Review article

Aspartyl proteinases of *Candida albicans* and their role in pathogenicity

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Among the putative virulence factors of *Candida albicans*, secreted aspartic proteinases (Sap, encoded by a family of at least nine genes) continue to attract the attention of many investigators studying the pathogenesis of candidiasis. Several early studies documented a correlation between the levels of Sap secretion and the virulence of different strains, but much stronger support for this role has been provided by more recent data on differential SAP gene(s) expression in *ex vivo* and *in vivo* models, the outcome of infections with SAP-deleted mutants, and use of Sap immunogens. In particular, some SAP-deleted strains suffered a substantial loss of virulence, and, more interestingly, this was specifically associated with selected gene products and selected experimental pathologies. Moreover, anti-Sap antibodies have been shown to mediate a degree of protection in an experimental mucosal candidiasis model. There is now initial evidence that distinct Saps are differentially produced in various *Candida* illnesses or stages of them. The exact mechanisms of each Sap involvement in any particular *Candida* disease, with special regard to human infections, and how the immune system deals with Sap, are critical issues for future research. An answer to these questions will possibly facilitate the generation of Sap-based anticandidal drugs or immunotherapeutics.

**Keywords** *Candida albicans*, pathogenicity, Sap, virulence

**Introduction**

Infections caused by the fungus *Candida albicans* and other related species, ranging from mucosal diseases, almost invariably observed in acquired immune deficiency syndrome (AIDS) patients, to fatal systemic episodes in severely neutropenic subjects, have increased in prevalence world-wide [1–4]. The wide spectrum of candidiasis and its recognised clinical importance has stimulated interest in understanding the mechanisms of *Candida* pathogenicity and the identification of its virulence factors. For *C. albicans*, the most virulent and most frequently isolated species of *Candida*, the widely advocated virulence traits include dimorphism, adherence, enzyme secretion, rapid phenotypic switch, antigenic variation and other various immunoevasion mechanisms [5]. However, despite intense effort, the actual contribution of each of these factors to the pathogenesis and severity of one or more of the diseases caused by this fungus still awaits elucidation.

Among the putative virulence factors of *C. albicans*, enzymes with proteolytic activity have long attracted the attention of investigators. Extracellular proteolytic activity, first described by Staib [6], enables *C. albicans* to utilise exogenous protein as the sole nitrogen source. This activity was subsequently ascribed to an acidic proteinase [7] which was then purified and classified as an aspartic proteinase [8–10]. High proteolytic activity has also been found *in vitro* in most isolates of *C. tropicalis* and *C. parapsilosis* and to a lesser extent in *C. glabrata* [11,12]. Activity is now attributed to a
multigene family of secreted aspartic proteinases (Sap), enzymes with at least nine members [13]. All Sap genes encode a signal sequence, a pro-peptide section with a Kex-2 like peptidase cleavage site and a mature (secreted) protein in the range of 35–48 kDa [14]. Saps exhibit broad substrate specificity, being able to hydrolyse a range of both pure and conjugated proteins [9]. Unlike other aspartic proteinases, the Candida Sap genes can hydrolyse collagen, keratin and mucin [15]. Saps also degrade antibodies and cytokines. Being typical members of the aspartic proteinase family, the Sap isoenzymes are inhibited by pepstatin A and synthetic derivatives, which also inhibit the human immunodeficiency syndrome (HIV) aspartic proteinase [16]. They generally have optimal activity at pH 2.5–4.5 but are still active up to pH 7.0, although this depends upon the specific Sap (see below). Increasing the pH above 7.0 causes irreversible alkaline denaturation that involves dimerisation [17]. Denaturation also occurs rapidly at temperatures above 45 °C [18].

To date, nine C. albicans Sap genes have been cloned and sequenced [13,19–23]. Alignments show that Sap1, Sap2, and Sap3 are approximately 75% identical. Sap4, Sap5, and Sap6 represent a distinct subgroup, showing 90% identity to each other, which falls to 65% when compared with Sap1–3. Sap8 is most similar to Sap1 (65%) and Sap9 has a C-terminal extension, which appears to encode a glycosylphosphatidylinositol (GPI) anchor. Sap7 is the most diverged member of the family; it exhibits only 44% sequence identity to Sap1–6 and has an open reading frame (ORF) which is 500 nucleotides longer than the other Sap genes [13,23].

