Intracellular and secreted proteases fulfill multiple functions in microorganisms. In pathogenic microorganisms extracellular proteases may be adapted to interactions with host cells. Here we describe two cell surface-associated aspartic proteases, Sap9 and Sap10, which have structural similarities to yapsins of Saccharomyces cerevisiae and are produced by the human pathogenic yeast Candida albicans. Sap9 and Sap10 are glycosylphosphatidylinositol-anchored and located in the cell membrane or the cell wall. Both proteases are glycosylated, cleave at dibasic or basic processing sites similar to yapsins and Kex2-like proteases, and have functions in cell surface integrity and cell separation during budding. Overexpression of Sap9 in mutants lacking Kex2 or Sap10, or of Sap10 in mutants lacking Kex2 or Sap9, only partially restored these phenotypes, suggesting distinct target proteins of fungal origin for each of the three proteases. In addition, deletion of Sap9 and Sap10 modified the adhesion properties of C. albicans to epithelial cells and caused attenuated epithelial cell damage during experimental oral infection suggesting a unique role for these proteases in both cellular processes and host-pathogen interactions.

Proteases possess multiple functions in nature ranging from regulation of subtle cellular processes by activating distinct proproteins to nonspecific degradation of proteins for recycling of biomolecules. Several pathogenic microorganisms have adapted this biochemical property to fulfill a number of specialized functions during the infective process. The substrate specificities of these proteases may be very narrow, as in the case of bacterial toxins responsible for botulism or anthrax. In contrast, facultative pathogens may secrete proteases that have more general and much broader effects and play important roles in nonspecific degradation of proteins for recycling of biomolecules. Several Kex2-like processing systems were identified in other eukaryotic microorganisms. In pathogenic microorganisms extracellular proteases may be adapted to interactions with host cells. Here we describe two cell surface-associated aspartic proteases, Sap9 and Sap10, which have structural similarities to yapsins of Saccharomyces cerevisiae and are produced by the human pathogenic yeast Candida albicans. Sap9 and Sap10 are glycosylphosphatidylinositol-anchored and located in the cell membrane or the cell wall. Both proteases are glycosylated, cleave at dibasic or basic processing sites similar to yapsins and Kex2-like proteases, and have functions in cell surface integrity and cell separation during budding. Overexpression of Sap9 in mutants lacking Kex2 or Sap10, or of Sap10 in mutants lacking Kex2 or Sap9, only partially restored these phenotypes, suggesting distinct target proteins of fungal origin for each of the three proteases. In addition, deletion of Sap9 and Sap10 modified the adhesion properties of C. albicans to epithelial cells and caused attenuated epithelial cell damage during experimental oral infection suggesting a unique role for these proteases in both cellular processes and host-pathogen interactions.

Glycosylphosphatidylinositol-anchored Proteases of Candida albicans Target Proteins Necessary for Both Cellular Processes and Host-Pathogen Interactions*

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Antje Albrecht1, Angelika Felk1,2, Iva Pichova3, Julian R. Naglik3, Martin Schaller4,5,6, Piet de Groot7,8,9, Donna MacCallum10, Frank C. Odds11, Wilhelm Schäfer12, Frans Klis13, Michel Monod14, and Bernhard Hube15

From the 1Robert Koch-Institut, D-13353 Berlin, Germany, 2Molecular Phytopathology and Genetics, University of Hamburg, D-22609 Hamburg, Germany, 3Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, 166 10 Prague 6, Czech Republic, 4Department of Oral Medicine and Pathology, Kings College London, SE1 9RT London, United Kingdom, 5Department of Dermatology, University of Tuebingen, D-72076 Tuebingen, Germany, 6University of Amsterdam, 1018 WV Amsterdam, The Netherlands, 7Aberdeen Fungal Group, School of Medical Sciences, University of Aberdeen, AB25 2ZD Aberdeen, Scotland, United Kingdom, and 8Centre Hospitalier Universitaire Vaudois, CH-1011 Lausanne, Switzerland

