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COMMENTARY

Unveiling Protein Kinase A Targets in *Cryptococcus neoformans* Capsule Formation

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ABSTRACT **The protein kinase A (PKA) signal transduction pathway has been associated with pathogenesis in many fungal species. Geddes and colleagues [mBio 7(1):e01862-15, 2016, doi:10.1128/mBio.01862-15] used quantitative proteomics approaches to define proteins with altered abundance during protein kinase A (PKA) activation and repression in the opportunistic human fungal pathogen *Cryptococcus neoformans*. They observed an association between microbial PKA signaling and ubiquitin-proteasome regulation of protein homeostasis. Additionally, they correlated these processes with expression of polysaccharide capsule on the fungal cell surface, the main virulence-associated phenotype in this organism. Not only are their findings important for microbial pathogenesis, but they also support similar associations between human PKA signaling and ubiquitinated protein accumulation in neurodegenerative diseases.**

Microorganisms that cause human disease need to survive stresses encountered in the infected host. Stress-responsive signaling pathways often mediate adaptive changes that favor microbial survival and propagation of the infection. Therefore, many investigators have attempted to define microbial signaling pathways activated in response to the host. One of these pathways, the cyclic AMP (cAMP)/protein kinase A (PKA) pathway, is closely associated with pathogenesis in many fungal species. Although the central PKA signaling elements are highly conserved in divergent fungi, the downstream effects of PKA activation are often species specific. For example, *Candida albicans* requires intact PKA signaling to undergo the yeast-hyphal transition, a morphological change associated with its pathogenesis (1). Similarly, many endemic fungal pathogens use this pathway to initiate a temperature-regulated transition from an environmental mold to a pathogenic, yeast-like form (2). In contrast, the opportunistic fungal pathogen *Cryptococcus neoformans* employs PKA to regulate the induction of phenotypes required for full virulence, such as polysaccharide capsule formation and laccase/melanin expression (3). In each case, diverse fungal species appear to have coopted this conserved signaling pathway to regulate specific phenotypes required for their survival within the host. However, we have lacked a comprehensive understanding of the downstream targets of the PKA pathway in pathogenic fungi.

In a recent article in *mBio*, Geddes et al. present an important series of investigations identifying PKA-responsive proteins in *C. neoformans* (4). Using quantitative proteomics approaches, that study offers us significant insight into PKA-dependent processes regulated by the fungal cell in response to various stresses, including many host-relevant signals. Far from being a simple story, the downstream targets of PKA in *C. neoformans* reported in these studies are components of multiple, varied cellular processes. The main highlighted result was the importance of PKA in the ubiquitin-proteasome pathway and other processes related to the control of protein abundance. The authors specifically linked proteasome activation to expression of the main virulence determinant in this microorganism: polysaccharide capsule expression. Additionally, the authors used various chemical inhibitors to explore translational prospects for their findings.

Interestingly, these observations in a unicellular, eukaryotic pathogen are consistent with recent reports of human neurodegenerative diseases in which PKA signaling is similarly linked to proteasome activation. Defects in human PKA signaling result in altered protein turnover, contributing to increases in ubiquitin-protein aggregates associated with conditions such as amyotrophic lateral sclerosis and Alzheimer's disease (5, 6). The effects of similar dysregulation of PKA signaling in eukaryotic microorganisms have yet to be deeply explored, especially since most microbial studies to date have considered primarily the effects of PKA in single cells growing in planktonic cultures. However, similarities in PKA signaling between simple and multicellular eukaryotes might be better appreciated by studying microorganisms living in complex communities, such as biofilms.

There is often inherent complexity in defining outputs from such a centrally acting signaling pathway. For example, in this paper, the authors note instances of similar cellular changes resulting from either activation or repression of PKA signaling in *C. neoformans*. Furthermore, the investigators noted the paradoxical observation that there was not always a direct correlation between transcript abundance and protein levels for individual, PKA-responsive gene products. Additionally, this type of proteomic analysis can be biased toward identifying the most abundant proteins in the cell, perhaps missing important changes in transient or low-abundancy proteins. However, the study by Geddes et al. uses sensitive techniques to define microbial processes that are controlled by PKA and, therefore, likely respond to host-derived stresses. Their detailed proteomics analysis will be a considerable resource for investigators exploring PKA signaling in varied eukaryotic pathogens and their infected hosts. As we better appreciate these adaptive responses of microorganisms attempting to survive within the host, we will gain new insight into novel

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diagnostic and therapeutic strategies to address challenging infectious diseases.

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