Published in final edited form as:

Methods Enzymol. 2011 ; 491: 91–109. doi:10.1016/B978-0-12-385928-0.00006-7.

Animal Models in the Study of the Unfolded Protein Response

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Abstract

The endoplasmic reticulum, a highly dynamic and complex organelle, is the site for synthesis, folding, and modification of transmembrane and secretory proteins. Any disruptions to the endoplasmic reticulum such as an accumulation of misfolded or unfolded proteins results in activation of the unfolded protein response (UPR). The UPR is comprised of three distinct signal transduction pathways that work to restore homeostasis to the endoplasmic reticulum. This review summarizes select mouse models available to study the UPR and the information learned from the analyses of these models.

1. INTRODUCTION

The unfolded protein response (UPR) is activated when the endoplasmic reticulum (ER) is exposed to stressful conditions, such as accumulation of unfolded or misfolded proteins. UPR signaling emanates from the ER and functions to maintain ER homeostasis during ER stress. Three mechanisms exist to respond to the accumulation of improperly or incompletely folded proteins. First, there is a decrease in the protein load through transient translational inhibition (Harding et al., 1999). Secondly, the ER increases in size and upregulates the expression of folding enzymes and chaperone proteins to accommodate the existing protein load (Kozutsumi et al., 1988; Shaffer et al., 2004; Sriburi et al., 2004). Thirdly, there is enhanced clearance of unfolded proteins through induction of ER-associated degradation (ERAD) components (Travers et al., 2000). Three ER transmembrane proteins, PKR-like ER kinase (PERK), activating transcription factor 6 (ATF6), and inositol-requiring enzyme 1 (IRE1), mediate ER stress detection, serving as proximal transducers of the UPR. During normal conditions, BiP (immunoglobulin (Ig)-binding protein) associates with the luminal domains of PERK, IRE1, and ATF6; however, upon ER stress, BiP dissociates from these UPR regulators, resulting in their activation (Bertolotti et al., 2000). Together, these three pathways of the UPR alter the translational and transcriptional program of the cell, helping to alleviate ER stress and restore ER homeostasis. Nevertheless, severe or chronic ER stress leads to apoptosis.

This review focuses on select mouse models available to study the UPR (Table 6.1) and summarizes the invaluable information learned from these models. Knockout animals are generated through the inactivation of the endogenous gene by replacing it with a disrupted version via homologous recombination. One potential limitation of knockout technology is embryonic lethality, which can be overcome through the utilization of the Cre/*loxP*DNA recombination system to generate tissue-specific knockout (conditional knockout) mice.

This system requires two different genetically modified mouse lines. The first contains the target gene flanked by two *loxP* sites in the same orientation (floxed gene), and the second line bears a transgene expressing the Cre recombinase under the control of a cell or tissue-specific transcriptional control region. When these two mouse lines are crossed, the floxed gene is deleted in a cell- or tissue-specific manner through the Cre-mediated excision of the targeted gene segment between the *loxP* sites.

2. ACTIVATING TRANSCRIPTION FACTOR 6

ATF6 exists in two isoforms, α and β , both of which are ubiquitously expressed and localized in the ER (Haze et al., 2001; Yoshida et al., 1998). ATF6 is a transmembrane protein whose activation and signaling is controlled by BiP. Upon ER stress, BiP dissociates from ATF6, revealing Golgi localization sequences (GLS; Shen et al., 2002). This results in translocation of ATF6 to the Golgi apparatus, where it undergoes regulated intramembrane proteolysis. Site 1 protease (S1P) cleaves ATF6 within the luminal domain and site 2 protease (S2P) cleaves within the phospholipid bilayer-spanning domain, releasing the transcriptionally active N-terminus cytosolic portion (Haze et al., 1999; Ye et al., 2000). Active ATF6 is a basic leucine zipper transcription factor that translocates to the nucleus, binds the ER stress response element (ERSE), and upregulates transcription of ER chaperone genes such as BiP (Yoshida et al., 1998, 2000, 2001b).

The function of ATF6 during ER stress was recently defined using ATF6-deficient mice. Single knockouts of *Atf6a* or *Atf6b* did not have any obvious defects, and the pups were born at the expected Mendelian frequency. *Atf6a/Atf6b* double knockout mice exhibited embryonic lethality, suggesting redundant functions for ATF6 α and ATF6 β during embryonic development (Yamamoto et al., 2007). *Atf6b*^{-/-} mouse embryonic fibroblasts (MEFs) subjected to ER stress did not demonstrate a compromised ability to upregulate ER chaperone proteins. In contrast, *Atf6a*^{-/-} MEFs are severely defective, upon ER stress, in the induction of ER chaperone proteins and folding enzymes such as BiP, GRP94, ERp72, and P5. Severe defects in the upregulation of ER-associated degradation components such as EDEM, HRD1, and Herp were also observed in *Atf6a*^{-/-} MEFs during ER stress (Wu et al., 2007; Yamamoto et al., 2007).

