THE CELL WALL OF *Candida albicans*: ARCHITECTURE AND BIOSYNTHESIS IN RESPONSE TO STRESS.

Rafael Sentandreu
Department of Microbiology and Ecology, University of de Valencia, Valencia, Spain.

Cells of *Candida albicans* are surrounded by a wall responsible for providing their shape and protection against physical and chemical aggressions. The cell wall makes up close to 30% of the cell dry weight , 80–90% of which corresponds to polysaccharides while the rest is represented mainly by proteins. The most important polysaccharides are chitin and β-1,3- and β-1,6-glucans. Despite the low amounts of proteins, their functional activities make their role in the wall exceedingly important. The cell wall is not a static structure as its chemical composition and the assembly of the different macromolecules are modified during cell growth and morphogenesis.

In order to learn how the different macromolecules are synthesized and incorporated to the wall, we have analyzed how the cells recover from the stress introduced by removal of the wall (protoplasts). Regeneration involves two phenomena: recovery from the stress imposed during cell wall removal, and synthesis of a completely new cell wall. Transcription profiling of the elimination of the wall and posterior regeneration was explored using DNA microarrays to measure changes in the expression of 6039 genes. Upregulated genes during regeneration at 28 °C were assigned to fourteen categories. A total of 407 genes were upregulated during the process, of which 144 reached a maximum after 1 h. Time-dependent expression divided the genes into 40 clusters. Clusters 1–19 were highly expressed initially (time 0) and downregulated following incubation, whereas transcription of the genes grouped into clusters 20–40 showed the opposite behaviour. These results suggest that the first clusters group genes permitting the cell adaptation to a sub-optimal environment due to removal of the wall, whereas the second group represents genes required for protoplasts regeneration after shifted to optimal conditions. Analysis the T-profiler showed that the group of ‘‘structural components of the wall’’ was up-regulated after two hours and remained as such during the process.

We carried out also a systematic and exhaustive analysis of the cell wall subproteome by LC coupled to MS (LC-MS) using samples obtained by different techniques aimed at a stepwise extraction of bound proteins. A total of 21 cell wall proteins predicted to contain a signal peptide were identified, together with a high content of potentially glycosylated Ser/Thr residues, and the presence of a GPI motif in 19 of them. We also identified 66 “atypical” cell wall proteins that lack the above-mentioned characteristics. After tryptic removal of the most accessible proteins in the cell wall, several of the same expected GPI proteins and the most commonly found “atypical” wall proteins were identified. This result suggests that proteins are located not only at the cell wall surface, but are embedded within the cell wall itself. These results, which include new identified cell wall proteins, and comparison of proteins in blastospore and mycelial walls, will help to elucidate the *C. albicans* cell wall architecture.