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CLINICAL IMPLICATIONS

Intermedin and the unfolded protein response

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Our topics for this *J Mol Med* issue are extremely exciting. However, for clinicians in the audience, should there be any left, we will have to address some background definitions. Nucleated cells synthesize many proteins, perhaps 5,000 or so. As in any industrial complex, quality control is extremely important. Proteins are synthesized by ribosomes along the endoplasmic reticulum (ER). Successful protein folding requires a tightly controlled environment, including metabolic energy, molecular chaperones, calcium, redox buffers, and numerous other components. Misfolded proteins are identified, labeled, moved out of the ER, ubiquitinated, and then undergo disposal via the 26S proteasome. When this process is overwhelmed, the unfolded protein response (UPR) is initiated. The UPR is unleashed as a response to ER stress and is primarily a protective mechanism. The UPR has two primary purposes. First, UPR should initially restore normal function in cells by halting protein translation. Second, UPR should activate signaling pathways that lead to increased production of chaperone proteins that are involved in protein folding. If this garbage-detail function fails, cells are advised to consider apoptosis and caspases are activated.

Conditions interfering with the function of the ER are induced by accumulation of unfolded protein aggregates, excessive protein traffic (viral infections), or ER-overload responses. ER stress possesses unique signaling pathways, caused by UPR aggregates. A special inositol-requiring kinase family with endonuclease activity (Ire 1α and β) is activated in mammals that processes the transcription factor XBP1. XBP1-splice is then translocated to the nucleus to induce chaperones including the glucose-regulated protein (Grp78 and Grp94) family. Grp78 is also known as the binding immunoglobulin protein (BiP) and is a heatshock protein HSP70 chaperone. The chaperones prevent protein aggregation and keep Ca^{2+} in the ER lumen. Furthermore, the protein kinase receptor-like ER kinase (PERK; or eIF2 kinase) phosphorylates the subunit of the eukaryotic initiation factor 2 (eIF2) that leads to attenuation of general protein synthesis. Finally, the third ER-stress molecule is moved to action, namely ATF6, a transcription factor that is essential for XBP1 mRNA expression and chaperone induction upon ER stress. ATF6 is activated after a brief sojourn to Golgi, where the 50 kDa portion is released for nuclear trafficking. A crude facsimile of ER stress and UPR are given in Fig. 1.

Calcitonin is a 32-amino acid linear hormone that is produced by parafollicular C cells of the thyroid. In humans, calcitonin is still looking for a home, but in fish, reptiles, and birds, the hormone may be important in calcium homeostasis. Calcitonin gene-related peptide (CGRP) is a member of the calcitonin peptide family, which in humans exists in two forms (α-CGRP and β-CGRP). CGRP is abundant in peripheral and central neurons. The peptide is a potent vasodilator and functions in the transmission of pain. CGRP mediates its effects through a heterotrimeric G protein-coupled receptor called calcitonin-like receptor (CALCRL) and a receptor activity-modifying protein (RAMP1). These receptors are found throughout the body. Currently, the clinical importance of CGRP is particularly focused on the pathogenesis of migraine headaches and temporalmandibular joint disorder.

Adrenomedullin (ADM) is an ubiquitously expressed peptide that was discovered in a pheochromocytoma in 1993. ADM forms a 52-amino acid, 6-amino acid ring that

