The endoplasmic reticulum unfolded protein response and neurodegeneration

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Abstract Proteostasis in the lumen of the endoplasmic reticulum is defended by signalling pathways that match the load of unfolded proteins to the complement of chaperones in the organelle. This balancing act is attained via a transcriptional program that activates genes encoding chaperones and other proteins that function in the secretory pathway and a translational program that transiently attenuates protein synthesis by regulating translation initiation. The key features of this unfolded protein response (UPR) will be reviewed, with an emphasis on those strands that are known to intersect with neurophysiology and neuropathology. A particular focus for review will be the evidence that activity of the translational arm of the UPR impacts on diverse phenomena ranging from memory consolidation to myelination and that the transcriptional arm of the UPR is in equilibrium with other pathways that defend proteostasis. Specific consideration will be given to points for intervention in the working of the UPR and how these might be harnessed for treatment of neurological disease.

Overview of the unfolded protein response

Protein secretion is key to cells' interaction with their environment and is at the core of multicellularity. This is conspicuously the case for the immune, endocrine and nervous systems of higher eukaryotes, where intercellular communication depends on the secretion and presentation on the cell surface of multiple protein ligands and receptors. Secreted and transmembrane proteins undergo early steps of their biogenesis in the lumen of the endoplasmic reticulum (ER), where a host of chaperones and protein-modifying enzymes assist the unfolded nascent chain to attain its

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R.I. Morimoto and Y. Christen (eds.), *Protein Quality Control in Neurodegenerative Diseases*, Research and Perspectives in Alzheimer's Disease,
DOI 10.1007/978-3-642-27928-7_2,

proper three-dimensional and oligomeric structure. The ER maintains a quality control function, retaining unfolded or misfolded proteins and selectively allowing only properly folded and assembled proteins to egress from the organelle and progress down the secretory pathway (Ellgaard and Helenius 2003). Proteins persistently engaged by this quality control machinery are eventually translocated back into the cytosol and degraded in a process known as ER associated protein degradation (ERAD; Tamura et al. 2010).

The mass of unfolded proteins translocated into the ER is modulated by diverse physiological exigencies. Furthermore, physiological variables impact on the functional state of the protein folding machinery in the ER lumen. To cope with these changing circumstances, eukaryotes have evolved dedicated signal transduction pathways that couple the capacity of the ER protein folding machinery and the load of unfolded proteins. These can be triggered experimentally by manipulations that promote protein misfolding in the ER and consequently have been named the unfolded protein response(s), or UPR (Kozutsumi et al. 1988).

Whilst there is a debate remaining in regard to the molecular mechanisms by which the UPR signal transducers are activated, it is clear that, directly or indirectly, the balance between unfolded proteins and chaperones is sensed (Kimata and Kohno 2011). Thus, an imbalance favoring unfolded proteins defines ER stress. It also follows that cells experience periodic low levels of ER stress under quite normal circumstances and that the study of its consequences is as relevant to physiology as it is to pathophysiology.

Animal cells respond to ER stress with two distinct adaptations: a rapid and transient reduction in rates of new protein synthesis and a more gradual alteration of gene expression programs (Ron and Walter 2007; Fig. 1). The former is an immediate rectifying response that indiscriminately attenuates the flux of new proteins entering the ER, whereas the latter globally up-regulates genes encoding proteins that function at all levels of the secretory pathway. The gene expression arm of UPR, which is conserved in all eukaryotes, enhances the ability of cells to cope with their secretory mission. This gene expression program, which includes many ER chaperones and enzymes, represents a signature of the UPR and serves to distinguish it from other stress pathways (Travers et al. 2000; Murray et al. 2004).

