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Endoplasmic reticulum stress and the unfolded protein response in disorders of myelinating glia

Benjamin L.L. Clayton^a and Brian Popko^{a,*}

^aDepartment of Neurology, The University of Chicago Center for Peripheral Neuropathy, The University of Chicago, Illinois, 60637

Abstract

Myelin is vital to the proper function of the nervous system. Oligodendrocytes in the CNS and Schwann cells in the PNS are the glial cells responsible for generating the myelin sheath. Myelination requires the production of a vast amount of proteins and lipid-rich membrane, which puts a heavy load on the secretory pathway of myelinating glia and leaves them susceptible to endoplasmic reticulum (ER) stress. Cells respond to ER stress by activating the unfolded protein response (UPR). The UPR is initially protective but in situations of prolonged unresolved stress the UPR can lead to the apoptotic death of the stressed cell. There is strong evidence that ER stress and the UPR play a role in a number of disorders of myelin and myelinating glia, including multiple sclerosis, Pelizaeus-Merzbacher disease, Vanishing White Matter Disease, and Charcot-Marie-Tooth disease. In this review we discuss the role that ER stress and the UPR play in these disorders of myelin. In addition, we discuss the progress that has been made in our understanding of the effect genetic and pharmacological manipulation of the UPR has in mouse models of these disorders and the novel therapeutic potential of targeting the UPR that these studies support.

Keywords

ER Stress; Unfolded Protein Response; Myelin; Oligodendrocytes; Schwann Cells

1. Introduction

Oligodendrocytes of the central nervous system (CNS) and Schwann cells from the peripheral nervous system (PNS) are glia cells responsible for producing the myelin sheath that wraps axons (Fig 1). Myelin is a multi-lamellar structure that wraps axons in multiple layers of compact lipid membrane with a cytoplasmic channel next to the periaxonal space. Oligodendrocytes simultaneously myelinate multiple axonal segments while Schwann cells myelinate a single segment of axon. The original function attributed to the myelin sheath was simply that of an electrical insulator to promote fast conduction of action potentials by increasing electrical resistance and decreasing capacitance (Bercury and Macklin, 2015).

*Corresponding author.: bpopko@neurology.bsd.uchicago.edu.

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Nevertheless, myelin is also essential in establishing the nodes of Ranvier, paranodes, and juxtaparanodes - axonal domains which are necessary for saltatory conduction (Fig 2) (see Buttermore et al., 2013 for review). The presence of myelinating glia is necessary for clustering of sodium channels within the axonal membrane at nodes of Ranvier (Ching et al., 1999; Rasband et al., 1999) and potassium channels at the juxtaparanode region (Fig 2) (Baba et al., 1999). Moreover, myelin and myelinating glia are critical for the development and maintenance of axonal integrity through metabolic support (see Simons and Nave, 2015 and Morrison et al., 2013 for review). Disruption of certain oligodendrocyte proteins can lead to axonal pathology even in the absence of gross demyelination. For example, ablation of the oligodendrocyte specific protein 2',3'-cyclic-nucleotide 3'-phosphodiesterase (CNP) in mice causes axonal damage such as axonal swelling, despite the presence of compact myelin (Griffiths et al., 1998; Lappe-Siefke et al., 2003). In addition, myelinating glia play an important role in providing metabolic energy to the axon through the myelin sheath. Although local energy production along the axon is likely necessary to maintain proper function, compact myelin covers much of the axonal surface area, thus limiting access of the axon to extracellular glucose for energy production. Recent findings indicate that myelinating glia act as energy providers via monocarboxylate transporters (MCTs) transport lactate and pyruvate across the cell membrane. MCT1 is localized to oligodendrocytes while MCT2 is found in neurons (Lee et al., 2012; Rinholm et al., 2011). Importantly, oligodendrocyte-specific downregulation of Mct1 causes axonal pathology (Lee et al., 2012) without death of oligodendrocytes which is likely due to the ability of oligodendrocytes to survive on aerobic glycolysis (Fünfschilling et al., 2012). These results suggest a system in which lactate, a product of glycolysis, is transported from myelin to axons via MCT1 and MCT2, such that myelinating glia provide metabolic support for axons isolated from extracellular glucose (Fig 2). The dependence on myelinating glia for formation of axonal domains and metabolic support clearly demonstrate that myelin is more than a passive electrical insulator. Indeed, myelination has been shown to be a major step in the evolution of the vertebrate nervous system with critical roles in motor, sensory, and cognitive function (Bercury and Macklin, 2015).

The importance of myelin and myelinating glia is unmistakable given the devastating effects of disorders of myelin including multiple sclerosis (MS), Pelizaeus-Merzbacher disease (PMD), Vanishing White Matter Disease (VWMD), and Charcot-Marie-Tooth disease (CMT) (Table 1). MS is a common CNS demyelinating disorder involving T cell-mediated autoimmunity that results in inflammation, loss of oligodendrocytes, demyelination, and axonal damage leading to worsening neurological outcomes that significantly decrease quality of life (Franklin et al., 2012). PMD is a genetic demyelinating disorder of the CNS with childhood onset caused by mutations in the PLP1 gene, which encodes proteolipid protein (PLP), a major protein component of myelin; prognosis varies by mutation from gait abnormalities to progressive neurological decline until death (Garbern, 2007). VWMD is an autosomal-recessive disorder characterized by CNS hypomyelination and abnormal "foamy" oligodendrocytes that occurs following minor head trauma or febrile infection causing episodes of rapid and progressive neurological deterioration (van der Knaap et al., 2006). CMT is a genetic disorder that affects PNS myelin and one form, CMT1, is caused by dominant mutations in genes that encode peripheral myelin proteins. CMT affects both

motor and sensory nerves but is not fatal (Theocharopoulou and Vlamos, 2015). Studies have suggested that endoplasmic reticulum (ER) stress is involved in all of these diseases. Because myelin is lipid-rich with large amounts of critical proteins, estimates from morphometric analysis indicate that myelinating glia have a surface area thousands of times greater than a typical mammalian cell (Pfeiffer et al., 1993). Therefore, production of these lipids and proteins during active myelination asserts a high burden on the secretory pathway and studies suggest that this high demand makes myelinating glia highly susceptible to ER stress (D'Antonio et al., 2009; Lin and Popko, 2009).

