The Unfolded Protein Response

By Annika Vuppala

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Introduction

The Unfolded Protein Response (UPR) is one of the many important processes carried out in a cell. It occurs in the Endoplasmic Reticulum, a network of membrane tubes attached to the nucleus. It is not only the protein maker of the cell, but also has many other important functions such as lipid synthesis and transportation. UPR is concerned with unfolded or misfolded proteins accumulating in the ER, which could become toxic and lead to mutations, diseases, and tumors. There are three main branches of UPR: IRE1α-XBP1, PERK-eIF2α-ATF4-CHOP, and ATF6.

Other processes and responses that will be repeatedly mentioned in the following sections include Apoptosis, the Integrated Stress Response, and the ER-Associated Degradation. Apoptosis is programmed cell death, and is triggered when external or internal factors determine that the cell has to self destruct. Apoptosis is different from Necrosis which is cell death by injury. The Integrated Stress Response (ISR) is a stress response which responds to all kinds of stress in the cell, including ones caused by unfolded proteins. Lastly, ER-Associated Degradation is a pathway that targets misfolded proteins in the ER for ubiquitination and subsequent degradation as they could become toxic and pose issues towards maintaining homeostasis in the cell.

IRE1α: Protein Portfolio

IRE1α, Inositol Requiring Kinase 1α, is a protein kinase and an endoribonuclease. It is an ER stress sensor and the central mediator of the IRE1α-XBP1 pathway. It is also a Type 1 Transmembrane protein, meaning that it is partly in the ER Lumen and partly in the cytosol and is anchored to the ER membrane with a stop-transfer anchor sequence. A stop-transfer anchor sequence is a hydrophobic sequence in the protein which can trigger the opening of a pore in the ER membrane. The protein can then slide into the pore with it's N-Terminal targeted toward the lumen during protein synthesis. This is because it has its luminal domain on the N-Terminal. The luminal domain acts as a key sensor for the activation of the IRE1α-XBP1 pathway and UPR. IRE1α also has a C-Terminal Endoribonuclease domain, and it is used for the splicing of XPB1. It also has a serine-threonine kinase domain in its cytosolic portion (C-Terminal) which is used to trans-autophosphorylate itself. Both of these topics will be covered in the IRE1α-XBP1 Pathway section of this paper.

In unstressed cells, BiP is bound to IRE1α through its nucleotide-binding domain (NBD). It is released upon misfolded/unfolded proteins binding to its substrate-binding domain (SBD). While there isn't much evidence, it is suggested that BiP also inhibits IRE1α's UPR signaling. It has also been shown that in vitro, there is a peptide binding

groove on the luminal domain of IRE1α. This could be used for misfolded proteins to bind directly to IRE1 to induce dimerization and eliminate the need for BiP. The only problem is that this binding groove isn't solvent accessible, making it almost useless. A mutant form of IRE1α isn't able to bind to BiP, but can dimerize and autophosphorylate without any ER stress. This suggests that IRE1α can be released from BiP, but doesn't need to be bonded to a misfolded peptide to be activated. Obviously this information is all just experimental and these mutations don't pose immediate issues.

As a protein kinase, IRE1α integrates UPR with other inflammatory responses. The trans-autophosphorylation of IRE1α's cytosolic domain causes it to interact with TRAF2 (TNF-Response Associated Factor 2). TRAF2 interacts with other TNF receptors and forms complexes with other TRAF proteins. It is required for and strengthens TNF induced apoptosis. TNF (Tumor Necrosis Factor) is an inflammatory cytokine produced during acute inflammation and can start a pathway leading to apoptosis.

This IRE1α-TRAF2 complex then recruits IKK which leads to the degradation of IκBα which then leads to the upregulation of the NF-κB inflammatory pathways. If that sounds complicated, that's because it is. First, the IRE1α-TRAF2 complex recruits IKK (IκB Kinase Enzyme Complex) which is a serine/threonine kinase and is part of the upstream NF-κB inflammatory pathway. An upstream signaling pathway is when the pathway is triggered by the binding of a signaling molecule to a receptor. IKK phosphorylates IκBα which then leads to its degradation. IκBα (Inhibitor of NF-κB Kinase Complex) is the master regulator of the NF-κB Pathway, as it inhibits NF-κB, and masks its Nuclear Localization Signals (NLS) so it stays inactive. So, with the degradation of IκBα, you have effectively activated the NF-κB Pathway. NF-κB (Nuclear Factor κB) is part of one of the major signaling pathways activated when cells are exposed to inflammatory stimuli like TNF, UV radiation, stress, etc. Once this whole signaling transduction is complete, NF-κB's NLS are unblocked, allowing it to travel to the nucleus and activate its target genes. Its target genes regulate the production of inflammatory cytokines, chemokines, and adhesion molecules. It also regulates cell proliferation, apoptosis, and differentiation.

The IRE1α-TRAF2 complex also recruits ASK1 in another pathway to stimulate inflammatory responses so buckle up, it's gonna be a long ride… ASK1 (Mitogen Activated Protein Kinase Kinase Kinase 5, also known as MAP3K5) is a serine/threonine kinase and is part of multiple signal transduction pathways including apoptosis signal transduction through caspase activation, signal transduction through various stressors and receptor mediated inflammatory signals, the p38 pathway, and lastly the JNK pathway. It activates the p38 and JNK pathways by phosphorylating MAP2K4 and MAP2K7. Both of these proteins are serine/threonine kinases as well as tyrosine kinases. These two proteins then phosphorylate JNK1 (Mitogen Activated Protein Kinase 8) which, like everyone else, is a serine/threonine kinase. It

phosphorylates and binds to the components of the AP1 complex transcription factor. JNK1 isoforms express different binding patterns, for example the β-1 isoform preferentially binds to c-Jun, while α-1, α-2, and β-2 have a similar level of binding to c-Jun as to ATF2.

