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A family of oligopeptide transporters is required for growth of *Candida albicans* on proteins

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Summary

The human fungal pathogen *Candida albicans* can use proteins as the sole source of nitrogen for growth. The secretion of aspartic proteinases, which have been shown to contribute to virulence of *C. albicans*, allows the fungus to digest host proteins to produce peptides that must be taken up into the cell by specific transporters. To understand in more detail how *C. albicans* utilizes proteins as a nitrogen source, we undertook a comprehensive analysis of oligopeptide transporters encoded in the *C. albicans* genome. We identified eight *OPT* genes encoding putative oligopeptide transporters, almost all of which are represented by polymorphic alleles in strain SC5314. Expression of these genes was differentially induced when *C. albicans* was grown in YCB-BSA medium, which contains bovine serum albumin as the sole nitrogen source. Whereas deletion of single *OPT* genes in strain SC5314 did not affect its ability to utilize proteins as a nitrogen source, *opt123*Δ triple mutants had a severe growth defect in YCB-BSA which was rescued by reintroduction of a single copy of *OPT1*, *OPT2* or *OPT3*. In addition, forced expression of *OPT4* or *OPT5* under control of the *ADH1* promoter also restored growth of an *opt123*Δ mutant, demonstrating that at least *OPT1-OPT5* encode functional peptide transporters. The various oligopeptide transporters differ in their substrate preferences, as shown by the ability of strains expressing specific *OPT* genes to grow on peptides of defined length and sequence. We present evidence that in addition to the known role of oligopeptide transporters in the uptake of tetra- and pentapeptides these proteins can also transport longer peptides up to at least eight amino acids in length, ensuring an efficient utilization of the various peptides produced via endoproteolytic digestion of proteins by the secreted aspartic proteinases. As even transporters encoded by polymorphic alleles of a single gene exhibited differences in their efficiency to take up specific peptides, the oligopeptide transporters represent an example for how the evolution of gene families containing differentially expressed and functionally optimized members increases the nutritional versatility and, presumably, the adaptation of *C. albicans* to different host niches.

Introduction

The yeast *Candida albicans* is a member of the microflora on mucosal surfaces of most healthy people, but it can also cause serious infections, especially in immunocompromised patients (Odds, 1988). *C. albicans* exhibits a variety of characteristics that contribute to its capacity to colonize and infect many different host tissues (Calderone and Fonzi, 2001). Among these virulence factors are secreted aspartic proteinases (Saps), which are encoded by a multigene family (Naglik *et al*., 2003). The Saps are differentially expressed at various stages of an infection (Staib *et al*., 2000) and are thought to have different roles in disease progression, for example, by contributing to adherence (Watts *et al*., 1998), by degrading tissue barriers to facilitate invasion (Colina *et al*., 1996; Morschhäuser *et al*., 1997), by destroying host defence molecules (Rüchel, 1986; Kaminishi *et al*., 1995) or by providing nutrients through the digestion of host proteins (Staib, 1965). Of the 10 *SAP* genes, *SAP2* appears to be dedicated to enable *C. albicans* to grow on proteins, as its expression is specifically induced in media containing protein as the sole nitrogen source and *C. albicans sap2*Δ mutants are unable to grow in such media (Hube *et al*., 1994; 1997).

The *C. albicans* Saps are endoproteinases and at least Sap2p has a broad substrate specificity (Monod *et al*., 2002; Naglik *et al*., 2003). Therefore, the degradation of proteins by Sap activity results in the production of peptides that must be taken up into the cell by specific transporters to allow fungal growth when proteins are the only available nitrogen source. Two distinct, proton-coupled peptide transport systems have been described in yeast, the PTR (peptide transport) system, which is specific for di- and tripeptides, and the OPT (oligopeptide transport)

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system, which transports tetra- and pentapeptides. Homologues of the di-/tripeptide transporter Ptr2p are found in virtually all organisms, whereas the oligopeptide transporters are limited to fungi and plants (reviewed in Hauser *et al*., 2001; Wiles *et al*., 2005). Both a di-/tripeptide transporter (*PTR2*) and an oligopeptide transporter (*OPT1*) have been cloned from *C. albicans* by their capacity to allow growth of a *Saccharomyces cerevisiae ptr2* mutant on a dipeptide or a normally non-utilized tetrapeptide, respectively, as a sole source of auxotrophic requirements (Basrai *et al*., 1995; Lubkowitz *et al*., 1997). These peptide transporters may therefore enable *C. albicans* to utilize peptides generated by proteolytic digestion of proteins as a nitrogen source.

Oligopeptides produced from proteins by basal Sap activity have also been suggested to be inducers of *SAP2* expression, because Sap activity was required for *SAP2* induction by bovine serum albumin (Hube *et al*., 1994; Staib *et al*., 2002) and oligopeptides of eight or more amino acids in length induced proteinase expression (Lerner and Goldman, 1993). It has been proposed that peptides may be sensed at the cell surface by specific receptors which then activate a signal transduction event that results in the induction of proteinase expression (Lerner and Goldman, 1993). In addition to transporting peptides, oligopeptide transporters might therefore also serve as sensors which in the presence of peptides activate a signalling pathway that induces *SAP2* expression. Such a nutrient-sensing function has been described for other members of yeast transporter families, for example, the ammonium permease Mep2p (Lorenz and Heitman, 1998), the glucose transporter homologues Snf3p and Rgt2p (Özcan *et al*., 1996), the general amino acid permease Gap1p (Donaton *et al*., 2003), and the amino acid permease homologue Ssy1p (Didion *et al*., 1998; Iraqui *et al*., 1999; Klasson *et al*., 1999). Some of these proteins (Rgt2p, Snf3p, Ssy1p) are similar to other members of the respective family but have lost their transport function and serve only as sensors.

We had previously investigated the importance of the *OPT1* gene in mediating peptide uptake and growth of *C. albicans* on proteins. Inactivation of *OPT1* in the *C. albicans* model strain SC5314 resulted in resistance of the mutants to the toxic tetrapeptide KLLEth, suggesting that Opt1p mediated most or all of the transport of this peptide into the cell. However, the *opt1*Δ mutants grew as well as the wild type in YCB-BSA medium and produced comparable amounts of proteinase, indicating that *OPT1* is not essential for *SAP2* induction and for uptake of peptides resulting from the proteolytic degradation of BSA (Reuß *et al*., 2004). Inspection of the published *C. albicans* genome sequence (Jones *et al*., 2004) revealed that *C. albicans* possesses additional genes encoding putative oligopeptide transporters which might have partially redundant functions. Therefore, we performed a functional analysis of all these genes to elucidate their role in proteinase expression and in the ability of *C. albicans* to grow on proteins.

Results

Identification of a C. albicans *gene family encoding oligopeptide transporters*

A BLAST search of the *C. albicans* genome sequence (http://www-sequence.stanford.edu/group/candida) identified a number of open reading frames (ORFs) encoding putative proteins with similarity to Opt1p. Close inspection of these ORFs and flanking sequences allowed the assignment of alleles and truncated reading frames to specific genes and suggested that the *C. albicans OPT* gene family consists of eight members located on four different chromosomes. The new genes were designated as *OPT2* to *OPT8* according to decreasing similarity of the encoded proteins to Opt1p (see Fig. 1). The results of this *in silico* analysis were experimentally confirmed by cloning and sequencing of all eight *OPT* genes (for details see *Experimental procedures*), which also identified additional polymorphic alleles of several genes that were missing in the genome sequence (see Table 1).

OPT1. As reported previously (Reuß *et al*., 2004), strain SC5314 possesses two polymorphic alleles of the *OPT1* gene which differ at 35 nucleotides within the coding region, five of which result in amino acid exchanges. In addition, the *OPT1-2* allele contains a 58 bp intron that is not present in the *OPT1-1* allele. The sequence of the two identical ORFs 19.2584 and 19.10116 is a mixture of the two cloned alleles but is more similar to *OPT1-1* (four nucleotide differences).

OPT2. The two identical ORFs 19.3746 and 19.11231 represent the *OPT2* gene, whose encoded product has 48% similarity to Opt1p. The *OPT2* gene cloned by us using different sets of primers is identical to these ORFs, suggesting that the two *OPT2* alleles in strain SC5314 are identical.

OPT3. The *OPT3* gene is located 3.3 kb downstream of *OPT2* and runs in the same direction. The encoded protein has very high (96%) similarity to Opt2p, suggesting that the two genes arose by tandem duplication. Strain SC5314 contains two polymorphic *OPT3* alleles that exhibit 86 nucleotide differences within the coding region, eight of which result in amino acid exchanges. The two cloned *OPT3* alleles are identical with ORFs 19.3749 (*OPT3-1*) and 19.11233 (*OPT3-2*).

	Opt1p	Opt2p	Opt3p	Opt4p	Opt5p	Opt6p	Opt7p	Opt8p
Opt1p		37.2	36.2	35.6	34.6	32.5	28.3	22.1
Opt2p	48.3		93.7	90.0	74.1	46.4	27.4	n. s.
Opt3p	47.1	95.9		92.3	74.8	45.0	26.7	n. s.
Opt4p	46.4	92.9	95.0		74.4	45.8	27.5	n. s.
Opt5p	46.2	79.6	80.4	80.2		46.9	27.3	n. s.
Opt6p	43.8	58.0	58.0	57.9	58.4		26.9	n. s.
Opt7p	38.0	38.0	37.8	37.7	37.8	37.3		n. s.
Opt8p	31.9	n. s.	n. s.	n. s.	n. s.	n. s.	n. s.	

Fig. 1. Per cent similarity (lower left) and identity (upper right) among the proteins encoded by the *C. albicans OPT* gene family. For clarity, only one of the two proteins encoded by polymorphic alleles (allele 1) has been included in the figure. For allelic differences see Table 1. n.s., no recognizable similarity.

OPT4. The *OPT4* gene is located about 47 kb upstream of *OPT2* and runs in the opposite direction. We cloned two polymorphic *OPT4* alleles that exhibit 74 nucleotide exchanges within the coding region, 13 of which result in amino acid differences. There is one complete ORF (19.2292) in the *C. albicans* genome sequence, which consists of the N-terminal part of the *OPT4-1* allele and the C-terminal part of the *OPT4-2* allele. In addition, truncated partial ORFs can be found in the genome sequence on different contigs. The identical ORFs 19.3718 and 19.11202 at the end of contigs 19-10190 and 19-20190 correspond to the N-terminal part (amino acids 1–436) of the *OPT4-2* allele, whereas the identical ORFs 19.176 and 19-7809 located at the end of contigs 19-10040 and 19-20040 correspond to the C-terminal part (amino acids 466–904) of the *OPT4-1* allele. The cloned *OPT4-2* allele also contains four nucleotide exchanges not present in any of the ORFs found in the

Table 1. Characteristics of *OPT* genes and encoded proteins.

		Genomic location			Allelic differences		
Gene	ORF names	Contigs	Chromosome	Length in amino acids	Nucleotides[a]	Amino acids	Restriction site polymorphisms[b]
OPT1	19.2602 19.10133	19-10251 19-20251	R	783	35[c]	5	HindIII cod, HindIII down
OPT2	19.3746 19.11231	19-10190 19-20190	R	929	–	–	SpeI down
OPT3	19.3749 19.11233	19-10190 19-20190	R	905	86	8	SpeI up
OPT4	19.2292 19.3718 19.11202 19.176 19.7809	19-1887 19-10190 19-20190 19-10040 19-20040	R	904	74	13	EcoRI up, EcoRV cod, NdeI cod
OPT5	19.5121 19.12587 19.1347 19.8927	19-10218 19-20218 19-10111 19-20111	2	921	–	–	NsiI up, NsiI down
OPT6	19.4655 19.12125	19-10212 19-20212	4	945	12	8	ClaI up, ClaI down
OPT7	19.5673 19.13118	19-10231 19-20231	4	747	5	1	EcoRI down
OPT8	19.5770 19.13192	19-10233 19-20233	6	718	7	–	BglII cod

a. Only nucleotide differences within the coding region are listed.
b. Restriction site polymorphisms in the upstream, downstream and coding regions are labelled as up, down and cod respectively. These restriction site polymorphisms were used to distinguish the two alleles of each gene in Southern hybridizations.
c. As reported previously (Reuß *et al.*, 2004), the *OPT1-2* allele also contains a 58 bp intron that is not present in the *OPT1-1* allele.

genome sequence. The proteins encoded by the *OPT4* alleles are highly similar to Opt2p and Opt3p (between 92% and 96% similarity).