ER stress also resulted in decreased survival of *Atf6a*^{-/-} MEFs as compared to wild-type (WT) MEFs (Wu et al., 2007; Yamamoto et al., 2007). *In vivo* studies using intraperitoneal injection of the ER stress inducing agent tunicamycin (TM) yielded an 80% decrease in the survival of *Atf6a*^{-/-} mice as compared to WT mice, demonstrating that ATF6 α is critical for protection against ER stress (Wu et al., 2007). Taken together, these studies demonstrate that ATF6 α deficiency increases sensitivity to ER stress both *in vitro* and *in vivo*.

The generation of animals with a conditional (floxed) *Atf6* allele to allow for the tissue-specific ablation of ATF6 will serve as a great resource to determine if this UPR-regulated transcription factor has cell-specific functions.

3. IRE1/X-Box-BINDING PROTEIN-1

IRE1 is an ER transmembrane protein found to have cytosolic kinase and endoribonuclease domains (Tirasophon et al., 1998). Two IRE1 isoforms are present in mammals, IRE1 α and IRE1 β . IRE1 α is expressed ubiquitously, whereas IRE1 β expression is limited to intestinal epithelial cells and the stomach (Tirasophon et al., 1998; Wang et al., 1998). Similar to ATF6, IRE1 is activated in response to BiP depletion following ER stress. BiP dissociation from IRE1 allows for IRE1 dimerization, which results in trans-autophosphorylation and activation of the endoribonuclease domain (Shamu and Walter, 1996; Welihinda and Kaufman, 1996). The site-specific endoribonuclease cleaves a 26-base pair intron from X-box binding protein 1 (*Xbp1*) mRNA, which encodes the transcription factor XBP1 (Calton et al., 2002; Yoshida et al., 2001a). This excision and subsequent religation of the *Xbp1* transcript alters the reading frame to increase the transcriptional activity of XBP1. Unspliced *Xbp1* encodes a 33-kDa protein [XBP1(U)] and the spliced form of *Xbp1* encodes a 54-kDa protein [XBP1(S)]. XBP1(U) and XBP1(S) share the same N-terminus but differ in their C-terminal transactivation domains. XBP1(S) is a stronger and more stable transcription factor than XBP1(U) (Calton et al., 2002; Lee et al., 2002; Yoshida et al., 2001a).

Ire1^{-/-} MEFS undergoing ER stress also display a defect in the ability to upregulate ERAD components, suggesting that both ATF6 α and XBP1 are required for optimal expression of ERAD components, perhaps through heterodimerization (Lee et al., 2003). Immunoprecipitation studies revealed that active ATF6 α and XBP1(S) heterodimerize during ER stress, suggesting that ATF6 and XBP1(S) work cooperatively during the UPR (Wu et al., 2007; Yamamoto et al., 2007).

Both *Ire1* α ^{-/-} and *Xbp1*^{-/-} mice exhibit embryonic lethality, demonstrating that both factors are necessary for embryonic development (Reimold et al., 2000; Zhang et al., 2005). XBP1 deficiency causes embryonic lethality due to liver hypoplasia and apoptosis (Reimold et al., 2000). Therefore, studying the role of the UPR transcriptional activator XBP1 in secretory cells has been difficult for the past several years. A liver-specific *Xbp1* transgene was able to rescue *Xbp1*^{-/-} mice (*Xbp1*^{-/-}; *Liv*^{*Xbp1*}) from dying *in utero*. While these mice were born at a normal frequency, they died a few days after birth. *Xbp1*^{-/-}; *Liv*^{*Xbp1*} mice display growth retardation and hypoglycemia that are suggestive of poor nutritional status. Additionally, these mice had undigested milk in their intestines and a poorly developed pancreas. Digestive enzymes, such as amylase and trypsin, were markedly decreased in *Xbp1*^{-/-}; *Liv*^{*Xbp1*} mice. Electron microscopy revealed a severely diminished amount of ER in *Xbp1*^{-/-}; *Liv*^{*Xbp1*} mice as compared to WT. There was also a decrease in the expression of genes encoding the ER-associated proteins SEC61 α , EDEM, and PDI, as measured by real-time RT-PCR. These data correlated with an increased apoptosis of pancreatic acinar cells, which secrete digestive enzymes that facilitate breakdown of food in the small intestine, suggesting that XBP1 is necessary for the survival of these cells; however, the pancreatic islet cells, which produce the hormones insulin and glucagon, were unaffected. Salivary glands in the mutant mice were also underdeveloped, displayed poorly developed ER, and produced less amylase (Lee et al., 2005). These data reveal a critical role for XBP1 in the proper development and function of pancreatic acinar cells and salivary gland cells, suggesting that XBP1 is required for high-rate secretory cell function.

