Proteolytic cleavage of covalently linked cell wall proteins by *Candida albicans* Sap9 and Sap10.

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Abstract

The cell wall of the human-pathogenic fungus Candida albicans is a robust but also dynamic structure which mediates adaptation to changing environmental conditions during infection. Sap9 and Sap10 are cell surface-associated proteases which function in C. albicans cell wall integrity and interaction with human epithelial cells and neutrophils. In this study, we have analyzed the enzymatic properties of Sap9 and Sap10 and investigated whether these proteases cleave proteins on the fungal cell surface. We show that Sap9 and Sap10, in contrast to other aspartic proteases, exhibit a near-neutral pH optimum of proteolytic activity and prefer the processing of peptides containing basic or dibasic residues. However, both proteases also cleaved at nonbasic sites, and not all tested peptides with dibasic residues were processed. By digesting isolated cell walls with Sap9 or Sap10, we identified the covalently linked cell wall proteins (CWPs) Cht2,

Ywp1, Als2, Rhd3, Rbt5, Ecm33, and Pga4 as in vitro protease substrates. Proteolytic cleavage of the chitinase Cht2 and the glucan-cross-linking protein Pir1 by Sap9 was verified using hemagglutinin (HA) epitope-tagged versions of both proteins. Deletion of the SAP9 and SAP10 genes resulted in a reduction of cell-associated chitinase activity similar to that upon deletion of CHT2, suggesting a direct influence of Sap9 and Sap10 on Cht2 function. In contrast, cell surface changes elicited by SAP9 and SAP10 deletion had no major impact on the phagocytosis and killing of C. albicans by human macrophages. We propose that Sap9 and Sap10 influence distinct cell wall functions by proteolytic cleavage of covalently linked cell wall proteins.

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