Backtracking a little, the p38 subfamily of MAPK proteins is associated with phosphorylating c-Fos, the other main part of AP1. Specifically, p38α and β also known as MAPK14 and MAPK11 respectively. So now, with the activated c-Jun and c-Fos, they form the complex known as AP1. AP1 (Activator Protein 1) is a heterodimer composed of c-Jun and c-Fos and is a leucine zipper. It binds specifically to the DNA motif *5'-TGAG/CTCA-3'* which is a palindromic DNA sequence present in the promoter region of target genes. A palindromic gene sequence is when you read the gene sequence on one strand, it matches the sequence reading in the opposite direction on the other strand. AP1 regulates proteins involved in cellular differentiation, apoptosis, and cell growth and proliferation. There is a very long list of possible target genes activated by AP1, the full list is here -> AP1 Target Genes This protein is also linked to cancer as members of the Jun and Fos families are homologs of viral oncogenes. c-Fos is also linked specifically to cancer as it is a proto-oncogene, meaning that it is a gene that, when altered by mutation, becomes an oncogene. It has been identified as an independent predictor of decreased survival of breast cancer.

A IRE1α-JNK complex might activate pro-apoptotic pathways indirectly by inducing insulin resistance by phosphorylating IRS1 and 2 (Insulin Resistance Substrate Proteins). They are adaptor proteins, which contain a variety of protein binding modules that link protein binding partners together and facilitate the synthesis of larger signaling complexes. Insulin suppresses apoptosis as was found in a study which noted that insulin suppresses the accumulation of ROS which would lead to the activation of caspase-3. ROS (reactive oxygen species) are chemical molecules, not proteins, which contain a lot of oxygen and easily react with other molecules in a cell. A build up of ROS can cause damage to DNA, RNA, and proteins. Caspases are a family of proteases (enzymes that catalyze the breakdown of proteins) which play essential roles in apoptosis. Precisely, caspase-3 is an effector caspase which is essential to the initiation and execution of apoptosis.

XBP1: Protein Portfolio

XBP1, X-Box Binding Protein 1, is a transcription factor during ER stress and helps mediate the IRE1α-XBP1 branch of UPR in a PIK3R and p38 MAPK dependent manner. p38 MAPK's are a subfamily of Mitogen Activated Protein Kinases which are specifically activated by the phosphorylation of a Thy-Gly-Thy motif in their activation loops. PIK3R's, Phosphatidylinositol 3-Kinase Regulatory Proteins, are a subfamily of the Phosphatidylinositol 3-Kinase family and bind to activated tyrosine kinases through their SH2 domain. It contains a Basic Leucine Zipper (bZIP) as it is a transcription

factor, and activates the target genes of the IRE1α-XBP1 Pathway. It also has two different isoforms.

Isoform 1 plays a role in the production of XBP1 Isoform 2, more of which will be talked about in the IRE1α-XBP1 Pathway section. Isoform 2 is a big part of this branch of UPR, as it is the transcription factor that travels to the nucleus to activate the target genes of this pathway. It also plays a role as an oncogene since it promotes tumor progression. This is because it stimulates Zinc Finger Protein SNAI1 transcription, which then induces the Epithelial-to-Mesenchymal Transition along with the cell migration and invasion of breast cancer cells. The epithelial-to-mesenchymal transition (EMT) is when epithelial cells (cells that form surfaces like skin, organs, etc) lose their polarity and cell to cell adhesion, which makes them gain migratory and invasive properties turning them into mesenchymal stem cells (cells typically found in bone marrow that can differentiate into many different cell types). This has been shown to occur in the initiation of metastasis in cancer progression. Keep in mind that this doesn't have anything to do with UPR, but is just background on the proteins involved.

<u>IRE1α-XBP1 Pathway</u>

The IRE1α-XBP1 is the most important branch of UPR. It starts in the Endoplasmic Reticulum where BiP is bound to IRE1 and is effectively inhibiting its UPR signaling. During ER stress, many misfolded and unfolded proteins accumulate in the ER Lumen, which causes BiP to bind to a misfolded substrate through its SBD. BiP chaperones misfolded or unfolded proteins so they don't oligomerize and will be prepared for folding. BiP leaving IRE1 causes it to trans-autophosphorylize itself with its serine-threonine kinase domain which leads to the activation of its endoribonuclease domain. Once the RNase domain is activated, it catalyzes the removal of a 26-base intron from the XBP1 mRNA. XBP1 Isoform 1 facilitates this process and plays a role in XBP1 Isoform 2 production. The emerging polypeptide chain (Isoform 1) is part of a mRNA-ribosome-nascent chain complex and simultaneously recruits its own unprocessed mRNA through temporary docking to the ER and translational pausing. With that, XBP1 Isoform 1 facilitates IRE1-mediated XBP1 Isoform 2 production.

Sidestepping a little, the splicing of mRNA also results in a translational frameshift of the mRNA, which produces 41 kDa CREB. A translational frameshift (aka ribosomal frameshift) is a really cool concept that allows for multiple, different proteins to be produced from the same mRNA. You can change the ribosomal reading frame either right or left, so the codons are different therefore giving you different amino acids and a different protein. Sometimes a frameshift will result in a string of amino acids that are useless, but every once in a while you can get a new protein. CREB, the cAMP Response Element-Binding Protein, is a transcription factor that contains a bZIP and binds to the cAMP response elements in the promoter regions of it's target genes.