In addition to pancreatic studies, *Xbp1*^{-/-} mouse models have been used to focus on high-rate secretory cells in the immune system. Because XBP1-deficient mice are embryonic lethal, the RAG2 (recombination-activating gene-2) complementation system was implemented to study the role of XBP1 in B cells. RAG2-deficient animals are unable to rearrange their antigen receptor genes; therefore, they lack B and T lymphocytes. *Xbp1*^{-/-} embryonic stem (ES) cells were injected into RAG2-deficient blastocysts. The blastocysts were implanted into pseudopregnant females, generating chimeric mice. The lymphoid compartments in these chimeric mice could only be derived from XBP1-deficient ES cells, resulting in T and B cells that lacked XBP1. The B cell numbers, percentages, and phenotype were normal; however, baseline serum Ig levels were decreased in the chimeric mice (Reimold et al., 2001). There was also a marked decrease in Ig secretion upon *in vitro* stimulation of *Xbp1*^{-/-} B cells with LPS. Further studies revealed that the initial levels of IgM synthesis were similar in LPS-stimulated WT and *Xbp1*^{-/-} B cells; however, over a 3-day time course there was a significant decrease in the level of IgM synthesis in *Xbp1*^{-/-} B cells (Tirosh et al., 2005). These data suggest that XBP1 is not critical for initial onset of IgM synthesis upon stimulation, yet it is required for enhanced and sustained IgM synthesis and secretion. Interestingly, Ig light chain synthesis is normal in these cells, demonstrating that XBP1 is not required for light chain synthesis, but is critical for heavy chain synthesis (Tirosh et al., 2005). All other aspects of B cell functioning, aside from antibody secretion, were normal.

Consistent with the marked decrease in serum Ig, histological analysis of the jejunum revealed a 70-fold decrease in antibody-secreting plasma cells in the chimeric mice as compared to the control mice. Retroviral transduction with a construct expressing the spliced form of XBP1 [XBP1(S)] rescued the ability of *Xbp1*^{-/-} B cells to secrete IgM. Transduction with this construct in mature B cells was sufficient to drive plasma cell differentiation (Reimold et al., 2001). These data demonstrate that XBP1 is required for terminal B cell differentiation and antibody production.

XBP1 is necessary for another type of immune cell, dendritic cells (DCs), which are critical in initiating the adaptive immune response. A specialized subset of DCs, plasmacytoid DCs (pDCs), secretes copious amounts of the cytokine interferon- α (IFN- α) upon activation by a virus or bacterial DNA (Liu, 2005). Similar to the terminal B cell differentiation process, pDCs expand the ER upon activation. *Rag2*^{-/-} mice reconstituted with *Xbp1*^{-/-} ES cells possessed severely reduced numbers of pDCs. *Xbp1*^{-/-} pDCs also demonstrated decreased IFN- α production and poorly developed ER as compared to control chimeric mice (Iwakoshi et al., 2007). These data illustrate the need for XBP1 in the maintenance and proper development of pDCs.

Recently, floxed *Xbp1* animals were generated, allowing for the production of tissue-specific knockout mice that have been utilized to delineate the function of XBP1 in various diseases, including Crohn's disease and amyotrophic lateral sclerosis (ALS; Hetz et al., 2008, 2009; Kaser et al., 2008). XBP1(S) and BiP levels were increased in biopsies from patients suffering from ulcerative colitis and Crohn's disease as compared to healthy individuals, implicating XBP1 in disease pathogenesis. Deletion of *Xbp1* specifically in murine intraepithelial cells (IECs) led to spontaneous enteritis (inflammation of the small intestine;

Kaser et al., 2008). Spontaneous enteritis in these animals correlates with an absence of Paneth cells due to apoptosis. Paneth cells are responsible for producing antimicrobial peptides and maintaining the gastrointestinal barrier. Moreover, chemically induced colitis using dextran sodium sulfate resulted in enhanced inflammation of the colon in mice lacking XBP1 in IECs as compared to WT littermates (Kaser et al., 2008). These data implicate the ER stress factor XBP1 in protection from inflammatory bowel disease by preservation of Paneth cells. Consistent with these data, mice deficient in *Ire1 β* , which is exclusively expressed in the GI tract, develop more rapid and severe colitis as compared to WT mice in response to challenge with dextran sodium sulfate (Bertolotti et al., 2001). This suggests that IRE1 β plays a protective role against colitis. These data suggest that the ER stress response IRE1/XBP1 pathway is protective against inflammatory bowel disease, likely through preservation of Paneth cells.

A link also exists between neurodegenerative diseases characterized by misfolded proteins/protein aggregates and UPR activation. One such disease is ALS, also referred to as Lou Gehrig's disease. *Xbp1* was specifically deleted in the CNS of a mouse model for ALS. While no difference was found in male mice, female mice lacking XBP1 in the CNS exhibited a delay in onset of disease and experienced a prolonged life span. This correlated with enhanced autophagy and significantly reduced protein aggregates in the spinal cord of female mice lacking XBP1 in the CNS (Hetz et al., 2009). It will be interesting to determine how gender, in combination with the absence of XBP1, affects the amelioration of disease symptoms, and to elucidate XBP1's role in other neurodegenerative diseases.

