PROTEOSTATIC SIGNALING & CONTROL OF PROTEIN SYNTHESIS

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Highlights:

- The Integrated Stress Response (ISR) functions to maintain proteostasis by stalling translation.
- The mitochondrial UPR (mtUPR) operates through the ISR.
- mTORC1 mediated translation is lost during proteostasis
- Stress granule formation can also cause translation stalling

ABBREVIATIONS

UPS, Ubiquitin Proteasome System; UPR, Unfolded Protein Response; mtUPR, Mitochondrial Unfolded Response; ER, Endoplasmic Reticulum; ISR, Integrated Stress Response; eIF2a, Eukaryotic Initiation Factor 2 Alpha; uORF, Upstream Open Reading Frame; ATF4, Activating Transcription Factor 4; PKR, Protein Kinase R; PERK, protein kinase R (PKR)-like endoplasmic reticulum kinase; ARE, Antioxidant Response Elements; CRT, Calreticulin; TOP, Terminal Oligopyrimidine Tract; RIDD, Regulation Ire1 Dependent Decay; HSR, Heat Shock Response; HSF1, Heat Shock Factor 1; MAMs, Mitochondrial Associated Membranes; IDR, Intrisically Disordered Region.

ABSTRACT

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The tremendous diversity and complexity of proteins invariably results in protein misfolding, to which cells have evolved numerous mechanisms of mitigating. Degrading misfolded proteins is perhaps the most intuitive strategy, but also critical to managing proteostasis are the elaborate mechanisms of translational control. Attenuated rates of translation ameliorate protein misfolding by downregulating the flux of new protein and conserving ATP. Loss of translational control constitutes a major proteostatic dysfunction capable of causing or exacerbating protein misfolding diseases, while interventions aimed at downregulating protein synthesis are generally protective. In this review, I examine the critical signaling networks employed to control translation with an emphasis on current research related to neurodegeneration. This includes the Unfolded Protein Response (UPR), the mitochondrial UPR (mtUPR), mTORC1 signaling, and stress granule formation.

INTRODUCTION

Cellular proteostasis relies on highly regulated coordination between protein translation, chaperone-assisted folding, and protein degradation. This "central dogma" of protein homeostasis encompasses a wide range of interconnected pathways such as the Ubiquitin-Proteasome

System (UPS), the UPR, and autophagy. Although a great deal of research has interrogated the mechanisms of degrading proteotoxic protein, cells also employ strict regulations of protein synthesis during proteostatic emergencies.

Cells possess multiple proteostatic sensing networks aimed at attenuating translation when challenged by the accumulation of misfolded protein. The importance of these networks is illustrated by experiments that have uncoupled these translational regulations during altered proteostasis, and this consistently aggravates protein misfolding and compromises cell viability [2, 3, 4]. In contrast, genetic, dietary, and pharmacological means of downregulating translation is sufficient to extend lifespan and protect against protein misfolding diseases by improving proteostasis [6-13]. The cytoprotection offered by attenuated translation is attributed to three principles. First, slowing the flux of new protein affords chaperones and degradative machinery more time to restore proteostasis [14]. Temporarily halting translation is also an effective strategy of maintaining ATP concentrations since translation is energetically expensive [15]. Calculations have estimated that translation is responsible for consuming 50-70% of a cell's pool of ATP [16, 17]. Lastly, translation is an error-prone process and requires chaperones that, during proteostatic disruption, could be preoccupied or sequestered in aggregates resulting in protein misfolding during translation. The inaccuracy of translational machinery is estimated to result in point mutations every 10^3 to 10^4 codons, affecting about 18% of all proteins [18, 19]. And of these mutations, roughly 10-50% of them are loss of folding mutations [20]. Furthermore, stress-induced deficiencies in translation fidelity increases the rate of protein misfolding, underscoring the importance of chaperone surveillance during translation [121, 123]. Many proteins, and protein complexes such as mTORC1, also require an available pool of chaperones in order to simply adopt correctly folded conformations, regardless of the presence of mutations. Indeed, up to 30% of newly synthesized proteins emerge from the ribosome misfolded, and, in total, about 12-15% of them are promptly degraded in a process termed cotranslational ubiquitination [21]. Thus deregulated translation during cellular stress challenges metabolic homeostasis by depleting ATP, while additionally straining proteostasis due to the unsupervised production of misfolded protein that may exceed the capacity of refolding and degradative networks.

Although transient translational stalling can ameliorate protein misfolding, it is vital that a cell restores proteostasis expeditiously, since sustained translation inhibition due to unresolved protein misfolding leads to cellular dysfunction and, eventually, cell death [22, 23]. The physiological consequence of persistent translation inhibition is exemplified in various developmental neurological diseases, such as Vanishing White Matter Syndrome, caused by mutations in the eIF2B translation initiation factor, resulting in significant defects in translation and early onset neurodegeneration [24]. There is also evidence that cells derived from patients with conventional protein misfolding disorders, like Alzheimer's Disease (AD), Parkinson's Disease (PD), and polyglutamine repeat disorders, are deleteriously affected by chronic translation inhibition [124]. Translation inhibition becomes unsustainable as the proteome becomes increasingly damaged, such as by oxidation, or depleted of essential proteins from degradation, and culminates in p53 dependent apoptosis [LINK [LINK LINK]. Proteostatic control of protein translation is likely beneficial during the early stages of disease while proteotoxic stress is manageable, but contributes to cellular dysfunction when the cell becomes

incorrigibly overwhelmed by misfolded protein. Thus dynamic monitoring and control of translation, along with efficient proteome turnover, are both critical facets to healthy cellular proteostasis.

Protein misfolding and aggregate formation is nearly ubiquitous across neurodegenerative diseases and, not surprisingly, significant perturbations in the major proteostatic signaling networks (UPS, autophagy, UPR, and mtUPR) are common. Many genetic manipulations of these networks in mouse models, or SNPs in humans, cause or increase susceptibility to neurodegenerative disease [27]. Neuronal vulnerability to proteotoxic stressors stems primarily from their status as post-mitotic cells, which are unable to dilute aggregates through cell division. The accumulation of aggregates may also be uniquely neurotoxic since neurons must facilitate vesicular transport through axons that can become physically obstructed by aggregated protein. Additionally, neurons are metabolically demanding, and even transient lapses in ATP production lead to cell death, as illustrated by their susceptibility to oxygen deprivation or oxidative phosphorylation failure. Neurodegenerative diseases are fundamentally disorders of altered proteostasis, thus a general understanding of how proteostatic signaling networks are activated, regulated, and communicated within the neuron may yield important discoveries and therapeutic opportunities.

