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The Unfolding Tale of the Unfolded Protein Response

Minireview

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Surface and secreted proteins are synthesized in the endoplasmic reticulum where they must fold and assemble before being transported. Changes in the ER that interfere with their proper maturation initiate the unfolded protein response pathway. New studies have filled in a missing link between the yeast and mammalian pathways.

Initial Discovery of the Unfolded Protein Response Pathway

This story begins in the mid-1970s with the identification of two proteins, p78 and p94, that were induced in RNA tumor virus transformed cells. Shortly thereafter, Pastan's group demonstrated that induction of these proteins was not a direct effect of cellular transformation, but instead was due to the depletion of glucose from the medium of rapidly growing tumor cells (Lee, 1987); hence their designation as glucose-regulated proteins (GRP) 78 and 94. However, a number of other conditions or agents that altered the ER environment were also shown to increase their expression. GRP78 was independently identified in 1983 by Haas and coworkers as an ER-localized protein that bound to nonsecreted Ig heavy chains, which they named BiP (Haas and Wabl, 1983). This finding, together with related studies by Bole et al. showing that BiP served to retain incompletely assembled Ig intermediates, led to the designation of BiP/GRP78 as the first ER molecular chaperone. BiP was subsequently shown to bind to many different unfolded proteins in the ER and prevent their transport. The group of Gething and Sambrook connected the two stories by suggesting that the alteration of the ER environment by GRP-inducing agents might affect protein folding, and that perhaps the signal for GRP induction was the accumulation of unfolded proteins in the ER. This would, in turn, lead to the upregulation of chaperones to prevent the aggregation of unfolded proteins. They elegantly demonstrated that simply overexpressing an unfolded variant of the influenza hemagglutinin protein (HA) was sufficient to induce BiP and GRP94 expression (Gething and Sambrook, 1992), leading to the designation of this signaling pathway as the *u*nfolded *p*rotein *r*esponse (UPR). Meanwhile, Lee and coworkers cloned the promoters of these two genes and identified a number of regions that contributed to the transcriptional upregulation of the ER chaperones, as well as some of the transcription factors that bound to them. Through their studies it became clear that all UPR inducers signaled through the same regulatory regions, and therefore presumably the same pathway (Lee, 1987). However, the promoters appeared functionally redundant and complex, and no clear ER stress inducible element(s) was identified. Thus, delineation of the components of the mammalian UPR was stalled, with an upstream signal (unfolded proteins) and a downstream response (induction of ER chaperones), but no idea of the pathway linking them.

The Yeast UPR Pathway: A Novel Signaling Cascade

The big breakthrough in identifying the components of the UPR pathway came in 1992 when the Gething and Sambrook labs identified a 22 bp *cis*-acting element in the yeast BiP promoter (UPRE) that was sufficient to confer ER stress inducibility on a heterologous reporter gene (Mori et al., 1992). The UPRE was then used by this group and the Walter lab in genetic screens to identify yeast that were defective in signaling the UPR. Both screens identified an ER-targeted, serine/threonine kinase (Ire1p/Ern1p) (Mori et al., 1993; Cox et al., 1993). Ire1p is essential for viability during ER stress and is required for the induction of yeast BiP and other ER chaperones. *IRE1* encodes an N-terminal ER targeting sequence followed by a lumenal "stress sensing" domain, an ER transmembrane domain, a kinase domain, and finally, a C-terminal domain of, at that point, unknown function. Both kinase inactive mutants and mutants lacking the C-terminal domain are unable to signal the response. A picture emerged in which the mechanism of Ire1p activation resembled that of many other transmembrane receptor kinases. In response to its appropriate signal (ER stress in this case), Ire1p oligomerizes and is activated by phosphorylation in *trans* (Patil and Walter, 2001). However, the similarities ended there.

