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Letters to the Editor

The Echinocandin "Target" Identified by Cross-Linking Is a Homolog of Pil1 and Lsp1, Sphingolipid-Dependent Regulators of Cell Wall Integrity Signaling

Echinocandins such as caspofungin, recently approved for treatment of specific yeast and mold infections, represent an important group of antifungals with a distinct mechanism of action (2). Early studies demonstrated that echinocandins inhibit the synthesis of β-1,3-glucan, a major cell wall component in many fungi (3). Initial reports in 1994 and 1995 of efforts to identify genes involved in β-1,3-glucan synthesis employed genetic characterization of echinocandin-resistant *Saccharomyces cerevisiae* mutants. One such study identified *GNS1*, disruption of which decreased β-1,3-glucan synthase activity 90 to 95% (5). However, this gene is allelic to *FEN1/ELO2* encoding a fatty acid elongase involved in sphingolipid long-chain base (LCB) biosynthesis. Other studies associated β-1,3-glucan synthase with the *FKS* family of genes encoding high-molecular-weight membrane proteins that localize to sites of cell wall remodeling (1, 4). Partial purification by glucan product entrapment supported this association (8).

Since further purification of β-1,3-glucan synthase proved difficult, direct proof that *FKS*-encoded proteins represent the echinocandin target was lacking. In a 1998 study, Radding et al. (10) sought to define the echinocandin target within *Candida albicans* membrane preparations by photoaffinity labeling with an echinocandin analog that retained antifungal activity. Two major proteins of 40 and 18 kDa were identified, cross-linking to which was dependent on irradiation and was competed out with unmodified echinocandin. Labeling of the 40-kDa protein was sufficient to allow purification, and peptide sequences of 12 and 17 amino acids were obtained. The *C. albicans* genome sequence was not yet available, but Radding et al. identified by BLAST analysis a pair of related *S. cerevisiae* proteins, Ypl004 and Ygr086, with 50 to 76% identity to both peptide sequences. We performed a BLAST search of the *C. albicans* sequence database (www-sequence.stanford.edu/group/candida) and confirmed that both peptides derive from a Ypl004-Ygr086 homolog within contig6-1742; a further search identified a second closely related *C. albicans* protein within contig6-1965. The *S. cerevisiae* and *C. albicans* protein pairs are highly conserved, with 70 to 76% identity to each other.

The echinocandin-cross-linked 40-kDa *C. albicans* protein and its partner are uncharacterized, but functions for their *S. cerevisiae* homologs have recently come to light and, surprisingly, there is again a link to sphingolipids. By two different assays, Ypl004 and Ygr086 were shown to physically interact with Pkh1 or Pkh2 (7, 9). These protein kinases are regulated by LCBs such as phytosphingosine (12). Indeed, Pkh2 phosphorylation of Ygr086 is inhibited by LCBs (hence the gene name *PIL1*) whereas LCBs stimulate phosphorylation of Ypl004 (hence the name *LSP1*). In a complex network of signaling pathways, LCB-regulated phosphorylation of Pil1 and Lsp1 controls activation of the well-characterized protein kinase C-MAP kinase pathway and the poorly characterized Ypk1 kinase pathway (12); both pathways mediate cell wall integrity in response to various stresses. In particular, the protein kinase C-MAP kinase pathway plays a major protective role in the yeast response to echinocandins (11; T. Edlind and S. Katiyar, Abstr. 42nd Intersci. Conf. Antimicrob. Agents and Chemother., abstr. M-19, 2002).

These data raise the possibility that Pil1-Lsp1 associate with β-1,3-glucan synthase and, along with Rho1 (2, 3), play a role in its regulation. Indeed, a protein complex containing both Pil1 and Fks1 has been previously identified (6). Moreover, these data raise questions regarding the echinocandin target which have implications for further development of these antifungals and identification of potential resistance mechanisms.

REFERENCES

1. **Castro, C., J. C. Ribas, M. H. Valdivieso, R. Varona, F. del Rey, and A. Duran.** 1995. Papulacandin B resistance in budding and fission yeasts: isolation and characterization of *PBR1*, a gene involved in (1,3)-β-D-glucan synthesis in *Saccharomyces cerevisiae*. J. Bacteriol. **177:**5732–5739.
2. **Denning, D. W.** 2003. Echinocandin antifungal drugs. Lancet **362:**1142–1151.
3. **Douglas, C. M.** 2001. Fungal β(1,3)-D-glucan synthesis. Med. Mycol. **39**(Suppl. 1)**:**55–66.
4. **Douglas, C. M., F. Foor, J. A. Marrinan, N. Morin, J. B. Nielsen, A. M. Dahl, P. Mazur, W. Baginsky, W. Li, M. el-Sherbeini, et al.** 1994. The *Saccharomyces cerevisiae FKS1* (*ETG1*) gene encodes an integral membrane protein which is a subunit of 1,3-β-D-glucan synthase. Proc. Natl. Acad. Sci. USA **91:**12907–12911.
5. **El-Sherbeini, M., and J. A. Clemas.** 1995. Cloning and characterization of *GNS1*: a *Saccharomyces cerevisiae* gene involved in synthesis of 1,3-β-glucan in vitro. J. Bacteriol. **177:**3227–3234.
6. **Gavin, A.-C., M. Bosche, R. Krause, et al.** 2002. Functional organization of the yeast proteome by systematic analysis of protein complexes. Nature **415:**141–147.
7. **Ho, Y., A. Gruhler, A. Heilbut, G. D. Bader, L. Moore, S. L. Adams, A. Millar, P. Taylor, K. Bennett, K. Boutilier, et al.** 2002. Systematic identification of protein complexes in *Saccharomyces cerevisiae* by mass spectrometry. Nature **415:**180–183.
8. **Inoue, S. B., N. Takewaki, T. Takasuka, T. Mio, M. Adachi, Y. Fujii, C. Miyamoto, M. Arisawa, Y. Furuichi, and T. Watanabe.** 1995. Characterization and gene cloning of 1,3-β-D-glucan synthase from *Saccharomyces cerevisiae*. Eur. J. Biochem. **231:**845–854.
9. **Ito, T., T. Chiba, R. Ozawa, M. Yoshida, M. Hattori, and Y. Sakaki.** 2001. A comprehensive two-hybrid analysis to explore the yeast protein interactome. Proc. Natl. Acad. Sci. USA **98:**4569–4574.
10. **Radding, J. A., S. A. Heidler, and W. W. Turner.** 1998. Photoaffinity analog of the semisynthetic echinocandin LY303366: identification of echinocandin targets in *Candida albicans*. Antimicrob. Agents Chemother. **42:**1187–1194.
11. **Reinoso-Martin, C., C. Schuller, M. Schuetzer-Muehlbauer, and K. Kuchler.** 2003. The yeast protein kinase C cell integrity pathway mediates tolerance to the antifungal drug caspofungin through activation of Slt2p mitogen-activated protein kinase signaling. Eukaryot. Cell **2:**1200–1210.
12. **Zhang, X., R. L. Lester, and R. C. Dickson.** 2004. Pil1p and Lsp1p negatively regulate the 3-phosphoinositide-dependent protein kinase-like kinase Pkh1p and downstream signaling pathways Pkc1p and Ypk1p. J. Biol. Chem. **279:**22030–22038.

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