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REVIEW ARTICLE

Candida albicans proteinases: resolving the mystery of a gene family

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Overview

Fungal infections of mucosal surfaces are extremely common, debilitating and often recurring diseases, which are frequently caused by the yeast *Candida albicans*. Furthermore, in the severely immunocompromised host, *C. albicans* may also cause deep-seated or even life-threatening systemic infections. In order to colonize, infect and evade host defence mechanisms, *C. albicans* possesses a repertoire of virulence attributes. In particular, the secreted aspartic proteinases (Saps), encoded by the *SAP* gene family with ten members, appear to play a major role in *C. albicans* virulence. The *SAP* family is differentially regulated and distinct members are expressed under a variety of laboratory growth conditions and during experimental *C. albicans* infections *in vitro* and *in vivo*. The contribution of the Saps to *C. albicans* pathogenesis has been clearly demonstrated using *SAP*-deficient mutants and proteinase inhibitors. These studies demonstrated that different *SAP* genes appear to be crucial for mucosal and systemic infections, and are involved in *C. albicans* adherence, tissue damage and evasion of host immune responses. Therefore, the Sap isoenzymes appear to have a variety of functions *in vivo*, which are probably called upon at different stages and in different types of *C. albicans* infection. This review aims to summarize the more recent data regarding the contribution of the secreted proteinases to *C. albicans* virulence and strives to explain why *C. albicans* possesses such a gene family.

Opportunistic fungal infections: from commensal to pathogen

C. albicans is a ubiquitous mucosal commensal yeast that is commonly isolated from the oral cavity, the gastro-intestinal tract and the vagina, where it resides in equilibrium with the microbial flora and the host immune system. The physiological status of the host is the primary factor governing the aetiology of candidosis. However, the observation that only slight alterations in this physiological state can turn normally harmless commensal yeasts into aggressive pathogens causing mucosal, superficial or even life-threatening systemic infections in the immunocompromised host points to the pathogenic potential of *Candida* species (Fidel *et al*., 1999). An increasing number of immunocompromised individuals, such as those with HIV infection, neutropaenia, intensive care patients and those treated with immunosuppressive drugs after organ transplantation, experience some form of mucosal *Candida* infection. In addition, nearly three-quarters of all healthy women experience at least one vaginal yeast infection (Sobel, 1985). Furthermore, while the high mortality rate associated with systemic bacterial infections has declined with the early administration of broad-spectrum antibiotics, systemic fungal infections have become increasingly significant in causing morbidity and mortality in immunocompromised patients (Fidel *et al*., 1999). As a result, *Candida* has become the fourth most common nosocomial bloodstream pathogen in the USA (Jarvis, 1995). Yet despite the growing importance of *Candida* infections in medicine, many of its interactions with the host are still poorly understood.

Virulence attributes of *C. albicans*

The process, development and course of microbial infections may be regarded as an encounter between the virulence of a micro-organism and the ability of the host to prevent or resist microbial colonization or invasion. Pathogenic bacteria, having a relatively small genome, often develop very specific ways of causing host infections. For example, several bacteria have developed highly specialized virulence factors, which are adapted to intracellular survival within a specific host cell type, and in a few cases single toxins may cause disease. Conversely, *C. albicans* is not a prokaryote but a

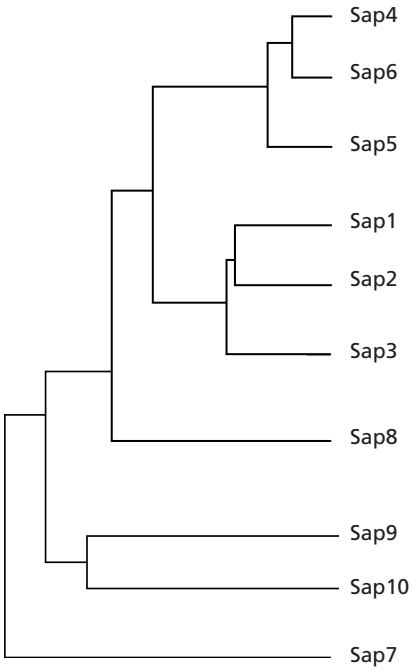

Fig. 1. Dendrogram of the *C. albicans* Sap isoenzyme family based on amino acid sequences. Three distinct groups are clustered within the family. Sap1–3 are up to 67 % and Sap4–6 up to 89 % identical, while Sap7 is only 20–27 % identical to other Saps. Sap9 and Sap10 both have C-terminal consensus sequences typical for GPI proteins. Reprinted from Stehr *et al.* (2000) with permission of the publisher.

