotactic factor 2	ALECT2 amyloidosis	Kidney
Lysozyme variants	Lysozyme amyloidosis	Kidney
Serum amyloid A	Secondary amyloidosis	All organs (except central nervous system)
Transthyretin variants	Familial amyloid polyneuropathy, Familial amyloid cardiomyopathy	Heart, peripheral nerves, leptomeninges
Transthyretin wild type	Senile systemic amyloidosis	Heart

Adapted from ref. (3).

fragments thereof) deposit as amyloid in association with multiple systemic amyloid diseases (Table 1; ref. (3)). The majority of these diseases are caused by inherited or acquired mutations in an amyloidogenic protein that destabilize the native protein structure and promote its extracellular misfolding and/or aggregation into proteotoxic soluble oligomers and amyloid fibrils. Despite the similar involvement of proteotoxic aggregation and distal deposition of amyloidogenic proteins, the pathologies of systemic amyloid diseases are highly variable, presenting with distinct ages of onset, organ involvement, and severity. This heterogeneity challenges the development of therapeutic approaches to intervene in these diseases.

Currently, few strategies exist to treat systemic amyloid diseases. Highly invasive liver transplantation is used to treat systemic amyloidoses caused by destabilized, amyloidogenic variants of proteins including transthyretin (TTR), α-fibrinogen, and apolipoprotein A–II (4–7). In this strategy, a liver expressing a destabilized, amyloidogenic protein is replaced with a liver secreting the wild-type protein, avoiding hepatic synthesis of the amyloidogenic aggregation-prone protein responsible for the distal proteotoxicity. Similarly, chemotherapeutic ablation of dyscratic plasma cells expressing an amyloidogenic immunoglobulin light chain (LC) reduces circulating concentrations of proteotoxic LC sequences and improves patient prognosis in LC amyloidosis (8,9). Alternatively, the small molecule Tafamidis, a kinetic stabilizer of the native TTR tetramer, is approved in Europe and Japan as a noninvasive strategy to treat familial amyloid polyneuropathy (FAP) caused by the secretion and subsequent proteotoxic aggregation of destabilized, amyloidogenic TTR variants (10–12). In this strategy, Tafamidis binding to the TTR tetramer prevents TTR tetramer dissociation and subsequent misfolding required for proteotoxic TTR aggregation. The establishment of similar strategies to prevent misfolding and proteotoxic protein aggregation in other systemic amyloidoses is challenged by the lack of small molecule binding sites on many amyloidogenic proteins. Furthermore, small molecule strategies to ameliorate proteotoxicity at distal tissues are limited by our poor understanding of the proteotoxic mechanism(s) by which amyloidogenic proteins induce toxicity, although it is clear that misfolded proteins and/or small soluble oligomers are the predominant proteotoxic species (13–16). The lack of noninvasive strategies to ameliorate distal proteotoxicity involved in many systemic amyloidoses has led to a significant amount of experimental effort to identify new biologic pathways and processes that can be therapeutically targeted to intervene in these disorders.

A critical factor in dictating systemic amyloid disease pathogenesis is the secretion of amyloidogenic proteins from effector tissues (*i.e.*, tissues that synthesize the amyloidogenic protein). The secretion of amyloidogenic proteins defines their serum concentrations available for proteotoxic, concentration-dependent aggregation and distal deposition. The importance of amyloidogenic protein serum concentrations in disease pathogenesis is evident in patients receiving liver transplantation. Replacing a liver secreting destabilized, amyloidogenic variants of proteins such as TTR, apolipoprotein A-II, or α-fibrinogen with a liver secreting the corresponding wild-type protein decreases serum concentrations of the amyloidogenic protein variant and corresponds with marked improvement in patients (4–7). Similarly, reducing hepatic TTR synthesis, and subsequently serum levels, of amyloidogenic TTRs using siRNA or antisense RNA technologies also shows significant potential to attenuate distal toxicity and to improve patient prognosis (17–19). As secretion of amyloidogenic proteins from effector tissues is a primary determinant in dictating circulating serum concentrations of amyloidogenic proteins, the activity and regulation of biologic pathways that mediate amyloidogenic protein secretion is both relevant to the pathogenesis of systemic amyloid diseases and represents a potential therapeutic target to reduce serum concentrations of amyloidogenic proteins causatively associated with distal proteotoxicity in these disorders.