The principal aim of this review is to consider the roles of Saps in Candida virulence and pathogenicity. Those seeking more detailed information about the genetics and biochemistry of Saps should consult the other excellent reviews already published on this subject [2,5,24–26].

**Sap and candidiasis: a general outline**

The evidence implicating Sap activity in C. albicans virulence is now convincing, but this position has not been achieved without controversy. Moreover, the biochemical mechanisms whereby these enzymes confer virulence upon the fungus remain almost totally obscure. As stated above, C. albicans Saps have broad substrate specificities and it is intuitive that the ability of these enzymes to degrade epithelial and mucosal barrier proteins as well as antibodies, complement and cytokines may be critical in the mechanism of pathogenicity. However, no proof has been provided that this is really so. In addition, most of the early speculation on the roles of Saps in virulence were only been based on circumstantial or correlative evidence.

**Correlative studies**

The demonstration of a microbial substance in the patient and/or of the host response elicited is preliminary and crucial in its evaluation as a virulence factor. Therefore, particular efforts were directed at demonstrating Saps in human sera and tissue during Candida infections.

Secreted proteinase has been detected in the tissue of infected hosts by immunofluorescence and high titres of anti-Sap antibodies have been found in patients with disseminated candidiasis [8,27–29], indicating that Saps are indeed produced in vivo. More recent studies of Sap gene expression have unequivocally confirmed this conclusion (see below).

Several early studies documented a correlation between the levels of Sap secretion and virulence of different strains [8,11,30]. In particular, Sap secretion has been correlated with the ability to adhere to host tissue. For example, Ghannoum & Abu Elteen [31] demonstrated that highly proteolytic strains of C. albicans adhered more readily to human buccal epithelial cells. Sap(s) have also been shown to associate with the cell wall, which suggests a role for these enzymes in the attachment of C. albicans to host surfaces. This proposal has been confirmed by pepstatin A inhibition of C. albicans adhesion to the mucosal surface and epidermal cells [32–35]. Moreover, close association of one or more Saps with the cell wall of C. albicans in close apposition with the host cell has also been demonstrated by immunoelectron microscopy (Fig. 1). Potential mechanisms whereby Saps might promote adherence include enzymatic activity, which generates a binding ligand, a non-enzymatic role, in which a Sap provides a ligand for attachment to host cells, or indirectly by degradation of secretory anti-adhesin immunoglobulins.

A correlation has also been found between resistance to phagocytosis and Sap production, since C. albicans cells induced for Sap production showed a relatively higher resistance to phagocytic killing compared with uninduced cells [37]. Further support for a role for Saps in the resistance to phagocytosis was provided by studies with cultured phagocytic human tumour cells (V-937) infected with Candida cells [38]. A cytotoxic effect was noted which largely reflected the proteolytic activity of the yeast, and pepstatin A provided a dose-dependent inhibitory effect.

Stronger correlative evidence for the involvement of Saps in virulence has been provided with Sap-deficient mutants. These mutants were significantly less virulent in
animal infections than the parental wild-type strains or partial revertants [37–41]. However, these earlier protease-deficient, pleiotropic mutants were obtained by chemical mutagenesis and, as such, may have harboured additional genetic lesions at Sap-unrelated loci. These data, therefore, did not provide unequivocal evidence for the involvement of Saps in pathogenesis.

Phenotypic switching and Sap secretion

Phenotypic switching, first described by Soll [42], is the ability of C. albicans to change its phenotype rapidly and reversibly, a phenomenon which allows the fungus to adapt to changing environmental conditions, and appears to be of some importance for the host-adapted commensal. Phenotypic switching affects some putative virulence traits of Candida including bud and hyphal growth transition [42], cell hydrophobicity and adherence [43,44], resistance to phagocyte killing [45] and drug sensitivity. In addition, it affects Sap secretion in ‘white’ and ‘opaque’ colony forming phenotypes [46,47]. ‘WO-1’ opaque cells in particular were more proteolytic than the white phenotype, and it was subsequently shown that the switching-regulated expression of SAP1 (opaque-specific) was responsible for this activity [47,48]. More recently, misexpression of SAP1 in the white-cell phenotype was shown to confer an enhanced ability to adhere to, and cavitate the skin in a cutaneous mouse model of infection [49]. Other independent observations suggest that SAP1 is particularly relevant for Candida pathogenicity to skin and the mucosal epidermis (see below).

