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Review

Endoplasmic reticulum stress and fungal pathogenesis

*Karthik KRISHNAN, David S. ASKEW**

Department of Pathology & Laboratory Medicine, University of Cincinnati, 231 Albert Sabin Way, Cincinnati, OH 45267-0529, USA

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ABSTRACT

The gateway to the secretory pathway is the endoplasmic reticulum (ER), an organelle that is responsible for the accurate folding, post-translational modification and final assembly of up to a third of the cellular proteome. When secretion levels are high, errors in protein biogenesis can lead to the accumulation of abnormally folded proteins, which threaten ER homeostasis. The unfolded protein response (UPR) is an adaptive signaling pathway that counters a buildup in misfolded and unfolded proteins by increasing the expression of genes that support ER protein folding capacity. Fungi, like other eukaryotic cells that are specialized for secretion, rely upon the UPR to buffer ER stress caused by fluctuations in secretory demand. However, emerging evidence is also implicating the UPR as a central regulator of fungal pathogenesis. In this review, we discuss how diverse fungal pathogens have adapted ER stress response pathways to support the expression of virulence-related traits that are necessary in the host environment.

1. Introduction

The ability of fungi to sense environmental stress and mount an appropriate response is essential for survival in the diverse biological niches occupied by these organisms. The endoplasmic reticulum (ER) is important for many adaptive responses because of its role as the initial folding and processing center for proteins that are destined for delivery into, or across, the plasma membrane, or to other parts of the endomembrane system. Nascent polypeptides enter the ER in an unfolded state, but must be folded accurately before they can transit to their target organelles. The ER lumen provides an oxidizing environment that is conducive to protein folding, and contains an abundance of ER-resident chaperones, foldases and a variety of other enzymes to help proteins achieve their native conformation (Braakman and Hebert, 2013). However, the high concentration of proteins in this milieu increases the risk for illegitimate interactions during the folding process, which can lead to misfolding and aggregation events that are detrimental to cell physiology. Thus, when the demand for secretion is high, or when a fungus encounters environmental conditions that impair ER function beyond the folding capacity of the ER, aberrantly folded or unfolded proteins may accumulate in the ER lumen. The ensuing ER stress initiates a complex series of adaptive events that collectively form the unfolded protein response (UPR)

* *Corresponding author.*
E-mail address: David.Askew@uc.edu (D. S. Askew).
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(Moore and Hollien, 2012). The UPR restores protein folding homeostasis by increasing the folding capacity of the ER, in addition to regulating the disposal of irreparably damaged proteins by ER-associated degradation (ERAD).

2. The UPR in model fungi

The mammalian UPR is comprised of a tripartite signaling system, each of which is triggered by a separate ER stress sensor embedded in the ER membrane: Ire1, Atf6, and Perk (Moore and Hollien, 2012). When confronted by unfolded proteins, the first two proteins work together to reprogram the transcriptome into a state that bolsters the folding capacity of the ER. This is accomplished by transcription factors that induce the expression of genes that directly influence the secretory pathway at multiple levels. The transcriptional rewiring mediated by Ire1 and Atf6 works in conjunction with Perk, an ER transmembrane kinase that reduces the workload imposed on the ER by phosphorylating eukaryotic translation initiation factor 2 subunit α (eIF2 α), resulting in widespread translation attenuation (Harding *et al.*, 1999). Current evidence suggests that fungi rely on Ire1 as the sole ER stress sensor, although accumulating data suggests that there is substantial divergence in pathway output within the fungal kingdom (Mori, 2009).