The translational arm of the UPR

PERK

ER stress is linked to attenuated rates of translation initiation by an ER localized transmembrane protein, PERK (Harding et al. 1999). PERK's lumenal stress-sensing domain monitors the balance of unfolded proteins and chaperones and

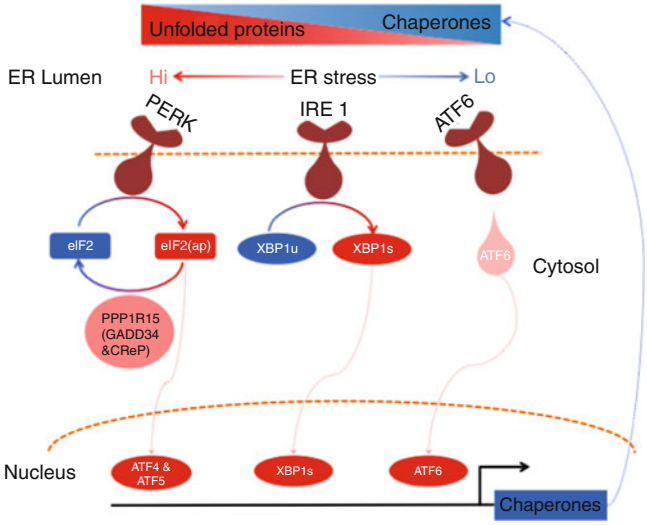

Fig. 1 The endoplasmic reticulum unfolded protein response (UPR). The balance between chaperones and unfolded proteins in the ER lumen establishes the levels of ER stress, which is recognized by three known transmembrane stress receptors. The translation strand of the UPR is mediated single handedly by the transmembrane eIF2α kinase PERK (*left*). Phosphorylation of eIF2α attenuates new protein synthesis, lowering the supply of unfolded proteins to the ER while, at the same time promoting the translation of the transcription factors ATF4 and ATF5. IRE1 signalling (*center*) promotes the unconventional splicing of the XBP1 mRNA, converting it from the inactive unspliced (XBP1u) form to the XBP1s form, encoding a transcriptional activator. ATF6 (*right*) is cleaved in ER stressed cells to generate an active transcription factor that, together with ATF4, ATF5 and XBP1, activates UPR target genes to enhance the capacity of the ER to cope with unfolded proteins

promotes oligomerization of PERK in the plane of the membrane in response to ER stress. PERK's cytosolic effector domain is a protein kinase that undergoes activating trans-autophosphorylation by this clustering. Activated PERK recruits and phosphorylates translation initiation factor 2 (eIF2) on serine 51 of its alpha subunit (Harding et al. 1999; Marciniak et al. 2006). In its GTP-bound form, eIF2 plays an essential role in translation initiation, and the phosphorylation of its alpha subunit inhibits the exchange of GDP for GTP on eIF2, inhibiting rates of protein synthesis (Hinnebusch 2000). Thus PERK single-handedly couples ER stress to attenuated rates of protein synthesis (Harding et al. 2000a).

In the absence of PERK, the flux of proteins into the ER is no longer responsive to the levels of ER stress, exposing cells to enhanced risk of protein misfolding in the ER and promoting higher levels of activity in the parallel strands of the UPR (Harding et al. 2000a). The loss of ER stress-regulated eIF2α phosphorylation is an especially great problem for professional secretory cells, leading to impairment of the endocrine and exocrine pancreas, salivary glands, liver and bone (Harding et al. 2001; Scheuner et al. 2001; Zhang et al. 2002).

The integrated stress response

In addition to negatively regulating rates of protein synthesis, eIF2α phosphorylation affects gene transcription (Harding et al. 2000b). This transcription is attained by the seemingly paradoxical translational upregulation of transcription factors such as ATF4 and ATF5, under conditions of high levels of phosphorylated eIF2α, abbreviated eIF2(αP) (Lu et al. 2004a; Vattem and Wek 2004; Zhou et al. 2008). A detailed discussion of the underlying molecular mechanism is beyond the scope of this review. It is, however, worth noting that guanine nucleotide exchange is the focal point of eIF2(αP) action, both in terms of regulating global translation and in terms of transcriptional activation (via ATF4, ATF5 and similarly regulated factors). eIF2(αP) inhibits the guanine nucleotide exchange activity of the GEF, eIF2B (Siekierka et al. 1981; Clemens et al. 1982) and strengthens the anti-exchange (GDI) activity of eIF5 (Jennings and Pavitt 2010), leading to lower levels of GTP-bound eIF2 complexes available to promote translation initiation.