An impressive series of papers by the Walter lab in the mid 90s (Patil and Walter, 2001; Kaufman, 1999) revealed a completely unique pathway for signaling the UPR in yeast (Figure 1). First, using a multicopy library, they identified a basic leucine zipper transcription factor, Hac1p, that when overexpressed activated the UPR. Hac1p binds directly to the yeast UPRE and is required for signaling ER stress. Surprisingly, *HAC1* mRNA is expressed even in the absence of stress, but Hac1 protein is only synthesized after ER stress. Activation of the UPR results in the excision of a 252 nucleotide intron from the *HAC1* message and re-ligation of the ends, via a nonspliceosome-mediated reaction. Stimulated by the homology between Ire1's C-terminal domain and mammalian RNaseL, they demonstrated that Ire1p also possesses endoribonuclease activity, which is dependent upon activation of its kinase domain in response to ER stress (Sidrauski and Walter, 1997). Activated Ire1p cleaves the *HAC1* mRNA within the predicted stem-loop structures at either end of the 252 nucleotide intron and requires the activity of Rlg1p, a tRNA ligase, to re-ligate the transcript. Without the inhibitory intron, the spliced *HAC1* message is now efficiently translated. As summarized in Figure 1, UPR activation in yeast requires only

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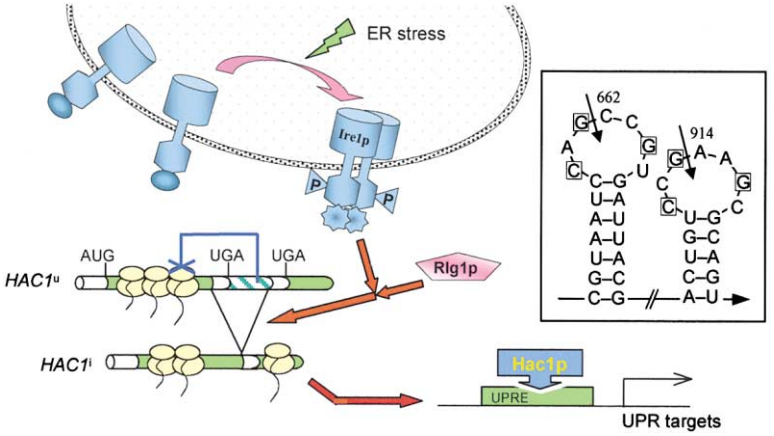

Figure 1. Schematic Illustration of the Yeast UPR Pathway

The yeast UPR requires only three gene products: Ire1p, Rlg1p, and Hac1p. First, during ER stress, Ire1p oligomerization leads to the activation of its kinase domain. This in turn activates the endoribonuclease activity at the C terminus of Ire1p, which removes the 252 nucleotide intron from *HAC1* mRNA that inhibits its translation. Rlg1p then re-ligates the ends of *HAC1* mRNA back together in a spliceosome-independent reaction. Finally, Hac1p is produced and binds to the UPREs present in the ER chaperone promoters, which leads to the transcriptional upregulation of these genes. The insert represents the cleavage site in the stem-loop sequence at the two ends of the *HAC1* intron.

these three gene products: Ire1p, Rlg1p, and Hac1p. Together they upregulate ER chaperone expression to protect the yeast ER from the accumulation of unfolded proteins. It appears that the main and perhaps only target of this remarkable splicing pathway is the *HAC1* mRNA, as cDNAs encoding the spliced form of Hac1p are able to rescue Δ*ire1* cells that have been treated with ER-stress-inducing agents, and no other genes have been identified in the *S. cerevisiae* genome that appear to undergo Ire1p-dependent splicing. Although only three gene products are needed to induce the UPR in yeast, microarray studies reveal that nearly 400 yeast genes are either directly or indirectly affected by its activation (Patil and Walter, 2001).