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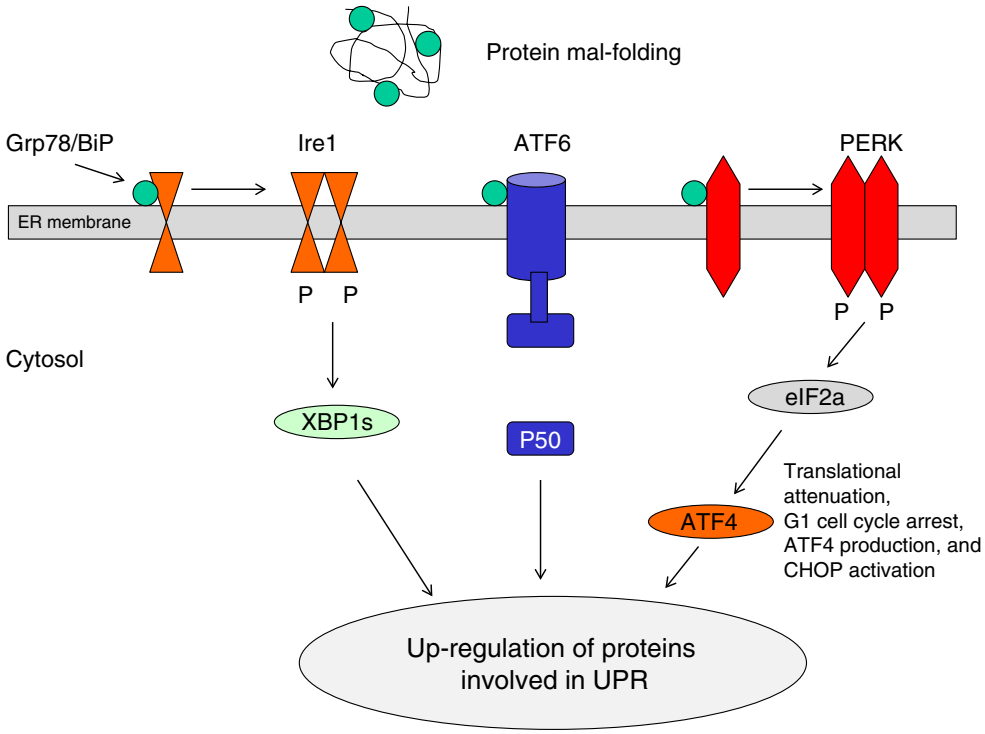

Fig. 1 A simplified diagram of UPR initiation by prolonged and overwhelming protein mal-folding is shown. Grp78/BiP recruitment chaperones the mal-folded proteins, resulting in Grp78 dissociation from its conformational binding state on the transmembrane receptor proteins IRE1, ATF6, and PERK. Dissociation results in receptor homodimerization and oligomerization to an active state. The activated cytosolic domain of PERK phosphorylates the eIF2alpha, inhibiting translation and resulting in cell cycle arrest. The activated cytosolic domain of IRE1 cleaves the 252 bp intron from its substrate XBP1, forming the transcription factor XBP1splice (XBP1s). Activated ATF6 translocates to the Golgi, cleaved by proteases to form an active 50-kDa fragment (ATF6 p50). ATF6 p50 and XBP1 bind ER stress promoters in the nucleus to produce upregulation of UPR proteins

shares similarity to the calcitonin-CGRP-amylin peptide family mentioned above. ADM is believed to function through CALCRL and to modify RAMP2 complexes. Fujisawa et al. identified a second member of the ADM family that they termed ADM2 [1]. Roh et al. identified intermedin as a CGRP-related family peptide that acts through the CALCRL to produce a CRLR/RAMP receptor complex [2]. Intermedin was a nonselective agonist for the RAMP coreceptors. Kuwasako et al. focused on the separate functions of ADM receptors and observed that the ADM2 receptor binds intermedin [3]. Intermedin and ADM2 appear to be the same peptide. Further information on intermedin and its function could be important to cardiovascular research.

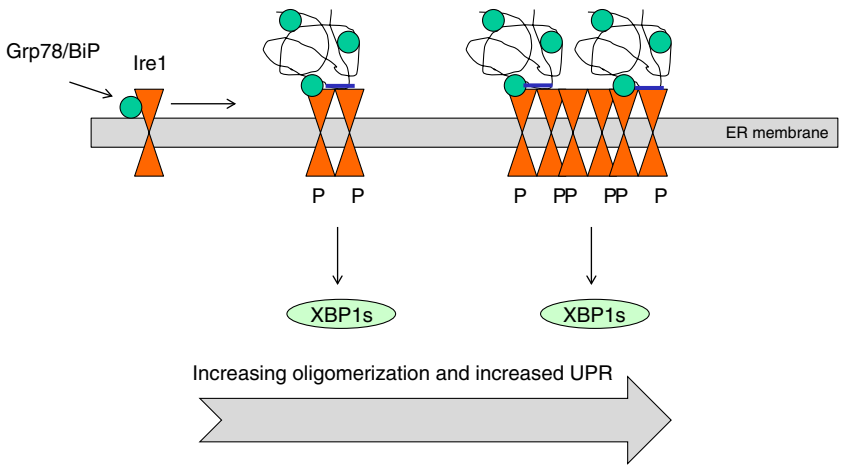

Fig. 2 Direct binding of unfolded proteins to a sensor in the ER, namely Ire1, triggers Ire1 oligomerization, and amplifies the cellular stress response. *Blue line* represents specific peptide region that binds to the luminal cleft of Ire1 [5] (figure adapted from Ref. [6])

