A first line of defense against ER stress

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BiP is the predominant DnaK/Hsp70-type chaperone protein in the ER. It is required for folding and assembling newly synthesized ER client proteins, yet having too much BiP inhibits folding. In this issue, Chambers et al. (2012. *J. Cell Biol.* doi:10.1083/jcb.201202005) report that ADP ribosylation of BiP provides a reversible switch that fine tunes BiP activity according to need.

Too much of a good thing can be bad. Just as an overzealous parent can impede a child's development, so can overactive molecular chaperones slow protein folding. Chaperones are ancient and universally conserved machines that are required at nearly every stage of a protein's life: they assist in the initial folding of polypeptides, assembly of protein complexes, inhibition of toxic aggregation, and stabilization of unfolded states so that they can be degraded (Bukau et al., 2006). Perhaps counterintuitive, a too-high concentration of chaperones inhibits protein folding (Dorner et al., 1992). This effect is a result of overstabilization of the unfolded state and results in increased degradation (Otero et al., 2010). Accordingly, translational efficiency of chaperones can be feedback regulated (Gülow et al., 2002).

In eukaryotes, transmembrane and secreted proteins are folded and assembled in the ER. Cells confront the challenge of a variable flux of proteins entering the ER. Perturbations in protein flux can result from rapid environmental changes, such as fluctuating nutrients that vary with feeding and fasting cycles, or long-term physiological programs, such as differentiation. To meet fluctuating demands and maintain optimal homeostasis of protein maturation, the ER must continually monitor and adjust its protein folding capacity.

Chaperone proteins and enzymes that add posttranslational modifications assist in the folding and maturation processes in the ER (Sitia and Braakman, 2003). When the flux of unfolded proteins entering the ER surpasses the capacity of the folding machinery, a condition termed ER stress arises. In response, ER resident transmembrane sensors activate a network of intracellular signaling pathways, collectively called the unfolded protein response (UPR; Walter and Ron, 2011). The UPR induces a comprehensive transcriptional program that leads to enhanced expression of genes encoding machinery to increase the folding capacity of the organelle. Additionally, the UPR inhibits protein translation and initiates the degradation of some ER-bound mRNAs, thus decreasing the load of unfolded proteins entering the compartment. The increase of the folding capacity of the ER mediated by the transcriptional response, however, takes hours to take appreciable effect, and the reduction in load afforded by translational attenuation and mRNA degradation has no effect on the accumulated unfolded proteins already present in the ER. Thus, a need exists for mechanisms allowing rapid fine tuning of the ER's folding capacity.

In this issue, Chambers et al. (2012) report a mechanism that acts to respond quickly to changing conditions in the ER lumen before the UPR takes effect. It was noticed in the 1980s that a fraction of the major ER resident chaperone BiP, a DnaK/Hsp70 family member, exists in an ADP-ribosylated form and that this fraction is inversely proportional to the folding load in the ER (Carlsson and Lazarides, 1983; Ledford and Jacobs, 1986; Hendershot et al., 1988; Leno and Ledford 1989). Though it had been proposed that ADP ribosylation could serve as a rapid regulator of BiP activity, only correlative evidence was reported. Now, in the current work, Chambers et al. (2012) characterize the physiology of BiP-ADP ribosylation, map the modification sites, provide insight into the biophysical mechanism by which ADP ribosylation can inactivate BiP, and lend compelling quantitative support for the notion that this modification provides a mechanism of regulating BiP activity. The results of the study lead to the working model that partitioning BiP between an active and a latent ADP-ribosylated pool allows the cell to adapt quickly (Fig. 1).

To assess the physiological regulation of BiP-ADP ribosylation, the authors monitored the modification state of BiP in extracts from mouse pancreas after periods of feeding or fasting. After feeding, when secretory demand on the pancreas is high, ADP-ribosylated BiP was below the limit of detection. In contrast, after fasting, when the secretory load in the pancreas is low, ~50% of BiP was ADP ribosylated. Moreover, the ADP-ribosylated form of BiP was depleted from a high–molecular weight multichaperone complex in which the unmodified form was enriched, suggesting that the modified form is not engaged in folding substrates.