The Mammalian UPR Machinery: Increased Complexity and Some Baffling Gaps

With the identification of the yeast components of the UPR, the search was on once again with renewed vigor for the mammalian counterparts. In 1998, the labs of Kaufman and Ron independently identified two different mammalian Ire1 homologs, which were named Ire1α and Ire1β (Wang et al., 1998; Tirasophon et al., 1998). Both proteins show a high degree of homology to each other and to the yeast Ire1p kinase, including the endonuclease domain. Ire1α is expressed ubiquitously, while Ire1β appears to be expressed predominantly in the gut epithelium. Overexpression of either protein in a number of different cell lines is sufficient to upregulate ER chaperones. This requires the kinase as well as endoribonuclease activity, as overexpression of kinase and RNase inactive mutants inhibits induction of the UPR. Kaufman's lab demonstrated that immunoprecipitated exogenously expressed Ire1α can cleave yeast *HAC1* mRNA in vitro, verifying that the endonuclease domain is functional. In separate studies, Ron's lab demonstrated that ER stress induces oligomerization and phosphorylation of endogenous Ire1α (Kaufman, 1999). Despite these findings, no *HAC1* homologs were forthcoming in any other organisms, and none of the transcription factors that bound to ER chaperone promoters appeared to be processed like *HAC1* mRNA. Although some reports suggested that Ire1α mRNA and 28S ribosomal RNA might be targets of Ire1's endoribonuclease activity, it was unclear what role these effects might play in inducing downstream components of the mammalian ER stress response. Even more puzzling was the finding that although Ire1α was essential for mouse embryonic development, MEFs from both *IRE1*$\alpha^{-/-}$ and *IRE1*$\alpha^{-/-}$:*IRE1*$\beta^{-/-}$ mice displayed a normal ER stress response.

Shortly after the discovery of the two Ire1 proteins, the labs of Ron and Wek independently discovered a third ER-localized, stress-induced kinase in mammals, which they named PERK and PEK, respectively (Harding et al., 1999; Kaufman, 1999). This kinase shows some homology to the lumenal domain of Ire1, but has no endonuclease domain and is not present in yeast. PERK/PEK is a member of the eIF2α family of kinases, which are activated in response to a variety of cellular stresses. Phosphorylation of eIF2α prevents the assembly of 80S ribosomal initiation complexes and serves to inhibit protein translation during ER stress. Unlike the Ire1 knock outs, interruption of the PERK signaling pathway, in *PERK*$^{-/-}$ mice or in mice bearing a homozygous eIF-2α mutant (S^{51}→A) that can no longer be phosphorylated by PERK, results in profound alterations in the ER stress signaling pathway (Harding et al., 2001; Scheuner et al., 2001). Both mutants exhibit severe disturbances in glucose metabolism and are particularly sensitive to ER-stress-induced apoptosis, owing to their inability to limit the accumulation of unfolded proteins. In addition, cells from these animals are unable to induce the CHOP transcription factor (another target of the UPR pathway) and show a reduced level of ER chaperone induction.

Concomitantly, Mori's lab revisited the mammalian ER chaperone promoters and identified a conserved 19 bp ERSE sequence (CCAAT-9bp-CCACG) that responds to ER stress (Yoshida et al., 1998). Although different from the yeast UPRE, the mammalian ERSE is also found in invertebrates, plants, and fungi. Using an ERSE from the human *GRP78* promoter in a yeast one-hybrid screen, they cloned two genes (Yoshida et al., 1998): ATF6, a bZIP transcription factor that had been previously identified by the Prywes lab, and XBP-1, an *X* box *b*inding *p*rotein. Although ATF6 has some homology to Hac1p in its basic leucine zipper region and, like *HAC1*, *ATF6* mRNA is constitutively expressed, the *ATF6* transcript does not undergo a similar cleavage reaction. Instead, ATF6 is synthesized as an ER-localized transmembrane protein with a lumenal "sensing" domain and