SAP expression

The multiplicity of genes coding for various Saps has, on the one hand, made it more difficult to understand the role of these enzymes in the pathogenesis of infection but, on the other, it has provided the exciting appeal of identifying possible correlations between specific diseases caused by Candida and specific Saps. As discussed below, the question is no longer indeed whether Saps play a role in disease, but rather which Sap plays a role in which disease. Unfortunately, it is still unclear which iso-enzymes are produced by each strain in vivo and whether during the course of the same disease different members of the SAP family are differentially expressed, and have different functions. Finally, it should be noted that the evidence for the selective involvement of different Sap isoenzymes has been gleaned almost completely from experimental models of candidiasis and, as yet, little has been done in humans (see, however, the section on HIV-protease and Sap). In this context, the studies of SAP gene expression have played an important role in the elucidation of the role in of Saps in pathogenicity. These analyses of differential expression in vivo usefully complement and integrate the results of the infection experiments (tissue damage) in animals challenged with SAP-deleted mutants.

SAP gene expression is differentially regulated according to strain, cell type and environmental conditions [50]. When bovine serum albumin (BSA) is the sole nitrogen source for budding yeast cells in vitro, SAP2 messenger RNA (mRNA) is the dominant transcript in most strains, and mRNA levels are unaffected by temperature or carbon source [50]. Yeast-form cells of some strains produce low levels of SAP3 mRNA in response to standard protease-inducing medium [51] and as previously mentioned, SAP1 mRNA expression is associated with the white-opaque switch in strain WO-1. Log-phase opaque cells contained high levels of SAP1 mRNA that rapidly decreased upon conversion to the white-phase [52]. SAP1 misexpression studies confirmed that Sap1 is produced during growth with protein as the sole nitrogen source.

Fig. 1 Immuno-electron micrograph of a hyphal apex of Candida albicans adhering to keratinocyte material from rat vaginal infection. Gold particles mark secretory aspartyl proteinase in the fungus cell wall as well as within the keratinocytes. Scale bar = 0.1 μm. For technical details, see [64] (Courtesy of A.R. Stringaro).
source [49]. The \textit{SAP3} transcript was also detected in both the log and stationary phases of opaque cells. \textit{SAP2} mRNA is expressed in the log phase of both the white and opaque switch phenotypes. Expression of \textit{SAP1} and \textit{SAP3} is strain-specific, compared to \textit{SAP2} which was expressed by all strains tested [49].

Transcription of \textit{SAP4}, \textit{SAP5} and \textit{SAP6} is also strain dependent and pH $\geq 6$ is required to achieve high levels of expression. Transient expression of \textit{SAP4–6} mRNA has been detected during serum-induced hyphal production and it has been shown that \textit{efg} (enhanced filamentous growth) mutants do not make germ-tubes and do not transcribe \textit{SAP4–6} genes [53]. Incidentally, this question of the relationship between Saps and other putative virulence traits of \textit{C. albicans} is a particularly important one. Note that the transfer of \textit{SAP2} to \textit{Saccharomyces cerevisiae} did not confer to this fungus any apparent virulence potential [54], suggesting that Sap must act in concert with other factors for virulence expression. Sap6-specific antibodies have been used to demonstrate high expression of Sap6 antigen on the surface of \textit{C. albicans} cells following phagocytosis by murine macrophages. Further, the triple \textit{SAP4–6} mutant was more sensitive than the parental strain to the phagocytic killing [53].

To date, \textit{SAP7} mRNA has not been detected under any laboratory conditions in any of the strain tested, but its expression has been noted in some patients with oral candidiasis [55]. \textit{SAP8} mRNA is expressed preferentially in the early exponential growth phase of yeast cells at 25 °C, but is also detectable at lower levels in cultures at 37 °C. The \textit{SAP8} transcript was also abundant in WO-1 opaque phase cells at 25 °C but a low level of \textit{SAP9} mRNA was detected in some culture conditions, when \textit{SAP8} mRNA decreased [13].

