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Protein quality control—linking the unfolded protein response to disease

Conference on 'From Unfolded Proteins in the Endoplasmic Reticulum to Disease'

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The Summer Research Conference 'From Unfolded Proteins in the Endoplasmic Reticulum to Disease' took place between 7 and 12 June 2009, in Saxtons River, Vermont, USA, and was organized by L. Hendershot and R. Hegde.

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See Glossary for abbreviations used in this article.

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Introduction

The correct folding of proteins is essential for cellular homeostasis and the prevention of disease. During times of stress or changes in demand for newly synthesized proteins, signalling pathways cooperate with networks of protein-folding and protein-degradation factors to maintain the pools of non-native proteins within a tolerable range. Such events are crucial for survival, as aberrant protein homeostasis can result in diseases that range from cancer and diabetes to neurodegeneration. Protein flux through the secretory pathway is controlled through the UPR signalling system. Recent breakthroughs in this field were presented at a Summer Research Conference, and the varied talks included studies of basic and clinical relevance. Here, we discuss the highlights of this important conference.

Chronic UPR signalling and disease

The plenary lecture was delivered by D. Ron (New York, NY, USA), who provided an overview of how transmembrane resident ER proteins signal from the ER lumen to the cytoplasm and nucleus to modulate translation and control transcriptional events to fine-tune protein flux through the ER (Fig 1). The signalling cascades that constitute the different UPR pathways have been known for some time, but what was evident from several presentations in Saxtons River is that UPR signalling is an adaptive response that helps cells to survive acute stress, but that is not always beneficial during chronic stress. CHOP is a transcription factor that is induced through PERK signalling and induces apoptotic cell death in response to chronic ER stress. Apoptosis is prevalent in atherosclerosis and Ron and I. Tabas (New York, NY, USA) reported that less apoptosis and necrosis occurs in atherosclerotic lesions in *Chop*$^{-/-}$ mice (Thorp *et al*, 2009).

Obesity is a metabolic state associated with chronic ER stress in liver cells and adipocytes that can lead to type II diabetes. G. Hotamisligil (Boston, MA, USA) discussed mechanisms by which obesity causes chronic induction of UPR and the IRE1-mediated activation of JNK, thereby inducing the expression of pro-inflammatory genes (Gregor *et al*, 2009). Chronic inflammation is