ER stress is caused by the accumulation of unfolded or misfolded proteins, disruption to lipid synthesis, or depletion of calcium stores, and the cell responds to ER stress through the unfolded protein response (UPR) in order to maintain ER homeostasis (Walter and Ron, 2011). The UPR is a highly conserved eukaryotic signaling network orchestrated by three transmembrane UPR signal transducers: inositol requiring enzyme 1 (IRE1), activating transcription factor 6 (ATF6), and PKR-like endoplasmic reticulum kinase (PERK; also known as eIF2 α kinase 3 [EIF2AK3])(Cao and Kaufman, 2012). The UPR coordinates a response that decreases protein folding load, increases folding capacity of the ER, and induces cytoprotective genes (Walter and Ron, 2011). Activation of the UPR protects the cell while the insult which initiated ER stress is corrected; however, if ER stress is left unresolved, the UPR can become pro-apoptotic (Li et al., 2014). The IRE1 pathway is the most evolutionarily conserved branch of the UPR and acts to decrease protein folding load and increase ER protein folding capacity (Cao and Kaufman, 2012). IRE1 splices X-box binding protein 1 (XBP-1) mRNA to produce an active transcription factor that induces expression of genes encoding protein-folding chaperones, proteins involved in ER biogenesis, and proteins necessary for ER associated degradation of proteins (Calfon et al., 2002; Peschek et al., 2015). In addition, IRE1 degrades ER resident mRNA through a stress dependent process named regulated IRE1-dependent decay of mRNAs, or RIDD, to decrease the ER load (Hollien and Weissman, 2006). In response to ER stress, ATF6 is trafficked to the Golgi complex where it is cleaved by Site-1 and Site-2 proteases. Cleaved ATF6 is then able to translocate to the nucleus as an active transcription factor (Ye et al., 2000). ATF6 can act as a homodimer to activate protein chaperone genes to aid in protein folding (Shen et al., 2005). In addition, ATF6 can also form heterodimers with spliced XBP-1 to activate ER associated degradation components (Cao and Kaufman, 2012). PERK activation occurs when it disassociates from the protein chaperone binding immunoglobulin protein (BiP; also known as heat shock 70kDa protein 5 [HSPA5]), leading to PERK autophosphorylation and kinase activity which decreases global protein translation through phosphorylation of eukaryotic initiation factor 2 alpha (eIF2 α) (Harding et al., 1999). The importance of the UPR in myelinating cells is highlighted in studies where Bip is specifically deleted from oligodendrocytes or Schwann cells, as both situations result in death of the myelinating cell and hypomyelination (Hussien et al., 2015). Phosphorylated eIF2 α inhibits global protein translation while allowing for increased translation of a subset of transcripts including the mRNA encoding for activating transcription factor 4 (ATF4), which increases the expression of cytoprotective genes and the transcription factor CAAT enhancer binding protein homologous protein (CHOP; also known as DNA-damage-inducible transcript 3 [DDIT3]) (Harding et al., 2003). CHOP transcriptionally activates growth and arrest DNA-damage 34

(GADD34; also known as protein phosphatase 1 regulatory subunit 15A [PPP1R15A]), which complexes with protein phosphatase 1 (PP1) to dephosphorylate eIF2 α and terminate the PERK arm of the UPR (Marciniak et al., 2004; Novoa et al., 2001). In situations of unresolved ER stress, CHOP accumulation induces apoptosis directly by repressing expression of the anti-apoptotic protein Bcl-2 and indirectly by increasing the expression of GADD34, which terminates the UPR prematurely and increases the protein folding demand within the stressed cell (Li et al., 2014; Marciniak et al., 2004; Zinszner et al., 1998). Together PERK, IRE-1, and ATF6 of the UPR play an important role in promoting cell survival in the face of ER stress. Each arm of the UPR has been shown to participate in disorders of myelin and myelinating glia.

In this review we will discuss the current understanding of the role that ER stress and the UPR play in disorders of myelin and myelinating glia. We will discuss ER stress in oligodendrocytes in MS, PMD, and VWMD, and in Schwann cells in CMT. Finally, we will highlight the considerable advances made in the genetic and pharmacological manipulation of the UPR to protect myelinating glia in these disorders and the potential of UPR-targeted therapeutics being brought to the clinic.

2. ER Stress in Multiple Sclerosis

MS is a demyelinating disorder that affects approximately 2.3 million individuals worldwide, occurring in both children and adults (Karussis, 2014). MS is an autoimmune disease that involves inflammation, loss of oligodendrocytes, demyelination, and axonal damage (Franklin et al., 2012). Different forms of MS range in severity from relapsing-remitting MS, in which patients experience transient immune-mediated attacks on myelin followed by remission and remyelination, to primary progressive MS, where neurological symptoms steadily worsen and no remission occurs (Barnett and Prineas, 2004). Although the etiology of MS remains controversial, studies have found the presence of apoptotic oligodendrocytes prior to immune infiltration (Barnett and Prineas, 2004). In addition, a recent report showed that early experimentally-driven death of oligodendrocytes resulted in a late-onset, immune-driven demyelinating disorder in mice (Traka et al., 2015). Together these findings suggest that MS may be initiated by oligodendrocyte death and myelin damage in the CNS. A number of studies have also established ER stress and the UPR as participants in MS and its primary animal model experimental autoimmune encephalomyelitis (EAE) (Stone and Lin, 2015; Way and Popko, 2016). Evidence of UPR activation, including increased CHOP and ATF4 mRNA, is found in demyelinating lesions of MS patients (Cunnea et al., 2011) and CHOP immunoreactivity is seen in multiple cell types, including oligodendrocytes, in active MS lesions (McMahon et al., 2012; Mhille et al., 2008). In agreement with the human MS findings, studies have detected evidence of UPR activation such as increased levels of phosphorylated eIF2 α and CHOP protein in multiple cell types, including oligodendrocytes, within white matter lesions of EAE mice and rats (Chakrabarty et al., 2005, 2004; Lin et al., 2007; N Fhlathartaigh et al., 2013). Nevertheless, Chop gene ablation has been reported to have no effect on the EAE disease course (Deslauriers et al., 2011).

Considerable work has focused on genetic manipulation of cells and mice to examine the role of the UPR in EAE and inflammatory-mediated demyelination (Way and Popko, 2016).

To model inflammation, interferon-gamma (IFN- γ), a cytokine that is expressed by T cells and plays a crucial role in MS and EAE, is often used (Lees and Cross, 2007; Popko et al., 1997). IFN- γ causes ER stress and apoptosis in cultured rat oligodendrocytes, and when ectopically expressed by astrocytes in the mouse CNS during development, the cytokine induces ER stress in oligodendrocytes and hypomyelination (Lin et al., 2005). Using IFN- γ it was also shown that UPR signaling through PERK protects oligodendrocytes. When mice heterozygous for a Perk-null mutation were exposed to IFN- γ during developmental myelination they had more severe hypomyelination and increased mortality compared to Perk wild-type mice exposed to IFN- γ (Lin et al., 2005). Likewise, the UPR was found to play a protective role in EAE. When IFN- γ was administered at different times during the EAE disease course, contrasting effects on EAE disease severity were found (Lin et al., 2007, 2006). Genetically-driven CNS-specific expression of IFN- γ prior to EAE onset protects oligodendrocytes and myelin and ameliorates EAE disease severity (Lin et al., 2007). Nevertheless, CNS expression of IFN- γ later in the EAE disease course inhibits remyelination and recovery (Lin et al., 2006). Importantly, the UPR plays a protective role in both situations. IFN- γ expression prior to EAE disease onset, protects oligodendrocytes and myelin and ameliorates EAE disease course. Nevertheless, these protective effects are lost in heterozygous Perk-null mice (Lin et al., 2007), demonstrating that the protective effects of prophylactic IFN- γ expression are at least in part dependent upon PERK. Using a similar approach, when the IFN- γ was expressed later in Perk haploinsufficient mice, the detrimental effects of the cytokine on remyelination and recovery were exacerbated, demonstrating again that activating PERK protects oligodendrocytes and myelin (Lin et al., 2006). These studies laid the foundation for the UPR as a potential therapeutic target in MS. Further studies have built upon these studies to examine the cell-specific role of PERK-mediated UPR activation in oligodendrocytes in both active EAE disease and recovery. Conditional deletion of Perk from oligodendrocytes worsens EAE disease severity and increases oligodendrocyte loss (Hussien et al., 2014). In addition, using a combined small molecule and genetic approach, it was shown that oligodendrocyte-specific activation of PERK protects mice against EAE and protects remyelinating oligodendrocytes during recovery from EAE (Lin et al., 2013). These studies demonstrate that the protective effects of UPR activation are, at least in part, due to a cell autonomous effect within oligodendrocytes.