OPT5. The two identical ORFs 19.5121 and 19.12587 represent the *OPT5* gene. In addition, two identical truncated ORFs (19.1347 and 19.8927) at the end of contigs 19-10111 and 19-20111 correspond to the C-terminal part of *OPT5* (amino acids 792–921). We cloned only one *OPT5* allele that exactly matched these ORFs, suggesting that the two *OPT5* alleles in strain SC5314 are identical; however, they can be distinguished by NsiI restriction site polymorphisms in the flanking regions. The encoded protein has about 80% similarity to Opt2p-Opt4p.

OPT6. We cloned two polymorphic *OPT6* alleles that exhibit 12 nucleotide differences within the coding region, eight of which result in amino acid exchanges. The two identical ORFs 19.4655 and 19.12125 are a mixture of the *OPT6-1* and *OPT6-2* alleles. The proteins encoded by these alleles have about 58% similarity to Opt2p-Opt5p.

OPT7. We cloned two polymorphic *OPT7* alleles that differ by five nucleotides within the coding region; one of them results in an amino acid exchange. The two identical ORFs 19.5673 and 19.13118 correspond to the *OPT7-1* allele. Opt7p displays only 37–38% similarity to all of Opt1p-Opt6p.

OPT8. The *C. albicans* genome sequence contains one gene, represented by the identical ORFs 19.5770 and 19.13192, whose encoded product exhibits 32% similarity to Opt1p but no recognizable similarity to any of the other Opt proteins. Therefore, the inclusion of this gene, which was named *OPT8*, into the *OPT* gene family should be considered tentative. *OPT8* displays high similarity to *YGL114w* from *S. cerevisiae*, which is described as a putative member of the oligopeptide transporter family, but it has recently been suggested that *YGL114w* probably does not encode an oligopeptide transporter (Wiles *et al*., 2005). In addition to the *OPT8-1* allele, which is identical to the ORFs found in the genome sequence, we cloned a second *OPT8* allele that differs from *OPT8-1* by seven nucleotide exchanges within the coding region, none of which results in an amino acid exchange.

Pseudogenes. Additional partial reading frames with similarity to the *OPT* genes can be found in the *C. albicans* genome sequence. About 25 kb downstream of the *OPT1* gene there is a reading frame (containing the identical ORFs 19.2584 and 19.10116) that is almost identical to the C-terminal part of *OPT1* (amino acids 534–783), including the intron found in the *OPT1-2* allele. This sequence is preceded by an inverted sequence corresponding to amino acids 434–531 of Opt1p and another sequence corresponding to amino acids 362–429 of Opt1p in the correct orientation. The region of identity to *OPT1* extends into the downstream region; however, the N-terminal part of *OPT1* and the upstream sequence are completely missing, as confirmed by Southern hybridization. Therefore we termed this pseudogene Ψ*OPT1*. Another region of about 0.8 kb with high similarity to the *OPT2-OPT4* genes (80% on the amino acid level) is located on contigs 19-10161 and 19-20161. However, there is no complete ORF in this region, which contains several stop codons, indicating that it also represents a pseudogene.

Oligopeptide transporters are required for utilization of proteins as a nitrogen source

To investigate whether the putative oligopeptide transporters encoded by the *OPT* gene family are required for *C. albicans* to utilize proteins as a nitrogen source, we constructed a series of knockout mutants lacking specific *OPT* genes. As described previously for *OPT1* (Reuß *et al*., 2004), we used the *SAT1* flipping strategy to delete the other *OPT* genes in the wild-type *C. albicans* strain SC5314. The tandemly arranged, highly homologous *OPT2* and *OPT3* genes were simultaneously deleted (see Fig. 2A), whereas the *OPT4-OPT8* genes were inactivated individually (see *Experimental procedures*). The *OPT2-OPT3* intergenic region, which was deleted together with these genes, contains two overlapping hypothetical short ORFs located on opposite DNA strands. We did not consider these ORFs as true genes, as one of them is found on only one of the two allelic contigs and none of them displays significant similarity to other proteins in the databases.

For all genes, two independent homozygous mutants were constructed (see Table S1) and tested for their ability to grow on BSA as the sole source of nitrogen. Like the wild-type strain SC5314, all the mutants grew well in YCB-BSA medium (data not shown), demonstrating that no single *OPT* gene is required for growth of *C. albicans* on BSA. We hypothesized that the oligopeptide transporters encoded by the *OPT* genes may have at least partially redundant functions in the uptake of peptides and/or the induction of proteinase expression and therefore constructed mutants lacking multiple *OPT* genes. The *OPT2* and *OPT3* genes were deleted in two previously constructed *opt1*Δ mutants (Reuß *et al*., 2004) to generate two independent *opt1*Δ *opt2*Δ *opt3*Δ triple mutants (hereafter referred to as *opt123*Δ mutants for simplicity). The *opt123*Δ mutants had a severe growth defect in YCB-BSA and, similar to a *sap2*Δ mutant, showed hardly any growth after 18 h of incubation, indicating that at least one of these oligopeptide transporters is required for normal

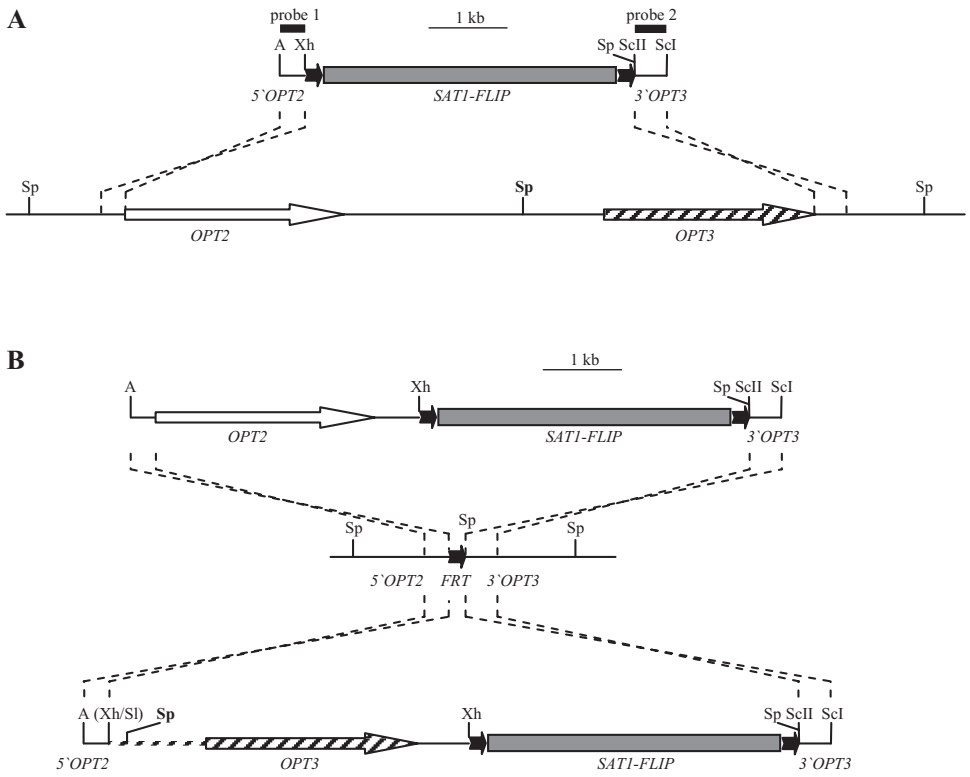

Fig. 2. Construction of *opt123*Δ triple mutants and complemented strains.
A. Structure of the deletion cassette from plasmid pOPT23M2 (top), which was used to delete the *OPT2* and *OPT3* genes, and genomic structure of the *OPT2-OPT3* locus in strain SC5314 (bottom). The *OPT2* and *OPT3* coding regions are represented by the white and hatched arrows respectively, and the upstream, downstream and intergenic regions by the solid lines. Details of the *SAT1* flipper [grey rectangle bordered by *FRT* sites (black arrows)] have been presented elsewhere (Reuß *et al*., 2004). The 34 bp *FRT* sites are not drawn to scale. The probes used for Southern hybridization analysis of the mutants are indicated by the black bars.
B. Structure of the DNA fragments from pOPT23K2 (top) and pOPT23K3A and B (bottom), which were used for reintegration of an intact copy of the *OPT2* gene or the *OPT3-1* and *OPT3-2* alleles, respectively, into one of the inactivated *opt23*Δ loci of the *opt123*Δ triple mutants (middle). The *OPT3* upstream region is represented by the thick hatched line in this scheme to distinguish it from the flanking 5′ *OPT2* region. Reinsertion of an intact copy of the *OPT1* gene into the *opt123*Δ triple mutants was performed as described previously for *opt1*Δ single mutants (Reuß *et al*., 2004). After verification of the correct insertion, the *SAT1* flipper cassette was excised again by FLP-mediated recombination. Only relevant restriction sites are given. A, ApaI; ScI, SacI; ScII, SacII; Sl, SalI; Sp, SpeI; Xh, XhoI. Sites shown in parenthesis were destroyed by the cloning procedure. The SpeI site marked in bold in the *OPT2-OPT3* intergenic region is present in only one of the two alleles (upstream of the *OPT3-1* allele).

growth of *C. albicans* in this medium (Fig. 3A). In fact, reintroduction of an intact copy of either *OPT1*, *OPT2* or *OPT3* into the *opt123*Δ mutants (see Fig. 2B) restored growth. The poor growth of the *opt123*Δ mutants was not caused by a failure to induce expression of the *SAP2* gene, as integration of a P_{SAP2} *-GFP* reporter gene confirmed that the *SAP* promoter was induced normally (Fig. 3B, left and middle panels) and the *opt123*Δ mutants produced similar levels of Sap2 protein and degraded the BSA in the growth medium as efficiently as the wild type (Fig. 3C, lanes 1–3). In addition, the expression of an additional copy of the *SAP2* gene from a Tet-inducible promoter (Park and Morschhäuser, 2005) in the *opt123*Δ mutants did not complement their growth defect (data not shown, for strains see Table S1). These results indicated that the growth defect of the *opt123*Δ mutants was caused by an inability to efficiently take up peptides produced by proteolytic degradation of BSA.

The individual members of the OPT *gene family are differentially expressed*

The fact that the remaining *OPT* genes, especially *OPT4* which is highly homologous to *OPT2* and *OPT3*, did not compensate for the loss of the *OPT1-OPT3* genes suggested that either the encoded oligopeptide transporters do not efficiently transport peptides produced by Sap-mediated degradation of BSA or the genes are not sufficiently expressed under these conditions. To distinguish between these possibilities, we analysed the expression pattern of the *OPT* genes. As the high similarity of the *OPT2-OPT4* genes would not allow discrimination of their

