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Cotranslocational Degradation: Utilitarianism in the ER Stress Response

Recently, a new layer of the unfolded protein response was discovered that supports the cotranslocational degradation of nascent chains stalled in endoplasmic reticulum translocons (Oyadomari et al., 2006).

Disruption of protein folding in the endoplasmic reticulum (ER) activates the unfolded protein response (UPR) signal transduction pathway, causing temporary remodeling of the ER (Schroder and Kaufman, 2005). The balance of ER resident proteins is shifted to remove aberrant substrates and restore the ER to an efficiently operating maturation compartment. The UPR pathway functions as a tripartite signal that involves (1) increasing the expression of housekeeping proteins that can work toward properly folding the misfolded proteins, (2) attenuating the secretory pathway load by decreasing the expression of secretory cargo, and (3) increasing the capacity for ER-associated protein degradation or ERAD (Figure 1). A study in the August 25, 2006, issue of *Cell* by Oyadomari et al. (2006) reveals a new layer in the UPR pathway that permits the cotranslocational degradation of secretory proteins (Oyadomari et al., 2006). This function diminishes the biosynthetic burden on the ER by degrading proteins at a stage earlier than previously envisioned.

The recruitment of the ER Hsp70 chaperone BiP to misfolded proteins in the lumen of the ER sequesters the chaperone away from three different ER membrane proteins that act as UPR transducers known as IRE1, ATF6, and PERK (Schroder and Kaufman, 2005). BiP release from these signaling proteins triggers a cascade of events that initiates the UPR. The enhanced transcription of folding and degradation machinery messages is initiated by IRE1 through its endonuclease activity and ATF6, which is itself a transcription factor. In addition, the kinase PERK attenuates general protein synthesis through its phosphorylation of eIF2α.

The quality control machinery of the ER monitors the integrity of protein maturation and sorts misfolded proteins to the ERAD pathway (Hebert et al., 2005). This process involves the posttranslational targeting of terminally misfolded proteins to a retrotranslocation channel whose identity is an area of vigorous concern and debate (Tsai et al., 2002). With the aid of the cytosolic AAA-ATPase p97/Cdc48, the misfolded protein is extruded through the membrane conduit, where it is then polyubiquitinated and delivered to the proteasome for degradation. The cotranslocational selection of nascent chains for degradation while they are still associated with the Sec61 translocon seemingly bypasses the necessity for the targeting of proteins to a translocon (Oyadomari et al., 2006). This mechanism of degradation also frees up anterograde channels for the translocation of housekeeping and ERAD machinery induced by UPR. While it still remains to be determined if Sec61 is the primary route for misfolded proteins to travel to the cytoplasm for ERAD, this study demonstrates that Sec61 can work in the reverse retrograde direction to retrotranslocate proteins that are stalled during the translation and translocation processes (Oyadomari et al., 2006).

P58^{IPK}/DNAJC3 was initially linked to UPR as an inhibitor of PERK (Yan et al., 2002). Thus, P58^{IPK}/DNAJC3 was thought to be part of a negative feedback loop involved in restoring translation to normal physiological levels once the stress had dissipated (Figure 1, dotted line). However, Oyadomari et al. (2006) demonstrated that P58^{IPK}/DNAJC3 is peripherally associated with the ER membrane and exists in large molecular weight complexes with the Sec61 translocon and cytosolic Hsp70 (Figure 2) (Oyadomari et al., 2006). P58^{IPK}/DNAJC3 binds Hsp70 chaperones and stimulates its ATPase activity through its nine TPR repeat motifs and its single J domain, both well-characterized Hsp70 binding motifs (Melville et al., 1999).

