[Frontiers in Bioscience 13, 7227-7242, May 1, 2008]

Secreted proteins of *Candida albicans*

Olga Hruskova-Heidingsfeldova

Gilead Sciences Research Center, Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, Flemingovo namesti 2, 166 10 Prague 6, Czech Republic

TABLE OF CONTENTS

1. ABSTRACT

The predicted secretome of the opportunistic fungal pathogen *Candida albicans* contains more than 200 ORFs of diverse and often unknown function. Majority of the secreted proteins that have been experimentally evaluated to date are hydrolytic enzymes (proteinases, phospholipases and lipases). Acting on the interface between the pathogen and the host, the secreted hydrolases may enable invasion of host tissues, help the pathogen to avoid host defense mechanisms, or allow the microorganism to utilize host cell macromolecules as a source of nutrients. Aspartic proteinases constitute the best-characterized family of the *C. albicans* secreted proteins. Number of studies addressed also secreted phospholipases. Lipases and N-acetylhexosaminidase have received less attention thus far. Many ORFs that are predicted to encode secreted proteins await characterization.

2. INTRODUCTION

The genus *Candida* comprises about 150 yeast species, and approximately 17 of them are associated with opportunistic infections of humans and other mammals (1). Of these, *C. albicans* is the most frequently encountered species in clinical practice. It causes a wide spectrum of diseases, ranging from superficial mycoses to disseminated infections with high mortality rates. Pathogenic *Candida* spp. can live in human or animal hosts as harmless commensals, being kept under the control by the host immune system. However, in a susceptible host, a transition of *Candida* from commensal to pathogen can occur. This transition is governed by host-pathogen interactions and is likely to require not only impairment of the host immune system but also an active contribution of the fungus (2).

Table 1. Summary of the genes encoding secreted hydrolases of *C. albicans*

Gene name	Chromosome	Most closely related family member (BLAST)	Size of the gene product [amino acid residues]	Posttranslational modifications	3D structure
SAP1	6	SAP2	391, mature enzyme 341	putative N-glycosylatin site probably not used	n.d.
SAP2	R	*SAP3*	398, mature enzyme 342	no	38,39
SAP3	3	*SAP2*	398, mature enzyme 340	putative N-glycosylatin site probably not used	40
SAP4	6	*SAP6*	417, mature enzyme 342	no	n.d.
SAP5	6	*SAP6*	418, mature enzyme 342	no	n.d.
SAP6	6	*SAP4*	418, mature enzyme 342	no	n.d.
SAP7	1	*SAP6*	588	putative N-glycosylation	n.d.
SAP8	3	*SAP3*	405	putative N-glycosylation	n.d.
SAP9	3	*SAP10*	544	GPI-anchor, N-glycosylation	n.d.
SAP10	4	*SAP3*	453	GPI-anchor, N-glycosylation	n.d.
PLB1	6	*PLB2*	605	N-glycosylation, possible Y phosphorylation	n.d.
PLB2	6	*PLB1*	609	putative N-glycosylation	n.d.
PLB3	R	*PLB5*	632	putative GPI-anchor	n.d.
PLB4	2	*PLB1*	304	putative GPI-anchor	n.d.
PLB4.5	2	*PLB3*	700	putative GPI-anchor	n.d.
PLB5	1	*PLB3*	754	putative GPI-anchor	n.d.
LIP1	1	*LIP3*	468	putative N-glycosylation	n.d.
LIP2	1	*LIP6*	466	putative N-glycosylation	n.d.
LIP3	1	*LIP1*	471	putative N-glycosylation	n.d.
LIP4	6	*LIP8*	459	putative N-glycosylation	n.d.
LIP5	7	*LIP8*	463	putative N-glycosylation	n.d.
LIP6	1	*LIP2*	463	putative N-glycosylation	n.d.
LIP7	R	*LIP1*	426	putative N-glycosylation	n.d.
LIP8	7	*LIP5*	460	putative N-glycosylation	n.d.
LIP9	7	*LIP5*	453	putative N-glycosylation	n.d.
LIP10	1	*LIP2*	465	putative N-glycosylation	n.d.
HEX1	5	n.d.	562	N-glycosylation	n.d.

Data are derived from ref 7 and from www.candidagenome.org. n.d., not determined.

The reasons to study the pathogenic *Candida* spp. are related in the first place to the needs of medical treatment and protection of the individuals with weak or impaired immune systems. Population of the immunocompromised individuals has expanded and the frequency of infections due to the *Candida* spp. has increased over the past two decades. This fact has prompted the research that resulted in a progress of the development of antifungal drugs and diagnostic tools (3). Although various antimycotics have been marketed, the number of therapeutical strategies for critically ill patients is limited. While the superficial mycoses can be relatively mild and easy to cure, we still have problems to prevent and treat the systemic infections.

The efforts aimed at the design of novel antifungals and diagnostic approaches produced a remarkable pool of scientific data. Thus, pathogenic *Candida* spp., and particularly *C. albicans* has become an attractive and powerful model to study a range of biological phenomena. *C. albicans* was one of the first eukaryotic pathogens whose genome was sequenced. The completed diploid genome sequence was published in 2004 (4) and the annotations in 2005 (5, 6). The unfinished sequences were made available to the community earlier to advance the understanding of the *C. albicans* pathogenicity. The latest and the most complete *C. albicans* genome sequence (Assembly 21) was released in September 2007. The haploid genome consists of 15.845 Mb of DNA organized into the 8 chromosomes, and contains 6109 predicted protein-coding genes (7). The main repository of genomic sequence data, and information on genes/proteins that have been studied so far is the Candida Genome Database (CGD, www.candidagenome.org).

According to the CGD annotation, 41 genes have been identified to date that encode proteins secreted to the extracellular space. However, the secretion has not been experimentaly demonstrated for all of these proteins. In some cases, the extracellular localization is inferred from electronic annotation. The list of predicted extracellular proteins was further extended by application of bioinformatics methods. Lee *et al.* identified 283 *C. albicans* ORFs with N-terminal signal peptide but without transmembrane domains, GPI-anchor and mitochondrial or other localization signal (8). These ORFs encode proteins of diverse and often unknown functions. Only 38 ORFs of this set were found to encode known *C. albicans* proteins or members of known families. In fact, gene/protein families form a large part of the list of known ORFs in the predicted *C. albicans* secretome.

The set of secreted proteins in principle comprises not only the proteins that can be recovered from the cell-free culture supernatants, but also those proteins that are displayed extracellularly, but remain linked to the cell surface. This review is, however, focused mostly on the entirely secreted proteins. Although they may be temporarily retained in the cell wall, they do not form covalent bonds to the cell surface structures.

All of the proteins falling into this category are hydrolytic enzymes (Table 1). Pathogenic microbes often possess a repertoire of secreted hydrolases, namely proteinases, lipases and phospholipases, which facilitate the pathogen invasion of the host cells. These enzymes may also allow microorganisms to utilize host cell macromolecules as a source of nutrients. The secretion of

extracellular proteinases, lipases and phospholipases by *C. albicans* was first reported in 1960s (9,10). The enzyme secretion was observed when the yeasts were cultivated on solid media containing either bovine serum albumin (proteinases) or egg yolk (lipases, phospholipases). Breakdown of the protein or lipid/phospholipid substrates resulted in a halo or precipitation zone, proportional to the activity of the hydrolases secreted by the yeast colonies. The optimized and simplified plate assays are still being used for rapid, gross screening of hydrolase activities, particularly in the studies monitoring a large number of clinical isolates (11-16).

The observation of extracellular proteolytic and lipolytic activities initiated extensive studies of secreted hydrolases, especially proteinases of *C. albicans* and other pathogenic *Candida* spp. The gene families encoding these enzymes were described, and the individual genes were found to be expressed differentially, depending on environmental factors and life stage of the fungus. While the phospholipase B activity has been detected also in *Saccharomyces cerevisiae* culture supernatants (17), secreted proteinases and lipases do not have any *S. cerevisiae* counterparts. Some of the hydrolase isoenzymes were studied on enzymological level and crystal structures of several secreted proteinases were solved. The reader is directed to excellent reviews discussing the topics of secreted proteinases (18-21), phospholipases (22-24), or hydrolazes in general (25). The aim of this review is to put forward the recent advancement of the research focused on secreted hydrolases of *C. albicans*, and to bring together the information on known representatives of the secretome of this fungus.

3. PROTEINASES

Proteinases, proteases or peptidases are ubiquitous enzymes that catalyze hydrolysis of peptide bonds. They are responsible for protein processing or degradation. Proteinases are classified according to the catalytic type, i.e. according to the characteristic amino acid residues in the active site. Aspartic, cystein, glutamic, serine, threonine and metallopeptidases have been described to date (26). Aspartic proteinases have been found in viruses, plants, vertebrates and fungi, but not in bacteria. They are active at acidic pH and are specifically inhibited by pepstatin. Two aspartic acid residues that are responsible for the catalytic activity occur within the conserved sequence motifs Asp-Thr/Ser-Gly. Aspartic proteinases play important roles in a range of diseases including hypertension, cancer, Alzheimer's disease, or viral and microbial infections (27). Extracellular aspartic proteinases are produced by *Aspergillus fumigatus* (28,29) and several pathogenic *Candida* species. The family of secreted aspartic proteinases (*SAP*) is undoubtedly the best-characterized set of proteins secreted by *C. albicans*.

While the *C. albicans SAP* family consists of ten members, four genes encoding secreted aspartic proteinases have been identified in *C. tropicalis* (30) and three in *C. parapsilosis* (31,32). As the *C. parapsilosis* and *C. tropicalis* genomes are currently being annotated, the number of the aspartic proteinases secreted by these species will soon become more accurate. *C. glabrata* does not produce significant levels of extracellular proteolytic activity. Nevertheless, it possesses a family of orthologs of the *S. cerevisiae* extracellular GPI-linked aspartic proteases that play a role in virulence (33). The extracellular GPI-anchored aspartic proteinases have been identified also in *C. albicans*. They are denominated Sap9 and Sap10, and are likely to play a role in the cell wall integrity and in the *C. albicans* adhesion to the host cells (34). The isoenyzmes Sap1-6 and Sap8 are secreted to the extracellular space and can be recovered from the culture supernatants (35-37). The extracellular localization of Sap7 was predicted (8).

The Sap-producing *Candida* spp. secret one major aspartic proteinase *in vitro*, in culture media containing exogenous protein as a sole source of nitrogen. In *C. albicans*, this isoenyzme is denominated Sap2. The *C. tropicalis* and *C. parapsilosis* orthologs are termed Sapt1p and Sapp1p, respectively. These proteinases are relatively easy to obtain in laboratory conditions and therefore they have been characterized in more detail than the other Sap isoenzymes.