eukaryote, with a larger genome and thus greater resources. Accordingly, *C. albicans*, although highly adapted to humans as a commensal organism, possesses a larger repertoire of virulence attributes in order to colonize the host, and inflict damage directly or inactivate, resist or misdirect host defence mechanisms. However, in principle, the main aim of a microorganism is not to cause death or even damage the host but to survive and reproduce, during which damage may only be a side effect. Indeed, in *C. albicans* it appears that the transition from harmless commensal to disease-causing pathogen is finely balanced, and is attributable to an extensive repertoire of virulence determinants selectively expressed under suitable predisposing conditions.

The virulence factors expressed or required by *C. albicans* to cause infections may well vary, depending on the type of infection, the stage and site of infection, and the nature of the host response. Thus, *C. albicans* must be highly adapted to an existence on and within the host, which indicates that this fungus possesses virulence attributes distinct from those of the closely related, but non-pathogenic yeast *Saccharomyces cerevisiae*. Although a number of potential virulence factors have been suggested for *C. albicans*, cell morphology, adhesion factors, phenotypic switching and extracellular lipolytic or proteolytic activity have long been recognized as the most credible (Odds, 1994). Extracellular proteolytic activity had already been discovered in the mid-sixties (Staib, 1965) but it was not until the early nineties, when molecular methods were introduced into the *Candida* field, that scientists began to understand the genetic complexity of this fungus. For instance, a gene (*SAP1*) encoding an extracellular proteinase was cloned in 1991 and was thought to be responsible for the observed secretory aspartic proteinase (Sap) activity of *C. albicans* (Hube *et al.*, 1991). However, a detailed study of the *C. albicans* genome in recent years indicated that *SAP1* was just the tip of an iceberg and revealed that the fungus possesses an arsenal of ten *SAP* genes that encode extracellular proteinases. The fact that *C. albicans* can encounter a large number of different tissues during the infection process *in vivo* may provide clues as to why it has evolved to possess such a large repertoire of *SAP* genes.

The *SAP* gene family

Shortly after *SAP1* was discovered, more genes encoding extracellular aspartic proteinases were identified, by PCR-based cloning strategies (Wright *et al.*, 1992), by using *SAP1* as a probe (Monod *et al.*, 1994, 1998; White *et al.*, 1993), by sequencing the promoter region of *SAP1* (Miyasaki *et al.*, 1994) or by BLAST searches and sequence alignments in the *Candida* genome databases (e.g. *SAP10*, accession number AF146440). The deduced proteins are all aspartic proteinases and share a number of Sap-specific characteristics (Fig. 1). All ten *SAP* genes encode preproenzymes approximately 60–200 amino acids longer than the mature enzyme. The N-terminal secretion signal is cleaved by a signal peptidase in the endoplasmic reticulum (ER) (Fig. 2). The propeptide is removed to activate the proteinases by the subtilisin-like Kex2 proteinase in the Golgi before being transported via vesicles to the cell surface for secretion or glycosylphosphatidylinositol (GPI)-anchoring. Although Kex2 may be a key regulatory proteinase of Saps (Newport & Agabian, 1997), other alternative processing pathways are thought to exist (Togni *et al.*, 1996), and autoactivation was shown to occur extracellularly for Sap1–3 and Sap6 at certain pH values (Koelsch *et al.*, 2000). The mature enzyme contains sequence motifs typical for all aspartic proteinases, including the two conserved aspartate residues of the active site. Conserved cysteine residues are probably implicated in maintaining the three-dimensional structure of the enzyme (Hube, 1996). Unlike Sap1–8, Sap9 and Sap10 both have C-terminal consensus sequences typical for GPI proteins (Fig. 2).

