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Review

Endoplasmic reticulum quality control and dysmyelination

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Abstract

Dysmyelination contributes to several human diseases including multiple sclerosis, Charcot-Marie-Tooth, leukodystrophies, and schizophrenia and can result in serious neurological disability. Properly formed, compacted myelin sheaths are required for appropriate nerve conduction velocities and the health and survival of neurons. Many different molecular mechanisms contribute to dysmyelination and many of these mechanisms originate at the level of the endoplasmic reticulum. The endoplasmic reticulum is a critical organelle for myelin biosynthesis and maintenance as the site of myelin protein folding quality control, Ca^{2+} homeostasis, cholesterol biosynthesis, and modulation of cellular stress. This review paper highlights the role of the endoplasmic reticulum and its resident molecules as an upstream and dynamic contributor to myelin and myelin pathologies.

Keywords: dysmyelination; endoplasmic reticulum; myelin.

Introduction

Myelin is a highly specialized entity unique to the nervous system. A defining feature of vertebrates, the evolutionary development of myelin reduced the energy required for neuronal communication and boosted the speed of impulse propagation to allow the timely and efficient operation of complex nervous systems (1). Aberrant formation or maintenance of the myelin sheath results in a morphology collectively termed dysmyelination and contributes to several diseases including multiple sclerosis (MS), Charcot-Marie-Tooth (CMT), leukodystrophy, schizophrenia, Alzheimer's disease, and Parkinson's disease. Myelin pathologies have been modeled in various murine models but complete molecular mechanisms of dysmyelination and therapeutic targets remain elusive. Recently, the importance of the endoplasmic reticulum (ER) and its resident molecules in myelination has been realized. A site for myelin protein synthesis, specialized lipid synthesis, Ca^{2+} storage, and protein folding quality control checks, the response of the ER to the unique demands of myelinating systems is crucial in the maintenance of a healthy nervous system. This review paper highlights the role of the ER in general and, more specifically, the previously unrecognized role of the ER protein quality control molecules, such as calnexin, in myelin and myelin diseases.

Myelin

Myelination is a membranous extension of embryological and morphologically distinct glial cells of the central nervous system (CNS) and peripheral nervous system (PNS); an oligodendrocyte myelinates multiple axons and internodes in the former, whereas a Schwann cell is dedicated to the ensheathment of a single axon/internode in the latter. Myelin membranes have a unique composition of lipids and proteins that maintain myelin structure and integrity. This integrity is crucial for the timely and precise conduction of action potentials in axons with myelin increasing nerve conduction velocity up to 100-fold. Furthermore, myelinating glia are required for the long-term integrity and survival of axons (2). A process initiated postnatally, myelinating glia are directed to axons with a diameter of 1 μM or greater (3). In humans, myelin formation takes place during the second half of fetal life, peaking in the first year but continuing through to 20 years of age (4). In mice, myelination is detectable at embryonic day 16.5 and peaks at postnatal day 18 (5). During myelination, Schwann cells or oligodendrocytes wrap membranous spiral extensions around axons with a thickness correlating to axon diameter. Appropriate myelin thickness can be determined using a simple measurement known as the g-ratio, defined as a ratio of the inner axonal diameter to the total outer diameter (axon+myelin sheath). A g-ratio of 0.6 is expected in the PNS, whereas a slightly higher g-ratio of 0.72–0.81 is appropriate in the CNS (6, 7). To initiate myelination, axon-derived signals relative to the axon size recruit and promote the differentiation of myelinating glia. In the PNS, myelination is initiated when a threshold level of axonal neuregulin-1 type III expression is reached and the expression of this protein is important for the subsequent regulation of myelination membrane growth (8). Myelination in the CNS is more complex and appears to be regulated by a balance of positive and negative neuronal signals (9). For example, expression of polysialylated-neural cell adhesion molecule has been shown to inhibit myelination on the neuronal cell surface (10), whereas expression of the L1 adhesion molecule on the neuronal cell surface is required for the initial stage of adhesion, after which L1 is downregulated (9). Regardless of how myelination is initiated, the appropriate timing of myelin formation is crucial for nervous system development.

Myelin membranes are distinctive with cholesterol, phospholipids, and glycosphingolipids accounting for 70% of the dry weight of the membrane. The unique lipid composition is enriched in galactosylceramide, sulfatide, ethanolamine, plasmalogen, and cholesterol; with cholesterol comprising 25% of the total lipid content (11). Cholesterol is not imported from the circulation, but is instead synthesized locally and cholesterol concentration is rate-limiting for myelin biogenesis (12, 13). It has been reported that during active myelination in rats, the myelin-membrane surface area expands at an estimated rate of $5–50\times10^3$ μm^2/cell/day, a daily rate of more than 100-fold the surface area of the cell soma (i.e., plasma membrane) of approximately 0.3×10^3 μm^2 (14). From an immature to a fully myelinated oligodendrocyte, this expansion corresponds to an estimated 6500-fold increase in membrane surface (15, 16). Supply must meet demand, and such an astounding rate of growth requires specialized cellular mechanisms. The large amounts of membrane and myelin specific proteins generated depend on vesicular trafficking for movement to the plasma membrane. Generation of myelin depends on the exocytosis of myelin lipids and proteins from the biosynthetic pathway and an endosomal pool. Changes in the balance of exocytosis/endocytosis provide a mechanism of myelin generation where downregulation of endocytosis contributes to membrane deposition (17).

The final architecture of a myelin sheath provides the structure to support fast, saltatory conduction down an axon in an insulated environment (Figure 1). The sheaths provide an insulated and metabolically isolated environment for the axon that is interrupted by exposed axonal membranes known as nodes of Ranvier. Saltatory conduction takes place at the nodes of Ranvier where Na^+ channels are clustered in the axonal membrane between paranodal axoglial junctions that provide a diffusion barrier. Fast K^+ channels are concentrated in the adjacent juxtaparanodal region. Compact myelin forms a periodic structure around the axon with the innermost part of the sheath being the farthest extension of the myelinating glial cell. The axon and myelin sheath are separated by a periaxonal space, and the compact myelin is