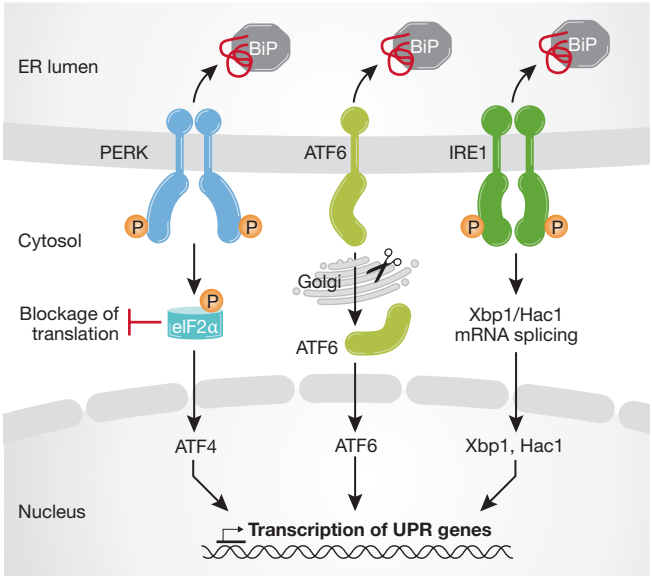

Fig 1 | Branches of the UPR signalling pathway. The ER resident BiP (Hsp70) binds the luminal tails of PERK, ATF6 and IRE1 to suppress their activity. When the levels of unfolded protein are elevated, BiP is titrated away, enhancing PERK-dependent phosphorylation of the translation initiation factor eIF2α, which leads to the suppression of protein synthesis and the activation of the transcription factor ATF4. Concomitantly, IRE1 is activated to mediate the expression of Xbp1/Hac1, which drives a global transcriptional induction of chaperones and quality control factors that maintain unfolded protein levels within a narrow range. When the stress that triggers UPR is chronic or extremely severe, PERK signalling drives the ATF4-dependent expression of the transcription factor CHOP, and its downstream products trigger apoptotic cell death. ATF4/6, activating transcription factor 4/6; BiP, immunoglobulin heavy-chain-binding protein; CHOP, CCAAT/enhancer-binding protein (C/EBP) homologous protein; ER, endoplasmic reticulum; eIF2α, eukaryotic initiation factor 2α; Hac1, homologous to ATF6/CrebI; Hsp70, heat-shock protein 70; IRE1, inositol-requiring enzyme 1; PERK, double-stranded RNA-dependent protein kinase-like ER; UPR, unfolded protein response; Xbp1, X-box binding protein 1.

Glossary

α1-AT	α1-antitrypsin
ATF4	activating transcription factor 4
CFTR	cystic fibrosis transmembrane conductance regulator
CHIP	carboxyl terminus of Hsc70-interacting protein
CHOP	CCAAT/enhancer-binding protein (C/EBP) homologous protein
DP1	deleted in polyposis locus protein 1
E3	enzyme 3, ubiquitin ligase
EDEM	ER degradation-enhancing α-mannosidase-like
eIF2α	eukaryotic initiation factor 2α
ER	endoplasmic reticulum
ERAD	endoplasmic-reticulum-associated degradation
Ero1	ER membrane oxidoreductase 1
Hsp	heat-shock protein
HSF1	heat-shock factor 1
HRD1	HMG-CoA reductase degradation 1
IRE1	inositol-requiring enzyme 1
JNK	c-jun N-terminal kinase
LULL1	luminal domain like LAP1
NAD	nicotinamide adenine dinucleotide
OS9	osteosarcoma 9
PERK	double-stranded RNA-dependent protein kinase-like ER
PDI	protein disulphide isomerase
RMA1	ring domain membrane-associated protein 1
SEL1L	Sel1-like repeat
shRNA	short hairpin RNA
SIRT1	Sirtuin 1
UPR	unfolded protein response
XTP3-B	XTP3-transactivated protein B
Yop1	YIP one partner

toxic and Hotamisligil suggested several approaches to attenuate IRE-1 signalling as a method to suppress ER stress in inflammatory diseases.

Free radicals as UPR signals

ER protein folding often involves disulphide bond formation, which occurs through the electron shuffling reactions catalysed by PDI family members. Ero1 catalyses the oxidation of PDI by linking disulphide formation with the reduction of oxygen to generate hydrogen peroxide. It now seems that hydrogen peroxide provides a source of reactive oxygen species in the ER. R. Kaufman (Ann Arbor, MI, USA) suggested that free radicals generated in the ER serve as UPR signals (Malhotra *et al*, 2008). Using a β-cell model, he showed that the regulation of eIF2α phosphorylation is required for glucose tolerance and cell viability as unregulated biosynthesis can lead to the lethal accumulation of oxidative damage. Chronic high-level secretion of proteins such as insulin is toxic to β-cells when the capacity of the cell to dispose of the by-products of oxidative protein folding is exceeded. Therefore, the UPR must strive to maintain both unfolded proteins and free radicals in a tolerable range. How the UPR detects the accumulation of reactive oxygen species to adjust protein flux remains a mystery.

The removal of hydrogen peroxide from cells is facilitated by catalase, glutathione and thioredoxin peroxidases and other non-enzymatic means. N. Bulleid (Manchester, UK) suggested that an ER-localized peroxiredoxin (PrxIV; Tavender *et al*, 2008) is able to metabolize the hydrogen peroxide produced by Ero1. In addition, he showed that hydrogen peroxide is indeed produced by Ero1 *in vivo*, thereby providing the first indication of a mechanism for the disposal of ER-generated hydrogen peroxide.