**SAP expression studies in vaginal candidiasis**

As emphasized above, there are many different forms of \textit{Candida} infections, involving various host tissues and predisposing conditions, and it would therefore be unwise to assume that all these diseases involve a common virulence trait. Several years ago before the existence of a Sap family was fully appreciated, it was hypothesized that Sap production might be more important when the organism colonizes the mucosal surface than in systemic infections [56]. The basis for this conjecture was the acidic pH required for the optimal enzymic activity of Sap2, the only enzyme of the family characterized at that time. We investigated the pathogenic role of the \textit{C. albicans} Saps in vulvovaginal candidiasis as the vaginal fluid of estrogen-sensitive mucosa has a normal pH close to that required for the activity of the isoenzymes which had been identified at that time. It was also recognized that mucosal fluid contains many potential inducers of Sap expression (proteolytic cleavage products and proteins themselves). In the initial search for a suitable experimental model of investigation, it was noted that highly proteolytic strains of \textit{C. albicans} were isolated more frequently from vaginitis subjects than from carriers [56], independent of the capacity for the dimorphic transition and other putative virulence attributes. It was also shown that Sap was secreted \textit{in vivo} in vaginal fluids, in an active form, and at higher concentrations in vaginitis patients than in carriers [41]. Thus, we selected a rat model of oestrogen-dependent candidal vaginitis because the ability of \textit{C. albicans} strains to secrete Sap \textit{in vivo} and to cause disease was strictly correlated, as follows: (i) a stable non-proteolytic mutant of \textit{C. albicans} was less vagino-pathic than the proteolytic parent strain [41]; (ii) only Sap-producing \textit{Candida} species were vaginopathic [57].

This model provided the first demonstration of the expression of the two \textit{SAP} genes that were first cloned (\textit{SAP1} and \textit{SAP2}), by Northern blot analysis with RNA extracted from the vaginal fluid of rats infected with highly vaginopathic strains. In contrast, neither gene was expressed during infection by a non-vaginopathic strain. The expression of \textit{SAP} genes during infection of the rat vagina was also detected by a polymerase chain reaction (PCR) with primers selected for each of the \textit{SAP1–6} genes. RNA extracted from vaginal fluid taken from rats at different days post-infection with \textit{C. albicans} was converted to cDNA, amplified by PCR and detected by hybridization with the corresponding probes (Fig. 2). Thus, \textit{SAP1}, \textit{SAP2} and \textit{SAP3} were expressed by \textit{C. albicans} during vaginal infection while no \textit{SAP4}, \textit{SAP5} and \textit{SAP6} vaginal transcripts were found in these infections. Overall, these results demonstrated for the first time the differential \textit{in vivo} expression of multiple \textit{SAP} genes and suggested their association with the establishment of a vaginal infection [58]. The rat vaginitis model proved to be the first animal model of candidiasis where the expression of \textit{SAP} genes could be rapidly and conveniently monitored during infection [58,59].

**Recent studies which formally demonstrate a role for Sap in \textit{Candida} pathogenicity**

Until recently it has been difficult to assign a precise virulence trait to any \textit{C. albicans} factor because of the lack of available molecular genetic protocols for use with this asexual, diploid organism. By contrast, molecular genetics has proven to be an incisive tool when applied...
to *S. cerevisiae* which has a haploid phase and a sexual cycle [60]. However, the development of the ‘Ura-blast’ protocol for *C. albicans* [61] has now made it possible to sequentially disrupt both alleles of a target gene. This technology has now been applied to the *SAP* multigene family [62,63].

Hube et al. [62] described the construction of three homozygous null mutant strains by targeted disruption of *SAPI*, *SAP2* and *SAP3* genes. Concomitantly, Sanglard et al. [63] constructed mutants with deletions in *SAP4*, *SAP5*, *SAP6* and a strain harbouring a triple deletion in the *SAP4*, *SAP5* and *SAP6* genes. *SAPI* and *SAP3* mutants grew slowly in proteinase-inducing medium and proteinase production was delayed compared with the parent strain. *SAP2* null mutants also grew poorly, tended to clump and produced the lowest amount of proteolytic activity. This is consistent with earlier results which indicated that Sap2 is the dominant isoenzyme in protein induced-culture media [18]. Surprisingly, the *SAP4–6* mutant showed the same growth deficiency as the *SAP2* mutant in Sap2-inducing medium, conditions under which *SAP4–6* expression had not previously been detected. This led the authors to suggest that Sap4, Sap5 or Sap6 may contribute in the induction of Sap2.