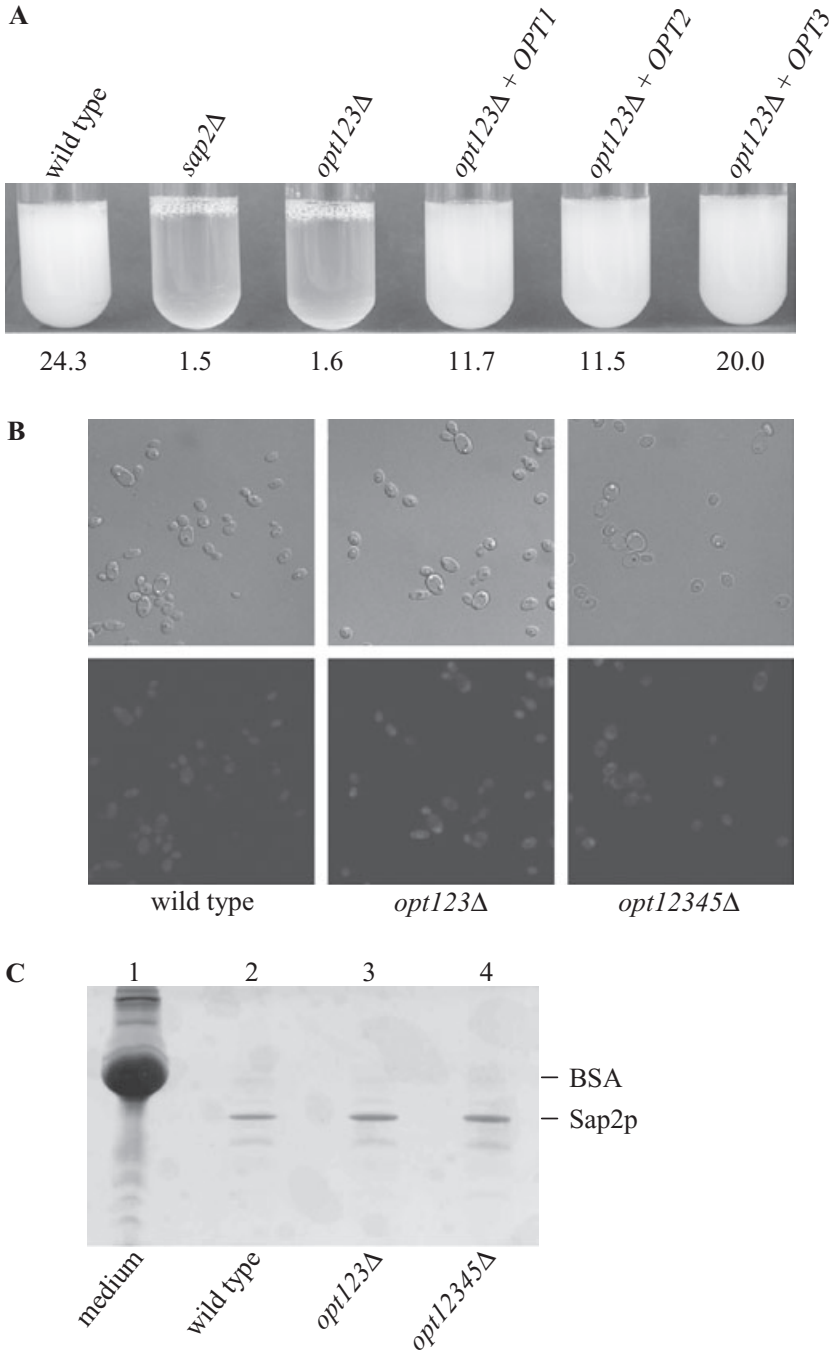

Fig. 3. Oligopeptide transporters are required for growth of *C. albicans* on proteins but not for *SAP2* expression.
A. Growth of the wild-type strain SC5314, a *sap2*Δ mutant, *opt123*Δ triple mutants, and complemented strains in YCB-BSA. Overnight cultures of each strain in YPD medium were diluted 1:100 in YCB-BSA, incubated for 18 h at 30°C, and photographed. The optical densities (OD_{600}) of the cultures are given below each tube. The following strains were used: SC5314 (wild type), SAP2MS4 (*sap2*Δ), OPT123M4A and B (*opt123*Δ), OPT123MK12A and B (*opt123*Δ + *OPT1*), OPT123MK22A and B (*opt123*Δ + *OPT2*), OPT123MK32A and B (*opt123*Δ + *OPT3*). The two independently constructed series of *opt123*Δ mutants and complemented strains behaved identically and only one of them is shown.
B. Expression of a P_{SAP2} -*GFP* reporter fusion in wild-type, *opt123*Δ and *opt12345*Δ backgrounds. Overnight cultures of the strains in YPD medium were diluted 1:100 in YCB-BSA-YE and grown for 18 h at 30°C to induce *SAP2* expression. Shown are corresponding phase contrast (top panels) and fluorescence micrographs (bottom panels) of the cells. The following strains were used: SCSAP2G1A and B (wild type), OPT123MSAP2G1A and B (*opt123*Δ) and OPT12345MSAP2G1A and B (*opt12345*Δ). The two independently constructed series of reporter strains behaved identically and only one of them is shown.
C. Expression of Sap2p in the wild type and *opt* mutants. Strains SC5314 (wild type) OPT123M4A and B (*opt123*Δ), and OPT12345M4A and B (*opt12345*Δ) were grown for 2 days in YCB-BSA-YE at 30°C and culture supernatants were analysed by SDS-PAGE. Growth medium was loaded for comparison, the bands corresponding to BSA and Sap2p are labelled. The two independently constructed series of *opt123*Δ and *opt12345*Δ mutants behaved identically and only one of them is shown in each case.

transcripts in Northern hybridization experiments, we constructed a set of reporter strains that expressed the *GFP* gene from the promoter of any of the 16 *OPT* alleles (see Table S1) and analysed the strains by fluorescence microscopy and flow cytometry (Fig. 4). None of these reporter strains showed detectable *GFP* expression in YPD medium, indicating that the *OPT* genes, like *SAP2*, are expressed at low levels or not at all in this rich medium. In contrast, expression of a subset of *OPT* genes was clearly induced when the cells were grown in YCB-BSA medium (Fig. 4A and B). The strains carrying the P_{OPT3} -*GFP* fusion showed the strongest fluorescence, followed by the *OPT1* reporter strains, and the *OPT5* gene was also expressed at low, but detectable levels. Fluorescence of these cells was detectable about 6 h after transfer from YPD to YCB-BSA medium, increased in the following hours, and remained detectable after overnight growth. These results demonstrated that the individual members of the *OPT* gene family are differentially expressed. That the *OPT2* promoter was not detectably

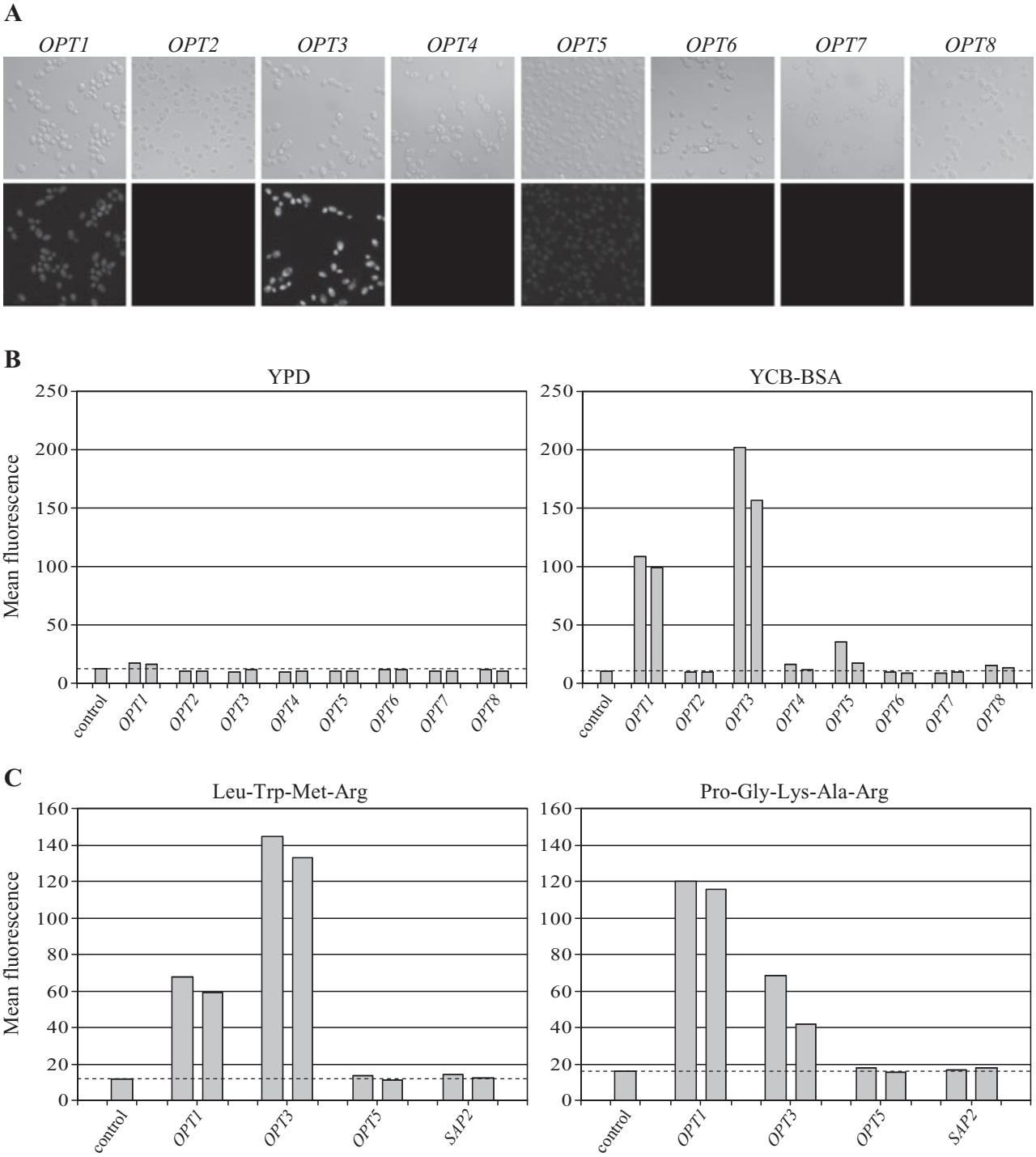

Fig. 4. Expression of the *GFP* reporter gene from the promoter of the indicated *OPT* genes.
A. Cells from an overnight culture of each reporter strain in YPD medium were diluted 1:100 in YCB-BSA and incubated overnight at 30°C. Shown are corresponding phase contrast (top) and fluorescence micrographs (bottom) of the cells. Only one of the two independently constructed reporter strains is shown for each *OPT* gene. Cells grown in YPD medium did not exhibit detectable fluorescence and are not shown.
B. Cells from an overnight culture of each reporter strain in YPD medium were diluted 1:100 in fresh YPD (left) or YCB-BSA medium (right), incubated for 8 h at 30°C, and analysed by flow cytometry. The mean fluorescence of each population of cells is shown. For each *OPT* gene, the first columns show the results obtained with the A series and the second columns show the results obtained with the B series of reporter strains (see Table S1). The parental strain SC5314, which does not contain *GFP*, was used as a negative control.
C. Cells from overnight cultures of the indicated reporter strains in YPD medium were diluted 1:100 in YCB medium containing the tetrapeptide Leu-Trp-Met-Arg (left) or the pentapeptide Pro-Gly-Lys-Ala-Arg (right) as the sole nitrogen source, incubated for 10 h at 30°C, and analysed by flow cytometry as described in (B).

induced in YCB-BSA medium although reinsertion of a single *OPT2* copy under control of its own promoter rescued the growth defect of *opt123*Δ mutants (see Fig. 3) was surprising, but may be explained by post-transcriptional control mechanisms. The weak (*OPT5*) or non-detectable expression (*OPT4* and *OPT6-OPT8*) of the other *OPT* genes is in line with their inability to compensate for the loss of *OPT1-OPT3* in the *opt123*Δ mutants.

We also tested whether the genes that were detectably expressed in YCB-BSA medium (*OPT1*, *OPT3*, *OPT5*, *SAP2*) were also induced by peptides. For this purpose, the corresponding *GFP* reporter strains were grown in YCB medium in which BSA was replaced by commercially available peptides of defined length and sequence (Fig. 4C). Both the tetrapetide Leu-Trp-Met-Arg and the pentapeptide Pro-Gly-Lys-Ala-Arg induced *OPT1* and *OPT3* expression. In contrast, neither the *OPT5* nor the *SAP2* promoter were detectably induced by the peptides used in these assays.

OPT4 *and* OPT5 *encode functional peptide transporters*

To investigate whether the putative oligopeptide transporters encoded by the *OPT4-OPT8* genes would allow growth on BSA when expressed from an active promoter, we placed all eight *OPT* genes under control of the *ADH1* promoter and integrated the fusions into an *opt123*Δ mutant. For each gene, two independent transformants were tested for growth in YCB-BSA medium (Fig. 5). As expected, expression of the *OPT1-OPT3* genes from the *ADH1* promoter restored growth of the *opt123*Δ mutant, although the strains grew less well than the wild-type strain SC5314 and required longer incubation to reach the same optical density. Interestingly, forced expression of the *OPT4* or *OPT5* gene from the *ADH1* promoter also partially rescued the growth defect of the *opt123*Δ mutants, indicating that these two genes encode functional oligopeptide transporters (Fig. 5). After prolonged incubation, the *opt123*Δ mutants also showed increased growth as compared with a *sap2*Δ mutant, which is unable to degrade the BSA in the medium (Fig. 5, bottom panel, see also below). In contrast to the *OPT1-OPT5* genes, expression of the other *OPT* genes from the *ADH1* promoter did not further enhance growth of the *opt123*Δ mutant.

