

Endoplasmic reticulum stress and fungal pathogenesis converge

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The endoplasmic reticulum (ER) forms an elaborate membranous network that functions as the major processing center for the folding and assembly of secreted and membrane proteins. Several environmental conditions can overwhelm the protein folding capacity of the ER, resulting in the accumulation of unfolded proteins. Since unfolded proteins can seed the formation of protein aggregates that are toxic to cells, their accumulation is tightly linked to the activation of stress response pathways that restore protein folding homeostasis.¹ In this special focus of *Virulence* we highlight recent advances in fungal biology that demonstrate the interplay between ER stress responses and virulence in four species of pathogenic fungi (two molds and two yeasts). Despite the fact that these organisms have very different lifestyles, and deploy unique species-specific virulence factors during infection, they all share the common characteristic of relying upon ER stress responses for their ability to cause disease.

In metazoans, ER stress signaling is orchestrated by at least three ER transmembrane proteins: IRE1, ATF6, and PERK. Each sensor contains an ER luminal domain that detects unfolded proteins, together with a cytosolic effector domain that transduces a signal to the cytoplasm and/or nucleus. Together, these sensors coordinate a series of interconnected adaptive responses known as the unfolded protein response (UPR).¹ No fungal homologs of ATF6 or PERK have been identified however,² so the current dogma is that fungi rely on the Ire1 branch to maintain ER homeostasis.² The prototype of fungal Ire1 signaling was established in the model fungus *Saccharomyces cerevisiae*.^{3,4} Activation of yeast Ire1 occurs when its luminal domain detects unfolded proteins in the ER. This triggers a conformational change that activates an RNase domain located in the cytoplasmic portion of the protein. Once activated, the RNase removes an intron from the cytoplasmic mRNA *HAC1*, which shifts the reading frame to allow the translation of a bZIP transcription factor that migrates to the nucleus and coordinates transcriptional changes to augment ER protein folding (see Gardner et al.¹ for a recent review). All of the fungal pathogens discussed in this collection of reviews follow the yeast paradigm of ER stress signaling to some extent. However, intriguing variations demonstrate that each species has optimized the pathways to suit specialized needs.

When pathogenic fungi enter a host they encounter ER stress from a variety of sources. For example, the need for a constant

supply of nutrients requires enzymatic digestion of host tissues. Like all eukaryotic cells specialized for secretion, the UPR helps the fungal secretory pathway meet the demand for increased secretory output.⁵ Second, fungal pathogens are exposed to hypoxic microenvironments in host tissues as well as oxidative damage from the host immune system,⁶ both of which are conditions that have been shown to trigger the UPR in other systems.⁷ Finally, an invading fungus may encounter membrane and cell wall damage in the host, due to either naturally occurring immune defenses or antifungal drugs that are used for therapeutic intervention.^{8–10} Since the repair of membrane and cell wall damage involves the secretory pathway, the UPR is likely to be involved in supporting the repair process.

The manuscript by Krishnan reviews the contribution of the UPR to the pathogenic mold *Aspergillus fumigatus*.¹¹ *A. fumigatus* is the predominant mold pathogen of humans, responsible for causing a severe pneumonia that can progress to a disseminated infection with very high mortality. The infection is acquired by inhalation of spores, which rapidly develop into hyphae that damage human tissues through the secretion of numerous hydrolytic enzymes. The manuscript discusses how *A. fumigatus* exploits the UPR to counter the ER stress of the host environment.¹² The mechanism is multifactorial, involving the support of key virulence-related attributes that include growth at mammalian body temperature, growth under hypoxia, growth in an iron-limited environment, the assimilation of nutrients from the polymers in mammalian tissues, and the ability to withstand membrane and cell wall damage mediated by antifungal drugs. This supports a model in which the *A. fumigatus* UPR acts as a regulatory hub for the expression of multiple clinically relevant traits that act in concert to support fitness within the host.¹³

Cryptococcus neoformans is an encapsulated yeast that is the causative agent of cryptococcosis, a life-threatening infection of immunocompromised patients. Although the infection is acquired by inhalation, the organism has a propensity to disseminate to the central nervous system and is the most common cause of fungal meningoencephalitis. The review by Cheon et al. summarizes the important role that the UPR plays in the virulence and antifungal drug resistance of *C. neoformans*. The pathway is comprised of an evolutionarily conserved Ire1 protein kinase that drives the expression of a transcription factor

that is surprisingly divergent in sequence relative to other Ire1-dependent transcription factors.¹⁴ Evidence is also presented that *C. neoformans* Ire1 has evolved unique functions that are independent of its downstream transcription factor. These Ire1-dependent functions include host-temperature adaptation and capsule biosynthesis, both of which are well established virulence factors for this pathogen.¹⁵ Interestingly, a similar bifurcation in Ire1 signaling has been reported in *A. fumigatus*,¹³ but not in *S. cerevisiae*, suggesting that the unique demands of pathogenic fungi may have driven the need for expanded Ire1 functions.