The pathogenicity of the various Sap-deleted mutants was tested in a model of systemic candidiasis. When *SAPI*, *SAP2* and *SAP3* mutants were injected intravenously into guinea pigs and mice, the survival rates of test animals were slightly higher than those of controls infected with the parental wild type strain SC5314. However, this partial loss of pathogenic potential was not commensurate with the marked reduction in proteolytic activity *in vitro*. A more marked decrease in virulence for systemic infection was noticed with the *SAP4–6* deleted mutants.

We compared all these mutants with the parental strain and some *SAP*-revertants for their virulence potential in the vaginal candidiasis model [64]. Null *SAPI–SAP3* but not *SAP4–SAP6* mutants lost most of the virulence of the parental strain SC5314. In particular, the *SAP2* mutant was almost avirulent in this model and this loss of virulence was now commensurate with loss of Sap activity. Importantly, reinsertion of the *SAP2* gene into the *SAP2* deleted mutant led to a full recovery of the vaginopathic potential (Fig. 3). The vaginal fluids of the animal infected with the wild type strain or the *SAPI* or *SAP3* mutants expressed a pepstatin-sensitive proteinase activity *in vitro*. No traces of this activity were found in the vaginal fluids of rats challenged by the *SAP2* mutant. All the strains were equally capable of developing true hyphae during infection (Fig. 4), and the initial adherence to epithelial cells of the rat vagina was comparable. We therefore concluded that Sap1–3 and, in particular, Sap2 plays a clear pathogenic role in vaginitis, by mechanisms which do not appear to be dependent on either dimorphic growth or adherence [64]. In addition,
these results showed a higher reduction of virulence of the \textit{SAP1–3} null mutant, especially \textit{SAP2}, in the rat vaginitis model compared to the systemic, disseminated infections in rodents. Interestingly, and as mentioned above, the triple \textit{SAP4–6} deleted mutant lost some virulence properties in a systemic infection model [63]. This again emphasises that different virulence factors come into play in vaginal and in disseminated infections as was suggested previously by the observations that strains of \textit{C. albicans} with a low potential for systemic infections can have an elevated vaginopathic potential and vice versa [36]. Similarly, deletion of the pH-regulated genes, \textit{PHR1} and \textit{PHR2}, independently affected systemic and vaginal infection [65].

The relevance of \textit{SAP2} in vaginal infections by \textit{Candida} has been confirmed, albeit indirectly, by the protective effect when animals were immunized with Sap2, and by transfer experiments with vaginal fluids containing anti-Sap2 antibodies [66]. All these studies clearly demonstrated the validity of previous assumptions that the multigene SAP family is differentially involved in multifaceted \textit{Candida} diseases.

Some research is now being directed towards different models of \textit{Candida} colonization and infection. For instance, in a model of murine peritonitis Kretschmar \textit{et al.} [67] measured virulence as the release of organ-specific enzymes into the plasma of infected mice. Alanine amino-transferase (ALT) and \(\alpha\)-amylase (AM) were used as parameters for damage of the liver and pancreas, respectively. When Sap activity was inhibited \textit{in vivo} with pepstatin A, there was a significant reduction of ALT and AM activities. Although pepstatin may have inhibited some host aspartic proteinases, these data suggest that Saps contributed to virulence in this model. Furthermore, strains of \textit{C. albicans} with disruptions in \textit{SAP1}, \textit{SAP2}, \textit{SAP3} and \textit{SAP4–6} showed considerable variation in virulence in this system. In fact, only the triple \textit{SAP4–6} mutant showed a significantly reduced ALT production in comparison to the parental strain. This again emphasises a role for \textit{SAP4–6} genes in systemic infection, adding further evidence that different Saps exert distinct roles in the various forms of candidiasis.