As a corollary to studies on redox-dependent folding in the ER, R. Morimoto (Evanston, IL, USA) presented an interesting model of how changes in cellular redox state can regulate the action of the cytosolic stress sensor HSF1. Morimoto's group found that HSF1 is acetylated at a key residue required for DNA binding and that SIRT1 deacetylates this site to activate HSF1 (Westerheide *et al*, 2009). SIRT1 is activated by NAD, the levels of which increase in response to reactive oxygen species and other metabolic conditions. Therefore, reactive oxygen species of ER origin possibly act to modify a UPR sensor either directly or indirectly, and thereby regulate protein flux through the ER. Another possibility is that the reactive oxygen species generated during ER stress act through a redox couple to activate HSF1. Such an event could explain the apparent links between ER stress and the cytosolic stress response (Mu *et al*, 2008).

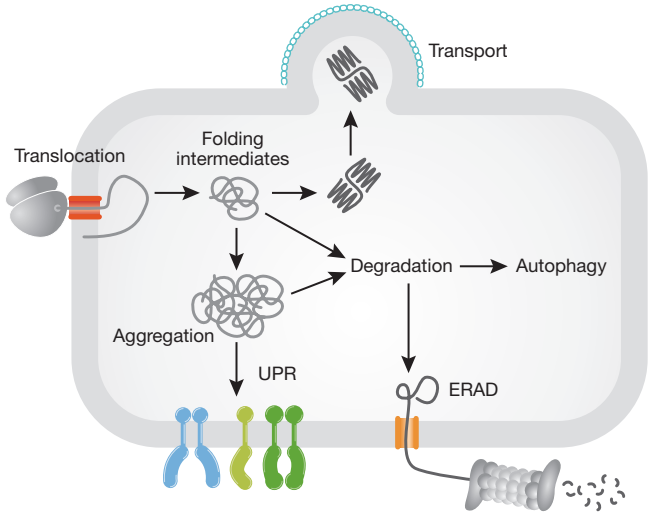

Fig 2 | The general ERAD pathway for degradation of misfolded ER proteins. ERAD is initiated on the recognition of non-native proteins as aberrant molecules by quality control receptors. These terminally misfolded or unassembled proteins are then sorted to an ER-membrane-associated dislocation/ubiquitination complex containing adaptor proteins that recognize the quality control receptor and/or the ERAD substrate directly. A translocon then acts as a conduit for the retrotranslocation of the protein to the cytosol, and this process is often coupled with ubiquitination by an ER-associated E3 ubiquitin ligase. Cytosolic components are also recruited to the retrotranslocation complexes to aid in the extraction of the ERAD substrate from the ER, and prepare the substrate for proteasomal degradation. E3, enzyme 3, ubiquitin ligase; ER, endoplasmic reticulum; ERAD, endoplasmic-reticulum-associated degradation; UPR, unfolded protein response.

Protein misfolding in the ER and disease

The UPR and the heat-shock response are designed to prevent the accumulation of misfolded proteins. However, there are several instances in which the high level of activity of such systems leads to the premature degradation of proteins by the proteasome, therefore causing loss-of-protein-function diseases. In other cases, mutant disease proteins escape protein quality control and accumulate in a proteotoxic state. Several presentations at the meeting highlighted treatments that are being developed to either facilitate the folding of disease proteins or promote the clearance of protein aggregates.

Lysosomal storage disorders are a class of around 40 inherited metabolic diseases caused by the loss of a specific lysosomal protein. H. Do (Cranbury, NJ, USA) suggested that active-site analogues can act as pharmacological chaperones that improve the stability of mutant lysosomal enzymes in the ER. This stabilization allows the transport of mutant enzymes to the lysosome where they are functional (Benjamin *et al*, 2009). Thus, enzyme ligands can act as folding correctors in some protein-folding disorders.

The mutation that causes the classical form of α1-AT deficiency causes nascent forms of α1-AT to assemble in large aggregates that are difficult to degrade by the ERAD pathway (Fig 2). D. Perlmutter (Pittsburgh, PA, USA) described that autophagy is an alternative pathway by which the cell prevents the accumulation of insoluble α1-AT. Autophagy is a catabolic process in which cellular components are degraded through the lysosome. The study of α1-AT degradation indicates that autophagy acts as a back-up system to the ERAD pathway by disposing of ER luminal protein aggregates (Perlmutter, 2009). Drugs that enhance autophagy can decrease the hepatic load of α1-AT in animal models that harbour the z-variant of α1-AT, which is prone to form large aggregates in the ER lumen, and this could benefit patients with α1-AT deficiency.