3.1. Properties and inhibition of the secreted aspartic proteinases

The crystal structures of three aspartic proteinases secreted by two pathogenic *Candida* spp. have been published: Sap2 and Sap3 from *C. albicans* and Sapt1p from *C. tropicalis* (38-41). These structures reveal a typical pepsin-like two-domain architecture, formed mainly by beta-strands (Figure 1A). At the junction of the two domains is an extended substrate-binding cleft, which contains the two catalytic aspartate residues, each contributed by one domain.

While the GPI-anchored proteinases Sap9 and Sap10 are highly glycosylated, no glycosylation has been reported for Sap1-Sap8 isoenyzmes. Sap1-Sap3 have optimum activity at pH 3-5 and Sap4-Sap6 at pH 5-7 (21).

Substrate specificity studies have been performed with Sap1, Sap2, Sap3 and Sap6. Although they preferentially hydrolyze bonds formed by hydrophobic residues, the charged amino acids can be accommodated in P1 and P1' positions as well (42,43, Figure 1B). In general, the specificity of these enzymes is rather broad, reflecting their role in degradation of the host cell substrates, such as fibronectin, laminin (44) casein, denatured collagen (45) or Fc portion of IgG (46). Recently, degradation of E-cadherin in oral epithelial cell junctions has been described and attributed to Sap5 (47).

Pepstatin inhibits most of the secreted aspartic proteinases in nanomolar concentrations. Several studies demonstrated a protective effect of pepstatin in early stages of the *C. albicans* attack. However, the results obtained in systemic infection models were contradictory (48, 49, other studies are reviewed in 20). Panels of the active site peptidomimetic inhibitors were synthesized and tested in attempts to find a lead structure for the design of novel antimycotics. Although no unambiguously convincing compound has been found, the Sap inhibition and structural

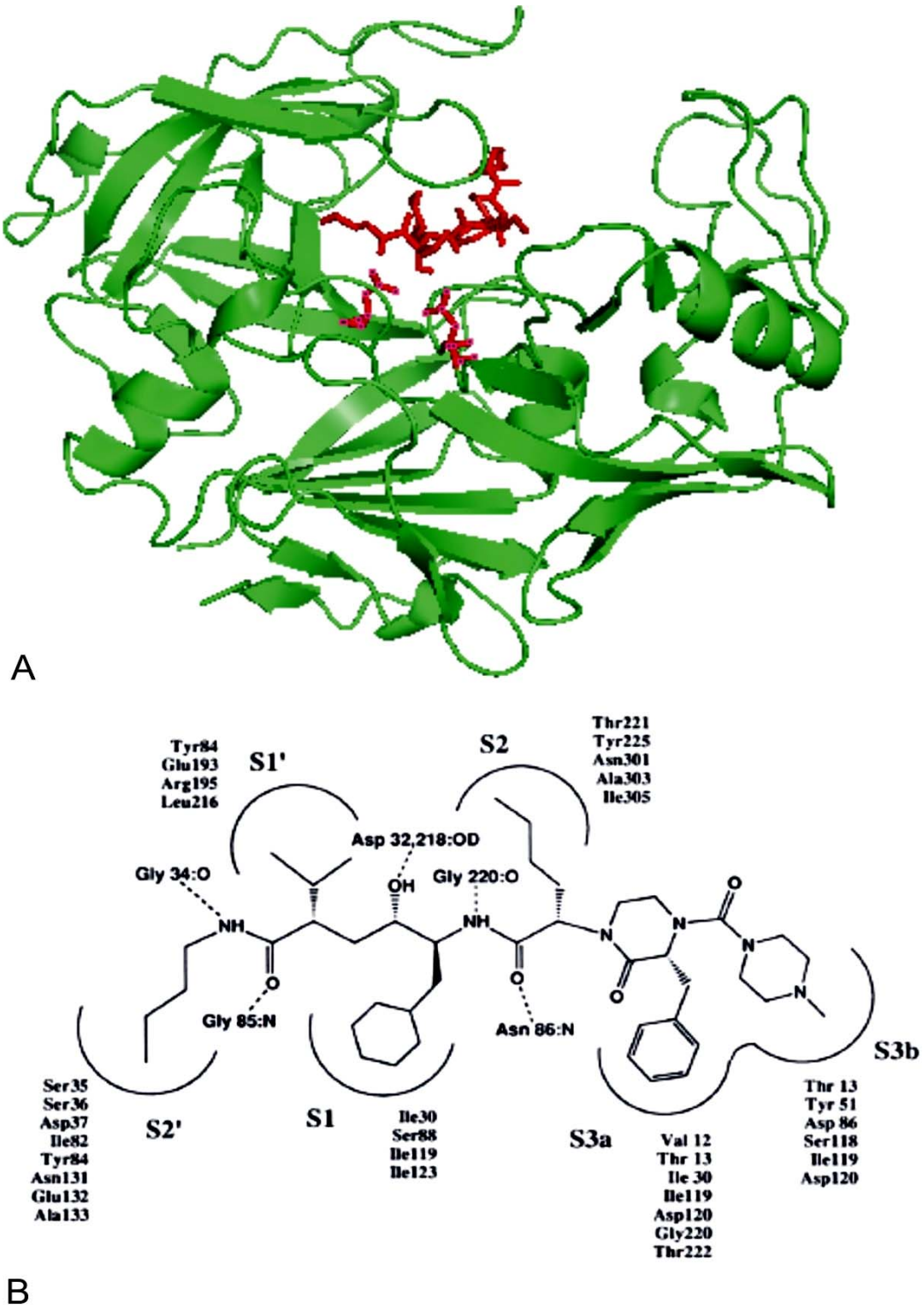

Figure 1. A - Ribbon representation of Sap2 complexed with the inhibitor A70450. Active-site aspartates as well as the inhibitor are highlighted in red. The PDB code of this structure is 1zap, and its detailed description is in ref. 39; B - Schematic representation of the hydrogen bonding interactins of the A-70450 inhibitor with the atoms of different enzyme pockets corresponding to the inhibitor subsites. Reproduced with permission from (39).

studies helped to deeply understand this class of enzymes and to map subtle differences in the active sites of proteinases secreted by several pathogenic *Candida* spp. (50-52).

The fact that the proteinases secreted by pathogenic *Candida* spp. and HIV proteinase belong to the same family of aspartic proteinases lead to an idea that HIV proteinase inhibitors may act also against *Candida* infection. The clinical inhibitors ritonavir, saquinavir, indinavir and nelfinavir interact mostly with S2-S2′ subsites of HIV-1 proteinase (53). In contrast, the binding cleft of the *Candida* aspartic proteinases is relatively spacious, forming the interactions with pepstatin in S4-S2' subsites (38-40). Thus, the inhibitors of HIV proteinase do not have sufficient contacts with the substrate-binding cleft of the *Candida* Sap enzymes. The inhibition constants measured for the HIV proteinase inhibitors and the *Candida* secreted protinases are within micromolar range or poorer (50, 54). Moreover, the inhibitors of HIV proteinase display differential activities against the individual Sap isoenzymes, inhibiting rather Sap1-Sap3 than the other proteinases (55). Saquinavir, indinavir and ritonavir had anticandidal effect in models of oral and vaginal candidiasis, when micromolar concentrations of the inhibitors were used (56-58). On the other hand, only ritonavir was able to block the *C. albicans* adherence to human endothelial cells, while the other HIV proteinase inhibitors did not have any effect in this experimental setting (59). These data further support the concept of possible application of the proteinase inhibitors only in the initial stages of the infection. The present-day compounds directed at Sap isoenzymes appear unsuitable for treatment or alleviation of the systemic disease. It is questionable whether the *SAP* family should be considered as a potential drug target, since most of the successful current antifungals act against functions that are essential rather for growth, than for virulence (3).

3.2. Regulation of the proteinases secreted by *C. albicans*

C. albicans inhabits diverse host niches and many genes are therefore expressed differentially in response to a specific environment. A number of studies have been performed in order to dissect the regulation of the *SAP* gene family, and the reader is directed to the excellent reviews that summarize results of this research (20,21,25). Expression of the *SAP* genes depends on many factors including ambient pH, temperature, and nutrient sources which vary according to the type and stage of the disease. The *SAP* gene control is related also to the morphological transition from yeast to hyphae, and to the cell-type switch between the commonly observed white phase of *C. albicans* and the opaque, mating-competent phase. The *SAP* genes are located on five different chromosomes and their promoter regions are distinctive. This allows differential regulation of their transcription, in which several transcription factors are involved. Moreover, the differential regulation may occur even on an allelic level (60).

SAP1 and *SAP3* are regulated by phenotypic switching and were found to be opaque-phase-specific (61,62). *SAP2* is expressed in both white and opaque phenotypes. Its expression is induced by presence of an exogenous protein and controlled via a positive feedback mechanism (60,63). Presence of peptides longer than 7 amino acids can elicit the *SAP2* expression as well (64). *SAP2* is activated by transcription factor Stp1, which also activates expression of the genes encoding oligopeptide transporters *OPT1* and *OPT3*. Thus, the degradation of exogenous proteins is co-regulated with the oligopeptide uptake (65). *SAP4-SAP6* are almost exclusively expressed during the hyphal formation at near neutral pH values. These genes are regulated by the factors that also regulate the yeast-hypha transition, particularly by the activator of hypha formation Efg1 (66). Conversely, the repressor of hyphal development Nrg1 exerts negative effect on the transcription of *SAP5* (67). Hyphal cells are likely to require the expression of the hypha-associated *SAP* genes during invasion (66). Expression of *SAP5* and *SAP6* was increased also during the formation of biofilm (68). The *SAP7* gene and the respective protein are rather enigmatic. The *SAP7*-specific RNA has not been detected under all the laboratory growth conditions tested and no studies of Sap7 on the protein level have been published. Nevertheless, the *SAP7* expression appears to be controlled by the transcription factor Ssn6, as the *SAP7* upregulation was observed in the *ssn6* null mutant (69). *SAP7* is repressed also by the repressor of yeast-hypha morphogenesis Tup1 (67). *SAP8* is temperature-regulated *in vitro*, it is expressed at higher levels at 25 °C than at 37 °C and the *SAP8*-specific mRNA is more abundant in opaque cells (70).