With the establishment of the protective role of the UPR in the MS model EAE, there has been considerable interest in finding compounds that could pharmacologically enhance the UPR for therapeutic benefit. Such an approach would be particularly beneficial because current MS therapies are primarily immunomodulatory, and there are no approved therapies that directly protect oligodendrocytes and/or myelin. In addition, genetic manipulations of a second major UPR target, Gadd34, were performed to better understand its role in the protective pathway. GADD34 complexes with protein phosphatase 1 (PP1) to dephosphorylate eIF2 α , terminating the UPR. As predicted, a loss of function mutation in Gadd34 resulted in enhancement of the UPR in oligodendrocytes (Lin et al., 2008). Therefore, it was postulated that functional deletion of Gadd34 should inhibit the dephosphorylation of eIF2 α and prolong the protective effects of the UPR. In agreement with this hypothesis, GADD34 inactivation increases the percentage of phosphorylated eIF2 α positive oligodendrocytes and

protects oligodendrocytes and myelin from IFN- γ -induced immune mediated hypomyelination (Lin et al., 2008). In addition, treatment of rat hippocampal slice cultures exposed to IFN- γ with the small molecule salubrinal, which blocks formation of eIF2 α phosphatase complexes, increases phosphorylated eIF2 α protein levels and protects oligodendrocytes and myelin (Lin et al., 2008). Unfortunately, salubrinal is not desirable for use in humans due to its low solubility in aqueous solutions, which requires use of potentially toxic DMSO as a solvent. In addition, prolonged inhibition of both eIF2 α phosphatase complexes can be lethal, making salubrinal a potentially dangerous drug with a narrow window of utility (Boyce et al., 2005; Pavitt and Ron, 2012). Importantly, another small molecule, guanabenz, was found that inhibits GADD34-PP1 complex formation, prolongs eIF2 α phosphorylation, and protects cells against ER stress (Tsaytler et al., 2011). Guanabenz prolongs the UPR and protects mice against IFN- γ -induced oligodendrocyte loss in the CNS. Guanabenz also ameliorates the disease course in EAE mice, which correlates with the protection of oligodendrocytes and decreased CNS T-cell presence (Way et al., 2015). Disease amelioration is also seen in a relapsing-remitting EAE model of MS when guanabenz is given after the initial onset of disease (Way et al., 2015). In addition, a highly specific inhibitor of GADD34 has been developed called Sephin1 (Das et al., 2015). It will be of interest to determine whether Sephin1 can improve upon the protective effects of guanabenz in EAE. With the success of GADD34 inhibition in mouse models of MS, guanabenz has entered into clinical trials (<https://clinicaltrials.gov/ct2/show/NCT02423083?term=guanabenz&rank=1>).

3. ER Stress in Pelizaeus-Merzbacher Disease

Pelizaeus-Merzbacher disease (PMD) is an X chromosome-linked disease that causes dysmyelination of the CNS due to one of more than 60 known mutations in Plp1, which encodes for proteolipid protein (PLP) and its alternative spliced isoform DM20 (Garbern, 2007). PLP is a major myelin protein in the CNS, making up approximately 50% of total CNS myelin protein (Greer and Lees, 2002). Severity of PMD varies broadly depending upon the mutation, and studies in PMD animal models have suggested that severity depends upon the ability of PLP/DM20 to translocate out of the ER, with more severe disease caused by mutations in which PLP/DM20 are abnormally sequestered within the ER (Garbern, 2007; Gow and Lazzarini, 1996). Two genetic mouse models of PMD that carry authentic human PLP mutations illustrate the role of mutant PLP/DM20 ER retention on disease severity. The myelin-synthesis deficient mouse has a severe phenotype caused by an A242V amino acid substitution that causes severe PMD in humans (Gow and Lazzarini, 1996). This A242V mutation in Plp1 causes an increase in PLP localized to the ER and DM20 localized to the Golgi, with neither mutant protein reaching the cell surface (D'Antonio et al., 2009; Gow and Lazzarini, 1996). In contrast, in the rumpshaker mouse, which carries an I186T mutation that causes mild disease in both mouse and humans, DM20 does not accumulate in the ER while PLP is still retained in the ER (D'Antonio et al., 2009; Gow and Lazzarini, 1996). Further evidence that PLP/DM20 ER retention is central to disease severity is provided by Plp1-null mice in which PLP/DM20 protein is absent, instead of misfolded, and the phenotype is mild (Klugmann et al., 1997; Rosenbluth et al., 2006). It is important to note that while classical PMD is caused by mutations in Plp1 there are also PMD-like

diseases caused by mutations in other genes coding for myelin proteins. One example comes from a recent report in which the authors describe a mutation in the gene encoding the myelin associated glycoprotein (MAG) protein, which was found in three siblings exhibiting PMD-like disease phenotype (Lossos et al., 2015). In these patients, mutant MAG protein did not properly fold and was retained in the ER and caused an increase in the UPR marker BiP, suggesting a role for ER stress in PMD-like disorders (Lossos et al., 2015).

Evidence from human patients, mouse models, and in vitro studies of PMD support the initial observation and hypothesis that ER stress plays a central role in PMD. In mice with mutant PLP, increases in CHOP protein localize to mature oligodendrocytes, which recapitulates the increase in oligodendrocyte-localized CHOP expression in PMD patients (Southwood et al., 2002). Indeed, microarray analysis in optic nerves from mice with mutant PLP show a significant increase in expression of the UPR genes encoding CHOP, ATF4, and BiP protein (Southwood et al., 2013). In addition, a recent study using induced pluripotent stem cells (iPSCs) harvested from PMD patients and differentiated into mature oligodendrocytes shows PLP accumulation in the ER (Numasawa-Kuroiwa et al., 2014). Interestingly, there is no increase in UPR markers in the PMD derived oligodendrocytes compared to those derived from a healthy patient. Nevertheless, PMD derived oligodendrocytes from a patient with a severe phenotype have increased sensitivity to mild ER stress, such that might occur during active myelination (Numasawa-Kuroiwa et al., 2014). In support of the role of ER stress in PMD severity, PMD derived oligodendrocytes from a second patient with a more mild disease were more resistant to ER stress compared to the oligodendrocytes from the severe PMD patient (Numasawa-Kuroiwa et al., 2014). Oligodendrocytes derived from both PMD patients also showed increased apoptosis, produced abnormal myelin structure, and had disrupted ER morphology compared to cells from a healthy patient (Numasawa-Kuroiwa et al., 2014).