CREB has been found to regulate around 4000 target genes in the human genome, all with diverse functions.

Going back to the main part of the IRE1α-XBP1 Pathway, as a transcription factor, XBP1 has many target genes. It binds to the UPR-Responsive Element (UPRE) and ER-Stress Response Elements I and II (ERSE-I and ERSE-II) in the promoter region of target genes. UPRE is a cis-acting transcription element, and may control the expression of ERAD components that can degrade misfolded proteins in the ER. It can be fully activated by XBP1, even without ATF6 present. ERSE-I is also a cis-acting transcription element which controls the expression of chaperones that are localized in the ER which can refold unfolded proteins (like BiP). ERSE-II is a cis-acting transcription element which mediates the ATF6-dependent branch of UPR. These target genes all produce DnaJ/Hsp40 related proteins, as those are the proteins which help dampen ER stress with protein folding and chaperoning functions.

The first target gene produces p58IPK (58 kDa Protein Kinase Inhibitor) which prevents the phosphorylation of eIF2α (at serine 52), therefore reducing protein synthesis. It also is a co-chaperone for HSPA8 and stimulates its ATPase activity. More about this protein and its job will come in the PERK-eIF2α-ATF4-CHOP Pathway section.

The next target gene produces ERdj4 (ER Localized DNAJ 4) which is a co-chaperone for BiP and plays a role in ER Associated Degradation (ERAD). It induces high affinity substrate binding by interacting with the NBD on BiP and facilitates the folding or degradation of misfolded substrates. Affinity with substrates is just how strong the bond between the enzyme and the substrate is, so high affinity substrate binding is just a strong bond between enzyme and substrate. It also stimulates BiP's ATPase activity. It has an HPD (His-Pro-Asp) motif in its J domain which allows it to bind to BiP. It interacts with it's misfolded substrates through a C-terminal glycine/phenylalanine-rich region.

ERdj3 (ER localized DNAJ 3), also known as DnaJB11, is a co-chaperone for BiP and is required for the proper folding, trafficking, or degradation of proteins. It binds directly to unfolded proteins that are substrates for ERAD, but disassociate from the BiP-unfolded protein complex before the folding is completed. It also stimulates ATPase activity for BiP.

EDEM (ER Degradation-Enhancing α-Mannosidase-like Proteins), specifically extracts misfolded glycoproteins and targets them for ERAD in an N-glycan dependant manner. It catalyzes mannose trimming either from Man8GlcNAc2 (N-glycan) to Man7GlcNAc2 (N-glycan) in the case of EDEM1 and 3, or it catalyzes mannose trimming from Man9GlcNAc2 (N-glycan) to Man8GlcNAc2 (N-glycan) in the case of EDEM2. α-Mannosidase is an enzyme involved in the cleaving of the α form of mannose. A glycoprotein is a protein that has oligosaccharides attached to some of its amino acids' side chains. An N-glycan is an oligosaccharide that forms a glycosidic

bond with asparagine (an amino acid) in an Asn-X-Ser/Thr sequon. The specific N-glycans EDEM deals with regulate immunity and neural cell migration. The more branched they become is an indication that the cell might be cancerous, as this extra branching helps facilitate cancer progression.

PDIA6 (Protein Disulfide Isomerase A6) is a chaperone that inhibits the aggregation of misfolded proteins in the ER. As the build up of misfolded/unfolded proteins in the ER increases, sometimes they might clump together causing them to become toxic. It also negatively regulates (inactivates their signaling) UPR sensors like IRE1α and PERK.

RAMP4 (Ribosome-Associated Membrane Protein 4), also known as SERP1, protects unfolded proteins from degrading during translocation into the ER Lumen. After ER stress has ended, it may facilitate glycosylation by modulating the use of N-Glycosylation sites on its target proteins. Glycosylation is the attachment of a carbohydrate to an amino acid side chain. N-Glycosylation is attachment of an N-glycan (an oligosaccharide that attaches to nitrogen) to a nitrogen atom on the amino acid, typically the nitrogen on asparagine residues.

PERK: Protein Portfolio

PERK, Protein Kinase R (PKR)-like Endoplasmic Reticulum Kinase, is also known as Eukaryotic Translation Initiation Factor 2α Kinase 3 (EIF2AK3). It is a serine/threonine kinase and also a Type 1 Transmembrane Protein meaning that it passes through the ER once with part of it inside the lumen of the ER and the other half in the cytosol. The structure of PERK's luminal domain is very similar to that of IRE1α and has the same binding functions. Once bound to a misfolded or unfolded protein, it phosphorylates eIF2α to continue the pathway.

eIF2α: Protein Portfolio

eIF2α, Eukaryotic Translation Factor 2α, is a key player not only in the PERK pathway but also in all of UPR and other apoptotic pathways. In a non-stress situation, this protein mediates the binding of tRNA to the ribosome for the process of translating the mRNA. To do this, it forms a complex with GTP (guanosine triphosphate) and the initiator tRNA to start the translation. GTP is an energy rich molecule, like ATP, that powers chemical reactions in your cells. When these molecules are hydrolysed, the free energy from the hydrolysis powers reactions between proteins and other molecules that otherwise wouldn't have been possible. The initiator tRNA plays a key role in recognizing the start codon on the mRNA. This eIF2α-GTP-tRNA complex then binds to the small and large ribosomal subunits to form the initiation complex and then begins translation. Once the GTP hydrolyses and provides the energy for the reaction, you are left with a eIF2α-GDP complex which is completely useless. This is where eIF2B, or Eukaryotic Translation Factor 2β, comes into play. It is the GEF (guanine exchange

factor) for eIF2α, meaning that it exchanges a GDP for a GTP making another chemical reaction possible.