Proteases possess multiple functions in nature ranging from regulation of subtle cellular processes by activating distinct proproteins to nonspecific degradation of proteins for recycling of biomolecules. Several pathogenic microorganisms have adapted this biochemical property to fulfill a number of specialized functions during the infective process. The substrate specificities of these proteases may be very narrow, as in the case of bacterial toxins responsible for botulism or anthrax. In contrast, facultative pathogens may secrete proteases that have more general and much broader effects and play important roles in nonspecific degradation of proteins for recycling of biomolecules. Several Kex2-like processing systems were identified in other eukaryotic microorganisms. In pathogenic microorganisms extracellular proteases may be adapted to interactions with host cells. Here we describe two cell surface-associated aspartic proteases, Sap9 and Sap10, which have structural similarities to yapsins of Saccharomyces cerevisiae and are produced by the human pathogenic yeast Candida albicans. Sap9 and Sap10 are glycosylphosphatidylinositol-anchored and located in the cell membrane or the cell wall. Both proteases are glycosylated, cleave at dibasic or basic processing sites similar to yapsins and Kex2-like proteases, and have functions in cell surface integrity and cell separation during budding. Overexpression of Sap9 in mutants lacking Kex2 or Sap10, or of Sap10 in mutants lacking Kex2 or Sap9, only partially restored these phenotypes, suggesting distinct target proteins of fungal origin for each of the three proteases. In addition, deletion of Sap9 and Sap10 modified the adhesion properties of C. albicans to epithelial cells and caused attenuated epithelial cell damage during experimental oral infection suggesting a unique role for these proteases in both cellular processes and host-pathogen interactions.

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The yeast Saccharomyces cerevisiae has served as a eukaryotic model organism to study functions of regulatory proteases including the Kex2 protease; GFP, green fluorescent protein; BEC, buccal epithelial cell; SD medium, synthetic defined medium; RHE, reconstituted human epithelium.

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1 To whom correspondence should be addressed: Robert-Koch-Institut, FG16, Nordufer 20, D-13353 Berlin, Germany. Tel.: 49-18887542116; Fax: 49-18887542605; E-mail: HubeB@rki.de.

2 The abbreviations used are: GPI, glycosylphosphatidylinositol; SAP, secretory aspartic protease; GFP, green fluorescent protein; BEC, buccal epithelial cell; SD medium, synthetic defined medium; RHE, reconstituted human epithelium.

EXPERIMENTAL PROCEDURES

Strains and Growth Conditions—C. albicans strains SC5314 (19) and CAI-4 carrying Clp10 (20) were used as wild type controls. CAI-4 (21)
was used to produce mutants either lacking or overexpressing SAP genes. For phenotypic screening, cells were diluted to give concentrations of 2000, 200, 20, and 2 cells/μl. Five μl of these cell suspensions were dropped onto agar supplemented as indicated. To determine differences in cell separation cells were grown in liquid SD medium until mid-log phase.

Real-time Reverse Transcription-PCR—Cells were harvested in mid-log phase in liquid SD medium. Total RNA was extracted and reverse transcribed into cDNA, and SAP9 and SAP10 were amplified using the Qiagen QuantiTect™ reverse transcription-PCR kit as described by the manufacturer. The gene ACT1 was used as an internal standard. Amplification and detection of specific products was performed with the ABI Prism 7000 sequence detection system (PE-Applied Biosystems).

In Vivo SAP Expression in Patient Oral Samples—Whole saliva samples were collected from 40 patients with active C. albicans infection (clinical signs of disease and >2000 C. albicans colony-forming units/ml of saliva) and 29 asymptomatic C. albicans carriers (no clinical signs and harboring 50–800 C. albicans colony-forming units/ml of saliva) attending the Oral Medicine clinic at Guy’s Hospital, London. Upon collection, the total RNA was isolated and examined for expression of SAP9, and SAP10 expression using a modified radio-active 32P-based reverse-transcriptase-PCR system (Access, Promega) as described previously (23, 24).

