Secreted proteinases and *Candida albicans* virulence

The association of secreted proteolytic activity with virulence in *Candida albicans* is a story that has developed over the past 40 years. From the earliest biochemical work which tacitly assumed that this major fungal pathogen secreted a single proteinase enzyme to the genomic revelation of a family of 10 genes encoding secreted aspartyl proteinases (Saps), the presumption has been that externally secreted *C. albicans* hydrolases of broad substrate specificity ought to contribute to the pathogenesis of disease. Many excellent studies, including reverse genetics and *in vivo* expression technologies, have contributed to detailed knowledge of the roles of the various *SAP* gene products in the pathogenesis of superficial and disseminated *Candida* infections (Naglik et al., 2004).

Now two papers in the present issue of *Microbiology* (Lermann & Morschhäuser, 2008; Naglik et al., 2008) force a rethink of the dogma that Saps are major virulence factors in *C. albicans*; at least for superficial infections and their *ex vivo* model, reconstituted human epithelium (RHE). In two laboratories, different experimental approaches with the same RHE model were used to measure gene expression *in vivo*. Only SAP5 (among SAP1–6) appeared to be activated significantly at any stage of infection. And both studies, using independently generated single and multiple mutants, showed that SAP1–6 did not play an important role in the invasion of RHE.

The coincidental appearance of the two papers in the same journal at the same time is a welcome event, since the main message from both studies is a resolution of the growing list of published discrepancies from experiments designed to indicate which of the Saps are important at what stages and in what types of infection. The candour of Naglik and colleagues is admirable in their acknowledgement of the error of their earlier work that showed sequential upregulation of SAP1, SAP3 and SAP6, with SAP2 and SAP8 expression detected only in late stages of RHE and oral infection. The two new publications concur that no Sap appears to play an essential role in mucosal invasion. The possibility of a nonessential contribution of Sap5 is left open. The role of SAP9, which was strongly upregulated in RHE and in human oral and vaginal samples (Naglik et al., 2008), remains unclear, but since Sap9 is not actively secreted by growing *C. albicans* (Naglik et al., 2004) it seems unlikely that this enzyme will have a redundant function similar to the other Sap family enzymes.

Do the new publications signal the end of the association of *C. albicans* Saps with virulence? Most certainly not. For a start, both the new studies involve mutants prepared from the same parental strain, SC5314, which is well known from animal experiments to be a poor colonizer and invader of mammalian epithelia (Taylor et al., 2000). (SC5314 is highly virulent by intravenous challenge.) Emerging technology for gene disruption in other strains will allow a re-evaluation of SAP expression in RHE with isolates having a greater inherent propensity to invade superficial tissues. Use of SC5314 in experimental superficial infection models has been inevitable since for many years only this strain background was available for specific gene disruptions. And because SC5314 can invade RHE this model can validly be used to compare effects of parental and mutant strains. The difficulty lies in extrapolation of RHE data to whole-animal infections. A role for Saps in one or more aspects of deep tissue invasion processes has not yet been excluded. Moreover, Saps 7–10 have received much less experimental attention than Saps 1–6, so continued investigation of these enzymes is still justified.

The scientific value of the RHE model is called into question by the new studies. It seems logically inconsistent to experiment with SC5314 invading RHE as a model for invasion of natural epithelia when the same *C. albicans* strain is unable to cause sustained superficial infections in experimental animals. From the sections of RHE illustrated in both new papers (and in prior publications) it seems that RHE thickness varies from run to run, which complicates efforts to quantification of damage caused from a fixed fungal inoculum, and – a possibly more serious criticism – that most of the fungal growth occurs in the medium outside the RHE. All model systems have inherent limitations (including rodent models of *C. albicans* infection), but the risk of irrelevance to the real disease inevitably increases the further away a model lies from that disease. It is to the credit of Naglik, Hube, Schaller and their colleagues, whose research is the most extensive in the *C. albicans*–RHE arena, that they have consistently sought to relate their RHE data to samples taken directly from infected patients. In the present study of Naglik et al. (2008) the RHE expression data were compared with results from 17 infected oral samples and 17 infected vaginal samples; however, the comparison generated no exciting findings.

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Charles J. Dorman, Editor-in-Chief
These two new Sap publications represent a landmark in the history of investigation of virulence in *C. albicans*. Science is a self-correcting process, and the correction now required to our thinking about the role of *C. albicans* Saps in the RHE model is a welcome step of real progress. The papers show the importance of quantification of experimental data, with lactate dehydrogenase assays and real-time PCR being more reliable markers of gross tissue damage and gene expression, respectively, than subjective histological examination and reverse-transcriptase PCR. Investigations now need to move to models closer to the reality of superficial infections. While the new data may disappoint a community that has acquired, for many years, a growing confidence in the role of Saps in superficial *Candida* infections, they pave the way for novel approaches to investigation of *C. albicans* virulence. The term ‘*C. albicans* virulence’ is almost an oxymoron, since the fungus is not capable of invading tissues in an immunologically intact human host. However, virulence differences in many types of experimental infection can be easily demonstrated between isolates of *C. albicans* and between different *Candida* species; the search for the molecules that determine these differences remains a fascinating challenge.

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**References**


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