4. PKR-LIKE ER KINASE

The serine/threonine PERK mediates the reduction of the protein load in the ER in order to alleviate ER stress. Upon stress to the ER, BiP dissociates from PERK, resulting in PERK dimerization and transautophosphorylation. Activated PERK phosphorylates the alpha subunit of eukaryotic translation initiation factor 2 (eIF2 α). Phosphorylated eIF2 α binds to and inhibits the activity of the guanine nucleotide exchange factor eIF2B, thus preventing formation of the translation initiation complex (Harding et al., 1999). This process results in the attenuation of global protein translation. Upon treatment with ER stress inducing agents, *Perk*^{-/-} cells are unable to phosphorylate eIF2 α and cannot slow the rate of protein translation, making them more sensitive to ER stress, as demonstrated by their decreased ability to survive (Harding et al., 2000b). These data demonstrate that PERK is critical for cell survival and inhibiting translation during the UPR.

Phosphorylation of eIF2 α results in a transient global decrease in translation initiation; however, it also facilitates selective translation of particular transcripts such as ATF4 (Harding et al., 2000a).

Two known mechanisms exist to restore normal protein translation over the course of the UPR. GADD34, downstream of ATF4, associates with protein phosphatase 1 (PP1) and counters PERK activity by dephosphorylating eIF2 α and restoring translation (Ma and Hendershot, 2003; Novoa et al., 2001, 2003). The second mechanism is thought to involve

the protein P58IPK, which is upregulated by XBP1(S). P58IPK binds to and inhibits PERK phosphorylation and activation (Lee et al., 2003; van Huizen et al., 2003; Yan et al., 2002).

PERK has been found to be critical for pancreatic function. *Perk*^{-/-} mice are hyperglycemic and develop diabetes mellitus (Harding et al., 2001). At ~ 4 weeks of age, there is a decrease in the number of insulin-producing islet cells in *Perk*^{-/-} mice. This correlates with diminished serum insulin levels and increased blood glucose levels. However, assessment of insulin production in prediabetic *Perk*^{-/-} mice demonstrated increased insulin synthesis by islet cells. There was also increased apoptosis of pancreatic acinar cells. Electron microscopy analysis revealed disrupted ER morphology in *Perk*^{-/-} islet cells and acinar cells as compared to WT cells (Harding et al., 2001). The pancreases of PERK-deficient mice appeared histologically normal at birth. However, after postnatal week 4, *Perk*^{-/-} animals possessed very few detectable pancreatic β cells. These data reveal the importance of PERK for normal pancreatic function.

Another group also generated *Perk* null animals by creating floxed *Perk* animals and mating them to transgenic mice expressing Cre recombinase under the control of the EIIa promoter, resulting in the loss of PERK activity in all cells of the resulting animals (Zhang et al., 2002). These animals also demonstrated a loss of glucagon-secreting pancreatic α cells. *Perk*^{-/-} animals display growth retardation, severe spinal curvature, splayed hind limbs, and reduced locomotor activity. At birth, *Perk*^{-/-} mice exhibit osteoporosis and deficient mineralization throughout the entire skeletal system. Like the pancreatic cells, osteoblasts from the null animals also displayed abnormal ER morphology. PERK-deficient animals also had reduced levels of collagen type I $\alpha 1$ and $\alpha 2$, which are major components of the extracellular matrix of compact bones (Zhang et al., 2002). These data demonstrate an essential function for PERK in bone development. Floxed *Perk* mice have been generated and crossed to cell-specific Cre transgenic animals to gain further understanding of PERK functioning in the pancreas and osteoblasts (Iida et al., 2007; Wei et al., 2008).

5. eIF2 α

The ability of PERK to inhibit global protein synthesis is dependent on the phosphorylation of serine 51 of murine eIF2 α . Mutating serine 51 to alanine creates a nonphosphorylatable form of eIF2 α . TM treatment of homozygous A/A eIF2 α -mutant MEFs resulted in significant cell death in comparison to WT MEFs, demonstrating that the phosphorylation of eIF2 α is required for cell survival in response to ER stress (Scheuner et al., 2001). Furthermore, MEFs with the nonphosphorylatable form of eIF2 α were unable to produce ATF4, demonstrating that eIF2 α phosphorylation is required for optimal ATF4 translation.