While in the early nineties efforts concentrated on the collection of sequence data, the increasing numbers of cloned *SAP* genes soon shifted the interest towards the role and function of the *SAP* gene family during the infection process. The fact that the presence of a *SAP* gene family was unique to only the most pathogenic *Candida* species, such as *C. albicans* (Magee *et al.*, 1993, 1994), *C. dubliniensis* (Gilfillan *et al.*, 1998), *C. tropicalis* (Zaugg *et al.*, 2001) and *C. parapsilosis* (Monod *et al.*,

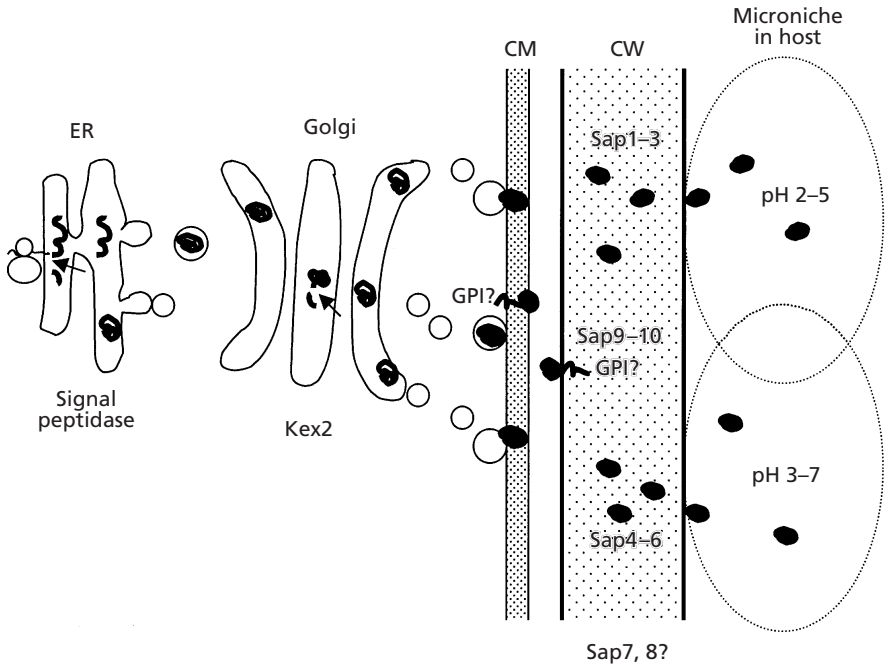

Fig. 2. Processing and secretion of Saps via the secretory pathway and their optimal pH activity ranges. *SAP* genes are translated as preproprotein into the ER. The signal peptide is processed by the signal peptidase (arrow) in the ER. The propeptide contains two Lys-Arg processing sites, which are targeted by the proteinase Kex2 (arrow) in the Golgi apparatus or by alternative activation processes (see text). The propeptide was found to be essential for proper folding of Saps (Beggah *et al.,* 2000). The mature Sap proteins are transported by vesicles via the cell membrane into the cell wall and secreted into the extracellular space. Sap1–3 are mostly active between pH 2 and 5 while Sap4–6 are active at a higher pH range (3–7) and thus may act in different host environments. Sequence analysis of Sap9 and Sap10 suggested that both proteinases are GPI-proteins anchored in the cell membrane or cell wall (Caro *et al.,* 1997).

1994), but was absent in the non-pathogenic yeast *S. cerevisiae*, supported the view that these proteinases may be involved in virulence. However, in the light of the discovery that only a single isoenzyme, Sap2, was necessary and sufficient to allow rapid growth of *C. albicans* in media containing protein as the sole source of nitrogen (Hube *et al*., 1994; White & Agabian, 1995), it remained a mystery as to why this fungus possessed a whole family of *SAP* genes. As one possible explanation may be that different proteinases are required to act upon different host proteins and tissues *in vivo*, a number of studies dealt with the possible targets of Saps.