Another caveat in the search for virulence factors is the possible association between germ-tube formation and \textit{SAP} expression. In this context the recent work by Staib \textit{et al.} [68] is particularly interesting. These investigators fused a reporter gene (\textit{FLP}) with \textit{SAP} promoters and monitored the \textit{in vivo} differential expression of the individual \textit{SAP} genes at various stages of the infection process. \textit{SAP} expression was clearly dependent on the type of infection and different \textit{SAP} genes were activated during systemic disease as compared with mucosal infections. In addition, the expression of individual \textit{SAP} genes depended on the progress of the infections, some being induced immediately after contact with the host, whereas others were expressed only after dissemination into deep organs (this applied particularly to \textit{SAP2}). Notable was the absence of \textit{SAP2} expression in an oesophageal model, a clear mucosal infection. This is at odds with our observations with another mucosal infection, the vaginal model (see above), as well as with the findings by Schaller \textit{et al.} [69] and by Naglick \textit{et al.} [55] with oral infections. These data prompt two suggestions: (i) specific factors of different mucosa are involved in \textit{SAP1–3} expression, or
(ii) differential expression of the SAP genes cannot simplistically be linked to either systemic or mucosal infections. It should also be noted that oral and vaginal candidiasis are achieved with high Candida burdens \((10^7 - 10^8)\) cells), whereas considerably fewer fungal cells are usually recovered in models of oesophageal infections. The importance of the size of the cell culture or cell colonization on SAP expression should therefore be more closely investigated. Another model where differential expression of distinct SAP genes was clearly demonstrated is with reconstituted human epithelium. In this system, SAP1 and SAP2 were predominant in the initial invasion of the stratum corneum whereas SAP3, SAP6 and SAP8 were only expressed later [69]. SAP6 expression was concomitant with germ-tube formation, when the fungal cells invaded the inner layers of the epidermis. The SAP-deleted mutants were attenuated in virulence in this model [69]. In this context it is noteworthy that de Repentigny et al. [70] attributed a role to Sap2 in the degradation of the mucin which overlays and protects the epithelial cells of the oral cavity and the small intestine.

**HIV proteinase inhibitors and Candida virulence in the oral cavity**

As mentioned above, pepstatin A, the prototypic inhibitor of aspartic proteinases, exerts a curative effect in experimental vaginal candidiasis and infections of epidermis. This effect, together with the recognition of the common properties of all aspartic proteinases, led us to investigate whether the HIV proteinase inhibitors indinavir and ritonavir inhibited Candida Saps and if this would have, in principle, justified the use of these drugs in anti-Candida therapy. Part of the interest in this approach also came from well established clinical observations that anti-retroviral therapy combining HIV-protease inhibitors with nucleoside anti-retrotranscriptional inhibitors (highly active anti-retroviral therapy; HAART) have a significant preventative and potent curative effect in oral candidiasis of HIV-infected
patients [71,72]. We have shown [73] that HIV-protease inhibitors were active against Sap in vitro and that they exerted a therapeutic effect in the rat vaginal candidiasis model (which is SAP1–3 expression-dependent) at concentrations achievable in the normal course of anti HIV-therapy. Others have confirmed these observations [74–76]. For example, Borg von Zeppelin et al. [74] demonstrated that some HIV-protease inhibitors inhibited Candida adherence to epithelial cells in culture, and purified Saps were able to compete in this effect. It is still unclear to what extent these findings explain the beneficial effect of HAART in candidiasis. It is however clear that Saps are easily detected in the salivary fluid and on epithelial cells of the human oral cavity as confirmed by the detection of anti-Sap antibodies in the saliva [77]. Interestingly, SAP1 and SAP3 were expressed only in patients with oral candidiasis, whereas all the other SAP genes were, to different extents, expressed during colonization [78]. It is pertinent that Wu & Samaranayake [79] noted that Sap expression was inversely correlated with total salivary protein concentrations and pH fluctuation, suggesting that salivary proteins might have been enzymically degraded by the Saps.

The expression of the SAP genes, the enzymes and relevant antibodies in the oral cavity demonstrates that this environment provides a favourable pH niche for the expression of several of these isoenzymes. Similarly, the vaginal cavity appears to be very favourable for the expression of these proteinases. As with the vaginal rat model, SAP1–3 deleted strains were more attenuated in virulence than other SAP mutants in reconstituted human epithelium [80].