Whilst PERK is the only kinase to couple eIF2α phosphorylation to ER stress, cells have three other kinases that affect eIf2α phosphorylation in response to other stresses: GCN2 to amino acid starvation, PKR to viral infection and HRI to heme depletion. Thus, eIF2(αP) integrates signaling in several stress responses and the subordinate gene expression program is referred to as the integrated stress response (ISR). The ISR constitutes one strand of the UPR, but it makes an important contribution to the overall gene expression program activated by ER stress, with genes involved in resistance to oxidative stress as particularly important ISR targets (Harding et al. 2003). It has been reported that PERK couples ER stress to phosphorylation and activation of NRF2, a transcription factor with a prominent role in resistance to oxidative stress (Cullinan et al. 2003); however, PERK-dependent gene expression is completely abolished in fibroblasts by mutating serine 51 of eIF2α to alanine (Lu et al. 2004b). Therefore, it would appear that PERK employs a rather private pathway, utilizing a single substrate to promote ER stress signaling.

eIF2(αP) dephosphorylation

Phosphorylation of eIF2α is reversed by PP1 phosphatase complexes that are directed to eIF2 by one of two regulatory subunits: the constitutively expressed PPP1R15B (also known as CReP; Jousse et al. 2003) and the ISR-inducible PPP1R15A (also known as GADD34; Novoa et al. 2001; Brush et al. 2003). The latter functions in a homeostatic negative feed-back loop that restores translation in stressed cells. Consequently, PPP1R15A/GADD34-deficient cells have higher and more sustained levels of eIF2(αP) with delayed translational recovery (Novoa et al. 2003). Deletion of the constitutive PPP1R15B/CReP leads to perinatal lethality in mice (Harding et al. 2009), but mice lacking PPP1R15A/GADD34 appear healthy.

Interestingly, the PPP1R15A/GADD34 mutant mice and cells derived from them are partially resistant to the lethal effects of toxins that promote ER stress (Marciniak et al. 2004), a phenotype shared by mice lacking the transcription factor CHOP (Zinszner et al. 1998). CHOP is transcriptionally induced by the ISR and has PPP1R15A/GADD34 as one of its target genes (Marciniak et al. 2004).

These observations suggest that the pathway for translational recovery mediated by eIF2(αP) dephosphorylation is subject to failure of homeostasis, in that attenuating this negative feedback loop has beneficial effects in some circumstances. This feature of the ISR also explains the recently described survival-promoting effects of guanabenz (Wytensin™) when applied (at relatively high concentrations) to ER-stressed cells. Guanabenz was found to selectively disrupt the interaction of PPP1R15A/GADD34 with the PP1 catalytic subunit, enforcing in effect a GADD34-deficient state (Tsaytler et al. 2011). The basis for the selectivity for PPP1R15A/GADD34 over PPP1R15B/CReP is not understood, but it is a critical feature of the compound: whereas wildtype cells tolerate guanabenz well, PPP1R15B/CReP knock-out cells exposed to guanabenz succumb to mounting levels of eIF2(αP) (Tsaytler et al. 2011), a phenotype predicted by the lethality of combined deletion of the two eIF2(αP)-directed phosphatase regulatory subunits (Harding et al. 2009).

The genetic and pharmacological experiments described above show that extremes of eIF2(αP) levels are poorly tolerated. Cells lacking PERK are presumably at excess risk of protein misfolding and ER-based proteotoxicity and cells lacking eIF2(αP)-directed phosphatases are unable to sustain levels of protein synthesis. However, between these two extremes there is quite some latitude for manipulation, a point to return to later when considering the role of the ISR in neuropathophysiology.