ATF4: Protein Portfolio

ATF4, Activating Transcription Factor 4, otherwise known as cAMP Dependent Transcription Factor 4 or cAMP Response Element Binding Protein 2 (CREB2).

ATF4 is produced in response to ER stress signals and the phosphorylation of eIF2α. It promotes the production of proteins involved in amino acid metabolic processes, mRNA translation, and UPR. Essentially, it is the master transcription factor of stress responsive genes that promote cell recovery and homeostasis. It binds to the cAMP response elements (CRE) in the promoter regions of its target genes. cAMP, or Cyclic Adenosine Monophosphate, is a derivative of ATP and a second messenger in many signaling transduction pathways. Second messengers are intracellular signals released by the cell in response to extracellular signals (the first messengers) and normally trigger physiological changes in the cell like differentiation, apoptosis, proliferation, etc. The cAMP response elements contain the sequence *5'-TGACGTCA-3'* and are found in the promoter regions. They are present in approximately 750,000 genes in the human genome, but a lot of these genes are not accessible as they are blocked by cytosine methylation. This is when a methyl group is added to gene sequence, obstructing proteins from binding to it.

PERK-eIF2α-ATF4-CHOP Pathway

This pathway is an incredibly complex branch of UPR and is connected to many other inflammatory and pro-apoptotic pathways and processes in the cell. Its main job is to stop the translation and transcription of proteins so that the IRE1α-XBP1 pathway can do its job without extra stress being piled on. This pathway is also concerned with promoting pro-apoptotic factors and ultimately triggers apoptosis if needed.

Upon ER stress, BiP releases from PERK (as it does in the IRE1α pathway), which makes PERK oligomerize and trans-autophosphorylate. The phospho-PERK will now phosphorylate serine 51 on eIF2α, causing it to become a global translation inhibitor and therefore reduce the ER protein folding load. There are also other kinases with the job of phosphorylating eIF2α, which vary depending on the type of stress they are responding to. One of these proteins is PKR, Protein Kinase R, is a serine/threonine and tyrosine kinase that responds to viral infections, ER stress, and nutrient signals. It also plays a big role in the innate response to viral infections because it is the protein which activates eIF2-α, which results in the shutdown of cellular protein synthesis, and therefore viral protein synthesis, making it hard for the virus to spread and infect more cells. PKR also upregulates ISR target genes as part of fighting the viruses. It can also regulate inflammatory pathways such as MAPK and NF-κB pathways, as well as insulin signaling through kinase and adaptor protein activity. Finally, PKR regulates the

synthesis of transcription factors that express pro-inflammatory cytokines which are vital to inflammatory and pro-apoptotic pathways.

The second protein that activates eIF2α is a serine/threonine kinase known as GCN2, General Control Non-Depressible 2, which is also known as eIF2α Kinase 4. It responds to amino acid starvation and UV radiation. Activating the global translation inhibitor results in the reduced use of amino acids because mRNA isn't being translated. Along with this, GCN2 upregulates ATF4 which allows ATF4-mediated reprogramming of amino acid biosynthetic gene expression to alleviate nutrient depletion. In other words, by reprogramming the chemical reactions and pathways resulting in the formation of amino acids you can alleviate nutrient depletion. Like PKR, GCN2 can activate and regulate inflammatory pathways such as MAPK and NF-κB.

Thirdly, HRI or Heme-Regulated Inhibitor Kinase, also known as eIF2α Kinase 4, responds to heme deficiency. Heme is an iron-containing compound that forms the non-protein part of hemoglobin. Heme is necessary to bind to oxygen in the bloodstream and is produced in the bone marrow and liver. Hemoglobin is a vital oxygen-transport metalloprotein in all the blood cells in your whole body, and is also almost in every single living vertebrae. It carries oxygen from the lungs to the rest of your body. Because of heme deficiency, HRI also responds to oxidative stress. HRI is a sensor of heme deficiency and binds to hemin to detect it. Hemin is a small iron containing molecule with chlorine that can be derived from heme. HRI also responds to osmotic stress, which happens when there is a sudden change in the solution around the cell, mitochondrial dysfunction, and heat shock.

But stopping all cap-dependent translation of proteins is a big deal, so even when eIF2α becomes a global translation inhibitor, it doesn't last very long because of GADD34. GADD34 dephosphorylates eIF2α and therefore starts up translation again. While stopping protein synthesis is important to allow the ER to destress, in order to adapt to and address the problems causing the ER stress, whether extracellular or intracellular, you need to be able to make proteins. You need those adaptive functions to continue protein synthesizing and folding without problems, but you need to start translation in order to achieve that. GADD34 also induces the activation of TP53, Cellular Tumor Antigen p53, which acts as a tumor suppressor and can activate apoptosis and growth arrest under certain conditions. TP53’s induction of apoptosis is mediated by the expression of the antigens BAX and FAS. Both of these proteins take part in the host-virus interaction and can induce apoptosis by triggering the release of Cytochrome C from the mitochondria. Cytochrome C is a small hemeprotein that is a key participant in ATP synthesis, and when released from the mitochondria into the cytosol, can trigger apoptosis. This host-virus interaction related pathway is intertwined with the inflammatory Caspase pathway and uses both standpoints to start apoptosis.