Possible target proteins of *C. albicans* proteinases *in vivo*

At the most basic level, one role of the *C. albicans* Saps may be to simply digest host proteins to provide nitrogen for the cells. Since Sap2 activity enables the fungus to grow efficiently in media containing serum albumin or other proteins as a sole source of nitrogen, nutrient acquisition is a likely function of the proteinases. However, the Saps may have also adapted and evolved to have more direct virulence functions. For example, Saps could contribute to host tissue adhesion and invasion by degrading or distorting host cell surface structures and intercellular substances, or by destroying cells and molecules of the host immune system to avoid or resist antimicrobial attack. Such activities for Sap2 have been shown *in vitro* (reviewed by Rüchel, 1992; and Hube, 1996, 1998). Extracellular matrix and host surface proteins such as keratin, collagen, laminin, fibronectin and mucin are efficiently degraded by Sap2. Several host defence proteins, such as salivary lactoferrin, the proteinase inhibitor α-macroglobulin, enzymes of the respiratory burst of macrophages and almost all immunoglobulins, including secretory IgA, which is normally resistant to most bacterial proteinases, can also be hydrolysed by Sap2.

C. albicans Saps may also act on host proteolytic cascades with numerous effects, which have no obvious advantage for the fungus. For example, Sap2 can activate host protein precursors of the blood clotting cascade (Kaminishi *et al*., 1994), inactivate the epidermal cysteine proteinase inhibitor cystatin A (Tsushima *et al*., 1994), and cleave human endothelin-1 precursor (a vasoconstrictive peptide) to alter vascular homeostasis (Tsushima & Mine, 1995). Such activities may be responsible for the enhanced overall circulating proteolytic activity observed in traumatized mice challenged with *C. albicans* (Neely *et al*., 1994). Furthermore, *Candida* proteinases have been shown to activate the

proinflammatory cytokine interleukin-1β from its precursor, suggesting a role for Saps in the activation and maintenance of the inflammatory response at epithelial surfaces *in vivo* (Beausejour *et al*., 1998).

These studies suggested that Sap2, in contrast to the highly substrate-specific enzymes produced by certain bacteria, has very broad substrate specificity and may have multiple targets *in vivo*. However, this raises the question of why, if a single proteinase has such a range of activities and functions, does *C. albicans* need a family of ten *SAP* genes? Magee *et al*. (1993) postulated that the Sap isoenzymes might have a variety of functions *in vivo*, which may be called upon at different sites, and during different stages and types of *C. albicans* infection.

SAP gene expression *in vitro* and *in vivo*

The presence of ten *SAP* genes indicated that different proteinases might target a variety of host cells and tissues during the infection process. If this were the case, one would expect the various members of the *SAP* family to be differentially regulated and expressed under a variety of laboratory growth conditions and during *C. albicans* infections *in vivo*. Accordingly, a number of studies have addressed this issue (Hube, 2000).

Under most proteinase-inducing conditions in the laboratory, the major proteinase gene expressed in *C. albicans* yeast forms is *SAP2*, which was found to be regulated by a positive feedback mechanism: peptides resulting from proteolysis of high-molecular-mass proteins were proposed to lead to the induction of *SAP2* gene expression (Hube *et al*., 1994). In contrast, *SAP1* and *SAP3* were discovered to be differentially expressed during phenotypic switching in certain strains (Morrow *et al*., 1992; White *et al*., 1993). However, later studies indicated the regulation of *SAP3* during switching was not absolute (Hube *et al*., 1994; White & Agabian, 1995; Smolenski *et al*., 1997). Expression of *SAP8* is temperature-regulated (Monod *et al*., 1998) and *SAP9* and *SAP10* are constitutively expressed under most environmental conditions in both yeast and hyphal forms (A. Felk, W. Schäfer & B. Hube, unpublished results). Since most aspartic proteinases are only active under acidic conditions, it was perhaps a surprising discovery that the *SAP4–6* genes were almost exclusively expressed during hyphal formation at neutral pH, even in defined protein-free media (Hube *et al*., 1994; White & Agabian, 1995). These studies demonstrated that the *SAP* gene family was differentially expressed *in vitro* and further suggested that, in contrast to the induction of *SAP2*, expression of other *SAP* genes was not dependent on the presence of exogenous protein or peptides.

