# **RESEARCH ARTICLE**

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# Genomic evidence for mating abilities in the asexual pathogen Aspergillus fumigatus

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Abstract The filamentous fungus Aspergillus fumigatus is one of the causes of invasive lung disease in immunocompromised individuals. It is classified as asexual because no direct observation of mating or meiosis has been reported. Sequencing of the complete genome by an international collaboration, including the Wellcome Trust Sanger Institute (UK) and The Institute for Genomic Research (TIGR, USA), has made most of the genomic sequence information from A. fumigatus publicly available. By searching the incomplete genome sequence of A. fumigatus, I have identified the coding capacity for a set of proteins that could be involved in mating and the pheromone response pathway. These include one putative mating-type gene, one gene encoding a pheromone and two pheromone-receptor genes. The mating-type gene encodes a high-mobility group domain protein exhibiting significant similarity with mating-type proteins from sexually reproducing filamentous ascomycetes. The pheromone gene is predicted to encode a precursor pheromone that is processed by a KEX2-like protease to yield a pheromone that is structurally similar to the  $\alpha$ -factor of the veast *Saccharomyces cerevisiae*. In addition, the deduced gene products of the receptor genes are putative seven-transmembrane proteins, which display a high-level amino acid identity with the a-factor receptor Ste3p and the  $\alpha$ -receptor Ste2p of S. cerevisiae, respectively. The identification of these homologues suggests the existence of a sexual cycle in A. fumigatus.

**Keywords** Aspergillus fumigatus · Mating · Sexual reproduction · Pheromone · Receptor

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## Introduction

Aspergillus fumigatus is an opportunistic fungal pathogen causing severe and usually fatal invasive infections in immunocompromised individuals (Denning 1998; Vogeser et al. 1999). Its natural habitat is the soil, in which the fungus grows on organic debris. A. fumigatus reproduces by producing large numbers of airborne conidiospores (Raper and Fennell 1965). The conidiospores released into the atmosphere are small enough to reach the lung aveoli. Conidia inhaled by susceptible patients can lead to life-threatening invasive pulmonary aspergillosis (Latgé 1999). To rapidly identify genes in this fungus, including potential targets for chemotherapy, diagnostics, and vaccine development, in 2001 an international group of scientists initiated the sequencing of the A. fumigatus genome. The group selected a clinical isolate, Af293, as the strain to be sequenced (Denning et al. 2002).

To date, no sexual stage is known for *A. fumigatus*. A search for the sexual stages of *A. fumigatus* has been attempted among species of the perfect genus *Neosartorya*. However, secondary metabolites, sequencing data, and DNA–DNA reassociation values prove that *N. fischeri*, whose anamorphic stage is very closely related to *A. fumigatus*, is a separate species (Latgé 1999).

The inability to demonstrate a sexual cycle has significantly impeded conventional genetic analysis, since tetrad analysis supplies comprehensive information about segregation patterns, gene linkage, and recombinational events.

In fungi, mating typically occurs between morphologically identical partners that are distinguished by their mating type. Sexual reproduction is typically controlled by genes that reside in the mating-type locus. In most cases, the single mating-type locus conferring mating behavior consists of dissimilar DNA sequences (idiomorphs) in the mating partners (Coppin et al. 1997; Kronstad and Staben 1997). All filamentous ascomycete mating-type idiomorphs encode proteins with confirmed or putative DNA-binding motifs [high-mobility group

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(HMG) boxes and  $\alpha$ 1 domains; Pöggeler 2001]. These proteins are master regulatory transcription factors that control the pathways of cell speciation and sexual morphogenesis, including the regulation of pheromone genes and pheromone-receptor genes.

Although sexual reproduction is absent in a large number of filamentous ascomycetes, mating-type sequences have been isolated from several asexual fungi. The asexual fungal pathogens *Bipolaris sacchari*, a pathogen of sugarcane, the asexual loculoascomycete *Alternaria alternata*, and the asexual pyrenomycete *Fusarium oxysporum* were proven to have mating-type loci, which are structurally similar to those of their sexually reproducing relatives (Sharon et al. 1996; Arie et al. 2000; Yun et al. 2000).

Sequencing of the complete genome led to the surprising finding that mating-type genes exist in the pathogenic yeast *Candida albicans*, which was thought to be constitutively diploid and to reproduce only asexually (Hull and Johnson 1999). In fact, it was later demonstrated by two research groups that *C. albicans* can be forced to mate under certain conditions (Hull et al. 2000; Magee and Magee 2000).