Phospho-eIF2α also induces the transcription of anti-apoptotic cellular inhibitors such as XIAP, cIAP1, and cIAP2. XIAP, X-Linked Inhibitor of Apoptosis Protein,

regulates caspase activity, copper homeostasis, MAPK signaling, apoptosis, other inflammatory pathways, cell proliferation, cell invasion, and metastasis. This protein acts as a direct caspase inhibitor by binding to the active site of Caspase 3 and Caspase 7 and therefore obstructing substrate entry. XIAP can also inactivate Caspase 9. It regulates NF-κB signaling by being an E3 Ubiquitin Protein Ligase. This means that it recruits the E2 Ubiquitin Conjugating Enzyme that has already binded to Ubiquitin and catalyzes the transfer of the ubiquitin from the E2 enzyme to the substrate. Ubiquitin is a small regulatory protein that is present in almost all tissues of eukaryotic organisms, hence the name being derived from the word ubiquitous. It tags defective/misfolded proteins for destruction in the ER. Moreover, XIAP can regulate the innate immune system as it can regulate NLRs. NLRs are intracellular sensors of Pathogen Associated Molecular Patterns (PAMP) that enter the cell via phagocytosis or pores in the cell. When they see the PAMPs, they can activate the NF-κB pathway. Lastly, XIAP protects cells from the spontaneous formation of ripoptosomes by ubiquinating RIPK1 and Caspase 8. RIPK1, Receptor Interaction Protein Kinase 1, is a key regulator of TNF-initiated apoptosis and once binded to a substrate, can activate the NF-κB pathway. Ripoptosomes are large multi-protein complexes that can activate apoptosis and necrosis. They have the ability to kill cancer cells in both a caspase-dependent and caspase-independent manner. They can spontaneously form after the depletion of IAPs, Inhibitors of Apoptosis, because of cellular stress or external factors.

cIAP1 and 2, Cellular Inhibitors of Apoptosis 1 and 2, are also E3 Ubiquitin ligases and ubiquitinate NF-κB in order to stop apoptosis. They also suppress substrates of NF-κB in order to promote cell survival. The target proteins for their E3 Ubiquitinase activity are RIPKs, Caspases 3, 7, and 8, TRAF2, and some members of the MAPK family. They not only regulate caspase and apoptosis, but also other inflammatory signaling, immunity, cell proliferation, and metastasis among other things. cIAP1 and 2 also help stop the spontaneous formation of ripoptosomes. In short, XIAP and cIAP1 and 2 are all very similar and therefore there are a lot of similarities in their functions. I think that IAPs and ripoptosomes could both be key players in the search for finding new ways to get rid of cancer cells.

The last thing phospho-eIF2α does is induce the transcription of ATF4, and thereby carries on the pathway. Like I said previously, ATF4 is essentially the master transcription factor of stress responsive genes that promote cell recovery and homeostasis. It induces the transcription of ER chaperones like BiP and Endoplasmin. BiP, Binding Immunoglobulin, has been mentioned many times before and is a key player in all goings on in the ER. It binds new proteins as they are moved into the ER, and maintains misfolded ones until they can be refolded or discarded. Endoplasmin is also a very important ER chaperone that processes and transports secreted proteins into the ER. It also takes part in ERAD (ER Associated Degradation).

Additionally, ATF4 induces the transcription of UPR associated transcription factors (like XBP1 and CHOP) and some intracellular trafficking machinery for ER-to-Golgi transport of ATF6. ATF6 is like a little mini branch of its own in UPR, so it will be covered later. Briefly, it is a transcription factor that is released from the ER membrane and translocates the nucleus to activate UPR target genes.

ATF4 induces a lot of different processes that also promote cellular homeostasis including amino acid biosynthesis and function, anti-oxidative stress responses, and lastly, it stimulates the expression of autophagy genes. Autophagy is the process of removing harmful or disfunctional parts of the cell to promote the growth and regeneration of a healthier cell. The autophagy genes include MAP1LC3B, ATG12, and BECN1. MAP1LC3B, or Autophagy Related Protein LC3 B, is an ubiquitin-like modifier and is involved in the formation of autophagosomes. Autophagosomes are like containers for the victims of autophagy which then bind to lysosomes so the contents can be disposed of. The process in which a lysosome does this is really cool, because it basically decreases the pH of the interior drastically so it is very acidic and can destruct the contents without spreading the harmful acid throughout the rest of the cell. MAP1LC3B participates in the 'remodeling' of the ER due to nutrient stress and fuses with lysosomes to get rid of the parts that are not needed. MAP1LC3B also contributes to the regulation of the amount of mitochondria in a cell, and eliminates mitochondria so the cell can still reach energy requirements without producing too many ROS's and causing oxidative stress.

ATG12, Autophagy Related Protein 12, is an ubiquitin-like protein that is involved in autophagy vesicle formation, which are also known as autophagosomes. It forms a complex with ATG5, Autophagy Related Protein 5. This complex acts like an E3 Ubiquitin ligase, a protein that recruits the E2 conjugating enzyme so it can catalyze the transfer of ubiquitin from the enzyme to the substrate. This ATG12-ATG5 complex can be pro-viral because it negatively regulates the innate antiviral immune response. ATG5 is also crucial to the development, proliferation, and survival of B and T lymphocytes.

