Deconstructing and reconstructing the yeast cell wall: A top-down, bottom-up approach

T. E. S. Dahms[1], A. Körnig[2], E. Ndlovu[1], H. Martin-Yken[3,4] and E. Dague[4]*

(1) University of Regina, 3737 Wascana Parkway, Regina, Saskatchewan, Canada S4S 0A2
(2) JPK Instruments, JPK Instruments AG, Colditzstr. 34-36, 12099 Berlin, Germany
(3) LISBP INSA Université de Toulouse, UMR CNRS 5504, UMR INRA 792, 135 Avenue de Rangueil 31077 Toulouse, France.
(4) LAAS-CNRS, 7 avenue du colonel Roche, Toulouse, France.

Models of the *Saccharomyces cerevisiae* cell wall include a complex interplay of polysaccharides and proteins, with β-1,3- and -β-1,6- glucans representing major components in terms of dry mass. The cell wall network is decorated with proteins bound to the glucans through different types of molecular interactions, including modified GPI-anchors. Chitin is another key component, which appears at the inner surface and is concentrated in bud scars, while the outer surface is coated with mannoproteins.

There is a great body of evidence supporting such models; however, the precise contribution of each molecular component to the overall cell wall viscoelasticity and integrity is still an area of active investigation. The aim of this work was to examine the structural and physical properties of the *S. cerevisiae* cell wall during its enzymatic degradation and subsequent regeneration, using atomic force microscopy (AFM) quantitative (QI™) and high-speed (HS) imaging. We studied a wild-type strain and two well characterized cell wall mutants of *S. cerevisiae*, *kre6Δ*, deleted in a gene required for β-1,6-glucan synthesis and *knr4Δ*, deprived of a cell wall signaling protein notably involved in the transcriptional control of chitin synthase genes.

Clear differences between the mutant and wild type cell walls were already distinguishable by phase contrast microscopy. Spheroplasts of each strain were prepared using zymolyase and lyticase, but imaging of the perfectly round spheroplasts proved challenging, even when housed in the wells of plasma-treated PDMS stamps coated with Cell Tak. Here we present HS- and QI-AFM imaging of *S. cerevisiae* exposed to lyticase, showing changes to cell wall structure and physical properties in real time.

References

1- F. M. Klis, A. Boorsma, P. W. J. DeGroot, Journ. Yeast **23**, 185 (2006).
2- B. P. Reference, Rev. Name **76**, 7648 (2005).

Corresponding author email: tanya.dahms@uregina.ca