News & Views

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In case of stress, hold tight: phosphorylation switches PDI from an oxidoreductase to a holdase, tuning ER proteostasis

Joao PL Coelho & Matthias J Feige

Eukaryotic cells have evolved multiple responses that allow endoplasmic reticulum (ER) homeostasis to be maintained even in the face of acute or chronic stresses. In this issue, Yu *et al* (2020) describe how site-specific phosphorylation switches protein disulfide isomerase (PDI) from a folding enzyme to a holdase chaperone which regulates ER stress responses, thus highlighting PDI as a key player in ER homeostasis.

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See also: **J Yu *et al*** (May 2020)

Cells interact via secreted and membrane proteins. In every eukaryotic cell, these are produced in the ER. It is thus essential for cell and organism homeostasis that protein production in the ER proceeds with high fidelity. This is made possible through a comprehensive ER protein folding machinery that supports protein folding, accelerates slow reactions in protein structure formation, and inhibits side reactions (Ellgaard *et al*, 2016).

In light of these critical functions, the ER has to have fail-safe mechanisms that engage in the event that protein folding is compromised, be it due to acute stresses like sudden spikes in protein production or more persistent stressors like hypoxia or the presence of permanently misfolded proteins (Preissler & Ron, 2019). One of the mechanisms evolved to sense and safeguard the integrity of the ER is the unfolded protein response (UPR). UPR stress sensing in the mammalian ER is based on three sensors, IRE1, PERK, and ATF6 (Karagoz *et al*, 2019). The UPR mounts a transcriptional and translational response to re-establish ER homeostasis by increasing ER protein folding and quality control capacity while reducing the flux of new proteins into the organelle. If cells fail to overcome ER stress, apoptosis is induced to protect the organism at the expense of sacrificing individual cells (Hetz & Papa, 2018). However, UPR measures generally work on the hour-timescale, which may be insufficient to deal with acute stress scenarios. Indeed, cells appear to also be capable of inducing rapid responses to ER protein folding stress. This has been delineated for BiP, a major ER chaperone, which becomes inactivated by oligomerization and AMPylation. Upon ER stress, these processes are rapidly reversed, allowing BiP to quickly act as a chaperone when needed (Preissler & Ron, 2019). This provides a fast response independent of *de novo* protein synthesis.

Yu *et al* (2020) now show that protein disulfide isomerase (PDI) is another key protein in protecting cells against acute ER stress. PDI was the first folding enzyme to be identified in the ER (Goldberger *et al*, 1963) and even after more than 50 years of study it still holds surprises. Yu and colleagues take an approach from phenotype to molecular characterization; they show that deletion of the secretory pathway protein kinase Fam20C increases ER stress signaling by IRE1. Having identified Fam20C as a UPR modulator, the authors used mass spectrometry to identify a possible link between Fam20C and IRE1. This revealed PDI as a major stress-regulated interaction partner of Fam20C. The authors show that multiple ER stressors induce rapid phosphorylation of a major fraction of PDI within minutes—before any translational effects of the UPR. Three serines within PDI are phosphorylated by Fam20C, and among those, the authors focus on phosphorylation of Ser357. The evolutionarily conserved Ser357 is positioned in a strategically very interesting position in PDI, the flexible linker that connects the last two of its four thioredoxin domains. Why interesting? Because PDI can act as a disulfide isomerase as well as a chaperone, and how regulation of these functions is coupled to the intrinsic flexibility of this multidomain protein has remained poorly understood (Freedman *et al*, 2017). Yu *et al* now provide new insights into this issue (Fig 1). Biochemical and *in silico* data suggest that PDI adopts an open conformation once Ser357 is phosphorylated, exposing an extended hydrophobic patch. This goes hand in hand with an increased chaperone activity and a decreased oxidoreductase function for PDI. PDI phosphorylation thus has pronounced effects on PDI function—and also on ER homeostasis as a whole. In cells, this functional switch appears to counteract protein misfolding in the ER, which the authors find to strictly depend on Ser357 within PDI. Loss of PDI (or this critical Ser) rendered cells more susceptible to lethal ER