Attempts to genetically manipulate the UPR in PMD mouse models have been met with surprising results. As mentioned, in most cell types and contexts, CHOP is pro-apoptotic (Marciniak et al., 2004; Zinszner et al., 1998). Nevertheless, Chop ablation from rumpshaker mice exacerbates disease severity dramatically (Southwood et al., 2002). Rumpshaker mice without CHOP have increased oligodendrocyte apoptosis and die around 5 weeks of age, whereas control rumpshaker mice have lifespans similar to wildtype mice (Southwood et al., 2002). These results demonstrate that modulating the UPR can affect PMD disease severity, but that surprisingly, CHOP in oligodendrocytes is protective within this disease context. This paradoxical role of CHOP in oligodendrocytes is still not fully understood. A suggested hypothesis is that CHOP targets a different set of genes in oligodendrocytes compared to other cell types, which is supported by the lack of induced downstream CHOP genes in PMD (Southwood et al., 2002). More studies are needed to better understand the disparate roles of CHOP in oligodendrocytes and disease models.

4. ER Stress in Vanishing White Matter Disease

Vanishing white matter disease (VWMD) is an autosomal-recessive disorder characterized by hypomyelination and abnormal “foamy” oligodendrocytes (van der Knaap et al., 2006). The clinical course of VWMD is chronic and progressive with minor head trauma and

febrile infection, causing episodes of rapid neurological deterioration (van der Voorn et al., 2005). VWMD is caused by a mutation in any of the genes encoding the five subunits of eukaryotic initiation factor 2B (eIF2B), a guanine nucleotide exchange factor that promotes the formation of an active eIF2-GTP complex necessary for protein translation (van der Knaap et al., 2006). ER stress leading to phosphorylation of eIF2 α reduces the activity of eIF2B by forming an inactive phosphorylated eIF2 α -eIF2B complex that inhibits translation initiation (van der Voorn et al., 2005). It has been proposed that mutant eIF2B perturbs the oligodendrocyte response to ER stress (van der Knaap et al., 2006). Support for this hypothesis has been shown in vitro, expression of eIF2B mutations in an oligodendrocyte cell line causes a higher basal level of UPR markers and cells expressing mutant eIF2B have a heightened UPR response and increased cell death compared to controls (Chen et al., 2015; Kantor et al., 2008).

Markers of ER stress and activation of all three arms of the UPR have been seen in oligodendrocytes of VWMD patients (van der Voorn et al., 2005; van Kollenburg et al., 2006). Increased levels of BiP, ATF4, CHOP, and GADD34 mRNA is seen in postmortem tissue from VWMD patients as well as increased XBP-1 mRNA splicing and increased immunostaining for XBP-1, BiP, and ATF6 in oligodendrocytes (van Kollenburg et al., 2006). In another study, increased immunostaining for phosphorylated PERK, phosphorylated eIF2 α , and ATF4 was also seen in oligodendrocytes from VWMD patient tissue (van der Voorn et al., 2005).

Mouse models that reproduce the characteristics of VWMD have been difficult to produce (Lin et al., 2014b). Point mutations in eIF2B reproduce some white matter deficits but to a much milder degree than seen in VWMD. While the role that ER stress plays in the phenotype of these mouse models has not been examined (Geva et al., 2010), a recent study using transgenic mice provides evidence for a role of ER stress in VWMD (Lin et al., 2014b). PLP/Fv2E-PERK transgenic mice allow for oligodendrocyte-specific expression of a chimeric protein that contains the luminal eIF2 α kinase domain of PERK fused to a small molecule-driven artificial dimerizing domain. Using this transgenic mouse, the authors demonstrate that strong activation of PERK specifically in oligodendrocytes leads to impaired eIF2B activity in young developing mice, resulting in a VWMD-like phenotype including myelin loss and “foamy” oligodendrocytes (Lin et al., 2014b). Strong activation of PERK was accomplished by using mice homozygous for PLP/Fv2E-PERK with high doses of the small molecule dimerizer compared to moderate PERK activation, which has no effect on developmental myelination in hemizygous PLP/Fv2E-PERK mice with low doses of dimerizer (Lin et al., 2014a). This study provides evidence of cell autonomous decreased eIF2B activity in oligodendrocytes as causative in VWMD. In addition, it presents the basis for a hypothesis regarding one of the mysteries of VWMD, namely how do mutations in a gene necessary for protein translation cause selective damage to white matter and oligodendrocytes? The authors propose that inhibition of eIF2B is biphasic in nature. Modest inhibition of eIF2B is protective (Lin et al., 2013, 2014a) while strong inhibition of eIF2B, such as would happen with the combination of eIF2B mutation and phosphorylation of eIF2 α by ER stress, is damaging to oligodendrocytes during active myelination and may explain the severe demyelination seen in VWMD following acute stress (Lin et al., 2014b). This hypothesis deserves further investigation.

6. ER Stress in Charcot-Marie Tooth Disease

Charcot-Marie-Tooth disease is a group of dominant genetic disorders affecting PNS myelin and categorized based on whether the disease originates from mutations in genes expressed by myelinating Schwann cells (Charcot-Marie-Tooth 1 or CMT1) or neurons (Charcot-Marie-Tooth 2 or CMT2) (Theocharopoulou and Vlamos, 2015). Charcot-Marie-Tooth affects 1 in 2,500 individuals, making it one of the most common inherited neurological disorders. Most CMT1 cases are caused by mutations in genes that encode peripheral myelin proteins (Theocharopoulou and Vlamos, 2015). CMT1 is further categorized based on the affected protein. The most prevalent form of CMT1A is caused by defects in the gene that encodes peripheral myelin protein 22 (PMP22), with the most common form resulting from duplication of the chromosomal region containing PMP22 (Hoyle et al., 2015). Studies in Trembler mice, which carry the same missense mutations found in some forms of human CMT1A patients, have shown that mutant PMP22 is misfolded and abnormally localizes to the ER where it forms aggregates and associates with the ER chaperone calnexin (Dickson et al., 2002). Nevertheless, Trembler mice do not show evidence of UPR activation (Dickson et al., 2002). A second category of CMT1 is CMT1B, which is caused by one of over 120 mutations found in the gene that encodes myelin protein zero (MPZ), the most abundant protein in PNS myelin (Theocharopoulou and Vlamos, 2015). Similar to CMT1A, many of these mutations cause MPZ to be retained in the ER instead of being transported to the myelin sheath. In contrast to CMT1A, CMT1B mouse models display evidence of UPR activation in Schwann cells (Theocharopoulou and Vlamos, 2015).