Although the expression of the secreted proteinases appears to be controlled mainly on the level of transcription, the posttranslational modifications may also play a role in the regulation of the *C. albicans* extracellular proteolysis. All the *SAP* genes encode proteinase precursors that contain 60-200 amino acids long pre-prosequence flanking the mature proteinase at the N-terminus. The pre-sequence, i.e. the signal peptide consists of approximately 20 amino acids and is cleaved by a signal peptidase. The propeptide is considered to be removed by the subtilisin-like Kex2 proteinase in the Golgi. The mature aspartic proteinase is then transported to the cell surface and secreted. This concept is supported by the study of Newport and Agabian (71), who showed that in the *kex1* null mutant Sap2 was processed upstream the correct promature junction and its secretion was reduced. On the other hand, Sap1-3 and Sap6 were found to be capable of autocatalytic activation, which indicates that alternative processing pathways exist. Interestingly, in *C. parapsilosis* one of the Sapp isoenzymes requires activation by a Kex-like proteinase *in vitro*, while the other can be activated both autocatalytically and *in trans* (72,73). This suggests that distinct processing pathways may be used by the individual proteinases, and that the mode of activation may be a part of the Sap regulatory mechanism.

3.3. Role of the secreted aspartic proteinases in virulence of *C. albicans*

The role of the *SAP* family in the *C. albicans* virulence has been reviewed in (20,21,54). The Sap isoenzymes were reported to be involved in adherence,

colonization, nutrition and dissemination of *C. albicans*. Moreover, the secreted proteinases contribute to the induction of the host inflamatory response by the activation of interleukin-beta (74) and stimulating expression of other cytokines (75).

Sap1-Sap3 were found to participate mostly in adhesion of *C. albicans* to the host cells. The role of these proteinases in the initial stages of the fungal attack and their contribution to mucosal infections has been studied in various models including oral, vaginal or cutaneous infections or reconstituted epithelia (49, 76-78).

SAP4-6 gene products are required for systemic infections. Although temporally distinct patterns of the *SAP* genes were expresssed during the intraperitoneal infection, *SAP4-6* were found to be critical for invasion of parenchymal organs (66). The Sap4-Sap6 isoenzymes are likely to contribute to virulence by helping *Candida* to resist the phagocytic attack.

The *SAP7* expression was elevated during vaginal infection of mice (79) and in humans with oral candidiasis (80). The *sap7* null mutant exhibited reduced virulence in intravenously infected mice, but surprisingly not in the vaginal infection model (79). The mechanism by which Sap8 contributes to virulence is also unclear. *SAP8* expression in reconstituted human epithelium was found to be regulated temporally. The highest levels of *SAP8* transcript occurred only after *SAP1*, *SAP3*, *SAP6* and *SAP2* were already expressed (81).

The role of the *SAP* family in the *C. albicans* pathogenesis is still under investigation. The recent additions to the pathogenesis studies include the observations that Sap6 is specifically associated with the *C. albicans* keratitis (82) and that Sap2 may contribute to aggravation of duodenal ulcers (83). Nevertheless, the individual Sap isoenzymes appear not to be sufficient to cause the *C. albicans* invasion. Instead, synergistic effect of more isoenzymes is needed. Mutants lacking an individual *SAP* gene rarely exhibit a fully avirulent phenotype, because the activities and functions of the Sap isoenzymes overlap (84,85).

4. PHOSPHOLIPASES

Phospholipases catalyze hydrolysis of one or more ester bonds in glycerophospholipids. They perform a variety of important tasks including membrane maintenance or destabilization, and release of lipid second messengers. Phospholipases are classified into four major groups A-D (Figure 2). Phospholipases A1 and A2 (PLA) hydrolyze fatty acyl ester bond at *sn*-1 or *sn*-2 position, respectively. Group B phospholipases (PLB) display both phospholipase A1 and A2 activities, catalyzing the concomitant removal of both acyl chains from phospholipids. However, phospholipases B of many fungal species are multifunctional enzymes that possess also lysophospholipase activity (Lyso-PL; acylhydrolase activity on monoacylphospholipids), and lysophospholipase-transacylase activity (LPTA; addition of fatty acids to monoacylphospholipids to form corresponding diacylphospholipids). Phospholipases C and D (PLC, PLD) are phosphodiesterases. Phospholipases C hydrolyze glycerophosphate bond, while phospholipases D remove the base group of phospholipids.

Phospholipases of diverse origin are being used in the food industry such as cheese production or baking (86). On the other hand, they participate in a number of unfavourable phenomena. Phospholipase activity was identified in the snake venom and bacterial toxins (for review see 87,88). Extracellular phospholipases either secreted or membrane bound, are associated with virulence of many procaryotic pathogens, e.g. *Neisseria gonorrhoeae* (89), *Bacillus anthracis* (90), group A *Streptococcus* (91), *Listeria monocytogenes* (92) and many others (reviewed in 22,93). In several fungal pathogens secreted phospholipases have been identified as virulence factors as well. Phospholipase B, which possesses also the lyso-PL and LPTA activities, contributes to virulence of *Cryptococcus neoformans* in animal models (94). Multifunctional phospholipase B isoenzymes are produced also by *Aspergillus fumigatus* and considered as potential virulence determinants (95).

Candida albicans encodes a wide repertoire of phospholipases. All four types of activities, A, B, C and D have been reported. However, the articles published to date differ in the exact number of specific types of phospholipases and their role in virulence. This may be caused by a variety of experimental approaches used, by overlaps among the functions of the individual enzymes, and also by difficulties to distinguish among phospholipases A, B and lysophospholipases. (25,96). It seems that the genome of *C. albicans* does not contain clear sequence homologs of mammalian or bacterial phospholipase A1 and A2 genes. In the reports available up to now, the phospholipase A2 activity has been associated with the *PLB5* gene. However, the whole range of biochemical activities that the *PLB5* gene product may possess still has to be elucidated (96).

Five genes denominated *PLB* are listed in the Candida Genome Database (Table 1). All of them contain N-terminal signal sequence for secretion. Plb3p, Plb4p, and Plb5p additionally contain hydrophobic COOH termini with putative GPI anchor attachment sites. The genes *PLB1* and *PLB2* encoding proteins without a GPI anchor were cloned (97, 109, 116), however, only *PLB1* has received significant attention. To date, *PLB1* is the best-characterized member of the *C. albicans PLB* family, and its gene product is secreted to the extracellular space. Accordingly, *PLB1* will be emphasized also in this review.

At least three *PLC* genes have been identified in *C. albicans*. None of them contains a signal peptide, and therefore their gene products are not likely to be secreted. Phospholipases C are predicted to be localized in cytoplasm and nucleus. While the first *PLC* gene identified in *C. albicans* (named *CaPLC1*) is an orthologue of *PLC1* of *S. cerevisiae*, the other two genes contain structural elements found in bacterial phospholipases C and do not have any

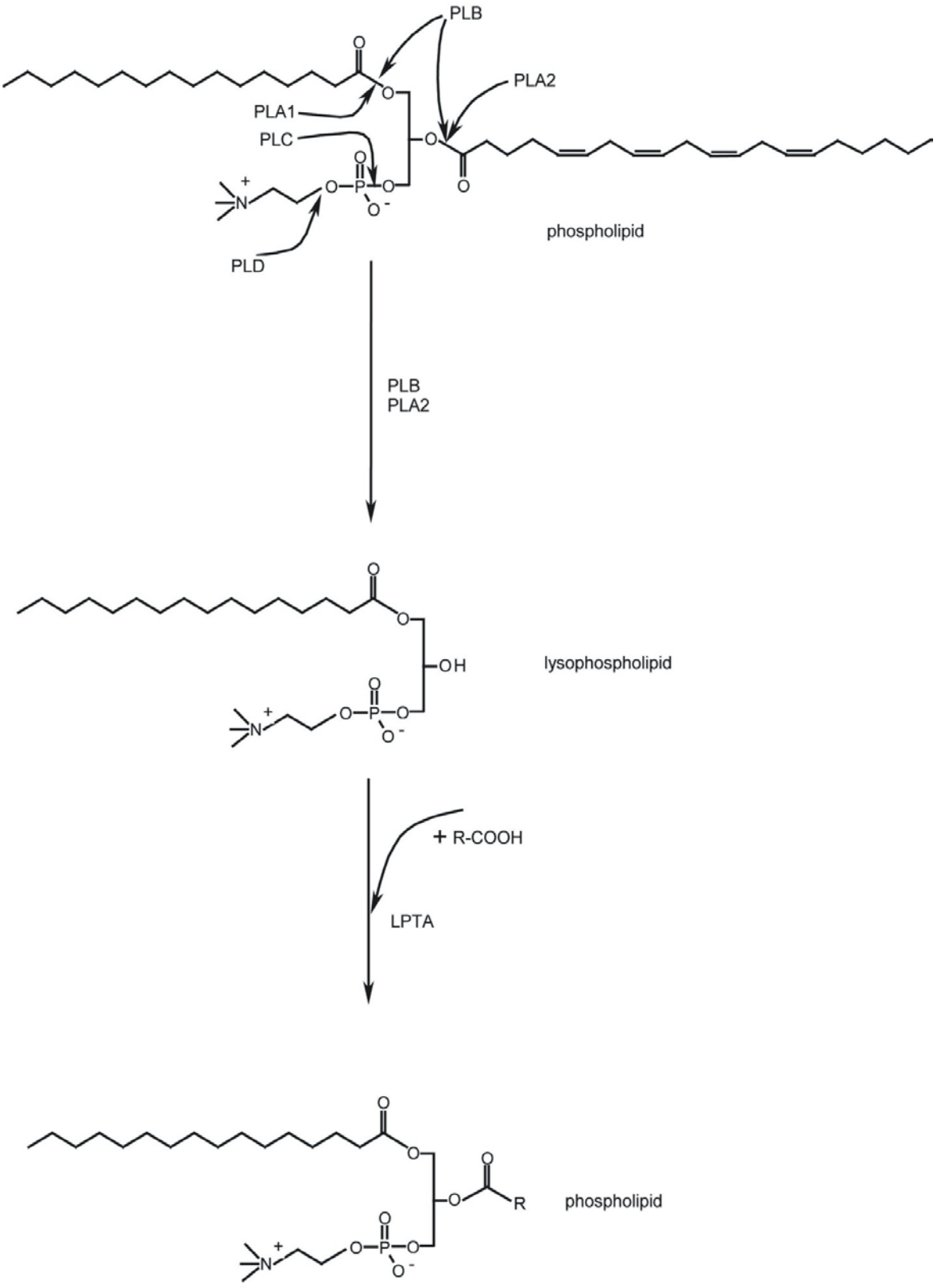

Figure 2. Reactions catalyzed by phospholipases A, B, C, D and lysophospholipase/transacylase. The bonds hydrolyzed by the individual types of enzymes are indicated by arrows. Lysophospholipase/transacylase catalyzes esterification of a free fatty acid to a lysophospholipid resulting in a phospholipid.

counterpart in *S. cerevisiae.* *CaPLC1* appears to be an essential gene; the *plc1* mutant is not viable. In contrast, *CaPLC2* and *CaPLC* 3 are not essential for growth and virulence (98).

