Minireview

A molecular portrait of the response to unfolded proteins

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Abstract

Using DNA microarrays, 381 genes have been found to be induced in response to unfolded proteins. The identity of the previously characterized 208 of these, and further experiments, have revealed new details on the scope of the unfolded protein response and its connection to the degradation of proteins at the endoplasmic reticulum.

The endoplasmic reticulum (ER) serves as a way station for the transit of secreted proteins, and specifically as a depot devoted to the folding and maturation of proteins and to the assembly of macromolecular protein complexes. Before the protein passenger can exit the station, however, it is subject to a 'search' by the quality-control manager of the ER. The quality-control manager, sensing trouble, can retain undesirable passengers in the ER, and in certain cases banish them from the station. Undesirable passengers - aberrant polypeptides that cannot fold properly or mature - are sent back to the cytoplasm from which they arrived, and there they are degraded by the proteasome. This brutal treatment clears the ER of polypeptides that have the potential to aggregate, and prevents potentially toxic molecules from being secreted. The process by which aberrant, secreted polypeptides are degraded by the cytoplasmic proteasome is known as ER-associated degradation, or ERAD [1].

ER-associated protein degradation

The mechanism of ERAD has been uncovered in recent years through the analysis of ERAD-defective yeast mutants [2,3], through the development of *in vitro* assays in which ERAD can be measured [1], and through analyses of the fates of misfolded proteins in the secretory pathway [4-8]. Many disease-causing mutations in secreted polypeptides escape ERAD, and in some cases the over-zealous actions of the quality-control manager can promote disease - for example, in cystic fibrosis [9,10]. In addition, viruses may co-opt ERAD to destroy plasma-membrane-targeted host proteins and to elude the immune system, for example in infection by HIV [11] or cytomegalovirus [12]. Bacterial toxins travel in reverse through the secretory pathway and finally exit from the ER, like ERAD substrates, to the cytoplasm [13].

The unfolded protein response

Foul conditions may increase the concentration of misfolded proteins in the ER and overwhelm the capacity of this way station. Unlike rail stations, though, overwhelmed eukaryotic cells simply increase the capacity of the ER to house the greater number of misfolded proteins, and synthesize molecular chaperones to help solubilize the denatured polypeptides. This unfolded protein response (UPR), a response to increased concentrations of misfolded polypeptides in the ER, is initiated by an ER-resident transmembrane protein, Ire1p [14,15], thought to sense the presence of aberrant proteins in the ER lumen. The cytoplasmic domain of this molecule, which has homology to both kinases and an RNase, then cleaves an intron from the primary transcript for the Hac1p transcription factor [16-18]. Ligation of the mRNA, which allows for subsequent translation, requires the tRNA ligase, Rlg1p [19]. Once made, the transcription factor transits into the nucleus and initiates the synthesis of genes

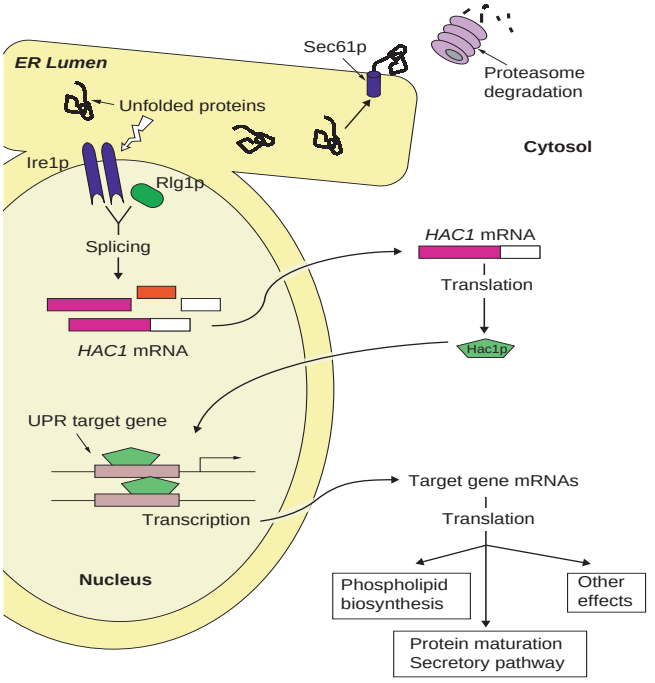

Figure 1
Unfolded proteins in the endoplasmic reticulum activate both ER-associated degradation (ERAD) and the unfolded protein response (UPR). Unfolded proteins are banished to the cytosol, by Sec61p-mediated retro-translocation, for degradation by the proteasome. Unfolded proteins also activate the ER transmembrane kinase/nuclease, Ire1p, which in conjunction with the tRNA ligase, Rlg1p, splices the *HAC1* primary transcript. *HAC1* mRNA passes to the cytoplasm for translation, and the newly synthesized transcription factor, Hac1p, enters the nucleus where it affects transcription of the UPR target genes. Translation of the target gene mRNAs provides proteins for secretory pathway functions and other cellular processes. The model was adapted from [20].

containing a 5′ 'unfolded protein response element' and other UPR target genes (Figure 1) [20].

