6. Summary

Candida albicans is normally a harmless commensal fungus of mucosal surfaces in healthy individuals but can cause several types of infections in predisposed patients, ranging from superficial to life-threatening disease. As the most common oral fungal pathogen of humans, *C. albicans* frequently causes oral infections in human immunodeficiency virus (HIV)-infected patients. During oral infections, fungal cells invade the oral mucosa and persist within the epithelium causing superficial lesions. However, the mechanisms by which *C. albicans* invades and persists within mucosal epithelium are not clear. To understand oral pathogenesis, cellular and molecular mechanisms of epithelial-fungus interactions were characterized using a model based on reconstituted human oral epithelial tissue (RHE). Based on the microscopical analysis of histological sections the experimental infection process was dissected into three different phases: an early attachment phase (1 h), a mid-invasion phase (3-6 h) and a late destruction phase (12–24 h). The early attachment phase was characterized by immediate hyphal formation and a strong adhesion of the fungal cells to the epithelial tissue. Furthermore, electron microscopy (SEM, TEM) revealed that hyphal formation facilitates epithelial invasion via both active (physical penetration) and passive (induced endocytosis) processes. The late phase was characterized by a dense network of hyphal cells invading and disseminating within the tissue. Only the late phase was correlated with strong tissue damage, reflected by the release of an epithelial marker enzyme (LDH). Based on these observations genome-wide transcript profiling of *C. albicans* was performed at five time-points (1, 3, 6, 12 and 24 h) aiming to identify phase-specific genes. To show that the data obtained from the *in vitro* infection model reflect the *in vivo* situation, transcriptional profiling of *C. albicans* cells isolated from patients suffering from oral *Candida* infections was also analyzed. Although the transcriptional profiles of the 11 patient samples showed an unexpected high heterogeneity, a set of genes was identified which were similarly regulated under *in vitro* and *in vivo* conditions. The expression profiles reflected the morphological switch and an adaptive response to neutral pH, non-glucose carbon sources and nitrosative stress. To identify genes, potentially associated with fungal virulence, eight genes were

targeted to create knock-out mutants. Thereby only genes whose expression was phase-specific and/or expressed in both patients and the RHE model were chosen. All eight mutants were analyzed in the RHE model and under a number of *in vitro* growth conditions. Four of the mutants had moderate or strongly reduced abilities to damage epithelial tissue. One gene, up-regulated in both RHE infection and patients, named *EED1*, was essential for maintenance of hyphal elongation. Mutants lacking *EED1* showed transient cell elongation on epithelial tissue, which enabled only superficial invasion of epithelial cells. Once inside an epithelial cell, *Δeed1* cells could proliferate as yeasts or pseudohyphae but remained trapped intracellular. These results suggest that the adaptive response and morphology of *C. albicans* play specific roles for host-fungal interactions during mucosal infections.