Candida isolates from HIV-positive subjects are extremely high Sap producers [81,82] and the administration of HAART has a specific inhibitory effect with these strains [A. Cassone et al., unpublished results]. Some authors have proposed the use of HIV-protease inhibitors for anti-mycotic refractory mucosal infections even in non-HIV-positive patients, but the adverse effects of these drugs warrants a careful evaluation of risks and benefits.

Conclusions and perspectives

Sap activity has long enjoyed a putative role in Candida virulence. The recent data on SAP gene expression and the pathogenicity of SAP-deleted mutants have not only validated the previous predictions but have also greatly emphasized the general importance of this gene/enzyme family in the biology and pathogenicity of the fungus. There is now ample evidence that distinct Saps are differentially produced in various states of Candida commensals and disease. Some members of the family, in particular SAP1–3, have critical roles in pathogenicity in experimental models of mucosal infection, a conclusion formally based on the attenuated virulence of isogenic mutants and the protection achieved in the rat vaginitis model by the passive transfer of Sap antibodies and active immunization with Sap2 [66]. Evidence for other members of the family (SAP4–SAP9) playing direct roles in virulence is less compelling but numerous studies of gene expression and mutant attenuation strongly suggest that the gene products contribute to some systemic infections [83,84]. The apparently contradictory findings on the involvement of various Saps in different experimental models of candidiasis, probably reflects the different niches and host regulatory factors which influence SAP expression.

The overall picture is therefore highly suggestive but not definitive. In addition to a precise definition of ‘which Sap, which disease or which stage of disease’, future research should be directed towards an understanding the mechanisms whereby Sap2 and the other Saps express their local or systemic virulence. Saps are potent enzymes with wide-substrate specificities. It is logical to predict that their primary role in virulence is the hydrolysis of the various structural and functional proteins, which bathe and defend mucosal surfaces and internal tissues. However, there is no proof for this hypothesis. Also lacking are in-depth studies on the substrate specificities of each Sap. However, this could now be achieved either by way of rigorous purification for each native Sap or via recombinant technology.

The relationship between Saps and other putative virulence factors is another issue which needs to be addressed. A role for Saps in provoking or augmenting fungal adherence has been widely advocated, and there are a few hints that this could indeed be the case. However, progress in this area is confounded by the plethora of Candida adhesins. Adhesion and enzymatic activity are not mutually exclusive, and Sap could also contribute non-enzymatically [5].

The relationship between Saps and hyphal formation is another issue for future research. Hyphal-repressed mutants lose their virulence in systemic (but not in mucosal) infections [85]. Overproduction of some Saps has been included among the many possible mechanisms whereby germ-tube formation increases virulence. The same might be true for the pH regulatory mutants, which express selective virulence properties by pH-regulated Sap expression in different sites in the host [65].

Thirty-five years have now elapsed since Staib [6] reported the discovery of extracellular proteinase activity in C. albicans. During the intervening years the structure of Sap2 has been solved [86] and it has been
established that this opportunistic pathogen possesses not one but at least nine SAP genes which are expressed in intriguing patterns in vivo. With this detailed knowledge of the biochemistry and genetics of the Saps it has been established that Saps are indeed virulence factors. This progress provides a compelling justification for more detailed research to unravel further the role of each isoenzyme in specific types of candidiasis.

Finally, we stress the need for rigorous research into the immune response to Saps and the potential use of these responses to control Candida infections. Aside from our own observations with the rat vaginitis model [66], very little if nothing at all is known about the dominance of Sap antigens, their prevalent B and T cell epitopes and the roles that Sap antibodies and cell-mediated immune responses play in the defence against Candida. Research in this area will provide further insights into the roles of Saps in Candida diseases. It will also generate further interest in the prevention and control of Candida infections by pharmacological and immunological tools specifically directed at inhibiting SAP expression and pathogenicity, or even to the use of Saps or their genes as a vaccine.

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References


Monod M, Hube B, Hess D, Sanglard D. Differential regulation of SAP8 and SAP9, which encode two new members of the secreted aspartic proteinase family in Candida albicans. Microbial 1998; 144: 2731–2737.
