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Review Article

Starting at the beginning: endoplasmic reticulum proteostasis and systemic amyloid disease

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Systemic amyloid diseases are characterized by the deposition of an amyloidogenic protein as toxic oligomers and amyloid fibrils on tissues distal from the site of protein synthesis. Traditionally, these diseases have been viewed as disorders of peripheral target tissues where aggregates are deposited, and toxicity is observed. However, recent evidence highlights an important role for endoplasmic reticulum (ER) proteostasis pathways within tissues synthesizing and secreting amyloidogenic proteins, such as the liver, in the pathogenesis of these disorders. Here, we describe the pathologic implications of ER proteostasis and its regulation on the toxic extracellular aggregation of amyloidogenic proteins implicated in systemic amyloid disease pathogenesis. Furthermore, we discuss the therapeutic potential for targeting ER proteostasis to reduce the secretion and toxic aggregation of amyloidogenic proteins to mitigate peripheral amyloid-associated toxicity involved in the onset and progression of systemic amyloid diseases.

Proteostasis, extracellular protein aggregation, and systemic amyloid disease pathogenesis

Systemic amyloid diseases are a class of disorders associated with the aggregation and deposition of amyloidogenic proteins on peripheral target tissues distal from the site of protein synthesis (Figure 1) [1–8]. Hundreds of thousands of individuals are affected by these diseases worldwide; however, this likely represents a significant underestimate of disease prevalence as many undiagnosed renal and cardiac disorders show pathology consistent with systemic amyloid diseases [6,9–11]. Destabilizing mutations in >15 different proteins, including, but not limited to, transthyretin (TTR), immunoglobulin light chains (LCs), lysozyme, apolipoproteins, and leucocyte cell-derived chemotaxin-2 (LECT2), predispose individuals to systemic amyloid diseases [1–8,12]. Additionally, systemic amyloidosis can occur as secondary co-morbidities to inflammatory disease (serum amyloid A) and renal failure (β2 microglobulin) [13,14]. Despite the common mechanism of toxic protein aggregation, systemic amyloid diseases present with complex disease progression involving varying ages of onset, penetrance, and tissue involvement [1–8].

Traditionally, systemic amyloid diseases have been viewed as disorders of peripheral target tissues such as the kidney, heart, and peripheral nerves where amyloid is deposited, and toxicity is observed. However, significant clinical and biological evidence now highlights a critical role for tissues that synthesize amyloidogenic proteins (e.g. liver) in the pathogenesis of systemic amyloid diseases, most notably, the TTR-related amyloidoses. TTR amyloid diseases are associated with the deposition of liver-derived TTR as protein aggregates on distal tissues including the heart and peripheral nerves [15,16]. Over 100 different TTR variants have been identified that predispose individuals to TTR amyloid disease, presenting with diverse pathology, penetrance, and ages of onset [15,16]. Wild-type TTR also deposits as aggregates and induces toxicity within the heart in an age-associated amyloid disease [15,16].

Clinical and *in vivo* evidence highlights an important role for the liver in the aggregation and distal toxicity of TTR in disease pathogenesis. Recipients of domino liver transplants wherein a liver from a TTR amyloid disease patient expressing a destabilized, aggregation-prone TTR variant is transplanted

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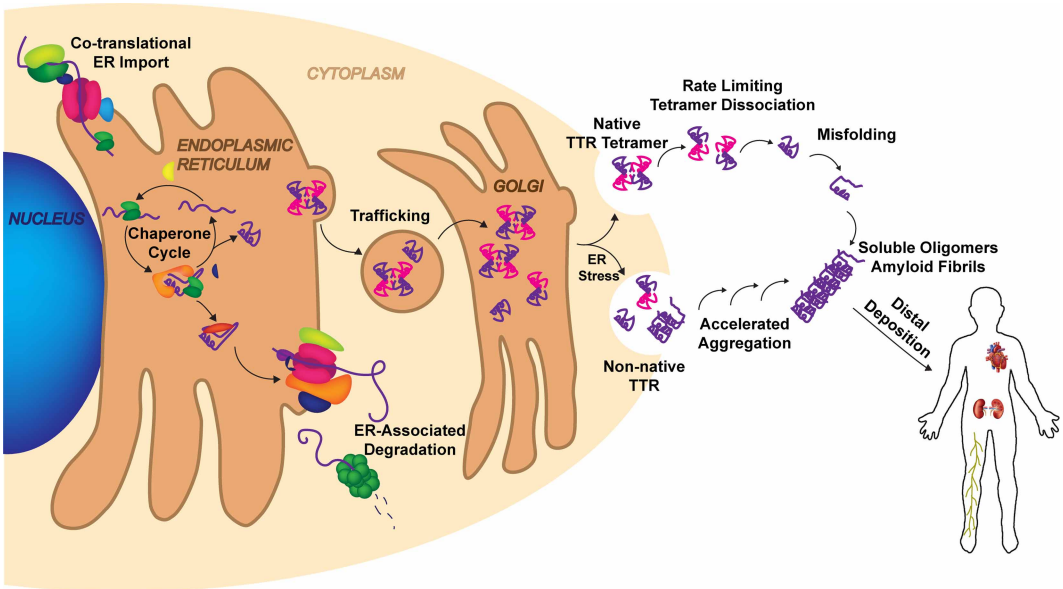

Figure 1. Importance of ER proteostasis and quality control in the extracellular aggregation of the model amyloidogenic protein TTR.

