SUMMARY

The incidence of life-threatening mycoses has dramatically increased in recent years. Among other human pathogenic fungi, *Candida albicans* belongs to those fungi which most commonly cause mycoses. *C. albicans* is an opportunistic pathogen which is harmless to healthy individuals and is part of the normal microbial flora of skin and mucosa. Only under certain circumstances, such as when the immune system is impaired or after administration of broad-spectrum antibiotics, do deep-seated or systemic infections with *C. albicans* occur. A major part of the research on *C. albicans* is focussed on those virulence factors that enable the fungus to penetrate into the host. The secreted aspartate proteases are one of the most discussed virulence factors of *C. albicans*. They can digest host proteins and may therefore help the fungus to invade host tissue. *C. albicans* possesses a large gene family (*SAP*s) with ten members encoding the secreted aspartate proteases (Saps) which may be differentially activated during infection. The different members of this gene family were identified within the last decade and no systematic research analysing all ten genes has been performed so far.

The initial objective of this thesis was to analyse the expression profiles of all ten *SAP* genes under different growth conditions *in vitro*. Thereafter, studies were extended to investigate *in vivo* (*SAP*) expression during oral and intra-peritoneal (i. p.) infections of mice. The second objective of this thesis was to describe and characterise the genes *SAP9* and *SAP10* and the proteolytic properties of the corresponding proteases.

The conditions chosen for the *in vitro* expression analysis were hyphal induction, growth in media containing protein or ammonium sulfate as a sole source of nitrogen, and phenotypic switching. The *SAP* gene expression pattern was investigated using RT-PCR. The highest level of transcription for all the *SAP* genes was detected in minimal media supplemented with protein, which is known to induce protease activity of *C. albicans*. In contrast, a general down-regulation of *SAP*s was observed in the absence of protein in minimal medium with ammonium sulfate as the sole source of nitrogen. Only the transcripts of *SAP2*, *SAP8*, *SAP9* and *SAP10* could be detected at all

time points in this protein-lacking medium. Different media that induced hyphal production had different influences on the expression of *SAP*s. In contrast to hyphal induction in defined media, no transcripts for *SAP1*, *SAP2* and *SAP3* were observed in the presence of serum, suggesting that serum compounds may have inhibited the expression of these genes and that *SAP*s may be differentially expressed in different niches of the host. The hyphal specific genes *SAP4* and *SAP6* were shown to have contrasting expression profiles in both media. The strongest transcription of *SAP6* was detected in defined media, while *SAP4* was most strongly expressed in serum-containing media. During phenotypic switching of *C. albicans* strain WO-1, the expression of *SAP* genes in the "opaque" form was generally stronger. Particularly, *SAP1, SAP3, SAP6* and *SAP8* transcripts were expressed at their highest level in this switching form as compared with the "white" form.

SAP expression analysis in the oesophagus of orally infected DBA/2 and Balb/c mice, indicated a direct correlation between the defence capacity of the host and the expression of *SAP* genes. In the complement factor C5-deficient DBA/2 mice, *Candida* cells showed stronger proliferation and expressed *SAP2*, *SAP3*, *SAP8* and *SAP10* at high levels. Transcription of *SAP4-6* and *SAP9* was detected in most infected oesophagus samples of both mice strains.

The kinetics of *SAP* expression during intra-peritoneal infections (i. p.) of mice was analysed in infected livers at 4, 8, 24, and 72 h post infection and in infected kidneys 72 h after initiation of the infection. In all investigated organs, *SAP2* and *SAP4-6* transcripts were detected. The transcripts for *SAP3* and *SAP10* were mostly detected at the time point of *C. albicans* invasion into the liver (8 h), but not at later stages of liver infection (72 h) or in secondary infected kidneys. Transcripts of *SAP7* were never detected. These results confirm the contribution of *SAP4-6* during *C. albicans* invasion. A plasmid constructed in this thesis, designed to retransform a Δ*sap6* mutant, was used to further demonstrate that *SAP6* in particular is required for invasion (Felk *et al.*, submitted).

Furthermore, it could be shown that expression of the hyphal-specific genes *SAP4-6* is likely to be regulated via the MAP-kinase and cAMP cascades.

The inactivation of both cascades by deletion of the transcriptional factors Efg1p and Cph1p, which causes a deficiency in *C. albicans* hyphal transformation, strongly reduced the expression of *SAP5* and *SAP6* in serum containing medium *in vitro*. During i. p. mice infections with Δ*efg1*, Δ*cph1* and Δ*efg1* / Δ*cph1* mutants of *C. albicans*, the inhibition of *SAP4-6* expression was less significant in comparison with the wild type strain. Twenty-four hours after infection no transcripts for *SAP1* or *SAP3* were found in the Δ*cph1* mutant, and no *SAP8* transcripts were detected in the Δ*efg1* mutant. This indicates that not only hyphal deficiency, but also the lack of other factors, such as secreted aspartate proteases, may influence the virulence properties of these mutants. These results verify the postulation that the simultaneous expression of several virulence attributes contribute to the virulence of *C. albicans* (CUTLER, 1991, ODDS, 1994b).

The *SAP10* gene was sequenced in this thesis. Due to the high homology to *SAP9*, *SAP10* was assigned as a member of the *SAP* gene family. The protein sequences of both Sap9p and Sap10p contain GPI attachment sequences, which indicate that both proteins may possibly localise either in the plasma membrane or in the cell wall. To functionally analyse *SAP9* and *SAP10*, the genes were disrupted and the corresponding mutants were characterised. The Δ*sap9* and Δ*sap10* single mutants had a reduced ability to form hyphae and showed a moderate osmotic sensitivity during growth on hyperosmotic media. The optimal proteolytic activity of Sap9p and Sap10p was determined to be pH 2.3 and 4.5, respectively. The high homologies of Sap9p and Sap10p to the processing proteases Yps1p and Yps2p of *S. cerevisiae* and the reduced ability of Sap9p and Sap10p to hydrolyse serum albumin, also indicate a processing function for both proteinases. Furthermore, using Southern analysis, it could be shown that similar genes also exist in non-proteolytic *Candida* species. However, the precise functions of Sap9p and Sap10p are still unknown and further investigations are required.