While homozygous A/A eIF2 α -mutant mice were born at the expected Mendelian ratio and were phenotypically normal, these mutant neonates died within 18 h of birth (Scheuner et al., 2001). Blood glucose analysis showed severe hypoglycemia at 6–9 h after birth in homozygous A/A eIF2 α mice. Hypoglycemia appeared to play a role in the lethality of the mutant neonates because injection with glucose for 2 days following birth rescued these animals for a short period. This hypoglycemia was linked to severely diminished insulin levels in the homozygous mutant embryonic and neonatal pancreas (Scheuner et al., 2001).

Interestingly, the disease is much more severe in homozygous A/A eIF2 α neonates than in *Perk*^{-/-} animals, indicating that perhaps other eIF2 α kinases, such as GCN2 or PKR, may compensate for the absence of PERK activity.

Heterozygous S/A eIF2 α -mutant mice appear phenotypically normal despite demonstrating an ~ 50% reduction in the phosphorylation of eIF2 α ; however, when these heterozygous mutant animals are fed a high fat diet, they become obese and develop diabetes (Scheuner et al., 2005). S/A eIF2 α mice have decreased insulin secretion, which correlates with abnormal ER morphology and decreased quantity of insulin granules in β cells. Low levels of extracellular glucose inhibit translation in β cells, while high levels of extracellular glucose restore translation in β cells. Upon high glucose treatment, the rate of total protein synthesis did not fluctuate in WT animals, whether they were fed a low fat diet or a high fat diet. In contrast, there was an increased rate of translation in heterozygous mutant animals fed a high fat diet as compared to those fed a low fat diet. These data suggest that proper eIF2 α phosphorylation is required to limit protein synthesis under increased glucose and high fat diet conditions. Interestingly, in mice receiving a high fat diet, there was enhanced binding of BiP to proinsulin in heterozygous S/A eIF2 α mice as compared to WT (Scheuner et al., 2005). Since BiP is an ER chaperone known to bind unfolded proteins, this suggests that the protein folding capacity in heterozygous S/A eIF2 α mice might be compromised.

6. ATF4

PERK activation and eIF2 α phosphorylation result in the inhibition of global protein synthesis; however, phosphorylated eIF2 α also leads to the preferential translation of *Atf4* mRNA. ATF4 is a transcription factor that upregulates genes involved in amino acid import, glutathione biosynthesis, and the antioxidative stress response (Harding et al., 2000a, 2003). ATF4 also upregulates CAAT/enhancer binding protein homologous protein (CHOP) (Ma and Hendershot, 2003).

Atf4 knockout mice demonstrate a high level of perinatal and postnatal mortality, with ~70% of animals dying between embryonic day 17.5 and postnatal day 14, which correlates with severe fetal anemia (Hettmann et al., 2000; Masuoka and Townes, 2002). The remaining 30% of mice lacking ATF4 survive until adulthood, but display growth retardation and exhibit severe microphthalmia, a developmental disorder of the eye (Hettmann et al., 2000). ER-stressed *Atf4*^{-/-} MEFs were severely impaired in the expression of genes involved in amino acid import, glutathione biosynthesis, and resistance to oxidative stress in comparison to WT MEFs. ATF4-deficient MEFs were unable to survive and grow in culture unless supplemented with amino acids and reducing substances in the growth media. In the absence of supplementation, *Atf4*^{-/-} cells displayed a buildup of intracellular peroxides followed by cell death (Harding et al., 2003). Taken together, these data suggest that the PERK/ATF4 pathway may be critical in protecting against oxidative damage during ER stress.

7. CHOP

CHOP is a bZIP transcription factor and its expression is strongly induced by ER stress (Wang et al., 1996). The PERK pathway is necessary for upregulating CHOP expression,

although the other pathways of the UPR also contribute. Studies implicate CHOP in ER stress-induced apoptosis. Enforced expression of CHOP results in cell cycle arrest and/or apoptosis (Barone et al., 1994; Matsumoto et al., 1996; Maytin et al., 2001). MEFs lacking CHOP display enhanced resistance to ER stress-mediated apoptosis (Zinszner et al., 1998). CHOP-deficient mice exhibit no developmental defects and displayed decreased cell death in the renal tubular epithelium upon intraperitoneal injection of TM as compared to control animals (Zinszner et al., 1998). These data demonstrate that CHOP promotes apoptosis during ER stress. CHOP has been shown to repress expression of the antiapoptotic protein BCL-2, which could contribute to CHOP-mediated apoptosis (McCullough et al., 2001). Nevertheless, apoptosis still occurs in *Chop*^{-/-} animals subjected to ER stress, suggesting that there are also CHOP-independent mechanisms of cell death that occur in response to ER stress. CHOP may also promote cell death through induction of the target genes *Gadd34* (discussed later) and *Ero1a* (Marciniak et al., 2004). *Ero1a*^{-/-} MEFs experience reduced cell death in response to ER stress (Li et al., 2009). ERO1 α creates a necessary oxidative environment in the ER, but in doing so produces reactive oxygen species that might promote apoptosis (Marciniak et al., 2004).