In this review, I describe how proteostatic dysfunction is communicated within the cell to achieve translational control and appropriate gene expression in order to ameliorate proteotoxic stress. Specifically, I will examine how protein misfolding in the endoplasmic reticulum (ER) and the mitochondria initiate proteostatic measures that regulate translation through the Integrated Stress Response (ISR), how the inactivation of mTORC1 complements this process, and how stress granules sequester latent mRNA and protein during translational shutdown.

The ISR

The ISR is an evolutionarily conserved cellular program that converges on the phosphorylation of ribosomal initiation complex subunit eIF2a (eukaryotic initiation factor 2 alpha). Phosphorylation of eIF2a at serine 51 blocks the formation of the ribosomal initiation complex on mRNA resulting in attenuated rates of translation (~80% decrease) and ATP conservation [LINK]. Paradoxically, p-eIF2a actually promotes the translation of certain transcripts bearing alternative upstream open reading frames (uORFs). Activating Transcription Factor 4 (ATF4) is the most well-understood p-eIF2a activated uORF and has earned a reputation as a stress-induced transcription factor capable of inducing proteostatic, autophagic, and antioxidant gene expression. By utilizing a basic leucine zipper domain, which requires dimerization for transcriptional activation, ATF4's transcriptional outcome can be fine-tuned by a diverse set of co-transcription factors. Although ATF4 typically homodimerizes, it also heterodimerizes with many other transcription factors such as AP-1, ATF3, or CHOP, and these interactions vastly expand ATF4's transcriptional versatility. ATF4/CHOP dimers, for example, are responsible for shifting the ISR's gene expression from cytoprotective to proapoptotic [28]. By utilizing stringent stress-activated translation through p-eIF2a, and heterodimerization with a diverse set of transcription factors, ATF4 has become a central player in the transduction of multiple stress pathways.

The critical checkpoint dictating this pathway is eIF2a phosphorylation, which is orchestrated by only four

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kinases; GCN2, HRI, PKR, and PERK. GCN2 is canonically activated by amino acid starvation, HRI by heme deficiency, PKR by double-stranded RNA, and PERK by ER protein misfolding (Reviewed in LINK). The activation of each of these kinases is derived from unique stimuli, thereby granting cells the ability to integrate diverse sources of stress into a single, but highly malleable, cytoprotective transcriptional response. Each of the following pathways discussed in this review involves, at least in part, this Integrated Stress Response, and thus the ISR has emerged as a recurring theme in proteostasis.

The UPR

Misfolded proteins are capable of triggering the ISR, and thus translational stalling and eIF2a induced ATF4 expression, through the PERK arm of the UPR. PERK, an ER membrane-embedded kinase activated by dimerization, is constitutively bound and inhibited by ER folding chaperone BiP. Misfolded proteins, however, compete BiP away from PERK enabling its homodimerization, activation, and phosphorylation of eIF2a. ER calcium depletion, a phenomenon observed in various neurodegenerative diseases like ALS, HD, and PD, has also been shown to activate PERK [29]. Since many ER folding chaperones, including BiP itself, require high calcium concentrations in order to function, protein misfolding could be exacerbated by chronic calcium depletion. Regardless, PERK-mediated phosphorylation of eIF2a triggers a rapid decrease in protein translation, and the noncanonical translation of ATF4, which subsequently translocates to the nucleus and upregulates cytoprotective gene expression. Prolonged ATF4 transcriptional activity leads to the upregulation of CHOP, a secondary transcription factor that promotes apoptotic gene expression and accounts for ATF4's duration dependent toxicity. In neurons, ATF4/CHOP dimers trigger ER stress-induced apoptosis through the BH3 protein PUMA [30]. CHOP also upregulates GADD34, a stress-induced subunit of the PP1A phosphatase that redirects its substrate specificity to P-eIF2a and restores normal translation [31]. Premature restoration of protein synthesis, through both GADD34 dependent and independent PP1A activation, has been shown to promote cell death by depleting ATP and increasing ROS generation [31, 32].

Although PERK is recognized most often for its role in connecting the UPR with the ISR and ATF4, the kinase is also important for initiating an antioxidant and autophagic response through the activation of NRF2. PERK-mediated phosphorylation of NRF2 displaces its negative regulator KEAP1, preventing NRF2 ubiquitination and unveiling a nuclear localization signal [33, 34]. After activation by PERK, NRF2 translocates to the nucleus and binds Antioxidant Response Elements (ARE) promoting cytoprotective gene expression. NRF2 promotes the expression of ER folding chaperones, antioxidant proteins, and various autophagic proteins like LAMP2A, for chaperone-mediated autophagy, and p62, for macroautophagy [35, 36, 37]. Interestingly, p62 is capable of reciprocally activating NRF2 by binding and displacing KEAP1, potentially fueling a positive feedback loop during NRF2 dependent autophagy.

Like NRF2, ATF4 is also responsible for activating autophagic gene expression including genes such as ATG5, p62, and Beclin1 [38, 39]. A study by B'Chir et al has even suggested that p-eIF2a/ATF4 is required for stress-induced autophagy [40]. In addition to the transcriptional upregulation of autophagic genes, PERK/eIF2a phosphorylation may also be critical to the processing of the autophagic cargo adaptor

receptor LC3 into LC3-II following expanded polyglutamine expression, but the mechanism is not understood [41]. The activation of autophagy is not only a consequence of translation inhibition but can also sustain it by promoting the degradation of ribosomes (ribophagy) [42]. The influence ATF4 and NRF2 have on autophagy could, in theory, function to prolong translation inhibition. Indeed, ATF4 activation results in decreased ribosomal subunit protein expression, an effect that could be related to ribophagy [43].