The transcriptional arm of the UPR

IRE1

The oldest and most conserved arm of the UPR is mediated by IRE1, an unusual ER localized transmembrane protein (Cox et al. 1993; Mori et al. 1993). IRE1 shares with its descendant, PERK, a functionally-interchangeable stress-sensing lumenal domain. IRE1's cytosolic effector domain is also a protein kinase; however, its only known substrates are other IRE1 molecules. In response to ER stress, IRE1 clusters in the plane of the membrane and undergoes activating *trans*-autophosphorylation (Shamu and Walter 1996). Phosphorylation unmasks a second catalytic function of IRE1: to cleave a pre-existing mRNA, HAC1 in yeast (Cox and Walter 1996) and XBP1 in animals, at precisely two locations (Yoshida et al. 2001; Calfon et al. 2002), liberating a small fragment (26 bases in mammals). The two ends of the XBP1 mRNA are joined together to complete the unconventional splicing event,

which results in a frame shift. The spliced XBP1 mRNA encodes a potent transcription factor that activates UPR target genes.

In addition to this highly conserved and unusual signal transduction pathway, IRE1 has been reported to engage in two other activities: phosphorylated mammalian IRE1 can recruit the TRAF2 adaptor, activating Jun N-terminal kinase (Urano et al. 2000; Nishitoh et al. 2002) or caspases (Yoneda et al. 2001). This strand of UPR activity has been proposed to link ER stress to cell death and dysfunction, but the physiological significance of these links has not been critically explored. IRE1 also possesses promiscuous RNase activity directed to ER membrane bound mRNA. Referred to as regulated IRE1-dependent degradation (or RIDD), it is believed to work alongside PERK in attenuating the load of unfolded proteins that enter the ER by degrading their encoding mRNAs (Hollien and Weissman 2006; Hollien et al. 2009). At present, there are no good genetic or pharmacological tools to separate XBP1 splicing from RIDD; therefore, it has not been possible to critically examine RIDD's contribution to protein folding homeostasis. However, observations made in an IRE1 over-expression model suggest that RIDD may contribute to the death of ER-stressed insulinoma cells (Han et al. 2009).

IRE1's kinase activity and RNase activity are mediated by different portions of the cytosolic effector domain and are separable (Lee et al. 2008). In vitro work with the yeast enzyme shows the importance of ligand engagement at the nucleotide binding pocket, not only to the kinase activity (which is expected) but also to the RNAse activity (Papa et al. 2003). The two are likely coupled by a ligand binding-dependent dimerization event (Lee et al. 2008), which may extend to a higher order, more active oligomer (Korennykh et al. 2009). Additional evidence for allosteric regulation of IRE1 is provided by the ability of the flavonol quercetin to activate yeast IRE1 in vitro by engaging a ligand-binding pocket at the dimer interface, which stabilizes the active dimeric form of the enzyme (Wiseman et al. 2010).

It remains to be seen to what extent these lessons from the yeast enzyme are applicable to mammalian IRE1. But the prospects of being able to tune and bias IRE1's signaling by ligands that engage its various allosteric and active sites are very enticing.

ATF6

The third known strand of the UPR is mediated by ATF6. In unstressed cells, ATF6 is found as a latent transcription factor, tethered to the ER membrane. However, under conditions of ER stress, regulated intra-membrane proteolysis liberates ATF6 from the ER membrane and the soluble transcription factor migrates to the nucleus to join ATF4 and the other mediators of the ISR and XBP1 in activating UPR target genes (Yoshida et al. 1998; Haze et al. 1999; Ye et al. 2000).

The proximate antecedent of ATF6 cleavage and activation is its migration from the ER to the Golgi. Once at the Golgi, ATF6 is cleaved by the same proteases that activate the sterol-response element binding proteins (SREBPs; Ye et al. 2000).