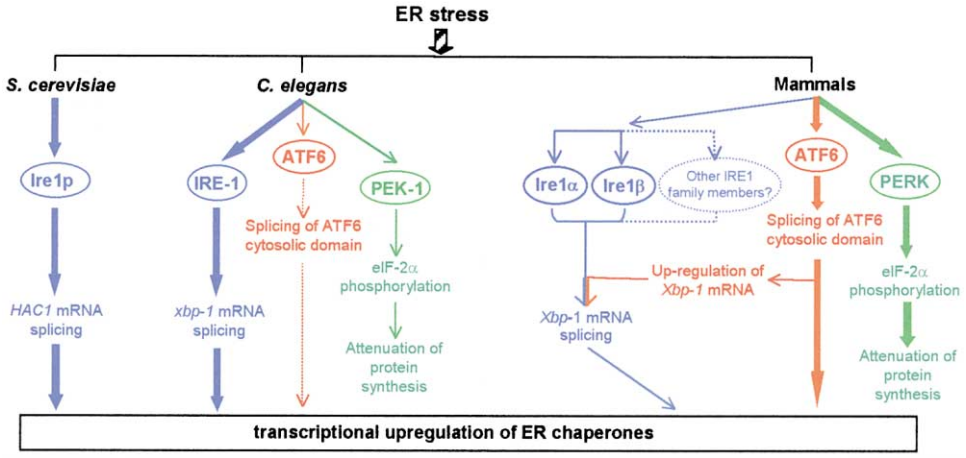

Figure 2. Comparison of UPR Pathway in *S. cerevisiae*, *C. elegans,* and Mammals

Broad lines represent pathway(s) that are predominant in activating the UPR.
Narrow lines represent pathway(s) that are functional in the UPR.
Dotted lines represent pathway(s) that are not required for the activation of ER chaperones during the UPR.

a cytosolic transcription transactivation domain. Upon ER stress, the transactivation domain of ATF6 is cleaved from the ER membrane and transported to the nucleus where it binds ERSEs (Yoshida et al., 1998; Kaufman, 1999). This cleavage was shown by the labs of Brown and Goldstein to be dependent upon the Golgi-localized S1P and S2P proteases, which also cleave the ER-localized sterol responsive element binding protein (SREBP) in response to changes in membrane cholesterol levels. Thus, it appeared that ATF6 fit the bill of the missing mammalian Hac1p transcription factor, but it was not activated in a manner that implicated Ire1 activity. Together, ATF6 and PERK seemed to account for most of the UPR signal transducing activities; there did not seem to be any indication of Ire1-dependent signaling outcomes that were critical to the UPR in mammals. This left the perplexing question of why such a unique bifunctional enzyme was not only retained, but actually duplicated through evolution.

A Substrate for Metazoan Ire1 Proteins!

Data about this missing Ire1 link are provided in two papers published in this issue of *Cell*. The labs of Kaufman (Shen et al., 2001) and Mori (Yoshida et al., 2001) now demonstrate, using *C. elegans* and mammalian model systems, that *XBP-1* mRNA undergoes an Ire1-dependent, *HAC1*-like splicing during UPR. Unlike the 252 nucleotide fragment deleted from yeast *HAC1* mRNA, the excised *Xbp-1* fragment is only 23 bases in *C. elegans* and 26 bases in mammals. In both of the latter cases, this deletion induces a frame-shift in the C-terminal portion of the protein. As demonstrated for mammals, instead of relieving a translation repression sequence, the resulting frame-shift now encodes a new "chimeric" protein having the original N-terminal DNA binding domain and a new C-terminal transactivation domain. Furthermore, *XBP-1* mRNA is actually induced by ATF6 during the mammalian UPR, and the "chimeric" XBP-1 protein binds to the ERSEs in target genes to upregulate their transcription, thus providing a link between the ATF6 and Ire1 systems. Using mutagenesis and RNA interference studies, the Kaufman lab demonstrates the existence of both IRE1 and PERK/PEK-dependent pathways in *C. elegans*. These two kinases provide separate, somewhat redundant signaling pathways that are essential for worm development as well as, in the case of IRE1, for *xbp-1*-mediated chaperone induction. However, although the *ire1*-deficient worms are growth retarded, *pek* null animals show no discernable phenotype, except for higher basal expression of chaperone genes. These findings suggest that normal development elicits an ER stress response, and that in nematodes the ability to upregulate ER chaperones is more important in dealing with ER stress than the ability to limit the load of unfolded proteins. Interestingly, the Kaufman group also mentions that inactivating a *C. elegans* ATF6 homolog in wild-type worms or in *ire-1* or *pek-1* mutants does not produce a significant phenotype. Thus, it remains unclear at the moment how *xbp1* transcription is regulated in *C. elegans* in response to ER stress.

