Commentary

Pausing to decide

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Sudden environmental changes and physiological stresses such as osmotic shock, nutrient availability, and heat shock demand that cells have the abilities to modulate their behavior to adapt and survive. Eukaryotic cells face the additional challenges of coordinating these changes between different cellular compartments, and this coordination necessitates interorganelle signaling pathways. For example, in times of stresses in the endoplasmic reticulum (ER), adaptive changes must be coordinated among protein folding capacity in the ER, transcription in the nucleus, and cytosolic synthesis of the proteins for the ER chaperons and protein processing enzymes. The pathway responsible for communicating these changes is called the unfolded protein response (UPR), ultimately leads to a significant remodeling of the entire secretory pathway in yeast. The UPR has proven remarkable both for the uniqueness of its signaling components and for the unprecedented mechanisms by which it is regulated (reviewed in refs. 1–3). In this issue of PNAS, the results of Brewer and Diehl (4) further our knowledge of mammalian UPR and its role in a complex cascade of cellular responses induced by unfolded proteins (5). More specifically, Brewer and Diehl (4) link a UPR-induced G_1 cell cycle arrest with a protein phosphorylation pathway known to globally repress protein translation.

To put the mammalian UPR work by Brewer and Diehl (4) into detailed context, it is best to begin by examining the simpler yeast UPR system. In *Saccharomyces cerevisiae*, the UPR is initiated when the N-terminal region of the transmembrane serine/threonine kinase Ire1p senses activating conditions in the ER lumen. Activation by autophosphorylation and oligomerization allows this signal to be transmitted across the ER membrane. Activated Ire1p functions as a site-specific endoribonuclease that excises a translation-inhibitory intron from the mRNA encoding the UPR-specific transcription factor Hac1p. tRNA ligase then joins the resulting exons to produce a translatable *HAC1* mRNA. It was recently shown that some of the salient features of this unusual splicing pathway, including the capacity to accurately splice the *HAC1* mRNA upon UPR induction, are conserved in mammalian cells (6).

As one might suspect, however, the mammalian UPR turns out to be far more complex (2). Rather than the single IRE1 kinase expressed in yeast, mammalian cells express at least three ER transmembrane kinases, IRE1α, IRE1β, and PERK, and are thought to function as the most ER-proximal effectors of the pathway (5–8). Furthermore, the observed consequences of UPR activation in mammalian cells include not only transcriptional induction of UPR target genes, but also an overall repression of translation and cell cycle arrest. Finally, the outcome of these responses is integrated to make a decision between two cell fates: survival by adaptation to the stressful conditions or elimination by apoptosis, or programmed cell death.

The initial branching of the signaling pathway into two arms, one affecting global translational repression and another leading to the up-regulation of UPR target genes, appears to take place at the ER membrane, immediately following UPR induction. The branch controlling translational repression is thought to be solely mediated by PERK (5, 9). Activated PERK phosphorylates the α subunit of eukaryotic translation initiation factor 2 (eIF2α), which leads to its inactivation and reduces translational initiation. Transcriptional activation of UPR target genes, on the other hand, appears to be mediated by both Ire1 and PERK (7, 8, 10). Thus, in an effort toward adaptation, the UPR increases ER capacity both by slowing down protein translation and protein traffic into the ER, and by transcriptional up-regulation of ER resident proteins involved in protein processing.

In addition to activating these adaptive responses, the UPR leads to the induction of some proapoptotic events such as transcriptional activation of the cell death genes, including CHOP, and activation of the ER-associated caspase-12 (5). Thus, before commitment to either adaptation or cell death, the UPR seems to induce the components necessary for cells to execute either choice expediently. What is critical then, is a cell's ability, at some particular point after UPR induction, to correctly assess the prevailing conditions to decide appropriately between survival and death.

Interestingly, Brewer and Diehl (4) found that UPR activation in tissue culture cells causes cell cycle arrest in G_1, suggesting a mechanism by which cells may expand the time window in which to make this crucial decision (11). The authors showed, mechanistically, that the UPR-induced G_1 arrest is caused by inhibiting translation of cyclin D1. Results presented in this issue of PNAS now extend these findings further by showing that translational inhibition of cyclin D1 is mediated by PERK, thus placing the G_1 arrest squarely on the PERK branch of the mammalian UPR pathway. UPR activation of PERK, thus, is thought to result in translational repression of cyclin D1 via phosphorylation of eIF2α.