Department of Chemistry and Institute for Advanced Study, Technical University of Munich, Garching, Germany
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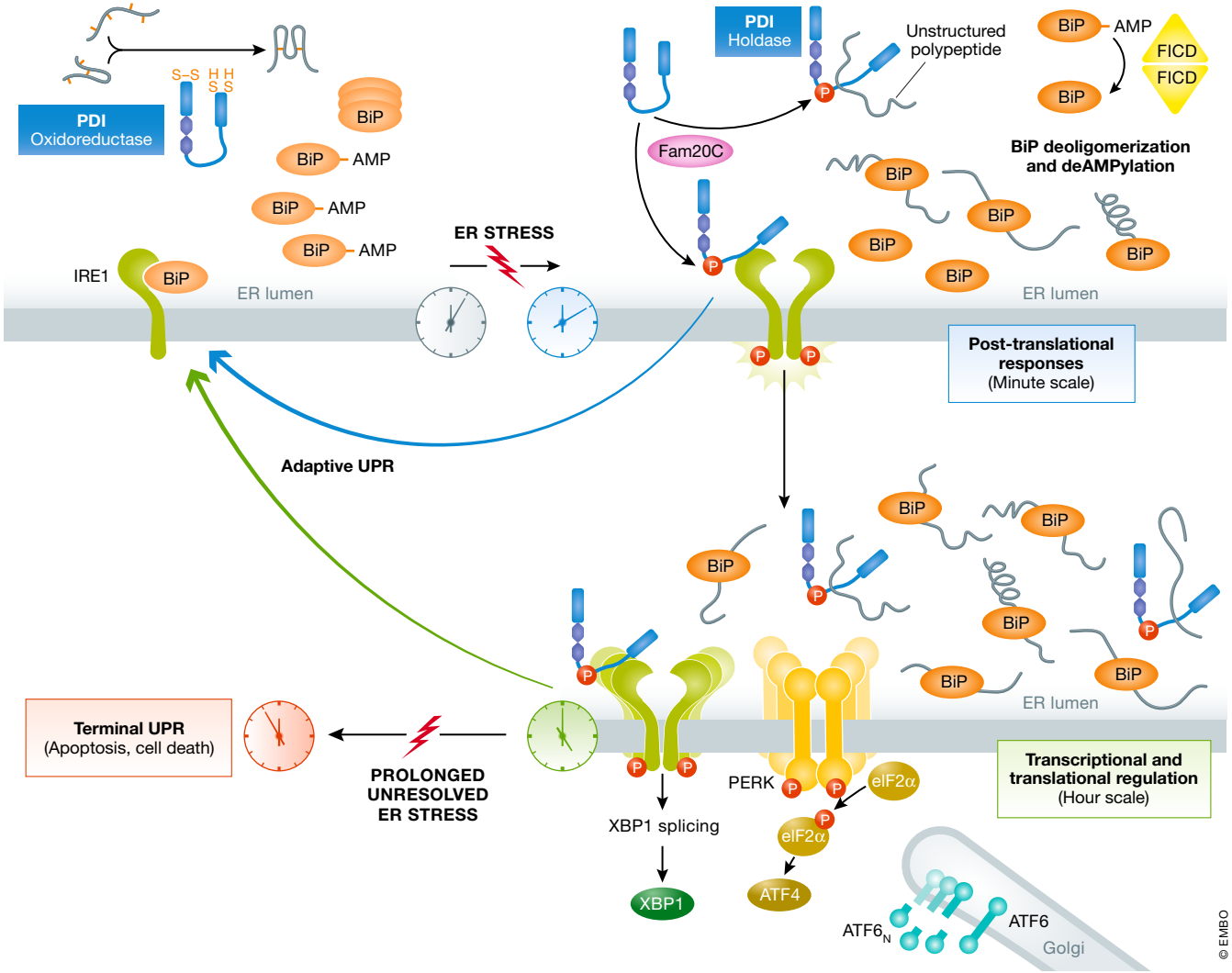