Gene Disruption and Overexpression—The Ura-blaster protocol (21) was used to disrupt SAP9 and SAP10. An internal fragment (bp +1169 to +1246) of SAP9 was replaced with the hisG-LIR3-hisG cassette to give pAF9ura. For deletion of SAP10, the hisG-LIR3-hisG cassette was flanked with the SAP10 region from positions +11 to +521 and +804 to +1206 to give pAA10ura. pAF9ura and pAA10ura were linearized and constructed by insertion of PCR products into the vector pKJ113 (28).

Production of Recombinant Proteases—Expression plasmids were constructed by insertion of PCR products into the P. pastoris expression vector pK1113 (28). P. pastoris transformation, and production of recombinant enzymes was described previously (29). Culture supernatants were harvested and dialyzed against 20 mM sodium citrate buffer, pH 6.5, containing 150 mM NaCl (buffer A). Proteases were purified on a Superdex™ 75 column equilibrated in buffer A using Acta explorer FPLC (Amersham Biosciences).

RESULTS

SAP9 and SAP10 Encode Aspartic Proteases with Structural Similarities to Yapsins—SAP9 and SAP10 code for preproenzymes with a signal peptide removed in the endoplasmic reticulum and a propeptide with lys-Arg residues, known as proteolytic processing sites for Kex2 (Fig. 1). Like all other members of the Sap family, the mature Sap9 and Sap10 proteins possess four conserved cysteine residues and two conserved aspartate residues. Sequence comparisons revealed that C. albicans Sap9 and Sap10 differ from the other Sap1–8 isoenzymes not only by sequence similarity (Fig. 2) but also by multiple N-glycosylation sites and putative GPI anchor attachment sequences at their C termini (28), a recognized structural property of yapsins.
Sap9 and Sap10 are N-Glycosylated and GPI-anchored on the Cell Surface—To determine whether Sap9 and Sap10 are in fact GPI proteins, we used a series of constructs to express native and C-terminal truncated versions of Sap9 and Sap10 in the yeast \( P. \) \( pastoris \) (Fig. 1). 

Expression of native Sap9 and Sap10 prevented secretion of these proteases. However, secretion was observed when parts of the C-terminal sequences of the GPI anchor consensus sequence were deleted and was highest when the complete consensus sequence was removed. These data suggest that the C termini prevent secretion of Sap9 and Sap10.

For cellular localization of the two proteases, we constructed Sap9- and Sap10-Gfp fusion proteins by inserting the \( GFP \) gene into the protease sequences directly after the Kex2 processing site and attaching the C-terminal sequences that prevented secretion in \( P. \) \( pastoris \). Immuno-electron microscopy using an anti-Gfp antibody revealed that both fusion proteins are located on the cell surface of \( C. \) \( albicans \). 

Sap10 with \( N \)-glycosidase \( F \) caused a clear band shift for both proteases, suggesting that Sap9 and Sap10 are \( N \)-glycosylated (Fig. 3). Thus, Sap9 and Sap10 are highly \( N \)-glycosylated proteases, which are GPI-anchored on the cell surface.

Sap9 and Sap10 Digest Peptides at Distinct Sites—Because Sap9 and Sap10 were exposed to the extracellular space but attached to the cell membrane or cell wall, we concluded that proteolysis by Sap9 and Sap10 must take place on the cell surface and that putative substrates of these proteases may be of either host or fungal origin.