Macroautophagy is responsible for the turnover of organelles such as the ER and mitochondria and also has a role in the clearance of protein aggregates from the cytoplasm. A. M. Cuervo (New York, NY, USA) described the chaperone-mediated autophagy pathway and showed that the cytosolic chaperone Hsp70 can target specific proteins for autophagic degradation. We are just beginning to appreciate the important housekeeping functions of autophagy. In addition, chaperone-mediated autophagy has recently been linked to defects in lysosomal function caused by aggregates of the microtubule-binding protein Tau in cell-based models for Alzheimer disease (Wang *et al*, 2009a).

The activity of the quality control machinery that is responsible for the recognition and degradation of slow-folding disease proteins varies between tissues and cell types. W. Balch (San Diego, CA, USA) suggested that the manipulation of the cellular folding environment can positively influence disease-protein-folding efficiency. Indeed, tools to manipulate the global expression pattern of the cellular folding machinery as a therapeutic avenue to treat protein-folding diseases are now being developed (Powers *et al*, 2009).

ER structure and interactome

A basic knowledge of ER structural dynamics and microdomain assembly are required to understand the UPR, as well as to identify the components of the pathways that are regulated by the UPR. G. Voeltz (Boulder, CO, USA) has characterized the functionally and structurally distinct subdomains of the continuous ER membrane system, and is identifying the ER proteins that have roles in controlling ER shape (English *et al*, 2009). Recent discoveries indicate that reticulon and DP1/Yop1 shape the tubular ER. By contrast, p180, polyribosomes and components of the translocon complex control the shape of the sheet-like ER domains. E. Snapp (New York, NY, USA) used quantitative single-cell fluorescence microscopy to visualize changes in the behaviour of ER luminal chaperones such as BiP under different cell stress conditions. The identification of the components that control ER ultrastructure and the introduction of methods to study ER protein dynamics are starting to reveal how the ER adapts to acute and chronic stress.

We are just beginning to uncover the nature of the protein complexes that are part of the different UPR-controlled pathways. To accelerate this discovery process, J. Weissman (San Francisco, CA, USA) has developed screens of the yeast genome that use a cell-based UPR sensor to identify several hundred ER-protein-folding genes (Jonikas *et al*, 2009). This approach has revealed a new protein complex that facilitates the insertion of tail-anchored membrane proteins into the ER. Weissman also described the development of high coverage shRNA libraries that can be used to screen for UPR-regulated genes in mammalian cells (Bassik *et al*, 2009).

The interactions between ER luminal chaperones have also been mapped using a unique yeast two-hybrid assay system developed by the group of D. Thomas (Montreal, QC, Canada). The assay is based on the Ire1 kinase in yeast and positives are verified by affinity purification with natively folded proteins as baits. By using this approach, Thomas showed that BiP and Hsp40s interact with the

luminal quality control factor EDEM1 to help select and retain misfolded proteins in the ER (Ushioda *et al*, 2008). J. Christianson (Oxford, UK) reported a complementary approach in which ER membrane protein complexes involved in ERAD were characterized by co-immunoprecipitation and mass spectroscopy (Christianson *et al*, 2008). Data obtained through this approach are being mined and have already unveiled a role for the ER resident glycoproteins OS9 and XTP3-B in the selection of substrates for the SEL1L/HRD1 E3 ligase complex. The successful development of these new methods is now allowing the field to use an array of complementary approaches to expand our understanding of the UPR machinery.