These findings prompted me to search for homologues of genes that function in mating and pheromonesignaling in *Aspergillus fumigatus*. Using the recently released genome of *A. fumigatus*, one mating-type gene has been identified that encodes a protein similar to the HMG-domain-containing master sexual regulator MAT a-1 of *Neurospora crassa*, mating-type proteins of other filamentous ascomycetes, and homologues of the *N. crassa* pheromone-precursor gene *ppg1* and pheromone-receptor genes.

# **Materials and methods**

Identification of sex-related genes in A. fumigatus

The preliminary sequence data from the *A. fumigatus* genome was used to identify homologues in *A. fumigatus* critical for mating and pheromone-signaling in other ascomycetes. *S. cerevisiae*, *N. crassa* and *Sordaria macrospora* protein sequences were used as query sequences in BLAST searches (Altschul et al. 1990; Gish and States 1993).

Preliminary A. fumigatus genome sequences were obtained from the TIGR website at http://www.tigr.org. The TIGR BLAST search engine runs the WU-BLAST 2.0 program (http://blast. wustl.edu). For homology search, the tblastn program was used and the statistical significance threshold for reporting matches against database sequences was 10. The e-value cutoff used to assign homologues was 1e–6. Sequences of Magnaporthe grisea were obtained from the Magnaporthe sequencing project (release I; R. Dean, Fungal Genomics Laboratory at North Carolina State University; http://www.fungalgenomics.ncsu.edu) and from the Whitehead Institute/MIT Center for Genome Research (http:// www.genome.wi.mit.edu).

For validation of the identified *A. fumigatus* genes, a bi-directional best hit analysis was performed, using the polypeptide sequence of the identified *A. fumigatus* ORFs as query for a blastp search at the Swiss Institute of Bioinformatics (http:// www.ch.embnet.org; http://SwissProt/TrEMBL/TrEMBL\_NEW database). *A. fumigatus* genes with a *Saccharomyces cerevisiae* homologue, listed in Table 1 and Table 2, were compared with the *S. cerevisiae* gene-set in the *Saccharomyces* genome database (http://genome-www.stanford.edu), using blastp. In order to filter out protein domains, a match was considered for a sub-alignment spanning 55% of the *A. fumigatus* ORF with an expected value lower than 1e–8.

#### Protein sequence analysis

Amino acid sequences and sequence alignments were done using the CLUSTAL W program (Thompson et al. 1994). Prediction of

Table 1	Pu	tative phe	eroi	no	ne-pi	roce	ssing er	ızyn	nes	encodec	l by	the
Aspergil	lus	fumigatus	s g	enc	ome,	as	shown	by	а	BLAST	` sea	arch
(http://b	olast	.wustl.edu	ı) (	of	the	A.	fumiga	tus	unf	inished	gen	ome

(http://tigrblast.tigr.org/ufmg/) In Stel4p and Ste6p, the size of the protein is only partial, because the ORF runs into the end of the contig. *aa* Amino acids

Saccharomyces cerevisiae protein (accession number)	Function	Contig/size of predicted protein	E-values interpreting BLAST results/identity; and positives in overlap
Ram1p (NP 010193)	CaaX Farnesyltransferase beta subunit; a-factor modification	884:a_fumigatus (14,487–17,753)/462 aa	4.1e-23/41%; 54% in 332 aa
Ram2p (NP 012906)	CaaX Farnesyltransferase alpha subunit; a-factor modification	457:a_fumigatus (23,981–26,607)/333 aa	4.8e-19/30%; 50% in 301 aa
Rce1p (NP 014001)	CaaX protease a-factor C-terminal processing	663:a_fumigatus (13.334–15.841)/315 aa	3.8e-11/32%; 46; in 218 aa
Ste24p (P47154)	CaaX prenyl protease N- and C-terminal a-factor processing	559:a_fumigatus (1,644-4,823)/481 aa	6.2e-88/42%; 61% in 462 aa
Ste14p (P32584)	Prenylcysteine carboxyl methyltransferase	1,090:a_fumigatus (125,082–125,795)/185 aa	7.5e-24/53%; 70% in 110 aa
Ste6p (CAA33467)	ATP-dependent multidrug efflux pump of a-factor	490:a_fumigatus (1-4,019)/1,220 aa	3.1e-108/28%; 48% in 627 aa
Kex1p (A29651)	Carboxypeptidase α-factor processing	1,050:a_fumigatus (8,010–10,360)/519 aa	1.2e-77/40%; 59% in 490 aa
Kex2p (NP 014161)	Endoprotease $\alpha$ -factor processing	616:a_fumigatus (4,858–8,318)/844 aa	2.8e-132/48%; 64% in 553 aa
Stel3p (NP 014862)	Dipeptidyl aminopeptidase $\alpha$ -factor processing	177:a_fumigatus (6,063–103,386)/765 aa	2.2e-85/32%; 50% in 680 aa