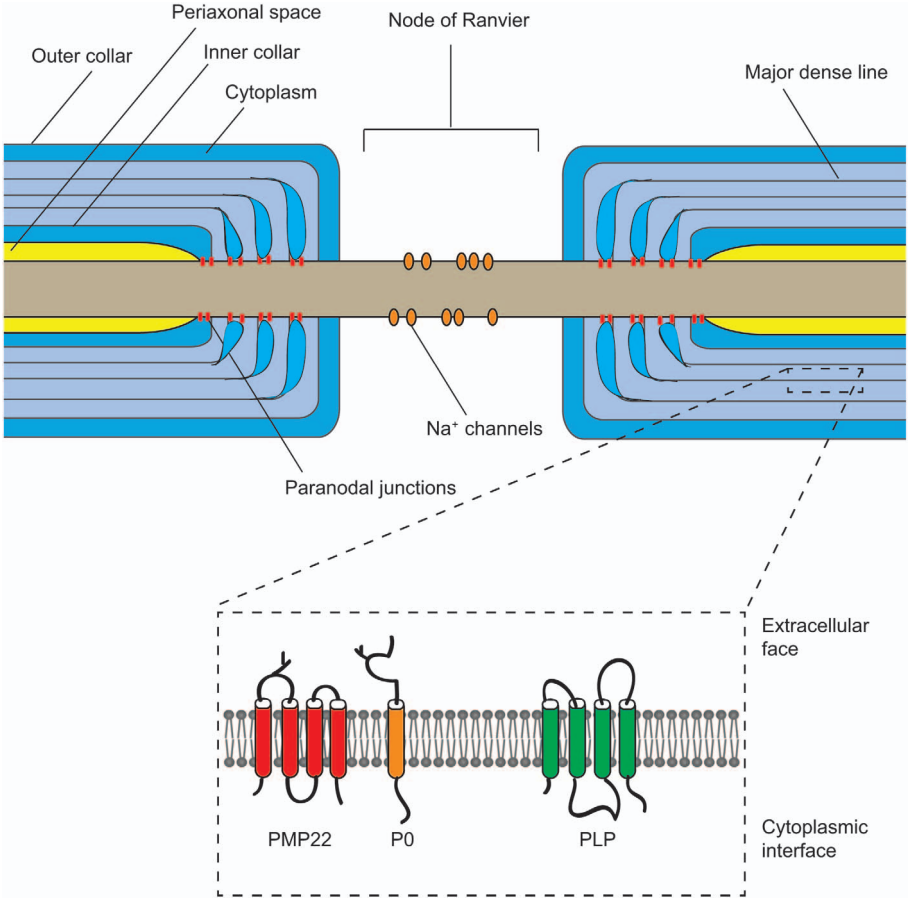

Figure 1 Compact myelin sheaths are required for nervous system function.
Schwann cells and oligodendrocytes wrap membranous extensions around axons to form compact myelin sheaths. Each Schwann cell forms a distinct internode, whereas oligodendrocytes myelinate multiple internodes. Voltage-gated Na^+ channels are clustered at the nodes of Ranvier, areas of unmyelinated axons between internodes. Paranodal junctions are found adjacent to the nodes of Ranvier to provide a diffusion barrier. Compact myelin sheaths are maintained through adhesive myelin proteins expressed at the cell surface, such as peripheral myelin protein 22 (PMP22) and myelin protein zero (P0) of the peripheral nervous system (PNS) and proteolipid protein (PLP) of the central nervous system (CNS).

connected through gap junctions in the Schmidt-Lanterman incisures or local stacks of non-compact myelin. The periodic ultrastructure of myelin is maintained by homophilic and heterophilic interactions of specific myelin proteins expressed in glia cells that confer the structural stability for compact, organized myelin sheaths. Two such proteins, peripheral myelin protein 22 (PMP22) and myelin protein zero (P0), are components of compact myelin in the PNS. Myelin protein zero (MPZ or P0) is the most abundant protein in peripheral myelin, accounting for approximately 50% of the total protein content (18, 19). A single-pass transmembrane protein, P0 has a glycosylated extracellular immunoglobulin-like domain stabilized by a disulfide domain (19, 20). P0 undergoes homophilic interactions to act as an adhesion protein connecting adjacent myelin lamellae (21). Both the extracellular and cytoplasmic domain of P0 are required for its adhesive function (22). PO also undergoes heterophilic interactions with PMP22 (23), a 22-kDa tetraspan transmembrane glycoprotein that constitutes 2–5% of the total myelin protein content (24).

Although myelin is essential for physiologically relevant nerve conduction velocities and motor function, another very important function of myelination is its contribution to neuronal development and survival (25). Secondary axon degeneration as a consequence of dysmyelination leads to the serious long-term disabilities seen in MS and CMT. Demyelination in the Trembler mouse (mutant PMP22) results in a decrease in neurofilament phosphorylation and reduces the rate of slow axonal transport (26). Reduced axonal caliber was also observed as a consequence of the reduced neurofilament phosphorylation (26). Although myelination is important for axonal preservation, the presence of the glia, independent of myelination, is also important. Myelinating glia regulate the microenvironment surrounding neurons, provide trophic support, and contribute to neuronal development independently of myelination (27, 28). Neurons and myelinating glia have a symbiotic relationship and defects in either one will have detrimental functional consequences on the other. Consequently, myelin disease could originate from problems with the neuron, and it has been proposed that in diseases, such as MS, aberrant expression of certain molecules on the neuronal surface inhibits the remyelination process (29).

Contributions of the ER to dysmyelination

Dysmyelination can take many different forms and is most simply defined as any perturbation to the myelin sheath as a result of impaired formation or maintenance of the myelin sheath. This differs from demyelination as a loss of the sheath. Perturbations of the myelin sheath can originate from multiple mechanistic sources including aberrant folding, expression, or trafficking of myelin specific proteins, the absence of lipid components, such as cholesterol, cellular stress, and/or Ca^{2+} homeostasis. These diverse contributors are mechanistically unique with one thing in common: they originate from the ER.

The ER and protein quality control

The ER is a centrally localized, dynamic cellular organelle that provides an oxidizing environment for the translation, folding, and post-translational modification of secreted and membrane proteins (30). The ER is also the site for the biosynthesis of steroids, cholesterol, and other lipids as well as being the storage site for the majority of intracellular Ca^{2+}. The protein folding capacity of the ER is linked to its role in Ca^{2+} homeostasis and depends on the high Ca^{2+} concentration of the ER lumen (31, 32). Protein folding quality control is essential to the survival of the cell as aberrant secretion, or accumulation of misfolded proteins is the hallmark of many diseases, including cystic fibrosis and cancer, and neurological diseases, such as Alzheimer's disease and CMT.