TTR is synthesized in the liver where it is co-translationally imported into the ER and interacts with ER proteostasis factors including the HSP70 chaperone BiP. Highly destabilized TTR variants are recognized by degradation factors and targeted to degradation pathways such as ERAD. This prevents secretion of these destabilized, aggregation-prone TTR variants to the extracellular space, limiting their concentration-dependent aggregation in extracellular environments. However, moderately destabilized, but still aggregation-prone, TTR variants are efficiently secreted to extracellular environments, increasing extracellular populations of protein available for rate-limiting tetramer dissociation and subsequent concentration-dependent aggregation into toxic oligomers and amyloid fibrils. These aggregates can then deposit on distal tissues such as the heart and peripheral nerves to induce toxicity. Disruptions in ER proteostasis (i.e. ER stress) can increase secretion of TTR in non-native conformations that accelerate extracellular protein aggregation by avoiding rate-limiting dissociation of the native tetramer.

into a patient suffering from a different liver disease, show a significant acceleration of distal TTR deposition and toxicity as compared with the donor who was suffering from hereditary TTR amyloidosis [17–20]. This accelerated disease onset suggests that the distal deposition and aggregation of amyloidogenic TTRs may be initiated through imbalances in protein homeostasis (or proteostasis) pathways within the donated liver. Consistent with this, transcriptional profiling of TTR amyloid disease patient livers suggests alterations in the hepatic expression of proteostasis factors that regulate the folding and secretion of amyloidogenic proteins [21]. Similarly, single-cell RNA-sequencing of TTR amyloid disease patient iPSC-derived hepatocytes expressing destabilized amyloidogenic TTRs shows altered expression of proteostasis factors relative to isogenic controls [22]. Altered expression of proteostasis factors within the livers of aged mice overexpressing TTR^{WT} also correlates with the distal cardiac deposition of TTR [23]. Collectively, these results highlight a previously unanticipated role for hepatic proteostasis in dictating the distal deposition of amyloidogenic proteins, such as TTR, in the pathogenesis of systemic amyloid diseases. This leads to an important question: *How does proteostasis within tissues secreting amyloidogenic proteins impact the toxic extracellular aggregation and distal deposition of amyloidogenic proteins on peripheral target tissues?* Here, we address this question primarily focusing on the implications of hepatic ER proteostasis on the secretion and extracellular aggregation of the amyloidogenic protein TTR.

Endoplasmic reticulum proteostasis dictates the secretion and extracellular aggregation of amyloidogenic proteins

Many aggregation-prone proteins implicated in systemic amyloid diseases (and other protein aggregation disorders) are produced in the liver and are secreted into extracellular environments such as the blood. This secretion is primarily regulated by proteostasis pathways localized within the ER comprised of ER chaperones, folding

factors, trafficking proteins, and degradation factors. These ER proteostasis factors integrate to dictate the partitioning of ER proteins between protein folding, trafficking, and degradation in a process called ER quality control.

In this process, secretory proteins co-translationally imported into the ER immediately engage a network of ER chaperones and folding factors including the ATP-dependent HSP70 chaperone BiP, BiP co-chaperones, protein disulfide isomerases (PDIs), and lectin chaperones [24–26]. These ER proteostasis factors facilitate the folding of secretory proteins into their functional three-dimensional conformation (Figure 1). Once folded, proteins are packaged into COPII vesicles for trafficking to downstream secretory environments including the Golgi apparatus, lysosomes, and the extracellular space [27]. However, proteins unable to attain a folded conformation within the ER are recognized by degradation factors that bind to non-native protein conformations or proteins containing degradation signals such as trimmed N-linked glycans [26,28]. These degradation factors then promote the removal of non-folded proteins from the ER through mechanisms such as ER-associated degradation (ERAD) — a process whereby non-native proteins are retro-translocated from the ER to the cytosol, ubiquitinated, and then degraded by the proteasome [28,29]. Non-native protein and ER-localized protein aggregates can also be degraded through other mechanisms including receptor-mediated lysosomal degradation of the ER or ER-phagy [28,30]. Through this partitioning between ER protein folding, trafficking, and degradation cells prevent the ER accumulation of non-native proteins that could disrupt ER function and the aberrant secretion of aggregation-prone proteins that could damage secretory environments including the extracellular space [27,29]. The importance of ER quality control in regulating secretory proteostasis is evident as, under certain conditions, up to 30% of newly synthesized secretory proteins are targeted to degradation, highlighting the urgency with which cells avoid the accumulation of newly synthesized, off-pathway folding products [31].