CHOP may play a central role in multiple mouse models of disease. In mouse models for type II diabetes, deletion of *Chop* resulted in decreased blood glucose levels and increased serum insulin levels. Additionally, improvement in pancreatic β cell function correlated with reduced cell death and decreased oxidative stress (Song et al., 2008). CHOP deficiency in two different atherosclerotic mouse models reduced the amount of necrosis and apoptosis in atherosclerotic lesions and decreased the overall lesion size, suggesting that CHOP activity may worsen atherosclerosis (Thorp et al., 2009). *Chop* ablation also reduced the severity of the neuropathy in a murine model of Charcot Marie Tooth disease, a neurological disorder affecting the peripheral nerves, as measured by enhanced motor capacity and increased nerve conduction velocity. Moreover, decreased neuropathy in *Chop*^{-/-} mice correlates with reduced demyelination, suggesting a deleterious role for CHOP in demyelination during neuropathy (Pennuto et al., 2008).

While many studies demonstrate a harmful role for CHOP during disease, one study suggests a protective function for this protein. The neurodegenerative disorder Pelizaeus–Merzbacher disease is the result of mutations in the gene encoding the abundantly expressed integral myelin membrane protein proteolipid protein and is characterized by hypomyelination in the CNS resulting in varying degrees of delayed motor and intellectual function. A mouse model of Pelizaeus–Merzbacher disease, *rumpshaker* (*rsh*), exhibits tremor, ataxia, occasional seizures, and hypomyelination but has normal oligodendrocyte numbers and a normal life span. In contrast, *rsh* mice deficient for CHOP exhibit frequent seizures and only have an average life span of 10 weeks. Further studies showed that CHOP deficiency in *rsh* mice seems to result in oligodendrocyte apoptosis and enhanced hypomyelination, suggesting a protective role for CHOP (Southwood et al., 2002). Floxed *Chop* animals are currently unavailable but would be very useful to gain further understanding into the function of CHOP during disease pathogenesis.

8. GADD34

GADD34 interacts with the protein phosphatase PP1C to dephosphorylate eIF2 α , resulting in a negative feedback loop. Dephosphorylation of eIF2 α allows for translational recovery from ER stress. *Gadd34*^{-/-} MEFs and *Gadd34*^{-/-} MEFs, in which mutant GADD34 is unable to bind PP1C, displayed sustained levels of eIF2 α phosphorylation and prolonged inhibition of protein synthesis during ER stress as compared to WT MEFs (Kojima et al., 2003; Novoa et al., 2003). *Gadd34*^{-/-} and *Gadd34*^{-/-} mice develop normally and were greatly protected from cell death in comparison to WT mice receiving an intraperitoneal TM injection, suggesting that GADD34 promotes cell death induced by ER stress (Kojima et al., 2003; Novoa et al., 2003). These results are similar to observations in *Chop*^{-/-} animals and MEFs, further supporting that CHOP is required for optimal GADD34 expression upon ER stress. The generation of floxed *Gadd34* animals will be of great benefit to gain further insight into the contribution of GADD34 in a cell-specific manner during pathological conditions.

9. P58IPK

P58IPK binds to PERK and inhibits its kinase activity (Yan et al., 2002). P58IPK is believed to be under the control of XBP1 since *Xbp1*^{-/-} MEFs displayed reduced P58IPK expression upon UPR activation by TM treatment (Lee et al., 2003). P58IPK-deficient mice are smaller and have significantly lower body weight in comparison to P58IPK heterozygous and WT animals. The decrease in body weight of *P58ipk*^{-/-} mice correlates with decreased body fat. Interestingly, adult *P58ipk*^{-/-} mice experience hypoglycemia, hypoinsulinemia, and increased apoptosis and loss of pancreatic β cells (Ladiges et al., 2005). The phenotype of these animals is not as severe as that of *Perk*^{-/-} or eIF2 α -mutant animals, suggesting that P58IPK does not solely attenuate eIF2 α phosphorylation.

P58IPK has been found to associate with SEC61, a component of the ER protein translocation channel (translocon). In *P58ipk*^{-/-} mice, decreased levels of the chaperone HSP70 associated with translocon components in TM treated *P58ipk*^{-/-} livers as compared to WT livers, suggesting that P58IPK functions in recruiting chaperones to the ER translocon. Studies also suggest that P58IPK plays a role in degrading proteins at the translocon during ER stress (Oyadomari et al., 2006). These data suggest another function by which P58IPK contributes to ER homeostasis in cells undergoing ER stress.

10. TRANSGENIC MOUSE MODELS FOR MONITORING ER STRESS

Currently, two different transgenic mouse models exist to monitor ER stress *in vivo*. The first is the ERSE—LacZ transgenic mouse model. The transgene consists of a LacZ reporter gene driven by the rat *Gp78* promoter, which contains ERSEs (Mao et al., 2006). A control strain of mice carries a D300LacZ transgene that lacks the promoter region containing the ERSEs. By utilizing both the ERSE—LacZ and D300LacZ transgenic animals, one can identify tissues that have undergone ERSE-mediated responses to various ER stresses *in vivo* by detecting β -galactosidase activity (Mao et al., 2006).