Protein folding quality control employs molecular chaperones that bind to nascent polypeptides and aid their folding and assembly processes while preventing unfolded protein aggregation. There are several chaperones dedicated to specific folding related functions including the classical chaperones [binding immunoglobulin protein (BiP), glucose regulated protein 94 (Grp94)], lectin chaperones (calnexin and calreticulin), redox enzymes [protein disulfide isomerase (PDI) and ERp57], proline isomerases, and sugar processing enzymes [Glucosidase I and II, ER Mannosidase I and II, and uridine diphosphate glucose glycoprotein glucosyltransferase (UGGT)] (30). These chaperones provide a way to ensure only properly folded and assembled proteins are trafficked from the ER. Proteins that cannot be properly folded are targeted to the ER associated degradation (ERAD) pathway.

Protein folding in the ER begins when a nascent polypeptide is translocated into the ER lumen, a process that takes place both co- and post-translationally. Proteins without a consensus N-glycosylation site early in their sequence associate with BiP and ERdj proteins (33). Conversely, proteins can be N-glycosylated and targeted to the calnexin/calreticulin cycle (Figure 2). N-glycosylation includes the assembly and transfer of two N-acetylglucosamine and nine mannoses with three terminal glucoses as a core oligosaccharide onto asparagine residues of the consensus site (Asn-X-Ser/Thr, where X is any amino acid except Pro) of a nascent polypeptide by oligosaccharyltransferase (OST). OST is associated with the translocon complex and adds the oligosaccharide when the consensus site is only 12–14 residues into the ER lumen (34). Lectin chaperones associate primarily with glycosylated polypeptides in the ER lumen (35–37). Several major myelin proteins are glycosylated including P0 and PMP22 of the PNS and the minor but highly immunogenic component of the CNS, myelin oligodendrocyte glycoprotein (MOG) (18, 38–41). Consequently, lectin chaperones are particularly important for myelin protein quality control. Calnexin is a type I integral membrane lectin quality control chaperone, working in conjunction with the analogous lectin calreticulin and PDI ERp57. Calnexin binds monoglucosylated polypeptides translated through the ER

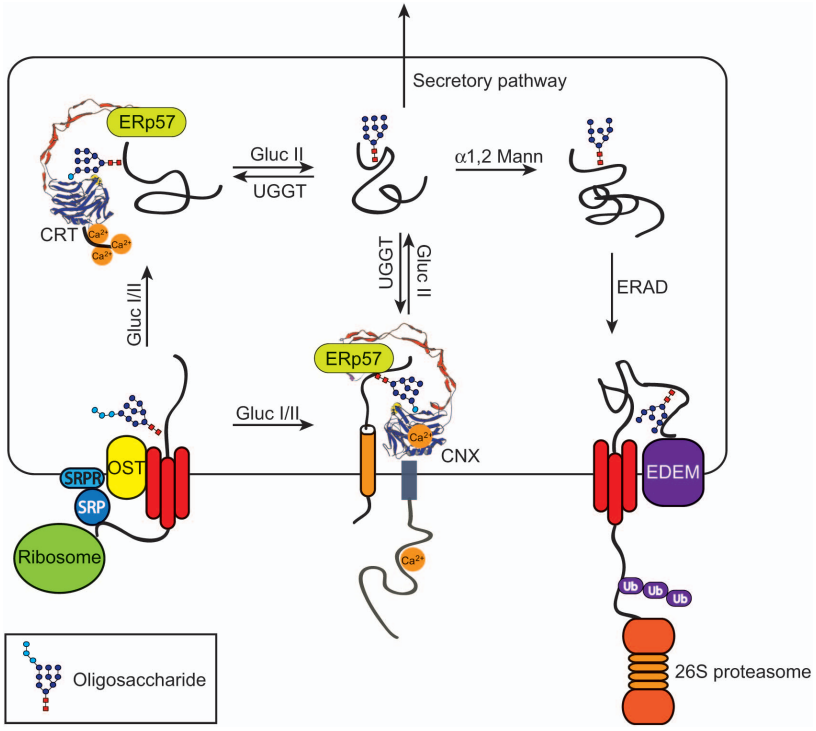

Figure 2 Quality control in the endoplasmic reticulum.
This schematic depicts how endoplasmic reticulum (ER) resident chaperones aid the folding of nascent polypeptides translocated into the ER lumen. Newly translated proteins containing a signal sequence are recognized by the signal recognition particle (SRP) and targeted to the ER, where SRP binds to its receptor (SRPR), bringing the translating protein and the ribosome as a complex to the Sec61 translocon at the ER membrane. Polypeptides are inserted through the heterotrimeric Sec61 complex. The transmembrane complex oligosaccharide transferase (OST) transfers a preassembled two N-acetylglucosamine, nine mannose, three terminal glucose ($Glc_3Man_9GlcNAc_2$) core oligosaccharide *en bloc* to N-X-S/T (where X is any amino acid except Pro) N-glycosylation sites. The $Glc_3Man_9GlcNAc_2$ oligosaccharide is trimmed sequentially by glucosidase I and II (Gluc I/II) to a monoglucosylated form ($Glc_1Man_9GlcNAc_2$). This monoglucosylated form is recognized by the lectin chaperones calreticulin (CRT) and calnexin (CNX). Calreticulin and calnexin are highly similar with calreticulin being a soluble protein and calnexin membrane anchored. Both CRT and CNX bind ER oxidoreductase ERp57 at the tip of their P-domain to aid folding of proteins requiring disulfide bonds. Gluc II removes the terminal glucose from the oligosaccharide, leaving $Man_9GlcNAc_2$. If the protein has achieved its proper conformation it exits the ER to be trafficked through the secretory pathway. Unfolded proteins are inserted back into the calnexin/calreticulin cycle when uridine diphosphate (UDP)-glucose glycoprotein glucosyltransferase (UGGT) reglucosylates the substrate. Proteins unable to achieve their appropriate structure (proteins containing mutations) are extracted from the calnexin cycle, and the carbohydrate on the unfolded substrate subjected to sequential cleavage of terminal mannose residues by α1,2-mannosidase, preventing reglucosylation by UGGT. This allows recognition by ER degradation enhancing α-mannosidase-like protein (EDEM) that transports the protein to the retrotranslocon where the unfolded protein is translocated across the ER membrane, ubiquitinated and targeted to the proteasome for destruction.

and assists their folding and assembly (37, 42, 43). The three-dimensional structure of luminal calnexin indicates that protein consists of a globular Ca^{2+}-binding domain (N-domain) responsible for interaction with monoglucosylated substrate, as well as an extended arm containing two conserved proline-rich sequence motifs each arranged in four tandem repeats (P-domain) (44). A cycle of glucose removal and addition by glucosidase I and glucosidase II and UGGT allows renewed calnexin/substrate interaction until the proper protein structure is attained and the substrate protein can be trafficked through the secretory pathway (37, 42, 43). Misfolded proteins are eventually targeted by α-mannosidase, which cleaves the mannose residue and targets the misfolded protein for ERAD (45, 46). In its capacity as a lectin quality control chaperone, calnexin is probably involved in the folding of all glycosylated integral membrane myelin proteins.