To identify possible target proteins of Sap9 and Sap10, several host proteins known to be substrates of other Saps (15) were exposed to heterologously expressed enzymes. None of the tested proteins, including serum albumin, collagen, hemoglobin, keratin, mucin, or immunoglobulins, were hydrolyzed in a Sap2-like manner (data not shown). In contrast, several synthetic peptides containing basic or dibasic amino acid motifs were digested by Sap9 or Sap10 or by both proteases (Table 1). Digests were similar to the activity of \( S. \) \( cerevisiae \) yapsins or Kex2-regulatory proteases with hydrolysis at KR, KK, or single Lys (K) sites (Table 1). Sap9 and Sap10 preferred cleavage after dibasic (KR, KK) or single Lys (K) sites (Table 1). Sap9 and Sap10 preferred cleavage after dibasic (KR, KK) or single Lys (K) sites (Table 1).
unknown for yapsin-like aspartic proteases (between Phe-Ser and His-Asn). Interestingly, the peptide KIHNLGF, which is identical to an internal sequence of Sap9 (Lys149 to Phe157 from the N terminus), was processed by Sap9 between Lys and Leu154. This suggests self-processing activity of Sap9 at this site, which possibly accounts for the two subunits observed in this study and by others (11). The N-terminal sequence of the larger β-subunit was previously shown to be Leu154, Phe-Gly-Phe (11), identical to the N-terminal sequence of one fragment of the digested peptide. These data suggest that Sap9 and Sap10 are proteases that hydrolyze polypeptides at distinct processing sites.

Sap9 and Sap10 Are Necessary for Cell Surface Integrity and Cell Separation during Budding—To determine the possible functions of the two proteases, we constructed Δsap9 and Δsap10 single mutants and a Δsap9/Δsap10 double mutant. All mutant strains grew normally on media containing different carbon sources, at different pH values, on hyphal inducing media or when incubated at different temperatures (data not shown). However, components that directly or indirectly target the fungal cell surface such as hygromycin B (Fig. 4A), amorolfine, calcofluor, Congo red, and itraconazole (not shown) caused significant growth defects for all mutants. In addition, nikkomycin inhibited growth of the Δsap9 mutant (not shown). Observed phenotypes were at least partially restored by mutants carrying plasmid-borne Sap9 or Sap10 (Fig. 4A). Together, these data suggest a role of Sap9 and Sap10 in cell surface integrity. Furthermore, all three mutants exhibited an abnormal budding phenotype, as daughter cells did not separate from the parent cell. All three mutants also showed increased levels of both protein (7.5 ± 0.6%; t test, p = 0.05) and chitin (3.4 ± 0.7%; t test, p = 0.05) as compared with the parental strain (protein 5.8 ± 0.7%; chitin 1.9 ± 0.5%).

Sap9, Sap10, and Kex2 Have Distinct Cellular Functions—Our results, together with previous data (18), suggest that Sap9 and Sap10 and the processing serine protease Kex2 in C. albicans all may be involved in the maintenance of cell surface integrity. To investigate whether Sap9, Sap10, and Kex2 have overlapping functions, we constructed SAP9 and SAP10 overexpression vectors, which were integrated into the single mutants (SAP9 into Δsap10 or Δkex2, respectively; SAP10 into Δsap9 or Δkex2, respectively). Phenotypic screening of these strains showed little or no overlapping function of the three proteases. Real-time reverse transcription-PCR analysis of the mutants revealed a 2-fold over-expression of SAP9 in the Δkex2 mutant, a 2-fold over-expression of SAP9 in the Δsap10 mutant, and a 4-fold over-expression of SAP10 in the Δsap9 mutant, suggesting that the loss of one processing protease gene induces up-regulation of the other genes (data not shown).

Deletion of SAP9 or SAP10 Modifies Adhesion to Epithelial Cells and Causes Attenuated Epithelial Cell Damage during Experimental Oral Infections—To investigate whether dysfunctions in cell surface integrity resulting from Sap9 or Sap10 deletion influenced virulence of C. albicans, we investigated the potential of Δsap9 and Δsap10 mutants to cause infections. Both mutants were only moderately attenuated in a mouse model of systemic infection after intravenous challenge (not shown). However, the same mutants had a significantly reduced ability to invade and damage epithelial cells in a model of oral infection based on RHE (Fig. 5).