P58^{IPK}/DNAJC3 appears to assist in the cotranslational/translocational degradation of nascent chains that are stalled on ribosomes and in ER translocons (Oyadomari et al., 2006). Pharmacological arrest of both ApoB100 and VCAM-1 within ER translocons led to their association with P58^{IPK}/DNAJC3. The cotranslocational degradation of these substrates was diminished in cells derived from *P58*$^{-/-}$ mice, accelerated when P58^{IPK}/DNAJC3 was overexpressed, and required a functional J domain. This supports the hypothesis that P58^{IPK}/DNAJC3 recruits Hsp70 to the cytosolic opening of the ER translocon stimulating its ATPase activity to assist in the extraction of stalled nascent proteins into the cytoplasm for proteasomal degradation (Figure 2).

UPR-induced intervention early in the maturation process by P58^{IPK}/DNAJC3 allows the maturation and quality control machinery to focus its attention on the pre-existing improperly folded proteins that triggered the initial UPR signal. A recent study from the Weissman laboratory has also uncovered a new early intervention posttranscriptional process that involves the translation branch of the UPR signal (Hollien and Weissman, 2006). RNA encoding secretory and membrane proteins was discovered to be degraded by the activated endonuclease, IRE1. The endonuclease activity of IRE1 was shown to be able to process numerous transcripts in addition to XBP-1 (or HAC1 in yeast) mRNA, the transcription factor message processed and activated by IRE1 (Schroder and Kaufman, 2005). The more promiscuous endonuclease activity of IRE1 provides an additional posttranscriptional method of lowering the workload of the ER by directly reducing the number of nascent proteins translated and translocated into the ER.

ER stress and UPR are associated with a number of human diseases (Kaufman, 2002). Studies using *P58*$^{-/-}$ mice found that these animals were greatly disrupted in their ability to respond to ER stress (Oyadomari et al., 2006). Hyperglycemic *P58*$^{-/-}$ mice possessing a mutation in the *Ins2* gene were unable to elevate their plasma insulin levels, causing β cells defects and death

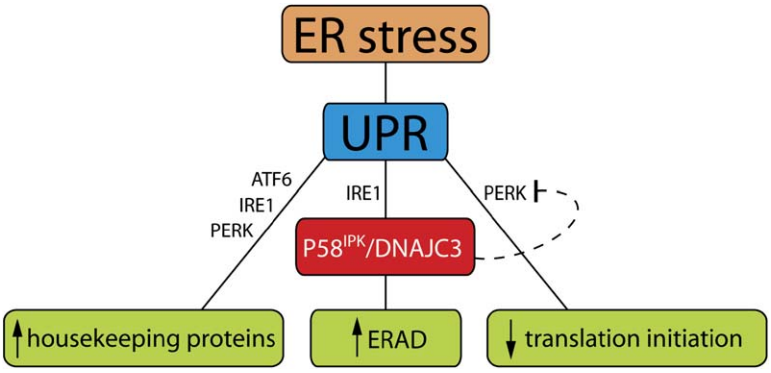

Figure 1. The Tripartite Unfolded Protein Response

Three primary transducers of the UPR signal, known as ATF6, IRE1, and PERK, seek to relieve ER stress through increased expression of housekeeping proteins, increased degradation of terminally misfolded proteins, and suppression of translational initiation until the aberrations have been alleviated. P58^{IPK}/DNAJC3 upregulation is triggered by the UPR, is initiated through IRE1 activity, and was originally found to act as a negative regulator of PERK-dependent translational attenuation (dotted line). However, a recent study demonstrates that P58^{IPK}/DNAJC3 assists in the cotranslational ER-associated degradation of translocon-stalled substrates (Oyadomari et al., 2006).

within 6 to 12 weeks of birth. This demonstrated that P58^{IPK}/DNAJC3 plays a central role in maintaining the homeostasis of the whole organism.

Many questions remain as to the molecular mechanism of P58^{IPK}/DNAJC3 substrate selection and its assistance in the extraction of translocon-associated polypeptides. Is the lumenal quality control process involved in substrate selection, or is this decision solely based on cytosolic cues? Does P58^{IPK}/DNAJC3 association with translocons require nascent chains? Is there a conformational change that takes place within P58^{IPK}/DNAJC3 that supports Hsp70 recruitment? Or is activation executed by the abundance of these complexes that is controlled by UPR? And finally, is the ability of P58^{IPK}/DNAJC3 to aid in the degradation of stalled nascent chains the key role of this multifunctional protein? Future studies will be required to increase our understanding of this powerful and intuitive stress response.