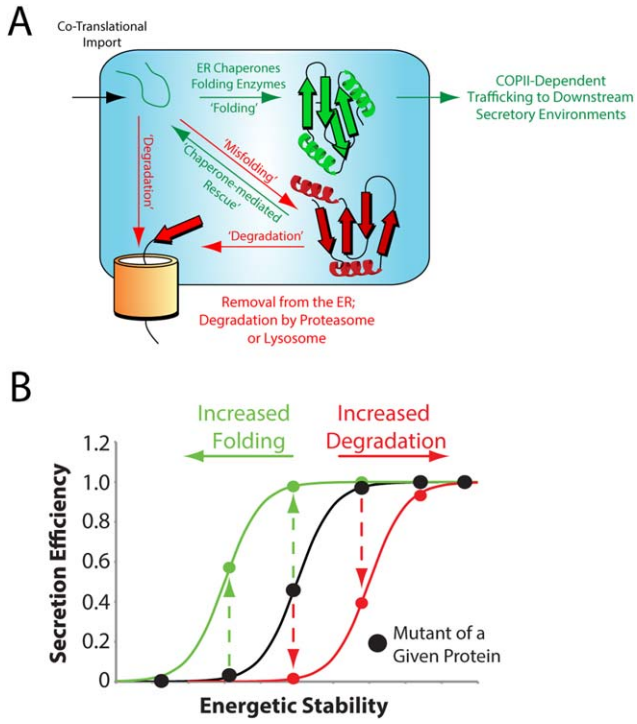

FIG 1 *Secretion of proteins through the secretory pathway is dependent on the relative activity of ER protein folding, trafficking, and degradation pathways. A: Illustration showing the partitioning of ER-targeted proteins between ER protein folding, trafficking, and degradation pathways. B: Plot relating the secretion efficiency from mammalian cells to the inherent energetic stability for a series of destabilized variants of a single protein. The impact of increasing ER folding capacity (green) or degradation capacity (red) on the secretion of these variants is also shown.*

Protein Secretion is Dictated by the Activity of Endoplasmic Reticulum Quality Control Pathways Involved in Protein Folding, Trafficking, and Degradation

Nearly one-third of the human proteome, including all proteins involved in systemic amyloid diseases, is targeted to the endoplasmic reticulum (ER) for folding and trafficking to downstream secretory environments such as the extracellular space. These proteins are directed to the ER by N-terminal targeting sequences that mediate their cotranslational import into the ER (Fig. 1A). In the ER, these newly synthesized unfolded proteins engage ER-localized folding enzymes and ATP-dependent chaperones that facilitate both post-translational modification of the polypeptide chain (*e.g.*, N-glycosylation, disulfide bond formation) and proper folding into the native three-dimensional conformation (20). Folded proteins are packaged into the COPII vesicles for trafficking to the Golgi where they are sorted and targeted to downstream secretory environments. Proteins unable to attain a folded conformation through interactions with ER chaperones and folding enzymes are retained in the ER and directed toward degradation through mechanisms such as ER-associated degradation (ERAD; refs. (21) and (22)). In ERAD, nonfolded or misfolded protein conformations are recognized by ERAD receptors in the ER lumen and targeted for retrotranslocation to the cytosol where they are ubiquitinated and degraded by the proteasome. Misfolded proteins can also be degraded through a mechanism involving trafficking to the lysosome via trafficking to the Golgi or autophagic removal of misfolded ER proteins (23).

