# Putting the brakes on the unfolded protein response

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The unfolded protein response is an ancient cellular pathway for rapidly responding to endoplasmic reticulum stress. Two studies in this issue (Rubio et al. 2011. *J. Cell. Biol.* doi:10.1083/jcb.201007077 and Chawla et al. 2011. *J. Cell. Biol.* doi:10.1083/jcb.201008071) provide insight into how the unfolded protein response is tamped down to restore normal endoplasmic reticulum function. Although both papers implicate the Ire1 kinase domain as the key effector of the off-switch mechanism, alternate models for how this is achieved are proposed.

The unfolded protein response (UPR) is triggered when the protein folding machinery in the ER becomes overwhelmed by an accumulation of misfolded or unfolded proteins. This occurs under a broad range of physiological conditions, such as viral infections, chemical disruption of ER calcium stores or disulfide bonds, and inhibition of protein glycosylation (Marciniak and Ron, 2006). The UPR signals a transcriptional response resulting in synthesis of resident ER protein folding and degradative enzymes (Travers et al., 2000), leading to ER expansion (Bernales et al., 2006; Schuck et al., 2009). The ER transmembrane protein, Ire1, is a key signaling element in the UPR consisting of an intra-ER lumenal domain that directly or indirectly senses misfolded proteins and a cytoplasmic tail with protein kinase and endoribonuclease domains (Sidrauski and Walter, 1997). Sensing of misfolded proteins causes lateral oligomerization of the Ire1 lumenal domains, trans-autophosphorylation, and activation of the nuclease (Shamu and Walter, 1996). In the yeast *Saccharomyces cerevisiae*, ER stress causes the ribonuclease domain of Ire1 to excise a 252-nt translational-inhibitory intron from *HAC1* mRNA (Cox and Walter, 1996). The separated *HAC1* exons are joined by nonconventional splicing in the cytoplasm by tRNA ligase. Finally, the mature, spliced mRNA is translated into Hac1 protein, a bZIP transcription factor for ER chaperones and protein folding enzymes that restores ER function. Although remarkable progress has been made in understanding UPR signaling, how the enhanced ER function is attenuated when protein folding is restored has remained a mystery. Two studies in this issue (Rubio et al. and Chawla et al.) both point toward a key role for the Ire1 kinase domain in the down-regulation of the UPR, though in surprisingly different ways.

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Previous studies showed that mutations in the catalytic site of the Ire1 kinase prevented *HAC1* mRNA splicing and UPR target gene expression (Cox et al., 1993; Mori et al., 1993). However, the kinase activity itself is not absolutely required for Ire1's RNase function. When the ATP-competitive drug, 1NM-PP1, binds to an Ire1 with a mutated nucleotide-binding pocket, the ribonuclease activity in response to ER stress is switched on in the absence of phosphorylation (Papa et al., 2003). Similarly, the antiviral endoribonuclease and pseudokinase, RNase L (Zhou et al., 1993), is highly homologous in its C-terminal half to the cytoplasmic portion of Ire1 (Dong et al., 2001). RNase L, which lacks protein kinase activity, has a ribonuclease domain that is activated by 2′-5′-oligo(rA) produced during viral infections (Dong and Silverman, 1999). Thus, RNase L activation/inactivation are uncoupled from kinase activity. So what, then, is the function of the Ire1 kinase?

In Rubio et al. (2011), a double mutant within the Ire1 kinase domain (D797N,K799N) was designed to block phosphoryl transfer while preserving ATP binding (Rubio et al., 2011). This Ire1 kinase-inactive mutant was nevertheless functional for ribonuclease activity. Interestingly, although the Ire1 kinase activity was dispensable for RNase function, it appeared critical for turning off Ire1 function. Mutant Ire1 oligomerized in the ER membrane, cleaved *HAC1* pre-mRNA, and promoted synthesis of Hac1 protein, induction of UPR target genes, and ER expansion in vivo. The mutant Ire1 was activated by misfolded proteins; however, unlike wild-type IRE1, the mutant Ire1 failed to deactivate late in the stress response, and its presence reduced cell survival of ER stressed yeast. The severe growth defect was linked to UPR overload of the ER import machinery with additional pleiotropic effects. Thus, Ire1 kinase activity appeared to be required for disassembly of the Ire1 signaling complex and enhanced cell survival in a homeostatic control mechanism. These studies reinforced the view that the Ire1 kinase regulates RNase activity while revealing a critical role for phosphorylation in the homeostatic feedback of the UPR. The authors propose that trans-autophosphorylation on sites within the Ire1 kinase domain, in a C-lobe region termed the hyperphosphorylation loop (HPL), is required for rapid de-oligomerization of Ire1 as a reset mechanism (Fig. 1 a). Consequently, it was proposed that phosphoryl transfer by the Ire1 kinase is important for Ire1 shutoff.