In the context of systemic amyloid disease, the ER proteostasis pathways that regulate the secretion of amyloidogenic proteins directly influence disease pathogenesis. Highly destabilized, aggregation-prone variants of amyloidogenic proteins such as TTR or lysozyme are efficiently recognized by ER quality control pathways, which increase their targeting to ERAD and reduce their secretion to downstream secretory environments [32–36] (Figure 1). This lessens extracellular populations of these destabilized highly aggregation-prone proteins available for concentration-dependent aggregation into soluble oligomers commonly associated with cytotoxicity [37–42]. Thus, patients expressing these types of highly destabilized mutations present with a moderate disease phenotype, despite the high aggregation propensity of these variants [32]. In contrast, moderately destabilized, yet still aggregation-prone, variants of amyloidogenic proteins escape ER quality control and are efficiently secreted to extracellular environments [32,34]. This increases extracellular populations of these proteins available for concentration-dependent aggregation into toxic oligomers and amyloid fibrils that deposit on peripheral target tissues. As such, TTR amyloid disease patients expressing moderately destabilized TTR variants can secrete the mutant to levels similar to the wild-type protein, resulting in high concentrations of circulating amyloidogenic TTR that enhances aggregation into toxic oligomer and amyloid fibrils. When compared with patients expressing highly destabilized variants, patients expressing these more moderately destabilized, yet still aggregation-prone TTR variants present with a more severe disease [32]. This indicates that the ability for amyloidogenic proteins to escape ER quality control directly influences toxic aggregation of amyloidogenic proteins by controlling serum populations available for concentration-dependent aggregation.

Considering the importance of ER quality control in regulating the secretion of amyloidogenic proteins, it stands to reason that imbalances in ER proteostasis (i.e. ER stress) can directly impact extracellular aggregation of amyloidogenic proteins by disrupting ER quality control. ER stress is largely defined by the accumulation of non-native, aggregation-prone proteins within the ER lumen. This accumulation of non-native proteins increases the burden on ER proteostasis pathways leading to a breakdown in ER quality control. As a consequence of ER stress, proteins in non-native conformations could be trafficked from the ER to downstream secretory environments as a mechanism to relieve the burden on ER proteostasis pathways. In fact, multiple cellular mechanisms have evolved to regulate ER stress-dependent increases in the trafficking of non-native proteins. For example, in a regulated process termed rapid ER-stress-induced export (RESET), ER stress increases the trafficking of GPI-anchored proteins, including the amyloidogenic prion protein, to the cell surface in non-native conformations [43,44]. At the plasma membrane, these proteins are then directed to the lysosome for degradation. Alternatively, in response to ER stress, ER-targeted co-chaperones such as ERdj3 are secreted to extracellular environments in complex with non-native proteins (e.g. TTR) to preemptively protect extracellular environments from these aggregation-prone protein conformations [45–47]. Both RESET and ERdj3 co-secretion provide mechanisms to remove non-native proteins from the ER environment in response to ER stress, preventing their potential toxic intracellular aggregation [48].

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Despite these mechanisms, ER stress can also increase the extracellular aggregation of amyloidogenic proteins. For example, inducing ER stress with the SERCA inhibitor thapsigargin (Tg) increases the extracellular accumulation of soluble TTR aggregates commonly associated with distal toxicity in TTR amyloid disease pathogenesis [49,50]. This ER stress-dependent increase in TTR aggregates corresponds with a failure in ER quality control to address misfolded TTR, resulting in greater secretion of TTR in non-native conformations (Figure 1). TTR aggregates through a mechanism involving rate-limiting dissociation of the native tetramer, monomer misfolding, and subsequent concentration-dependent aggregation [15,16] (Figure 1). However, Tg-induced ER stress increases secretion of TTR in non-tetrameric conformations that can rapidly aggregate into soluble TTR oligomers by circumventing rate-limiting tetramer dissociation [49,50] (Figure 1). This ER stress-dependent increase in the secretion of non-native TTR provides a mechanism to explain how hepatic imbalances in the expression of ER proteostasis factors, a marker of ER stress, can promote toxic distal deposition of TTR oligomers and aggregates *in vivo* [21,23]. Furthermore, this work highlights a potential mechanism by which recipients of domino transplant livers from hereditary TTR amyloidosis patients experience a rapid onset of TTR amyloidosis due to transplantation of an 'aged' or 'stressed' liver that, as a result, may be secreting TTR in aggregation-prone conformations at a faster rate than early in the donor's life.

Collectively, the studies above show that ER proteostasis can directly influence extracellular aggregation of amyloidogenic proteins such as TTR through multiple mechanisms. The ability for moderately destabilized, amyloidogenic proteins to escape ER quality control and be efficiently secreted from mammalian cells increases extracellular populations of protein available for concentration-dependent aggregation. Furthermore, imbalances in ER proteostasis (i.e. ER stress) can promote the secretion of amyloidogenic proteins in non-native conformations that accelerate extracellular aggregation into toxic oligomers. Considering the importance of ER proteostasis in the context of extracellular protein aggregation, it is not surprising that mammals and other eukaryotes evolved mechanisms to regulate ER proteostasis in response to diverse types of pathologic insults that challenge ER quality control.