The P0S63del mutant mouse is a mouse model of CMT1B that carries the same S63 deletion in Mpz that causes CMT1B in humans (Wrabetz et al., 2006). Studies of this mouse show that mutant MPZ is retained in the ER and activates all branches of the UPR in Schwann cells. Increased levels of phosphorylated eIF2 α , ATF4, and CHOP indicate activation of the PERK pathway, spliced XBP-1 is evidence of IRE1 activation, and cleavage of ATF6 is also detected in P0S63del mice (Pennuto et al., 2008; Wrabetz et al., 2006). Moreover, activation of the UPR in the nerves of these mice is dose-dependent, strongly suggesting that increased activation of the UPR is directly related to increased levels of mutant MPZ protein in the ER (Wrabetz et al., 2006). As previously mentioned, CHOP is generally considered to be pro-apoptotic and Chop deletion protects cells from ER stress-induced apoptosis (Walter and Ron, 2011). In agreement with this classical role, Chop ablation from P0S63del mice reduces the number of demyelinated fibers and lessens the electrophysiological and behavioral defects typically found in P0S63del mice (Pennuto et al., 2008). Surprisingly, CHOP deletion neither decreases the number of apoptotic Schwann cells nor increases the myelin thickness of P0S63del nerves. Similarly, another mouse model of CMT1B that contains the human R98C mutation in the Mpz locus, also exhibits ER retention of mutant MPZ and activation of the UPR (Saporta et al., 2012). In addition, treatment of R98C mice with curcumin, which has been shown to relieve endoplasmic reticulum stress, is protective. However, Chop ablation in this model did not provide therapeutic benefit (Patzkó et al., 2012; Saporta et al., 2012). The results of Chop ablation in the CMT1B mouse models again point to an apparent distinct role that CHOP plays in myelinating glia, which requires further investigation (Pennuto et al., 2008). A more recent study continued to expand on the

therapeutic potential of UPR manipulation in cases of CMT1B. Utilizing microarray analysis of P0S63del nerves, D'Antonio et al. reported that UPR transcripts are upregulated in P0S63del nerves prior to the onset of myelination and prior to induction of any apoptotic or inflammatory transcripts (D'Antonio et al., 2013). These results, coupled with the protective effects of Chop deletion, suggest that the UPR is maladaptive in the P0S63del model of CMT1B. Interestingly, GADD34 mRNA and protein was increased in P0S63del nerves and returned to normal levels in P0S63del nerves absent CHOP (D'Antonio et al., 2013). This led the authors to hypothesize that premature termination of the UPR by GADD34 is maladaptive in P0S63del nerves. To examine the role of GADD34 in CMT1B nerves, P0S63del mice were crossed with GADD34-null mice. Removal of GADD34 function from P0S63del nerves restores motor performance, lessens the electrophysiological deficits in P0S63del mice, decreases the number of demyelinated axons, and increases myelin thickness (D'Antonio et al., 2013). Importantly, treatment of P0S63del mice with the GADD34 inhibitor salubrinal has the same effect as genetic removal of GADD34 function (D'Antonio et al., 2013). The protective effects of GADD34 inhibition are accompanied by increased levels of phosphorylated eIF2 α and ATF4, showing an increase in the PERK arm of the UPR, although XBP-1 splicing and ATF6 cleavage are decreased (D'Antonio et al., 2013). Treatment with the specific GADD34 inhibitor Sephin1 restores motor function and rescued myelin deficits in P0S63del nerves compared to vehicle treated controls (Das et al., 2015). These results in a CMT1B mouse model highlight the therapeutic potential of targeting the UPR in the treatment of CMT1B.

7. Therapeutic potential of targeting ER Stress in demyelinating disorders

There are currently no completely effective therapies for demyelinating disorders of the CNS or PNS. Many disorders of myelinating glia, however, involve disruptions in protein folding and trafficking, resulting in ER stress. The UPR is thus an exciting candidate for therapeutic intervention. Nevertheless, therapeutically targeting the UPR, a conserved pathway essential to efficient protein production, without causing severe side effects will not be trivial. In addition, manipulation of ER stress and the UPR can have seemingly contradictory effects within myelinating disorders. For example, deletion of Chop is detrimental in mouse models of PMD (Southwood et al., 2002) but protective in mouse models of CMT1B (Pennuto et al., 2008), and has no effect on EAE (Deslauriers et al., 2011). Therefore, it will be important to have a full understanding of ER stress and UPR signaling within each disease to better predict the effect that manipulating the UPR will have in patients. This is best displayed in two of the demyelinating disorders discussed, MS in the CNS and CMT1B in the PNS. Studies in both diseases began by using genetic and biochemical studies to build an understanding of the specific role ER stress and the UPR has in each disease, followed by studies utilizing pharmacological manipulation of the UPR that in the case of MS has resulted in a clinical trial. In addition, better and more varied pharmacological manipulators of the UPR are needed. The discovery of guanabenz as a GADD34 inhibitor (Tsaytler et al., 2011) was vital to translating the protective effects of GADD34-null mutations in MS models to a potential clinical therapy, and the increased specificity of Sephin1 holds the potential to improve upon guanabenz. As we gain a greater

understanding of ER stress and the UPR, we are hopeful that the results will translate into more meaningful therapies for myelinating disorders.

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References

- Baba H, Akita H, Ishibashi T, Inoue Y, Nakahira K, Ikenaka K. Completion of myelin compaction, but not the attachment of oligodendroglial processes triggers K⁺ channel clustering. *J Neurosci Res*. 1999
- Barnett MH, Prineas JW. Relapsing and remitting multiple sclerosis: pathology of the newly forming lesion. *Ann Neurol*. 2004; 55:458–468. [PubMed: 15048884]
- Bercury KK, Macklin WB. Dynamics and mechanisms of CNS myelination. *Dev Cell*. 2015; 32:447–458. [PubMed: 25710531]
- Boyce M, Bryant KF, Jousse C, Long K, Harding HP, Scheuner D, Kaufman RJ, Ma D, Coen DM, Ron D, Yuan J. A selective inhibitor of eIF2 α dephosphorylation protects cells from ER stress. *Science*. 2005; 307:935–939. [PubMed: 15705855]
- Buttermore ED, Thaxton CL, Bhat MA. Organization and maintenance of molecular domains in myelinated axons. *J Neurosci Res*. 2013; 91:603–622. [PubMed: 23404451]
- Calfon M, Zeng H, Urano F, Till JH, Hubbard SR, Harding HP, Clark SG, Ron D. IRE1 couples endoplasmic reticulum load to secretory capacity by processing the XBP-1 mRNA. *Nature*. 2002; 415:92–96. [PubMed: 11780124]
- Cao SS, Kaufman RJ. Unfolded protein response. *Curr Biol*. 2012; 22:R622–R626. [PubMed: 22917505]
- Chakrabarty A, Danley MM, LeVine SM. Immunohistochemical localization of phosphorylated protein kinase R and phosphorylated eukaryotic initiation factor-2 α in the central nervous system of SJL mice with experimental allergic encephalomyelitis. *J Neurosci Res*. 2004; 76:822–833. [PubMed: 15160394]
- Chakrabarty A, Fleming KK, Marquis JG, LeVine SM. Quantifying immunohistochemical staining of phospho-eIF2 α , heme oxygenase-2 and NADPH cytochrome P450 reductase in oligodendrocytes during experimental autoimmune encephalomyelitis. *J Neurosci Methods*. 2005; 144:227–234. [PubMed: 15910982]
- Chen N, Jiang YW, Hao HJ, Ban TT, Gao K, Zhang ZB, Wang JM, Wu Y. Different Eukaryotic Initiation Factor 2B ϵ Mutations Lead to Various Degrees of Intolerance to the Stress of Endoplasmic Reticulum in Oligodendrocytes. *Chin Med J*. 2015; 128:1772–1777. [PubMed: 26112719]
- Ching W, Zanazzi G, Levinson SR, Salzer JL. Clustering of neuronal sodium channels requires contact with myelinating Schwann cells. *J Neurocytol*. 1999; 28:295–301. [PubMed: 10739572]
- Cunnea P, Mhille AN, McQuaid S, Farrell M, McMahon J, FitzGerald U. Expression profiles of endoplasmic reticulum stress-related molecules in demyelinating lesions and multiple sclerosis. *Mult Scler*. 2011; 17:808–818. [PubMed: 21382862]
- D’Antonio M, Feltri ML, Wrabetz L. Myelin under stress. *J Neurosci Res*. 2009; 87:3241–3249. [PubMed: 19330777]
- D’Antonio M, Musner N, Scapin C, Ungaro D, Del Carro U, Ron D, Feltri ML, Wrabetz L. Resetting translational homeostasis restores myelination in Charcot-Marie-Tooth disease type 1B mice. *J Exp Med*. 2013; 210:821–838. [PubMed: 23547100]
- Das I, Krzyzosiak A, Schneider K, Wrabetz L, D’Antonio M, Barry N, Sigurdardottir A, Bertolotti A. Preventing proteostasis diseases by selective inhibition of a phosphatase regulatory subunit. *Science*. 2015; 348:239–242. [PubMed: 25859045]