One possible explanation for the attenuated virulence phenotype in the RHE model could be a reduced ability of the mutant strains to adhere to epithelial cells, thus resulting in reduced invasion and cell damage. This may indeed be the case for Δsap10, as adherence of this mutant to BECs was reduced (Fig. 6). However, the ability of the Δsap9 mutant to adhere to epithelial cells was dramatically increased as compared with the wild type, suggesting that properties other than reduced adhesion attributes were responsible for the decreased epithelial cell damage of Δsap9. Interestingly, the phenotype of the double mutant Δsap9/Δsap10 showed reduced adhesion resembling the phenotype of proteins involved in the production of alternative structural elements, we quantified the protein and chitin levels in the cell walls of the mutants. Chitin levels of the Δsap9 mutant were significantly increased (4.0 ± 0.9% S.D.; t test, p = 0.05), whereas the Δsap10 mutant showed increased levels of both protein (7.5 ± 0.6%; t test, p = 0.05) and chitin (3.4 ± 0.7%; t test, p = 0.05) as compared with the parental strain (protein 5.8 ± 0.7%; chitin 1.9 ± 0.5%).

### TABLE 1

**Digestion by Sap9 or Sap10**

Peptides are digested by Sap9 and/or Sap10 between amino acids (in bold). Asterisk, digestion; underline, digestion by Sap9; overline, digestion by Sap10; double underline, digestion by both Sap9 and Sap10.

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Processing after Arg and Lys</th>
<th>Processing after Lys, Phe, or His</th>
</tr>
</thead>
<tbody>
<tr>
<td>TTTKR<strong>SAGF</strong></td>
<td>FKV<strong>TPKA</strong></td>
<td><strong>KIH</strong>NLGF <strong>R</strong></td>
</tr>
<tr>
<td>P<strong>KISR</strong>NERS</td>
<td>N<strong>VTV</strong>KDL<strong>GSL</strong></td>
<td>RT<strong>NEK</strong>V<strong>ELQ</strong></td>
</tr>
<tr>
<td>R<strong>TVKR</strong>QAG**</td>
<td><strong>K</strong>T<strong>SKR</strong>Q<strong>AVP</strong></td>
<td>N<strong>TE</strong>K<strong>N</strong>TRT**</td>
</tr>
</tbody>
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![Image](http://example.com/image.jpg)

**FIGURE 4.** Hypersensitivity of mutants lacking SAP9 and/or SAP10 (Δsap9, Δsap10, and two isogenic Δsap9/Δsap10 mutants) during growth in the presence of hygromycin B (A) and dysfunction of these mutants during budding in liquid medium (B) as compared with the wild type (WT). Phenotypes were reversed by plasmid (Cip10)-borne Sap9 and Sap10 expression (Δsap9[SAP9], Δsap10[SAP10]). Hygromycin B, 800 μg/ml (Δsap9, Δsap9/Δsap10) and 1100 μg/ml (Δsap10).
the Δsap10 and not the Δsap9 single mutant (Fig. 6). Therefore, activity of Sap9 and Sap10 is necessary for wild type adhesion properties and invasion and cell damage of oral epithelial cells.

In Vivo SAP Expression in Oral Samples from Patients—If SAP9 and SAP10 play a virulence role during oral infections, both of these genes would be predicted to be expressed in vivo by C. albicans strains colonizing and infecting the oral cavity. To demonstrate this possibility, we analyzed SAP9 and SAP10 expression in total RNA samples isolated from the saliva of a large number of patients (40 symptomatic C. albicans infected and 29 asymptomatic C. albicans carriers). SAP9 and SAP10 transcripts were detected in 98 and 93% of C. albicans-infected patients, respectively, and in 83 and 86% of C. albicans carriers, respectively. This indicates that both SAP genes are frequently expressed both in the commensal stage and during infection, suggesting an important role for growth and/or survival in the oral cavity.