The *in vitro* demonstration of distinct *SAP* expression patterns in yeast, hyphal and phenotypically switched cells indicated that proteinase expression was a highly regulated and tightly controlled process. However, this did not address the issue of whether the Sap family contributed to the pathogenicity of *C. albicans in vivo*. For this reason it was crucial to first ascertain whether the proteinases were also differentially expressed during *C. albicans* infections; this was demonstrated using *in vitro* and animal infection experimental models.

In vitro experimental models of oral (Schaller *et al*., 1998) and cutaneous (Schaller *et al*., 2000) *C. albicans* infections suggested that *SAP1–3* were the main proteinases expressed during superficial infections. This supposition was supported by the detection of *SAP1* and *SAP2* transcripts in a rat vaginitis model (De Bernardis *et al*., 1995). In contrast to mucosal infection models, experimental models of systemic *C. albicans* infections correlated *SAP4–6* expression with systemic disease (A. Felk and others, unpublished results; Staib *et al*., 2000). However, using *in vivo* expression technology, Staib *et al*. (1999) also demonstrated *SAP2* expression in late stages of systemic infections. Taken together, these *in vitro* experimental and animal model data correlated *SAP* gene expression with *C. albicans* virulence and demonstrated differential *SAP* gene expression during different types of *C. albicans* infection.

Whether these models were representative of proteinase expression during human mucosal and systemic infections was not known. However, high titres of anti-Sap antibodies have been observed in sera of candidosis patients, indicating the presence of Sap antigen during infection (Rüchel, 1992). In addition, Sap antigens have been detected in biopsies of oral epithelial lesions collected from HIV-infected patients (Schaller *et al*., 1999a) and in almost all organs of immunocompromised patients who had died of systemic *C. albicans* infections (Rüchel *et al*., 1991). Nevertheless, only two studies have investigated the expression of the *SAP* gene family during human *C. albicans* infections. Schaller *et al*. (1998) showed that the *SAP* genes were indeed expressed during oral candidosis. Moreover, Naglik *et al*. (1999) and J. R. Naglik and others (unpublished results) were able to demonstrate differential expression of the *SAP* gene family, with *SAP1*, *SAP3* and *SAP7* transcripts predominantly being expressed in patients with oral *C. albicans* infection as opposed to oral *C. albicans* carriers.

Therefore, differential expression of the proteinase family has been demonstrated in culture media, during *in vitro* and animal experimental infection models and during human *C. albicans* infections. Such differential expression suggested a more specific role or function for the different *SAP* genes. However, although these studies correlated *SAP* gene expression with the ability of *C. albicans* to cause infection, they did not directly demonstrate that Sap proteinases actually contributed to the pathology or virulence of *C. albicans* infections.

Contribution of Saps to the pathogenesis of *C. albicans* infections

Strong evidence existed to indicate that *Candida* proteinases were differentially expressed *in vivo* and had the potential to degrade a large number of host proteins, but their direct contribution to pathogenesis was still unknown. However, recent studies using proteinase

Table 1. Use of *SAP*-deficient mutants to determine a role for the proteinases during *C. albicans* infections