BECN1, Beclin-1, plays a central role in autophagy as it is involved in the initiation and maturation of autophagosomes. BECN1 also takes part in endocytosis, the process of bringing large molecules into the cell by forming a vacuole around it with the cell membrane. It does this by being a core subunit of the PI3K complex that mediates the formation of Phosphatidylinositol 3-Phosphate. Phosphatidylinositol 3-Phosphate is an abundant phospholipid found in the cell membrane. They act as receptors and recruit a range of proteins, mostly related to protein trafficking, to the cell membrane. The PI3K Family, Phosphatidylinositol-3 Kinase Family, go hand in hand with this phospholipid. A PI3K's main job is to phosphorylate and activate the phospholipids, and then interact with the proteins brought in from the extracellular matrix. They are involved in a host of things, including cell growth, proliferation, motility (the ability to move), differentiation, survival, and intracellular trafficking.

Lastly, ATF4 promotes bone related processes like endochondral ossification. This is the process of building cartilage and then systematically replacing the cartilage with bone until there is no more cartilage left and you have a fully matured bone. ATF4 achieves this by mediating the proliferation and differentiation of chondrocytes, cells that secrete cartilage and then become embedded in it. Then, ATF4 drives the production of osteoblasts, cells that form new bone. It controls osteoblast functions like bone formation, the production of the extracellular matrix, and the bioactivity of osteocalcin. Osteocalcin, a protein hormone that constitutes 1-2% of total bone protein. It is found in bone and dentin.

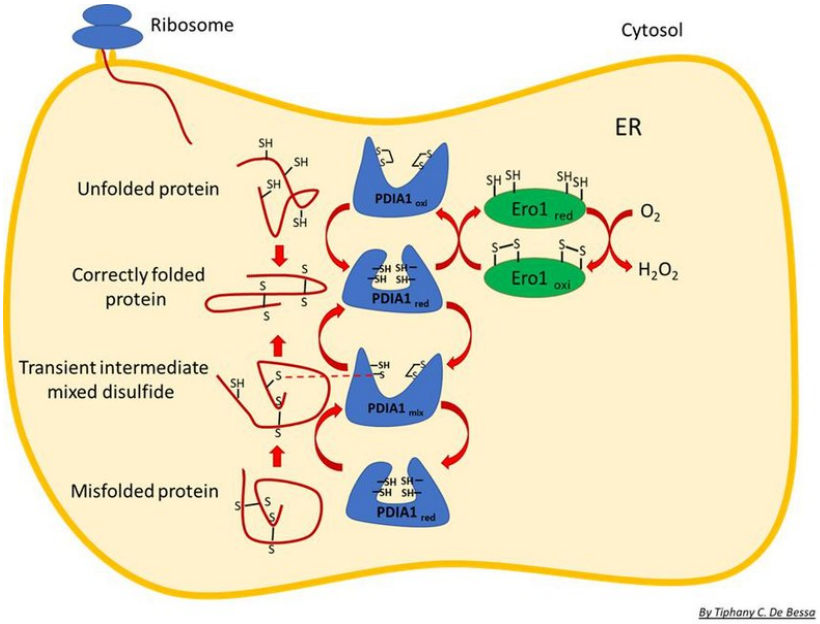
CHOP: Protein Portfolio

CHOP, CCAAT/Enhancer Binding Protein Homologous Protein, is a 29 kDa transcription factor also known as DNA Damage-Inducible Transcript 3 Protein (DDIT3). CHOP's production is induced by many proteins, but mainly ATF4. It regulates cell cycle arrest and apoptosis. Cell cycle arrest is when the cell stops being involved in processes having to do with duplication and division. CHOP is an inhibitor of C/EBP, a transcription factor that regulates genes involving inflammatory and immune responses. It also negatively regulates the expression of Bcl-2, which is a key player in apoptosis as it's main function is controlling mitochondrial permeabilization. As we all know, the mitochondria is the powerhouse of the cell (haha) and synthesizes ATP and GTP among other things, which power chemical reactions. Mitochondrial permeabilization is the process of making incredibly small holes in the membrane so molecules and chemicals can pass through. Permeabilization can also be used in favor of apoptosis by making larger holes in the mitochondria so that pro-apoptotic signaling molecules can flow through. Damage to Bcl-2's gene is shown in many cancers, including breast cancer, and this has been shown to be a cause of the resistance of cancer treatments.

CHOP also negatively regulates proteins related to iron transport and distribution as well as the activation of Asparagine Synthetase (ASNS), a cytoplasmic enzyme that produces asparagine (an amino acid) from aspartate and glutamine (two other amino acids) in an ATP dependent reaction in the mitochondria. The expression of ASNS is dependent on UPR and the Amino Acid Response.

To create more of a reason to induce apoptosis, CHOP causes oxidative stress by inducing the transcription of ERO1α which transfers electrons from the Protein Disulfide Isomerase to O2 to produce H2O2. The Protein Disulfide Isomerase (PDI) actually has a really fascinating job, and an even cooler way of going about it. It's job is to catalyze the formation and destruction of disulfide bonds between cysteine residues in the tertiary and quaternary forms of proteins. PDI allows the protein to find the correct configuration of disulfide bonds to be folded properly, and if this fails, you end up with a misfolded protein. First, PDI takes the hydrogen molecules from the cysteine residues on an unfolded protein so the now positively charged cysteines can form disulfide bonds

with each other. PDI is now negatively charged with all the extra hydrogens, so it passes these hydrogens on to ERO1α. ERO1α passes these hydrogens to O_2 making it H_2O_2, thereby increasing oxidative stress. Refer to this diagram for clarity:



ERO1α also promotes the release of Ca^{2+} from the ER through the Inositol 1,4,5-Trisphosphate Receptor, a membrane glycoprotein that acts as a Ca^{2+} channel. Ca^{2+} is required for protein folding, ER chaperones, and the depletion of it further disrupts the folding and increases ER stress. Once Ca^{2+} is released from the ER, it goes on to activate oxidative stress and pro-apoptotic signaling in the mitochondria.