Figure 1. Timecourse of ER stress responses and the role of PDI.
In non-stress conditions, PDI (in blue) is acting as a pro-folding enzyme, through the reduction, oxidation, and reshuffling of disulfide bonds. The UPR is kept inactivate e.g. by the stabilization of monomeric IRE1 (in green) through BiP binding (in orange); BiP itself is partially inactivated through oligomerization and AMPylation. Minutes after the begin of ER stress, Fam20C kinase (in pink) phosphorylates PDI at Ser357 promoting an open conformation which enhances PDIs holdase activity, thus enabling interaction with unfolded peptides and protection against aggregation in the ER lumen. In the same timescale, the pool of inactive BiP is activated by (i) deoligomerization, and (ii) deAMPylation carried out by FICD dimers. BiP displacement from IRE1 leads to its dimerization, trans-autophosphorylation, and splicing of XBP1 transcripts. Phosphorylated PDI is able to bind IRE1, and this phosphorylation-dependent interaction modulates the adaptive UPR. Prolonged, unsolvable ER stress leads to (hyper)activation of the different UPR branches and will ultimately induce apoptosis.

stress. And this phosphorylation switch appeared to have yet another function; it increased association of PDI with IRE1 in a cysteine-independent, chaperone-like manner. IRE1 binding was paralleled by a reduction in its XBP1 mRNA splicing activity. Thus, PDI is the link that closes the loop between Fam20C deletion and increased ER stress. Finally, the weight of this study comes to light when the authors move to animal models, generating mice where PDI lacks this key serine residue. Cellular responses after ER stress show that lack of PDI phosphorylation led to an exacerbated UPR response, including upregulation of apoptosis markers, as well as pro-inflammatory pathways, resulting in aggravated liver damage.

By using comprehensive approaches from molecular simulations to mouse models, Yu *et al* make a major contribution to our understanding of ER proteostasis. The authors reveal how different functions of one of the best-understood ER folding enzymes/chaperones can be regulated, how they affect ER protein (mis)folding, and what the consequences on ER stress and cellular survival *ex* and *in vivo* are. PDI phosphorylation as a novel, fast means to control stress responses is in line with recent reports on other rapid ER stress responses. The study also further highlights the intimate connections between direct rapid cellular responses (PDI chaperone functions) and the regulation of slower adaptive measures cells take to maintain ER homeostasis (via the discovered PDI:IRE1 axis). It nicely fits into an emerging concept that under acute stress, the ER folding machinery first inhibits aggregation rather than supporting folding,

as also exemplified by BiP re-activation (Preissler & Ron, 2019), and inactivation of oxidative folding in the ER—if conditions become too oxidative (Sevier *et al*, 2007)—and now by PDI, which switches from promoting oxidative folding to protecting its clients from aggregation. Proteins involved in these rapid responses appear to often feed back into the UPR signaling branches. This likely allows cells a smoother transition from acute to chronic ER stress responses, without ever leaving a dangerous gap of strong protein misfolding that may irreversibly commit cells to apoptosis (Lam *et al*, 2020) when rescue was still possible.

Important findings always open up more questions. It will be very interesting to see if other Fam20C clients are also involved in ER stress responses, since abundance does not necessarily mean importance. A key question is also the regulation of Fam20C function and trafficking itself. The study additionally provides new perspectives on PDI regulation, which warrant further investigation, and further establishes the many ER PDIs not only as folding enzymes, but also as regulators of ER homeostasis (Oka *et al*, 2019). Lastly, the interplay between PDI and IRE1 adds a very important new aspect to the intricate regulation of this stress sensor (Karagoz *et al*, 2019). Future studies will now have to further refine the emerging picture of closely intertwined ER stress responses, which act on a very different timescale (Fig 1) and involve post-translational, as well as transcriptional and translational responses to protect this key organelle.

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