A more recently generated model to detect ER stress *in vivo* is the “ER stress-activated indicator” (ERAI) model, which was generated by fusing Venus, a reporter gene that is a variant of green fluorescent protein, downstream of a partial sequence of human *XBPI* (Iwawaki et al., 2004). Under normal conditions, the mRNA of the fusion gene should not be spliced, which results in termination of translation at the stop codon upstream of the Venus sequence. In contrast, during ER stress, the 26 base pair intron of *XBPI* is spliced out, leading to a frame shift that permits for translation of the Venus protein, which allows for monitoring of ER stress in various tissues by the detection of fluorescence. Intraperitoneal injection of TM into the ERAI transgenic mice resulted in strong fluorescence in the kidney, consistent with previous findings that the UPR is activated in the kidney after intraperitoneal injection of TM (Iwawaki et al., 2004). The ERAI mouse model might serve as a useful tool to monitor ER stress *in vivo* during development or pathophysiological conditions; however, limitations may include an undetectable signal from weak ER stress and a lack of ERAI transgene expression in certain cell types. Moreover, this model only monitors *XBPI* splicing and is not able to give insight into PERK and ATF6 activation. Nevertheless, the Venus system, in combination with *in vivo* imaging techniques, could prove to be a powerful tool for determining the timing and tissue-specific activation of the UPR in many disease models.

11. UPR AND LIPID METABOLISM

Studies from multiple labs demonstrate a link between the UPR and lipid metabolism and homeostasis. Intraperitoneal injection of the ER stress inducing agent TM in *Atf6a*^{-/-} animals resulted in hepatic steatosis (fatty liver; Rutkowski et al., 2008; Yamamoto et al., 2010). Ultrastructural analysis of the *Atf6a* null liver revealed increased intracellular triglyceride levels and accumulation of lipid droplets. Deletion of *Atf6a* resulted in the down-regulation of key genes involved in fatty acid oxidation, gluconeogenesis, and lipogenesis (Rutkowski et al., 2008; Yamamoto et al., 2010). Moreover, genes involved in lipoprotein synthesis and transport were also suppressed. Hepatic steatosis is not unique to *Atf6* null animals, but is also found in mice with liver-specific deletion of *Ire1*, mice lacking phosphorylatable eIF2 α in the liver, and *P58ipk*-deficient animals (Rutkowski et al., 2008). Intraperitoneal injection of TM led to hepatic steatosis in *Ire1a* null and mutant eIF2 α livers, suggesting that the absence of any of the three pathways of the UPR can result in lipid deregulation in the liver. Interestingly, there was persistent CHOP expression in the *Atf6a*^{-/-} mice, *P58ipk*^{-/-} mice, and mice harboring the liver-specific deletion of *Ire1* after TM treatment, suggesting an inability to resolve ER stress (Rutkowski et al., 2008). It is possible that chronic ER stress might promote hepatic steatosis in part through CHOP. Other studies found that blocking eIF2 α phosphorylation in the liver decreased hepatic steatosis in response to a high fat diet, suggesting that eIF2 α phosphorylation leads to fatty liver disease during a high fat diet (Oyadomari et al., 2008). Lee et al. (2008) found that selective and inducible deletion of *Xbp1* in the liver resulted in decreased lipid synthesis by the liver, leading to a marked decrease in cholesterol and triglyceride production, suggesting that *XBPI* might be required for normal hepatic lipogenesis. Taken together, it appears that all three pathways of the UPR are important for regulating lipogenesis in response to chronic unresolved ER stress and physiological ER stress induced by diet.

12. UPR, HYPOXIA, AND CANCER

Cancer is an uncontrolled growth of abnormal cells, which invade and destroy healthy tissues. Cancerous cells are able to divide aggressively in a hypoxic environment, making them more likely to metastasize and more resistant to radiation. Transformed *Xbp1*^{-/-} and WT MEFs were injected into the flanks of SCID mice, which lack B and T lymphocytes and are unable to reject tumors. Remarkably, transformed *Xbp1*^{-/-} cells are unable to grow into tumors when implanted into these mice. Consistent with this finding, *Xbp1*^{-/-} MEFs are compromised in their ability to survive hypoxic conditions as compared to WT cells (Romero-Ramirez et al., 2004). These data suggest that XBP1 may be required for tumor survival during hypoxic conditions. PERK has also been implicated in tumor growth. PERK-deficient cells exhibit decreased survival as compared to WT MEFs under hypoxic conditions (Koumenis et al., 2002). WT and *Perk*^{-/-} MEFs immortalized with SV40 large/small T-antigen and transformed with the oncogene Ki-RasV12 were injected into the flanks of nude mice that have severely decreased T cells and are unable to reject tumors. *Perk*^{-/-} tumors were markedly impaired in their ability to grow *in vivo*, suggesting that PERK is also essential for tumor growth (Bi et al., 2005; Blais et al., 2006). Active ATF6 is detected during hepatocellular carcinoma; therefore, it will be of great interest to determine if ATF6 also promotes tumorigenesis through utilization of ATF6-deficient MEFs (Shuda et al., 2003).