There are many animal models available to study different aspects and effects of dysmyelination (47), and several mouse models have led to the discovery of the genetic contributions underlying clinical disease. Notably, several of these dysmyelination mouse models are attributable to gene mutations that result in a loss of appropriate protein folding and trafficking quality control at the ER. One such example is the *trembler* (*Tr*) and *trembler-J* (*Tr-J*) mice. *Tr* and *Tr-J* mice carry natural autosomal dominant mutations in the gene encoding PMP22 (48) and led to the identification of a role for PMP22 in the clinical disease CMT (49). CMT, one of the most commonly inherited neurological disorders with more than 30 different contributing genes, affects the PNS. Clinically, the disease is categorized into four main types (CMT1–CMT4) and these are classified into further subtypes depending on the causative gene (for a list see http://www.

molgen.ua.ac.be/CMTMutations/Home/IPN.cfm). Since the identification of the trembler mice, PMP22 mutation and overexpression murine models have been used extensively to study various CMT subtypes. PMP22 duplication resembles CMT1 and mice overexpressing PMP22 demonstrate a slower rate of myelination restricted to small diameter axons with a resulting decreased myelin thickness (hypomyelination) (50). Mice devoid of PMP22 have a delayed onset of myelination and develop abundant tomocula (51), a term derived from the Latin word *tomaculum* (meaning sausage) that is used to describe the bulbous expansions of redundant myelin. PMP22 deletion is reminiscent of hereditary neuropathy with liability to pressure palsies (52, 53). Several PMP22 point mutants are retained in the ER and are not trafficked to the myelin membrane (54–56). $PMP22^{Tr}$ and $PMP22^{Tr\text{-}J}$ mutants are known to have prolonged association with calnexin in the ER and it has been postulated that this prolonged retention of mutant PMP22 provides a mechanism for the resulting neuropathies (57). Whether it is the absence of PMP22 or a point mutation causing it to be retained in

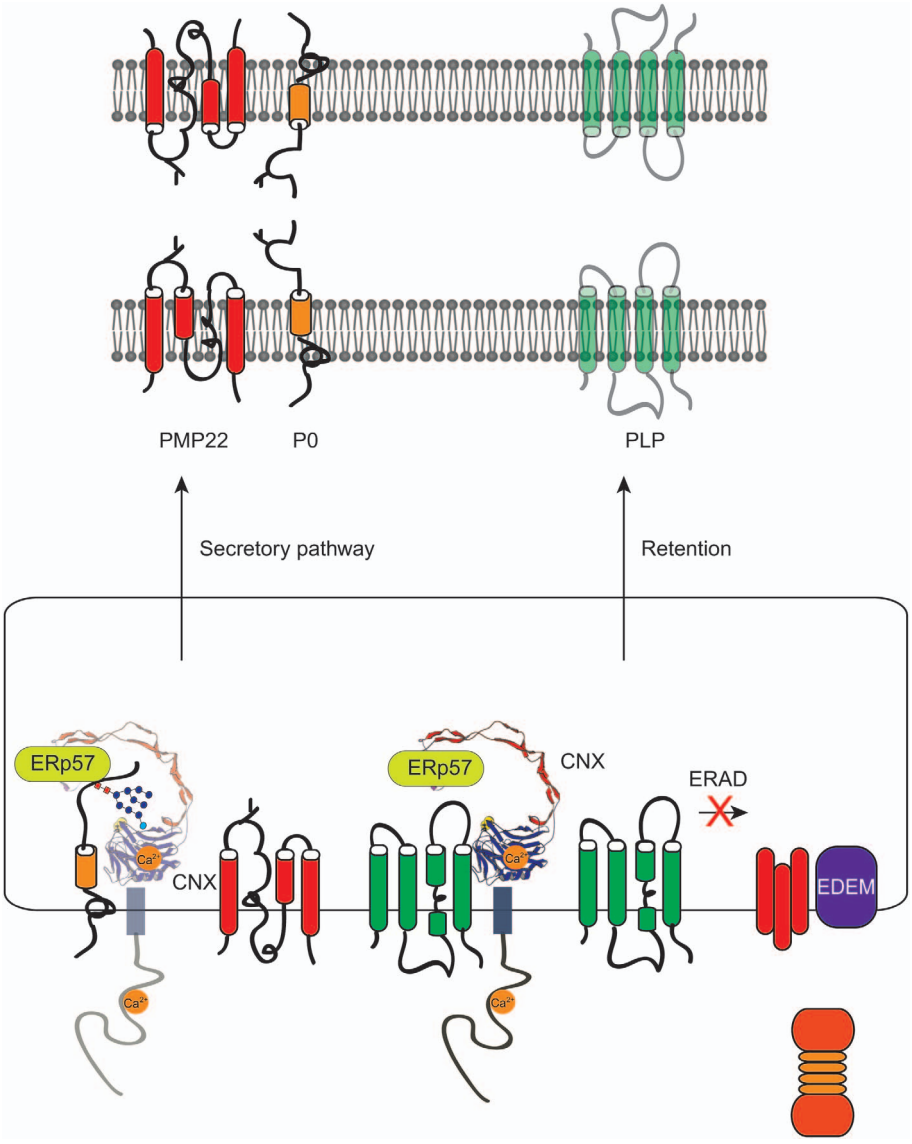

Figure 3 Quality control of myelin proteins.
Many myelin proteins are glycosylated and are known calnexin (CNX) substrates. Problems with the protein quality control apparatus can lead to two independent outcomes: misfolded proteins either escape the quality control system and enter the secretory pathway or are successfully, although potentially more detrimentally, retained in the ER. Misfolded protein myelin protein 22 (PMP22) and protein zero (P0) are known to traffic through the secretory pathway to the cell surface in the absence of calnexin. Mutant proteolipid (PLP) is shown as retained in the ER, leading to loss of its expression at the plasma membrane. If ER associated degradation (ERAD) is unable to cope with the misfolded protein load retained by quality control chaperones in the ER, it can lead to the induction of the unfolded protein response (UPR). Both trafficking of misfolded proteins through the secretory pathway and retention of misfolded proteins in the ER results in problems with crucial structural elements for compact myelin sheaths.

the ER, absence of PMP22 at the myelin membrane results in a loss of a crucial myelin structural element (Figure 3).