The partitioning of proteins between ER protein folding, trafficking, and degradation pathways, also referred to as ER quality control, serves a critical role in regulating downstream secretory environments including the extracellular space. ER quality control functions to prevent the trafficking of misfolded or nonfolded protein conformations to the extracellular environment where they could further misfold or aggregate into proteotoxic conformations. In this ER quality control mechanism, the secretion of proteins to the extracellular space is dictated by two primary determinants (24). The first is the inherent energetic stability of the protein fold, which includes both the thermodynamic stability (*i.e.*, the propensity to attain a folded conformation) and the kinetic stability (*i.e.*, the rate of folding) of the polypeptide chain—two parameters predominantly dictated by the genetically encoded amino acid

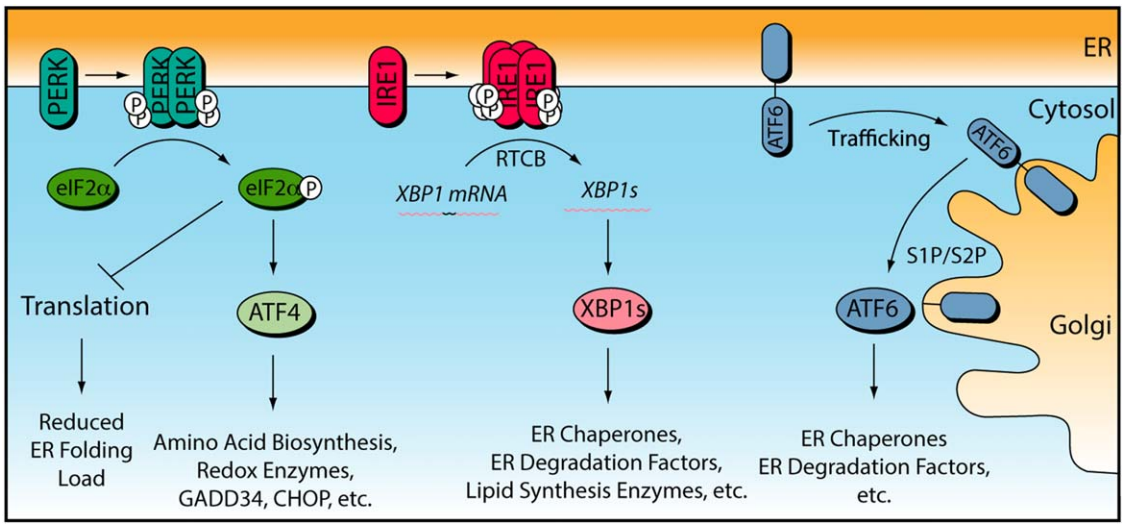

FIG 2 *Illustration showing the signaling pathways activated downstream of the three UPR sensor proteins PERK, IRE1, and ATF6.*

sequence. The energetic stability of a protein defines its ability to attain folded conformations in the steady-state ER environment, and thus is an important determinant in defining protein partitioning between ER protein folding/trafficking and degradation pathways. The importance of protein stability in secretion can be visualized by relating the energetic stability for a series of destabilized variants of a single protein to the secretion efficiency of these same protein variants from a mammalian cell (Fig. 1B). Above a certain energetic stability, protein variants are efficiently secreted to levels similar to that observed for the wild-type protein, reflecting their ability to attain a folded conformation in the ER environment. Alternatively, highly destabilized proteins are not efficiently secreted, but instead retained in the ER and/or targeted for degradation. This relationship between secretion and protein stability has been demonstrated for destabilized variants of amyloidogenic proteins including TTR and lysozyme (24–26).

The second factor that dictates protein secretion through the secretory pathway is the relative activity of ER protein folding and degradation pathways (24). These pathways compete for misfolded or nonfolded protein conformations in the ER to facilitate their partitioning toward either protein folding or degradation (Fig. 1A). Thus, altering the activity of ER protein folding or degradation pathways significantly influences the partitioning of nonfolded proteins between these two pathways. The impact of increasing ER folding or degradation pathway activity on protein secretion can be visualized using the relationship between the energetic stability and secretion for the destabilized protein variants shown in Fig. 1B. Increasing the activity of ER protein folding pathways can increase the ability for protein variants to attain a folded conformation through interactions with ER chaperones and folding enzymes, resulting in the more efficient secretion of moderately stable protein variants (Fig. 1B, green). Alternatively, increasing the activity of ER degradation pathways will increase the partitioning of misfolded or nonfolded protein conformations toward degradation, preventing their interactions with profolding ER chaperones and folding enzymes, and decrease the secretion of protein variants with moderate levels of stability (Fig. 1B, red). This ability for cells to influence protein secretion through altering the activity of ER protein folding, trafficking, and degradation pathways provides a mechanism to adapt ER quality control and secretory function to tissue-specific, environmental, or metabolic demands. This is achieved through the activation of stress-responsive signaling pathways such as the unfolded protein response (UPR).