The results presented above suggested that the residual slow growth of the *opt123*Δ mutants in YCB-BSA is caused by oligopeptide uptake mediated by Opt4p and Opt5p. To evaluate the contribution of these transporters to growth of *C. albicans* in YCB-BSA medium, we constructed additional mutants lacking *OPT1-OPT4* or *OPT1-OPT5*. For this purpose, the two *OPT4* alleles were sequentially deleted in both *opt123*Δ mutants to obtain

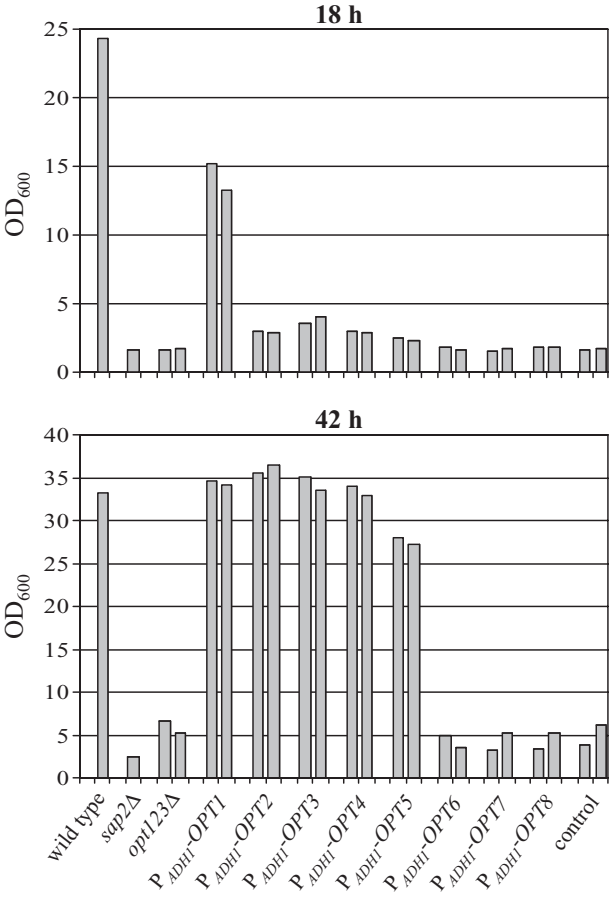

Fig. 5. Growth of the wild-type strain SC5314, a *sap2*Δ mutant, *opt123*Δ mutants and *opt123*Δ mutants expressing individual *OPT* genes under control of the *ADH1* promoter or carrying a control construct in YCB-BSA medium. Cells from an overnight culture in SD medium were diluted 1:100 in YCB-BSA and incubated at 30°C. Shown are the optical densities of the cultures after 18 h and 42 h of growth. The following strains were used: SC5314 (wild type), SAP2MS4 (*sap2*Δ), OPT123M4A and B (*opt123*Δ), OPT123ME1A and B (P_{ADH1}-*OPT1*), OPT123ME2A and B (P_{ADH1}-*OPT2*), OPT123ME3A and B (P_{ADH1}-*OPT3*), OPT123ME4A and B (P_{ADH1}-*OPT4*), OPT123ME5A and B (P_{ADH1}-*OPT5*), OPT123ME6A and B (P_{ADH1}-*OPT6*), OPT123ME7A and B (P_{ADH1}-*OPT7*), OPT123ME8A and B (P_{ADH1}-*OPT8*), OPT123MC1A and B (control).

*opt1234*Δ quadruple mutants. These mutants were then used to create two independent *opt12345*Δ quintuple mutants. The construction of these strains was carefully controlled by Southern hybridization with upstream and downstream probes from all previously inactivated *OPT* genes, taking advantage of restriction site polymorphisms that allowed discrimination of the two alleles of each gene, to exclude as far as possible undesired recombination events between *FRT* sites remaining in the disrupted gene loci after each round of insertion and excision of the *SAT1* flipper cassette (see Fig. 6).

The *opt1234*Δ and *opt12345*Δ mutants and control strains were grown for 3 days in YCB-BSA medium, a time

when the cultures of the *opt123*Δ mutants had reached the same density as that of the wild-type strain SC5314 (although the *opt123*Δ mutants grew much more slowly). Growth of the *opt1234*Δ quadruple mutants was clearly reduced as compared with the *opt123*Δ triple mutants and a further growth reduction was seen in the *opt12345*Δ quintuple mutants (Fig. 7). These results confirmed that Opt4p and Opt5p contribute to the ability of *C. albicans* to

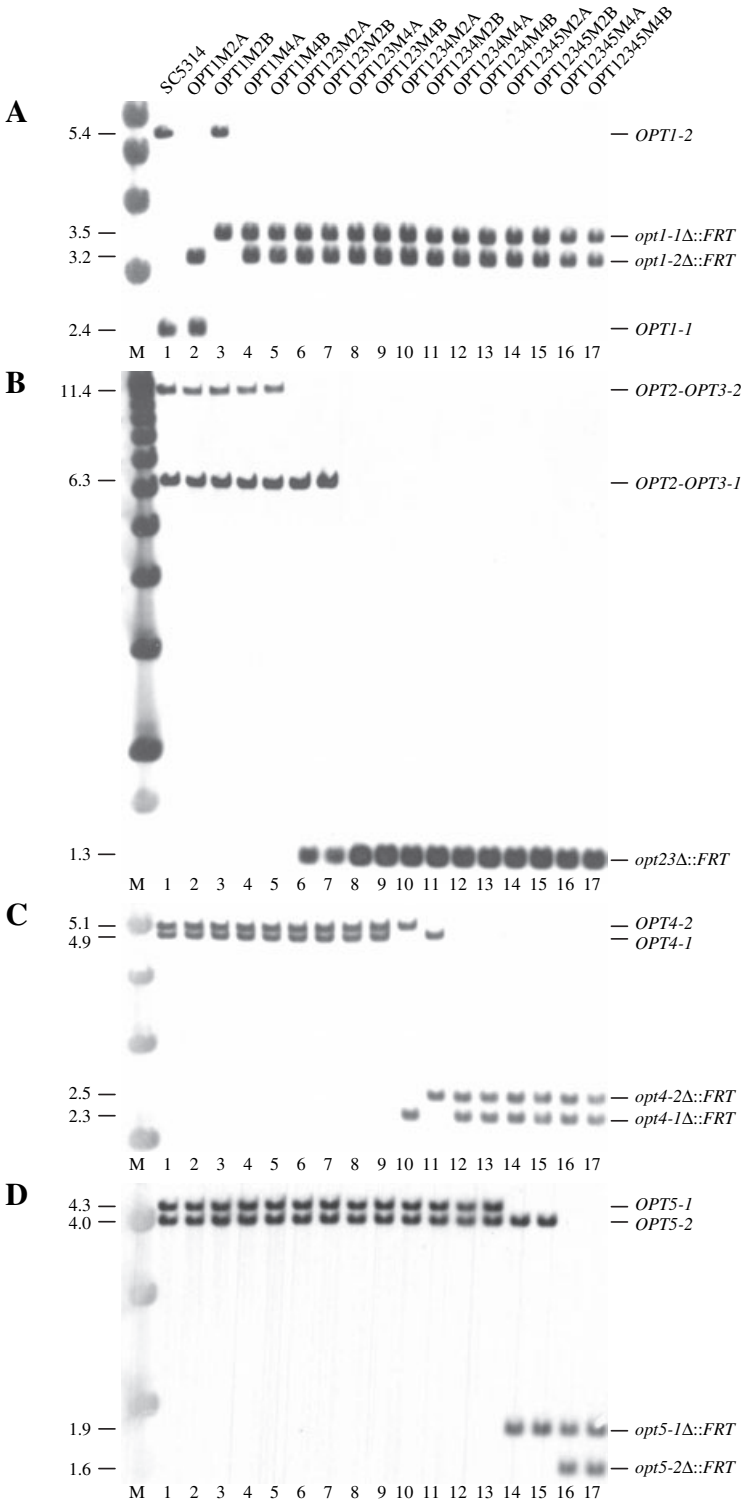

Fig. 6. Southern hybridization of genomic DNA of the wild-type strain SC5314 (lane 1) and heterozygous and homozygous *opt1*Δ single mutants (lanes 2–5), *opt123*Δ triple mutants (lanes 6–9), *opt1234*Δ quadruple mutants (lanes 10–13), and *opt12345*Δ quintuple mutants (lanes 14–17) with probes from the (A) *OPT1,* (B) *OPT2,* (C) *OPT4* and (D) *OPT5* upstream regions. An aliquot of labelled molecular marker was included in each hybridization to visualize the size marker (lane M). All strains were also verified with probes from the *OPT1*, *OPT3*, *OPT4* and *OPT5* downstream regions (not shown). The genomic DNA was digested with restriction enzymes that allowed discrimination of the two alleles of each gene, HindIII for *OPT1* (A), SpeI for *OPT2-OPT3* (B), EcoRI for *OPT4* (C), NsiI/XhoI for *OPT5* (D). For simplicity, only the heterozygous and homozygous mutants in which the *SAT1* flipper cassette was excised again after each round of gene deletion are shown. The sizes of the hybridizing fragments (in kb) are given on the left side of the blots and the corresponding *OPT* alleles are indicated on the right. Note that the polymorphic SpeI site in the *OPT2-OPT3* intergenic region is lost after deletion of these genes.

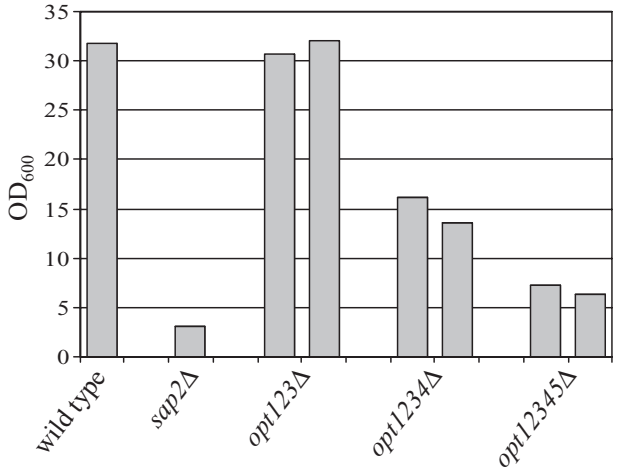

Fig. 7. Growth of the wild-type strain SC5314, a *sap2*Δ mutant, *opt123*Δ triple mutants, *opt1234*Δ quadruple mutants and *opt12345*Δ quintuple mutants in YCB-BSA medium. Cells from an overnight culture in SD medium were diluted 1:100 in YCB-BSA and incubated for 77 h at 30°C. The following strains were used: SC5314 (wild type), SAP2MS4 (*sap2*Δ), OPT123M4A and B (*opt123*Δ), OPT1234M4A and B (*opt1234*Δ), OPT12345M4A and B (*opt12345*Δ).

use proteins as a nitrogen source and that at least *OPT1-OPT5* encode functional oligopeptide transporters. Even the *opt12345*Δ quintuple mutants still grew somewhat better than a *sap2*Δ mutant, suggesting that they have retained some capacity to use proteolytic degradation products from BSA for growth. This weak residual growth may be due to less efficient uptake of oligopeptides by the remaining oligopeptide transporters, Opt6p-Opt8p, which may have more specialized functions, uptake of shorter peptides (two to three amino acids in length) by di-/tripeptide transporters, or uptake of amino acids, which have also been suggested to be produced in minor amounts by Sap activity (Martinez and Ljungdahl, 2005), by amino acid permeases.

Individual oligopeptide transporters differ in their substrate preferences

The evolution of a gene family encoding oligopeptide transporters in *C. albicans* suggested that, in addition to their differential regulation, the individual members of the family may have functionally diversified. We tested this possibility by growing *opt123*Δ mutants expressing specific *OPT* genes under control of the *ADH1* promoter in media containing tetrapeptides of defined sequence, Leu-Ser-Lys-Leu (LSKL) or Leu-Trp-Met-Arg (LWMR), as the sole nitrogen source (Fig. 8). It should be noted that, although all extra copies of the *OPT* genes were expressed from the same promoter, this does not necessarily mean that the different proteins are expressed at the same level and differences in growth between strains may also be caused by differences in the amount of the expressed transporter. Nevertheless, the experiments shown in Fig. 8 support the idea that the *C. albicans* oligopeptide transporters differ in their substrate preferences. For example, while the LWMR peptide was clearly a better substrate than LSKL for strains expressing *OPT3*, *OPT4* or *OPT5*, the reverse was true for strains expressing *OPT2*, which grew only on LSKL (albeit not very efficiently) but not at all on LWMR. Proteinase activity was not required for *C. albicans* to grow on these peptides, because a *sap2*Δ mutant grew as well as the wild-type strain SC5314. Like in YCB-BSA, strains expressing *OPT6*, *OPT7* or *OPT8* did not grow on the tested tetrapeptides and could not be distinguished from the parental triple mutants or strains carrying a control construct. Interestingly, these experiments also suggested that the allelic differences observed in specific *OPT* genes may have functional consequences. The strain expressing the *OPT3-1* allele reproducibly grew better than the strain expressing the *OPT3-2* allele on the LSKL peptide (see Fig. 8, upper left panel), and this result was confirmed with two additional, independent transformants carrying either of the two alleles.