Study	Main findings
Mucosal infections	
Watts *et al.* (1998)	*sap1*, *sap2* and *sap3* null mutants were less adherent to buccal epithelial cells than the parental strain. The *sap4–6* triple mutant showed significantly increased adherence.
Schaller *et al.* (1999b)	*sap1*, *sap2* and *sap3* mutants caused less tissue damage than the parental strain. A *sap1/3* double mutant caused fewer lesions than the two *sap1* or *sap3* mutants individually. *sap4–6* mutant showed equal or enhanced tissue damage.
De Bernardis *et al.* (1999)	*sap1*, *sap2* and *sap3* mutants, but not the *sap4–6* mutant, were less virulent in a rat vaginitis model compared with the parent strain. The *sap2* mutant was almost avirulent.
Systemic infections	
Ibrahim *et al.* (1998)	No difference in adherence to endothelial cells between the *sap1*, *sap2* and *sap3* null mutants and the parental strain. The *sap2* mutant caused less damage to endothelial cells.
Kretschmar *et al.* (1999)	The *sap4–6* mutant, but not *sap1*, *sap2* or *sap3* mutants, caused less tissue damage and invasion in peritoneal infections.
Hube *et al.* (1997); Sanglard *et al.* (1997)	*sap1*, *sap2*, *sap3* and, in particular, *sap4–6* mutants were less lethal in two animal models compared with the parent strain. The *sap4–6* mutant displayed the greatest attenuation.
Interactions with immune system	
Borg-von Zepelin *et al.* (1998)	The *sap4–6* triple mutant was eliminated 53% more effectively after phagocytosis by macrophages than the parent strain.

inhibitors and *SAP*-disrupted or *SAP*-overexpressing mutants have compellingly demonstrated that Saps indeed contribute to the virulence of *C. albicans*. Inhibition of Saps with the aspartic proteinase inhibitor pepstatin A prevented the initial penetration of *C. albicans* through mucosal surfaces, but not the dissemination of the fungus once the cells had already reached the blood vessels (Fallon *et al.*, 1997). Moreover, pepstatin A prevented *C. albicans* invading and causing tissue damage in oral, vaginal and skin experimental infection models (Schaller *et al.*, 1999b; De Bernardis *et al.*, 1997). In addition, HIV aspartic proteinase inhibitors, which also act on the *C. albicans* Saps (Gruber *et al.*, 1999; Borg-von Zepelin *et al.*, 1999; Cassone *et al.*, 1999; Korting *et al.*, 1999), can inhibit *C. albicans* adherence to epithelial cells (Borg-von Zepelin *et al.*, 1999). Taken together, these data indicate that Sap activity may be crucial during the early stages of *C. albicans* infections.

Although Sap2 was the dominant *C. albicans* extracellular proteinase *in vitro*, the *in vivo* expression pattern of *SAP* genes suggested that Sap2 was not the only proteinase and certainly not the most dominant proteinase acting *in vivo*. Therefore, to determine to what extent the different Saps contributed to the different types of *Candida* infection, mutants lacking (Table 1) or overexpressing (Dubois *et al.*, 1998; Kvaal *et al.*, 1999) distinct *SAP* genes were created and used in experimental infections. These studies showed that not only were distinct Sap isoenzymes required during different types of infection, but also that not all *Candida* proteinases were important for virulence *per se* (Dubois *et al.*, 1998). Using *SAP*-deficient mutants, Schaller *et al.* (1999b) demonstrated that *SAP1–3*, but not *SAP4–6*, contributed significantly to experimental *C. albicans* infections of artificial oral mucosa. A major role for *SAP1–3*, but not *SAP4–6*, was also demonstrated in experimental vaginal infections using *SAP*-disrupted mutants (DeBernardis *et al.*, 1999). Using a gene misexpression strategy in the switching strain WO-1 in which white-phase cells misexpressed the opaque-specific gene *SAP1*, Kvaal *et al.* (1999) demonstrated in a cutaneous mouse model that *SAP1* caused a dramatic increase in cutaneous infection, probably as a result of increased adherence to, and 'cavitation' of, the skin.

In contrast, *SAP4–6*, but not *SAP1–3*, appeared to be critical for systemic infections, as mutants lacking *SAP4–6* were strongly attenuated in two rodent models of intravenous infection (Hube *et al.*, 1997; Sanglard *et al.*, 1997) and during intraperitoneal infection (Kretschmar *et al.*, 1999) (Table 1). In this model, *SAP4–6* may contribute to virulence by resisting phagocytic attack, since the corresponding proteinases were mostly expressed by *C. albicans* in phagolysosomes of murine peritoneal macrophages and neutrophils (Borg-von Zepelin *et al.*, 1998; A. Felk and others, unpublished

results). Furthermore, *SAP4–6*-deficient mutants were shown to be hypersensitive to phagocytic killing by macrophages (Borg-von Zepelin *et al*., 1998).