ER Quality Control is Regulated by the Unfolded Protein Response

The UPR is a stress-responsive signaling pathway that regulates ER quality control in response to developmental cues or genetic, environmental, or aging-related insults that increase accumulation of misfolded proteins in the ER (*i.e.*, ER stress; refs. (27–29). The UPR is a collective term for three stress signaling pathways activated downstream of the ER stress-sensing proteins protein kinase R-like ER kinase (PERK), inositol requiring enzyme 1 (IRE1), and activating transcription factor 6 (ATF6; Fig. 2). These three signaling pathways function to relieve ER stress and reestablish efficient ER quality control in response to pathologic insult by adjusting two regulatory parameters that dictate ER protein homeostasis: protein load and ER quality control capacity (30–32).

PERK is activated through a mechanism involving PERK autophosphorylation and dimerization (27–29). Activated PERK contains an active cytosolic kinase that phosphorylates the α-subunit of eukaryotic initiation factor 2 (eIF2α). Phosphorylated eIF2α inhibits the eIF2B GTP exchange factor required for

translation initiation, resulting in a reduction in new protein synthesis. Reducing protein synthesis through PERK activation decreases the load of newly synthesized, unfolded proteins entering into the ER during the initial phase of ER stress. This functions to promote ER protein homeostasis by freeing ER quality control factors including chaperones, folding enzymes, and degradation factors to alleviate the misfolded protein load in the ER that initiated UPR activation (*i.e.*, the ER stress).

PERK-dependent eIF2α phosphorylation also leads to the downstream activation of stress-responsive transcription factors such as ATF4. These transcription factors induce genes involved in global cellular protein homeostasis maintenance including amino acid biosynthesis enzymes and cellular redox factors (33,34). PERK-regulated transcription factors also induce expression of the eIF2α phosphatase regulatory subunit GADD34 that associates with protein phosphatase 1 to dephosphorylate eIF2α and restore translational integrity in a negative feedback loop of PERK signaling (35,36). Furthermore, ATF4 induces the transcription factor CHOP, which is involved in the ER stress-dependent expression of proapoptotic factors including death receptor 5, PUMA, Bax, and Bak during prolonged or severe ER stress (37,38). Importantly, PERK-dependent eIF2α phosphorylation is activated by ER stress as part of the UPR, whereas other stress-regulated eIF2α kinases phosphorylate eIF2α in response to other stresses such as amino acid deprivation, viral infection, and oxidative stress (39,40). This ability for eIF2α phosphorylation to be activated by a variety of cellular insults is consistent with the global impact of eIF2α phosphorylation on cellular proteome maintenance.

The IRE1 pathway is the most conserved arm of the UPR found in all eukaryotes from yeast to humans (27–29). IRE1 activation proceeds through a mechanism involving autophosphorylation and oligomerization. Active IRE1 contains a cytosolic endoribonuclease domain that cleaves *XBP1* mRNA at two sites 26 nt apart. Cleaved *XBP1* is religated by the RtcB tRNA ligase, resulting in a new mRNA encoding the active transcription factor spliced XBP1 (XBP1s; refs. (41–43). XBP1s localizes to the nucleus and induces expression of genes involved in a variety of ER functions including lipid homeostasis, protein folding, and protein degradation (44–46). Apart from XBP1s activation, active IRE1 also functions to degrade mRNA localized to the plasma membrane in a process called regulated IRE1-mediated mRNA decay (RIDD; refs. (47–50). Although the functional implications of RIDD remain to be defined, RIDD has been proposed to influence ER function through multiple mechanisms including reducing the load of newly synthesized proteins entering into the ER, regulating the activity of UPR signaling pathways, and promoting apoptosis (51). Active IRE1 can also promote apoptosis through recruitment of TRAF2, activating the JNK signaling pathway (37,52).

