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EDITORIAL

Unmasking fungal pathogens by studying MAPK-dependent cell wall regulation in *Candida albicans*

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Fungal infections are a worldwide health problem and are a leading cause of death in developing countries. They contribute to pneumonia,[1] asthma,[2] and blindness.[3] Fungal infections also compound the morbidity of other diseases like tuberculosis.[4] In developed countries, invasive fungal infections underlie mortality in immune-compromised individuals with HIV/AIDS [5] and cancer patients undergoing chemotherapy,[6] and are the fourth largest cause of blood infections. Improved diagnosis and new anti-fungal drugs are recognized as key routes to improving patient survival.[7] Identifying and increasing the efficacy of anti-fungal drugs is a world-wide health research priority.[8]

The cell wall is an attractive therapeutic target for fungal pathogens. This extracellular organelle is accessible to anti-fungal drugs and is the first site of contact by the immune system. The current picture of the cell wall comes from extensive research in multiple areas, including studies in model organisms where genetic and biochemical approaches have identified cell wall proteins and polysaccharides.[9] The major polysaccharides in the cell wall are β-1,3-glucans consisting of long linear chains of β-1,3-linked glucose. Less abundant polysaccharides include chitin and β-1,6-glucan. There is extensive cross-linking between the polysaccharides that comprise the cell wall. The cell wall is also composed of glycoproteins, which are glycosylated by covalently modified N- and O-linked oligosaccharide addition.[10,11] The principle oligosaccharide modification of cell wall proteins is mannosylation. Mannosylation can impact the function, stability, and localization of proteins and can also lead to the cross linking of glycoproteins to polysaccharides in the cell wall.[12]

The cell wall is a dynamic structure. It is rigid enough to provide support and protection, yet is malleable enough to allow for growth and differentiation. What's more, the cell wall can be reorganized in response to environmental perturbations to promote adaptability and disguise cells from recognition by the immune system.[13] Among the pathways that regulate cell wall biosynthesis and remodeling are mitogen-activated protein kinase (MAPK) pathways. MAPK pathways are evolutionarily conserved signaling pathways that control stress responses, cell proliferation, and cell differentiation in eukaryotes.[14,15] In *C. albicans*, one MAPK pathway implicated in cell wall regulation is the Cek1 pathway.[16]

The Cek1 pathway is regulated by transmembrane sensors that detect alterations in the environment and initiate cellular responses to these changes. The mucin-like glycoprotein Msb2 is one of the regulators of the Cek1 pathway. Msb2 is a single-pass transmembrane protein with a cytosolic signaling domain and a heavily glycosylated extracellular domain.[17,18] The extracellular domain of Msb2 is processed by secreted aspartic proteases (SAPs). Release of the inhibitory glycosylated domain is required for MAPK signaling.[19,20] Msb2 detects (by some mechanism) limiting nutrients in the environment and can also sense elevated temperatures.[21]

Other signaling pathways also impact cell wall integrity. One is the cell wall integrity or Protein Kinase C (PKC) pathway.[22-24] Another is the Unfolded Protein Response (UPR) pathway,[21,25-27] whose major function is to control protein folding in the endoplasmic reticulum.[28] Not surprisingly, problems with folding and glycosylation of secreted and cell wall associated proteins can impact the integrity of the cell wall. The fact that multiple pathways regulate the cell wall underscores its critical role in regulating fungal homeostasis.

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Landmark discoveries have opened up the field by identifying receptors in the immune system and their substrates on the fungal surface.[29] Mannose-binding lectin is a serum protein of the innate immune system that binds to and promotes clearance of fungal microorganisms.[30] The dectin-1 receptor on macrophages recognizes β-1,3-glucan in the fungal cell wall.[31,32] The dectin-2 receptor is a pattern recognition receptor on dendritic and other cells that recognizes fungal α-mannan.[33,34] The pattern recognition receptor galectin-3 on macrophages detects phospholipomannan,[35] a sphingolipid in *C. albicans* [36] that when recognized elicits a pathogen-specific response.[37] Importantly, dectin-1 and galectin-3 interact synergistically to improve the outcome of host immune response to *C. albicans* β-glucans.[38]

In this issue, Román et al. [39] build on the picture of Cek1 pathway-mediated control of cell wall regulation in *C. albicans*. They show that cells lacking an intact Cek1 pathway (*cek1* mutant) exhibit increased exposure of α-1,2-mannosides and β-1,2-mannosides. They reach this conclusion by examining cell wall proteins with antibodies against α-1,2- and β-1,2-mannosides. Under different conditions and treatments, the authors see a striking increase in the electrophoretic mobility of proteins with these modifications between wild-type cells and the *cek1* mutant. This may indicate that cell wall proteins become under-glycosylated in the *cek1* mutant or alternatively fail to become linked to the cell wall. Together with the fact that the *cek1* mutant has abnormally appearing cell walls by transmission electron microscopy, and that *cek1* mutants have increased β-1,3-glucan exposure,[40,41] their findings support the idea that the cell wall is generally disorganized in cells lacking the Cek1 pathway. This idea is supported by a second set of experiments. The authors show that tunicamycin, an inhibitor of N-linked glycosylation that triggers the UPR pathway and leads to under-glycosylation of cell wall glycoproteins,[42] exacerbates the growth and cell wall defects of the *cek1* mutant. Their conclusion is in line with other reports[18,21,43] and may be due to the misfolding, misincorporation, and/or problems with the processing and release of under-glycosylated cell wall proteins.[44]

The authors go on to test the role of the immune system in Cek1-mediated cell wall control. Because galectin-3 can bind β-1,2-mannosides,[37] the authors examined whether Gal3 $^{-/-}$ mice showed increased fungal susceptibility in a Cek1-dependent manner. As expected from previous studies,[16] *C. albicans* lacking the Cek1 pathway showed reduced virulence in systemically infected mice. However, mice lacking Gal3 did not restore the virulence of the *cek1* mutant. Unexpectedly, Gal3 $^{-/-}$ mice survived even better than wild-type mice systemically infected with *C. albicans*. Thus, this work from the Pla group points to gaps in understanding the relationship between Gal3 and the molecular basis of *C. albicans* recognition in the immune response.

This study by Román et al.[39] identifies directions for future research. An important question moving forward is to understand how the Cek1 pathway produces a cell wall that is 'invisible' to the immune system. The CEK pathway helps maintain an organized and healthy cell wall by regulating proteins that control cell-wall biosynthesis and remodeling. Who are these proteins? One class of proteins are O-mannosyltransferases (Pmt proteins) that initiate O-mannosylation and selectively glycosylate mucins and other cell wall proteins.[41,45,46] Other target proteins continue to be uncovered by gene expression profiling by the Pla group and other laboratories.[47] Biochemical analysis of these cell wall remodeling enzymes may elucidate the precise role the CEK pathway plays in regulating the cell wall. Innovative approaches, like multivariate analysis of morphometric data,[48] may accelerate progress in this area. These proteins themselves represent attractive drug targets, because they function outside the cell and may be accessible to antifungal compounds.

A second question has to do with understanding the functions of cell wall regulatory pathways. At least 3 major pathways control the cell wall: Cek1, PKC, and UPR. Genetic buffering between these pathways complicates assignment of the functions of each pathway in regulating cell wall function. Further complicating matters is the fact that Cek1, PKC, and UPR show cross-regulation.[21,49-51] Therefore, new approaches are needed to decipher the precise functions of the pathways in regulating the cell wall. Ultimately, studies on MAPK-dependent cell wall regulation and immune recognition promises to elucidate the functions of MAPK pathways and their unique targets with the long-term goals of reducing virulence and improving human health.

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