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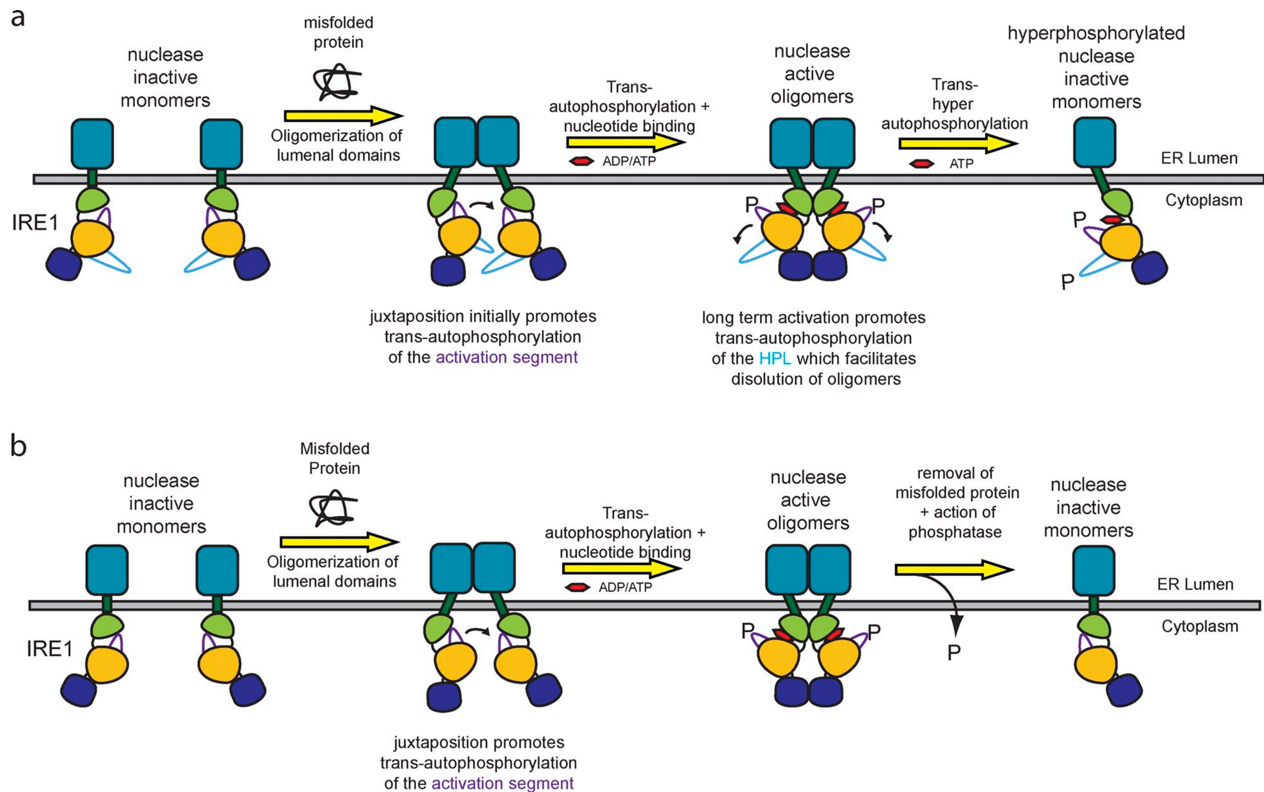


Figure 1. **Turning off the UPR.** Schematics of phosphorylation (a)- and dephosphorylation (b)-driven models for attenuation of Ire1 activity. Domains colored light blue, green, yellow, and dark blue correspond to Ire1 lumenal, kinase N-lobe, kinase C-lobe, and endoribonuclease domains, respectively. Kinase domain loop elements in purple and light blue correspond to the activation segment and hyper phosphorylated loop (HPL), respectively. P corresponds to one or more phosphate moieties.