After mapping two potential ADP ribosylation sites, the authors took an in vitro approach to understand the effect of the modification on BiP function. The crystal structure of the

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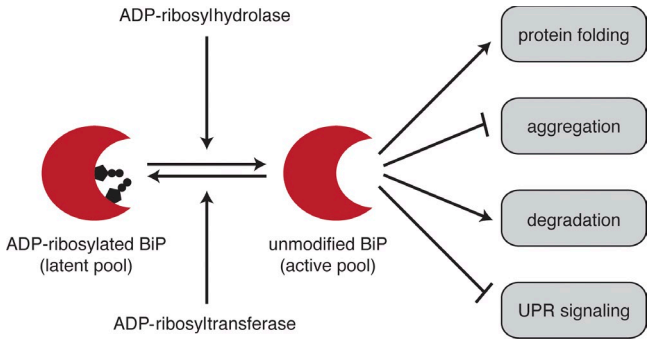

Figure 1. **ADP ribosylation provides a reversible switch that fine tunes BiP activity.** The unmodified active pool of BiP contributes to protein folding and degradation and inhibits aggregation and UPR signaling. If too much unmodified BiP is present, then folding is inhibited as the unfolded state is stabilized and degradation is increased. To improve efficiency of protein folding, excess BiP is ADP ribosylated by an unknown ADP-ribosyltransferase. This pool of inactive BiP can be reactivated by removal of the modification by an unknown ADP-ribosylhydrolase.

substrate-binding domain of the BiP homologue DnaK provided some hints: one of the mapped ADP ribosylation sites is predicted to make an intramolecular ionic interaction with the lid domain involved in substrate engagement and in the allostery underlying the chaperone's cycle of substrate binding and release (Schlecht et al., 2011). Thus, the authors hypothesized that ADP ribosylation would destabilize the closed-lid conformation of BiP, thereby diminishing its ability to bind to substrates. To test this idea, the authors designed an ADP ribosylation mimetic. Though the mimetic lacks the bulk of the true ADP-ribosyl moiety, it mimics the negative charge of the modification and, hence, is likely to underestimate the destabilizing effect that ADP ribosylation would have on the closed-lid conformation.

Nevertheless, the mimetic mutant BiP displayed a 40-fold decrease in the stability of the substrate-bound complex compared with the wild type, supporting the idea that ADP ribosylation would impair substrate binding. Furthermore, in the presence of ATP and substrate peptide, both the mutant and the ADP-ribosylated form of BiP were resistant to the specific BiP protease SubA (which preferentially cleaves the closed-lid, substrate-bound form of wild-type BiP), suggesting that both mostly populate the open-lid, unbound conformation.

To gain quantitative insight into the potential benefits of the modification, the authors built a mathematical model based on kinetic theory. The model reports on protein folding, aggregation, and degradation as a function of fluctuations in secretory load from feeding and fasting cycles. The model compared the consequences of BiP up-regulation through the UPR alone or in combination with reversible ADP ribosylation. Importantly, including ADP ribosylation resulted in 10% less aggregation and 25% less degradation. The predicted reduction in protein aggregation resulted from the quick recruitment of the inactive pool of BiP through removal of the ADP-ribose, whereas the predicted decrease in degradation resulted from a rapid inactivation of BiP by modification after it was no longer required. The model reveals the value of sequestering excess BiP from the active pool, which otherwise impairs protein folding by wasteful degradation. This result underscores the importance of the often-overlooked facet of homeostasis, the deactivation of the response.

As is the case for all advances in our understanding, many more questions arise. What are the enzymes responsible for adding and removing the ADP-ribose? Once we know the enzymes that regulate BiP, it will be important to understand their regulation that must reflect conditions in the ER. How universal is this mechanism? It will be valuable to delineate the scope of cell types and organisms in which BiP-ADP ribosylation occurs. How important is the transcriptional activity of the UPR during normal physiological fluctuations? In light of the quick and acute response afforded by BiP modification, the role of the UPR may need to be recast primarily as a longer-term adaptation process. What are the limits of the response? How much of an increase in unfolded protein load can the pool of latent BiP cope with? What are the physiological consequences of removing the ability for BiP to be ADP ribosylated (i.e., what is the fitness cost of the predicted 10% increase in aggregation and the 25% increase in degradation)? What is the role, if any, of ADP ribosylation in regulating BiP's interaction with the UPR sensor proteins? BiP binds to the ER stress sensors, so ADP-ribosylated BiP may be ideally suited to tune UPR activity. Do inactive pools of other chaperones exist in the ER or other compartments in the cell?

Finally, this work epitomizes the power of multidisciplinary and multiscale approaches to distill functional insight from complex biological systems. It provides an elegant example of a synergistic combination of in vivo, in vitro, and in silico techniques, connecting a descriptive physiological correlate to a molecular mechanism and embedding the interpretation of the results in a formal theoretical framework.

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