In this issue, Teng and colleagues examined putative cardioprotective actions of intermedin [4]. The authors first prepared myocardial slices that were incubated in 24-well plates. These slices were placed in media and then exposed to dithiothreitol or tunicamycin. Both compounds interfere with the synthesis of N-linked glycoproteins and induce ER stress. GRP78, activated caspase-12, and the C/EBP homologous protein (CHOP) were promptly upregulated in the slice system, providing strong evidence that UPR was activated. Furthermore, lactic acid dehydrogenase (LDH) appeared in the supernatant of the slices. However, preincubation with intermedin, (10^{-9} M) reduced the ER stress markers. The intermedin effect could be blocked with an intermedin receptor antagonist. Since GRP78 and CHOP expression are influenced by ATF6, the authors also inspected ATF6 and ATF4 expression. The expression of both transcription factors followed a similar pattern.

Teng et al. [4] next investigated phosphoinositide 3-kinase and Akt signaling (PI3K/Akt). Akt is also known as protein kinase B. The purpose of these experiments was to implicate this signaling cascade mechanistically in the actions of intermedin. A PI3K inhibitor (LY 294002) was also used. The PI3K inhibitor blocked the effects of intermedin, while an ERK inhibitor and a protein kinase A inhibitor had no effect. The authors next moved to an in vivo experiment, namely ischemia/reperfusion injury by tying off a main coronary artery transiently. Intermedin was used as a treatment in these experiments. An intermedin infusion decreased severe cardiac impairment and lowered LDH activity, suggesting ameliorated target-organ damage. The levels of GRP78, caspase-12, and the CHOP complex were reduced in the intermedin-treated hearts. The findings are exciting for two reasons. First, they provide novel information into the actions of intermedin. Second, they underscore a role for ER stress in ischemia/reperfusion injury and suggest an ameliorative treatment.

The authors showed convincingly that the diminution of ER stress provided by intermedin somehow involves activation of PI3K/Akt signaling. How this activation ameliorates ER stress is not entirely clear. Possibly effectors such as active caspase 12 and CHOP are involved. However, perhaps the production of unfolded proteins is ameliorated directly. A recent report sheds light on how Ire1 is activated by unfolded proteins, at least in yeast. Once activated, the Ire1 protein forms oligmers. The oligomerization activates a cytoplasmic kinase and ribonuclease domain of Ire1, which perform the splicing reaction of XBP1 mRNA. XBP1-splice is then translated to produce the transcription factor, which upregulates protein folding material. Gardner and Walter recently showed how unfolded proteins are able to directly bind the core ER-luminal domain of yeast Ire1 [5]. In yeast, the ER-luminal chaperone BiP (called Kar2 in yeast) binds to inactive monomeric Ire1. When the protein is activated and forms oligomers, Kar2 no longer binds. However, the Ire1 oligomer when modeled revealed a cleft in the dimeric structure that could bind peptides. Furthermore, direct binding of unfolded proteins drives oligomerization. Gardner and Walter used a yeast strain engineered to produce a carboxy-peptidase Y (CPY*) variant that is misfolded, termed CPY* [5]. CPY* triggers the UPR by binding to Ire1, while properly folded CPY does not bind. The authors were able to analyze the peptide sequences and identified the relative binding affinities. They could then mutate these sequences, which impaired activation. This model is established for yeast. An imperfect schematic is given in Fig. 2. Whether or not the process is conserved to include mammals is currently unknown. However, the peptide grove of oligomerized Ire1 and the oligomerization interfaces could serve as drug targets that could alleviate faulty regulation of Ire1 and other aspects of the UPR. We could someday modulate ER stress directly. In the mean time, we have intermedin.

Respectfully,
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