A contrasting view of Ire1 de-activation is presented in Chawla et al. (2011), obtained through characterization of an extensive panel of Ire1 kinase domain mutants. These authors found that mutation of some but not all catalytic residues and residues in the kinase activation segment, including sites of autophosphorylation, resulted in a prolonged presence of spliced *HAC1* mRNA after UPR induction in yeast by tunicamycin (Tm). Homeostasis of unspliced *HAC1* mRNA, *BIP* mRNA, and protein and carboxyl peptidase Y activity was restored at late times after treatment with Tm in yeast with wild-type Ire1, but not in ΔIre1 cells. Recovery occurred more rapidly upon Tm removal from culture medium in the wild type than in mutant Ire1 cells. Chawla et al. (2011) reasoned that because the activation segment of Ire1 is autophosphorylated during UPR (Shamu & Walter, 1996; Lee et al., 2008), dephosphorylation might deactivate Ire1 (Fig. 1 b). This model is in contrast to Rubio et al. (2011), in which phosphorylation not dephosphorylation is the driver of Ire1 deactivation. To test their model, Chawla et al. (2011) generated a phosphomimetic version of Ire1 (S840D/S841D/T844D within the activation segment), predicted to leave kinase phosphotransfer function intact but render the enzyme unresponsive to the action of phosphatases (Chawla et al., 2011). Strikingly, the triple mutation resulted in an Ire1 that could still respond to Tm and could autophosphorylate robustly, but which did not return to baseline after resolution of the ER stress (i.e., *HAC1* mRNA continued to be spliced even after removal of Tm). This suggested that phosphorylation activity was not the principle driver for the shutoff of Ire1 ribonuclease activity.

Characterization of additional single site and multisite Ire1 mutants and their behavior in combination provided valuable insight into how dephosphorylation of the activation segment might serve as a shutoff switch for the UPR (Chawla et al., 2011). The simplest model that can explain the aggregate behavior of mutants tested in the two studies, anchored by precedents set in other protein kinase systems (for review see Nolen et al., 2004), centers on the phosphorylation-dependent transition of the activation segment between alternate conformation states: one compatible, the other incompatible with Ire1 ribonuclease function. The ribonuclease-incompatible state is likely organized by interaction of the dephosphorylated activation segment, with a subset of active site residues in the kinase catalytic cleft. Phosphorylation of hydroxyl-bearing residues within the activation segment (namely Ser840, S841, and Thr 844) disrupts this off-state conformation, transitioning the activation segment into a conformation supportive of Ire1 ribonuclease function. With this model, the behavior of the characterized mutants can be rationalized by the sum of perturbatory effects on the ability of Ire1 to transfer phosphate versus effects on the stability of the off-state conformation of the activation segment.

In closing, the newfound understanding of how the autokinase activity of Ire1 regulates the turn-on and shutoff of Ire1 ribonuclease function helps make sense of two striking features

that differentiate RNase L from Ire1. As noted, although RNase L shares many of the architectural features of a bona fide protein kinase domain, it has dispensed with phosphoryl transfer function (Dong and Silverman, 1999). Coincident with this loss, RNase L appears to lack a recognizable activation segment and substrate recognition infrastructure including the P + 1 loop, helix αEF, and likely helix αG. Lacking an activation segment that transitions between productive and nonproductive conformations in response to autophosphorylation signals removes the premium on maintaining protein kinase function or substrate recognition infrastructure. But this begs the question, what was lost first, the activation segment or kinase activity? A remaining conundrum is why shutoff of compromised Ire1 mutants requires ER stress to become active in the first place? One might imagine constitutive activation in the absence of ER stress. The shutoff of compromised mutant behavior is reminiscent of 1-NM-PP1 activatable mutant Ire1 proteins (Papa et al., 2003). In the absence of ER stress, this small molecule is not sufficient to activate Ire1 ribonuclease activity but is absolutely required for mutant Ire1 activation in the presence of ER stress. One would expect that if both cytoplasmic (autophosphorylation or ligand binding) and lumenal (detection of unfolded protein) signals are required for Ire1 activation, then loss of either one should be sufficient for shutoff. However, this is not the case and will surely be fodder for future studies.

Finally, it is worth noting that these two papers may be relevant to a long list of human diseases characterized by ER stress, including cancer, neurodegenerative diseases, diabetes mellitus, and atherosclerosis (Marciniak and Ron, 2006). Therefore, the findings could eventually provide a basis for novel therapeutic strategies that regulate UPR shutdown. For instance, if the Chawla et al. (2011) model turns out to be correct, phosphatases that dephosphorylate Ire1 might be therapeutic targets for inhibitor drugs to sustain the UPR.

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