Increasing Complexity and Unresolved Issues

In addition to finding the perplexing missing substrate of Ire1's endoribonuclease activity in higher eukaryotes, these two exciting papers underscore the increased complexity that occurs during evolution (Figure 2). Yeast cells are able to activate the entire UPR pathway with three proteins. Instead of resorting to protein synthesis inhibition to limit damage to the cell, they respond by increasing the degradative machinery to eliminate unfolded proteins and by increasing the volume and components of the entire secretory pathway to accommodate this burden and prevent aggregation of affected proteins. All of this is achieved through the single Ire1-dependent pathway (blue arrows). This pathway is maintained in *C. elegans* but is supplemented with the PEK pathway (green arrows), which, based on homology to mammalian counterparts (PERK), would be expected to limit protein synthesis during ER stress. The data suggest that IRE1 is the dominant signaling pathway in *C. elegans*, but the PEK pathway is functional and contrib-

utes to survival mechanisms during ER stress. Although an ATF6 homolog (red arrows) exists in nematodes, it is evidently not essential for UPR induction. By contrast, mammals have expanded the Ire1 pathway to include two homologs, one of which is essential for embryonic development, but both of which appear to be dispensable for UPR induction, at least under the conditions tested. The PEK/PERK signaling pathway instead has expanded functions, in which it not only plays a major role in limiting the accumulation of unfolded proteins but also contributes to the upregulation of UPR target genes and serves to induce cell cycle arrest and translation of ATF4 in response to ER stress. In addition, the ATF6 transcription factor represents a third mammalian signaling pathway that appears to be essential to UPR induction, based on the overexpression of constitutively active or dominant-negative forms of this protein. However, direct proof awaits the generation and characterization of a mouse lacking ATF6 activity.

So what is Ire1 doing in mammals? The apparent lack of a requirement for Ire1 in UPR induction is somewhat surprising. It is possible that a third Ire1 homolog exists, which could be revealed by examining *XBP-1* mRNA processing in the *IRE1*α*:IRE1*β null mice. However, if *XBP-1* mRNA remains unprocessed, it would appear that mammals do not rely on the Ire1-XBP-1 pathway to signal ER stress. If not, then what is the function of Ire1 proteins and why do mammals have two of them? The fact that both *IRE1*$^{-/-}$ and *XBP-1*$^{-/-}$ mice show an embryonic lethal phenotype and that XBP-1 is essential for both hepatocyte development and the terminal differentiation of B cells to plasma cells (Reimold et al., 2001) might imply that these kinases could play a role in ER expansion during the development and differentiation of certain ER-rich cell types. In support of this idea, yeast Ire1p was independently identified through its role in ER membrane biosynthesis. If the Ire1 proteins can be activated by differentiation signals, this might provide a mechanism for increasing ER chaperones and the secretory capacity of the cell without the coordinate inhibition of protein synthesis and growth arrest, as reported by Brewer et al. (1997). The fact that gut epithelium has a separate Ire1β protein is consistent with the concept that Ire1s could respond to specific differentiation signals. Along these lines, it will be interesting to determine if *XBP-1* mRNA is spliced in B cells undergoing differentiation to plasma cells, and if targeted disruption of *IRE1*α in B cells also prevents differentiation.

These two papers convincingly demonstrate that the novel signaling pathway that regulates the UPR in yeast is indeed maintained in multicellular organisms. Further experiments will be needed to determine if this signaling pathway has taken on new functions in mammalian cells. Obviously, there remains much yet to do, but the pathways are rapidly unfolding.