The *PLD1* gene encodes the major phosphatidylcholine-specific phospholipase D in *C. albicans*. The corresponding activity has been localized in the membrane fraction (99). *PLD1* is upregulated during the yeast to hypha transition. When cultivated in liquid media, the *pld1* mutant did not show any growth or morphological defects. However, a defect in hyphal growth was observed when the *pld1* mutant was subjected to an agar invasion test (100). *PLD1* was found to be essential for the *C. albicans* virulence in the mouse model (101).

4.1. Properties of the extracellular phospholipase

Although many phospholipases from various sources have been structurally characterized, no structure of *Candida* phospholipase has been published to date. Phospholipases B from *C. albicans* display a significant homology to Plbs from *S. cerevisiae*, *P. notatum* or *S. pombe* (22). The *C. albicans PLB1* gene encodes a protein 605 amino acids long, containing N-glycosylation and possible tyrosine phosphorylation sites. The acylhydrolase activity is usually assayed at pH 4-5. On the other hand, Mirbod *et al.* reported isolation of a *C. albicans* secreted enzyme with acylhydrolase and LPTA acitivities exhibiting pH optima at pH 6 (102). Optimum activity for the *S. cerevisiae* secreted Plb enzymes was observed at pH 2.5 - 3.5 (17).

Substrates of phospholipases are either phospholipids or lysophospholipids consisting of a polar head group (ethanolamine, choline or inositol esterified to phosphoric acid) and one or two non-polar fatty acyl chains esterified to a glycerol backbone. The substrate specificity of phospholipases is determined both by phospho-head groups and by the chain length and saturation of the fatty acyl side chains. Plb1 is strongly inhibited by compounds in which two cationic headgroups are linked by an alkyl spacer. Bisammonium, bispyridinium or bisquinolinium compounds appear to inhibit only Plb1 activity, but not Lyso-PL or LPTA activities. It is not known, which of the three activities is most important for virulence. Nevertheless, some of these compounds have an antifungal effect against *C. albicans* and *Cryptococcus neoformans* (103).

The extracellular LPTA but not Lyso-PL activity was inhibited by palmitoyl carnitine. Presence of palmitoyl carnitine at 300 μM concentration blocked the adhesion of *Candida* to immobilized lysophospholipids and HEp-2 cells (104). The LPTA activity of the *Cryptococcus* Plb1was inhibited by the alkyl phosphocholine drug Miltefosine. When assayed in microdilution test, this compound exhibited antifungal activity against a range of species, including *C. albicans* (105).

Antitumor drugs doxorubicin and daunorubicin were reported to block the extracellular Plb activity at concentrations higher than 17 μM. However, the experiments were performed with the whole cell culture and not with purified enzymes. Therefore, the mode of action of these compounds has not been fully clarified (106).

4.2. Regulation of the phospholipase secreted by *C. albicans*

Expression of *PLB1* is affected by multiple environmental and physiological stimuli, including pH, temperature and concentration of nutrients. Nevertheless, the reports published to date differ in the opinion of whether a particular set of ambient conditions causes induction or repression of this gene. Mukherjee *et al.* detected *PLB1*-specific mRNA in the cells cultivated at 30 °C, both in a rich medium (Sabouraud dextrose broth) containing at least 2 % glucose, and in the yeast nitrogen base supplemented with 0.5 % glucose. At 37 °C, the *PLB1* expression was always lower, and required presence of serum and a mixture of phospholipids (107). Conversely, Samaranayake *et al.* reported that *PLB1* was expressed at 37°C, in a rich medium (YPD) without addition of serum or phospholipids (108). Hoover and colleagues detected the highest level of *PLB1* in blastospores and pseudohyphae, while hyphal form expressed reduced levels of *PLB1* mRNA (109). In contrast, Theiss *et al.* observed high expression of *PLB1* in pseudohyphae and hyphae that were formed during the growth of *C. albicans* in the Lee's medium at 37 °C. The yeast form expressed one order of magnitude less *PLB1* mRNA (96). The concept of the *PLB1* expression in the hyphae is supported by observations that in many cells the phospholipase activity is concentrated to the initial bud sites and hyphal tips (110,111). Plb1 was shown to be localized mostly to the hyphal tips also by immunofluorescence microscopy (112).

The Samaranayake group analyzed the expression of *PLB1* in a set of *C. albicans* isolates from HIV infected individuals. Although the *PLB1* gene was present in all of these strains, a considerable subset of isolates behaved as phospholipase-negative, exhibiting neither phospholipase secretion, nor *PLB1* transcription (113). These observations indicate that an extensive variability in the *PLB1* regulation may exist among the *C. albicans* strains. The differences in the reported data may thus be due to the properties of the strains under investigation.

Genome-wide and mutation studies using the strains ATCC 32354, SC5314 and CAF2–1 revealed that *PLB1* is controlled by the transcriptional repressor *TUP1*. *PLB1* was upregulated in the *tup1* mutants (67,109,114). Moreover, in the *tup1* mutant the *PLB1* expression became constitutive and did not vary in response to environmental stimuli (109). *PLB1* expression appears to be affected also by the signaling of the stress-activated protein kinase Hog1. Higher levels of *PLB1* mRNA were detected in the parental strain than in the *hog1* mutant (115).

Secreted phospholipase is likely to be regulated also on posttranslational level. However, potential

regulatory role of the protein modifications has not been addressed as yet.

4.3. Role of the secreted phospholipase in virulence of *C. albicans*

As phospholipases are able to destroy the components of the host cell membranes, they have been regarded as virulence determinants. Of the *PLB* genes studied thus far, *PLB1* and the respective protein appear to be the most important contribution to the virulence. Leidich *et al.* reported that disruption of *PLB1* did not affect growth and morphology of the *C. albicans* cells, or their adherence to epithelial and endothelial cells. However, virulence of *plb1* mutants in murine model of hematogenously disseminated candidiasis was significantly reduced. This was probably due to increased clearance rates of the *PLB1*-deficient strains, combined with their decreased ability to penetrate host cells (116). The reduced invasivness of the *plb1* strain was confirmed by histological analysis of stomach sections of infected mice. Without functional *PLB1*, *C. albicans* was unable to penetrate gastric mucosa. In comparison with parental strain the *plb1* mutant was also less efficient in colonizing the host liver (112). Surprisingly, *PLB1* was not expressed during the growth of *C. albicans* on the reconstituted human oral epithelium (108). On the other hand, elevated levels of the *PLB1* mRNA were detected in the samples collected from individuals diagnosed with oral or vaginal candidiasis. Expression of *PLB1* in oral or vaginal *C. albicans* carriers was lower and less frequent (80).

5. LIPASES

Lipases and esterases catalyze the hydrolysis of ester bonds of triacylglycerols. Lipases are capable of identifying and hydrolysis of insoluble or aggregated substrates and act on oil/water interface. Esterases preferentially hydrolyze more water-soluble substrates containing shorter fatty acid chains. Lipases and esterases have been identified in a wide range of organisms, and many of them have been characterized on enzymological and structural level (117). Exploration of lipases is stimulated by the fact that they have become one of the most popular tools in biocatalysis (118,119).

Lipases are studied also because of their contribution to virulence of pathogenic bacteria and fungi, such as *Helicobacter pylori*, *Pseudomonas aeruginosa*, and pathogenic *Candida* spp. (25, 120-122). However, secreted lipases of *C. albicans* have attracted less attention than proteinases and phospholipases.

The *LIP1* gene was first cloned by Fu *et al.* (123). This gene was transcribed only in a minimal medium supplemented with Tween or triacylglycerols as the sole carbon source. Both Tween and triacylglycerols were hydrolyzed by the *LIP1* gene product, a protein consisting of 351 amino acids and containing Gly-X-Ser-X-Gly motif characteristic of lipases. *C. albicans* genome sequencing project revealed that the *LIP1* gene product consists of 468 amino acids.

The genes *LIP2 –LIP10* of *C. albicans* were characterized by Hube *et al.* (124). These genes are strongly similar to *LIP1*, but no homologous genes were found in *S. cerevisiae*. They encode 48 - 52 kDa proteins containing the Gly-X-Ser-X-Gly motif and potential N-glycosylation sites (Table 1). With the exception of *LIP7*, all the *LIP* genes contain putative signal peptide. *LIP3*, *LIP4*, *LIP5*, *LIP6* and *LIP8* were induced in presence of Tween 40 as the sole source of carbon. When proline was used as a source of carbon, all the *LIP* genes with the exception of *LIP7* were upregulated. Although the *LIP* genes were found to be expressed during the yeast-hypha transition, they appear not to depend on the morphology of the fungus. Transcripts of *LIP5*, *LIP6*, *LIP8* and *LIP9* were detected in organs of mice infected in a model of *C. albicans* peritonitis. Expression of the *C. albicans LIP* genes in human clinical specimens was observed by Stehr *et al.* (125), who also confirmed that the transcription of these genes depends on the stage of infection. The differential expression of the *LIP* genes was further studied by Schofield *et al.* (126), who analyzed presence of the *LIP*-specific mRNA during colonization and infection of alimentary tract of mice. Transcripts of *LIP4-8* were detected in all the samples tested. *LIP1*, *LIP3* and *LIP9* were expressed in gastric and not in oral tissues. Importantly, *LIP2* was expressed rather in colonized tissues than in infected ones. Conversely, *LIP*1 and *LIP*3 were expressed in infected gastric tissues but to a much lesser extent during colonization. Gacser *et al.* (127) constructed *C. albicans* mutants carrying different copy numbers of the *LIP8* genes. The *lip8* strain had a diminished lipolytic activity and was significantly less virulent in the mice model of hematogenously disseminated candidiasis.

It appears that not all the *C. albicans LIP* gene products display lipolytic activity. When expressed in *S. cerevisiae*, only *LIP4, LIP6, LIP8*, and *LIP10* genes produce active enzymes (128). Of all the *C. albicans* lipases, only Lip4 has been studied on an enzymological level. It preferred saturated, C14-C16 acyl chains and did not hydrolyze esters of short-chain fatty acids. The data on enzymological activities and specificities of other *LIP* gene products have not been published to date. It is therefore unclear, whether these enzymes display lipase or rather esterase activities. Extracellular esterase (monoester hydrolase) activity of *C. albicans* was described by Tsuboi *et al.* (129). However, it is not known, if this esterase is encoded by a member of the *LIP* gene family, or by some other gene. The potential posttranslational modifications of the *C. albicans* lipases have not been elucidated either.