ATF6 activation proceeds through a mechanism distinct from IRE1 and PERK. In response to ER stress, ATF6 is trafficked to the Golgi (27–29). The signals that induce ATF6 trafficking are largely undefined, but have been proposed to involve alterations in ATF6 disulfide bonding and oligomerization (53). In the Golgi, ATF6 is proteolytically processed by Site 1 and Site 2 proteases, releasing the active N-terminal ATF6 transcription factor domain. The active cleaved N-terminal ATF6 transcription factor (henceforth referred to as ATF6) induces expression of genes involved in ER functions including ER protein quality control (45,46).

XBP1s and ATF6 transcriptionally induce overlapping, but distinct, sets of genes involved in ER quality control (44–46). These UPR-associated transcription factors can also heterodimerize to synergistically induce certain ER quality control factors including those involved in protein degradation (45,46). As such, differential activation of XBP1s and/or ATF6 results in a continuum of ER quality control environments with distinct capacities that can be used to sensitively adapt ER quality control and function to specific cellular demands.

ER Quality Control is a Critical Determinant In Systemic Amyloid Disease Pathology

ER quality control regulates the folding, trafficking, and degradation of all proteins involved in systemic amyloid diseases. This indicates that ER quality control could significantly impact the secretion and subsequent distal proteotoxicity of destabilized, amyloidogenic proteins associated with these disorders. The importance of ER quality control in systemic amyloid diseases has been best demonstrated for the familial TTR amyloidoses. These diseases are causatively associated with the expression and secretion of >100 destabilized TTR variants (54). Amyloidogenic TTR variants are predominantly secreted from the liver and deposit as proteotoxic oligomers and amyloid fibrils on distal tissues including the heart and peripheral nerves in association with familial amyloid cardiomyopathy (FAC) and FAP, respectively (17,54,55).

Clinical presentation of TTR amyloid diseases is influenced by the ability for ER quality control pathways to identify destabilized TTR variants and to prevent their hepatic secretion. Patients expressing highly destabilized, highly aggregation-prone TTR variants such as TTR^{D18G} present with a relatively mild systemic amyloid disease pathology that is inconsistent with the extremely high aggregation propensity of these variants (25,56–58). Interestingly, these highly destabilized, highly aggregation-prone TTR variants are recognized by ER quality control pathways in the liver and targeted for degradation (25,59,60). The recognition of these variants decreases their secretion and subsequently serum concentrations, slowing proteotoxic aggregation of these highly destabilized TTRs on distal tissues (56–58).

Alternatively, moderately destabilized, but still aggregation-prone, TTR variants such as TTR^{L55P} escape ER quality control and are secreted from the liver at levels identical to those observed for the stable wild-type TTR (25,60). This leads to high serum concentrations for these amyloidogenic variants, which facilitates proteotoxic aggregation in the serum. Patients expressing these TTR variants present with a severe, early onset disease pathology (61). These results indicate that the ability for

destabilized, aggregation-prone TTRs to escape ER quality control influences the onset and severity of TTR amyloid disease pathology. Similar relationships between ER quality control efficiency and destabilized protein secretion have been proposed to influence disease pathology in other systemic amyloid diseases such as lysozyme amyloidosis (26,62), although further studies are required to better correlate secretion efficiency, amyloidogenic protein serum concentration, and disease severity for these other disorders.

As ER quality control dictates amyloidogenic protein secretion, imbalanced regulation of ER quality control pathways in effector tissues, through mechanisms such as impaired UPR activity, could also contribute to systemic amyloid disease pathology. Correlative evidence suggests that alterations in hepatic ER quality control regulation can influence distal deposition of amyloidogenic proteins. A longitudinal study measuring hepatic gene expression in mice overexpressing wild-type TTR—a model of senile systemic amyloidosis (Table 1)—showed that aging-dependent cardiac deposition of TTR correlates with reduced hepatic expression of stress-responsive genes including the UPR markers *XBP1s* and *TRIB3* (63). Similarly, ER quality control genes showed differential expression in livers of patients with FAP when compared with controls, suggesting that altered ER quality control regulation may be involved in the proteotoxic TTR deposition observed in patients (64). These results suggest that stress signaling in the liver directly impacts distal TTR deposition.