Regulating extracellular protein aggregation through the unfolded protein response (UPR)

The main mechanism to regulate ER proteostasis in response to pathologic insults is through activation of the UPR. The mammalian UPR comprises three integrated signaling pathways activated downstream of the ER transmembrane proteins IRE1, PERK, and ATF6 [51–53] (Figure 2). These three pathways are activated in response to ER stress and promote adaptive remodeling of ER proteostasis and global cellular physiology through both transcriptional and translational signaling. In the context of the secretory pathway, this remodeling functions to alleviate ER stress, restore ER quality control, and prevent the aberrant secretion of non-native, aggregation-prone proteins to downstream secretory environments. Thus, in response to acute ER insults, the UPR functions as a protective mechanism to regulate proteostasis within the ER and throughout the secretory pathway. However, in response to chronic or severe ER insults, IRE1 and PERK signaling promote apoptosis, highlighting an important role for the UPR in dictating cell fate in response to varying levels of ER stress [51–53]. Considering the focus of this review on the importance of ER proteostasis on the secretion and extracellular aggregation of amyloidogenic proteins, we specifically focus our discussion on the role of the UPR in regulating secretory proteostasis of amyloidogenic proteins, most notably our model protein TTR.

A primary way in which the UPR regulates secretory proteostasis is through the transcriptional remodeling of ER proteostasis pathways. This is achieved mainly through the activation of two UPR-associated transcription factors: XBP1s (activated downstream of IRE1) and ATF6 (a cleaved product of full-length ATF6) [51–53] (Figure 2, middle and right). These transcription factors induce overlapping, but distinct, subsets of ER proteostasis factors (e.g. chaperones, folding enzymes, ERAD factors) that differentially influence ER quality control and function [54–56]. The differential impact of IRE1/XBP1s and/or ATF6 signaling on ER proteostasis has been shown to distinctly influence the folding, trafficking, or degradation of destabilized, aggregation-prone proteins [54]. For example, selective activation of XBP1s or ATF6 differentially influences the degradation of different destabilized, non-secreted variants of α1-antitrypsin (A1AT), demonstrating the unique impacts of these two transcription factors on ER quality control [54,57,58]. This ability to influence ER quality control of destabilized proteins through IRE1/XBP1s or ATF6 activation offers unique opportunities to correct pathologic imbalances in ER proteostasis implicated in disease. Consistent with this, activation of IRE1/XBP1s or ATF6 signaling has been shown to reduce the trafficking and intracellular aggregation of disease-associated variants of rhodopsin [59–61].

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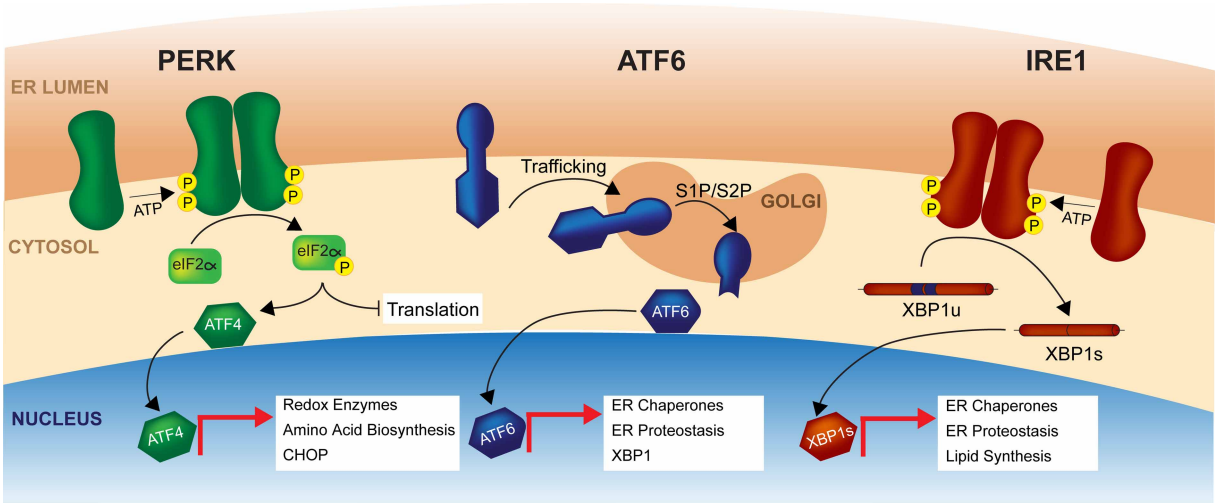

Figure 2. The mammalian unfolded protein response.

This illustration, adapted from [27], shows simplified mechanisms for ER stress-dependent activation of the three UPR signaling arms regulated downstream of PERK, ATF6, and IRE1. In response to ER stress, PERK undergoes dimerization and autophosphorylation, activating its cytosolic kinase domain (left). Upon activation, PERK selectively phosphorylates eIF2α, resulting in both transient translation attenuation and activation of transcription factors such as ATF4. ATF4 induces expression of proteins involved in diverse functions including redox, amino acid biosynthesis, and apoptotic signaling (e.g. *CHOP*). ATF6 is activated in response to ER stress through a mechanism involving increased trafficking to the Golgi, where it is proteolytically processed by site 1 and site 2 proteases (S1P and S2P, respectively; middle). This releases the active ATF6 N-terminal bZip transcription factor domain that translocates to the nucleus and induces expression of ER chaperones and proteostasis factors. Finally, in response to ER stress, IRE1 is activated through a mechanism involving oligomerization and autophosphorylation (right). This activates a cytosolic endoribonuclease domain involved in the non-canonical splicing of *XBP1* mRNA. Spliced *XBP1* (*XBP1s*) encodes an active transcription factor that regulates the expression of diverse ER chaperones and ER proteostasis factors. For further reading on the activation and functional implications of UPR signaling, please see [51–53].