ATF6 is co-activated with the other strands of the UPR, and BiP binding has been proposed to play a role in repressing its trafficking to the Golgi in unstressed cells (Shen et al. 2002). Mammals have two ATF6 genes, deletion of which significantly compromises resistance to ER stress (Yamamoto et al. 2007). In addition, mammals possess several other proteins with similar architecture and (likely) similar activation mechanisms (e.g., CREB-H and OASIS). Their role in the ER stress response is currently under study in several labs.

UPR signaling in neurophysiology

Gross brain development is tolerant of deletion of key branches of the UPR (unlike the sensitivity of secretory organs to such genetic manipulation). PERK knockout mice and humans with mutations in the PERK-encoding *EIF2AK3* gene (leading to the Walcott-Rallison syndrome) have relatively normal CNS function (Harding et al. 2001), whereas conditional deletion of XBP1 in brain tissue is also compatible with normal development of the organ (Hetz et al. 2009). However, before considering the role of the UPR in neuropathophysiology it is worth noting two interesting contributions that regulated eIF2α phosphorylation makes to neurophysiology.

eIF2(αP), feeding behavior and memory formation

In yeast, the single eIF2α kinase GCN2 couples amino acid availability to an eIF2 (αP)-dependent gene expression program, akin to the ISR. The gene products of this program adapt the yeast to an environment limited in amino acids (Dever et al. 1992). A conceptually similar response is induced by GCN2 in the piriform cortex of mice fed a diet deficient in a single amino acid (Hao et al. 2005; Maurin et al. 2005). The basis of GCN2 activation by amino acid deprivation is fairly well understood; however, the molecular events triggered by GCN2-mediated eIF2 (αP) in the responding neurones are not understood and likely involve both transcriptional and post-transcriptional events.

The regulated phosphorylation of eIF2α also plays an important role in memory formation. In brain slices of mice lacking GCN2, long-term potentiation by a single train of electrical stimulation is enhanced, compared with the wildtype (Costa-Mattioli et al. 2005). This likely hinges on the interplay between the eIF2(αP)-dependent expression of ATF4 and the related transcription factor CREB, but neither the upstream mediators of GCN2 activation, in this context, nor the downstream events that connect eIF2(αP) to synaptic plasticity are known.

The uncertainty as regards to mechanism notwithstanding, the experimental indictment of eIF2(αP) in fundamental aspects of neurophysiology has an important implication as we consider the role of the UPR in neuropathology because it suggests that the phenotypic consequences of UPR signaling, of which eIF2α

phosphorylation is an important component, may be established not only through effects on cellular health and viability but also through the impact of UPR signaling on functional circuitry.

UPR signaling in neuropathophysiology

Control of translation initiation and the syndrome of childhood ataxia and cerebral hypomyelination (CACH)

Rare, loss-of-function mutations affecting the guanine nucleotide exchange factor eIF2B are strongly associated with a syndrome of episodic progressive childhood ataxia with CNS hypomyelination (Fogli and Boespflug-Tanguy 2006; Schiffmann and Elroy-Stein 2006). eIF2B activity is a target for repression by phosphorylated eIF2α, which mediates the effects of eIF2(αP) on both global translation and on gene-specific induction in the ISR. As expected, fibroblasts of individuals with CACH have heightened activity in their ISR when exposed to conditions that impose mild levels of ER stress (Kantor et al. 2005).

The notion that a misregulated ISR contributes to the pathophysiology of CACH is further supported by the clinical history. Patients are reported to experience bouts of demyelination in the context of acute febrile illness or head trauma, events that are likely to enhance ER stress signaling and promote transient increases in levels of eIF2(αP). The notion that CACH is a disease caused by a de-regulated ISR remains an unproven hypothesis but, if correct, may have interesting implications for therapy for diseases of aging and protein misfolding.