Similar relationships are also suggested by the clinical presentations of acquired systemic amyloid disease pathology in recipients of domino liver transplantations. In these types of transplantations, patients suffering from severe liver damage receive a liver from a patient suffering from a TTR amyloid disease caused by the hepatic secretion of a destabilized, amyloidogenic TTR variant. Interestingly, the recipients of the mutant TTR expressing liver present with TTR deposition on the heart or peripheral nerves on a timescale considerably faster than that observed in the donor (65–68). This suggests that alterations in the ability for the liver to regulate secretion of amyloidogenic TTR may contribute to the more rapid distal deposition of proteotoxic TTR conformations observed in patients suffering from this acquired form of disease.

Although we are still learning of the relationship between ER quality control and distal proteotoxicity in systemic amyloid disease pathology, this relationship provides a potential mechanism to explain the involvement of aging in the onset and pathology of systemic amyloid diseases. The ability to regulate ER quality control through UPR signaling declines during aging (69,70). Furthermore, overexpression of destabilized, disease-associated variants of amyloidogenic proteins including TTR and lysozyme can induce ER stress and UPR activation in model systems (59,71,72), suggesting that the expression of these amyloidogenic proteins can challenge ER quality control in effector tissues. Thus, age-dependent reductions in the ability for cells to regulate ER quality control could impact the secretion of destabilized, amyloidogenic proteins and facilitate their distal deposition. As new models and approaches are being developed to probe the role of ER quality control in the distal proteotoxic aggregation of amyloidogenic proteins, we anticipate that ER quality control and the maintenance thereof will continue to be identified as a critical factor in defining the onset and pathogenesis for systemic amyloid diseases.

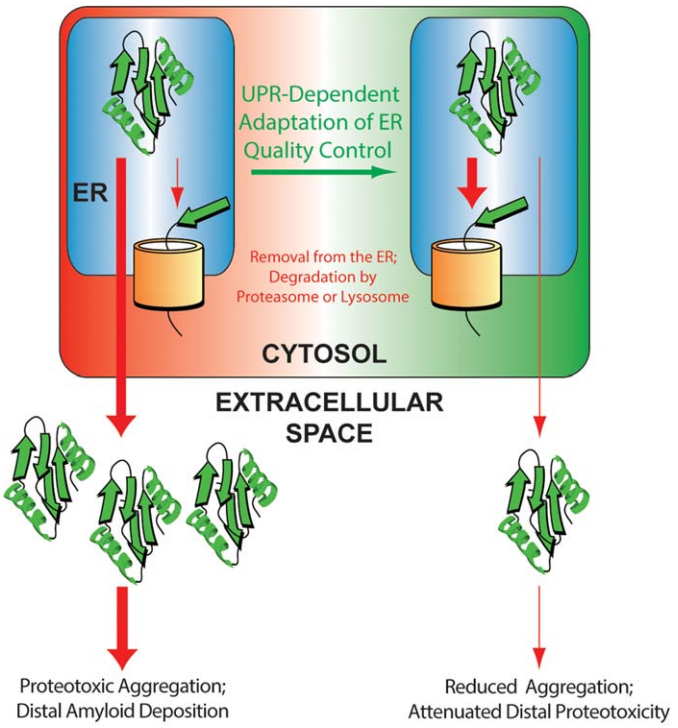

FIG 3

Illustration showing the therapeutic potential for UPR-dependent adaptation of ER quality control to attenuate secretion of destabilized, aggregation-prone proteins. In disease pathophysiology (left), destabilized proteins are folded and efficiently secreted. The efficient secretion of these proteins increases their extracellular concentrations available for the proteotoxic aggregation and distal deposition involved in disease pathogenesis. UPR-dependent remodeling of ER quality control pathways (right) can reduce secretion of these destabilized proteins through increased ER retention or increased partitioning to degradation pathways. This reduced secretion decreases extracellular populations of destabilized, aggregation-prone protein available for proteotoxic aggregation and attenuates the distal proteotoxicity causatively associated with systemic amyloid disease pathogenesis.