In the context of systemic amyloid diseases, significant evidence has shown that ATF6 offers a unique opportunity to reduce the secretion and extracellular aggregation of structurally diverse amyloidogenic proteins through multiple mechanisms. Stress-independent activation of ATF6, but not XBP1s, selectively reduces the secretion of destabilized, aggregation-prone variants of TTR without impacting secretion of stable, non-amyloidogenic TTRs or the endogenous secretory proteome [33,54,62]. This ATF6-dependent reduction in destabilized TTR secretion corresponds with increased targeting of TTR to ERAD, preventing the potentially toxic accumulation of aggregation-prone TTR within the ER lumen. Importantly, the ATF6-dependent reduction in the secretion of destabilized TTR decreases the extracellular populations of protein available for concentration-dependent aggregation, thus lessening the accumulation of soluble TTR oligomers in extracellular environments [33,54,62]. This highlights how improving ER quality control to promote the degradation of destabilized amyloidogenic proteins such as TTR can reduce their toxic extracellular aggregation.

ATF6 activation also reduces secretion and extracellular aggregation of destabilized, aggregation-prone immunoglobulin LCs implicated in LC amyloidosis (AL). However, this reduction in secretion does not correspond with increased targeting of LCs to ER degradation pathways. Instead, ATF6 activation reduces the secretion of destabilized aggregation-prone LCs through a mechanism involving increased interactions with ATF6-regulated chaperones such as BiP [63,64]. These increased interactions function to retain destabilized LCs within the ER. Similarly, BiP overexpression also results in ER retention of destabilized TTR variants [36]. While the increased ER retention of destabilized amyloidogenic proteins, even in chaperone bound complexes, could lead to disruptions in ER function and induce ER stress [35], these results highlight the potential for ER proteostasis remodeling to reduce the secretion and toxic aggregation of amyloidogenic proteins through a mechanism independent of increased degradation. Apart from regulating the secretion of destabilized, aggregation-prone proteins, stress-independent activation of ATF6 also increases secretion of the co-chaperone ERdj3, which can directly regulate extracellular proteostasis through its extracellular chaperoning activity [45–

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47]. Together these results indicate that ATF6-dependent remodeling of ER proteostasis pathways broadly improves ER quality control for multiple disease-associated, aggregation-prone proteins including TTR and amyloidogenic LCs, albeit through different mechanisms.

IRE1/XBP1s activation also has significant potential for reducing the secretion and toxic aggregation of amyloidogenic proteins involved in systemic amyloid diseases. For example, stress-independent IRE1/XBP1s activation reduces the extracellular accumulation of the toxic cleavage product Aβ implicated in Alzheimer's disease through a mechanism involving increased ERAD of amyloid precursor protein (APP) — the protein that is proteolytically processed to produce Aβ [65,66]. While there is no evidence to date of a systemic amyloid disease-associated protein that is sensitive to IRE1/XBP1s activation, it is clear that this UPR pathway has significant potential to influence the secretion and toxic aggregation of amyloidogenic proteins and should be further considered as a strategy for reducing the secretion and toxic protein aggregation associated with systemic amyloid disease pathogenesis.

In contrast with ATF6 and IRE1/XBP1s, the PERK arm of the UPR regulates cellular physiology during ER stress through both transcriptional and translational signaling [51,53] (Figure 2, left). In response to ER stress, the cytosolic PERK kinase domain is activated resulting in the selective phosphorylation of the α subunit of eukaryotic initiation factor 2 (eIF2α). This leads to both a transient reduction in protein synthesis and the activation of the UPR-associated transcription factor ATF4. ATF4 induces expression of multiple genes involved in biological functions including amino acid biosynthesis, redox regulation, and apoptotic signaling [51,53]. Through this combination of PERK-regulated translational and transcriptional signaling, PERK functions to dictate cellular proteostasis and survival following varying levels of ER stress.

Not only does PERK have an important role in regulating global cellular physiology, but PERK signaling also significantly influences ER proteostasis and quality control. For example, translational attenuation induced upon PERK activation reduces the co-translational import of newly synthesized proteins into the ER during conditions of ER stress [51–53]. This reduces the load of nascent, unfolded peptides entering the ER, freeing ER proteostasis factors to engage with the increased population of non-native proteins induced by the ER insult. Furthermore, PERK signaling is critical for regulating proper UPR-dependent remodeling of ER proteostasis pathways in response to ER stress. For example, transcriptional regulation of ER proteostasis factors in cells expressing pro-insulin was dysregulated upon PERK inhibition, highlighting an important role for PERK in the adaptive remodeling of ER proteostasis pathways within these cells [67]. PERK signaling also regulates two other aspects of ER proteostasis, ER-to-Golgi trafficking and ERAD [68,69], further highlighting an important role for PERK in regulating secretory proteostasis during ER stress.