Sequences similar to *LIP1-10* were found also in *C. parapsilosis*, *C. tropicalis* and *C. krusei*, but not in *C. glabrata*. *C. parapsilosis* possesses two *LIP* genes, but only one of them appears to encode a functional lipase. This enzyme preferentially hydrolyzes substrates containing C12 acyl chains. Biofilm formation as well as virulence was reduced in lipase-negative *C. parapsilosis* mutants (122).

6. MISCELLANEOUS

For a number of proteins listed in CGD or NCBI, the evidence of secretion is based on a single observation published in no more than one article. This diverse set of proteins includes e.g. hyaluronidase and chondroitin sulphatase (130), alcohol dehydrogenase encoded by *ADH1* (131), alpha-glucosidase encoded by *MAL2* (132), chitinase encoded by *CHT3* (133), or a member of *IFF* family comprising cell-wall proteins, Iff11 that unlike other *IFF* members lacks a potential GPI-anchor or transmembrane domain (134). Future experiments may either confirm or disprove extracellular localization of these proteins. In contrast, multiple evidence of secretion is available for beta-*N*-actylhexosaminidase.

6.1. Beta-N-Acetylhexosaminidase

The HEX1 gene encodes beta-N-acetylhexosaminidase, an enzyme that is secreted to periplasm and to the extracellular space. This hydrolytic enzyme was previously denominated chitobiase, because it acts on the chitin oligomers N,N'-diacetylchitobiose and N,N'N''-triacetylchitobiose, releasing N-acetylglucosamine. But since this enzyme can cleave a broad range of substrates (135), the name was changed to beta-N-acetylhexosaminidase, also known as HexNAcase. HexNAcases are produced by a variety of fungi, but also by bacteria and mammals. Among pathogenic Candida species, high levels of extracellular HexNAcase activity were detected in C. albicans and C. dubliniensis. The HexNAcase plate assay can thus be used for an easy distinction of these two species from C. glabrata, C. guillermondii, C. krusei or C. tropicalis, that do not produce this enzyme (136).

The HexNAcase from C. albicans is a highly glycosylated, relatively stable enzyme with a maximum activity at pH 4 (135). Its production is induced by presence of N-acetylglucosamine, which can serve as the only source of carbon and nitrogen (137). HexNAcase is considered to be a part of a nutrient-scavenging system, that may give the cells a growth advantage. It has been suggested that extracellular HexNAcase may contribute to the C. albicans pathogenicity by cleaving host glycoproteins and releasing N-acetylglucosamine that may serve as a nutrient source. It is also likely that HexNAcase plays a role in the chitin turnover (137). HexNAcase-defficient mutant was less pathogenic in a mouse model than the parental strain (138).

7. SUMMARY AND PERSPECTIVE

Some of the secreted proteins mentioned in this review are considered as highly relevant for the *C. albicans* virulence. However, the contribution of a particular gene/protein to the fungus invasion and pathogenesis is difficult to dissect, especially because of a high degree of functional redundancy within the families. The continuing efforts of many laboratories may therefore result in reinterpretation of the relative importance of the individual proteins.

Although secreted aspartic proteinases of *C. albicans* have been characterized thoroughly, some pieces of information are still missing. The structures of Sap2 and Sap3 have been published, but comparative data addressing Sap2 and Sap3 specificity are scarce (40). Other members of the *SAP* family have not been studied so extensively. Sap7 is rather enigmatic, and the precise roles of Sap9 and Sap10 remain unknown. The information on the secreted phospholipases are less detailed in comparison with the *SAP* family. No structural studies have been published and the amount of data concerning potential inhibitors of these enzymes is rather limited. Possible role of the *Candida* phospholipases in signaling between the pathogen and the host has to be elucidated. Lipases secreted by *C. albicans* have been marginalized, and clearly deserve more attention. Biological and biochemical data concerning the *LIP* family are sparse.

This review obviously covers only a part of the *C. albicans* secretome. Future experiments are likely to provide information on more secreted proteins. Some of them might be encoded by ORFs whose function is currently unknown.

8. ACKNOWLEDGMENT

This work was a part of a research project conducted at the Institute of Organic Chemistry and Biochemistry under the code number AV 0Z4 055 0506, and was also supported by the Czech Science Foundation (grant 203/05/0038) and by the Ministry of Education of the Czech Republic (grant LC531). I thank Jan Snasel and Jiri Dostal for their kind help with the figures.

9. REFERENCES

1. R. Calderone: *Candida* and Candidiasis. ASM Press, Washington, DC (2002)

2. B. Hube: From commensal to pathogen: stage- and tissue-specific gene expression of *Candida albicans*. *Curr Opin Microbiol* 7, 336-341 (2004)

3. M. Weig and A. J. P. Brown: Genomics and the development of new diagnostics and anti-*Candida* drugs. *Trends Microbiol* 15, 310-317 (2007)

4. T. Jones, N. A. Federspiel, H. Chibana, J. Dungan, S. Kalman, B. B. Magee, G. Newport, Y. R. Thorstenson, N. Agabian, P. T. Magee, R. W. Davis and S. Scherer: The diploid genome sequence of *Candida albicans*. *Proc Natl Acad Sci* 101, 7329-7334 (2004)

5. B. R. Braun, M. van het Hoog, C. d'Enfert, M. Martchenko, J. Dungan, A. Kuo, D. O. Inglis, M. A. Uhl, H. Hogues, M. Berriman, M. Lorenz, A. Levitin, U. Oberholzer, C. Bachewich, D. Harcus, A. Marcil, D. Dignard, T. Iouk, R. Zito, L. Frangeul, F. Tekaia, K. Rutherford, E. Wang, C. A. Munro, S. Bates, N.A. Gow, L. L. Hoyer, G. Kohler, J. Morschhauser, G. Newport, S. Znaidi, M. Raymond, B. Turcotte, G. Sherlock, M. Costanzo, J. Ihmels, J. Berman, D. Sanglard, N. Agabian, A.

P. Mitchell, A. D. Johnson, M. Whiteway and A. Nantel: A human-curated annotation of the *Candida albicans* genome. *PLoS Genet* 1, 36-57 (2005)

6. C. d'Enfert, S. Goyard, S. Rodriguez-Arnaveilhe, L. Frangeul, L. Jones, F. Tekaia, O. Bader, A. Albrecht, L. Castillo, A. Dominguez, J. F. Ernst, C. Fradin, C. Gaillardin, S. Garcia-Sanchez, P. de Groot, B. Hube, F. M. Klis, S. Krishnamurthy, D. Kunze, M. C. Lopez, A. Mavor, N. Martin, I. Mozser, D. Onesime, J. Perez Martin, R. Sentandreu, E. Valentin and A. J. Brown: CandidaDB: a genome database for *Candida albicans* pathogenomics. *Nucleic Acids Res* 33, D353-357 (2005)

7. M. van het Hoog, T. J. Rast, M. Martchenko, S. Grindle, D. Dignard, H. Hogues, C. Cuomo, M. Berriman, S. Scherer, B. B. Magee, M. Whiteway, H. Chibana, A. Nantel and P. T. Magee: Assembly of the *Candida albicans* genome into sixteen supercontigs aligned on the eight chromosomes. *Genome Biol* 8, R52 (2007)

8. S. A. Lee, S. Wormsley, S. Kamoun, A.F.S. Lee, K. Joiner and B. Wong: An analysis of the *Candida albicans* genome database for soluble secreted proteins using computer-based prediction algorithms. *Yeast* 20, 595-610 (2003)

9. F. Staib: Serum-proteins as nitrogen source for yeastlike fungi. *Sabouraudia* 4, 187-193 (1965)

10. H. Werner: Studies on the lipase activity in yeasts and yeast-like fungi (German). *Zentbl Bakteriol Mikrobiol Hyg 1 Abt Orig A* 200, 113-124 (1966)

11. F. C. Odds and A. B. Abbott: A simple system for the presumptive identification of *Candida albicans* and differentiation of strains within the species. *Sabouraudia* 18, 301-317 (1980)

12. M. F. Price, I. D. Wilkinson and L. O. Gentry: Plate method for detection of phospholipase activity in *Candida albicans*. *Sabouraudia* 20, 7-14 (1982)

13. J. Dostal, P. Hamal, L. Pavlickova, M. Soucek, T. Ruml, I. Pichova and O. Hruskova-Heidingsfeldova: Simple method for screening *Candida* species isolates for the presence of secreted proteinases: a tool for the prediction of successful inhibitory treatment. *J Clin Microbiol* 41, 712-716 (2003)

14. A. Borst and A. C. Fluit: High levels of hydrolytic enzymes secreted by *Candida albicans* isolates involved in respiratory infections. *J Med Microbiol* 52, 971-974 (2003)

15. E. G. Birman, S. Kignel, F. R. Da Silveira and C. R. Paula: *Candida albicans*: Frequency and characterization in oral cancer (Stage I) from smokers and drinkers. *Rev Iberoam Micol* 14, 101-103 (1997)

16. T. Kadir, B. Gumru and B. Uygun-Can: Phospholipase activity of *Candida albicans* isolates from patients with denture stomatitis: The influence of chlorhexidine gluconate on phospholipase production. *Arch Oral Biol* 52, 691-696 (2007)

17. O. Merkel, M. Fido, J. A. Mayr, H. Pruger, F. Raab, G. Zandonella, S. D. Kohlwein and F. Paltauf: Characterization and function *in vivo* of two novel phospholipases B/lysophospholipases from *Saccharomyces cerevisiae*. *J Biol Chem* 274, 28121-28127 (1999)

18. B. Hube: Extracellular proteinases of human pathogenic fungi. *Contrib Microbiol* 5, 126-137 (2000)

19. F. De Bernardis, P. A. Sullivan and A. Cassone: Aspartyl proteinases of *Candida albicans* and their role in pathogenicity. *Med Mycol* 39, 303-313 (2001)

20. J. R. Naglik, S. J. Challacombe and B. Hube: *Candida albicans* secreted aspartyl proteinases in virulence and pathogenesis. *Microbiol Mol Biol Rev* 67, 400-428 (2003)

21. J. Naglik, A. Albrecht, O. Bader and B. Hube: *Candida albicans* proteinases and host/pathogen interactions. *Cell Microbiol* 6, 915-926 (2004)