Lastly, CHOP activates many pro-apoptotic factors, including DR5, BIM, and TRB3 along with GADD34 (which isn't a pro-apoptotic factor, but still very important). DR5, or Death Receptor 5, is part of the TNF-receptor superfamily and is located on the cell membrane. As you might have guessed, it is activated by TNF-Ligand Superfamily Member 10 (TNFSF10). DR5 is also part of the Death Inducing Complex (DISC) along with adapter molecule FADD and Caspase 8. DISC is a complex that activates the caspase signal transduction is formed by FADD, Caspase 8, and a death receptor, which in this case was DR5.

BIM, or Bcl-2 like Protein 11, belongs to the Bcl-2 family and interacts with other members of that family. In the Bcl-2 family, there are pro-apoptotic proteins and apoptotic inhibiting proteins, BIM is pro-apoptotic. Cancer cells suppress BIM expression to allow for tumor progression and metastasis, as BIM poses a threat to that. It has been shown that a mutagenesis on position 156 of BIM changing the amino acid from Glycine to Glutamic Acid abolishes BIM's ability to induce apoptosis. Production of this protein is induced by NGF (Nerve Growth Factor) and FOXO3 (Forkhead Box Protein O3). NGF and FOXO3 promote neuronal apoptosis, with FOXO3 promoting apoptosis in the case of oxidative stress. FOXO3 also participates in the

post-translational activation of proto-oncogene MYC. While MYC isn't too important specifically to BIM, it is an important protein as one of its main functions is to activate growth factors and it is a proto-oncogene. A proto-oncogene means that it's gene, when altered by a mutation, can contribute and even play a crucial role in turning a cell cancerous.

TRB3, or Tribble Homolog 3, is a protein kinase which regulates the ISR (Integrated Stress Response). Once TRB3 is phosphorylated, on serine 12, it localizes to the nucleus and interacts with CHOP on residues 1-127 to inhibit it. It is also a negative feedback regulator of ATF4 dependent transcription activity in ISR, which seems a little traitorous because TRB3 expression is promoted by ATF4, and then TRB3 goes and stabs ATF4 in the back by inhibiting its activity. TRB3 also inhibits NF-κB transcription factors, p65 RELA, phosphorylation therefore inhibiting NF-κB. p65 RELA is a very important protein in NF-κB signaling because it basically controls the whole pathway. It is involved in the homodimerization and nuclear translocation of NF-κB. The activation of p65 RELA has been shown to be positively associated with many cancers, and p65 RELA seems to have a connection between AhR and the subsequent transcription of proto-oncogene MYC in breast cancer cells. AhR is a ligand activated transcription factor that regulates xenobiotic chemicals (compounds that aren't produced in the body).

GADD34, or Growth Arrest and DNA Damage Inducible Protein, is a receptor protein that forms a complex with Protein Phosphatase 1 (PP1) to dephosphorylate eIF2α. This removes it from the role of global translation inhibitor, and essentially reverses the work we did with eIF2α as a result of ER stress. It may also promote apoptosis by interacting with various apoptosis and growth factors.

In short, when CHOP is produced and released it compromises protein folding and just the cell in general because it increases oxidative stress and pro-apoptotic signaling. Because of this, the deletion of CHOP is protective against ER induced cell death in multiple cell types. Therefore, I believe that CHOP should be considered as a target for treating diseases like cancer, type 2 diabetes, and other diseases related to misfolded proteins and ER stress.

ATF6 Pathway

This pathway is definitely the smallest of the UPR branches, but that doesn't make it any less important. ATF6α, or the Activating Transcription Factor 6α, is a type II transmembrane protein and has a bZIP domain in its N-terminal. Once it is activated, on account of ER stress, it is released from its chaperone (BiP) keeping it at the ER. This allows it to freely travel to the Golgi body to be cleaved into its active form. This process is called Regulated Intramembrane Proteolysis (RIP). Once it has reached the Golgi, it is cleaved at its transmembrane site into an active transcription factor, ATF6 p50, by Site 1 and Site 2 proteases. Site 1 and 2 proteases are serine proteases and cleave

after hydrophobic or small residues. Once ATF6 has been cleaved and activated, it can heterodimerize with XBP1 during ER stress to induce the transcription of ERAD components (like EDEM, HERP, etc.). Independently, ATF6 is a transcription factor that binds to ER stress response elements (ERSE) I and II in the promoter of UPR and ERAD target genes. Its target genes are essential for protein folding, secretion, and their degradation. One type of protein that is promoted by ATF6 are ER quality control proteins. They monitor proteins after they have been synthesized to weed out any inadequate or mutated proteins before they can be released from the ER. To do this, ATF6 has to recruit CRTC2 to the promoter of those genes. CRTC2 is a transcriptional coactivator for CREB1, which together can regulate gluconeogenesis genes. With ATF6 bound to CRTC2, it can disrupt this process and therefore downregulate hepatic gluconeogenesis. Hepatic gluconeogenesis is the metabolic process of producing sugars (mostly glucose) in the liver.