Consistent with this, genetic or pharmacologic inhibition of PERK significantly impairs ER quality control of multiple disease-associated proteins. Inhibiting PERK signaling reduces the ER-stress dependent trafficking of proteins such as collagen, insulin, and mutant rhodopsin, increasing their intracellular accumulation and aggregation [67,70–72]. Furthermore, pharmacologic inhibition of PERK signaling increases the ER stress-dependent secretion of TTR in non-native, aggregation-prone conformations, directly increasing the extracellular accumulation of toxic soluble TTR aggregates [50]. While it is difficult to explicitly define the contributions of PERK translational and transcriptional signaling on the disruptions of ER quality control described above, these results clearly define an important role for PERK in protecting the ER and secretory environments against toxic protein aggregation.

The dependence of secretory proteostasis on the UPR indicates that dysregulation of UPR signaling in cells synthesizing disease-associated amyloidogenic proteins could unexpectedly sensitize extracellular environments to ER stress-induced accumulation of toxic protein aggregates. The capacity for cells to regulate ER proteostasis through UPR activation declines with age [73,74]. This suggests that aging-dependent disruptions in metabolism and/or UPR signaling in tissues secreting amyloidogenic proteins could increase extracellular protein aggregation through mechanisms such as increased secretion of proteins in non-native conformations. Consistent with this, livers of aged mice with cardiac TTR^{WT} deposition demonstrated decreased expression of select UPR target genes when compared with livers of aged mice with no TTR deposition [23]. While a causative relationship between dysregulated UPR signaling in tissues such as the liver and distal deposition of amyloidogenic proteins remains to be further established for TTR and other systemic amyloid diseases, these results highlight the potential for aging-dependent changes in UPR-dependent ER regulation to significantly contribute to systemic amyloid disease pathogenesis by promoting imbalances in ER quality control that enhance extracellular protein aggregation and distal deposition.

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Therapeutic targeting of ER proteostasis in systemic amyloid diseases

Considering the importance of amyloidogenic protein serum concentrations for systemic amyloid disease pathogenesis, it is not surprising that many therapeutic strategies to mitigate these diseases focus on the tissues that produce the amyloidogenic protein. For years, the primary standard of care for many systemic amyloid diseases involved liver transplantation, where a liver synthesizing a destabilized, amyloidogenic protein was replaced with a liver synthesizing the wild-type protein [16]. Transplantation prevents the hepatic secretion of destabilized, aggregation-prone proteins (e.g. TTR), reducing their extracellular aggregation and distal deposition implicated in disease pathogenesis. siRNA and antisense RNA strategies have also been developed to reduce the hepatic synthesis of destabilized, amyloidogenic proteins, similarly reducing their hepatic secretion and subsequent concentration-dependent aggregation into toxic oligomers and amyloid fibrils [75–77].

As a less invasive treatment, small molecule-based strategies have been developed to mitigate the pathogenesis of systemic amyloid diseases. Notably, the small molecule tafamidis was developed to bind to the native TTR tetramer and prevent the rate-limiting tetramer dissociation required for toxic TTR aggregation [78]. This small molecule has proven effective in reducing TTR-associated toxicity and has been approved by multiple regulatory agencies including the FDA and EMA to treat TTR induced cardiomyopathy and hereditary TTR induced polyneuropathy [77,79]. Other compounds such as the non-steroidal anti-inflammatory compound diflunisal have shown similar potential for stabilizing TTR tetramers and preventing toxic TTR aggregation in patients [80,81].

Tafamidis has generally been thought to protect against TTR amyloid disease by stabilizing secreted TTR tetramers, thereby preventing tetramer dissociation and subsequent toxic TTR aggregation in extracellular environments. However, tafamidis is cell-permeable and can stabilize TTR tetramers within the ER [33,50]. This suggests that tafamidis-dependent stabilization of TTR tetramers within the liver could contribute to the clinical protection observed for this compound. The ability for compounds like tafamidis to bind to destabilized TTRs within the ER offers significant advantages for preventing TTR-associated aggregation and toxicity. For example, stabilization of TTR tetramers within the ER will increase the population of TTR secreted as stabilized TTR tetramers that are unable to undergo tetramer dissociation and subsequent aggregation (Figure 3A). Moreover, the presence of compounds that bind to TTR tetramers intracellularly can increase the population of TTR secreted as tetramers, reducing the secretion of TTR in non-native, aggregation-prone conformations that can rapidly aggregate in extracellular environments (Figure 3A). Recent results support these predictions showing that treatment with tafamidis increases the secretion of a destabilized TTR variant as native tetramers and reduces extracellular TTR aggregates, when compared with the cell impermeable tafamidis analog tafamidis-sulfonate [49,50]. Tafamidis was also shown to significantly reduce the ER stress-dependent extracellular accumulation of TTR aggregates relative to tafamidis-sulfonate, further highlighting the importance of intracellular TTR tetramer stabilization by tafamidis in lowering extracellular TTR aggregates [50]. While the clinical benefits of intracellular tafamidis-dependent stabilization of TTR tetramers have not been demonstrated to date, these results highlight a distinct advantage for intracellular stabilization of amyloidogenic proteins such as TTR using small molecules, which should be considered when developing similar approaches for other amyloidogenic proteins.