As noted above, loss-of-function mutations in PPP1R15A/GADD34 are associated with resistance to cell death and dysfunction caused by ER stress. This is attributed to a sustained induction of eIF2(αP) levels in the mutant and attenuated unfolded protein load confronting the ER. A recent study suggests that the potential benefits of sustained ISR activity may extend to protein misfolding in other compartments, too. The anti-hypertensive drug Guanabenz (Wytensin™) was found to have anti-prion activity (Tribouillard-Tanvier et al. 2008) and also to protect cells against ER stress. The latter activity is well explained by the selective dissociation of PPP1R15A/GADD34 from the catalytic subunit of the phosphatase by Guanabenz (Tsaytler et al. 2011). This observation and previous reports that Salubrinol, a compound that inhibits eIF2(αP) dephosphorylation by yet-to-be determined mechanism(s), protects against ER stress (Boyce et al. 2005) nominate GADD34 as a target for inhibition to treat/prevent diseases of protein misfolding. It remains to be determined whether the protection afforded by Guanabenz against prions is also mediated by inhibition of GADD34.

Together, these disparate strands suggest that protein folding is subject to a failure of homeostasis that can be rectified, in some circumstances, by inhibition of GADD34 and an accentuated and sustained ISR. According to one very simple

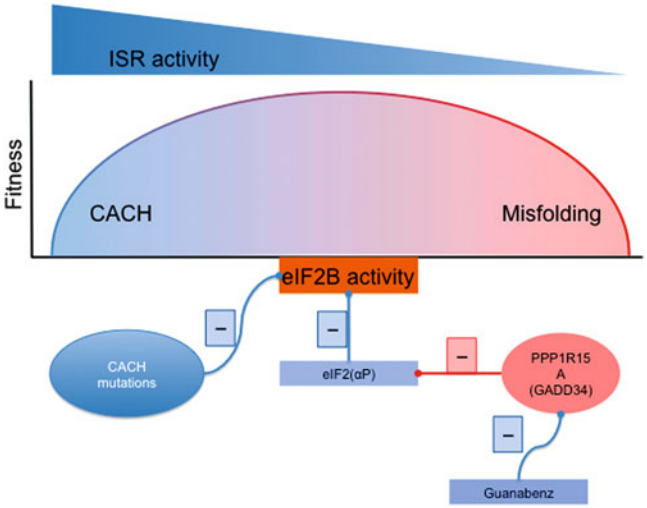

Fig. 2 Extremes of ISR signalling are detrimental. Shown is a schema of the hypothesized relationship between fitness (in terms of health of CNS myelin) and ISR activity, which is regulated by eIF2B activity. The latter is attenuated both by mutations associated with the syndrome of childhood ataxia and cerebral hypomyelination (CACH) and by elevated eIF2α phosphorylation. The PPP1R15A/GADD34 phosphatase regulatory subunit promotes eIF2(αP) dephosphorylation and attenuates ISR signalling. This action can be reversed by Guanabenz, which has recently been demonstrated to protect cells from ER stress (Tsaytler et al. 2011)

model, CACH reports on the costs of an inappropriately robust and sustained ISR, paid for by a catastrophic collapse of myelin homeostasis (Fig. 2). If these ideas are correct, CACH defines one of the limitations of the benefits of a therapeutic strategy aimed at strengthening the ISR.

Inflammatory CNS demyelination and the ISR

The ISR also appears to play an important role in the defense against inflammatory demyelination in the CNS. In this context, it is an insufficiently robust ISR (imposed by haploid insufficiency of PERK) that exposes mice to enhanced demyelination in a model of experimental allergic encephalitis (EAE; Lin et al. 2007) or cytokine-induced CNS inflammation (Lin et al. 2006), and GADD34 deletion protects against oligodendrocyte loss in these models (Lin et al. 2008).