There is also evidence that ATF4 and NRF2 physically interact, and do so in such a way to prevent ATF4 from binding and initiating transcription at the promoter of proapoptotic CHOP [44]. In addition to preventing apoptotic gene expression, ATF4/NRF2 cotranscription potentiates antioxidant gene expression [45]. The significant overlap between the cytoprotective transcriptional outcomes of NRF2 and ATF4, and their common mechanism of activation through PERK, suggests that the UPR likely relies on both transcription factors for proteostatic gene expression in response to ER protein misfolding.

Loss of BiP due to the presence of misfolded protein can also activate the ER stress sensor Ire1, resulting in its multimerization and formation of an atypical endoribonuclease. UPR induced Ire1 oligomerization communicates to the nucleus via the removal of an inhibitory mRNA motif in the 5' UTR of the XBP1 transcription factor. Following Ire1 dependent splicing, XBP1 is translated and undergoes nuclear translocation to trigger proteostatic gene expression, including folding chaperones and machinery involved in ER-associated degradation.

Dimerized Ire1 can participate directly in translational control by a process termed RIDD, Regulated Ire1 Dependent Decay, whereby mRNA, miRNA, and even rRNA critical to translation, are cleaved at consensus sites that share homology to XBP1 resulting in their degradation [LINK]. RIDD has been demonstrated to target and degrade CReP mRNA, a regulatory subunit of eIF2a that contains constitutive phosphatase activity and maintains a low basal level of p-eIF2a [46]. Ire1 deletion nearly doubles the expression of CReP and, as a result, greatly decreases p-eIF2a in response to ER stress. By facilitating the degradation of CReP, RIDD functions to amplify PERK/eIF2a/ATF4 signaling.

Despite their similarities, RIDD mediated RNA decay and XBP1 cleavage appear to be distinct and pharmacologically separable mechanisms. Generally, dimerized Ire1 is associated with RIDD and cell death, while oligomerized Ire1 (four or more) is associated with XBP1 splicing and cell survival [47]. Interestingly, the proapoptotic membrane pore-forming proteins Bak and Bax, which localize to the ER membrane in addition to the mitochondria, may critically regulate Ire1 multimerization status. During normal conditions, Bak and Bax bind to Ire1 and promote its oligomerization in order to induce cytoprotective XBP signaling. During chronic ER stress, however, BH3 proteins are upregulated, which compete for Bak/Bax binding sites. Ire1's ability to bind and sequester Bak/Bax is then diminished leading to pore formation and cytotoxic release of ER contents, an event partially dependent on the expression of the BH3 protein Bnip3 [48]. ER membrane permeabilization, like mitochondrial membrane permeabilization, promotes apoptosis by facilitating the release of cytotoxic molecules such as calcium, ROS, and apoptotic proteins like Calreticulin (CRT). CRT can be repurposed and relocated from the ER, where it functions as a critical folding chaperone, to the plasma membrane, where it serves as an immunogenic "eat

me" signal, and has been shown to promote the phagocytosis of live viable neurons by microglia [49, 50]. Although the details are complex, current models suggest Bak/Bax interactions facilitate Ire1 clustering during proteotoxic stress and promotes cytoprotective XBP1 splicing, but prolonged proteotoxic stress, and increased BH3 protein expression, exhausts Ire1's capacity to bind Bak/Bax. This results not only in Bak/Bax pore formation, but also Ire1 declustering and a shift from cytoprotective XBP splicing to proapoptotic RIDD [51].

It is not clear how Ire1 is declustered during proteotoxic stress in order to release Bak/Bax, but one possibility is that XBP1 mediated gene expression upregulates folding chaperones that monomerize Ire1, and possibly facilitates the release of Bak/Bax. ER co-chaperone ERdj4, for example, is upregulated by XBP1 and functions to disaggregate Ire1 clusters by reattaching BiP, which could, in the process of Ire1 monomerization, enhance Bak/Bax oligomerization [52]. Besides its endoribonuclease activity, oligomerized Ire1 also serves as a signaling platform that recruits ASK1 and TRAF2, which collectively lead to the recruitment and activation of the stress signaling kinase JNK, a stress signaling kinase [53]. In sum, Ire1 dimerization or oligomerization is induced following the loss of BiP to misfolded protein, leading to several distinct processes including RIDD, XBP1 cleavage, Bak/Bax induced pore formation, and JNK activation.

The mtUPR

The ISR is also activated by the mitochondrial unfolded protein response, mtUPR. Despite its name, and the suggestion of a regulated response to misfolded protein, studies examining tissues from patients with bonafide mitochondrial protein misfolding diseases have not found patterns of gene expression marked by the induction of mitochondrial chaperones or proteases, as the classic mtUPR program was envisioned [55]. The inability to clearly define the mtUPR probably results from the interconnectedness of mitochondrial dysfunctions. For example, mitochondrial defects, such as import inhibition, oxidative phosphorylation (OXPHOS) deficiency, or mitochondrial or nuclear DNA mutations, can all result in misfolded proteins, and thus the mtUPR. Likewise, the accumulation of misfolded proteins in the mitochondria can cause import inhibition, OXPHOS deficiency, and mtDNA mutations. The term mtUPR is thus used here to describe any mitonuclear signaling event occurring during mitochondrial dysfunction that functions to restore mitochondrial homeostasis.

The mtUPR triggers a host of cytoprotective genes that, like the ISR, appear to rely primarily on p-eIF2a mediated ATF4 gene expression. A recent high throughput evaluation of the mitochondrial stress response in HeLa cells by Quiros et al, found many conventional mitochondrial toxins, including uncoupling agent FCCP, OXPHOS inhibitors, mitochondrial ribosomal inhibitor doxycycline, or a mitochondrial import inhibitor, each triggered broad cytoprotective gene expression that was largely dependent on p-eIF2a induced ATF4 activation [43]. Interestingly, none of the conventional eIF2a kinases were implicated, suggesting a unique mechanism of ISR activation (discussed below). Analysis of KEGG pathways affected by these toxins found mitochondrial ribosomal biogenesis to be the most significantly down-regulated pathway, while mitochondrial folding chaperones (the classic mtUPR) were neither upregulated or downregulated. In mouse skeletal muscle the

mtUPR, caused by a genetic autophagy deficiency that results in the accumulation of dysfunctional mitochondria, upregulates p-eIF2a and ATF4 dependent expression of Fgf21 [56]. Fgf21 is a critical stress induced mitokine that promotes β-oxidation and glucose uptake. ATF4 is also required for the expression of genes involved in one carbon metabolism, a metabolic program upregulated during mitochondrial stress [LINK, 59, 58]. Collectively, these results suggest mitochondrial stress is commonly dependent on the eIF2a/ATF4 axis in order to activate cytoprotective gene expression.