DISCUSSION

The model yeast S. cerevisiae and the human pathogenic fungus C. albicans share a large portion of similar genes, which may, however, have different functions because of the different natural environments of these two fungal species. Data presented in this study suggest that C. albicans is equipped with 10 extracellular aspartic proteases, Sap1–10, which are either secreted (Sap1–8) or associated with the cell surface (Sap9 and Sap10). However, the functions of these two subclasses of extracellular aspartic proteases seem to be fundamentally different. Proteases Sap1–6 are known to hydrolyze host proteins and consequently to cause tissue damage (15). In contrast, our data suggest that Sap9 and Sap10 are linked to regulatory processes on the fungal cell surface that are essential for maximal pathogenicity during interaction with oral epithelial tissue.

Cell surface-associated proteases that are exposed to the extracellular space with such regulatory functions are rare in nature. The most prominent examples are the yapsins, first discovered in S. cerevisiae. Sap9 and Sap10 of C. albicans share a number of structural characteristics with yapsins. Both proteins show high sequence similarities to Yps1, Yps2, Yps3/4, Yps6, and Yps7 (www.yeastgenome.org) and, in contrast to other Saps, are glycosylated. C-terminal sequences of all of these aspartic proteases contain putative GPI anchor sequences. As has been
shown for Yps1 and Yps2 (31), only C-terminally truncated versions of Sap9 and Sap10 are secreted to the extracellular space. Furthermore, using a Gfp reporter protein fused to the C-terminal sequences of Sap9 and Sap10, along with immunoelectron microscopy, we showed that these proteases are targeted predominantly to either the cell membrane (Sap9) or to both the cell membrane and the cell wall (Sap10). In addition, our data shed light on a previous study, which showed that native Sap9 consists of two subunits (11) linked by a disulfide bridge. We demonstrate that Sap9 is able to digest a peptide identical to the putative processing site of the Sap9 precursor, indicating an autocatalytic processing ability for Sap9.

The function of yapsins has yet not been elucidated. Several lines of evidence in this study suggest that Sap9 and Sap10 target proteins of fungal origin with roles in cell surface integrity and cell separation, which they probably share with yapsin-like proteases in S. cerevisiae and other fungi. In pathogenic fungi these proteases may have gained functions with specific roles during host-pathogen interactions. In addition to C. albicans, yapsin-like proteases exist in other pathogenic fungi; for example, as many as nine genes encoding yapsin-like aspartic proteases exist in the genome of the emerging pathogen Candida glabrata (32).

Although Sap9 and Sap10 are required for cell surface integrity and both cleave at similar or identical processing sites, they also seem to have unique processing sites. This view is supported by the fact that the single mutants showed phenotypes that were only partially compensated for by overexpression of its paralog Sap isoenzyme, suggesting that each protease has distinct target proteins.

What are the target proteins of Sap9 and Sap10? Because both proteases are exposed to the extracellular space, processing must occur on the cell surface, and thus the fungal target proteins must have been transported, presumably via the secretory pathway, to the outer face of the cell membrane. These may be proteins of the cell membrane (facing the extracellular space), the cell wall, or secreted proteins. Other Sap5 may also be target proteins. However, total proteolytic activity of Δsap9 and Δsap10 mutants was not reduced as compared with wild type strains (not shown), suggesting that processing of Sap2 (the dominant secreted protease expressed in vitro (33)) can occur independently of Sap9 and Sap10. GPI-anchored proteases are an abundant class of cell surface proteins. As many as 104 putative GPI proteins were identified by in silico approaches in C. albicans (34). Some of them were identified by biochemical analysis (26, 35), including proteins such as Pfr1, Ecm33, and Crc1, which are also present in S. cerevisiae. In addition, several proteins and protein families with no significant homology to S. cerevisiae proteins were identified, including the cell wall-associated proteins Als1 and Als4, Rbt5, and Hwp1. Several studies showed that yeast cell wall proteins are associated with a number of functions in cell physiology including cell surface integrity, flexibility and remodeling, cell morphology, growth, and budding (36). In pathogenic yeasts like C. albicans, some of these proteins have been shown to be directly or indirectly involved in virulence functions, in particular adhesion and immune evasion (37). Therefore, proteases such as Sap9 and Sap10 that process proteins of the cell wall may be associated with these functions. In fact, C. albicans strains lacking certain cell wall proteins show characteristics resembling the phenotypes of Δsap9 and Δsap10. For example, mutants lacking Ecm33 show sensitivity to compounds that target the cell wall, including calcofluor white, Congo red, and hygromycin B, similar to the phenotypes of Δsap9 and Δsap10 (38). In addition, it has been shown that the chitin content is increased in cells that are exposed to environmental stresses or that have dysfunctions in the biosynthesis or remodeling of structural elements as a compensatory mechanism (36). For example, mutants lacking KRE5, which encodes an endoplasmic reticulum protein involved in glucan synthesis, had strongly reduced levels of glucan but higher chitin content (39), and disruption of GBP7, encoding a GPI anchor-modifying activity, showed increases in chitin deposition (40). Because mutants lacking Sap9 or Sap10 have higher chitin contents, it is possible that some of the target proteins are associated with the integrity of other structural elements of the cell wall such as glucan. Processing of Sap9 and Sap10 target proteins is not essential for normal growth. However, it is a prerequisite for normal separation of cells after budding. Cell separation defects similar to those found for Δsap9 and Δsap10 in this study have also been observed for mutants lacking the chitin synthase gene CHS1 (41) or ACE2, which encodes a key regulator of cell wall metabolism (42).