It is unclear if CACH and the EAE/cytokine-induced CNS inflammation are exposing different facets of a special role for the ISR in myelin homeostasis. In the case of the CACH syndrome, it is not even clear in which cell type(s) the drama of attenuated eIF2B activity is played out, while the role of the ISR in inflammatory demyelination is likely played out at the level of the myelin producing cell (the oligodendrocyte, in case of the CNS). The latter is supported by evidence

from the peripheral nervous system, where mutations that lead to misfolding of abundantly expressed myelin constituents affect the viability and health of the Schwann cell and lead to a peripheral neuropathy of the Charcot-Marie-Tooth type. In a mouse model of Charcot-Marie-Tooth type 1B, caused by a misfolding-prone mutation in P0, deletion of CHOP restores motor function and reduces demyelination (Pennuto et al. 2008). It will be interesting to learn what role, if any, other mutations that affect the ISR (e.g., PERK haploid insufficiency and GADD34 mutation) will have on this condition. A coherent phenotype, with ISR loss-of-function exacerbating and ISR gain-of-function ameliorating demyelination, would support the notion that the myelin-producing cells are especially sensitive to ER stress.

Parkinson's disease, defective vesicular transport and chaperone inactivation

Evidence of enhanced UPR activity in the affected brain regions of Parkinson's disease (PD) patients and of patients suffering from the related multi-systems atrophy syndrome has been noted (Hoozemans et al. 2007; Makioka et al. 2010), and experimental manipulations that model aspects of PD in cultured cells and mouse tissues have been noted to promote UPR signaling (Ryu et al. 2002; Holtz and O'Malley 2003; Silva et al. 2005). This finding engenders two important questions: how are ER stress signaling pathways engaged in PD, and what roles do the underlying ER stress or the response to it play in the pathophysiology of PD?

Studies in yeast have shown that synuclein over-expression interferes with ER to Golgi transport of vesicles (Cooper et al. 2006), an effect that appears to be direct, as it can be reproduced in an in vitro transport assay (Gitler et al. 2008). The defect can be corrected by over-expression of Rab1, a small GTPase that regulates aspects of vesicular transport. Remarkably, Rab1 over-expression also attenuates the dysfunction of dopaminergic neurons over-expressing synuclein (Cooper et al. 2006), supporting a role for defective vesicular transport in the toxicity of synuclein. But is this related to ER stress?

ER to Golgi transport is key to secretion and interfering with it may compromise diverse cellular functions, besides imposing a measure of ER stress (presumably due to back-up of proteins in the ER). Thus, it is unclear if the ER misfolded protein stress that likely accompanies a mutant synuclein expression-mediated block to vesicular transport (Smith et al. 2005) contributes to the pathophysiology of PD. The point would be mooted by interventions that rectify the underlying defect; therefore, coming up with such interventions seems a priority. If this proves challenging, then it may be worth pausing to consider the role of ER stress and ER stress signaling in the pathophysiology of PD.

Altered vesicular transport may not be the only mechanism linking PD with ER stress; inactivating S-nitrosation of the ER-localized protein disulfide isomerase has

been reported in brain samples from individuals with sporadic PD (Uehara et al. 2006). This post-translational modification is predicted to affect disulfide bond formation and re-shuffling and thus has the potential to compromise protein folding in the ER and promote ER stress signaling.

Genetic studies point to a role for ER stress in the demise of dopaminergic neurons in toxigenic models of PD in mice. CHOP deficiency protects the neonatal striatum from 6-hydroxydopamine (Silva et al. 2005), whereas targeted expression of spliced XBP1 protected dopaminergic neurons against 1-methyl-4-phenylpyridinium (MPP+) in vitro (Sado et al. 2009) and ATF6α deficiency sensitized the striatum to similar insults in vivo (Egawa et al. 2010). These experiments strongly support a role for ER stress in the death and dysfunction of dopaminergic neurons exposed to toxigenic models of PD; however, the relevance of ER stress and the response to it in the pathogenesis of human PD remain unclear.