These data clearly demonstrate that Saps contribute to the pathogenesis of *Candida* infections. However, it should be noted that mutants lacking individual *SAP* genes rarely exhibit a full avirulent phenotype in a particular infection model. This may indicate a synergistic effect of a number of proteinases during certain infections, or the involvement of other virulence factors. Nonetheless, these studies illustrate that not all of the proteinases are required at the same time or stage of the infection process and not all contribute to the same types of infection. Determining the precise roles and functions of the *C. albicans* proteinases *in vivo* should provide a big step forward in identifying which proteinases, or set of proteinases, may contribute to, or even be responsible for, specific types of *C. albicans* infection.

Why does *C. albicans* possess a *SAP* gene family?

There are several possible reasons or circumstances that would make the assembly of a family of proteinases necessary for *C. albicans*.

Firstly, the different Sap isoenzymes may have adapted and evolved to function in different tissues and environments. Such a view may also explain how the gene family evolved: *C. albicans* may have duplicated a successful factor (an ancestral proteinase), which in turn adapted to different host environments and thus evolved into homologous but functionally distinct Sap isoenzymes. This hypothesis may be supported by the fact that all the *SAP* genes encode similar amino acid sequences (Fig. 1). However, since only *SAP1* and *SAP4* are clustered in tandem and all other *SAP* genes are located on five different chromosomes, this gene duplication event probably occurred sometime in the distant past. Alternatively, it is possible that the high genetic flexibility of *C. albicans* may have accelerated this widespread distribution of the *SAP* genes.

Although the overall similarity of the *SAP* genes is high, differences in their promoter sequences indicate that expression of the various *SAP* genes is controlled by different *SAP*-specific transcriptional regulators. This would suggest that the *SAP* genes might have evolved to possess distinct properties and functions, which indeed appears to be the case. For example, different Saps have been shown to have different pH optima for activity (Borg-von Zepelin *et al*., 1998). Sap2 acts mainly at acidic pH values around pH 4·0, Sap4–6 are significantly active at physiological pH and Sap3 still has activity at pH 2·0. This provides *C. albicans* with a range of proteolytic activity from pH 2·0 to 7·0, a property that may prove essential for the specific adaptation of individual Saps to different host environments.

It seems surprising that all secreted proteinases of *C. albicans* are aspartic proteinases and that neither extracellular serine-, metallo- nor cysteine proteinases have been identified in pathogenic strains of *C. albicans*. In contrast, other human pathogenic fungi, such as the filamentous fungus *Aspergillus fumigatus*, secrete several classes of different proteinases, including aspartic, serine and metalloproteinases, although none of these enzymes has been definitively associated with virulence (Monod *et al*., 1999). This may disadvantage *C. albicans* since aspartic proteinases (with a few exceptions, such as human renin) are usually only active in acidic pH ranges. However, this may be compensated for not only by the adaptation of particular Sap isoenzymes to function at higher pH values, but also by the ability of *C. albicans* to actively acidify its surrounding environment to provide microniches optimal for Sap activity during infection. These properties may be essential for the fungus when infecting the vaginal mucosa or the oral cavity.