If the target proteins of Sap9 and Sap10 are transported to the surface via the secretory pathway and have processing sites similar to Kex2, why does Kex2 (43) not cleave them? There are at least two possible explanations. First, processing sites of the target proteins may not be exposed to processing by Kex2 in the Golgi because of folding of these proteins. Second, recent studies of GPI-anchored protein sorting have led to the surprising finding that, during their delivery to the surface, different types of plasma membrane proteins are sorted from each other early in this pathway in the endoplasmic reticulum. Therefore, it may be possible that these putative target proteins are bypassing Kex2-containing Golgi compartments and thus are not processed by Kex2 (44).

One of the most interesting and surprising observations was the fact that Δsap9 and Δsap10 mutants had oppositely altered adhesion properties; whereas mutants lacking Sap9 were more adherent to BECs, the loss of SAP10 caused reduced adherence, and mutants lacking both genes behaved like the Δsap10 mutant. This intriguing observation led us to speculate that Sap9 and Sap10 may have defined functions during infection similar to surface-associated proteases of malaria and Toxoplasma parasites (45). Here, distinct surface proteins are trimmed to activate or enhance adhesion to host receptors, whereas other proteases shave resident surface proteins or break connections between surface ligands and the host receptor during the penetration process. A similar function of Sap9 may explain why Δsap9 had reduced abilities to invade epithelial tissue and to cause damage despite enhanced adhesion properties.

Although we cannot exclude the theoretical possibility that the Sap10 protein itself may have a direct adhesion function, this seems very unlikely in the case of Sap9 because disruption of SAP99 caused increased adhesion. Removal of an adhesion protein should not increase the adhesion properties but rather should reduce adhesion. Furthermore, adherence of C. albicans to human mucosa (46), epidermal corneocytes (47), and epidermal keratinocytes (48) has been shown to be inhibited by pepstatin A, suggesting that proteolytic activity of aspartic proteinases is necessary for the adhesion properties of C. albicans.

In C. albicans, loss of the surface protein Als3 or the cell wall regulatory protein Aec1 was shown to reduce adherence to epithelial cell or plastic surfaces (42, 49). However, the loss of the putative adhesin Eap1 or the GPI-anchored cell wall protein Ywp1/Pga24 caused increased adherence, suggesting that aberrant, misprocessing of, or removal of certain cell wall proteins may cause either enhanced or attenuated adhesion (50, 51). Interestingly, Granger et al. (50) observed that Ywp1/Pga24 seems to be processed by proteases other than Kex2 and suggested an expansive role for processing of cell surface proteins in regulating cellular adhesion. The data presented in our study suggest that Sap9 and Sap10 of C. albicans are key enzymes for such activities.

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GPI-anchored Proteases of Candida albicans

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