Myelin P0 is the most abundant protein component of peripheral myelin and more than 90 different point mutations in the gene encoding P0 are known to contribute to inherited neuropathies including CMT and Dejerine-Sottas syndrome. Similar to PMP22 mutant proteins, many P0 mutants are retained in the ER. Interestingly, curcumin, a dietary supplement, has been shown to stimulate the translocation of ER retained mutant P0 and subsequently reduce apoptosis induced by P0 mutant accumulation (58). Thus, overcoming the ER retention of mutant P0 and PMP22 could be beneficial (Figure 3).

Pelizaeus-Merzbacher disease (PMD) is a dysmyelinating disease of the CNS. Reminiscent of CMT, PMD is caused by heterogeneous missense mutations, gene duplication or deletion of a myelin protein, proteolipid protein (PLP). Similar to P0 in the PNS, PLP is the major protein component of the CNS. It was the similarities between PMD and CMT1A (caused by a duplication of PMP22) that led to the identification of PLP duplications in PMD patients (59). Correlations can be made between the type of mutation and the clinical phenotype with deletions resulting in mild disease, duplications in moderate PMD, and missense mutations leading to severe disease (60). However, the clinical severity of PMD cannot be explained by the size of PLP duplication (61), and it has been proposed that modifier genes within the duplicated region or elsewhere in the genome can affect PLP expression and subsequent disease pathogenesis (60). In addition, the disease caused by missense mutations can range in phenotypic severity from mild PMD and late-onset spastic paraplegia type 2, characterized by hypomyelination (62, 63), to the severe connatal PMD, characterized by a severe deficit of myelin sheaths and widespread oligodendrocyte apoptosis (64, 65). The pathogenic mechanism underlying PMD was thought to be related to the accumulation of misfolded PLP in the ER, leading to the activation of the unfolded protein response (UPR) and oligodendrocyte apoptosis (66–68). However, this did not address the range in phenotypic severity seen with various missense mutations. A recent study examined two different mutations within PLP, the $A^{242}V$ mutant associated with severe PMD and the $G^{245}A$ mutant associated with mild disease (69). Both mutants were localized with calnexin in the ER, indicating ER retention. However, the $G^{245}A$ mutant associated with mild disease was more efficiently cleared from the ER for degradation than the severe disease-associated $A^{242}V$ mutant. It was also observed that the $A^{242}V$ mutant formed oligomeric structures. The decreased ERAD of the $A^{242}V$ led to the induction of the UPR and reduced cell viability under high mutant expression conditions, whereas the mild phenotype $G^{245}A$ mutant did not activate the UPR. Similar to PMP22 and P0 mutants, ER retention of PLP mutants appears to contribute to the resulting clinical disease.

Recently, dysmyelination has been implicated in schizophrenia (70). Imaging techniques, including magnetic transfer imaging and diffusion tensor imaging (DTI) have indicated changes in the structural integrity of white matter in schizophrenic patients (71–73). DTI measures anisotropy, a reflection of the coherence of structures within a given region with a decreased value indicating a less uniform orientation. Decreased anisotropy was observed in schizophrenic patients and was reminiscent of the decrease in anisotropy seen in MS patients (70). The mechanism underlying the white matter dysfunction in schizophrenia remains elusive; however, myelin-specific proteins have been implicated in the disease. The dorsolateral prefrontal cortex (DLPFC) has been implicated in schizophrenia pathology. To identify schizophrenia related changes in gene transcription, DNA microarray analysis was performed on DLPFC postmortem tissue from 12 chronic schizophrenic and 12 control patients (74). Changes in 89 transcripts were observed, with 35 of the 89 transcripts known to be involved in myelination. Six of these transcripts had decreased expression levels: myelin and lymphocyte protein (MAL), gelsolin, 2′,3′-cyclic nucleotide 3′-phosphodiesterase (CNP), myelin-associated glycoprotein (MAG), transferrin, and human epidermal growth factor receptor 3 (Her3/ErbB3). MAL is a transmembrane protein found predominantly in compact myelin clustered with the structural proteins myelin basic protein (MBP) and PLP (75). Gelsolin is a highly expressed cytosolic protein in oligodendrocytes (76) and CNP is a transmembrane protein in oligodendrocyte membrane sheets associated with cytoskeletal components (77). MAG is involved in the initiation and maintenance of myelination in the CNS and PNS and is expressed early when oligodendrocytes first interact with axons (78–82). MAG-deficient mice have delayed formation of compact myelin (78) and hypomyelination of the optic nerve, along with an increased number of unmyelinated axons (80). MAG also plays a role in the long-term maintenance of myelin integrity. In contrast to young 4- and 10-day old MAG-deficient animals with no visible abnormalities in the myelin sheath, older 8- to 10-month MAG mouse mutants display degeneration of both axon and myelin components (83). Transferrin gene expression has been found to correlate with the postnatal development of oligodendrocytes in the mouse CNS (84) and impact the expression of specific myelin protein genes (85). ErbB3 (HER3) is a receptor tyrosine kinase that binds neuregulins resulting in a signal transduction cascade that dictates glial cell differentiation (86). The downregulation of any of the above molecules would have a large impact on myelination processes. The ER is very important for the synthesis and quality control of several molecular contributors to schizophrenia including MAL, MAG, and ErbB3, and similar to myelin proteins P0 and PMP22, the lectin quality control chaperones calreticulin and calnexin are responsible for the folding of transferrin (87).