- Deslauriers AM, Afkhami-Goli A, Paul AM, Bhat RK, Acharjee S, Ellestad KK, Noorbakhsh F, Michalak M, Power C. Neuroinflammation and endoplasmic reticulum stress are coregulated by crocin to prevent demyelination and neurodegeneration. *J Immunol.* 2011; 187:4788–4799. [PubMed: 21964030]
- Dickson KM, Bergeron JJ, Shames I, Colby J, Nguyen DT, Chevet E, Thomas DY, Snipes GJ. Association of calnexin with mutant peripheral myelin protein-22 ex vivo: a basis for “gain-of-function” ER diseases. *Proc Natl Acad Sci U S A.* 2002; 99:9852–9857. [PubMed: 12119418]
- Franklin RJ, ffrench-Constant C, Edgar JM, Smith KJ. Neuroprotection and repair in multiple sclerosis. *Nat Rev Neurol.* 2012; 8:624–634. [PubMed: 23026979]
- Fünfschilling U, Supplie LM, Mahad D, Boretius S, Saab AS, Edgar J, Brinkmann BG, Kassmann CM, Tzvetanova ID, Möbius W, Diaz F, Meijer D, Suter U, Hamprecht B, Sereda MW, Moraes CT, Frahm J, Goebbels S, Nave KA. Glycolytic oligodendrocytes maintain myelin and long-term axonal integrity. *Nature.* 2012; 485:517–521. [PubMed: 22622581]
- Garbern JY. Pelizaeus-Merzbacher disease: Genetic and cellular pathogenesis. *Cell Mol Life Sci.* 2007; 64:50–65. [PubMed: 17115121]
- Geva M, Cabilly Y, Assaf Y, Mindroul N, Marom L, Raini G, Pinchasi D, Elroy-Stein O. A mouse model for eukaryotic translation initiation factor 2B–leucodystrophy reveals abnormal development of brain white matter. *Brain.* 2010; 133:2448–2461. [PubMed: 20826436]
- Gow A, Lazzarini RA. A cellular mechanism governing the severity of Pelizaeus-Merzbacher disease. *Nat Genet.* 1996; 13:422–428. [PubMed: 8696336]
- Greer JM, Lees MB. Myelin proteolipid protein--the first 50 years. *Int J Biochem Cell Biol.* 2002; 34:211–215. [PubMed: 11849988]
- Griffiths I, Klugmann M, Anderson T, Yool D, Thomson C, Schwab MH, Schneider A, Zimmermann F, McCulloch M, Nadon N, Nave KA. Axonal swellings and degeneration in mice lacking the major proteolipid of myelin. *Science.* 1998; 280:1610–1613. [PubMed: 9616125]
- Harding HP, Zhang Y, Ron D. Protein translation and folding are coupled by an endoplasmic-reticulum-resident kinase. *Nature.* 1999; 397:271–274. [PubMed: 9930704]
- Harding HP, Zhang Y, Zeng H, Novoa I, Lu PD, Calfon M, Sadri N, Yun C, Popko B, Paules R, Stojdl DF, Bell JC, Hettmann T, Leiden JM, Ron D. An integrated stress response regulates amino acid metabolism and resistance to oxidative stress. *Mol Cell.* 2003; 11:619–633. [PubMed: 12667446]
- Hollien J, Weissman JS. Decay of endoplasmic reticulum-localized mRNAs during the unfolded protein response. *Science.* 2006; 313:104–107. [PubMed: 16825573]
- Hoyle JC, Isfort MC, Roggenbuck J, Arnold WD. The genetics of Charcot-Marie-Tooth disease: current trends and future implications for diagnosis and management. *Appl Clin Genet.* 2015; 8:235–243. [PubMed: 26527893]
- Hussien Y, Cavener DR, Popko B. Genetic inactivation of PERK signaling in mouse oligodendrocytes: normal developmental myelination with increased susceptibility to inflammatory demyelination. *Glia.* 2014; 62:680–691. [PubMed: 24481666]
- Hussien Y, Podojil JR, Robinson AP, Lee AS, Miller SD, Popko B. ER Chaperone BiP/GRP78 Is Required for Myelinating Cell Survival and Provides Protection during Experimental Autoimmune Encephalomyelitis. *J Neurosci.* 2015; 35:15921–15933. [PubMed: 26631473]
- Kantor L, Pinchasi D, Mintz M, Hathout Y, Vanderver A, Elroy-Stein O. A point mutation in translation initiation factor 2B leads to a continuous hyper stress state in oligodendroglial-derived cells. *PLoS ONE.* 2008; 3:e3783. [PubMed: 19023445]
- Karussis D. The diagnosis of multiple sclerosis and the various related demyelinating syndromes: A critical review. *J Autoimmun.* 2014
- Klugmann M, Schwab MH, Pühlhofer A, Schneider A, Zimmermann F, Griffiths IR, Nave KA. Assembly of CNS myelin in the absence of proteolipid protein. *Neuron.* 1997; 18:59–70. [PubMed: 9010205]
- Lappe-Siefke C, Goebbels S, Gravel M, Nicksch E, Lee J, Braun PE, Griffiths IR, Nave KA. Disruption of *Cnp1* uncouples oligodendroglial functions in axonal support and myelination. *Nat Genet.* 2003; 33:366–374. [PubMed: 12590258]