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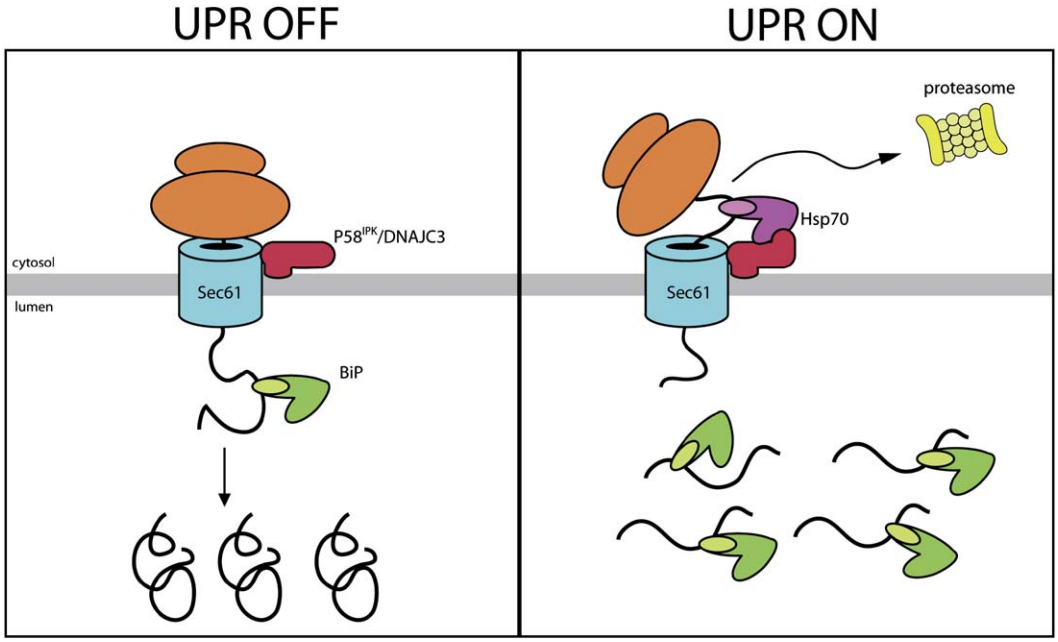

Figure 2. Cotranslational/Translocational Degradation Regulates the Secretory Load during the ER Stress Response

Under normal conditions in the ER, nascent chains are cotranslationally translocated into the ER lumen through the Sec61 translocon (blue). As the polypeptide emerges from the translocon channel, it is engaged by numerous chaperones such as BiP (green) that support proper substrate folding. Under ER stress conditions and initiation of the UPR, P58^{IPK}/DNAJC3 (red) recruits cytosolic HSP70 chaperones (purple) to the Sec61 translocon to extract nascent chains for subsequent proteasomal degradation (yellow). This cotranslational/translocational degradation decreases the amount of secretory cargo entering the ER lumen, allowing UPR-induced chaperones such as BiP to more effectively clear misfolded proteins from the ER.

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SnoRNP Biogenesis Meets Pre-mRNA Splicing

In vertebrates, hundreds of small nucleolar RNAs (snoRNAs) are processed from pre-mRNA introns. In the September 1 issue of *Molecular Cell*, Hirose et al. (2006) demonstrate that a spliceosomal intron binding protein, IBP160, couples box C/D snoRNA processing with pre-mRNA splicing in the C1 splicing complex.