We recently published a study on calnexin-deficiency in mice. Calnexin-deficient mice demonstrate a neurological phenotype characterized by a rolling walk, gait disturbance, and ataxia (88). This phenotype was the result of dysmyelination of the CNS and PNS. The peripheral dysmyelination was attributable to the misfolded and non-functional myelin proteins, PMP22 and P0 (Jung J, Coe H, Michalak M, unpublished data). The specificity of calnexin, a ubiquitous ER chaperone, for myelin proteins and the lack of compensatory

redundancy in neurological systems was an unexpected finding. Calnexin has been implicated in the quality control of several myelin specific proteins, including wild-type and mutant PMP22, PLP, and recently MOG (57, 89–91). This highlights calnexin as an upstream, central regulator of myelin structural proteins that might provide a novel therapeutic target in dysmyelinating pathologies. However, one must consider the dichotomy of retention versus release of mutant proteins: overcoming myelin protein retention could alleviate the severity of disease but the insertion of misfolded myelin proteins at the surface membrane could lead to a very detrimental demyelinating inflammatory immune response. Curcumin, a component of the dietary spice turmeric, has been shown to release ER retention of select mutant P0 sufficiently to ameliorate their toxic apoptotic effect (58). However, experimental autoimmune encephalomyelitis (EAE) is a mouse model used to study autoantibody mediated demyelination reminiscent of MS and is induced with injection of MOG or $MOG_{35–55}$ peptide emulsified in complete Freund's adjuvant and pertussis toxin to open the blood-brain barrier (92). Further studies in animal models are required to examine the benefits of overcoming ER retention of mutant proteins in the context of an immune system.

ER stress in dysmyelination

ER stress is thought to contribute to dysmyelinating pathologies. The ER is an essential site of secreted and transmembrane protein synthesis and folding and maintenance of ER homeostasis is critical for cellular function and survival. Glial cells require large amounts of lipids and proteins to form their myelinating processes and such a system would be particularly sensitive to any aberrations in the secretory organelle or its components that supplied the necessary building blocks for myelin. Protein translation at the ER needs to meet cellular requirements while still maintaining quality control of the proteins that exit the ER. During episodes of cell stress or insult, protein folding in the ER could be perturbed, and in an effort to restore homeostasis a pathway known as the UPR is activated (93). UPR employs three ER transmembrane sensors, pancreatic ER kinase (PERK), inositol-requiring kinase 1 (IRE1), and activating transcription factor 6 (ATF6) to coordinate a compensatory cellular response to increased protein load (Figure 4). Activation of PERK leads to the phosphorylation of eukaryotic translation initiation factor-α (eIF2α) and a subsequent attenuation of general translation while concomitantly upregulating the translation of proteins involved in amino acid metabolism, protein secretion, and the antioxidant response. Activation of IRE1 and ATF6 leads to the transcription of genes encoding components that increase ER protein folding capacity, export, and degradation. (93). Sustained or overwhelmed UPR leads to apoptosis and cell death (94). Recently, components and/or activation of UPR have been implicated in dysmyelination. It was proposed that the rate of which different PLP mutants are cleared from the ER and the extent of UPR activation could explain the varying disease severity of the mutants where increased UPR correlates to increased severity (69). In fact, ablation of CHOP [CCAAT/enhancer-binding protein (C/EBP) homologous protein], a downstream effector of UPR signaling, restores the motor function and reduces demyelination 2-fold in P0S63del mice, a model of CMT1B (95).

Vanishing white matter disease (VWM) is an autosomal recessive disease that affects oligodendrocytes and is characterized by cystic white matter degeneration (96). Although it affects people of all ages, it is one of the most prevalent inherited childhood leukoencepholopathies (97). Myelin stains of the affected white matter show sparse myelin sheaths with the remaining sheaths being thin and dispersed by vacuoles that correspond to focal areas of uncompacted myelin close to the axonal membrane (98, 99). Biochemical analysis indicates the major myelin proteins (i.e., MBP, PLP, cyclic nucleotide phosphodiesterase, and MOG) are present but in markedly reduced quantities (99). VWM is caused by mutations in the eukaryotic initiation factor (eIF) 2B (eIF2B) (100). Phosphorylation of eIF2α, an event downstream of ER stress PERK activation, allows the formation of an inhibitory eIF2α-P/eIF2B complex (101, 102). Furthermore, all three UPR pathways are activated in glia of patients with VWM (103, 104).

Activation of ER stress signals has also been identified in MS patient lesions, areas of myelin damage readily visible by MRI. Investigation of active, chronic active, and chronic inactive MS lesions revealed an increase in the expression of ER stress-associated C/EBP homologous protein at the edges of chronic active lesions. Increased expression of X-box binding protein 1 (XBP1) was detected in oligodendrocytes, astrocytes, T cells, and microglia present in active MS lesions (105). It has recently been proposed that the ER stress response might even provide a therapeutic target in MS (106). Using an EAE murine model of MS, it was found that activation of the ER stress response through CNS-specific expression of interferon-γ (IFN-γ) was protective, a result mediated by the enhanced survival of oligodendrocytes after PERK activation (107). However, in another study, IFN-γ expression has been associated with oligodendrocyte death during demyelinating insult (108). During the first study, it is important to note that IFN-γ expression was induced before EAE. The timing of ER stress is probably crucial in determining its benefits as ER stress preconditioning has a known protective effect (109).

Antibodies to MOG are elevated in MS patients and this is thought to be part of the autoimmune destruction of myelin (110–112). Interestingly, when the molecular effects of antibody crosslinking of MOG on the surface of oligodendrocytes were investigated, changes in stress response-related proteins were observed (113). Anti-MOG antibodies led to the increased phosphorylation of elongation factor-2 (EF-2), heat shock protein-74 (HSP74), and α-enolase, and subsequent activation of cellular stress pathways. This implies that antibody recognition of MOG in autoimmune demyelinating diseases such as MS can activate an ER stress response.