ATF6α is essential to cell survival as it has been shown in mice that ATF6α deficient cells did not survive acute ER stress. However important it may be, ATF6 can't activate genes all by itself, and requires NF-Y to also be binded to ERSE in order for the gene to be transcripted.

NF-Y, Nuclear Transcription Factor Subunit Y, is a heterotrimer composed of 3 parts: NFYA, NFYB, and NFYC. As a transcription factor, it binds to CCAAT motifs in the promoters of its target genes. NF-Y is a pioneer factor, meaning that it can bind directly to the DNA instead of through another protein. It can bind to the DNA with high affinity and specificity, and is a cofactor with many proteins including ATF6. It can either act as an activator or a repressor towards its cofactors, but in the case of ATF6, it is an activator. Its function is to promote chromatin accessibility and help make the job of other transcription factors easier, and therefore has to be localized to the nucleus. Chromatin is just things that are made of nucleic acid or amino acids, so basically RNA, DNA, and proteins. I think that NF-Y is essential to all nucleus functions.

The second homolog of ATF6 is ATF6β, which is seemingly less important and less abundant. It has all the same general functions of ATF6α, and almost the same structure, but is not essential. It was shown that ATF6β deficient cells in mice showed no significant difference when faced with acute ER stress and handled it appropriately. Also, the genes regulated by ATF6β have not been discovered yet and are currently a mystery.

In addition to ATF6, there have been other regulators of apoptosis that have been identified that are similar to ATF6. The first is CREB3, cAMP Regulated Element Binding Protein 3, which is also more commonly referred to as Luman. It is a transmembrane protein in the ER. Its transcription proteolytic processing is induced by ER stress. Like ATF6, it travels to the Golgi body to be cleaved into its active form before going to the nucleus to activate its target genes. It is a pioneer factor that can bind directly to the DNA, and binds to the 5'-CCAC[GA]-3' half of the ERSE-II element,

CRE's, UPRE's, and C/EBP sequences in the promoter region. It can induce the production of HERP, an important player in ERAD and UPR as it reduces the amount of proteins the ER has to fold, therefore improving its performance before the ER can return to normal capacity.

The next regulator of apoptosis is CREB3L1, also known as OASIS (Old Astrocyte Specifically Induced Substance). It is a type II transmembrane protein, like everyone else, with its N-terminal in the cytosol. Its N-terminal also contains its DNA binding and transcription activation domains. In response to ER stress, similar to ATF6 and CREB3, it performs Regulated Intramembrane Proteolysis by going to the Golgi to be cleaved by Site 1 and 2 proteases. It then goes to the nucleus to go through with its transcription factor duties and binds to the DNA consensus sequence 5'-GTGXGCXGC-3' in the promoter. CREB3L1 is also required for TGFB1 to activate genes related to the extracellular collagen matrix. Collagen is a fibrous protein that is actually the main structural element of the extracellular matrix. It is in charge of regulating cell adhesion and providing tensile strength. TGFB1, Transforming Growth Factor β-1, regulates the differentiation and growth of lots of different cell types and has a part in lots of processes including immune function and microglia function. Microglia are cells that function as macrophages in the central nervous system. Coming back to CREB3L1, independently, its target genes are part of bone formation and the secretion of bone matrix proteins. Specifically to astrocytes in the brain, where it gets its name from, CREB3L1 activates BiP and prevents ER stress induced apoptosis. Astrocytes are non-neuronal cells in the central nervous system that are in charge of glutamate and water homeostasis and energy storage. Glutamate is a chemical that lets other cells transmit/receive information through the nervous system.

The third regulator of apoptosis similar to ATF6 is CREBH, cAMP Regulated Element Binding Protein-like Protein 3. CREBH is a liver specific transcription factor whose production is induced by inflammatory cytokines like Interleukin-6 and TNFα. It then becomes activated by cAMP stimulation. cAMP, Cyclic Adenosine Monophosphate, is a second messenger derived from ATP that induces lots of pathways through PKA. After being activated, CREBH performs Regulated Intramembrane Proteolysis. Once it is in its active form, it goes to the nucleus and binds to the CRE and Box-B elements in promoters. It has a vast variety of target genes with different purposes. CREBH mediates transcription related to gluconeogenesis and lipid metabolism in your liver. For example, it can cause the production of FGF21 (Fibroblast Growth Factor 21) which stimulates glucose intake in fat cells, or adipocytes. It has also been shown that people with little or no CREBH in their livers experience extreme hypertriglyceridemia, which occurs when you have too many triglycerides in your bloodstream. One thing that differentiates CREBH from the other apoptosis regulators is that instead of activating UPR genes, it regulates the expression of the Acute Phase Response (APR) genes. APR is a branch of the innate immune response. It is essentially all the different

processes that are initiated right after the tissue is infected or injured, for example, a burn, a surgery, or an infection. It can either be positive where it increases the inflammatory response or negative where it decreases it, but is typically positive and can take the form of a fever, swelling, etc.

Conclusion

In short, the Unfolded Protein Response is a complicated and exhaustive web of proteins intertwined with about a million different functions. Not only does it amass about 140 proteins, but it also connects with different responses and pathways in the cell with even MORE proteins. Overall, one thing I learned from researching the UPR is that proteins are most definitely the most useful molecules in your body. Without them, absolutely nothing would happen because everything is controlled by them. Because of this I feel that the UPR is one of the most important responses in your body, because you can forget about fighting extracellular threats if you can't even keep your own cells healthy and working like well-oiled machines.