A different perspective on the importance of ER stress responses to *C. neoformans* is reviewed by Glazier and Panepinto.¹⁶ The manuscript integrates the current understanding of the *C. neoformans* UPR with recent developments in post-transcriptional regulation of ER stress resolution. The authors highlight evidence that mutants that are deficient in mRNA decay are unable to downregulate ER stress mRNAs at the termination of an ER stress response, supporting a role for mRNA decay in turning off the pathway. Intriguingly, these mutants fail to adapt to host body temperature and lose virulence, indicating that an appropriate resolution of an ER stress response is perhaps as crucial for the ability of *C. neoformans* to cause infection as is the initial engagement of the response.

Alternaria brassicicola is unique among the fungal pathogens discussed in this special focus because it is a member of a genus of plant pathogens. *A. brassicicola* infects cultivated *Brassica* species such as broccoli, cabbage, canola, and mustard, and is associated with severe reductions in crop yield that have important economic impact. As a necrotrophic fungus, *A. brassicicola* acquires nutrients from plant tissue using a battery of toxins and hydrolytic enzymes to actively kill plant cells.¹⁷ The review by Guillemette et al. addresses the importance of the UPR to this unique pathogenic lifestyle.¹⁸ In the absence of a functional

UPR, *A. brassicicola* loses its ability to secrete the arsenal of toxic compounds that it uses for virulence, becomes hypersensitive to plant antifungal defense compounds, and is avirulent. The necrotrophic lifestyle of this plant pathogen is thus highly dependent on UPR signaling, suggesting that plant pathogens that use similar infection strategies may also depend on support from the UPR.

Candida glabrata is a human commensal yeast that is a potent opportunistic pathogen, second only to *Candida albicans* as a cause of candidemia. The manuscript by Miyazaki and Kohno offers an intriguing exception to the archetypal UPR pathway of *S. cerevisiae*.¹⁹ Despite a close phylogenetic relationship to *S. cerevisiae*, *C. glabrata* appears to have lost the canonical Ire1-Hac1 UPR. Instead, it uses Ire1 to relieve ER stress by degrading ER-associated mRNAs and exploits other signaling pathways for the transcriptional upregulation of ER stress response genes. However, Ire1 is essential for the pathogenesis of *C. glabrata*, suggesting that the contribution of ER stress responses to fungal virulence is evolutionarily conserved, even though the precise mechanisms involved may differ.

In summary, the review articles in this special focus highlight the importance of the secretory pathway to fungal pathogens and reveal that ER stress responses are deeply entwined with the regulation of cellular functions that impact virulence. It is worth noting that strategies to target the regulatory mechanisms that underpin ER stress signaling are already in the development pipeline for the treatment of human secretory cancers that depend on these pathways for survival.²⁰ The inability of pathogenic fungi to cause disease when ER stress responses are impaired suggests that analogous approaches to disrupt these pathways could be useful additions to the antifungal armamentarium.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