If mitochondrial stress, and compensatory transcriptional program, are signaled through eIF2a phosphorylation and ATF4, what kinase is mediating this pathway? Celardo et al found PERK was essential in mediating eIF2a phosphorylation and ATF4 dependent expression of one carbon metabolism genes [58]. In a Drosophila model of mtUPR induced dendritic degeneration, researchers Tsuyama et al discovered eIF2a phosphorylation by PERK was essential in mediating dendritic retraction [61]. PERK phosphorylation was required for dendritic retraction in response to mitochondrial dysfunction or protein misfolding. This suggests that dendritic degeneration in response to mitochondrial dysfunction, a degenerative but stress adaptive response aimed at preserving ATP, may function through a PERK initiated ISR signaling network. Authors Michel et al found mtDNA depletion triggers the ISR and, interestingly, discovered this process to be dependent on GCN2 expression [60]. In addition to PERK and GCN2, PKR has also been implicated in the transduction of mitochondrial stress. The mtUPR induced by mutant OTC, a protein that misfolds during mitochondrial import, activated a transcriptional response in intestinal cells that was contingent on PKR mediated eIF2a phosphorylation [62]. The convergence of various mitochondrial stressors onto eIF2a phosphorylation suggest global translational inhibition, and downstream ATF4 transcription, are critical factors orchestrating the mtUPR.

Besides the traditional eIF2a kinases, a recent study by Khan et al provided evidence that mTORC1 can activate ATF4 translation following mitochondrial dysfunction [63]. Despite this seemingly paradoxical relationship (mTORC1 promotes translation while eIF2a/ATF4 inhibits translation), this study provides compelling evidence that mTORC1 can, at least in some circumstances, function upstream of ATF4 activation during mitochondrial stress. Indeed, the Quiros et al study discussed above did find significant upregulation of mTORC signaling components following the mtUPR. The implication of each eIF2a kinase, or none at all, in mediating the mtUPR suggests there are multiple mechanisms by which mitochondrial dysfunction is relayed to the ISR.

In addition to ATF4, the structurally and functionally related ATF5 transcription factor has also been implicated in the mtUPR. Research by Fioerese et al discovered that ATF5, a transcription factor also induced by eIF2a phosphorylation, is essential to the transduction of the mtUPR in C. elegans [64]. ATF5 is an intriguing candidate due to its partial homology with ATFS-1, a previously validated mtUPR transcription factor in C. elegans. Mammalian ATF5, but not ATF4, can functionally replace ATSF-1 in C. elegans and ameliorate mitochondrial dysfunction. In mammalian cells, ATF5 expression can be induced by proteostatic stress derived from proteasome inhibition and mitochondrial depolarization, both of which trigger its expression through eIF2a phosphorylation. Following translation, ATF5 translocates to the nucleus where it upregulates various mitochondrial folding chaperones and ultimately restores mitochondrial proteostasis.

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Since ATF4 and CHOP have both been confirmed to bind the promoter of ATF5 and activate its transcription, it is plausible that ATF5 functions secondary to ATF4 activation [65].

The mechanistic link between mitochondrial stress and eIF2a kinases, and thus ATF4 and ATF5 translation, has not been identified, but several candidate mechanisms exist. Since the mtUPR is marked by membrane depolarization and import deficiency, it is possible that the well-characterized PINK1/PARKIN signaling cascade could be involved. In this pathway, mitochondrial membrane depolarization prevents the PINK1 kinase from translocating into the mitochondria and being degraded and instead results in PINK1 accumulating on the outer mitochondrial membrane. PINK1 then phosphorylates and activates both PARKIN1, an E3 ubiquitin ligase, and its preferred species of ubiquitin, resulting in protein phoshoubiquitination and mitophagy. Induction of the mtUPR by supplying cells with mutant OTC does indeed induce mitophagy suggesting PINK1/PARKIN1 is activated in response to the mtUPR [66]. The stress-induced expression of PARKIN1 is also critically reliant on PERK/ATF4 mediated gene expression, but any mechanistic link between these processes has not been identified [67].

The loss of mitochondrial electrochemical potential prevents not just PINK1 from entering the mitochondria, but many other mitochondrial preproteins as well. In C. elegans, the cytosolic accumulation of mitochondrial preproteins results from depolarization induced deficits in preprotein import, or actively facilitated by mitochondrial matrix peptide exporter HAF1 that functions during the mtUPR to expel partially degraded peptides [68]. The release of these short mitochondrial peptides are required in some way for ATSF1 mediated gene expression and mtUPR resolution. Recent work in mice by Liu et al discovered mitochondrial import inhibition resulted in the toxic accumulation of metastable mitochondrial preproteins in the cytosol that formed LC3/ubiquitin positive aggregates and, in mouse models, caused neurodegeneration [69]. Perhaps physiological levels of preprotein accumulation during mitochondrial stress could somehow function to activate the ISR, and thus mediate the mtUPR. Mitochondrial preprotein accumulation in yeast does indeed downregulate cytosolic protein translation, in addition to stimulating proteasomal degradation, but a link to eIF2a has yet to be identified [70]. Interestingly, stress-induced mitochondrial import deficiencies have been linked to the activation of PERK, which can trigger the degradation of TIM17A, a critical subunit of the mitochondrial import receptor [71].

Although typically associated with transducing the UPR, PERK also influences mitochondrial dynamics at mitochondrial-associated membranes (MAMs), where it is functionally involved in tethering the two membranes, independent of its kinase activity [72]. Owing to the extensive communication that occurs at these MAMs, it is possible that the mtUPR could be launched through MAMs, thus linking PERK to the mtUPR. Since mitochondrial stress does indeed activate the UPR, it would be intriguing to explore whether MAMs are essential in mediating this process, and perhaps yield important insight into how the mtUPR triggers the ISR [73]. Another ISR kinase, GCN2, has been shown to be activated by ROS signaling in addition to uncharged tRNA [74]. Although the exact mechanism is not understood, ROS production during mitochondrial dysfunction can facilitate eIF2a phosphorylation through GCN2.