- Lee Y, Morrison BM, Li Y, Lengacher S, Farah MH, Hoffman PN, Liu Y, Tsingalia A, Jin L, Zhang PW, Pellerin L, Magistretti PJ, Rothstein JD. Oligodendroglia metabolically support axons and contribute to neurodegeneration. *Nature*. 2012; 487:443–448. [PubMed: 22801498]
- Lees JR, Cross AH. A little stress is good: IFN-gamma, demyelination, and multiple sclerosis. *J Clin Invest*. 2007; 117:297–299. [PubMed: 17273549]
- Li Y, Guo Y, Tang J, Jiang J, Chen Z. New insights into the roles of CHOP-induced apoptosis in ER stress. *Acta Biochim Biophys Sin (Shanghai)*. 2014
- Lin W, Bailey SL, Ho H, Harding HP, Ron D, Miller SD, Popko B. The integrated stress response prevents demyelination by protecting oligodendrocytes against immune-mediated damage. *J Clin Invest*. 2007; 117:448–456. [PubMed: 17273557]
- Lin W, Harding HP, Ron D, Popko B. Endoplasmic reticulum stress modulates the response of myelinating oligodendrocytes to the immune cytokine interferon-gamma. *J Cell Biol*. 2005; 169:603–612. [PubMed: 15911877]
- Lin W, Kemper A, Dupree JL, Harding HP, Ron D, Popko B. Interferon-gamma inhibits central nervous system remyelination through a process modulated by endoplasmic reticulum stress. *Brain*. 2006; 129:1306–1318. [PubMed: 16504972]
- Lin W, Kunkler PE, Harding HP, Ron D, Kraig RP, Popko B. Enhanced integrated stress response promotes myelinating oligodendrocyte survival in response to interferon-gamma. *Am J Pathol*. 2008; 173:1508–1517. [PubMed: 18818381]
- Lin W, Lin Y, Li J, Fenstermaker AG, Way SW, Clayton B, Jamison S, Harding HP, Ron D, Popko B. Oligodendrocyte-specific activation of PERK signaling protects mice against experimental autoimmune encephalomyelitis. *J Neurosci*. 2013; 33:5980–5991. [PubMed: 23554479]
- Lin W, Popko B. Endoplasmic reticulum stress in disorders of myelinating cells. *Nat Neurosci*. 2009; 12:379–385. [PubMed: 19287390]
- Lin Y, Huang G, Jamison S, Li J, Harding HP, Ron D, Lin W. PERK activation preserves the viability and function of remyelinating oligodendrocytes in immune-mediated demyelinating diseases. *Am J Pathol*. 2014a; 184:507–519. [PubMed: 24269558]
- Lin Y, Pang X, Huang G, Jamison S, Fang J, Harding HP, Ron D, Lin W. Impaired eukaryotic translation initiation factor 2B activity specifically in oligodendrocytes reproduces the pathology of vanishing white matter disease in mice. *J Neurosci*. 2014b; 34:12182–12191. [PubMed: 25186761]
- Lossos A, Elazar N, Lerer I, Schueler-Furman O, Fellig Y, Glick B, Zimmerman BE, Azulay H, Dotan S, Goldberg S, Gomori JM, Ponger P, Newman JP, Marreed H, Steck AJ, Schaeren-Wiemers N, Mor N, Harel M, Geiger T, Eshed-Eisenbach Y, Meiner V, Peles E. Myelin-associated glycoprotein gene mutation causes Pelizaeus-Merzbacher disease-like disorder. *Brain*. 2015; 138:2521–2536. [PubMed: 26179919]
- Marciniak SJ, Yun CY, Oyadomari S, Novoa I, Zhang Y, Jungreis R, Nagata K, Harding HP, Ron D. CHOP induces death by promoting protein synthesis and oxidation in the stressed endoplasmic reticulum. *Genes Dev*. 2004; 18:3066–3077. [PubMed: 15601821]
- McMahon JM, McQuaid S, Reynolds R, FitzGerald UF. Increased expression of ER stress- and hypoxia-associated molecules in grey matter lesions in multiple sclerosis. *Mult Scler*. 2012; 18:1437–1447. [PubMed: 22354737]
- Mháille AN, McQuaid S, Windebank A, Cunnea P, McMahon J, Samali A, FitzGerald U. Increased expression of endoplasmic reticulum stress-related signaling pathway molecules in multiple sclerosis lesions. *J Neuropathol Exp Neurol*. 2008; 67:200–211. [PubMed: 18344911]
- Morrison BM, Lee Y, Rothstein JD. Oligodendroglia: metabolic supporters of axons. *Trends Cell Biol*. 2013; 23:644–651. [PubMed: 23988427]
- Ní Fhlathartaigh M, McMahon J, Reynolds R, Connolly D, Higgins E, Counihan T, Fitzgerald U. Calreticulin and other components of endoplasmic reticulum stress in rat and human inflammatory demyelination. *Acta Neuropathol Commun*. 2013; 1:37. [PubMed: 24252779]
- Novoa I, Zeng H, Harding HP, Ron D. Feedback inhibition of the unfolded protein response by GADD34-mediated dephosphorylation of eIF2alpha. *J Cell Biol*. 2001; 153:1011–1022. [PubMed: 11381086]

- Numasawa-Kuroiwa Y, Okada Y, Shibata S, Kishi N, Akamatsu W, Shoji M, Nakanishi A, Oyama M, Osaka H, Inoue K, Takahashi K, Yamanaka S, Kosaki K, Takahashi T, Okano H. Involvement of ER stress in dysmyelination of Pelizaeus-Merzbacher Disease with PLP1 missense mutations shown by iPSC-derived oligodendrocytes. *Stem Cell Reports*. 2014; 2:648–661. [PubMed: 24936452]
- Patzkó A, Bai Y, Saporta MA, Katona I, Wu X, Vizzuso D, Feltri ML, Wang S, Dillon LM, Kamholz J, Kirschner D, Sarkar FH, Wrabetz L, Shy ME. Curcumin derivatives promote Schwann cell differentiation and improve neuropathy in R98C CMT1B mice. *Brain*. 2012; 135:3551–3566. [PubMed: 23250879]
- Pavitt GD, Ron D. New insights into translational regulation in the endoplasmic reticulum unfolded protein response. *Cold Spring Harb Perspect Biol*. 2012; 4
- Pennuto M, Tinelli E, Malaguti M, Del Carro U, D'Antonio M, Ron D, Quattrini A, Feltri ML, Wrabetz L. Ablation of the UPR-mediator CHOP restores motor function and reduces demyelination in Charcot-Marie-Tooth 1B mice. *Neuron*. 2008; 57:393–405. [PubMed: 18255032]
- Peschek J, Acosta-Alvear D, Mendez AS, Walter P. A conformational RNA zipper promotes intron ejection during non-conventional XBP1 mRNA splicing. *EMBO Rep*. 2015; 16:1688–1698. [PubMed: 26483401]
- Pfeiffer SE, Warrington AE, Bansal R. The oligodendrocyte and its many cellular processes. *Trends Cell Biol*. 1993; 3:191–197. [PubMed: 14731493]
- Popko B, Corbin JG, Baerwald KD, Dupree J, Garcia AM. The effects of interferon-gamma on the central nervous system. *Mol Neurobiol*. 1997; 14:19–35. [PubMed: 9170099]
- Rasband MN, Peles E, Trimmer JS, Levinson SR, Lux SE, Shrager P. Dependence of nodal sodium channel clustering on paranodal axoglial contact in the developing CNS. *J Neurosci*. 1999; 19:7516–7528. [PubMed: 10460258]
- Rinholm JE, Hamilton NB, Kessaris N, Richardson WD, Bergersen LH, Attwell D. Regulation of oligodendrocyte development and myelination by glucose and lactate. *J Neurosci*. 2011; 31:538–548. [PubMed: 21228163]
- Rosenbluth J, Nave KA, Mierzwa A, Schiff R. Subtle myelin defects in PLP-null mice. *Glia*. 2006; 54:172–182. [PubMed: 16802387]
- Saporta MA, Shy BR, Patzko A, Bai Y, Pennuto M, Ferri C, Tinelli E, Saveri P, Kirschner D, Crowther M, Southwood C, Wu X, Gow A, Feltri ML, Wrabetz L, Shy ME. MpzR98C arrests Schwann cell development in a mouse model of early-onset Charcot-Marie-Tooth disease type 1B. *Brain*. 2012; 135:2032–2047. [PubMed: 22689911]
- Shen X, Ellis RE, Sakaki K, Kaufman RJ. Genetic interactions due to constitutive and inducible gene regulation mediated by the unfolded protein response in *C. elegans*. *PLoS Genet*. 2005; 1:e37. [PubMed: 16184190]
- Southwood CM, Fykolodziej B, Dachet F, Gow A. Potential For Cell-mediated Immune Responses In Mouse Models Of Pelizaeus-Merzbacher Disease. *Brain Sci*. 2013; 3:1417–1444. [PubMed: 24575297]
- Southwood CM, Garbern J, Jiang W, Gow A. The unfolded protein response modulates disease severity in Pelizaeus-Merzbacher disease. *Neuron*. 2002; 36:585–596. [PubMed: 12441049]
- Stone S, Lin W. The unfolded protein response in multiple sclerosis. *Front Neurosci*. 2015; 9:264. [PubMed: 26283904]
- Theocharopoulou G, Vlamos P. Modeling protein misfolding in charcot-marie-tooth disease. *Adv Exp Med Biol*. 2015; 820:91–102. [PubMed: 25417019]
- Traka M, Podojil JR, McCarthy DP, Miller SD, Popko B. Oligodendrocyte death results in immune-mediated CNS demyelination. *Nat Neurosci*. 2015
- Tsaytler P, Harding HP, Ron D, Bertolotti A. Selective inhibition of a regulatory subunit of protein phosphatase 1 restores proteostasis. *Science*. 2011; 332:91–94. [PubMed: 21385720]
- Van der Knaap MS, Pronk JC, Scheper GC. Vanishing white matter disease. *Lancet Neurol*. 2006; 5:413–423. [PubMed: 16632312]
- Van der Voorn JP, van Kollenburg B, Bertrand G, Van Haren K, Scheper GC, Powers JM, van der Knaap MS. The unfolded protein response in vanishing white matter disease. *J Neuropathol Exp Neurol*. 2005; 64:770–775. [PubMed: 16141786]