The paradigm of fungal UPR signaling was elucidated in the model yeast *Saccharomyces cerevisiae* (Gardner *et al.*, 2013). Like all Ire1 orthologs examined to-date, yeast Ire1 has a lumenal ER stress-sensing domain and a cytosolic tail that contains both a kinase and an endoribonuclease (RNase) domain (Fig 1). The ER chaperone BiP/Kar2 binds to Ire1 in unstressed cells, but dissociates to assist with protein folding during ER stress conditions (Bertolotti *et al.*, 2000; Okamura *et al.*, 2000). Unfolded proteins activate Ire1 by direct interactions with the lumenal sensing domain (Credle *et al.*, 2005; Gardner *et al.*, 2013); the Ire1-BiP/Kar2 interaction is thought to play a regulatory role by desensitizing Ire1 to low levels of ER stress, thereby ensuring that the level of activation is in proportion to the magnitude and duration of ER dysfunction (Pincus *et al.*, 2010). Ire1 activation is associated with the formation of higher-order oligomeric Ire1 complexes in the ER membrane, resulting in trans-autophosphorylation and a conformational change that activates the RNase domain. Once active, the RNase catalyzes the spliceosome-independent removal of an unconventional intron from the cytoplasmic mRNA *HAC1*, which shifts the reading frame to allow translation of a bZIP transcription factor known as Hac1p. Hac1p then moves to the nucleus and increases the expression of UPR target genes (Travers *et al.*, 2000). However, even with the intervention of the UPR, a substantial fraction of polypeptides inevitably fail to achieve the appropriate conformation (Hartl and Hayer-Hartl, 2009). These aberrant proteins are eliminated by ERAD, a signaling pathway that retrotranslocates misfolded proteins back into the cytosol, ubiquitinates them on the cytosolic face of the ER and releases them for degradation by the proteasome (Ruggiano *et al.*, 2014). The UPR is required for efficient ERAD, indicating a tight coordination between the protein folding and disposal machineries (Travers *et al.*, 2000).

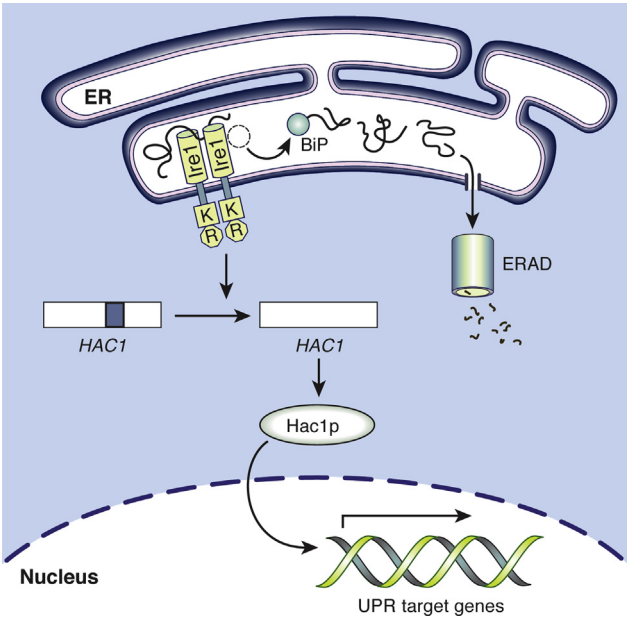

Fig 1 – The canonical UPR pathway established in *S. cerevisiae*. The chaperone BiP/Kar2 is bound to the ER stress sensor Ire1 in unstressed cells, but dissociates during ER stress to assist with protein folding. Yeast Ire1 is activated by direct interactions with unfolded proteins, and the binding to BiP/Kar2 plays a regulatory role by fine-tuning the sensitivity and shutoff kinetics for Ire1 activation. Activated Ire1 forms oligomeric complexes in the ER membrane, resulting in trans-autophosphorylation by the kinase (K) domain and a conformational change that activates the RNase domain (R). The RNase mediates the splicing of an intron from the cytosolic *HAC1* mRNA, causing a frame-shift that is a pre-requisite for translation of *HAC1* mRNA. Hac1p translocates to the nucleus and increases the expression of UPR target genes that boost ER protein folding capacity. Proteins that fail to achieve the appropriate conformation are disposed of by ERAD, a UPR-linked pathway that retrotranslocates aberrant proteins back into the cytoplasm for proteasomal degradation. In some fungal species, Ire1 may participate in 'regulated Ire1'dependent decay (RIDD)', a process in which mRNAs encoding ER-targeted proteins are degraded as a way to reduce the workload of the ER. RIDD is not present in *S. cerevisiae*, but has been reported in *S. pombe* and *C. glabrata*.

Although Ire1 is present in other fungal species, divergence exists in the mechanism it uses to mitigate ER stress. For example, although the fission yeast *Schizosaccharomyces pombe* has a clear Ire1 ortholog, bioinformatic analyses have failed to identify Hac1 orthologs in *S. pombe* or other yeasts of the same genus (Kimmig *et al.*, 2012). Instead of using the canonical Ire1-Hac1 transcriptional program to modulate the abundance of mRNAs during ER stress, *S. pombe* exploits the Ire1 RNase to initiate the selective decay of a large subset of mRNAs encoding ER-localized proteins. This 'regulated Ire1-dependent decay' (RIDD) has been shown to relieve ER stress in human cells by reducing the load of proteins entering the ER, but it