Lastly, mtRNA has recently been shown to occupy and activate a large portion of endogenous PKR, which could be released as a result of mitochondrial rupture or facilitated

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export [75]. Supporting this notion, recent research has revealed that mitochondrial stress leads to the cytosolic accumulation of mtDNA [76, 77, 78]. Interestingly, PINK1/PARKIN1 mediated mitophagy in mice is required to suppress mtDNA release from stressed mitochondria, a phenomenon that subsequently leads to neurodegeneration through a cGAS/STING dependent proinflammatory response [77]. Thus deficiencies in mitophagy may lead to the deleterious rupture of mitochondria, releasing mtDNA/mtRNA and potentially activating PKR and p-eIF2a. It is important to emphasize that none of these mechanisms are mutually exclusive, and it is highly likely that mitochondria use multiple signaling routes to activate the ISR. Ultimately there are dozens of pathways activated by mitochondrial stress, and any number of them could potentially activate the ISR and stall translation, but that discussion exceeds the scope of this review.

mTORC1

Loss of proteostasis can influence translation and metabolism through the modulation of mTORC1. mTORC1 is a serine/threonine kinase composed of three primary subunits; RAPTOR, critical for substrate recognition, mLST8, required for mTORC1's active site formation, and mTOR, the functional kinase. mTORC1's regulation is complex, and reviewed elsewhere, but can generally be summarized as a kinase activated by growth signaling and nutrient availability, and inactivated by nutrient deprivation and stress [79]. When active mTORC1 controls translation initiation by phosphorylating and inactivating 4E-BP, a protein that, when relieved from mTORC1's negative regulation, potently inhibits eIF4e of the translation initiation complex. mTORC1 upregulates translation elongation as well by phosphorylating and activating S6K1, a kinase that increases the processivity of the ribosome. mTORC1 also negatively regulates eIF2a phosphorylation by phosphorylating eIF2B resulting in the recruitment of NCK1, which mediates eIF2a dephosphorylation (possibly by recruiting PP1A) [80]. In addition, mTORC1 has been shown to enhance the rate of rRNA biogenesis by phosphorylating the critical RNA polymerase I transcription factor TIF-1A, and the inhibition of mTORC1 disrupts ribosomal assembly [81, 82]. mTORC1 thus serves to initiate translation through its inhibition of 4E-BP and p-eIF2a, upregulate the rate of translation elongation through S6K1 activation, and promote ribosome biogenesis by TIF-1a activation. Besides its modulation of translation, mTORC1 phosphorylates and inactivates ULK1, the central kinase of the autophagic preinitiation complex. Autophagy is additionally downregulated at the transcriptional level by mTORC1 mediated phosphorylation of TFEB, a critical autophagic and lysosomal transcription factor, resulting in its negative regulation by binding inhibitory 14-3-3 class of phosphoserine-binding proteins [83]. Thus in order to restore proteostasis, it seems practical that mTORC1 would be inactivated during protein misfolding in order to simultaneously activate autophagy and inactivate translation, and indeed proteostatic disturbances consistently result in mTORC1 inhibition.

Considering mTORC1's pivotal role in modulating metabolic and degradative pathways, determining how proteostatic disruption affects mTORC1 signaling has become a central question. Generally, events that disrupt proteostasis are accompanied by the attenuation of mTORC1 signaling. For example, acute inhibition of the proteasome, a traditional method of disrupting proteostasis, rapidly deactivates

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mTORC1, even in the presence of translation inhibitors suggesting new protein synthesis is not required [LINK]. Since certain species of misfolded protein have been shown to either inhibit the proteasome, or sequester it in aggregates, understanding how this deactivates mTORC1, and thus translation may provide critical insight into neurodegenerative processes. The loss of proteasomal function could result in the accumulation of DEPTOR, a protein that potently inhibits mTORC1 but is normally ubiquitinated by SCF^{BTrCP} ubiquitin ligases and degraded in proteasomes [84, 85]. mTORC1 activity may also be limited by its rate of formation, a process that is critically reliant on the HSP90 chaperone [86]. The loss of HSP90 due to the presence of misfolded proteins, which can function as chaperone sinks, has been shown to limit the rate at which mTORC1 is constructed resulting in decreased mTORC1 signaling. The activation of the UPR is also accompanied with decreased mTORC1 signaling, and thus translation, an effect that occurs independently of eIF2a phosphorylation [87]. mTORC1 inhibition accounts for the downregulation of translation in eIF2a knockout cells following ER stress (although this effect takes hours, as opposed to minutes in WT cells) suggesting the UPR can mediate mTORC inhibition independent of the ISR. Interestingly, mTORC1 mediated translation following UPR stress could be restored by transducing cells with ATF4, suggesting ATF4 transcription positively regulates mTORC1 (supported by Quiros et al above). In contrast, another study found ATF4 mediated transcription sustained mTORC1 inhibition by upregulating Sestrin [88]. Whether ATF4 transcriptional activity promotes or inhibits mTORC1 is not clear, but regardless there appears to important transcriptional crosstalk between these pathways.

How does UPR signaling result in mTORC1 suppression independent of eIF2a phosphorylation? The activation of the UPR is associated with the activation of AMPK, an upstream suppressor of mTORC1 [89]. Although it is not understood exactly how the UPR activates AMPK, this would, in theory, result in diminished mTORC1 kinase activity. AMPK achieves this effect by phosphorylating the substrate recognition domain of mTORC1, RAPTOR, inducing its dissociation from mTOR and binding to 14-3-3 [LINK]. Alternatively, as discussed above, protein misfolding in the ER can lead to the activation of JNK from oligomerized Ire1 signaling platforms. JNK both associates with and phosphorylates mTORC1, an effect that may trigger the disassembly of the mTORC1 complex (discussed below).