22. M. A. Ghannoum: Potential role of phospholipases in virulence and fungal pathogenesis. *Clin Microbiol Rev* 13, 122-143, (2000)

23. M. Niewerth and H. C. Korting: Phospholipases of *Candida albicans*. *Mycoses* 44, 361-367 (2001)

24. G. A. Kohler, A. Brenot, E. Haas-Stapleton, N. Agabian, R. Deva and S. Nigam: Phospholipase A2 and phospholipase B activities in fungi. *Biochim Biophys Acta* 1761, 1391-1399 (2006)

25. M. Schaller, C. Borelli, H. C. Korting and B. Hube: Hydrolytic enzymes as virulence factors of *Candida albicans*. *Mycoses* 48, 365-377 (2005)

26. http://merops.sanger.ac.uk

27. J. B. Cooper: Aspartic proteinases in disease: A structural perspective. *Curr Drug Targets* 3, 155-173 (2002)

28. U. Reichard, H. Eiffert and R. Ruchel: Purification and characterization of an extracellular aspartic proteinase from *Aspergillus fumigatus*. *J Med Vet Mycol* 32, 427-436 (1994)

29. I. Vickers, E. P. Reeves, K. A. Kavanagh and S. Doyle: Isolation, activity and immunological characterisation of a secreted aspartic protease, CtsD, from *Aspergillus fumigatus*. *Protein Expr Purif* 53, 216-224 (2007)

30. C. Zaugg, M. Borg-von Zepelin, U. Reichard, D. Sanglard and M. Monod: Secreted aspartic proteinase family of *Candida tropicalis*. *Infect Immun* 69, 405-412 (2001)

31. P. A. de Viragh, D. Sanglard, G. Togni, R. Falchetto and M. Monod: Cloning and sequencing of two *Candida parapsilosis* genes encoding acid proteases. *J. Gen. Microbiol*. 139, 335-342 (1993)

32. C. Zaugg and M. Monod: direct submission to NCBI, http://www.ncbi.nlm.nih.gov/entrez/viewer.fcgi?db=protein&id=18478802

33. R. Kaur, B. Ma and B. P. Cormack: A family of glycosylphosphatidylinositol-linked aspartyl proteases is required for virulence of *Candida glabrata*. *Proc Natl Acad Sci USA* 104, 7628-7633 (2007)

34. A. Albrecht, A. Felk, I. Pichova, J. R. Naglik, M. Schaller, P. De Groot, D. Maccallum, F. C. Odds, W. Schafer, F. Klis, M. Monod and B. Hube: Glycosylphosphatidylinositol-anchored proteases of *Candida albicans* target proteins necessary for both cellular processes and host-pathogen interactions. *J Biol Chem* 281, 688-94 (2006)

35. T. C. White, S. H. Miyasaki and N. Agabian: Three distinct secreted aspartyl proteinases in *Candida albicans*. *J Bacteriol* 175, 6126-6133 (1993)

36. Y.-C. Chen, C.-C. Wu, W.-L. Chung and F.-J. S. Lee: Differential secretion of Sap4–6 proteins in *Candida albicans* during hyphae formation. *Microbiology* 148, 3743-3754 (2002)

37. C. J. Morrison, S. F. Hurst, S. L. Bragg, R. J. Kuykendall, H. Diaz, J. Pohl and E. Reiss: Heterogeneity of the purified extracellular aspartyl proteinase from *Candida albicans*: characterization with monoclonal antibodies and N-terminal amino acid sequence analysis. *Infect Immun* 61, 2030-2036 (1993)

38. S. M. Cutfield, E. J. Dodson, B. F. Anderson, P. C. E. Moody, C. J. Marshall, P. A. Sullivan and J. F. Cutfield: The crystal structure of a major secreted aspartic proteinase from *Candida albicans* in complexes with two inhibitors. *Structure* 3, 1261-1271 (1995)

39. C. Abad -Zapatero, R. Goldman, S. W. Muchmore, C. Hutchins, K. Stewart, J. Navaza, C. D. Payne and T. L. Ray: Structure of a secreted aspartic protease from *C. albicans* complexed with a potent inhibitor: implications for the design of antifungal agents. *Protein Sci* 5, 640-652 (1996)

40. C. Borelli, E. Ruge, M. Schaller, M. Monod, H. C. Korting, R. Huber and K. Maskos: The crystal structure of the secreted aspartic proteinase 3 from *Candida albicans* and its complex with pepstatin A. *Proteins* 68, 738-748 (2007)

41. J. Symersky, M. Monod and S. I. Foundling: High-resolution structure of the extracellular aspartic proteinase from *Candida tropicalis* yeast. *Biochemistry* 36, 12700-12710 (1997)

42. G. Koelsch, J. Tang, J. A. Loy, M. Monod, K. Jackson, S. I. Foundling and X. Lin: Enzymic characteristics of secreted aspartic proteases of *Candida albicans*. *Biochim Biophys Acta* 1480, 117-131 (2000)

43. M. Fusek, E. A. Smith, M. Monod, B. M. Dunn and S. I. Foundling: Extracellular aspartic proteinases from *Candida albicans*, *Candida tropicalis*, and *Candida parapsilosis* yeasts differ substantially in their specificities. *Biochemistry* 33, 9791-9799 (1994)

44. J. Morschhauser, R. Virkola, T. K. Korhonen and J. Hacker: Degradation of human subendothelial extracellular matrix by proteinase-secreting *Candida albicans*. *FEMS Microbiol Lett* 153, 349-355 (1997)

45. T. L. Ray and C. D. Payne: Comparative production and rapid purification of *Candida* acid proteinase from protein-supplemented cultures. *Infect Immun* 58, 508-514 (1990)

46. H. Kaminishi, H. Miyaguchi, T. Tamaki, N. Suenega, M. Hisamatsu, I. Mihashi, H. Matsumoto, H. Maeda and Y. Hagihara: Degradation of humoral host defense by *Candida albicans* proteinase. *Infect Immun* 63, 984-988 (1995)

47. C. C. Villar, H. Kashleva, C. J. Nobile, A. P. Mitchell and A. Dongari-Bagtzoglou: Mucosal tissue invasion by *Candida albicans* is associated with E-Cadherin degradation, mediated by transcription factor Rim101p and protease Sap5p. *Infect Immun* 75, 2126-2135 (2007)

48. K. Fallon, K. Bausch, J. Noonan, E. Huguenel and P. Tamburini: Role of aspartic proteases in disseminated *Candida albicans* infection in mice. *Infect Immun* 65, 551-556 (1997)

49. M. Schaller, M. Bein, H. C. Korting, S. Baur, G. Hamm, M. Monod, S. Beinhauer and B. Hube: The secreted aspartyl proteinases Sap1 and Sap2 cause tissue damage in an *in vitro* model of vaginal candidiasis based on reconstituted human vaginal epithelium. *Infect Immun* 71, 3227-3234 (2003)

50. I. Pichova, L. Pavlickova, J. Dostal, E. Dolejsi, O. Hruskova-Heidingsfeldova, J. Weber, T. Ruml and M. Soucek: Secreted aspartic proteases of *Candida albicans*, *Candida tropicalis*, *Candida parapsilosis*, *Candida lusitaniae*: inhibition with peptidomimetic inhibitors. *Eur J Biochem* 268, 2669-2677 (2001)

51. K. Stewart and C. Abad-Zapatero: *Candida* proteases and their inhibitors. *Curr Med Chem* 8, 941-948 (2001)

52. F. Majer, L. Pavlickova, P. Majer, M. Hradilek, E. Dolejsi, O. Hruskova-Heidingsfeldova and I. Pichova: Structure-based specificity mapping of secreted aspartic proteases of *Candida parapsilosis*, *Candida albicans*, and *Candida tropicalis* using peptidomimetic inhibitors and homology modeling. *Biol Chem* 387, 1247-1254 (2006)

53. M. Prabu-Jeyabalan, E. A. Nalivaika, N. M. King and C. A. Schiffer: Viability of a drug-resistant human immunodeficiency virus type 1 protease variant: structural insights for better antiviral therapy. *J Virol* 77, 1306-1315 (2003)

54. M. Monod and M. Borg- von Zepelin: Secreted aspartic proteases as virulence factors of *Candida* species. *Biol Chem* 383, 1087-1093 (2002)

55. P. Staib, U. Lermann, J. Blass-Warmuth, B. Degel, R. Wurzner, M. Monod, T. Schirmeister and J. Morschhauser: Tetracycline-inducible expression of individual secreted aspartic proteases in *Candida albicans* allows isoenzyme-specific inhibitor screening. *Antimicrob Agents Chemother* 52, 146-156 (2008)

56. H. C. Korting, M. Schaller, G. Eder, G. Hamm, U. Bohmer and B. Hube: Effects of the human immunodeficiency virus (HIV) proteinase inhibitors Saquinavir and Indinavir on *in vitro* activities of secreted aspartyl proteinases of *Candida albicans* isolates from HIV-infected patients. *Antimicrob Agents Chemother* 43, 2038-2042 (1999)

57. A. Cassone, F. De Bernardis, A. Torosantucci, E. Tacconelli, M. Tumbarello and R. Cauda: *In vitro* and *in vivo* anticandidal activity of human immunodeficiency virus protease inhibitors. *J Infect Dis* 180, 448-453 (1999)

58. J. Bektic, C. P. Lell, A. Fuchs, H. Stoiber, C. Speth, C. Lass-Florl, M. Borg-von Zepelin, M. P. Dierich and R. Wurzner: HIV protease inhibitors attenuate adherence of *Candida albicans* to epithelial cells *in vitro*. *FEMS Immunol Med Microbiol* 31, 65-71 (2001)

59. B. Falkensammer, G. Pilz, J. Bektic, P. Imwidthaya, K. Johrer, C. Speth, C. Lass-Florl, P. Dierich and R. Wurzner: Absent reduction by HIV protease inhibitors of *Candida albicans* adhesion to endothelial cells. *Mycoses* 50, 172-177 (2007)

60. P. Staib, M. Kretschmar, T. Nichterlein, H. Hof and J. Morschhauser**:** Host versus *in vitro* signals and intrastrain allelic differences in the expression of a *Candida albicans* virulence gene. *Mol Microbiol* 44, 1351-1366 (2002)

61. B. Morrow, H. Ramsey and D. R. Soll: Regulation of phase-specific genes in the more general switching system of *Candida albicans* strain 3153A. *J Vet Med Mycol* 32, 287-294 (1994)