13. UPR AND INFLAMMATORY-MEDIATED DEMYELINATION

Multiple sclerosis (MS) is a disease that affects the central nervous system and is characterized by inflammation and the extensive death of specialized cells called oligodendrocytes. Oligodendrocytes function to produce myelin that forms a protective sheath around axons, and myelin is essential for the proper functioning of the nervous system. Loss of oligodendrocytes and damage to the myelin sheath cause serious neurological disorders such as MS.

Our laboratory believes that the high secretory demand on myelinating oligodendrocytes likely makes these cells sensitive to disruptions in the secretory pathway (Lin and Popko, 2009). We have found that CNS inflammation, through targeted expression of the proinflammatory cytokine IFN- γ in the CNS of mice, leads to tremor, ataxia, oligodendrocyte death, and hypomyelination. Consistent with our hypothesis, IFN- γ treatment *in vitro* and *in vivo* results in increased levels of phosphorylated eIF2 α in oligodendrocytes (Lin et al., 2005). *Perk*^{+/-} mice with CNS inflammation exhibit reduced animal survival, increased loss of myelinating oligodendrocytes, and enhanced hypomyelination as compared to WT animals with CNS inflammation (Lin et al., 2005, 2007). These data demonstrate that the PERK/eIF2 α pathway is critical for dampening disease severity in response to enforced expression of IFN- γ in the CNS. Moreover, augmented activity of the PERK/eIF2 α pathway through inactivation of GADD34 results in protection from the effects of IFN- γ . *Gadd34*^{C/C}; *Ifn- γ* ^{CNS+} mice display a diminished loss of myelinating oligodendrocytes and less hypomyelination as compared to WT mice expressing IFN- γ in the CNS (Lin et al., 2008). Moreover, many of the *Gadd34*^{C/C}; *Ifn- γ* ^{CNS+} animals died by postnatal day 28, which correlated with enhanced medulloblastoma

formation, suggesting that GADD34 might be involved in cancer regulation. Taken together, these data suggest a function for the UPR during inflammation-mediated demyelination and oligodendrocyte loss. Future studies might shed further light on the potential contribution of the other pathways of the UPR during inflammation-mediated demyelinating disorders such as MS.

14. FUTURE CHALLENGES

As discussed above, the UPR serves to protect cells undergoing ER stress by orchestrating transcriptional and translational changes within the cell. If the UPR is unsuccessful in restoring ER homeostasis, then apoptosis is triggered. Through the genetic manipulation of mice, much progress has been made in understanding the contribution of the UPR during physiological and disease conditions; however, much still remains to be elucidated. Creation of tissue-specific mutant or knockout animals, as well as utilization of available murine models to study the UPR, will be critical in furthering our knowledge of the UPR. Current and future studies have the potential to shed further light on the effects of manipulating UPR signals, which could lead to novel therapeutic approaches to ameliorate disease through selective targeting of the distinct branches of the UPR.

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Table 6.1

UPR model systems in mouse

Gene name	Genetic models	References
<i>Atf6</i>	<i>Atf6</i> ^{-/-}	Wu et al. (2007), Yamamoto et al. (2007)
	<i>Atf6b</i> ^{-/-}	Wu et al. (2007)
<i>Ire1</i>	<i>Ire1a</i> ^{-/-}	Zhang et al. (2005)
	<i>Ire1b</i> ^{-/-}	Bertolotti et al. (2001)
<i>Xbp1</i>	<i>Xbp1</i> ^{-/-}	Reimold et al. (2000)
	<i>Xbp1</i> ^{flox/flox}	Hetz et al. (2008)
<i>Perk</i>	<i>Perk</i> ^{-/-}	Harding et al. (2001)
	<i>Perk</i> ^{flox/flox}	Zhang et al. (2002)
<i>eIF2α</i>	<i>eIF2α</i> ^{A/A}	Scheuner et al. (2001)
<i>Atf4</i>	<i>Atf4</i> ^{-/-}	Hettmann et al. (2000)
<i>Chop</i>	<i>Chop</i> ^{-/-}	Zinszner et al. (1998)
<i>Gadd34</i>	<i>Gadd34</i> ^{-/-}	Kojima et al. (2003)
	<i>Gadd34</i> ^{C/C}	Novoa et al. (2003)
<i>P58ipk</i>	<i>P58ipk</i> ^{-/-}	Ladiges et al. (2005)