Recent research by Su et al found JNK mediated phosphorylation of mTORC1's RAPTOR domain induces the disassembly of the mTORC1 complex [90]. However, during normal proteostatic conditions JNK is bound and sequestered away from mTORC1 by HSF1. Misfolded proteins, triggered through heat shock, proteasome inhibition, or HSP70 inhibition, bind to HSF1 and displace JNK resulting in the inhibitory phosphorylation of Raptor and mTORC1 disassembly. Additionally, HSF1 deletion in mice, which would, in theory, lead to constitutively active JNK and inhibited mTORC signaling, does indeed disrupt mTORC activity [91]. Thus JNK, activated by misfolded protein (or perhaps Ire1 platforms), leads to the inactivation of mTORC1 and translation. This also provides a mechanism by which proteasome inhibition results in mTORC1 deactivation, that is, the accumulation of misfolded proteins releases JNK from HSF1 to deactivate mTORC1.

Mitochondrial insults, including mitochondrial translation inhibition and ETC disruption, have also been

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shown to deactivate mTORC1, thus promoting autophagy, stalling translation, and preserving ATP [92, 93]. Although it is clear mTORC1 is inactivated by mitochondrial stress, the mechanistic details bridging these two phenomena are poorly understood. It is reasonable that the loss of ATP production, and thus AMP accumulation, could activate AMPK and mediate mTORC1 inhibition. It was also reported that mitochondrial stress induced depolarization resulted in the activation of PARKIN1, discussed above, that polyubiquitinates mTOR triggering its proteasomal degradation [94]. mTORC1 is indeed associated with mitochondrial outer membranes, cofractionates with mitochondria, and is typically inactive while localized to mitochondria (mTORC1 requires lysosomal localization for activation) [95, 96]. Also associated with the mitochondrial outer membrane is JNK, which translocates to mitochondria during stress and is anchored by the protein Sab [97, 98]. It would be intriguing to test if Sab mediated recruitment of JNK to the mitochondria facilitates mTORC1 phosphorylation and degradation. In sum, mTORC is inhibited by multiple proteostatic disturbances including the UPR, proteasome inhibition, chaperone depletion, and the mtUPR, although more research is needed to determine the underlying signaling networks.

STRESS GRANULES

Stress granules are membraneless ~200 nm pseudo organelles that form in the cytoplasm during proteostatic or ribostatic stress, and function to temporarily sequester mRNA until translation resumes. Stress granules are dynamic structures that generally contain dense immobile cores and gel-like outer shells, the latter of which turns over on the order of seconds as measured by FRAP studies [99]. Although the majority of a cell's pool of mRNAs (about 90%) are targeted to stress granules during stress, only about 10% of mRNAs are consistently nucleated into aggregates (perhaps the population of mRNA locked in insoluble cores) [100]. The rest appear to only transiently associate with stress granules before being released, or funneled into P bodies for degradation. Only 185 genes have greater than 50% of their mRNA localized within stress granules, suggesting stress granules serve a more specialized function than previously thought, rather than broad sequestration of mRNA. These mRNAs prone to aggregation in stress granules appear to be related by extended 3' UTRs and larger than average transcript size, leading to poor translatability. These criteria would encompass all viral RNAs and result in their preferential accumulation in SGs, which is likely the evolutionary purpose of stress granules [101]. Indeed, many viruses employ transcripts specifically evolved to inhibit stress granule nucleating machinery. Larger proteins are also more prone to misfolding or causing translational errors, thus their preferential targeting to stress granules may aid in proteostatic recovery. There is also evidence that certain classes of mRNA, such as the HSP folding chaperones, are preferentially excluded from stress granules resulting in their upregulation during stress. The inability to sequester mRNA in stress granules during proteotoxic stress can accelerate neuronal aggregate formation and rate of cell death [102, 103, 104]. The loss of stress granule nucleation machinery alone is sufficient to induce neurodegeneration, particularly in the hippocampus [105, 106]. Similarly, the irreversible aggregation of stress granules also constitutes a proteostatic dysfunction that can promote protein misfolding and cell death [107]. Thus a careful balance of stress granule formation and

dissolution in response to translation status is an important component of the proteostatic network.

Stress granules are a proteostatic response to translation inhibition derived from two primary physiological sources; mTORC inhibition and eIF2a phosphorylation. Since both of these events are fundamental to the proteostatic response, stress granules are nearly ubiquitous across protein misfolding disorders. The RNA binding proteins responsible for nucleating stress granules, including most notably TIA1 and G3BP1, bind through prion-related protein interactions between intrinsically disordered domains promoting their aggregation [108, 109]. During healthy proteostatic conditions, continuous ATP dependent chaperone activity and the absence of ribosome-free mRNA precludes the formation of stress granules [110, 111]. It is thus the non-association of mRNA with ribosomes due to p-eIF2a or mTORC inhibition, and loss of enzymatically active chaperones like HSP70 (perhaps due to the presence of misfolded protein or ATP depletion), that induces the aggregation of mRNA into stress granules. During these conditions, RNA binding protein TIA1 is recruited to 3' TOP sequences of mRNA (Terminal Oligopyrimidine Tracts), and G3BP to stagnated 40s small ribosomal subunits, thus increasing their local concentration, promoting interactions between IDRs (Intrinsically Disordered Region), and facilitating aggregation [112, 113].

Although stress granules nucleate around mRNA, certain proteins also become sequestered in stress granules, a regulated process that usually depends on the presence of an IDR (for interaction with TIA1/G3BP) or an RNA binding domain (for interaction with aggregated mRNA). For example, a cell's population of Ubiquilin-2 proteins are nearly completely shuttled into SGs owing to an IDR [114]. Interestingly, the ubiquilin family of proteins are responsible for binding transmembrane mitochondrial outer membrane proteins that fail to be inserted into the mitochondrial membrane and delivers them to the proteasome [115]. Since the loss of ubiquilins results in the cytosolic accumulation and aggregation of mitochondrial outer membrane preproteins, it may be possible that stress granule formation, and sequestration of ubiquilin proteins, promotes the mtUPR. In addition to Ubiquilin-2, critical RNA binding proteins required for nucleocytoplasmic shuttling, splicing, and snRNA biogenesis, are also sequestered in stress granules [116]. By sequestering a host of RNA shuttling proteins, including Ran, Exportins, and Importins, stress granules significantly downregulate the flux of new mRNA into the cytoplasm, serving as an additional mechanism of proteostatic control of translation [106]. Similarly, the sequestration of TDP-43 in stress granules, a major ALS associated protein normally localized to Cajal bodies, could result in abnormally spliced transcripts and RNA processing [117]. Collectively, the aggregation of RNA binding proteins in stress granules could collapse protein assembly lines, which, like other inhibitors of translation, can be beneficial or detrimental depending on duration.