Oligopeptide transporters can transport peptides longer than five amino acids

In contrast to the di-/tripeptide transporters, which use peptides of only two or three amino acids in length, oligopeptide transporters are usually described as transporters that take up tetra- and pentapeptides (Hauser *et al*., 2001). We tested whether the *C. albicans* oligopeptide transporters also transport longer peptides by using commercially available peptides of five, six, seven or eight amino acids in length as the sole nitrogen source for growth. While the wild-type strain SC5314 was able to grow on the pentapeptides RPPGF (Fig. 9A) and PGKAR (Fig. 9B), the heptapeptides RPPGFSP (Fig. 9C) and (weakly) DRVYIHP (Fig. 9D), and the octapeptides RPPGFSPL (Fig. 9E) and DRVYIHPF (Fig. 9F), the *opt123*Δ triple mutants grew much worse or not at all on these peptides. Except for DRVYIHP, the peptides did not need to be degraded before uptake, because the addition of pepstatin A, an inhibitor of the Saps that are thought to account for all extracellular proteolytic activity of *C. albicans* (Naglik *et al*., 2003), did not affect growth of the wild-type strain SC5314 on these peptides (whereas it completely blocked growth in YCB-BSA), and a *sap2*Δ mutant grew as well as the wild-type strain (Fig. 9). Two hexapeptides, 6xArg and the tropoelastin fragment PGAIPG, were also tested, but the wild-type strain SC5314 was unable to grow on these peptides (data not shown). These results provide strong evidence that the

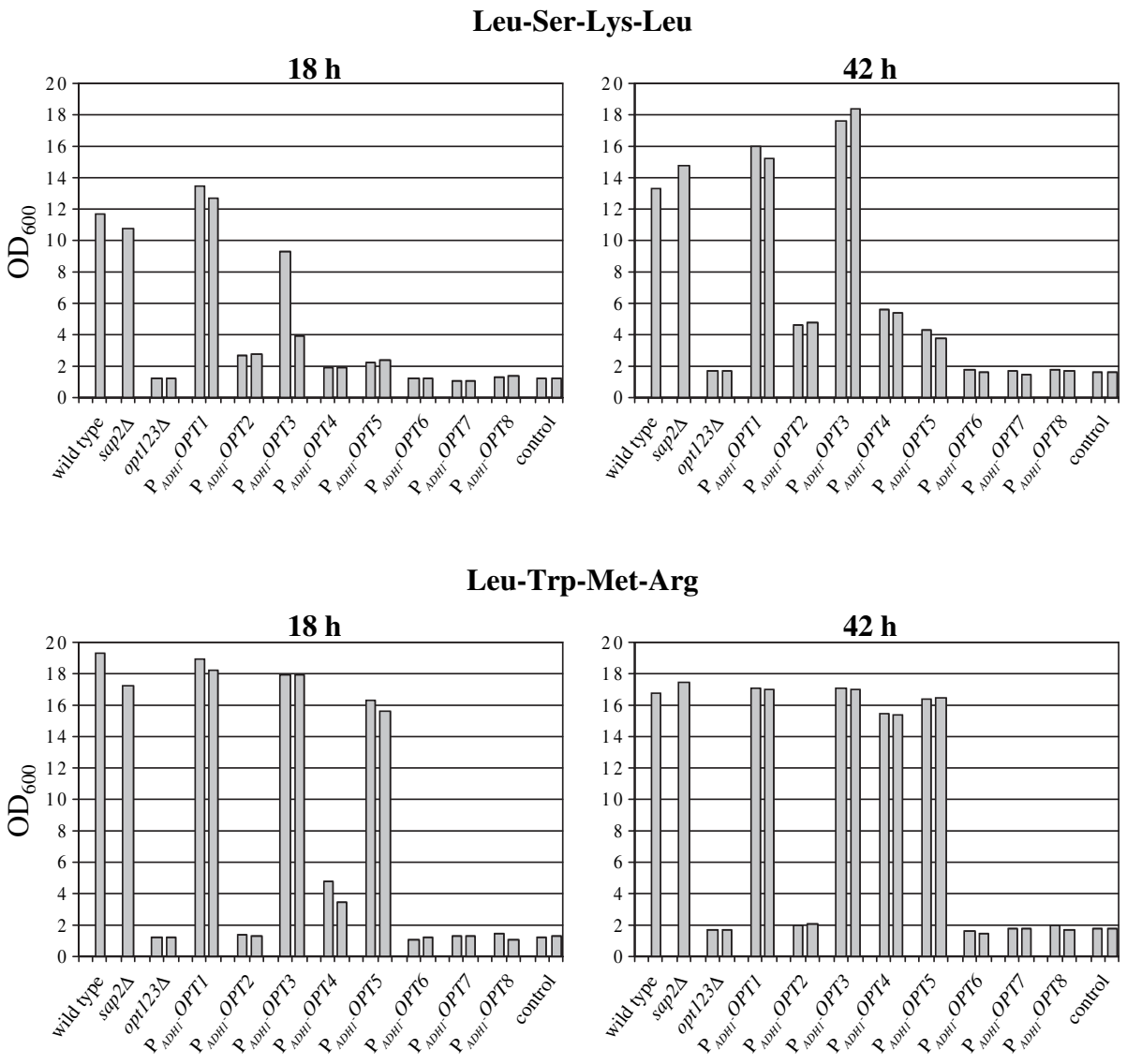

Fig. 8. Growth of the wild-type strain SC5314, a *sap2*Δ mutant, *opt123*Δ mutants and *opt123*Δ mutants expressing individual *OPT* genes under control of the *ADH1* promoter or carrying a control construct in YCB medium containing the indicated tetrapeptides as the sole nitrogen source. Cells from an overnight culture in SD medium were diluted 1:100 in the test medium and incubated at 30°C. Shown are the optical densities of the cultures after 18 h and 42 h of growth. For strain names see legend to Fig. 5.

C. albicans oligopeptide transporters can also transport longer peptides, at least up to eight amino acids in length.

Discussion

In the past years, considerable progress has been made in elucidating virulence mechanisms of *C. albicans* and other human pathogenic fungi, for example, by identifying adhesins, secreted hydrolytic enzymes, and other virulence factors, all of which contribute to a successful infection (Calderone and Fonzi, 2001). A complete understanding of fungal pathogenicity, however, also requires insights into how these organisms meet the nutritional requirements within their host. *C. albicans* colonizes and infects virtually all human body sites (Odds, 1988) and therefore must be able to utilize the carbon and nitrogen sources that are available in different host niches. One long-known characteristic of *C. albicans* is its ability to secrete aspartic proteinases which, among other functions, allow the fungus to utilize proteins as a nitrogen source (Staib, 1965). As at least Sap2p has a broad substrate specificity (Naglik *et al.*, 2003), the breakdown products of protein degradation are peptides of various lengths and sequences that must be taken up

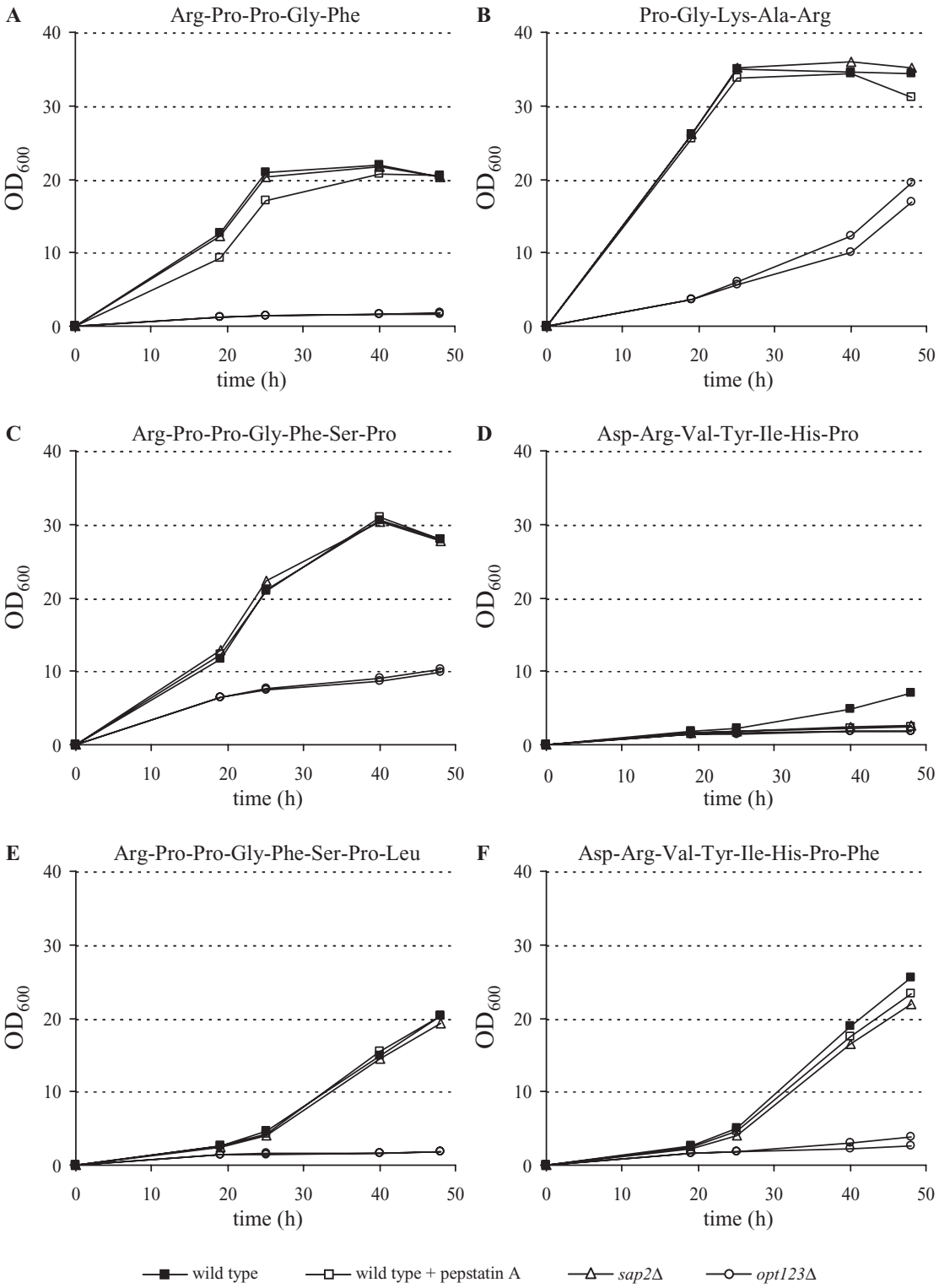

Fig. 9. Growth of the wild-type strain SC5314 (filled squares), the *sap2*Δ mutant SAP2MS4 (open triangles) and the *opt123*Δ mutants OPT123M4A and B (open circles) in a medium containing the indicated peptides as the sole nitrogen source. Growth of the wild-type strain SC5314 on these peptides was also tested in the presence of 5 μg ml^{-1} pepstatin A (open squares) to inhibit extracellular proteolytic activity. Cells from an overnight culture in SD medium were diluted 1:100 in the test medium and incubated at 30°C. The optical densities of the cultures were measured at the indicated times.