UPR-Dependent Regulation of ER Quality Control as a Potential Therapeutic Target in Systemic Amyloid Disease

Our view of systemic amyloid diseases is that imbalances in ER quality control influence the secretion and subsequent

distal proteotoxicity of amyloidogenic proteins. Thus, we view ER quality control as an upstream determinant in defining distal proteotoxicity in amyloid disease pathogenesis. This suggests the possibility that promoting ER quality control to reduce secretion of amyloidogenic proteins offers an opportunity to ameliorate the distal proteotoxicity involved in these diseases (Fig. 3).

ER quality control is predominantly regulated through the activity of the UPR-associated transcription factors XBP1s and ATF6 (Fig. 2; refs. (44–46)). These transcription factors have evolved to induce a transcriptional program that promotes ER quality control in response to challenges to the ER environment. As such, activation of these transcription factors globally adapts ER quality control pathways to prevent secretion of misfolding-prone protein conformations that accumulate during ER stress. This suggests that activating XBP1s and/or ATF6 offers a potential strategy to sensitively reduce secretion of destabilized, amyloidogenic proteins without globally impacting secretion of endogenous wild-type proteins.

Recently, the advantage of activating XBP1s and/or ATF6 to reduce secretion and extracellular aggregation of amyloidogenic proteins has been demonstrated. Stress-independent activation of the UPR-associated transcription factor ATF6 was shown to preferentially reduce secretion and extracellular aggregation of destabilized, amyloidogenic TTR variants from cell culture models (46,60). This decrease in TTR variant secretion corresponds to increased degradation of these destabilized proteins, indicating that ATF6 activation increases partitioning of destabilized TTRs toward degradation pathways. Alternatively, neither the secretion nor degradation of wild-type TTR was significantly affected by ATF6 activation. Interestingly, the decreased TTR variant secretion and increased TTR variant degradation observed following ATF6 activation correlates with the energetic stability, and thus aggregation propensity, for each variant, demonstrating that ATF6 activation increases ER quality control stringency for TTR secretion (60). This indicates that ATF6 activation could be broadly applied to reduce secretion of the >100 destabilized TTR variants involved in TTR amyloid diseases. Furthermore, ATF6-dependent reductions in destabilized TTR secretion synergizes with strategies to stabilize the native TTR tetramer using molecules such as Tafamidis, indicating that these two approaches could potentially used in combination to treat TTR amyloid disease *in vivo*.

Stress-independent activation of XBP1s and/or ATF6 also preferentially reduced secretion and extracellular aggregation of a destabilized, amyloidogenic immunoglobulin LC when compared with a stable, nonamyloidogenic LC (73). Interestingly, XBP1s- or ATF6-dependent remodeling of the ER environment reduced amyloidogenic LC secretion through distinct mechanisms. XBP1s activation increased amyloidogenic LC degradation. Alternatively, ATF6 activation increased ER retention of the amyloidogenic LC through a mechanism involving associations with ATP-dependent chaperones such as BiP or GRP94. The ability to influence the secretion and extracellular aggregation of amyloidogenic TTRs and LCs by activating ATF6 and/or XBP1s suggests that similar strategies can be used to attenuate secretion and extracellular aggregation of other destabilized amyloidogenic proteins involved in systemic amyloid diseases.

Similar strategies could also be used to intervene in other diseases caused by the aggregation of destabilized, aggregation-prone proteins targeted to the ER. ATF6 activation attenuates pathologic intracellular aggregation of the destabilized, aggregation-prone Z-variant of α-1-antitrypsin (A1AT) that induces hepatic dysfunction in association with A1AT deficiency (74). Similarly, activating IRE1/XBP1s or ATF6 reduces intracellular aggregation of the P23H rhodopsin variant involved in retinal degeneration (75,76). In neither case was trafficking of the wild-type protein significantly affected. Thus, adapting ER quality control through ATF6 and/or IRE1/XBP1s activation has the potential to decrease the intracellular and/or extracellular proteotoxic aggregation of multiple destabilized proteins involved in highly diverse human protein aggregation diseases.

