REVIEW

Unfolded Protein Response (UPR): Cellular control for our errors in life

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The unfolding protein response (UPR) is critical for the maintenance of cellular function under endoplasmic reticulum (ER) stress. Under stress conditions, proteins in the ER may misfold and accumulate into aggregates. To prevent aggregate accumulation, BiP is released from ATF6, IRE1 or PERK to bind to the misfolded proteins and assist in ATP mediated chaperone refolding. With the loss of BiP, ATF6 travels to the Golgi and is cleaved to a nucleus targeting form that promotes expression of UPR-responsive genes. IRE1 homodimerizes upon release of BiP to form an active RNase domain that removes an intron sequence from the XBP1 mRNA transcript. XBP1 is another promoter protein that is also responsible for UPR gene transcription. PERK homodimerizes upon BiP release and forms an active kinase domain that attenuates general translation and increases translation of specific UPR transcripts. Under prolonged UPR stimulation, IRE1 signals for proteins responsible for ER associated degradation. These proteins remove the misfolded proteins from the ER and bring them to the proteasome for degradation. If the misfolded proteins are not refolded or cleared from the ER, then the cell may be targeted for apoptosis. False UPR signals or accumulation of mutant or misfolded proteins in the ER lead to many diseases. Understanding the UPR pathway and signals that are involved may offer a template for designing novel therapeutics to help alleviate the symptoms of misfolding diseases or assist in refolding these proteins.

Introduction

The biosynthesis of proteins targeted for membranes or secreted from the cell takes place within the endoplasmic reticulum (ER). The ER is a reticular membranous network throughout the cytoplasm of eukaryotic cells that must correctly fold, assemble and glycosylate these proteins (1). Homeostasis within the ER is important for proper protein folding and stresses including perturbations in calcium homeostasis, elevated secretory protein synthesis, expression of misfolded or mutant proteins, nutrient or glucose reduction and overload of cellular cholesterol can lead to an accumulation of unfolded proteins or protein aggregates (2). To prevent the accumulation of unfolded proteins, the ER activates the unfolding protein response (UPR) system. UPR allows for an increase in the capacity for protein folding, reduction in newly translated proteins entering into the ER, increase in the degradation of misfolded proteins. If all else has failed, the UPR signals for cell apoptosis to preserve the surrounding eukaryotic cells.

The UPR system was first characterized in yeast, which provided a simple model system; however, translating this model to higher eukaryotes has shown to be difficult with more diverse signalling and a greater number of downstream responses (3). In recent years, great progress has been made in understanding the UPR in mammalian cells, much of which will be reviewed here. Understanding the UPR mechanism and proteins involved may aid in the therapeutic prevention or treatment of protein misfolding diseases.

Assistance in ER Protein Folding

The ER contains several chaperones to facilitate proper protein folding, including binding Ig protein (BiP). BiP, also know as glucose regulated protein 78 (GRP78), is a member of the heat shock 70 protein family that binds transiently to newly synthesized proteins (4, 5). BiP is also bound to the luminal domains of IRE1, ATF6 and PERK under normal conditions. However, in the event of an increase in unfolded proteins within the ER, BiP will preferentially

bind to the misfolded protein, freeing the luminal domains of these proteins to perform their functions. In mammals, two homologues of IRE1 are found: IRE1α is expressed in most cells while IRE1β is expressed in gastrointestinal epithelial cells (3). Upon the release of BiP, IRE1α/ β dimerizes to activate its cytosolic RNase domain, which cleaves a 26-nucleotide intron sequence from X-box DNA-binding protein (XBP1) mRNA (Figure 1) (5). The resulting XBP1 is a potent activator of UPR genes after it migrates to the nucleus and binds the upstream DNA UPR element (UPRE) (6). The UPR genes regulating XBP1 are essential for protein folding, maturation and degradation. In the disease hyperhomocysteinaemia, homocysteine induces apoptosis in endothelial cells by activating IRE1 (7). Homocysteine-induced cell death was suppressed with the overexpression of point mutants that inactivated the RNase domain of IRE1, suggesting that excessive homocysteine uncontrollably activates UPR though the false activation of XBP1.