Aside from pharmacologic chaperoning, extracellular aggregation of amyloidogenic proteins can also be reduced through the adaptive remodeling of ER proteostasis pathways to reduce the secretion and subsequent concentration-dependent extracellular aggregation of amyloidogenic proteins without affecting wild-type protein secretion [27]. A significant advantage of directly targeting ER proteostasis for systemic amyloid diseases is that one strategy could improve ER quality control for a variety of amyloidogenic proteins, potentially allowing for a single therapeutic approach to be applied broadly to reduce extracellular aggregation of structurally diverse, disease-associated proteins. One way to promote adaptive remodeling of ER proteostasis pathways is through activation of protective UPR signaling pathways such as ATF6. As indicated above, stress-independent ATF6 activation reduces the secretion and toxic aggregation of multiple amyloidogenic proteins including both TTR and immunoglobulin LCs [33,54,57,60,63,64]. This suggests that pharmacologic remodeling of ER proteostasis pathways by ATF6-activating compounds could similarly reduce secretion and toxic aggregation of these amyloidogenic proteins (Figure 3B).

ER proteostasis regulating compounds that selectively activate the ATF6 UPR signaling pathway through covalent targeting of a subset of ER-localized PDIs have recently been identified providing new opportunities to

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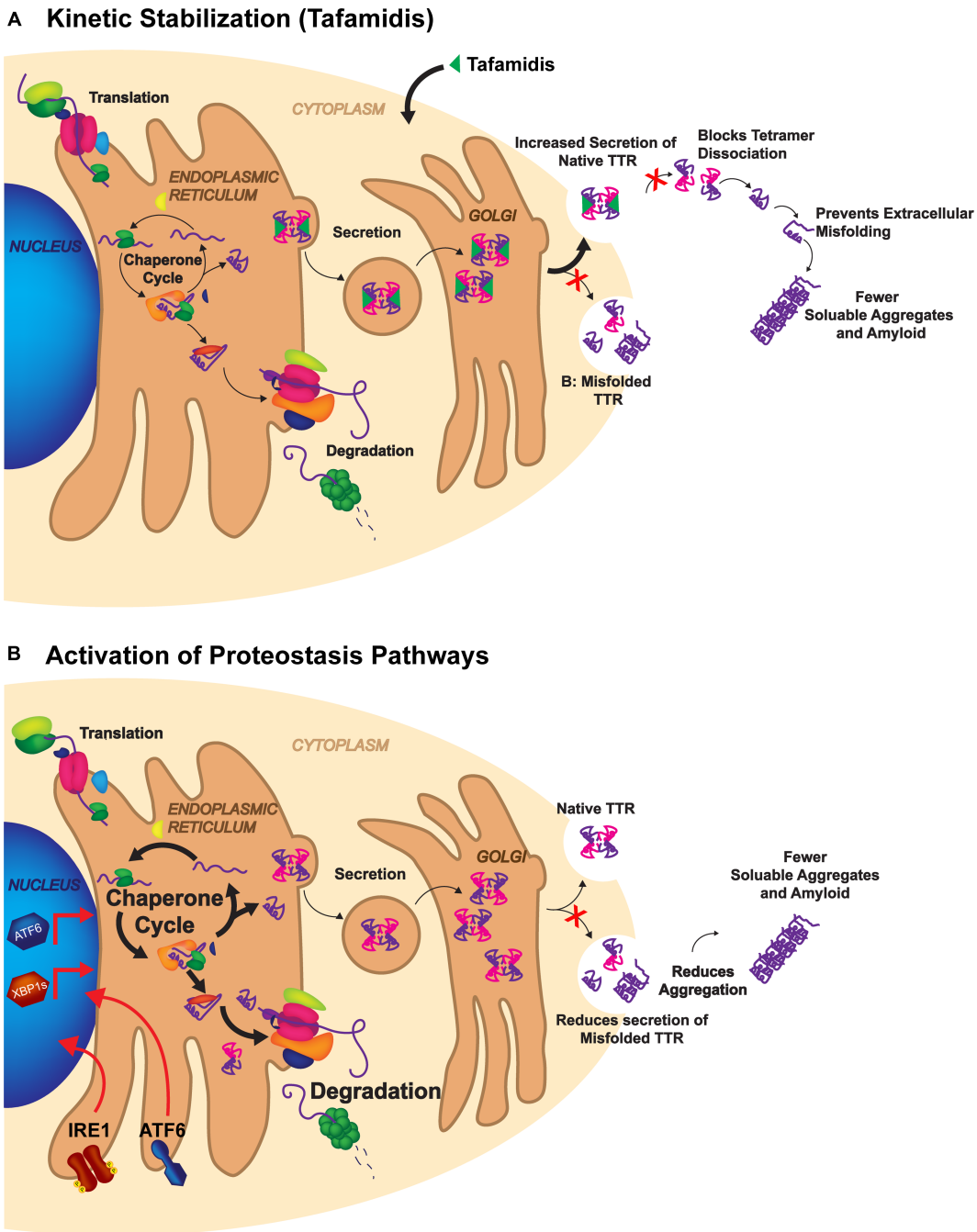

Figure 3. Therapeutically targeting ER proteostasis to reduce toxic TTR aggregation.