Establishing IRE1/XBP1s or ATF6 activation as a strategy to intervene in systemic amyloid diseases is challenged by the lack of small molecules available to preferentially activate these UPR-associated signaling pathways. Global activators of the UPR such as thapsigargin or tunicamycin are not therapeutically valuable as they induce global UPR activation and subsequent apoptotic signaling primarily through the PERK signaling pathway (37,38). Small molecules that bind to the IRE1 nucleotide binding pocket and activate IRE1 endoribonuclease activity and subsequent XBP1 splicing have been reported, although the selectivity of these molecules for the IRE1/XBP1s pathway remains to be established (48,77). Similarly, the small molecule BiX was reported to activate the ATF6 arm of the UPR, although the selectivity of this molecule also remains to be defined (78). Although these molecules offer a potential strategy to pharmacologically target IRE1/XBP1s or ATF6 signaling, new small molecule strategies to activate the IRE1/XBP1s or ATF6 transcriptional programs are required to define the therapeutic potential for UPR-dependent adaptation of ER quality control to intervene in systemic amyloid disease pathology.

A potential limitation in targeting UPR signaling pathways in the context of disease is the impact of adapting ER quality control on physiologic ER function. Initial experiments show that activating XBP1s or ATF6 does not globally impact the secretion of the endogenous secreted proteome from HEK293 cells (46). Furthermore, IRE1/XBP1s or ATF6 activation does not influence the secretion/trafficking of wild-type proteins including TTR, A1AT, or rhodopsin (46,60,74–76). Although this suggests that adapting ER quality control through UPR-associated transcription factor activation allows for preferential reduction in secretion of destabilized, amyloidogenic proteins when compared with stable, wild-type proteins, further studies will be required to define the potential impact for activating these pathways on the secretion and activity of the endogenous secreted proteome *in vivo*.

Activating UPR-associated transcriptional signaling pathways could also influence other aspects of ER function such as

lipid metabolism or the regulation of apoptotic signaling. High levels of ATF6 activation are sufficient to induce fatty liver disease in zebrafish (79). Alternatively, increasing IRE1 RIDD activity increases apoptosis in the INS-1 pancreatic β-cell model (48). Although therapeutic adaptations of ER quality control *in vitro* can be achieved with lower levels of ATF6 or IRE1/XBP1s activation that do not induce these detrimental consequences (46,60,73), evaluating the impact of activating these UPR-associated transcription factors on other aspects of ER function and organismal health will be critical for the establishment of this potential strategy to ameliorate proteotoxic protein aggregation involved in protein aggregation diseases.

Concluding Remarks

ER quality control is involved in defining the secretion efficiency of destabilized, amyloidogenic proteins associated with systemic amyloid diseases. Thus, ER quality control is an upstream determinant that can influence the extracellular, concentration-dependent aggregation of amyloidogenic proteins into the proteotoxic oligomers and amyloid fibrils that induce toxicity at distal tissues in systemic amyloid disease pathogenesis. Taking this view, it is clear that alterations in ER quality control can contribute to the onset and pathogenesis of systemic amyloid diseases. Furthermore, promoting ER quality control in effector tissues through mechanisms such as arm-selective UPR activation could reveal a broadly applicable therapeutic strategy to intervene in this class of protein aggregation diseases. This approach can synergize with other established and developing therapeutic approaches to intervene in these diseases such as small molecules that stabilize the native protein structure (*e.g.*, Tafamidis; refs. (10–12)) or molecules that disrupt fibril formation (*e.g.*, doxycycline; refs. (17) and (19)). As we continue to explore the pathologic and therapeutic involvement of ER quality control in systemic amyloid diseases, we will begin to learn more of the intricate relationship between ER function and extracellular protein homeostasis that can be applied to define pathologic and potentially therapeutic roles for ER quality control in other protein aggregation diseases such as Alzheimer's disease, retinal degeneration, and A1AT deficiency.

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