The production, transport and integration of myelin components into the membrane during development is a highly coordinated and regulated process that relies heavily on the endoplasmic reticulum (ER), a sub-cellular organelle that is the principal site of membrane assembly. Ribosomes on the rough ER allow translation of proteins such as proteolipid protein (PLP) prior to correct folding, post-translational modification and eventual complexing with nascent smooth ER-synthesised lipids.

A single oligodendrocyte can ensheath multiple axons during developmental myelination and this process has been shown to occur during a short time window (estimated between 12–18 hours) by which time the cell loses its myelinating capacity. It has been estimated that, in post-natal rat, these cells expand their surface area at a rate of 5–50×10^6 μm^2/cell/day during myelination (Baron and Hoekstra, 2010) and, as such, a considerable burden is placed on the ER.

Acting as a site of quality-control in the cell, the ER ensures conformational fidelity of all its products via the action of a range of chaperones, co-chaperones and foldases and, when molecules fail to attain the correct tertiary structure, they are targeted for degradation via the ER-associated protein degradation pathway (ERAD). The ER can initiate a complex homeostatic mechanism known as the unfolded protein response (UPR) when maximal biosynthesis is occurring in a cell, and its ER is approaching maximal capacity. The series of signalling pathways that comprise this response generally results in a slowing of traffic through the ER and an expansion in ER function, allowing the cell to regain balance, but under prolonged ER stress can eventually lead to cell death.

This homeostatic mechanism is mediated by three transmembrane sensors in the ER: protein kinase RNA-like endoplasmic reticulum kinase (PERK), activating transcription factor 6 (ATF6) and inositol requiring enzyme 1 (IRE1). Under conditions of physiological stress or pathological conditions, the sensors will initiate cell-signalling pathways that work together to reduce the stress in the ER and ultimately the cell. PERK dimerises, auto-phosphorylates and, in turn, induces the activation of eukaryotic translation initiation factor alpha (EIF2α) by addition of a phosphoryl group. The net effect of this is a transient global arrest in translation. However, in a few select mRNAs containing short open reading frames in their 5' untranslated region, EIF2α activation leads to increased expression of molecules, such as activating transcription factor 4 (ATF4), whose target gene encodes molecules involved in amino acid metabolism, protein secretion and regulation of redox homeostasis. IRE1 also responds to ER stress by oligomerisation and auto-phosphorylation effecting the activation of an endoribonuclease domain that splices X-box binding protein 1 (XBP1) mRNA to produce a potent pro-survival transcription factor for many molecules associated with ERAD, membrane biogenesis, ER chaperones and redox enzymes. IRE1 also has a function in regulated IRE1-dependent decay (RIDD), which results in degradation of mRNAs destined for the ER, thus alleviating ER burden. Unlike the other two sensors, ATF6 responds to ER stress by transiting to the Golgi where it is cleaved into a active transcription factor that has numerous targets, many shared with XBP1, including ER chaperones, anti-apoptotic proteins and ERAD proteins.

These homeostatic responses will generally tend towards production of molecules that can improve the capacity and efficacy of the organelle. Four such molecules, sometimes considered to be classical indicators of the UPR, are GRP78/Bip, GRP94, calreticulin and protein disulphide isomerase (PDI). All the molecules are multi-functional but have in common a chaperone function, in that they assist folding/unfolding and assembly/disassembly of proteins in the ER.

Somewhat surprisingly, the dynamics of the activation of all three arms of the UPR (as defined by the three transmembrane sensors) during myelination had not previously been studied. In our laboratory, we have previously seen upregulation of UPR-associated molecules in association with multiple sclerosis (MS) pathology in grey and white matter lesions and in acute pathological conditions of the spinal cord. In MS, a proteolytic cleavage product of an EAE induced by myelin oligodendrocyte glycoprotein (MOG) inculcation (Mhaille et al., 2008; McMahon et al., 2012; Ni Flháthartaigh et al., 2013). We postulated that knowledge of UPR activation in early developmental myelination of axons could provide us with insights into the role of these molecules in a disease characterised by rounds of myelin loss and subsequent remyelination.