Secondly, the *C. albicans SAP* gene family may have evolved to allow coordinated regulation of the proteinases with other virulence factors. This may not only explain why certain *SAP* genes are induced in the absence of exogenous protein or peptides, but also the differences between the *SAP* promoter sequences. Key transcriptional factors would permit the expression of specific Saps when *C. albicans* needs to call upon other virulence factors or when it encounters different host environments. Therefore, the same transcriptional factor may regulate several genes to allow the simultaneous expression of a combination of virulence factors to respond to local environmental challenges. For example, *SAP4–6* were found to be regulated during the yeast-to-hyphal transition, which in turn is known to be regulated by key transcriptional factors such as Efg1 (Ernst, 2000). It is currently not precisely known why mutants lacking *EFG1* are avirulent in several infection models. However, it seems that Efg1 is a global regulator not only of hyphal formation, but also of a number of other virulence attributes, including proteinase expression (Schröppel *et al*., 2000). A lack of this factor would subsequently cause severe defects in the virulence potential of *C. albicans*. In addition, *SAP1* and *SAP3* were shown to be regulated during phenotypic switching in particular strains, which involves the rapid change of a large number of different phenotypes and is presumably regulated via a master switch mechanism (Soll, 1997). This specific coordinated regulation of the *SAP* gene family with various virulence factors indicates that the proteinases may have evolved to specifically enhance the pathogenic ability of *C. albicans*.

A third possible reason for a family of proteinase genes is that the concomitant expression of a number of similar but functionally distinct *SAP* genes, rather than the expression of a single *SAP* gene, may result in a synergistic effect to promote colonization or infection. Thus, several Saps may act in unison to carry out a series of tasks to possibly provide *C. albicans* with a biological advantage.

Finally, providing an arsenal of *SAP* genes has the advantage of having a second enzyme in line when one

fails (i.e. the enzyme may be functionally redundant), is removed or is otherwise lost. There is evidence for such compensatory mechanisms in *C. albicans*. For instance, disruption of *SAP1* and *SAP3* genes, which may be required for oral infection, lead to increased expression of *SAP5* and *SAP8*, suggesting that *C. albicans* may be attempting to compensate for the loss of key genes by upregulating alternative genes (Schaller *et al*., 1999b). Such balanced regulation is likely to be a general phenomenon in *C. albicans*.

Conclusion and future directions

The presence of a *SAP* gene family in *C. albicans* clearly provides the fungus with an efficient and flexible proteolytic system that may prove vital to its success as an opportunistic pathogen. Furthermore, Sap production is a highly regulated and tightly controlled process, which appears to be a central factor in many aspects of *C. albicans* virulence and is indicative of the multiple functions this gene family possesses. These include the simple role of digesting molecules for nutrient acquisition, the contribution to host tissue invasion by digesting or distorting host cell membranes, the degradation of host surface molecules to enhance adhesion, and the digestion of cells and molecules of the host immune system to avoid or to resist antimicrobial attack.

However, there are still many unanswered questions that need to be addressed. (1) How is proteinase expression regulated? The signal transduction pathways that control the differential expression of *SAP* genes are currently unknown and no receptor has yet been implicated in the regulation of *C. albicans* proteinase secretion. (2) What are the actual targets of Saps during human infections? Although the possible targets of the proteinases have been deduced from *in vitro* studies, these need to be confirmed in the *in vivo* environment. Furthermore, as the substrate specificity of the Saps is so broad and most Sap antigen is detected within the *C. albicans* cell wall (Rüchel *et al*., 1991; Schaller *et al*., 1999a), how does the fungus manage to protect itself from proteolytic digestion? (3) The precise roles and functions of the *SAP* genes, especially *SAP7*, *SAP9* and *SAP10*, during human infections are currently unknown, as is the reason why Sap9 and Sap10 have C-terminal GPI-anchoring sequences. Do these two proteinases have functions similar to the GPI-anchored yapsins of *S. cerevisiae* (Komano *et al*., 1999) or do they contribute to *C. albicans* infection? (4) The immunological consequences of Sap secretion and other interactions of the proteinases with host factors *in vivo* are largely unknown and need to be addressed.

In summary, this review has endeavoured to elucidate the possible reasons why *C. albicans* is equipped with a family of proteinase genes and suggests a number of possible explanations for their evolution in light of the information available. However, there are several shortfalls that need to be tackled before the biological significance of this gene family is fully understood. With access to the complete genome sequence of *C. albicans* and with the development of new and exciting tools such as DNA microarray analysis, our understanding of the *SAP* gene family and its interaction with the human host should rapidly increase.

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