The UPR and cytosolic proteotoxicity

One of the themes to emerge from the study of PD is that pathological processes that occur in the cytosol, like the misfolding of α-synuclein, may have consequences on the ER and lead to (potentially) significant ER misfolded protein stress. This theme is echoed in cellular models of other neurodegenerative diseases. Elevated levels of ER stress markers have been noted in yeast (Bence et al. 2001) and mammalian cells (Nishitoh et al. 2002) over-expressing misfolding-prone poly-glutamine expanded proteins, like Huntingtin. This finding correlates with dysfunction of the ubiquitin proteasome system and suggests a model whereby attenuated degradation of misfolded/unfolded ER proteins (an ER-associated degradation, or ERAD, defect) triggers secondary ER stress in what is primarily a state of cytoplasmic proteotoxicity (Duennwald and Lindquist 2008).

The notion that normal function of the cytosolic ubiquitin-proteasome system is limiting to ERAD and ER protein folding homeostasis is further supported by high levels of ER stress noted in secretory cells exposed to proteasome inhibitors — such as Bortezomib, used in cancer therapy (Nawrocki et al. 2005; Obeng et al. 2006). A role for ER stress in the death and dysfunction of cells expressing misfolding-prone poly-glutamine expanded proteins is suggested by the observation that, in a cellular model of Huntington's Disease, disrupting the link between ER stress and Jun N-terminal kinase by inactivation of the upstream ASK1 kinase ameliorates proteotoxicity (Nishitoh et al. 2002). However, the extent to which these observations would carry over to tissue models, let alone the human disease, is unclear. Furthermore, it is unclear if the benefits of ASK1 inactivation accrue solely via disruption of this ER nexus.

An intriguing development in the study of the links between ER stress and cytoplasmic proteotoxicity concerns the case of a mouse model of amyotrophic lateral sclerosis (ALS)/ motor neuron disease, based on targeted expression of an ALS-associated mutant $SOD1^{G86R}$. SOD1 is not a secreted protein; its

misfolding-prone mutant versions accumulate intracellularly and, like the aforementioned cytosolic proteotoxicity diseases, experimental models of both ALS and the sporadic disease in humans are associated with activation of UPR markers in the motor neurons (Atkin et al. 2008). Therefore, the conceptual frame of a transmembrane equilibrium of proteostasis — whereby protein misfolding in the cytosol begets ER stress via attenuated ERAD — predicted that further compromise of the UPR would accelerate disease development caused by mutant SOD1 and enhance its severity. The opposite was observed. Deletion of XBP1 in the nervous system delayed degeneration of motor neurons in an $SOD1^{G86R}$ transgenic model (Hetz et al. 2009). This surprising observation correlated with enhanced clearance of the mutant SOD1 by autophagy and suggested a circuitous model whereby defective induction ERAD genes in the XBP1 mutant leads to a compensatory upregulation of autophagy. Mutant SOD1 is apparently caught up in this backup proteostatic mechanism as a bystander and its clearance is enhanced (Hetz et al. 2009).

The pathways linking ER stress to autophagy are incompletely worked up. In yeast, HOG1 links ER stress to autophagy (Bicknell et al. 2010) and, in mammalian cells, induction of autophagy by proteasome inhibitors is IRE1 — but not XBP1-dependent (Ding et al. 2007). Viewed narrowly, these observations suggest that inhibitors of IRE1's sequence-specific RNase activity may delay motor neuron loss in patients with SOD1 mutations. But the broader implication is that the extensive homeostatic feedback in the UPR makes it difficult to predict the consequences of manipulations that enhance or enfeeble specific strands of the response. At the same time, it suggests that tools to selectively (and transiently) inhibit UPR signaling may be applied to mobilize homeostatic responses to intervene in the pathophysiology of neurodegeneration. Because UPR signaling is conducted via rather private pathways through the activity of enzymes like IRE1 and PERK with few substrates, it presents several attractive drug targets with potential for considerable selectivity. It will be interesting to learn if efforts currently underway to exploit these nascent concepts will prove relevant to the treatment of neurodegenerative diseases.

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http://www.springer.com/978-3-642-27927-0

Protein Quality Control in Neurodegenerative Diseases
Morimoto, R.I.; Christen, Y. (Eds.)
2013, XII, 136 p., Hardcover
ISBN: 978-3-642-27927-0