into the cell to support growth in the absence of other nitrogen sources. Of the two types of peptide transport systems that have been described in yeasts, the di-/tripeptide transporters and the oligopeptide transporters (Hauser *et al*., 2001), we focused on the latter, because we hypothesized that they might act not only as transporters of the peptides generated from proteolytic digestion of proteins but also as sensors of longer extracellular peptides, which have been suggested to be inducers of proteinase expression (Lerner and Goldman, 1993). We identified a large family of *OPT* genes and it seemed possible that one or several members of the gene family might have a sensing function, as described for other members of yeast transporter families (Özcan *et al*., 1996; Didion *et al*., 1998; Lorenz and Heitman, 1998; Iraqui *et al*., 1999; Klasson *et al*., 1999; Donaton *et al*., 2003). Based on the previous finding that expression of the *SAP2* gene is required for the ability of *C. albicans* to grow in a medium containing protein as a sole nitrogen source (Hube *et al*., 1994; Staib *et al*., 2002), we expected that inactivation of such a sensor would abolish *SAP2* expression and render the corresponding mutant unable to grow under these conditions. Although no single *OPT* gene was required for growth of *C. albicans* on BSA, a mutant lacking *OPT1-OPT3* exhibited a severe growth defect under these conditions, which was exacerbated by the additional deletion of *OPT4* and *OPT5*. However, this growth defect was not due to an inability of the mutants to induce proteinase expression, as the *SAP2* promoter was normally induced even in the *opt12345*Δ quintuple mutants (compare left and right panels in Fig. 3B). Although we cannot exclude the (unlikely) possibility that all Opt proteins have a redundant function in oligopeptide sensing, our results suggest that the Opt proteins do not function as peptide sensors and are not required for the induction of *SAP2* expression. Indeed, it has recently been suggested that low concentrations of amino acids may also be released from proteins by Sap activity and induce *SAP2* expression (Martinez and Ljungdahl, 2005). The presence of extracellular amino acids is sensed by the SPS sensor in the cytoplasmic membrane and the signal is transmitted by proteolytic activation of the Stp1p transcription factor, which then migrates into the nucleus and induces expression of the *SAP2* proteinase, the di-/tripeptide transporter *PTR2*, and the oligopeptide transporters *OPT1* and *OPT3* (Martinez and Ljungdahl, 2005). We also found that *OPT1*, *OPT3* and *OPT5* were co-ordinately induced with *SAP2* in YCB-BSA medium. Therefore, instead of peptides it may in fact be micromolar concentrations of extracellular amino acids that are sensed by *C. albicans* and which, via the SPS sensor, signal the presence of proteins in the environment to induce the expression of the components that are necessary for their utilization as a nitrogen source, the secreted aspartic proteinase Sap2p and oligopeptide transporters. Although *OPT1* and *OPT3* expression was clearly induced by the LWMR and PGKAR peptides (Fig. 4C), we cannot rule out the possibility that trace amounts of amino acids may have been present in the peptide preparations used in these assays.

An interesting finding of our study was that oligopeptide transporters of the *OPT* family transport peptides longer than five amino acids, as shown by the ability of both the wild-type strain SC5314 and a *sap2*Δ mutant to grow on peptides of seven or eight amino acids in length, apparently without the necessity to degrade these peptides before uptake, and the loss of this capacity in the *opt123*Δ mutants. Therefore, the substrate range of this type of transporters seems to be considerably broader than known from previous work (Hauser *et al*., 2001). In addition to the oligopeptide transporters encoded by the *OPT* gene family, *C. albicans* also possesses two genes encoding di-/tripeptide transporters, *PTR2* (Basrai *et al*., 1995) and a second gene (orf 19.6937) that is even more similar to *PTR2* from *S. cerevisiae* and which we have termed *PTR22* (R. Franz, O. Reuß, and J. Morchhäuser, unpubl. results). The inability of the *opt123*Δ mutants and, even more, the *opt12345*Δ mutants to efficiently take up peptides resulting from the degradation of BSA suggests that proteolytic digestion of BSA produces mainly longer peptides that cannot be transported by the di-/tripeptide transporters.

In contrast to the normally non-pathogenic yeast *S. cerevisiae*, which contains three genes (*OPT1*, *OPT2*, *YGL114w*) encoding confirmed or putative oligopeptide transporters (Hauser *et al*., 2001), *C. albicans* possesses a much larger family of eight genes encoding oligopeptide transporters. This is similar to the situation in the plant *Arabidopsis thaliana* where nine genes encoding oligopeptide transporters have been found. The physiological roles of the *A. thaliana* oligopeptide transporters have not been resolved, but they are believed to play different functional roles because they exhibit a distinct, tissue-specific expression pattern (Koh *et al*., 2002). We found that at least *OPT1-OPT5* of *C. albicans* encode functional oligopeptide transporters that mediate the uptake of BSA-derived peptides. No peptide uptake activity could be demonstrated for the *OPT6-OPT8* genes in these assays, but they may encode oligopeptide transporters with a narrow substrate specificity and have more specific functions. Our results show that the transporters encoded by the various *OPT* genes and even by polymorphic alleles of the same gene exhibit differences in their substrate preferences, ensuring that a broad variety of peptides can be efficiently taken up into the cell. Together with the Saps, which are also encoded by a large gene family whose members are

differentially regulated, the evolution of the *OPT* gene family may therefore have contributed to an optimal adaptation of *C. albicans* to its mammalian host by enabling the fungus to grow on proteins when sufficient amounts of other nitrogen sources are lacking. Preliminary experiments showed that the *opt12345*Δ quintuple mutants were as virulent as the wild-type strain SC5314 in a mouse model of disseminated candidiasis (K. Schröppel, pers. comm.), suggesting that oligopeptide transporters are not required for a successful infection via the bloodstream. Therefore, one can assume that in the host niches encountered in this infection model, *C. albicans* has access to other nitrogen sources and does not depend on the uptake of oligopeptides for growth. In fact, it has been argued that *C. albicans* utilizes amino acids, which are abundant in blood, as nitrogen source during systemic infections (Martinez and Ljungdahl, 2004). Nevertheless, one can hypothesize that *C. albicans* colonizes and infects other host niches in which the ability to degrade proteins and use the resulting peptides as a nitrogen source facilitates growth of the fungus. The identification of such host niches, in which the expression of oligopeptide transporters is induced and in which these transporters are required for a successful infection, is a goal for future studies and will contribute to a better understanding of the adaptation mechanisms of *C. albicans* to a mammalian host.

Experimental procedures

Strains and growth conditions

Candida albicans strains used in this study are listed in Table S1. The strains were routinely grown in YPD medium (10 g yeast extract, 20 g peptone, 20 g glucose per litre) at 30°C. In some experiments, SD medium [6.7 g yeast nitrogen base without amino acids (YNB; BIO 101, Vista, CA), 20 g glucose] was used. To prepare solid media, 1.5% agar was added before autoclaving. For selection of nourseothricin-resistant (Nou^R) transformants, 200 μg ml^{-1} of nourseothricin (Werner Bioagents, Jena, Germany) was added to YPD agar plates. To obtain nourseothricin-sensitive (Nou^S) derivatives in which the *SAT1* flipper was excised by FLP-mediated recombination, transformants were grown for 6 h in YPM medium (10 g yeast extract, 20 g peptone, 20 g maltose per litre) without selective pressure to induce the *MAL2* promoter. One hundred to 200 cells were then spread on YPD plates containing 20 μg ml^{-1} of nourseothricin and grown for 2 days at 30°C. Nou^S clones were identified by their small colony size and confirmed by restreaking on YPD plates containing 100 μg ml^{-1} of nourseothricin as described previously (Reuß *et al.*, 2004). To test for growth on BSA as the sole nitrogen source, strains were grown at 30°C in YCB-BSA medium (23.4 g of yeast carbon base, 4 g of bovine serum albumin, pH 5.0). Expression of *SAP2* in *opt* mutants was monitored in YCB-BSA-YE medium (YCB-BSA + 0.2% yeast extract), which also induces *SAP2* expression (Staib *et al.*, 2002) and allowed growth of *opt123*Δ and *opt12345*Δ mutants. In growth assays with peptides, the following oligopeptides (Bachem, Weil am Rhein, Germany) were used at a concentration of 4 mg ml^{-1} instead of BSA: LSKL, LWMR, PGKAR, RRRRRR, the tropoelastin fragment PGAIPG, RPPGF (Bradykinin [1–5]), RPPGFSP (Bradykinin [1–7]), RPPGFSPL ([Des-Arg^9,Leu^8]-Bradykinin), DRVYIHP (Angiotensin I/II [1–7]) and DRVYIHPF (Angiotensin II). The aspartic proteinase inhibitor pepstatin A (Sigma, Deisenhofen, Germany) was added at a concentration of 5 μg ml^{-1} when required.

Plasmid constructions

Cloning of OPT genes. The *OPT2* gene including upstream and downstream flanking sequences was amplified from genomic DNA of strain SC5314 by polymerase chain reaction (PCR) with the primer pair OPT14 (5′-CATAAATT GTGTGGGCCCATTTATGGCG-3′) and OPT23 (5′-AAAAA CTCGAGGTGACACAAACGCGGC-3′). The PCR product was digested at the introduced ApaI and XhoI sites (underlined) and cloned in pBluescript (Stratagene, La Jolla, CA) to yield pOPT2. Similarly, the *OPT3* gene and flanking sequences were amplified with the primers OPT24 (5′-ATAG CATGTGTGTCGACCGCCGTGGGTCTTGG-3′) and OPT25 (5′-ATATAGGATC**CTCGAG**TGCAGCCAGGAAAGTAATGG-3′), digested at the introduced SalI and BamHI sites, and cloned in pBluescript (an additionally introduced XhoI site is marked in bold). The resulting plasmids pOPT3A and pOPT3B contained the *OPT3-1* and *OPT3-2* alleles respectively. The *OPT4* gene and flanking sequences were amplified with the primers OPT41 (5′-ATAGGTACTAAGA GAGCTCACTTGGGATATAG-3′) and OPT44 (5′-TAAAA GGGCCCGCGGAATCTTGAGATTGTTGATGG-3′), digested at the introduced SacI and ApaI sites, and cloned in pBluescript. The resulting plasmids pOPT4A and pOPT4B contained the *OPT4-1* and *OPT4-2* alleles respectively. For the other *OPT* genes, only the coding region was amplified by PCR and used to express it from the *ADH1* promoter (see below). Sequence differences between the two alleles of an *OPT* gene were confirmed by amplifying the allele that did not match the *C. albicans* genome sequence a second time in an independent PCR reaction.

OPT gene deletion constructs. For simultaneous deletion of the tandemly arranged *OPT2* and *OPT3* genes, the *OPT2* upstream region was PCR-amplified from genomic DNA of strain SC5314 with the primers OPT14 and OPT15 (5′-CATATCTCGAGTAAATTTTGCAAAAGGATGTTG-3′) and the *OPT3* downstream region was amplified with the primer pair OPT16 (5′-GGTTATTTTGGACCCGCGGTTGGTCACT TC CC-3′) and OPT17 (5′-TTGTTTAAACAGAGCTCTTTGA TAAGGGACTTGG-3′). The PCR products were digested at the introduced ApaI/XhoI and SacII/SacI sites respectively, and substituted for the flanking *OPT1* regions in the previously described plasmid pOPT1M3 (Reuß *et al.*, 2004), resulting in plasmid pOPT23M2 in which the *SAT1* flipper is flanked by *OPT2* upstream and *OPT3* downstream sequences (see Fig. 2A). To delete the other *OPT* genes, similar constructs containing flanking upstream and downstream sequences of individual *OPT* genes were made using the following primer pairs: OPT41 and OPT42 (5′-AATTC