References

- Gardner BM, Pincus D, Gotthardt K, Gallagher CM, Walter P. Endoplasmic reticulum stress sensing in the unfolded protein response. *Cold Spring Harb Perspect Biol* 2013; 5:a013169; PMID:23388626; <http://dx.doi.org/10.1101/cshperspect.a013169>
- Korennykh A, Walter P. Structural basis of the unfolded protein response. *Annu Rev Cell Dev Biol* 2012; 28:251-77; PMID:23057742; <http://dx.doi.org/10.1146/annurev-cellbio-101011-155826>
- Cox JS, Shamu CE, Walter P. Transcriptional induction of genes encoding endoplasmic reticulum resident proteins requires a transmembrane protein kinase. *Cell* 1993; 73:1197-206; PMID:8513503; [http://dx.doi.org/10.1016/0092-8674\(93\)90648-A](http://dx.doi.org/10.1016/0092-8674(93)90648-A)
- Mori K, Ma W, Gething MJ, Sambrook J. A transmembrane protein with a cdc2+/CDC28-related kinase activity is required for signaling from the ER to the nucleus. *Cell* 1993; 74:743-56; PMID:8358794; [http://dx.doi.org/10.1016/0092-8674\(93\)90521-Q](http://dx.doi.org/10.1016/0092-8674(93)90521-Q)
- Moore KA, Hollien J. The unfolded protein response in secretory cell function. *Annu Rev Genet* 2012; 46:165-83; PMID:22934644; <http://dx.doi.org/10.1146/annurev-genet-110711-155644>
- Hartmann T, Sasse C, Schedler A, Hasenberg M, Gunzer M, Krappmann S. Shaping the fungal adaptome--stress responses of *Aspergillus fumigatus*. *Int J Med Microbiol* 2011; 301:408-16; PMID:21565548; <http://dx.doi.org/10.1016/j.ijmm.2011.04.008>
- Kitamura M. The unfolded protein response triggered by environmental factors. *Semin Immunopathol* 2013; 35:259-75; PMID:23553212; <http://dx.doi.org/10.1007/s00281-013-0371-y>
- Lupetti A, Danesi R, Campa M, Del Tacca M, Kelly S. Molecular basis of resistance to azole antifungals. *Trends Mol Med* 2002; 8:76-81; PMID:11815273; [http://dx.doi.org/10.1016/S1471-4914\(02\)02280-3](http://dx.doi.org/10.1016/S1471-4914(02)02280-3)
- Sellam A, Dongo A, Guillemette T, Hudhomme P, Simoneau P. Transcriptional responses to exposure to the brassicaceous defence metabolites camalexin and allyl-isothiocyanate in the necrotrophic fungus *Alternaria brassicicola*. *Mol Plant Pathol* 2007; 8:195-208; PMID:20507491; <http://dx.doi.org/10.1111/j.1364-3703.2007.00387.x>
- Paiva JA, Pereira JM. New antifungal antibiotics. *Curr Opin Infect Dis* 2013; 26:168-74; PMID:23411420; <http://dx.doi.org/10.1097/QCO.0b013e32835ebcb7>
- Krishnan K, Askew DS. The fungal UPR: A regulatory hub for virulence traits in the mold pathogen *Aspergillus fumigatus*. *Virulence* 2014; 5:334-40; PMID:24189125; <http://dx.doi.org/10.4161/viru.26571>
- Richie DL, Hartl L, Amanianda V, Winters MS, Fuller KK, Miley MD, White S, McCarthy JW, Latgé JP, Feldmesser M, et al. A role for the unfolded protein response (UPR) in virulence and antifungal susceptibility in *Aspergillus fumigatus*. *PLoS Pathog* 2009; 5:e1000258; PMID:19132084; <http://dx.doi.org/10.1371/journal.ppat.1000258>
- Feng X, Krishnan K, Richie DL, Amanianda V, Hartl L, Grahl N, Powers-Fletcher MV, Zhang M, Fuller KK, Nierman WC, et al. HacA-independent functions of the ER stress sensor IreA synergize with the canonical UPR to influence virulence traits in *Aspergillus fumigatus*. *PLoS Pathog* 2011; 7:e1002330; PMID:22028661; <http://dx.doi.org/10.1371/journal.ppat.1002330>
- Cheon SA, Jung K-W, Bahn Y-S, Kang HA. The unfolded protein response (UPR) pathway in *Cryptococcus*. *Virulence* 2014; 5:341-50; PMID:24504058; <http://dx.doi.org/10.4161/viru.26774>

15. Alspaugh JA, Perfect JR, Heitman J. Signal transduction pathways regulating differentiation and pathogenicity of *Cryptococcus neoformans*. *Fungal Genet Biol* 1998; 25:1-14; PMID:9806801; <http://dx.doi.org/10.1006/fgbi.1998.1079>
16. Glazier VE, Panepinto JC. The ER stress response and host temperature adaptation in the human fungal pathogen *Cryptococcus neoformans*. *Virulence* 2014; 5:351-6; PMID:24253500; <http://dx.doi.org/10.4161/viru.27187>
17. Oliver RP, Solomon PS. New developments in pathogenicity and virulence of necrotrophs. *Curr Opin Plant Biol* 2010; 13:415-9; PMID:20684067; <http://dx.doi.org/10.1016/j.pbi.2010.05.003>
18. Guillemette T, Calmes B, Simoneau P. Impact of the UPR on the virulence of the plant fungal pathogen *A. brassicicola*. *Virulence* 2014; 5:357-64; PMID:24189567; <http://dx.doi.org/10.4161/viru.26772>
19. Miyazaki T, Kohno S. Unique evolution of ER stress response mechanisms in the pathogenic yeast *Candida glabrata* and its role in virulence. *Virulence* 2014; 5:365-70; PMID:24335436; <http://dx.doi.org/10.4161/viru.27373>
20. Hetz C, Chevet E, Harding HP. Targeting the unfolded protein response in disease. *Nat Rev Drug Discov* 2013; 12:703-19; PMID:23989796; <http://dx.doi.org/10.1038/nrd3976>