Although ER stress appears to be involved in certain dysmyelinating pathologies, dysmyelination does not always

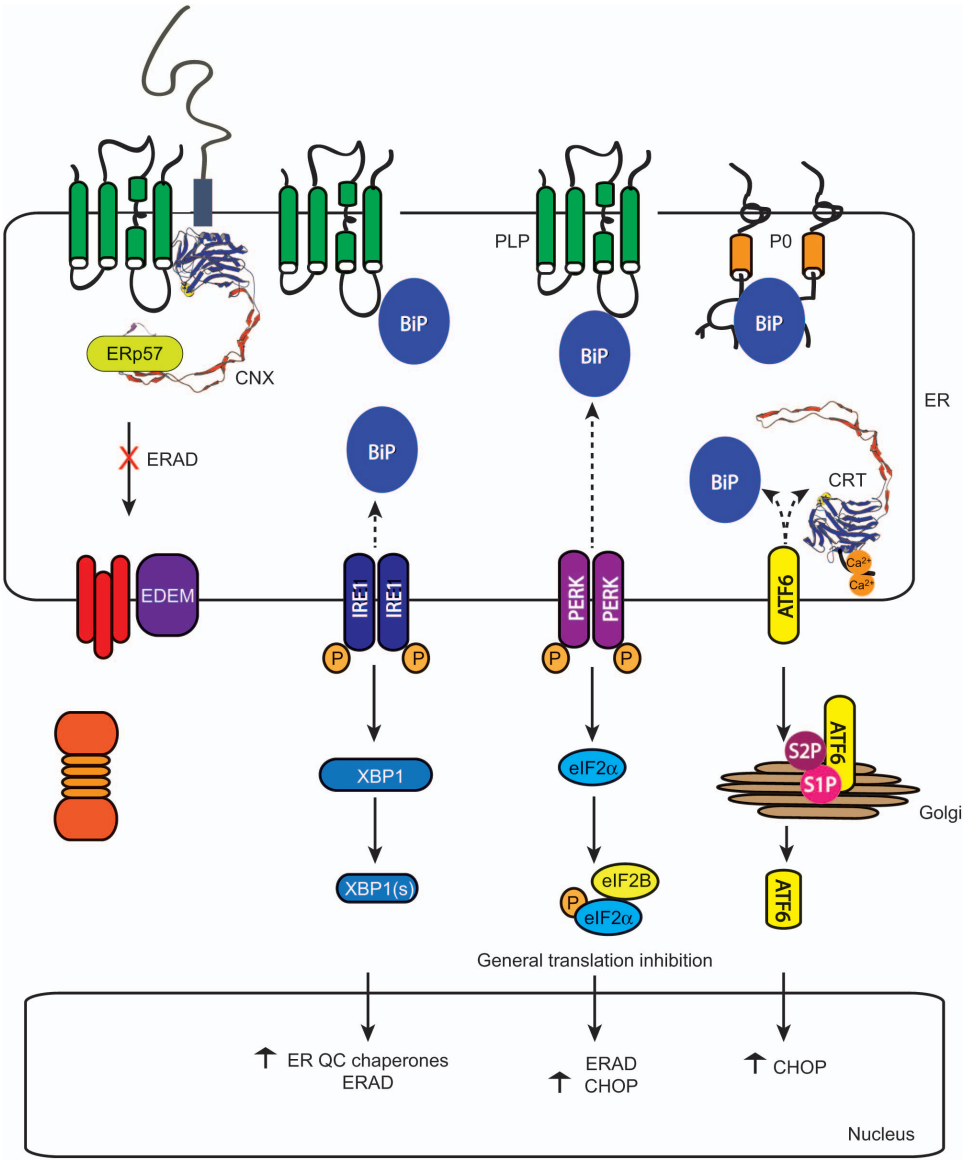

Figure 4 ER stress and myelin.
Sustained protein unfolding in the endoplasmic reticulum activates the unfolded protein response (UPR). This is modulated through three endoplasmic proteins sensors: inositol-requiring kinase 1 (IRE1), double-stranded RNA-activated protein (PKR)-like endoplasmic reticulum kinase (PERK), and activating transcription factor 6 (ATF6). Binding protein (BiP) binds to all three proteins and it is titrated away by unfolded proteins in the ER lumen. Upon the dissociation of BiP, IRE1 dimerizes and autophosphorylates, activating its RNase domain. X-box binding protein 1 (XBP-1) mRNA is cleaved with spliced XBP1 being an active transcription factor that induces the translation of ER quality control (QC) chaperones and folding machinery to deal with the increased protein load. Upon BiP titration, PERK similarly dimerizes and autophosphorylates. PERK also phosphorylates eukaryotic translation initiation factor 2 (eIF2α), leading to the inhibition of general translation. Along with BiP, calreticulin (CRT) also retains ATF6 in the ER, a retention mechanism that is overcome under ER stress conditions by underglycosylation of newly synthesized ATF6. Loss of BiP and CRT interaction allows ATF6 to translocate to the Golgi where it is cleaved by Site-1-protease (S1P) and Site-2-protease (S2P), releasing its cytosolic bZIP domain that translocates to the nucleus to activate transcription of proteins, such as CCAAT/enhancer-binding protein (C/EBP) homologous protein (CHOP), a pro-apoptotic transcription factor.

cause ER stress. Dysmyelination in the calnexin-deficient mouse appears to be directly attributable to the absence of quality control for several myelin specific proteins, including P0, PMP22, and MOG. Although calnexin is an ER resident chaperone, ER stress does not seem to play a large role in the observed dysmyelination. No significant ER stress was visible at the tissue level in calnexin-deficient animals, although activation of UPR was visible in cultured calnexin-deficient mouse embryonic fibroblasts (114). In addition, expression of MOG in the absence of the ER quality control

chaperones calnexin, calreticulin, and ERp57 does not induce the UPR (89). The contribution of calnexin to dysmyelination is surprisingly specific to the chaperone itself, and not attributable to secondary ER stress effects.

Cholesterol and myelin

Cholesterol, a major component of myelin, is not imported from the circulation, but instead synthesized locally and is rate-limiting for myelin biogenesis (12, 13). The rate-limiting effect of cholesterol was determined when a conditional mouse mutant was generated using Cre-mediated targeting of the squalene synthase (SQS) gene in oligodendrocytes and Schwann cells. SQS catalyzes the first step of the cholesterol synthetic pathway specific to the formation of sterols (115). The mutant mice lagged behind the controls in weight gain and developed motor function deficits at 2 weeks of age characterized by ataxia, initiation tremor, and impaired control of hindlimb movements. This was attributed to severe dysmyelination in the form of hypomyelinated axons in the CNS and PNS. Interestingly, there was a stringent preservation of the cholesterol to lipid stoichiometry in the mutant myelin despite the abrogated ability of the myelinating glia to synthesize cholesterol. This suggested horizontal cholesterol transfer, potentially from neighboring wild-type cells, such as astrocytes and demonstrated cholesterol is an essential rate-limiting factor in myelin biogenesis. It is important to note that critical components of cholesterol biosynthesis are located at ER membranes, including the rate-limiting step, 3-hydroxy-3-methyl-glutaryl-CoA reductase (HMGR), an integral ER membrane protein.