GTCGCTGCATTTTTATCCTCC-3′) to amplify *OPT4* upstream sequences and OPT43 (5′-GTTGGATGC CACTC GAGATGCACCAGATG-3′) and OPT44 to amplify *OPT4* downstream sequences; OPT52 (5′-GCATATATTGGTAAC CCGAGCTCATTAGCTT-3′) and OPT53 (5′-CAGGAAGAG CAGGCCGCGGTTTCTCTGTAG-3′) to amplify *OPT5* upstream sequences and OPT54 (5′-GGATATTTGAATG CTCGAGTACAGGCACCTG-3′) and OPT55 (5′-CTCTAATC TTACGGGCCCGGTTTAGTGATT-3′) to amplify *OPT5* downstream sequences; OPT69 (5′-GCAGTCTCAACGAGCTCT ATTTAGACGAGG-3′) and OPT600 (5′-GTATTCGTGTCCG CGGTTCGTCATTAG-3′) to amplify *OPT6* upstream sequences and OPT601 (5′-CGATACTGCTCGAGATGGT TATATTGG-3′) and OPT602 (5′-CTTGAACAATTGGGCCC TATTGCTATAGTTAATC-3′) to amplify *OPT6* downstream sequences; OPT76 (5′-GATCCACCTTGAGCTCAAACTTC AATG-3′) and OPT77 (5′-CAAATTCAGCCTCCGCGGACAT TATGATTGTG-3′) to amplify *OPT7* upstream sequences and OPT78 (5′-GCAACAATGGCTCGAGTGTGGAGAGATG-3′) and OPT79 (5′-CCCATTTCTCAGGGCCCGTCTCCACGA AG-3′) to amplify *OPT7* downstream sequences; OPT86 (5′-CGATTACTGTAGAGCTCCCAATTCGG-3′) and OPT87 (5′-GAGAGCCGTATTCCGCGGTTATTTGATATCGTC-3′) to amplify *OPT8* upstream sequences and OPT88 (5′-CACTA TGCTTTTAACTCGAGTGGGTGTTAAGC-3′) and OPT89 (5′-CAGATTAGTTGTGGGCCCAGGTATTCTC-3′) to amplify *OPT8* downstream sequences. The *OPT5*, *OPT6*, *OPT7* and *OPT8* flanking sequences were used to replace the *OPT2* and *OPT3* flanking sequences in pOPT23M2, resulting in plasmids pOPT5M2, pOPT6M2, pOPT7M2 and pOPT8M2. For deletion of the *OPT4* gene, we used a modified version of the *SAT1* flipper containing a longer region (about 2 kb) of the *MAL2* promoter, which was amplified from SC5314 genomic DNA with the primers MAL3 (5′-CCCATGTTT GGATCCTATCCTTGGTGCAC-3′) and MAL2 (5′-GTTCAC TCATTGTCGACGATTATTAGTTAAACC-3′). The PCR product was digested at the introduced BamHI and SalI sites and used to replace the shorter *MAL2* promoter in pSFS2 (Reuß *et al*., 2004), generating pSFS3. The *OPT4* flanking sequences were cloned on both sides of this modified *SAT1* flipper cassette to result in pOPT4M2. However, no difference in the performance of this cassette as compared with the original *SAT1* flipper were observed [in both cases the *MAL2* promoter was leaky (Reuß *et al*., 2004; and data not shown)].

Plasmids for reinsertion of the OPT2 and OPT3 genes. For reinsertion of the *OPT2* gene at its original locus in the *opt123*Δ triple mutants, the ApaI–XhoI fragment from pOPT2 was substituted for the *OPT2* upstream region in plasmid pOPT23M2, thereby generating pOPT23K2 (see Fig. 2B, top). To reinsert the *OPT3* gene at its original locus in the *opt123*Δ triple mutants, the ApaI–XhoI fragment from pOPT23M2 containing the *OPT2* upstream region was first inserted in front of the *OPT3* gene in the ApaI/SalI-digested plasmids pOPT3A or pOPT3B to generate pOPT31A and pOPT31B respectively. The ApaI–XhoI fragments from these plasmids were then substituted for the *OPT2* upstream region in plasmid pOPT23M2 to generate pOPT23K3A and pOPT23K3B containing the *OPT3-1* and *OPT3-2* alleles respectively (see Fig. 2B, bottom).

Plasmids for expression of individual OPT genes from the ADH1 promoter. To express individual *OPT* genes from the *ADH1* promoter, we constructed an expression cassette containing unique sites to allow convenient cloning of ORFs and excision of the whole cassette from the vector backbone for genomic integration at the *ADH1* locus. A fragment from the *ADH1* downstream region was first amplified with the primers ADH8 (5′-GGTGCTGAACCAAACTGCAGTGAAGCTGAC-3′) and ADH11 (5′-GAACCTTTGATTTCCGCGGATTTGACA ACAGC-3′), digested at the introduced PstI and SacII sites, and cloned together with an XhoI–PstI fragment containing the *caSAT1* marker (Reuß *et al*., 2004) into the XhoI/SacII-digested vector pBluescript to produce pSAT2. An XhoI–SacI *caSAT1-3′ADH1* fragment from pSAT2 was then cloned behind the *ACT1* transcription termination sequence (T_{ACT1}) in the SalI/SacI-digested plasmid pCBF1M4 (Biswas *et al*., 2003) to generate pSAT3. An *ADH1* promoter fragment (P_{ADH1}) was amplified with the primers ADH13 (5′-ATATAGG TACC**GGGCCC**ACTACCACTGCAGCTGCATC-3′) and ADH14 (5′-TATATAGATCTATA**CTCGAG**TTTTTGTATTTGTT GTTGTTGTTGTATGAC-3′), digested at the introduced KpnI and BglII sites (underlined, additionally introduced ApaI and XhoI sites are marked in bold), and cloned in front of T_{ACT1} in the KpnI/BglII-digested pSAT3 to produce pADH1E1. The insert from this plasmid was cloned into the vector pBC SK (Stratagene) to generate pADH1E2. The coding sequences of the different *OPT* genes were amplified by PCR using the following primer pairs (start and stop codons are marked in bold): OPT100 (5′-AAGTGTCGACT**ATG**GACAAAATAAGGG CAG-3′) and OPT101 (5′-TGTGTTTCTTCAGATCT**TTA**CCAG GAAGATG-3′) for *OPT1*, OPT200 (5′-GCAAAATTTACGTC GACT**ATG**GTTTTAAAAG-3′) and OPT201 (5′-CTCTTCAA CATCAGATCTC**CTA**AGGGAAATG-3′) for *OPT2*, OPT300 (5′-CACAATAAAATACACTAGTCGACA**ATG**GATG-3′) and OPT301 (5′-CTTCAATAAAGATCTATGT**CTA**AGGGAAGTG-3′) for *OPT3*, OPT400 (5′-CAAACTCGAGAAA**ATG**GAGGA TAAAAATGC-3′) and OPT401 (5′-ATAATAAATCCATCTAGA TCTTA**TCA**AGGG-3′) for *OPT4*, OPT50 (5′-CTAAGTCGA CAATAAT**ATG**TCTACAGAGAAACC-3′) and OPT51 (5′-CAATACAACAGGATCCAA**TCA**TGGGAAATGTCC-3′) for *OPT5*, OPT60 (5′-CCCTCCCAAAGTCGACA**ATG**ACGAAA AAAGGACACG-3′) and OPT61 (5′-CAATAAGGGGGGAGAT CTGGAAACC**CTA**TGGG-3′) for *OPT6*, OPT70 (5′-CAATC ACGTCGACA**ATG**TCCAAAGAGGCTGAATTTG-3′) and OPT71 (5′-CACAATGATACTGAGATCT**TCA**ACTCATGGC-3′) for *OPT7*, and OPT80 (5′-CTGGCTATTATTCTCGAGA**ATG**A CAATAGAGACG-3′) and OPT81 (5′-GACTCTAATGTAAAC AGATCTAT**TTA**CCAATGC-3′) or OPT801 (5′-GACTCTAAT GGATCCGTTTCTAT**TTA**CCAATGC-3′) for the *OPT8-1* and *OPT8-2* alleles respectively. After digestion at the underlined restriction sites, the various *OPT* ORFs were then cloned as SalI–BglII, XhoI–BglII, SalI–BamHI or XhoI–BamHI fragments between the *ADH1* promoter and *ACT1* transcription termination sequence in the XhoI/BglII-digested pADH1E2 to generate pOPT1E1 (containing the *OPT1-1* allele), pOPT2E1 (containing the *OPT2* gene), pOPT3E1A and B (containing the *OPT3-1* and *OPT3-2* alleles respectively), pOPT4E1 (containing the *OPT4-2* allele), pOPT5E1 (containing the *OPT5* gene), pOPT6E1A and B (containing the *OPT6-1* and *OPT6-2* alleles respectively), pOPT7E1A and B (containing the *OPT7-1* and *OPT7-2* alleles respectively), and

pOPT8E1A and B (containing the *OPT8-1* and *OPT8-2* alleles respectively).

Plasmids containing the GFP *reporter gene under control of* OPT *promoters.* To place the *GFP* reporter gene under control of the *OPT1* promoter, an ApaI–SalI fragment containing *OPT1* upstream sequences was amplified with the primers OPT5 (5′-CAAGTCAACGTAGGGCCCAAATTG AGACC-3′) and OPT108 (5′-CATAGTCGACACTTATAATAA GTGTTAGG-3′) and cloned in front of the *GFP* gene in the ApaI/SalI-digested pNIM1 (Park and Morschhäuser, 2005) to produce pOPT1G21. A PstI–SacI fragment containing *OPT1* downstream sequences was then amplified with the primers OPT109 (5′-TGGGCCATCTTCCTGCAGAGCTGACGAAG-3′) and OPT110 (5′-CATAGTCGACACTTATAATAAGTGTT AGG-3′) and cloned behind the *caSAT1* selection marker in the PstI/SacI-digested pOPT1G21 to generate pOPT1G22. To express *GFP* from the promoters of the other *OPT* genes, the upstream and downstream regions of these genes were amplified with the following primer pairs: OPT14 + OPT202 (5′-CTTTTAAAACCATTGTCGACGTAAATTTTGCAAAAGG ATGTTG-3′) and OPT203 (5′-CGGTCATTTCCCTGCAGA GTGTCGATGTTGAAG-3′) + OPT204 (5′-CTATTTGCATCTT GAGCTCGAAATAAACC-3′) for *OPT2*, OPT302 (5′-GTCT AATATAGGGCCCGCAACATTTATGTA-3′) + OPT303 (5′-CATCCATTGTCGACTAGTGTATTTTATTGTGTTATGGTAC-3′) and OPT304 (5′-CTTCCCTTAGCTGCAGGTGTCATTA TTGAAG-3′) + OPT17 for *OPT3*, OPT404 (5′-GCATAACAT AGAGCGGGGCCCAAGTTATAGGTAC-3′) + OPT405 (5′-CC TCCATTGTCGACGTTTGTATGGTATCTTGTATGATATGG-3′) and OPT406 (5′-GGTAATTTCCCTGCAGAAGATGGAGAT GG-3′) + OPT407 (5′-GATGACGTTGACGAGCTCAGTGTTT AGTTTG-3′) for *OPT4*, OPT503 (5′-CTCAATATCATAAGCA GGGCCCTAGCTACGC-3′) + OPT504 (5′-GTAGACATTGT CGACGCAACTTAGACAATCCCTGTTATC-3′) and OPT505 (5′-GGACATTTCTGCAGATTGTTTATTGTTG-3′) + OPT506 (5′-CAAAACCCAACAGAGCTCCATCTCTAATC-3′) for *OPT5*, OPT603 (5′-CTCTATCAAGGGCCCTTCATTTAATTC AG-3′) + OPT604 (5′-CGTCATTGTCGACTTTGGGAGGGA CAATTCTAATAGAGGG-3′) and OPT605 (5′-GAAAAGG TCATTTCCTGCAGGGTTTCCGCCCC-3′) + OPT606 (5′-GCTATAGGAGCTCAACAAATATTAAATGG-3′) for *OPT6*, OPT700 (5′-CATTAGAGTAGGGCCCATATCCAAAAGCCCC-3′) + OPT701 (5′-GGACATTGTCGACGTGATTGTTTTGATAT CACAAATAATAA-3′) and OPT702 (5′-GATGTTTTGCCATGA CTGCAGCGTACAG-3′) + OPT703 (5′-GGAAGCAATTGGG AGCTCAGTTAGCAGG-3′) for *OPT7*, and OPT803 (5′-GTC CATGATGGGCCCTGATTCAATTCG-3′) + OPT804 (5′-CTA TTGTCATTGTCGACAATAATAGCCAGCTGGTAAGTAAT-3′) and OPT805 (5′-GTTAAGCATTGGTCTGCAGAAACGTTT AC-3′) + OPT806 (5′-CTAATATCAGAAAGTGAGCTCTAACA GG-3′) for *OPT8*. The amplified upstream and downstream sequences of individual *OPT* genes were substituted for the *OPT1* flanking sequences in plasmid pOPT1G22 to result in pOPT2G22, pOPT3G22, pOPT4G22, pOPT5G22, pOPT6G22, pOPT7G22 and pOPT8G22.