**Table 2** Putative components of a pheromone-response pathway encoded by the *A. fumigatus* genome, as shown by a BLAST search (http://blast.wustl.edu) of the *A. fumigatus* unfinished genome

(http://tigrblast.tigr.org/ufmg/). In Ste7p, the size of the protein is only partial, because the ORF runs into the end of the contig

<i>S. cerevisiae</i> protein (accession number)	Function	Contig/size of predicted protein	E-values interpreting BLAST results/% identity; % positives in overlap
GBA1p (P08539)	Alpha-subunit G protein	691:a_fumigatus (5.939–9.082)/353 aa	1.6e-79/51%; 72% in 350 aa
Ste4p (P18851)	Beta-subunit G protein	966:a_fumigatus (1–3,457)/389 aa	8.5e-61/42%; 67% in 268 aa
Ste18p (NP 012619)	Gamma-subunit G protein	440:a_fumigatus (1,379–3,462)/86 aa	0.12/39%; 64%; in 28 aa
Ste20p (Q03497)	Serine/threonine protein kinase	385:a_fumigatus (10,791–14,177)/815 aa	2.8e-97/53%; 66% in 508 aa
Stel1p (P23561)	Serine/threonine protein kinase	18:a_fumigatus (4,912–9,661)/888 aa	2.1e-83/40%; 57% in 576 aa
Ste7p (P06784)	Serine/threonine protein kinase	730:a_fumigatus (11.927–13,387)/361 aa	2.2e-39/53%; 72% in 152 aa
Fus3p (P16892)	Mitogen-activated protein kinase	488:a_fumigatus (1,539–3,922)/358 aa	6.4e-80/60%;78% in 275 aa

transmembrane helices and topology of the proteins were performed with the HMMTOP server (http://www.enzim.hu/hmmtop; Tusnády and Simon 2001) and transmembrane detection based on CLU-STAL W alignments using the program TMAP (http://www.mbiki.se/tmap).

# **Results and discussion**

### Mating-type genes

Mating-type genes were cloned and sequenced from a number of heterothallic and homothallic ascomycetes, including the pyrenomycete, loculoascomycete, and

Fig. 1 Highly conserved high-mobility group domain found in fungal Aspergillus fumigatus MTLa-1 homologues: AfMTLa-1 (A. fumigatus MTLa-1), CpMAT-2 (Cryphonectria parasitica MAT1-2-1, Acc. No. AF380364), NcMATa-1 (Neurospora crassa MAT a-1, Acc. No. P36981), SmMATa-1 (Sordaria macrospora SMT a-1, Acc. No. CAA71624), PaFPR1 (Podospora anserina FPR1, Acc. No. CAA45520), GfMAT-2 (Gibberella fujikuroi MAT-2, Acc. No. AAC71056), SpMC (Schizosaccharomyces pombe M, Acc. No. S00555), PbMAT-2 (Pyrenopezziza brassicae MAT-2, Acc. No. CAA06843), ChMAT-2 (Cochliobolus heterostrophus MAT-2, Acc. No. AAB4004). Identical residues in a column are indicated in white on black, four out of five identical residues per column are indicated in white on gray, conserved changes are boxed in gray, and gaps are indicated as dashes. The position of the conserved intron is indicated with an arrow. The number at the right refers to the amino acid position in the corresponding protein

discomycete classes of ascomycete fungi (Pöggeler 2001). Using the recently released sequence of the A. fumigatus genome at TIGR, it was possible to identify a putative ORF in contig 1,148 showing significant similarity to the N. crassa mating-type protein Mat a-1 (Staben and Yanofsky 1990). Since there is no functional evidence that the identified ORF is a genuine mating-type gene, the A. fumigatus ORF was termed mtla-1 (for matingtype-like). The A. fumigatus mtla-1 gene encodes a predicted polypeptide of 322 amino acid residues, which contains a conserved HMG DNA-binding domain. The HMG domain is a DNA-binding motif that is shared by non-histone components of chromatin and by specific regulators of transcription and cell differentiation (Grosschedl et al. 1994). Two introns, of 55 bp and 54 bp, which exhibit typical fungal consensus splice sites, can be predicted in the A. fumigatus mtl a-1 ORF (Edelman and Staben 1994; Pöggeler 1997).