62. A. Strauss, S. Michel and J. Morschhauser: Analysis of phase-specific gene expression at the single-cell level in the white-opaque switching system of *Candida albicans*. *J Bacteriol* 183, 3761-3769 (2001)

63. B. Hube, M. Monod, D. A. Schofield, A. J. P. Brown and N. A. R. Gow: Expression of seven members of the gene family encoding secretory aspartyl proteinases in *Candida albicans*. *Mol Microbiol* 14, 87-99 (1994)

64. C. G. Lerner and R. C. Goldman: Stimuli that induce production of *Candida albicans* extracellular aspartic proteinase. *J Gen Microbiol* 139, 1643-1651 (1993)

65. P. Martinez and P. O. Ljungdahl: Divergence of Stp1 and Stp2 transcription factors in *Candida albicans* places virulence factors required for proper nutrient acquisition under amino acid control. *Mol Cell Biol* 25, 9435-9446 (2005)

66. A. Felk, M. Kretschmar, A. Albrecht, M. Schaller, S. Beinhauer, T. Nichterlein, D.Sanglard, H. C. Korting, W. Schafer and B. Hube: *Candida albicans* hyphal formation and the expression of the Efg1-regulated proteinases Sap4 to Sap6 are required for the invasion of parenchymal organs. *Infect Immun* 70, 3689-3700 (2002)

67. A. M. A. Murad, C. d'Enfert, C. Gaillardin, H. Tournu, F. Tekaia, D. Talibi, D. Marechal, V. Marchais, J. Cottin and A. J. P. Brown: Transcript profiling in *Candida albicans* reveals new cellular functions for the transcriptional repressors CaTup1, CaMig1 and CaNrg1. *Mol Microbiol* 42, 981-993 (2001)

68. S. Garcia-Sanchez, S. Aubert, I. Iraqui, G. Janbon, J. M. Ghigo and C. d'Enfert: *Candida albicans* biofilms: a developmental state associated with specific and stable gene expression patterns. *Eukayot Cell* 3, 536-545 (2004)

69. S. Garcia-Sanchez, A. L. Mavor, C. L. Russell, S. Argimon, P. Denisson, B. Enjalbert and A. J. P. Brown: Global roles of Ssn6 in Tup1- and Nrg1-dependent gene regulation in the fungal pathogen, *Candida albicans*. *Mol Biol Cell* 16, 2913-2925 (2005)

70. M. Monod, B. Hube, D. Hess and D. Sanglard: Differential regulation of *SAP8* and *SAP9*, which encode two new members of the secreted aspartic proteinase family in *Candida albicans*. *Microbiology* 144, 2731-2737 (1998)

71. G. Newport and N. Agabian: *KEX2* influences *Candida albicans* proteinase secretion and hyphal formation. *J Biol Chem* 272, 28954-28961 (1997)

72. J. Dostal, H. Dlouha, P. Malon, I. Pichova and O. Hruskova-Heidingsfeldova: The precursor of secreted aspartic proteinase Sapp1p from *Candida parapsilosis* can be activated both autocatalytically and by a membrane-bound processing proteinase. *Biol Chem* 386, 791-799 (2005)

73. M. Merkerova, J. Dostal, M. Hradilek, I. Pichova and O. Hruskova-Heidingsfeldova: Cloning and characterization of Sapp2p, the second aspartic proteinase isoenzyme from *Candida parapsilosis*. *FEMS Yeast Res* 6, 1018-1026 (2006)

74. A. Beausejour, D. Grenier, J. P. Goulet, N. Deslauriers: Proteolytic activation of the interleukin-1beta precursor by *Candida albicans*. *Infect Immun* 66, 676-681 (1998)

75. M. Schaller, H. C. Korting, C. Borelli, G. Hamm and B. Hube: *Candida albicans*-secreted aspartic proteinases modify the epithelial cytokine response in an *in vitro* model of vaginal candidiasis. *Infect Immun* 73, 2758-2765 (2005)

76. F. De Bernardis, S. Arancia, L. Morelli, B. Hube, D. Sanglard, W. Schafer and A. Cassone: Evidence that members of the secretory aspartyl protease gene family, in

particular *SAP2*, are virulence factors for *C. albicans* vaginitis. *J Infect Dis* 179, 201-208 (1999)

77. M. Schaller, H. C. Korting, W. Schafer, J. Bastert, W. Chen and B. Hube: Secreted aspartic proteinase (Sap) activity contributes to tissue damage in a model of human oral candidosis. *Mol Microbiol* 34, 169-180 (1999)

78. H. Sandovsky-Losica and E. Segal: Infection of HEp2 epithelial cells with *Candida albicans*: adherence and postadherence events. *FEMS Immunol Med Microbiol* 46, 470-475 (2006)

79. B. N. Taylor, H. Hannemann, M. Sehnal, A. Biesemeier, A. Schweizer, M. Rollinghoff and K. Schroppel: Induction of *SAP7* correlates with virulence in an intravenous infection model of candidiasis but not in a vaginal infection model in mice. *Infect Immun* 73, 7061-7063 (2005)

80. J. R. Naglik, C. A. Rodgers, P. J. Shirlaw, J. L. Dobbie, L.L. Fernandez-Naglik, D. Greenspan, N. Agabian and S. J. Challacombe: Differential expression of *Candida albicans* secreted aspartyl proteinase and phospholipase B genes in humans correlates with active oral and vaginal infections. *J Infect Dis* 188, 469-479 (2003)

81. M. Schaller, W. Schafer, H. C. Korting and B. Hube: Differential expression of secreted aspartyl proteinases in a model of human oral candidosis and in patient samples from the oral cavity. *Mol Microbiol* 29, 605-615 (1998)

82. B. E. Jackson, K. R. Wilhelmus and B. Hube: The role of secreted aspartyl proteinases in *Candida albicans* keratitis. *Invest Ophtalmol Vis Sci* 48, 3559-3565 (2007)

83. T. Nakamura, M. Yoshida, H. Ishikawa, K. Kameyama, G. Wakabayashi, Y. Otani, M. Shimazu, M. Tanabe, S. Kawachi, K. Kumai, T. Kubota, Y. Saikawa, K. Sano and M. Kitajima: *Candida albicans* aggravates duodenal ulcer perforation induced by administration of cysteamine in rats. *J Gastroenterol Hepatol* 22, 749-756 (2007)

84. M. Kretschmar, A. Felk, P. Staib, M. Schaller, D. Hess, M. Callapina, J. Morschhauser, W. Schafer, H. C. Korting, H. Hof, B. Hube and T. Nichterlein: Individual acid aspartic proteinases (Saps) 1-6 of *Candida albicans* are not essential for invasion and colonization of the gastrointestinal tract in mice. *Microb Pathog* 32, 61-70 (2002)

85. B. Hube, and J. Naglik: *Candida albicans* proteinases: resolving the mystery of a gene family. *Microbiology* 147, 1997-2005 (2001)

86. L. De Maria, J. Vind, K. M. Oxenboll, A. Svendsen and S. Patkar: Phospholipases and their industrial application. *Appl Microbiol Biotechnol* 74, 290-300 (2007)

87. B. Geny and M. R. Popoff: Bacterial protein toxins and lipids: role in toxin targeting and activity. *Biol Cell* 98, 633-651, (2006)

88. D. C. I. Koh, A. Armugam, K. Jeyaseelan: Snake venom components and their applications in biomedicine. *Cell Mol Life Sci* 63, 3030-3041 (2006)

89. J. L. Edwards and M. A. Apicella: *Neisseria gonorrhoeae* PLD directly interacts with Akt kinase upon infection of primary, human, cervical epithelial cells. *Cell Microbiol* 8, 1253-1271 (2006)

90. B. J. Heffernan, B. Thomason, A. Herring-Palmer, L. Shaughnessy, R. McDonald, N. Fisher, G. B. Huffnagle and P. Hanna: *Bacillus anthracis* phospholipases C facilitate macrophage-associated growth and contribute to virulence in a murine model of inhalation anthrax. *Infect Immun* 74, 3756-3764 (2006)

91. I. Sitkiewicz, K. E. Stockbauer and J. M. Musser: Secreted bacterial phospholipase A2 enzymes: better living through phospholipolysis. *Trends Microbiol* 15, 63-69 (2007)

92. A. Grundling, M. D. Gonzalez and D. E. Higgins: Requirement of the *Listeria monocytogenes* broad-range phospholipase PC-PLC during infection of human epithelial cells. *J Bacteriol* 185, 6295-6307 (2003)

93. T. S. Istivan and P. J. Coloe: Phospholipase A in Gram-negative bacteria and its role in pathogenesis. *Microbiology* 152, 1263-1274 (2006)

94. G. M. Cox, H. C. McDade, S. C. Chen, S. C. Tucker, M. Gottfredsson, L. C. Wright, T. C. Sorrell, S.D. Leidich, A. Casadevall, M. A. Ghannoum and J. R. Perfect: Extracellular phospholipase activity is a virulence factor for *Cryptococcus neoformans*. *Mol Microbiol* 39, 166-175 (2001)

95. D. K. Shen, A. D. Noodeh, A. Kazemi, R. Grillot, G. Robson and J.-F. Brugere: Characterisation and expression of phospholipases B from the opportunistic fungus *Aspergillus fumigatus*. *FEMS Microbiol Lett* 239, 87-93 (2004)

96. S. Theiss, G. Ishdorj, A. Brenot, M. Kretschmar, C.-Y. Lan, T. Nichterlein, J. Hacker, S. Nigam, N. Agabian and G. A. Kohler: Inactivation of the phospholipase B gene *PLB5* in wild-type *Candida albicans* reduces cell-associated phospholipase A_2 activity and attenuates virulence. *Int J Med Microbiol* 296, 405-420 (2006)

97. Y. Sugiyama, S. Nakashima, F. Mirbod, H. Kanoh, Y. Kitajima, M. A. Ghannoum and Y. Nozawa: Molecular cloning of a second phospholipase B gene, *CaPLB2* from *Candida albicans*. *Med Mycol* 37, 61-67 (1999)

98. D. Kunze, I. Melzer, D. Bennett, D. Sanglard, D. MacCallum, J. Norskau, D. C. Coleman, F. C. Odds, W. Schafer and B. Hube: Functional analysis of the

phospholipase C gene *CaPLC1* and two unusual phospholipase C genes, *CaPLC2* and *CaPLC3*, of *Candida albicans. Microbiology* 151, 3381-3394 (2005)

99. N. McLain and J. W. Dolan: Phospholipase D activity is required for dimorphic transition in *Candida albicans*. *Microbiology* 143, 3521-3526 (1997)