Other plasmids. To express *GFP* under control of the *SAP2* promoter in the *C. albicans* wild-type strain SC5314 and the *opt123*Δ triple and *opt12345*Δ quintuple mutants, an XbaI–SalI fragment from pSFL213 containing the promoter of the *SAP2-1* allele of strain SC5314 (Staib *et al*., 2000) was substituted for the *SAP2* promoter from a fosmid clone in the previously described plasmid pGFP41 (Morschhäuser *et al*., 1998) to generate pGFP55. The SalI–PstI fragment containing the *URA3* marker was then replaced by an XhoI–PstI fragment with the *caSAT1* marker to produce pSAP2G1. To express *SAP2* from a Tet-inducible promoter (P_{Tet}) (Park and Morschhäuser, 2005), the coding region of the *SAP2-1* allele was amplified with the primer pair SAP2ex1 (5′-ACCAGTC GACA**ATG**TTTTTAAAGAATATTTTCAT-3′) and SAP2ex2 (5′-ACCCCGGATCC**TTA**GGTCAAGGCAGAAATACTGGAAGC-3′), digested at the SalI and BamHI sites introduced in front of the start codon and behind the stop codon (bold) and cloned between P_{Tet} and T_{ACT1} in a SalI/BglII-digested derivative of pNIM1 (Park and Morschhäuser, 2005) to generate pSAP2ex4.

Candida albicans *transformation*

Candida albicans strains were transformed by electroporation (Köhler *et al*., 1997) with the following gel-purified linear DNA fragments: the ApaI–SacI fragments from pOPT23M2, pOPT4M2, pOPT5M2, pOPT6M2, pOPT7M2 and pOPT8M2 to inactivate the various members of the *OPT* gene family, the ApaI–SacI fragments from pOPT1K1 (Reuß *et al*., 2004), pOPT23K2 and pOPT23K3A and B to reinsert an intact copy of the *OPT1*, *OPT2* and *OPT3* genes into the *opt123*Δ triple mutants, the ApaI–SacII fragments from pOPT1E1, pOPT2E1, pOPT3E1A and B, pOPT4E1, pOPT5E1, pOPT6E1A, pOPT7E1A and B, pOPT8E1A and pADH1E2 to express individual *OPT* genes from the *ADH1* promoter or to insert a control construct without an *OPT* ORF in the same way, the ApaI–SacI fragments from pOPT1G22, pOPT2G22, pOPT3G22, pOPT4G22, pOPT5G22, pOPT6G22, pOPT7G22 and pOPT8G22 to express the *GFP* reporter gene under control of individual *OPT* promoters, an XbaI–HindIII fragment from pSAP2G1 to express *GFP* from the *SAP2-1* promoter, and the SacII–ApaI fragments from pSAP2ex4 and pNIM1 to express *SAP2* or *GFP* (as a control) from the Tet-inducible promoter. Selection of nourseothricin-resistant transformants was performed as described previously (Reuß *et al*., 2004). Single copy integration of each construct at the desired genomic locus was confirmed by Southern hybridization with specific probes.

Isolation of genomic DNA and Southern hybridization

Genomic DNA from *C. albicans* was isolated as described previously (Millon *et al*., 1994). Ten micrograms of DNA was digested with appropriate restriction enzymes, separated on a 1% agarose gel and, after ethidium bromide staining, transferred by vacuum blotting onto a nylon membrane and fixed by UV cross-linking. Southern hybridization with enhanced chemiluminescence (ECL)-labelled probes was performed with the ECL labelling and detection kit from Amersham (Braunschweig, Germany) according to the instructions of the manufacturer.

Fluorescence microscopy and flow cytometry

Fluorescence was detected using a Zeiss LSM 510 inverted

confocal laser scanning microscope equipped with a Zeiss Axiovert 100 microscope. Imaging scans were acquired with an Argon laser of 488 nm wavelength and corresponding filter settings for GFP and parallel transmission images. Observation was performed with a 63× immersion oil objective. FACS analysis was performed using a FACSCalibur cytometry system equipped with an argon laser emitting at 488 nm (Becton Dickinson, Heidelberg, Germany). Fluorescence was measured on the FL1 fluorescence channel equipped with a 530 nm bandpass filter. A total of 5×10^4 events were counted at the low flow rate. Fluorescence and forward scatter data were collected using logarithmic amplifiers. The mean fluorescence values were determined using CellQuest Pro (Becton Dickinson) software.

Analysis of Sap2p expression

Strains were grown for 2 days in YCB-BSA-YE medium and 18 μl of the supernatant was analysed on an SDS 12% polyacrylamide gel. Protein bands were visualized by staining with Colloidal Coomassie dye. The Precision Plus Protein Standards all blue size marker (Bio-Rad, München, Germany) was used to determine the molecular weight of the proteins.

Sequence analysis

The overall similarity of the proteins encoded by the *OPT* genes was determined using the program GAP from the Genetics Computer Group, Madison, WI.

Nucleotide sequence accession numbers

GenBank accession numbers for the *OPT1-1* (AY598824) and *OPT1-2* (AY598825) alleles have been provided previously (Reuß *et al*., 2004). The coding sequences of the remaining *OPT* alleles of *C. albicans* strain SC5314 described in the present work have been deposited in GenBank with the following accession numbers: DQ372671 (*OPT2*), DQ372672 (*OPT3-1*), DQ372673 (*OPT3-2*), DQ372674 (*OPT4-1*), DQ372675 (*OPT4-2*), DQ372676 (*OPT5*), DQ372677 (*OPT6-1*), DQ372678 (*OPT6-2*), DQ372679 (*OPT7-1*), DQ372680 (*OPT7-2*), DQ372681 (*OPT8-1*), DQ372682 (*OPT8-2*). Note that for the *OPT5-OPT8* genes the start and end of the coding sequence is derived from the primers used for amplification.

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References

Basrai, M.A., Lubkowitz, M.A., Perry, J.R., Miller, D., Krainer, E., Naider, F., and Becker, J.M. (1995) Cloning of a *Candida albicans* peptide transport gene. *Microbiology* **141:** 1147–1156.

Biswas, K., Rieger, K.J., and Morschhäuser, J. (2003) Functional characterization of *CaCBF1*, the *Candida albicans* homolog of centromere binding factor 1. *Gene* **323:** 43–55.

Calderone, R.A., and Fonzi, W.A. (2001) Virulence factors of *Candida albicans*. *Trends Microbiol* **9:** 327–335.

Colina, A.R., Aumont, F., Deslauriers, N., Belhumeur, P., and de Repentigny, L. (1996) Evidence for degradation of gastrointestinal mucin by *Candida albicans* secretory aspartyl proteinase. *Infect Immun* **64:** 4514–4519.

Didion, T., Regenberg, B., Jorgensen, M.U., Kielland-Brandt, M.C., and Andersen, H.A. (1998) The permease homologue Ssy1p controls the expression of amino acid and peptide transporter genes in *Saccharomyces cerevisiae*. *Mol Microbiol* **27:** 643–650.

Donaton, M.C., Holsbeeks, I., Lagatie, O., Van Zeebroeck, G., Crauwels, M., Winderickx, J., and Thevelein, J.M. (2003) The Gap1 general amino acid permease acts as an amino acid sensor for activation of protein kinase A targets in the yeast *Saccharomyces cerevisiae*. *Mol Microbiol* **50:** 911–929.

Hauser, M., Narita, V., Donhardt, A.M., Naider, F., and Becker, J.M. (2001) Multiplicity and regulation of genes encoding peptide transporters in *Saccharomyces cerevisiae*. *Mol Membr Biol* **18:** 105–112.

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

Hube, B., Sanglard, D., Odds, F.C., Hess, D., Monod, M., Schafer, W., *et al.* (1997) Disruption of each of the secreted aspartyl proteinase genes *SAP1*, *SAP2*, and *SAP3* of *Candida albicans* attenuates virulence. *Infect Immun* **65:** 3529–3538.

Iraqui, I., Vissers, S., Bernard, F., de Craene, J.O., Boles, E., Urrestarazu, A., and Andre, B. (1999) Amino acid signaling in *Saccharomyces cerevisiae*: a permease-like sensor of external amino acids and F-Box protein Grr1p are required for transcriptional induction of the *AGP1* gene, which encodes a broad-specificity amino acid permease. *Mol Cell Biol* **19:** 989–1001.

Jones, T., Federspiel, N.A., Chibana, H., Dungan, J., Kalman, S., Magee, B.B., *et al.* (2004) The diploid genome sequence of *Candida albicans*. *Proc Natl Acad Sci USA* **101:** 7329–7334.

Kaminishi, H., Miyaguchi, H., Tamaki, T., Suenaga, N., Hisamatsu, M., Mihashi, I., *et al.* (1995) Degradation of humoral host defense by *Candida albicans* proteinase. *Infect Immun* **63:** 984–988.

Klasson, H., Fink, G.R., and Ljungdahl, P.O. (1999) Ssy1p and Ptr3p are plasma membrane components of a yeast system that senses extracellular amino acids. *Mol Cell Biol* **19:** 5405–5416.

Koh, S., Wiles, A.M., Sharp, J.S., Naider, F.R., Becker, J.M., and Stacey, G. (2002) An oligopeptide transporter gene family in Arabidopsis. *Plant Physiol* **128:** 21–29.

Köhler, G.A., White, T.C., and Agabian, N. (1997) Overexpression of a cloned IMP dehydrogenase gene of *Candida albicans* confers resistance to the specific inhibitor mycophenolic acid. *J Bacteriol* **179:** 2331–2338.

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

Lorenz, M.C., and Heitman, J. (1998) The MEP2 ammonium permease regulates pseudohyphal differentiation in *Saccharomyces cerevisiae*. *EMBO J* **17:** 1236–1247.

Lubkowitz, M.A., Hauser, L., Breslav, M., Naider, F., and Becker, J.M. (1997) An oligopeptide transport gene from *Candida albicans*. *Microbiology* **143:** 387–396.

Martinez, P., and Ljungdahl, P.O. (2004) An ER packaging chaperone determines the amino acid uptake capacity and virulence of *Candida albicans*. *Mol Microbiol* **51:** 371–384.

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

Millon, L., Manteaux, A., Reboux, G., Drobacheff, C., Monod, M., Barale, T., and Michel-Briand, Y. (1994) Fluconazole-resistant recurrent oral candidiasis in human immunodeficiency virus-positive patients: persistence of *Candida albicans* strains with the same genotype. *J Clin Microbiol* **32:** 1115–1118.

Monod, M., Capoccia, S., Lechenne, B., Zaugg, C., Holdom, M., and Jousson, O. (2002) Secreted proteases from pathogenic fungi. *Int J Med Microbiol* **292:** 405–419.

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

Morschhäuser, J., Michel, S., and Hacker, J. (1998) Expression of a chromosomally integrated, single-copy *GFP* gene in *Candida albicans*, and its use as a reporter of gene regulation. *Mol Gen Genet* **257:** 412–420.

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

Odds, F.C. (1988) *Candida and Candidosis: A Review and Bibliography.* London: Bailliere Tindall.

Özcan, S., Dover, J., Rosenwald, A.G., Wölfl, S., and Johnston, M. (1996) Two glucose transporters in *Saccharomyces cerevisiae* are glucose sensors that generate a signal for induction of gene expression. *Proc Natl Acad Sci USA* **93:** 12428–12432.

Park, Y.N., and Morschhäuser, J. (2005) Tetracycline-inducible gene expression and gene deletion in *Candida albicans*. *Eukaryot Cell* **4:** 1328–1342.

Reuß, O., Vik, Å., Kolter, R., and Morschhäuser, J. (2004) The *SAT1* flipper, an optimized tool for gene disruption in *Candida albicans*. *Gene* **341:** 119–127.

Rüchel, R. (1986) Cleavage of immunoglobulins by pathogenic yeasts of the genus *Candida*. *Microbiol Sci* **3:** 316–319.

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

Staib, P., Kretschmar, M., Nichterlein, T., Hof, H., and Morschhäuser, J. (2000) Differential activation of a *Candida albicans* virulence gene family during infection. *Proc Natl Acad Sci USA* **97:** 6102–6107.

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

Watts, H.J., Cheah, F.S., Hube, B., Sanglard, D., and Gow, N.A. (1998) Altered adherence in strains of *Candida albicans* harbouring null mutations in secreted aspartic proteinase genes. *FEMS Microbiol Lett* **159:** 129–135.

Wiles, A.M., Naider, F., and Becker, J.M. Transmembrane domain prediction and consensus sequence identification of the oligopeptide transport family. *Res Microbiol* 2005 [Epub ahead of print].

Supplementary material

The following supplementary material is available for this article online:

Table S1. *C. albicans* strains used in this study.

This material is available as part of the online article from http://www.blackwell-synergy.com