The Unfolded Protein Response

The Unfolded Protein Response (UPR) is a cellular stress response related to the Endoplasmic Reticulum (ER). It is activated following an accumulation of unfolded or misfolded proteins in the lumen of the ER. The ER is a large organelle which is found in the cytoplasm of all eukaryotic cells. This extensive membranous structure is a site for lipid and sterol biosynthesis, is the main storage depot for intracellular calcium, and provides a compartment for protein folding and assembly (1, 2). The rates of protein synthesis, folding and trafficking are tightly regulated to ensure that only correctly folded proteins can exit the ER, and it is critical that a fine balance between the protein load and the folding capacity to process this load is maintained in the ER lumen. This balance can be disturbed by physiological and pathological factors such as high protein demand, viral infection and mutant protein expression, and the resulting accumulation of unfolded or misfolded proteins in the ER lumen, which leads to a condition known as ER stress, activates the UPR (3).

The ER responds to stress through a number of signalling cascades that are known collectively as the Unfolded Protein Response (UPR). The UPR performs three functions – adaptation, alarm and apoptosis. During the adaptation phase the UPR tries to re-establish folding homeostasis by inducing the expression of chaperones that enhance protein folding, and reducing translation. If this is not successful the alarm phase is activated, during which signal transduction events result in the down-regulation of pro-survival factors such as B-cell lymphoma 2 (Bcl2). Following the alarm phase, cells can undergo apoptosis (4).

In mammals three ER transmembrane proteins act as stress sensors - Inositol-Requiring kinase/EndoRNase 1 (IRE1), Pancreatic ER kinase (PKR)-like ER kinase (PERK) and Activating Transcription Factor 6 (ATF6). A major ER chaperone, Glucose-Regulated Protein 78 (GRP78), also known as Binding immunoglobulin Protein (BiP) or Heat Shock 70kDa Protein 5 (HSPA5), acts as a master regulator of all three of these proteins. Under normal conditions GRP78 binds to the luminal domains, maintaining the sensors in an inactive state. When unfolded or misfolded proteins accumulate in the ER lumen they sequester GRP78, and the dissociation of GRP78 from IRE1, PERK and ATF6 allows them to undergo conformational changes and drive the UPR (5). In addition to GRP78, other chaperone proteins include Glucose-Regulated Protein 94 (GRP94), Endoplasmic Reticulum resident Protein 57 (ERP57), Protein Disulphide Isomerase (PDI), Calnexin and Calreticulin, to name just a few (6).

Each of the three stress sensors is functionally distinct:

- IRE1 represents the most conserved branch of the UPR, and is present in organisms ranging from yeast to humans. Two isoforms of IRE1 exist– IRE1α and IRE1β. IRE1α is expressed in all cell types, and as a result has been much more extensively studied than IRE1β. The latter was originally thought to be confined to the intestinal epithelium, although a recent study has shown IRE1β also to be expressed in airway mucous cells (7). Upon sensing unfolded or misfolded proteins, IRE1α dimerises and autophosphorylates to become active. Activated IRE1α cleaves a 26-base fragment from the mRNA which encodes X-box Binding Protein 1 (XBP1), and this mRNA is then translated to give rise to the XBP1 transcription factor. XBP1 targets a wide range of genes, including those required for protein folding and secretion from the cell, as well as genes involved in the ER-Associated Degradation (ERAD) pathway

(2). ERAD is a complex process through which misfolded proteins are targeted for degradation by the ubiquitin-proteasome system. Once a protein has been selected for degradation it must be transferred across the ER membrane; ubiquitin ligases embedded in the ER membrane link recognition components in the ER lumen to the cytoplasmic ubiquitin-proteasome system (8).

- PERK, also known as Eukaryotic translation Initiation Factor 2-alpha Kinase 3 (EIF2AK3), provides another arm of the UPR. Like IRE1α, this sensor becomes active through dimerisation and autophosphorylation (9). Active PERK phosphorylates the α-subunit of eukaryotic Initiation Factor 2 (eIF2α) at Ser51, resulting in its inactivation. Inactivation of eIF2α reduces protein synthesis, and results in the preferential translation of selected mRNAs. One of these mRNAs encodes ATF4, which regulates the expression of many different genes. C/EBP homologous protein (CHOP), also known as DNA Damage-Inducible Transcript 3 protein (DDIT3), is regulated by ATF4, and is a pro-apoptotic transcription factor which is believed to exert its effects through suppressing transcription of the anti-apoptotic/pro-survival protein Bcl-2 (10,11).
- The third branch of the UPR involves ATF6 which, like IRE1, exists as two isoforms – ATF6α and ATF6β. ATF6α has been researched more comprehensively than the ATF6β isoform however both proteins are known to be ubiquitously expressed in the majority of tissues. While IRE1 and PERK remain in the ER and act via cytoplasmic effectors, ATF6α translocates to the Golgi apparatus following its release by GRP78. Here the full length (90kDa) protein is cleaved by serine protease site-1 (S1P) and metalloprotease site-2 (S2P) to release the N-terminal 50kDa transcription factor, known as ATF6N (2). This binds DNA at various ER stress response elements, including that which controls the expression of GRP78 (12).

Sustained over-activation of the UPR due to ER stress has been associated with many different disease conditions including cancer, diabetes, autoimmune conditions, liver disorders and neurodegeneration (6, 13). The role played by various individual protein components in these disorders has been subjected to a huge diversity of studies, for example:

- **Cancer** GRP78 expression is elevated in the vasculature derived from human glioma samples, suggesting a role for GRP78 in tumour angiogenesis (14).
- **Diabetes** The synthesis of insulin takes place in the ER of pancreatic β cells in response to elevated glucose levels in the blood. When the demand for insulin synthesis exceeds the capacity of the ER, the UPR would normally be initiated. In a PERK -/- mouse model however the apoptotic pathway is activated, resulting in the destruction of β cells and the development of type II diabetes. (15).
- **Autoimmunity** Intestinal secretory cells such as Paneth cells and goblet cells are particularly susceptible to ER stress, and depend upon a properly functioning UPR to maintain cellular viability and homeostasis (16). ER stress has been suggested to play a role in the development of inflammatory bowel disease (IBS) conditions such as Crohn's disease and Ulcerative Colitis. A study investigating the expression of UPR genes in colonic and ileal biopsies from IBS patients demonstrated significant activation of all three arms of the UPR in comparison to control samples (17).
- **Liver disorders** Fatty Liver Disease (FLD), commonly caused by obesity, diabetes or alcohol abuse, is characterised by the accumulation of lipids in hepatocytes, and is a condition that

can progress to cirrhosis. Affected cells have impaired protein secretion, which results in induction of the UPR. ATF6 over-expression in zebrafish hepatocytes has been shown to induce genes that increase lipid production, leading to FLD (18).

- **Neurodegeneration** Several neurodegenerative disorders are characterized by activation of the UPR, including Parkinson's disease, Alzheimer's disease and progressive retinal degeneration (19). Administration of the UPR-activating reagent tangeretin to mice has been shown to enhance the expression of a number of UPR target genes in neurons and astrocytes and to promote neuronal survival in a mouse model of Parkinson's disease (20).

The UPR is an extremely complex signal transduction pathway, and new functions are still being discovered. It is a target for therapeutic intervention in a wide variety of disease conditions, and will be the subject of considerably more research in the future. Novus Biologicals is a leading supplier of research tools for studying cellular responses to stress including the UPR. Go to www.novusbio.com and search on Unfolded Protein Response to find a list of quality products for UPR studies.

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