Activating transcription factor 6 (ATF6) was found in mammalian cells using yeast hybrid screens as another UPR transducer that promotes UPR responsive genes. Two forms of ATF6, ATF6α and ATF6β, both reside in the ER and can each travel to the Golgi upon release of BiP (2). The 50 kDa cytosolic region of ATF6 α/β is cleaved by site-1 protease (S1P) and site-2 protease (S2P) in the Golgi compartment (8). This active cytosolic ATF6 domain migrates to the nucleus and binds to the ER stress response element (ERSE) with CCAAT-binding factor (CBF) to promote the transcription of ER-resident molecular chaperones and other assistant folding enzymes (2, 6). ATF6 may work in parallel with XBP1 to promote proteins needed to assist in the folding of unfolded proteins in the ER (5). Since the timing of activation for either the XBP1 pathway or the ATF6 pathway is unclear, it is unknown if these pathways are redundant or are used for a UPR gradient response. Further study of these pathways is required to better understand their role in mammalian cells.

Translational Arrest

The luminal domain of double stranded RNA-activated protein kinase-like ER kinase (PERK) shares homology with the luminal domain of IRE1, suggesting that either can be readily activated by BiP release (4). PERK similarly homodimerizes but transiently attenuates protein synthesis with phosphorylation of α-subunit of eukaryotic translation initiation factor 2 (eIF2α) (Figure 2).

When eIF2α is phosphorylated, it can no longer exchange GDP for GTP in the eIF2-GDP-eIF2α complex, which prevents initiation events on almost all cellular mRNAs within the cell (2, 4). Since eIFα is required to bring the initiator methionyl-transfer RNA (Met-tRNAi) to the 40S ribosome, the absence of free eIF2 will prevent the 40S and 60S ribosomal subunits from forming the 80S initiation complex for translation (2, 4). However, selective mRNAs can be preferentially translated with eIF2α phosphorylated, provided that there are open reading frames within the 5' untranslated region of the mRNA (2). Activating transcription factor 4 (ATF4) is transcribed in unstressed cells, but is poorly translated until eIF2α is phosphorylated. ATF4 activates genes involved in amino acid

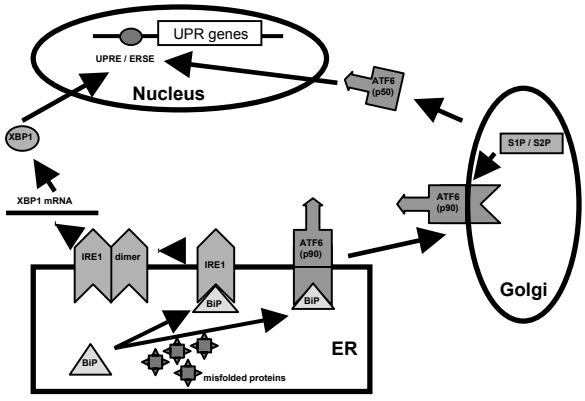

Figure 1: Activation of UPR genes by XBP1 and ATF6 with UPR initiation. IRE1 dimerizes to form an active RNase domain with loss of bound BiP. ATF6 then transports to the Golgi and is cleaved to a 50 kDa active domain after BiP loss.