Mechanisms of ERAD

Defective proteins are cleared from the ER through a process termed ER-associated degradation, or ERAD. A crucial step in ERAD is the selection and delivery of soluble misfolded proteins in the ER lumen to the membrane-associated translocation machinery (Fig 2). EDEM1 has been proposed to have a role in this process by recognizing mannose trimming of N-linked glycans attached to ERAD substrates. D. Hebert (Amherst, MA, USA) showed that EDEM1 recognizes protein-folding defects directly, independent of the glycosylation status of a protein (Cormier *et al*, 2009). Nevertheless, EDEM1 seems to bind to the downstream glycosylated membrane-associated components of the ERAD machinery through its mannosidase-like domain. These results indicate that EDEM1 functions as a quality control receptor, and that the mannose trimming of glycans attached to ERAD components could be used to mediate ERAD complex formation.

The quality control or ERAD selection process ensures that defective proteins are not improperly secreted. M. Molinari (Bellinzona, Switzerland) showed that the topology of the ERAD substrate determines—at least in part—the components that are needed for its efficient turnover. His group found that individual or combined knockdown of the mammalian ER E3 ligases HRD1 and gp78 delayed the turnover of membrane-anchored ERAD substrates. However, deletion of the membrane anchors to create soluble variants of the substrates led to the exclusive use of the HRD1 pathway, showing that protein topology is a crucial determinant for ERAD selection.

The selection of misfolded polytopic proteins for degradation by the ERAD machinery is complicated because such proteins expose surfaces in the ER lumen, ER membrane and in the cytosol. D. Cyr (Chapel Hill, NC, USA) presented studies on CFTR biogenesis that identify Hsp70/CHIP and RMA1/Derlin1 as E3 ubiquitin ligases that scan the cytosol and ER membrane for misfolded domains in different regions of CFTR. The RMA1/Derlin1 E3 seems to selectively recognize misfolding events in CFTR that cause cystic fibrosis. The inactivation of RMA1 allows mutant CFTR to fold and escape the ER and markedly enhances the effectiveness of small molecules that correct CFTR misfolding. Therefore, inactivation of the specific components of the ERAD machinery is sufficient to allow folding of disease-causing CFTR mutants.

To define the mechanism for the extraction and delivery of ubiquitinated ERAD substrates from the ER to the proteasome, W. Skach (Portland, OR, USA) has developed a cell-free ERAD assay comprising a rabbit reticulocyte translation system and rough ER-derived microsomes. The assay revealed that topologically distinct regions of CFTR are differentially dependent on the AAA-ATPase p97 for their extraction from the ER membrane. This reconstituted system should allow the events that mediate the delivery of ubiquitinated ERAD substrates to the proteasome to be defined in unprecedented detail.

Y. Ye (Bethesda, MD, USA) presented work on the development of chemical tools to modulate p97 action in ERAD. Ye reported that Eeyarestatin I inhibits ERAD, at least in part owing to its ability to inhibit the deubiquitinating activity of ataxin 3, the p97 interaction partner previously shown to be involved in ERAD. Interestingly, Eeyarestatin-I-mediated inhibition of ERAD induces cell death signalling cascades that involve ATF4 (Wang *et al*, 2009b). Inhibition of ERAD can induce cell death through apoptosis, and so ERAD inhibitors are now being developed as drugs to treat cancer.

TorsinA is a member of the AAA-ATPase family that is located in the ER lumen. It is mutated in patients that have early-onset torsion dystonia, which is a neurological movement disorder. P. Hanson (St Louis, MO, USA) reported that TorsinA interacts with the ER membrane protein LULL1 (Vander Heyden *et al*, 2009), resulting in the redistribution of TorsinA to the nuclear envelope where it is thought to modulate the structure of the nuclear membrane. This ability seems to be compromised in the dystonia-associated mutants. Whether mutations in TorsinA also cause defects in UPR-related processes is not yet known, but the studies on dystonia provide an example of a genetic disorder related to the dysfunction of an ER luminal chaperone.

Concluding remarks

Our understanding of the mechanisms of the UPR, ER protein folding and turnover have increased tremendously during the past decade. With this expanding knowledge, these cellular processes have been increasingly clearly associated with a number of human disease states. Remaining challenges in the field include the further understanding of the mechanisms that fine-tune protein flux through the secretory system, the identification of the molecules that mediate different aspects of UPR, and the development of therapeutics to modulate UPR signalling, which is a high priority in the field.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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