Many myelin proteins are known to partition into cholesterol-rich membrane rafts in myelin membranes and it was postulated that an interaction with cholesterol could be part of the ER protein quality control system (116). Consistent with this hypothesis, it was observed that the major peripheral myelin protein P0 requires cholesterol for its trafficking from the ER (116). Trafficking was dependent on a functional cholesterol recognition/interaction amino acid consensus motif in P0 (residues 142–151). Expression of myelin protein genes was also dependent on high intracellular cholesterol levels, as mRNA levels of MAG, MBP, P0, and PMP22 were significantly reduced in sciatic nerves from SQS mutant animals (116).

Cholesterol and dysmyelination have also been linked in human disease. Niemann-Pick type C is a very rare and often fatal lysosomal storage disease where mutations in the proteins Niemann-Pick C1 (NPC1) and Niemann-Pick C2 (NPC2) block the ability of the cell to transport cholesterol from the late endosome/lysosome to other organelles including the plasma membrane, endocytic recycling compartment, and ER (117). Clinical manifestations of NPC include effects on the liver, spleen, lungs, and neurological systems (118); and a peripheral demyelinating polyneuropathy has also been observed (119). Considering the importance of cholesterol for myelin protein trafficking and myelin membrane formation, it remains to be seen if defects in cholesterol biosynthesis, processing or trafficking contribute to human myelinopathies.

The role of ER Ca^{2+} stores in myelin

The ER is responsible for the storage of the majority of intracellular Ca^{2+} and this high Ca^{2+} concentration is required for protein folding and glycoprotein processing (31, 32). ER Ca^{2+} homeostasis is maintained by balancing Ca^{2+} release from the ER by the inositol 1,4,5-trisphosphate receptor ($InsP_3R$) and ryanodine receptor (RyR) (5, 6) with replenishment of the stores by sarcoplasmic/endoplasmic reticulum Ca^{2+}-ATPase (SERCA) (120). The total Ca^{2+} concentration in the ER is in excess of 2 mM but the ER employs several Ca^{2+} binding/buffering proteins, such as calreticulin, Grp94, and BiP to keep free ER Ca^{2+} in concentration ranges of 50–500 μM (121–123). The glutamatergic system of excitatory neurotransmission, where glutamate is the natural ligand for the ion-permeable channels α-amino-3-hydroxy-methyl-4-isoxazole propionic acid (AMPA), kainate and *N*-methyl-D-aspartate (NMDA) receptors, is critical for excitatory neurotransmission in the CNS and essential to plasticity and neuronal development. NMDA receptors are slow gating channels that are highly permeable to Na^+ and Ca^{2+} and activation of NMDA receptors is known to induce Ca^{2+}-induced Ca^{2+} release (CICR) from internal Ca^{2+} stores (i.e., ER) in hippocampal dendritic spines (124). It was previously thought that AMPA and kainate receptors, but not NMDA receptors, were expressed in white matter glia (125). Since then, several groups have demonstrated the presence of NMDA receptors in oligodendrocyte processes and the inner and outer loops of compact myelin where the NMDA receptor is shown to mediate Ca^{2+} accumulation in myelin during chemical ischemia (126–128). As previously discussed, resident chaperone molecules of the ER, such as calnexin, are crucial for the protein quality control and assembly of transmembrane proteins, including plasma membrane receptors, such as the NMDA and AMPA receptor. Calnexin has been shown to interact with the NR1 NMDA receptor subunit (129) and glycosylated AMPA receptors (130). In this case, the contributions of the ER to healthy myelin sheaths are 2-fold: first, quality control chaperones are responsible for the appropriate folding, assembly, and trafficking of the NMDA receptor subunits to the plasma membrane; and second, the appropriate modulation of potentially damaging ER Ca^{2+} stores downstream of NMDA receptor activation is essential.

Concluding remarks

The specialized requirements of the system and cells that maintain myelin include needs for large quantities of protein and lipid synthesis, complex inter-cell communication, and unique environments. Dysmyelination is not attributable to any one effector and can be caused by a variety of components. The ER is important for all of these aspects, making it an essential contributing organelle to myelination. Small

changes in any of these parameters can lead to dysmyelination phenotypes and disease. Implications of ER stress and ER chaperones, such as calnexin in the myelination process emphasize the importance of such a secretory organelle to myelinating cells. Although a great deal of study has been conducted on myelin proteins and their contribution to myelin formation and maintenance, the global role of the ER in myelination implicates that upstream events contribute to the resulting pathology of dysmyelination and only studying the downstream myelin proteins affected will not provide us with the whole picture. This provides the exciting possibility of therapeutic intervention at an upstream regulatory component. By controlling molecules such as calnexin or ER responses during myelination, we could affect myelin processes and dysmyelination in disease.

Outlook

Although the importance of the ER for myelinating systems is somewhat intuitive considering its role as the major secretory organelle for a vast system, the concept of the ER as the central regulator in myelination is new. Furthermore, that we could modulate how the ER deals with cell stress, protein retention or release, or general protein load, and thus its impact on myelination is a new approach that could have significant therapeutic potential. Targeting key molecules in ER processes that are specific to myelin systems could develop strategies that might lessen the degree of dysmyelination in disease or improve remyelination processes in pathologies, such as MS. In the near future, we can anticipate a shift in how myelin diseases are studied by investigating not only downstream effectors in myelin disease, such as the misfolded myelin proteins, but additionally the upstream effectors at the level of the ER where the problem initiated. Furthermore, with dysmyelination being newly implicated in diseases such as Alzheimer's disease and various psychiatric diseases, we anticipate the emergence of common underlying mechanisms in myelin diseases that will extend our knowledge beyond the identification of single gene contributions. It is an opportune time for neuroscientists investigating dysmyelination and other neuropathies to make the examination of the contribution of the ER quality machinery their next challenge.

Highlights

- Quality control of myelin proteins in the ER is often carried out by the calnexin cycle and calnexin in particular emerges as a critical chaperone for myelinating systems.
- It is possible that overcoming retention of mutant myelin proteins could alleviate the resulting myelin disease. However, this needs to be examined in the context of the immune system as misfolded proteins stably expressed at the cell surface could trigger an immune response.
- ER stress is implicated in dysmyelination pathologies. Controlling how the ER responds to unfolded protein load and the ER stress response could be beneficial in modulating myelin disease.
- Cholesterol is essential for myelin biogenesis and crucial components of cholesterol biosynthesis occur within the ER.
- Release of ER Ca^{2+} stores during chemical ischemia contributes to white matter injury.

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