The importance of stress granules in attenuating translation has been challenged by recent research demonstrating that G3BP knockout cells, which cannot form stress granules following eIF2a phosphorylation, are not defective in translational arrest [113]. Additionally, the majority of mRNA are not sequestered in stress granules, discovered following the high throughput analysis of stress granule transcriptomes [100]. Although stress granules are rapidly formed following eIF2a phosphorylation thus translational stalling, they may not contribute to the translational arrest. If not critical to translational attenuation,

why are stress granules central to maintaining proteostasis? By nucleating mRNA, ribosomes, and translation initiation factors, stress granules may, paradoxically, be critical for the rapid reinitiation of translation by colocalizing translational machinery [118]. Perhaps the sequestration of mRNA is simply a consequence of colocalizing RNA binding proteins and ribosomes. Another possibility is that stress granules shield latent mRNA from post transcriptional mutations or cleavage, perhaps derived from ROS or siRNA, thereby helping to mitigate subsequent translation errors. And lastly, stress granules may influence post-transcriptional modification dynamics. Storing mRNA in dense stress granules could serve as a mechanism for preventing potentially deleterious or otherwise unintended mRNA modifications. Regardless, the association of translational machinery, RNA binding proteins, and mRNA with stress granules strongly suggests they serve an important function in translation, although it may not be as straightforward as simply decreasing protein synthesis.

Once formed, a cell must efficiently disaggregate stress granules in order to resume proper translation, RNA processing, and recover from stress. The majority of stress granules in HeLa cells are cleared by the HSPB8/BAG3/HSP70 chaperone complex, although many other chaperones are also involved [111]. The efficient clearance of stress granules seems to require the recruitment of the 26S proteasome, a process conducted by ZFAND1/p97 [119]. As an ATPase segregase, p97 (also known as VCP) has been repeatedly implicated in the clearance of both stress granules and aggregates, and various mutations within p97 have been linked to ALS and FTD. In addition, p97's ATPase activity can remodel stress granules so as to expose ubiquitin moieties required for autophagic clearance (in yeast), although the importance of autophagic clearance of SGs in mammalian cells is disputed [120]. Regardless, since ZFAND1 is responsible for recruiting both p97 and the proteasome, it likely functions upstream of both clearance mechanisms. The current model of stress granule clearance involves the disaggregation of RNA binding proteins by chaperones like HSP70 and p97 (and many others) and degradation in the proteasome. In sum, stress granules aid proteostatic networks by sequestering mRNA, and proteins critical to mRNA splicing and nucleocytoplasmic shuttling, thereby downregulating protein synthesis.

CONCLUSION

Neurons have evolved complex mechanisms of translational control in order to manage the proteostatic and energetic challenges of neuronal function. However, in order to more completely integrate these mechanisms with the neurodegenerative process, a better understanding of how translation is affected by ageing is needed. Ageing is the most significant risk factor for neurodegenerative disease, and any attempt to understanding proteostasis must be examined through the lens of ageing. Old age is not only associated with aberrant protein accumulation, but also the accumulation of undegradable "biochemical garbage", such as lipofuscin in lysosomes, advanced glycation end products, or extracellular atherosclerotic cholesterol plaques. Why does cellular garbage accumulate as we age? The age-dependent accumulation of genetic mutations, known as the Somatic Mutation Theory of Ageing, could steadily escalate proteostatic pressures as protein function deteriorates and they become increasingly prone to misfold. Indeed, DNA damage syndromes have been linked to protein misfolding and proteostatic collapse [121]. Alternatively, a large body of evidence suggests the presence

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of various environmental toxins that accelerate aging. These "gerontogens" are diverse, cumulative in their effect, and are often related to the induction of DNA damage. The age-dependent loss of immune function has also been linked to the reactivation of dormant viruses, such a HSV6/7 in the brain of Alzheimer's patients [122]. Many of the proteostatic pathways discussed in this paper have evolutionary roots in the cellular response to viral RNA and protein, and so the possibility that these proteostatic disturbances are caused by viruses is not implausible. Future research will benefit significantly from examining interactions between these mechanisms of ageing and translational control, and could yield critical insight into the neurodegenerative process.

Figure Captions

Figure 1. The UPR Signaling Network

Protein misfolding in the ER activates PERK and Ire1 by competing BiP away from their lumenal domains and promoting multimerization. PERK phosphorylates eIF2a resulting in attenuated translation and ATF4 expression. PERK also activates NRF2, and in concert with ATF4, these transcription factors promote cytoprotective gene expression. Prolonged ATF4 transcription promotes cell death through the expression of CHOP and PUMA. Ire1 activates both proapoptotic and antiapoptotic pathways through RIDD and XBP1, respectively.

Figure 2. mTORC & mtUPR Signaling Network

Proteostatic networks activated by mtUPR and chaperone sequestration. mTORC inhibition, derived from JNK or chaperone depletion, results in downregulated translation. The mtUPR activates a myriad of pathways that converge of eIF2a phosphorylation, ATF4 expression, and mTORC inhibition. Both events lead to stress granule assembly which may also attenuate translation.

