

However, residues critical for S12 binding to survivin are not well conserved in AfBIR1 (Fig. 1). Thus, studies to verify direct binding of S12 to AfBIR1 are needed, as their interaction cannot be inferred solely on the basis of multiple sequence alignments.

The other hallmark of mammalian apoptosis used throughout the Shlezinger *et al.* study is nuclear DNA fragmentation, which is quantified using TUNEL (terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick end labeling) assays. The TUNEL assay is useful for labeling the new ends of nuclear DNA after caspase-dependent activation of the mammalian DNase CAD (caspase-activated deoxyribonuclease) (11). However, the TUNEL assay is not specific to apoptosis and detects DNA breaks resulting from nonapoptotic cell death (12). DNA fragmentation and many other features of dying mammalian cells have been observed in a diverse range of nonmetazoan taxa, including protists, microalgae, yeast, bacteria, and plants. However, the genes directly responsible are largely unknown. Thus, the central unanswered question is whether the fungal factors responsible for TUNEL reactivity, caspase-like activity, or other mammalian readouts play an active and causal

role in fungal cell death, or whether they only serve to mark dead and dying cells with degrading DNA, vacuolar permeability, and depletion of ATP.

Shlezinger *et al.* provide convincing genetic arguments that host phagocyte NOX [reduced nicotinamide adenine dinucleotide phosphate (NADPH) oxidase] mediates the demise of ingested fungal conidia. The possibility that a NOX-derived reactive oxygen species induces a FITC-VAD-FMK-traceable signal modulated by AfBIR is also intriguing, but more evidence will be required to distinguish this model from other possibilities, including direct killing of the engulfed pathogens by neutrophils without pro-death contributions from fungal “caspase-like activities.” Some fungal species have orthologs of the mammalian necroptosis mediator MLKL or the mammalian pyroptosis mediator DNFA5/gasdermin (13, 14). Even if these pro-necrotic death factors also do not promote regulated death pathways in fungi, this does not deny the existence of genetically controlled fungal cell death, especially in fungi with elaborate morphological forms (15).

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Comment on "Sterilizing immunity in the lung relies on targeting fungal apoptosis-like programmed cell death"

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