- Van Kollenburg B, van Dijk J, Garbern J, Thomas AA, Scheper GC, Powers JM, van der Knaap MS. Glia-specific activation of all pathways of the unfolded protein response in vanishing white matter disease. *J Neuropathol Exp Neurol*. 2006; 65:707–715. [PubMed: 16825957]
- Walter P, Ron D. The unfolded protein response: from stress pathway to homeostatic regulation. *Science*. 2011; 334:1081–1086. [PubMed: 22116877]
- Way SW, Podojil JR, Clayton BL, Zaremba A, Collins TL, Kunjamma RB, Robinson AP, Brugarolas P, Miller RH, Miller SD, Popko B. Pharmaceutical integrated stress response enhancement protects oligodendrocytes and provides a potential multiple sclerosis therapeutic. *Nat Commun*. 2015; 6:6532. [PubMed: 25766071]
- Way SW, Popko B. Harnessing the integrated stress response for the treatment of multiple sclerosis. *Lancet Neurol*. 2016; 15:434–443. [PubMed: 26873788]
- Wrabetz L, D'Antonio M, Pennuto M, Dati G, Tinelli E, Fratta P, Previtali S, Imperiale D, Zielasek J, Toyka K, Avila RL, Kirschner DA, Messing A, Feltri ML, Quattrini A. Different intracellular pathomechanisms produce diverse Myelin Protein Zero neuropathies in transgenic mice. *J Neurosci*. 2006; 26:2358–2368. [PubMed: 16495463]
- Ye J, Rawson RB, Komuro R, Chen X, Davé UP, Prywes R, Brown MS, Goldstein JL. ER stress induces cleavage of membrane-bound ATF6 by the same proteases that process SREBPs. *Mol Cell*. 2000; 6:1355–1364. [PubMed: 11163209]
- Zinszner H, Kuroda M, Wang X, Batchvarova N, Lightfoot RT, Remotti H, Stevens JL, Ron D. CHOP is implicated in programmed cell death in response to impaired function of the endoplasmic reticulum. *Genes Dev*. 1998; 12:982–995. [PubMed: 9531536]

Web References

<https://clinicaltrials.gov/ct2/show/NCT02423083?term=guanabenz&rank=1>

Highlights

- Myelinating glia are particularly sensitive to ER stress.
- A number of neurological disorders display evidence of myelinating cell ER stress.
- Guanabenz and Sephin1 enhance the UPR, thereby increasing protection to ER stress.
- Guanabenz protects oligodendrocytes in mouse models of multiple sclerosis.
- Sephin1 protects Schwann cells in a mouse model of Charcot-Marie-Tooth disorder.

Figure 1.

Oligodendrocyte and Schwann cell electron micrographs. (A) False color electron microscopy image and (C) schematic of a myelinated axon in the CNS with an oligodendrocyte process (blue) forming the myelin sheath. (C) Oligodendrocytes will myelinate multiple axon segments in the CNS. (B) False color electron microscopy image and (D) schematic of a myelinated axon in the PNS. The Schwann cell body (green) engulfs the axon and will only produce the myelin sheath for a single axon segment.

Figure 2.

Myelinated axon domains and metabolic support of axons by oligodendrocytes. Contact with oligodendrocytes is necessary for clustering of Na⁺ channels at nodes and K⁺ channels at juxtaparanodes, which are required for fast saltatory conduction of action potentials. Oligodendrocytes also metabolically support axons by shuttling lactate through oligodendrocyte expressed MCT-1 into the periaxonal space where it is taken up into the axon through the neuron expressed MCT-2.

Table 1

ER stress in myelin disorders.

Disease	Presence of UPR Markers		Effect of UPR Manipulation	References		
Multiple Sclerosis	•	CHOP mRNA in patients	•	Gadd34 deletion and pharmacological inhibition are protective in mouse models.	•	Cunnea et al., 2011
	•	ATF4 mRNA in patients			•	Way et al., 2015
	•	Phosphorylated eIF2 α in mouse models				
	•	CHOP protein in mouse models				
Pelizaeus-Merzbacher Disease	•	CHOP protein in patients	•	Chop deletion exacerbates disease in mouse models.	•	Southwood et al., 2002
	•	Chop mRNA in mouse models			•	Southwood et al., 2013
	•	Atf4 mRNA in mouse models				
	•	Bip mRNA in mouse models				
Vanishing White Matter Disease	•	ATF4 mRNA and protein in patients	•	Effects of UPR Manipulation on disease outcome have not been studied.	•	van Kollenburg et al., 2006
	•	CHOP mRNA in patients			•	van der Voorn et al., 2005
	•	GADD34 mRNA in patients				
	•	BiP mRNA in patients				
	•	XBP-1 splicing in patients				
	•	Phosphorylated PERK in patients				
	•	Phosphorylated eIF2 α in patients				
Charcot-Marie-Tooth	•	BiP mRNA and protein in mouse models	•	Chop deletion is protective in some mouse models.	•	Bai et al., 2013
	•	CHOP mRNA and protein in mouse models	•	Gadd34 deletion and pharmacological inhibition are protective in some mouse models.	•	Pennuto et al., 2008
	•	XBP-1 splicing in mouse models			•	D'Antonio et al., 2013