FUNGAL VIRULENCE

A cryptococcal global regulator?

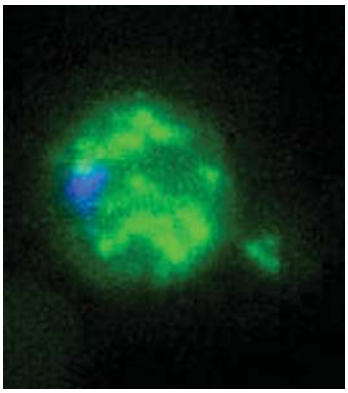
Results reported in the *Journal of Clinical Investigation* have identified Vad1, a DEAD-box RNA helicase, as a putative global virulence regulator in *Cryptococcus neoformans*.

C. neoformans is an important pathogen occuring in immunocompromised individuals, particularly in AIDS patients. Over the past decade, the development of genetic analysis techniques has led to the identification of several virulence factors in this basidiomycete. Efforts have also been made to identify the molecular mechanisms of virulence regulation by studying the model yeast *Saccharomyces cerevisiae*. In this work, however, Panepinto *et al.* were interested in identifying *C. neoformans* virulence regulators directly.

The authors used a random insertion mutagenesis and plasmid rescue technique to identify a putative virulence regulator. Analysis of the disrupted gene in a mutant that was deficient in the production of the virulence factor laccase showed that it encoded a 616-residue protein with homology to the RCK/p54 family of DEAD-box RNA helicases, which was termed Vad1.

Phenotypic analysis of a Δ*vad1* mutant revealed reduced laccase activity, and in a mouse model of cryptococcosis the virulence of this mutant was attenuated to a degree that indicated that, in addition to laccase, other virulence-associated traits must be involved. Further analysis of the Δ*vad1* mutant revealed the presence of cell-wall defects and a decreased ability to use non-fermentable substrates. Additionally, analysis of epitope-tagged Vad1 by HPLC gel filtration and deconvolution microscopy revealed that this protein is part of a large, cytoplasmic multiprotein complex.

Panepinto *et al.* then used differential display and RNAi to determine whether disrupting *vad1* caused alterations in the expression of any other genes and, if so, what their functions were. One gene, *NOT1*, was upregulated in Δ*vad1* cells and was shown to be a negative regulator of laccase expression. Three genes were downregulated — *TUF1*, which is essential for the synthesis of mitochondrial proteins; *MPF3*, which has a role in cell-wall integrity; and *PCK1*, which encodes the enzyme that catalyses the only irreversible step in gluconeogenesis. All four transcripts are thought to be involved in the response of *C. neoformans* to stress.



Epi-fluorescent image of *C. neoformans* expressing green fluorescent-tagged Vad1 in perinuclear RNA-degradation granules. Blue denotes nuclear staining with 4′,6-diamidino-2-phenylindole. Courtesy of J. Panepinto, J. Ramos and P. R. Williamson.

So, Vad1 is the first DEAD-box RNA helicase to be shown to have a role in fungal virulence. In *C. neoformans*, it regulates the expression of various different virulence traits and could potentially be a master regulator of the *C. neoformans* stress response.

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References and links

ORIGINAL RESEARCH PAPER Panepinto, J. *et al.* The DEAD-box RNA helicase Vad1 regulates multiple virulence-associated genes in *Cryptococcus neoformans*. *J. Clin. Invest.* **115**, 632–641 (2005)

WEB SITE

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