(**A**) Administration of cell-permeable TTR binding compounds like tafamidis reduces extracellular TTR aggregation by stabilizing TTR tetramers within the ER, reducing the population of TTR secreted in non-native conformations and preemptively preventing extracellular TTR tetramer dissociation. (**B**) Increasing ER proteostasis capacity through mechanisms such as ATF6 activation increases ER quality control to selectively reduce the secretion of destabilized, aggregation-prone TTRs. This reduces the extracellular populations of these proteins available for concentration-dependent aggregation.

address the potential for pharmacologically targeting ER proteostasis to reduce the secretion and toxic aggregation of amyloidogenic proteins [62,82]. Using these compounds, it has been shown that pharmacologic remodeling of ER proteostasis reduces the secretion and extracellular aggregation of multiple disease-associated amyloidogenic proteins including TTR and LCs in liver-derived cell models and AL patient-derived plasma

cells, respectively [62]. Importantly, these compounds do not globally disrupt the secretion of the endogenous secretory proteome or stable, non-amyloidogenic variants of TTR or LC [62]. While the specific mechanism by which these compounds reduce the secretion of amyloidogenic proteins remains to be established, these results demonstrate the broad potential for pharmacologic targeting of ER proteostasis to reduce the secretion and toxic aggregation of multiple destabilized, amyloidogenic proteins [27].

Apart from the remodeling of ER proteostasis pathways, these ATF6-activating compounds have also been shown to reduce hepatic ER stress in mice subjected to chemical insult through an ATF6-dependent mechanism [83]. This suggests that these compounds could decrease ER stress in tissues such as the liver, potentially reducing the ER-stress dependent secretion of proteins in non-native conformations that rapidly aggregate into toxic oligomers [49,50]. Thus, pharmacologically targeting ER proteostasis in cells secreting destabilized, amyloidogenic proteins offers many potential advantages for mitigating extracellular aggregation and distal toxicity of amyloidogenic proteins. However, the translational potential for this approach remains to be established.

The above results highlight how directly impacting amyloidogenic protein ER proteostasis using either protein-specific strategies (e.g. tafamidis-dependent TTR tetramer stabilization) or broad pharmacologic approaches to remodel ER proteostasis (e.g. small molecule ATF6-activating compounds) can reduce the secretion and toxic aggregation of destabilized amyloidogenic proteins through different mechanisms (Figure 3A,B). Moving forward, it will be interesting to determine how other pharmacologic strategies to manipulate ER proteostasis influence the secretion of amyloidogenic proteins. For example, pharmacologic enhancement of ERAD or other ER degradation pathways has the potential to increase degradation and reduce the secretion of amyloidogenic proteins, mimicking the results observed with ATF6 activation [33,54,62]. The development of new strategies to target ER proteostasis in systemic amyloid diseases will reveal new opportunities to therapeutically intervene in disease pathogenesis. Furthermore, it will be exciting to define potential synergistic benefits for combining the two strategies to reduce amyloidogenic protein aggregation shown in Figure 3B. While the benefits of combining protein-specific strategies (e.g. tafamidis) and ER proteostasis regulators has not been demonstrated as of yet, the ability to both target the protein and the ER environment to mitigate toxic extracellular aggregation offers a unique opportunity to enhance ER proteostasis and quality control on multiple levels to reduce the toxic protein aggregation causatively associated with systemic amyloid disease pathogenesis.

Concluding remarks

While peripheral toxicity has long been implicated in systemic amyloid diseases, it is becoming increasingly clear that dysregulation of ER proteostasis in tissues producing amyloidogenic proteins, such as the liver, has an important role in defining disease pathogenesis. As we continue to learn more about the importance of these tissues in the pathogenesis of amyloid diseases, we will identify key aspects of ER proteostasis, maintenance, and function that are critical determinants in dictating the onset and pathology associated with individual disorders apart from just the TTR-associated diseases. Through these efforts, we hope to identify specific biomarkers in tissues secreting amyloidogenic proteins that can be used to track disease progression and dictate treatment administration. Additionally, further defining the importance of ER proteostasis in the onset and pathogenesis of systemic amyloid diseases will allow identification of new opportunities to reduce the secretion and toxic aggregation implicated in these disorders by targeting different aspects ER proteostasis.

Competing Interests

The authors declare that there are no competing interests associated with the manuscript.

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Abbreviations

ER, endoplasmic reticulum; ERAD, ER-associated degradation; LCs, light chains; PDIs, protein disulfide isomerases; RESET, rapid ER-stress-induced export; TTR, transthyretin.

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