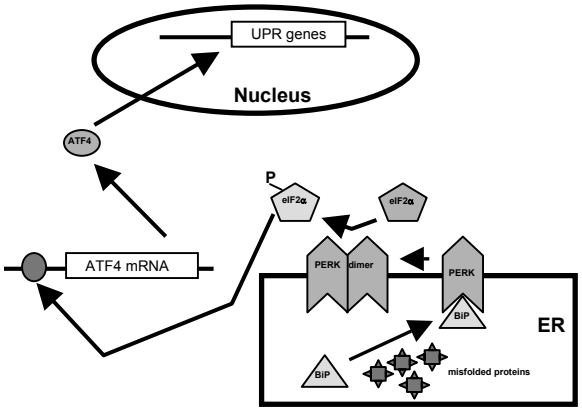

Figure 2: Translational arrest mediated by PERK through phosphorylation of eIF2α.

metabolism and transport, oxidative-reduction reactions and ER stress-induced apoptosis (5). ATF4 has been shown to bind to a C/EBP-ATF composite site and regulate CHOP (C/EBP homologous transcription factor) transcription (9). CHOP is implicated in both growth arrest and cellular apoptosis; with prolonged UPR activation ATF4 can induce expression of CHOP leading to caspase-3 activation and cell death (9).

Alternatively under prolonged UPR, ER calcium homeostasis is significantly disrupted, leading to activation of caspase-12. Caspase-12 is an ER-associated effector of caspase activity that leas to cellular apoptosis though the activation of caspases 9 and 3 (5). In G_{M1}-gangliosidosis, G_{M1}-ganglioside accumulates in the ER and leads to loss in ER calcium. This depletion signals activation of UPR in these cells resulting, in prolonged activation of UPR genes and UPR signalled apoptosis (10). Thus, to allow the upregulated chaperones to fold misfolded proteins in the ER, the UPR stimulates a decrease in newly translated mRNA transcripts. However, if the cell is delayed significantly in translation, then apoptosis will be signalled to protect the neighbouring cells.

Degradation of Misfolded Proteins

Before a cell commits itself to apoptosis through prolonged UPR activation, it coordinates with the ER associated degradation (ERAD) system to degrade misfolded proteins (11). As mentioned earlier, IRE1 is responsible for signalling the transcription of proteins required in ERAD and mutations or loss of IRE1 results in defective degradation (6). One such protein is EDEM (ER degradation-enhancing α-mannosidase-like protein), which is a membrane protein that interacts with calnexin (CNX) to help retrotranslocate misfolded proteins for degradation by the proteasome (Figure 3) (2, 6, 12).

Misfolded proteins targeted for degradation are marked with N-linked oligosaccharide mannose-N-acetylglucosamine ($Man_8GlcNAc_2$) isomer B. EDEM associated with CNX recognizes this modification and delivers the misfolded protein to the Sec61 channel for retrotranslocation (12). The mechanism of unfolding misfolded proteins remains unclear; however, once the polypeptide exits the ER, it undergoes ubiquitylation and is degraded by the proteasome. Proteins that are unable to be unfolded and accumulate in the ER lead to cellular apoptosis and some disease phenotypes. In liver disease with deficiency in α 1-antitrypsin (A1AT), the mutant Z form occurs in the majority of these individuals. The accumulation of mutant Z A1AT protein occurs in the ER, resulting in aggregation and over-stimulation of the UPR system, resulting in apoptosis (13). Also, proteins that are foreign to a cell may not be folded properly leading to disease. $FrCas^E$ is a mouse retrovirus that causes fatal noninflammatory spongiform neurodegenerative disease with the accumulation of misfolded viral envelope protein in the ER of brain stem cells (14). As a result of UPR-induced cell death, the disease results in a pathology similar to that of transmissible spongiform encephalopathy.

These diseases signify the importance of the cellular ERAD system that attempts to degrade misfolded proteins instead of just initiating apoptosis. If ERAD could be assisted in the removal of these proteins, then these diseases could be treated.