There remains, however, a gap in our knowledge. Although PERK-mediated eIF2α phosphorylation (and repression of global translation) occurs almost immediately (within 0.5–1 h), translational repression of cyclin D1 took place 16–20 h after the UPR induction. Remarkably, at this late time point, translational inhibition is specific to cyclin D1; production of proteins including CHOP and BiP are normal by this time. Thus, because the phosphorylation of eIF2α may not necessarily occur in the same time frame as cyclin D1 repression, the molecular basis for the specificity of cyclin D1 translational repression remains uncertain. Indeed, if the eIF2α phosphorylation is sustained over the course of the UPR activation, then there must be a mechanism to overcome inefficient translation initiation of proteins like BiP and CHOP. Alternatively, if eIF2α is dephosphorylated at later time points, it would raise the interesting question of how cells specifically target cyclin D1 for translational repression. In other words, what makes cyclin D1 mRNA special?

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Article published online before print: *Proc. Natl. Acad. Sci. USA*, 10.1073/pnas.250476097. Article and publication date are at www.pnas.org/cgi/doi/10.1073/pnas.250476097

In this regard, it is interesting to note that under a different kind of stress, namely amino acid starvation in yeast, eIF2α phosphorylation can modulate mRNA-specific translation. Here, eIF2α is phosphorylated by GCN2 kinase at the same residue that PERK targets in mammalian cells. During starvation, this phosphorylation results in overall repression of translation (12), but remarkably, the translation of an otherwise inefficiently translated *GCN4* mRNA, encoding for a transcription factor regulating the expression of biosynthetic enzymes, occurs rather efficiently. The ability of *GCN4* mRNA to escape translation repression caused by GCN2 activation is mediated by short open reading frames (uORFs) within the 5′ untranslated region (UTR) of the *GCN4* mRNA. Interestingly, depending on their relative position and spacing in the 5′ UTR, some uORFs can have positive effects while others can have negative effects on translation of the downstream ORF encoding the protein of interest. Similarly, a small uORF is present within the 5′ UTR of the yeast cyclin D1 homologue *CLN3* and this uORF has been shown to regulate translation of *CLN3* mRNA during the cell cycle (13). In light of these observations, then, it becomes particularly intriguing that cyclin D1 mRNA also has two suspected uORFs in the 5′ UTR, raising the possibility that those uORFs play active roles in PERK-mediated repression of cyclin D1 translation.

The cyclin D1–CdC28 complex initiates expression of other cyclins and genes involved in DNA replication. Because expression of cyclin D1 is strongly influenced by conditions affecting cell growth, cyclin D1 has been suggested to play a pivotal role in coupling growth rate to cell cycle progression (14). Thus, eIF2α phosphorylation by PERK would allow UPR-induced cells to use a more general stress response to regulate cell cycle progression. UPR-induced cell cycle arrest might therefore be the key to establishing a time window for cells to decide whether conditions are promising enough to proceed with the cell cycle or bleak enough to choose programmed cell death. Indeed UPR-induced cell cycle arrest may give cells an opportunity to pause and decide.

Phosphorylation of eIF2α, in fact, is a shared output of many stress pathway kinases, such as hemin-regulated inhibitor of protein synthesis (HRI) (15), the interferon-inducible RNA-dependent kinase (PKR) (protecting cells from viral infection) (16), and a general control of amino acid biosynthesis kinase (GCN2) (12). The common consequence of eIF2α phosphorylation by all of these kinases is the repression of translation initiation. As eIF2α is phosphorylated in each case at the very same residue, downstream responses cannot "know" where the signals originated. Thus, it is plausible that the PERK branch of the UPR signaling pathway leads to the induction of a general stress response to augment a more ER-specific response. In this view, this latter branch is likely to be activated by Ire1 and would induce a response to meet more specifically the protein folding needs of the ER. Thus, both branches induce responses that ultimately act cooperatively to induce beneficial changes, one by broadly adjusting cell physiology and the other by inducing changes specific to the ER and secretory pathway. What become important now are the identification of the mammalian components that lie downstream of Ire1 and deciphering the mechanism by which cells make their life-and-death decision.

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