100. B. Hube, D. Hess, C. A. Baker, M. Schaller, W. Schafer and J. W. Dolan: The role and relevance of phospholipase D1 during growth and dimorphism of *Candida albicans*. *Microbiology* 147, 879-889 (2001)

101. J. W. Dolan, A. C. Bell, B. Hube, M. Schaller, T. F. Warner and E. Balish: *Candida albicans PLD I* activity is required for full virulence. *Med Mycol* 42, 439-447 (2004)

102. F. Mirbod, Y. Banno, M. A. Ghannoum, A. S. Ibrahim, S. Nakashima, Y. Kitajima, G. T. Cole and Y. Nozawa: Purification and characterization of lysophospholipase-transacylase (h-LPTA) from a highly virulent strain of *Candida albicans*. *Biochim Biophys Acta* 1257, 181-188 (1995)

103. C. K. Ng, V. Singhal, F. Widmer, L. C. Wright, T.C. Sorrell and K. A. Jolliffe: Synthesis, antifungal and haemolytic activity of a series of bis(pyridinium)alkanes. *Bioorg Med Chem* 15, 3422-3429 (2007)

104. A. Prakobphol, H. Leffler, C. I. Hoover and C. J. Fisher: Palmitoyl carnitine, a lysophospholipase-transacylase inhibitor, prevents *Candida* adherence *in vitro*. *FEMS Microbiol Lett* 151, 89-94 (1997)

105. F. Widmer, L. C. Wright, D. Obando, R. Handke, R. Ganendren, D. H. Ellis and T. C. Sorrell: Hexadecylphosphocholine (Miltefosine) has a broad-spectrum fungicidal activity and is efficacious in a mouse model of cyptococcosis. *Animicrob Agents Chemother* 50, 414-421 (2006)

106. R. Arai, T. Sugita and A. Nishikawa: The anthracycline antitumor agents doxorubicin and danorubicin reduce the activity of *Candida albicans* phospholipase B. *Microbiol Immunol* 48, 665-667 (2004)

107. P. K. Mukherjee, J. Chandra, D. M. Kuhn and M. A. Ghannoum: Differential expression of *Candida albicans* phospholipase B (*PLB1*) under various environmental and physiological conditions. *Microbiology* 149, 261-267 (2003)

108. Y. H. Samaranayake, R. S. Dassanayake, B. P. Cheung, J. A. Jayatilake, K.W. Yeung, J. Y. Yau and L. P. Samaranayake: Differential phospholipase gene expression by *Candida albicans* in artificial media and cultured human oral epithelium. *APMIS* 114, 857-866 (2006)

109. C. I. Hoover, M. J. Jantapour, G. Newport, N. Agabian and S.J. Fisher: Cloning and regulated expression of the *Candida albicans* phospholipase B (*PLB1*) gene. *FEMS Microbiol Lett* 167, 163-169 (1998)

110. D. Pugh, and R. A. Cawson: The cytochemical localization of phospholipase in *Candida albicans* infecting the chick chorio-allantoic membrane. *Sabouraudia*15, 29-35 (1977)

111. J. A. Jayatilake, Y. H. Samaranayake and L. P. Samaranayake: An ultrastructural and a cytochemical study of candidal invasion of reconstituted human oral epithelium. *J Oral Pathol Med* 34, 240-246 (2005)

112. P. K. Mukherjee, K.R. Seshan, S.D. Leidich, J. Chandra, G.T. Cole and M. A. Ghannoum: Reintroduction of the *PLB1* gene into *Candida albicans* restores virulence *in vivo*. *Microbiology* 147, 2585-2597 (2001)

113. Y. H. Samaranayake, R. S. Dassanayake, J. A. Jayatilake, B. P. Cheung, J. Y.Yau, K. W. Yeung and L. P. Samaranayake: Phospholipase B enzyme expression is not associated with other virulence attributes in *Candida albicans* isolates from patients with human immunodeficiency virus infection. *J Med Microbiol* 54, 583-593 (2005)

114. D. Kadosh and A. D. Johnson: Induction of the *Candida albicans* filamentous growth program by relief of transcriptional repression: a genome-wide analysis. *Mol Biol Cell* 16, 2903-2912 (2005)

115. B. Enjalbert, D. A. Smith, M. J. Cornell, I. Alam, S. Nicholls, A. J. P. Brown and J. Quinn: Role of the Hog1 stress-activated protein kinase in the global transcriptional response to stress in the fungal pathogen *Candida albicans*. *Mol Biol Cell* 17, 1018-1032 (2006)

116. S. D. Leidich, A.S. Ibrahim, Y. Fu, A. Koul, C. Jessup, J. Vitullo, W. Fonzi, F. Mirbod, S. Nakashima, Y. Nozawa and M. A. Ghannoum: Cloning and disruption of *caPLB1*, a phospholipase B gene involved in the pathogenicity of *Candida albicans*. *J Biol Chem* 273, 26078-26086 (1998)

117. P. Fojan, P. H. Jonson, M. T. N. Petersen and S. B. Petersen: What distinguishes an esterase from a lipase: A novel structural approach. *Biochimie* 82, 1033-1041 (2000)

118. M. T. Reetz: Lipases as practical biocatalysts. *Curr Opin Chem Biol* 6, 145-150 (2002)

119. Y. Wache, F. Husson, G. Feron and J.-M. Belin: Yeast as an efficient biocatalyst for the production of lipid-derived flavours and fragrances. *Antonie van Leeuwenhoek* 89, 405-416 (2006)

120. H. Radosz-Komoniewska, T. Bek, J. Jozwiak and G. Martirosian: Pathogenicity of *Helicobacter pylori* infection. *Clin Microbiol Infect* 11, 602-610 (2005)

121. V. Majtan, A. Hostacka, L. Majtanova and J. Trupl: Toxinogenicity and markers of pathogenicity of *Pseudomonas aeruginosa* strains isolated from patients with tumor diseases. *Folia Micorbiol* 47, 445-449 (2002)

122. A. Gacser, D. Trofa, W. Schafer and J. D. Nosanchuk: Targeted gene deletion in *Candida parapsilosis* demonstrates the role of secreted lipase in virulence. *J Clin Invest* 117, 3049-3058 (2007)

123. Y. Fu, A. S. Ibrahim, W. Fonzi, X. Zhou, C. F. Ramos and M. A. Ghanoum: Cloning and characterization of a gene (*LIP1*) which encodes a lipase from the pathogenic yeast *Candida albicans*. *Microbiology* 143, 331-340, (1997)

124. B. Hube, F. Stehr, M. Bossenz, A. Mazur, M. Kretschmar and W. Schafer: Secreted lipases of *Candida albicans*: cloning, characterization and expression analysis of a new gene family with at least ten members. *Arch Microbiol* 174, 362-374 (2000)

125. F. Stehr, A. Felk, A. Gacser, M. Kretschmar, B. Mahnss, K. Neuber, B. Hube and W. Schafer: Expression analysis of the *Candida albicans* lipase gene family during experimental infections and in patient samples. *FEMS Yeast Res 4*, 401-408 (2004)

126. D. A. Schofield, C. Westwater, T. Warner and E. Balish: Differential *Candida albicans* lipase gene expression during alimentary tract colonization and infection. *FEMS Microbiol Lett* 244, 359-365 (2005)

127. A. Gacser, F. Stehr, C. Kroger, L. Kredics, W. Schafer and J. D. Nosanchuk: Lipase 8 affects the pathogenesis of *Candida albicans. Infect Immun* 75, 4710-4718 (2007)

128. J. L. Roustan, A. R. Chu, G. Moulin and F. Bigey: A novel lipase/acyltransferase from the yeast *Candida albicans*: expression and characterisation of the recombinant enzyme. *Appl Microbiol Biotechnol* 68, 203-212 (2005)

129. R. Tsuboi, H. Komatsuzaki and H. Ogawa: Induction of an extracellular esterase from *Candida albicans* and some of its properties. *Infect Immun* 64, 2936-2940 (1996)

130. M. T. Shimizu, N. Q. Almeida, V. Fantinato and C. S. Unterkircher: Studies on hyaluronidase, chondroitin sulphatase, proteinase and phospholipase secreted by *Candida* species. *Mycoses* 39, 161-167 (1996)

131. S. A. Klotz, M. L. Pendrak and R. C. Hein: Antibodies to alpha5beta1 and alpha(v)beta3 integrins react with *Candida albicans* alcohol dehydrogenase. *Microbiology* 147, 3159-3164 (2001)

132. K. J. Kwon-Chung, J. B. Hicks and P. N. Lipke: Evidence that *Candida stellatoidea* type II is a mutant of *Candida albicans* that does not express sucrose-inhibitable alpha-glucosidase. *Infect Immun* 58, 2804-2808 (1990)

133. A. Dunkler, A. Walther, C. A. Specht, J. Wendland: *Candida albicans CHT3* encodes the functional homolog of the Cts1 chitinase of *Saccharomyces cerevisiae*. *Fungal Genet Biol* 42, 935-947 (2005)

134. S. Bates, J. M. de la Rosa, D. M. MacCallum, A. J. P. Brown, N. A. R. Gow and F. C. Odds: *Candida albicans* Iff11, a secreted protein required for cell-wall structure and virulence. *Infect Immun* 75, 2922-2928 (2007)

135. C. Molloy, R. D. Cannon, P. A. Sullivan and M. G. Shepherd: Purification and characterization of two forms of *N*-actylhexosaminidase from *Candida albicans* showing widely different outer chain glycosylation. *Microbiology* 140, 1543-1553 (1994)

136. K. Niimi, M. G. Shepherd and R. D. Cannon: Distinguishing *Candida* species by beta-*N*-actylhexosaminidase activity. *J Clin Microbiol* 39, 2089-2097 (2001)

137. K. Niimi, M. Niimi, M. G. Shepherd and R. D. Cannon: Regulation of *N*-actylhexosaminidase production in *Candida albicans*. *Arch Microbiol* 168, 464-475 (1997)

138. H. F. Jenkinson, and M. G. Shepherd: A mutant of *Candida albicans* deficient in beta-*N*-actylhexosaminidase (chitobiase). *J Gen Microbiol* 133, 2097-2326 (1987)

Key words: *Candida albicans*, proteinase, phospholipase, lipase, beta-*N*-acetylhexosaminidase, Review

Send correspondence to: Olga Hruskova-Heidingsfeldova, Gilead Sciences Research Center, Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, Flemingovo namesti 2, 166 10 Prague 6, Czech Republic, Tel: 420 220 183 249, Fax: 420 224 310 090, E-mail: olga-hh@uochb.cas.cz

http://www.bioscience.org/current/vol13.htm