In eukaryotic cells, a large number of box C/D and H/ACA small nucleolar RNAs (snoRNAs) direct the site-specific 2′-*O*-ribose methylation and pseudouridylation of rRNAs, respectively. The snoRNAs function in the form of small nucleolar RNPs (snoRNPs), each of which consists of a box C/D or box H/ACA guide RNA and four associated C/D or H/ACA snoRNP proteins. Biogenesis of vertebrate snoRNPs is remarkable: instead of being transcribed from independent genes, the majority of box C/D and H/ACA snoRNAs are synthesized within pre-mRNA introns. The intron-encoded snoRNAs are processed from the spliced out and debranched host introns by exonucleolytic trimmings. In fact, the box C/D and H/ACA snoRNP proteins that bind to the intron-embedded snoRNA sequences protect the termini of mature snoRNAs from the processing exonucleases. However, vertebrate introns have a rapid turnover; they are degraded immediately upon cotranscriptional removal from the growing pre-mRNA chain. Recent studies revealed that efficient processing of intronic snoRNAs is promoted by active recruitment of snoRNP proteins to the nascent intronic snoRNAs during synthesis or before splicing of the host pre-mRNAs (Ballarino et al., 2005; Darzacq et al., 2006; Richard et al., 2006; Yang et al., 2005). These works also indicated that recruitment of snoRNP proteins and early assembly of intronic snoRNPs are physically and functionally linked to pre-mRNA synthesis or processing. However, the molecular mechanisms responsible for coupling intronic snoRNP assembly with mRNA biogenesis remained speculative.

In a recent issue of *Molecular Cell*, Hirose et al. identify a general splicing factor, the intron binding protein 160 (IPB160), as a crucial factor for splicing-dependent assembly of intronic box C/D snoRNPs (Hirose et al., 2006). Earlier, IBP160 had been reported to be associated with the purified C1 splicing complex (Jurica et al., 2002). In the current study, depletion of IBP160 from HeLa cells by RNA interference demonstrates that IBP160 is essential for both cell viability and intronic box C/D snoRNP expression. Using an in vitro system that couples pre-mRNA splicing with snoRNP processing, Hirose and coworkers show that binding of IBP160 to pre-mRNA introns takes place in the C1 splicing complex, in which assembly of intronic box C/D snoRNPs also occurs (Hirose et al., 2003). The authors demonstrate that IBP160 interacts with intronic nucleotides 33–40 upstream of the branch site in a sequence-independent manner. Binding of IBP160 to pre-mRNA introns does not depend on the presence of an intronic snoRNA, supporting the notion that IBP160 is a general splicing factor, although its function in pre-mRNA splicing remains unknown. The position-dependent binding of IBP160 to pre-mRNA introns may be guided by the SAP155 spliceosomal protein that, as supported by coimmunoprecipitation experiments, can specifically interact with IBP160. SAP155 is a component of the U2 snRNP-associated SF3b complex that interacts with intronic sequences up to 25 nucleotides upstream of the branch point. Thus, SAP155 may recruit and deposit IBP160 onto upstream intronic sequences.

How does IBP160 link intronic snoRNP assembly to pre-mRNA splicing? It was noticed earlier that most mammalian intronic box C/D snoRNAs are located about 70–80 nucleotides upstream of the 3′ splice site (Hirose and Steitz, 2001). Later, in vivo and in vitro processing studies confirmed the significance of this observation, showing that an optimal distance (about 50 nucleotides) between the intronic box C/D snoRNA and the branch point is crucial for efficient snoRNP assembly (Hirose et al., 2003). Since IBP160 contacts intronic sequences about 40 nucleotides upstream of the branch point, it may recruit, directly or indirectly, box C/D snoRNP proteins or snoRNP assembly factors to snoRNAs positioned about 50 nucleotides upstream of the branch point. According to another attractive scenario, upon binding to pre-mRNA introns, IBP160 may facilitate proper folding of neighboring intronic box C/D snoRNAs. Supporting this possibility, IBP160 contains a helicase-like domain, and, more tellingly, processing of intronic box C/D snoRNAs that are not optimally located within their host introns requires extended terminal stem structures that likely promote correct folding of these snoRNAs. The external stems, therefore, could compensate for the absence of IBP160 in the proximity