The rat cerebellum provides an excellent model for the study of events occurring during neonatal myelination for many reasons. Firstly, although oligodendrocyte progenitor cells (OPCs) are present in the cerebellum before birth, the process of myelination is not initiated until cortical neurones mature (at approximately P10). This is accompanied by a rapid expansion in oligodendrocyte cell density in cerebellar white matter tracts (from approximately 10% of cells at P10 to over 70%) due to maturations of OPCs, astroglial cell death and apoptosis of OPCs that fail to mature into functioning myelinating oligodendrocytes. Secondly, the laminar structure of the cerebellum has led to its use in studies of the oligodendrocyte phenotype and morphology during development, since prospective white matter tracts can be easily identified. Close monitoring of myelination milestones in tracts III and IV, as characterised by expression of different myelin-specific proteins (Figure 1), has allowed us to carry out a comprehensive study of the dynamics of various UPR-associated molecules during developmental myelination.

An initial immunohistochemical analysis of the activation status of the ER-associated sensors was carried out by determining the phosphorylation status of PERK, IRE1α and the α subunit of the inositol (indicative of ATF6-cleavage) (Figure 2). This indicated significant increases in both pIRE1 and nuclear-localised ATF6 in the cerebellum (Figure 1) has allowed us to carry out a comprehensive study of the dynamics of various UPR-associated molecules during developmental myelination.

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An initial immunohistochemical analysis of the activation status of the ER-associated sensors was carried out by determining the phosphorylation status of PERK, IRE1α and the α subunit of the inositol (indicative of ATF6-cleavage) (Figure 2). This indicated significant increases in both pIRE1 and nuclear-localised ATF6 immediately prior to, and during, active demyelination and with a return to low levels of both molecules in adult fully-myelinated white matter tracts. Conversely, pPERK was expressed at low levels throughout the myelination process and showed only a small, but significant, increase in adult tissue. The known downstream targets of PERK activation, EIF2α and CHOP also did not show any significant increase throughout the entire myelination process, suggesting that this arm of the UPR is not required for this developmental process. This is not entirely surprising given the results of a series of elegant studies from the Popko laboratory examining the role of PERK both in inflammatory demyelination and in normal neonatal myelin formation. Data from this group have shown that delivery of interferon-gamma prior to induction of EAE in mice can protect oligodendrocytes from undergoing EAE-induced apoptosis and demyelination and that this response is PERK-dependent (Lin et al., 2007). Similarly, they found that, in genetically-engineered mice, controlled stimulation of PERK signalling, in the absence of ER stress, could provide protection against apoptosis and ameliorate the severity of disease in EAE (Lin et al., 2013). Further experimentation by this group applying a drug called guanabenz, known to stimulate the PERK-EIF2α arm of the UPR, to cultured oligodendrocytes and cerebellar explants produced oligodendrocyte-protective effects similar to those achieved by stimulating PERK (Way et al., 2015) and has led to the proposition that the drug could be a potential therapeutic for MS. Yet, in spite of these insights into the role of this arm of the UPR in a pathological situation, what is of great interest to us is that they also found that neonatal myelination was completely unimpaired in PERK-null mice (Hussien et al., 2014). This suggests that the PERK arm is more crucial in the integrated stress response (ISR) that occurs in response to pathological tissue change, rather than normal physiological ER overload and highlights that different mechanisms may be occurring in reparative adult remyelination compared to during brain development. Of note, however, is that the IRE1 and ATF6 arms of the UPR were not examined in these studies.

Interestingly, immunohistochemical staining indicated that both ATF6 and IRE1 were activated at the P7 time-point, which is prior to the appearance of myelin, with maximal nuclear-localised ATF6 appearing at P10 and pIRE1 peaking at P14 (levels of pIRE1 being significantly higher at p17 and than at the pre- and post-myelination phases). ATF6’s appearance in the nucleus coincides with the earliest stages of myelination (just prior to appearance of myelin protein). Loss of expression during which there is a massive biosynthetic burst in the absence of cell stress. However, it is difficult to confirm the exact role that ATF6 plays in developmental myelination since the mouse seems to have redundant genetic compensation. However, this may be due to the compensatory mecha-
the increase in molecules that improve both the capacity and efficiency of the ER, afforded by signalling through ATF6 and IRE1, can only be beneficial during a period of such intense bioactivity. The question of whether or not activation of the IRE1 and ATF6 arms of the UPR can be boosted therapeutically in an effort to promote remyelination in diseases such as MS, merits further investigation.

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References