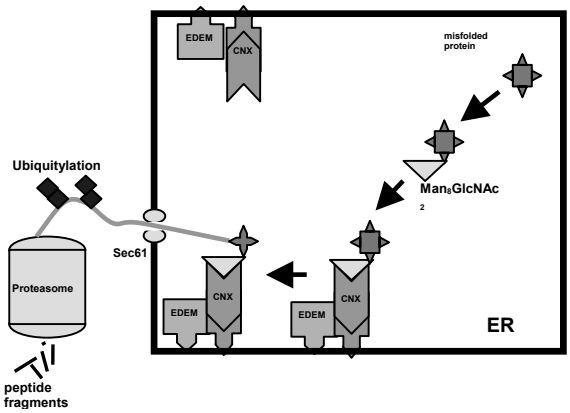

Figure 3: ER-associated degradation with misfolded protein marked with oligosaccharide that is recognized by calnexin (CNX) and EDEM for retrotranslocation and degradation by proteasome.

Concluding Remarks

Understanding the UPR delineates a signal transduction cascade that can be utilized for clearing misfolded proteins in the ER and is very valuable for learning about misfolding diseases. The timing and coordination of these components in this cascade appears to be relatively controversial. One possible cascade includes the release of BiP from ATF6 in the presence of misfolded proteins within the ER. ATF6 then signals the transcription of additional UPR components that may include XBP1 mRNA (5). At this point, IRE1 and PERK would have homodimerized with a maintained stress signal. PERK, with its similarity to IRE1, may be activated at the same time and allow for translational arrest while the initial ATF6-translated UPR genes attempted to refold any misfolded proteins. With prolonged IRE1 signalling, transcription of associated degradation proteins for the removal of the misfolded protein from the ER would be initiated. If degradation failed or required too much energy under certain stress conditions, then it is possible that the cell is targeted for apoptosis through either prolonged ATF4 expression or ER calcium loss. This possible progression is highly debated, as it is still unclear if ATF6 lies up- or down-stream of XBP1 (5).

Understanding the UPR pathway would allow for protein targeting to regulate the severity of a disease and to allow adequate time for attempts at protein refolding before prematurely activating apoptosis. This concept has been observed in leukodystrophy Pelizaeus-Merzbacher disease (PMD), a neurodegenerative disease that causes diffuse hypomylelination of the central nervous system and is characterized by splice site mutations, duplications of the PLP1 gene or null alleles. The severity of PMD is graded based on strong or weak UPR signalling and the activation of CHOP, leading to either cell survival (mild disease) or apoptosis (severe disease) (15). If the mechanistic protein recruitment sequence were known, then this disease, and many others, could be controlled by inhibiting these proteins to decrease the propensity for strong UPR activation and allow for the cell to survive. Pharmaceutics for misfolding diseases that result in irremovable misfolding aggregates could be designed to initiate the UPR system earlier to assist in the prevention of large aggregates that may eventually lead to apoptosis.

Future research in this field does not only need to determine the stepwise mechanism of the UPR response, but also the fundamental events that occur during protein refolding. There appears to be a gap in the knowledge between how the transcribed chaperones and proteins from the initiation of UPR act to refold detected misfolded proteins. With this understanding in hand, pharmaceutical interventions can be developed for earlier detection and stabilization of these proteins to prevent cell loss through apoptosis. This appears to be the logic behind designing chemical chaperones as specific small molecule drugs to help stabilize specific unfolded proteins in a disease condition. To advance this new pharmaceutical technology,the fundamentals behind UPR will need to be further examined.

Abbreviations:

ATF4, Activating transcription factor 4; ATF6, Activating transcription factor 6; BiP, Binding Ig protein; CBF, CCAAT-binding factor; CHOP, C/EBP homologous transcription factor; CNX, calnexin; EDEM, ER degradation-enhancing α-manosidase-like protein; eIF2α, eukaryotic translation initiation factor 2 α -subunit; ER, endoplasmic reticulum; ERAD, ER associated degradation; IRE1, inositol-requiring enzyme; PERK, protein kinase-like ER kinase; PMD, Pelizaeus-Merzbacher disease; S1P, site-1 protease; S2P, site-2 protease; UPR, unfolding protein response; UPRE, unfolding protein response element; XBP1, X-box DNA-binding protein.

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