Although the identities of a few of the genes up-regulated by the UPR have been known for several years (for example, ER lumenal molecular chaperones), the full spectrum of proteins induced directly or indirectly by the UPR has been ill-defined. A recent paper in *Cell* from the Walter and Weissman laboratories [21] rectifies this problem, and provides fascinating details on the breadth of the UPR and the connection between the UPR and ERAD.

A protein profile of the UPR

To establish the complete profile of genes induced in response to unfolded proteins, yeast cells (*Saccharomyces cerevisiae*) were incubated with compounds known to compromise polypeptide folding in the ER: dithiothreitol (DTT), a reducing agent, and tunicamycin, an inhibitor of *N*-linked glycosylation. Yeast genomic oligonucleotide arrays were screened using biotinylated cRNA probes in collaboration with researchers from Affymetrix, Inc. [www.affymetrix.com]. The criterion for identifying UPR-target genes was that their transcription should not be stimulated in *ire1* or *hac1* mutant strains, but in wild-type cells transcription should be induced to levels similar to that of a group of seven previously identified UPR targets. One of these target genes, *KAR2*, encodes the lumenal Hsp70 molecular chaperone, BiP, which is known to play a vital role in protein translocation into the ER, protein folding in the ER, and ERAD [22-25]. Thus, Travers *et al.* [21] chose genes as UPR targets only if the level of their over-expression correlated to the canonical set at least as well as that observed for BiP. The ultimate result of this analysis yielded 381 open reading frames (ORFs). Some information on the function of the corresponding proteins is available for 208 of these, and no information is available for the other 173. One of the previously uncharacterized genes uncovered from the screen, *PER100*, is homologous to a gene required for protein translocation in a related yeast, *Yarrowia lipolytica* [26], and a *per100* mutant was shown by Travers *et al.* [21] to exhibit ERAD defects. Clearly, continued analysis of the 173 previously uncharacterized ORFs will yield many exciting discoveries.

Links between the UPR and ERAD

About one half (103) of the previously characterized genes identified by Travers *et al.* [21] play roles in protein translocation, glycosylation, vesicular transport from the ER, protein targeting to the vacuole (which can also dispose of aberrant proteins in yeast), cell wall biosynthesis, and ERAD. In a related work, a screen to isolate yeast mutants that cannot survive in the absence of the UPR was performed, and the synthetic lethal mutants isolated from this screen uncovered genes similarly required for protein translocation, protein folding and glycosylation, and ERAD (D. Ng, personal communication). It is not difficult to imagine why these processes may be induced when the secretory way station is under siege.

Travers *et al.* [21] found further interactions between the UPR and ERAD. First, ERAD was less efficient in strains in which *IRE1* was deleted. Second, induction of the UPR increased the efficiency of ERAD. Third, if the UPR, or the concentration of misfolded proteins, was elevated to very high levels by the introduction of DTT, tunicamycin, or an over-expression system for an ERAD substrate, ERAD was reduced. Taken together, these results indicate that the UPR helps reduce the mass of unfolded proteins in the ER via ERAD, but when this mass raises above a threshold level the efficiency of ERAD is compromised. Fourth, deletion of genes that are not essential for viability but are required for ERAD led to a modest (around twofold) induction of the UPR. And fifth, strains lacking one of these ERAD genes and *IRE1* were nonviable at elevated temperatures, indicating

that cells survive either a certain level of aberrant proteins in the ER or an inability to respond to unfolded proteins, but they succumb when the two are combined.

Collectively, the results of Travers *et al*. [21] support a model in which the UPR and ERAD cooperate to eliminate misfolded proteins from the ER, and provide insights into the nature of this collective and compensatory action. What is remarkable about the findings is the breadth of the UPR. Half of the UPR target genes that have previously been characterized encode proteins generally associated with secretory pathway functions. These proteins are required to maintain the specialized environments of the ER and beyond, an environment necessary for protein folding and quality control, vesicle trafficking, vacuolar protein sorting, and cell wall biogenesis. Furthermore, the other half of the characterized UPR target genes encode proteins with functions in unidentified processes. So, more surprises are likely to emerge from studies of the UPR, studies that may reveal divergent intracellular signaling pathways linked to several, as yet unidentified, physiological responses.

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