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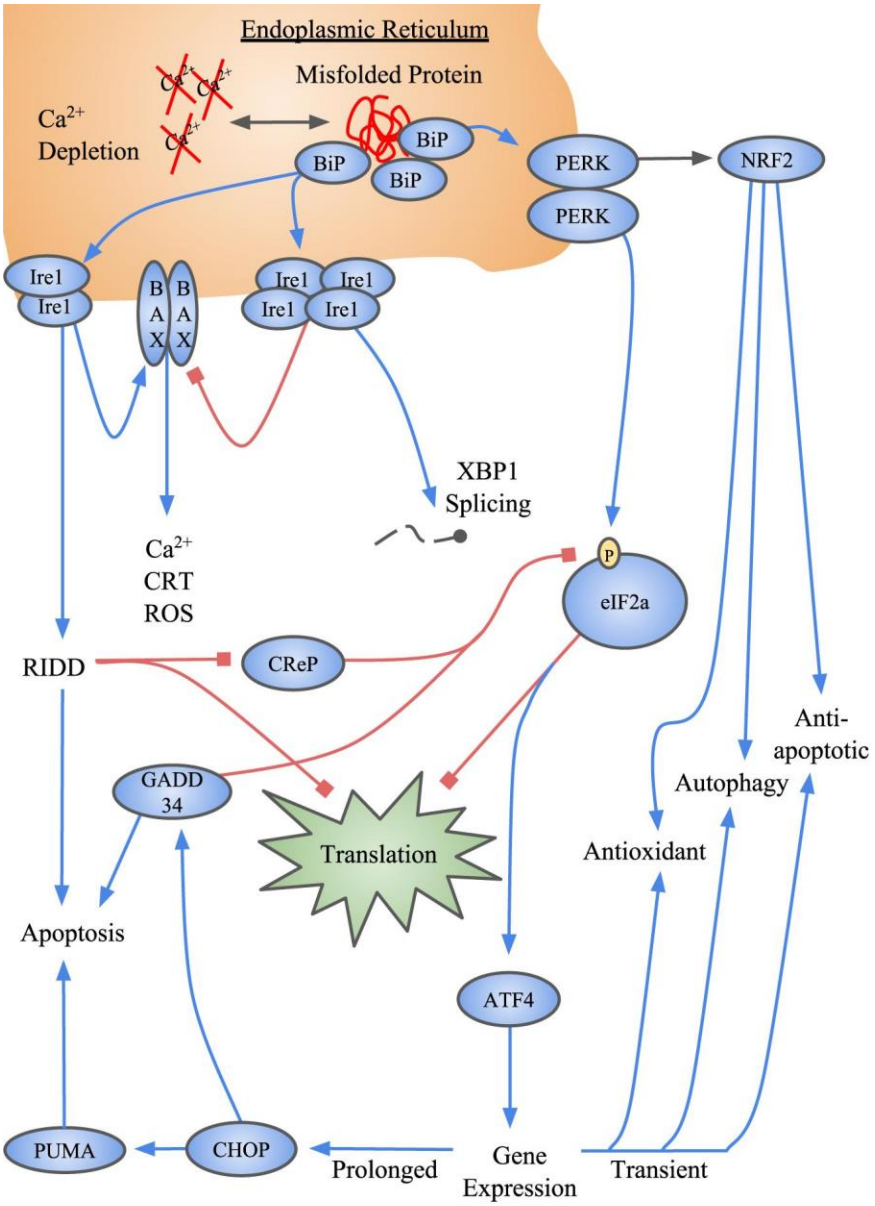
FIGURE2



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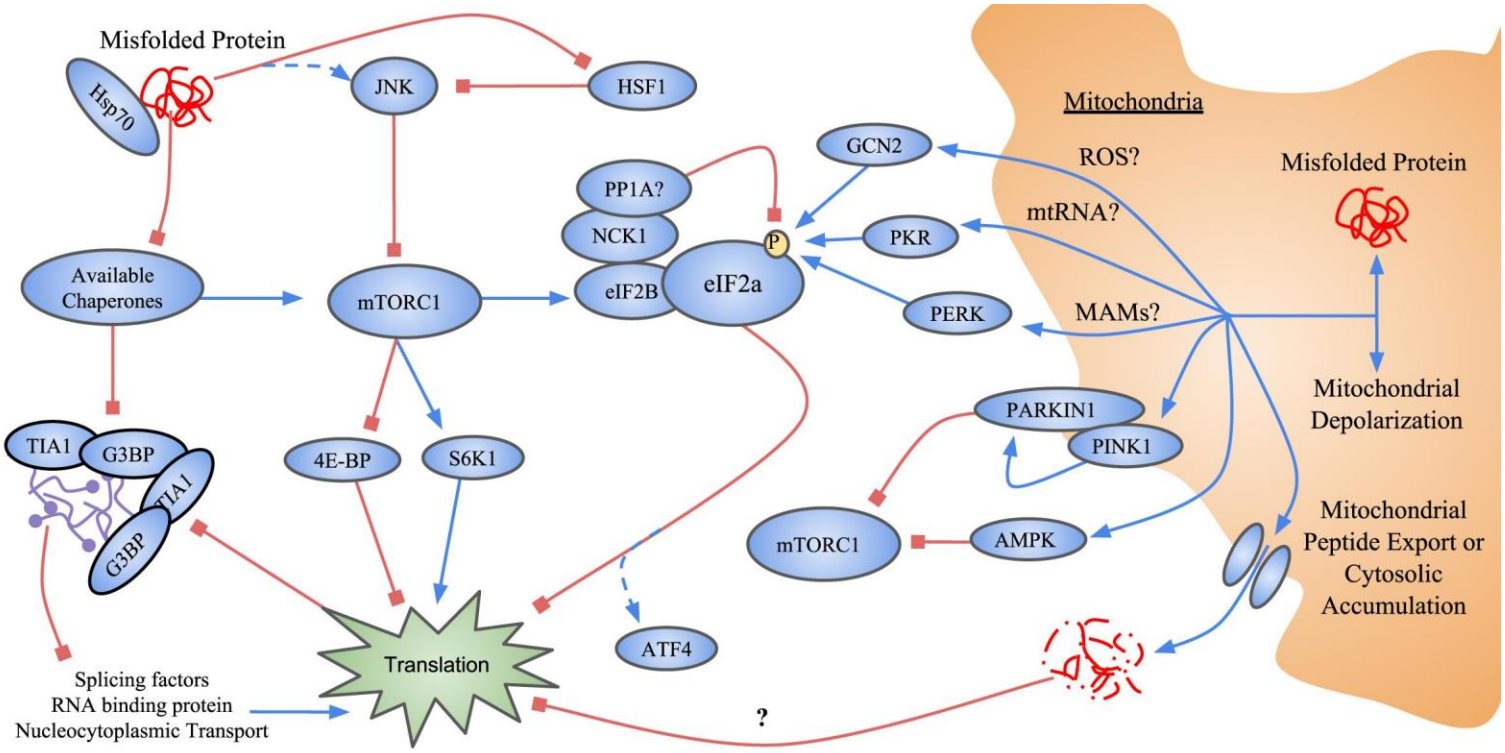

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