Selected Reading

Brewer, J.W., Cleveland, J.L., and Hendershot, L.M. (1997). EMBO J. *16*, 7207–7216.

Cox, J.S., Shamu, C.E., and Walter, P. (1993). Cell *73*, 1197–1206.

Gething, M.J., and Sambrook, J. (1992). Nature *355*, 33–45.

Haas, I.G., and Wabl, M. (1983). Nature *306*, 387–389.

Harding, H.P., Zeng, H., Zhang, Y., Jungries, R., Chung, P., Plesken, H., Sabatini, D.D., and Ron, D. (2001). Mol. Cell *7*, 1153–1163.

Harding, H.P., Zhang, Y., and Ron, D. (1999). Nature *397*, 271–274.

Kaufman, R.J. (1999). Genes Dev. *13*, 1211–1233.

Lee, A.S. (1987). Trends Biochem. Sci. *12*, 20–23.

Mori, K., Ma, W., Gething, M.J., and Sambrook, J. (1993). Cell *74*, 743–756.

Mori, K., Sant, A., Kohno, K., Normington, K., Gething, M.J., and Sambrook, J.F. (1992). EMBO J. *11*, 2583–2593.

Patil, C., and Walter, P. (2001). Curr. Opin. Cell Biol. *13*, 349–355.

Reimold, A.M., Iwakoshi, N.N., Manis, J., Vallabhajosyula, P., Szomolanyi-Tsuda, E., Gravallese, E.M., Friend, D., Grusby, M.J., Alt, F., and Glimcher, L.H. (2001). Nature *412*, 300–307.

Scheuner, D., Song, B., McEwen, E., Liu, C., Laybutt, R., Gillespie, P., Saunders, T., Bonner-Weir, S., and Kaufman, R.J. (2001). Mol. Cell *7*, 1165–1176.

Shen, X., Ellis, R.E., Kurnit, D.M., Liu, C.Y., Lee, K., Solomon, A., Morimoto, R.I., Yoshida, H., Mori, K., and Kaufman, R.J. (2001). Cell *107*, this issue, 893–903.

Sidrauski, C., and Walter, P. (1997). Cell *90*, 1031–1039.

Tirasophon, W., Welihinda, A.A., and Kaufman, R.J. (1998). Genes Dev. *12*, 1812–1824.

Wang, X.-Z., Harding, H.P., Zhang, Y., Jolicoeur, E.M., Kuroda, M., and Ron, D. (1998). EMBO J. *17*, 5708–5717.

Yoshida, H., Haze, K., Yanagi, H., Yura, T., and Mori, K. (1998). J. Biol. Chem. *273*, 33741–33749.

Yoshida, H., Matsui, T., Yamamoto, A., Okada, T., and Mori, K. (2001). Cell *107*, this issue, 881–891.

Note Added in Proof

As reported in a paper now in press in *Nature*, the Ron lab has independently identified XBP-1 as a target of Ire1's endonuclease activity in a genetic screen conducted in *C. elegans*. They show that the spliced form of mouse XBP-1 can be generated in vitro with the purified C-terminal tail of Ire1β and that the in vivo splicing of XBP-1 is not observed in fibroblasts from mice lacking both Ire1α and β. Together, these data provide evidence that XBP-1 is a direct target of Ire1 and strongly suggest that the lack of a UPR phenotype that was previously reported in the *Ire1*α*:Ire1*β null cells is not due to the presence of additional Ire1 homologs. In addition, their data reveal that the *XBP-1* transcript that is induced in LPS-treated B cells produces the spliced form of the XBP-1 protein. This supports the suggestion put forward in this review that mammalian Ire1 may have evolved to play a more important role in ER expansion during differentiation. (Calfon, M., Zeng, H., Urano, F., Till, J.H., Hubbard, S.R., Harding, H.P., Clask, S.G., and Ron, D. (2002). *Nature*, in press).