The first intron is present upstream of the HMG domain and the second putative intron is located at a conserved position in a serine codon of the HMG domain (Fig. 1). The position of this intron in the coding region of the HMG domain is precisely at the same position in all of the known ascomycete HMG mating-type genes. The MTL a-1 protein shows not only 21.4% identity with the corresponding sequence from *N. crassa* MAT a-1 but also a relatively high level of similarity with mating-type proteins of other ascomycetes. For

		r i	20	* 1	40		*	60	*	80	
AfMTLa-1:	KVPRPPNAFI	ILYR <mark>QHHHP</mark> I	KIKEAYPDYS®	INDI	SVML GKQW	KDE NEE IKT	QERNLAEEI	KKKHAEDHPD3	ZHYT <mark>PRK</mark> PS	ERKR	: 205
CpMAT-2 :	HIPRPPNPFI	HYRAAH R'	TVSEAHPDAS	IEI	SKKIGRQ	QSESEEVRD	AYRKKAADI	KAAFMIAHPDY	TKYHPRKSS	EVKR	: 276
NcMATa-1:	KIPRPPNAYI	ILYRKDHHRI	EIREQNPGLH	INE I	SVIVG <mark>NM</mark> #	RDEQPHIRE	KYFNMSNEI	KTRLLLENPD	TRYNPRRS Q	DIRR	: 195
SmMATa-1:	KIPRPPNAYI	ILYRKDH HR	QUREQNEGLH	INE I	SVIVGNM#	RDEQPHIRD	KYF SMANE I	KARLLLDNPD	TRYNPRRS Q	DIRR	: 199
PaFPR1 :	KIPRPPNAYI	LYRKD Q QAA	ALKAANPGIP	IDU	SVMTGGM	KKE SPEVR <mark>A</mark>	EYQRRASEI	KAKLMSAHPHI	7RY <mark>V</mark> PRRSS	EIRR	: 248
GfMAT-2 :	KIPRPPNAYI	LYRKERHH	SIKAQRPDITR	INEI	S <mark>QVLG</mark> RLM	NSE TREVRA	T AKÔWED Ő Þ	)KAEHRRQYPD)	70 YRP.RP S	ERRR	: 202
SpMC :	RTPRPPNAFI	LYRKEK A	TULKSNPSIN	SQ₹	S <mark>K</mark> LVG <mark>E</mark> MW	RNE SKEVRM	RYFKMSEFY	KAQHQKMYPG	KYQPRK-N	KVKR	: 181
PbMAT-2 :	VIARPPREEI	ilerqam ai	AVVAANPGVH	IVVI	SRL I SGMM	RESPPEIIE	HYKALAELA	KARHLHLYPN	TRF TPRKSS	DKKR	: 262
ChMAT-2 :	KAPREMNCWI	HERDAMHK	HUKAEFPHLTI	ιQΕΙ	STRCSHI	HNL SPDAKK	₽ <u>₩</u> QDAAQSA	KEEHLROHPHY	YKYTPRKPG	EKKK	: 210

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comparison, the *N. crassa* MAT a-1 and the homologue protein FPR1 of the close relative *Podospora anserina* exhibit an amino acid identity of only 38.2%. The highest degree of amino acid identity between the *A. fumigatus* MAT a-1 and HMG mating-type proteins from other ascomycetes can be observed in the HMG domain region (Fig. 1).

A functional analysis of the N. crassa MAT a-1 revealed that it is a sequence-specific DNA-binding protein. The HMG domain of MAT a-1 is sufficient for DNA-binding in vitro and necessary for mating in vivo (Philley and Staben 1994). The A. fumigatus MTL a-1 HMG box domain (amino acids 124-205) is in the same position as in the N. crassa MAT a-1 polypeptide, differs at only 12 out of 81 amino acids, and has 37 identical and 32 similar amino acids in this region. This similarity implies DNA-binding properties for the A. fumigatus MTL a-1 polypeptide. The identification of the A. fumigatus ORF was further validated by performing a bi-directional best hit analysis. A reciprocal blastp search using the polypeptide sequence of the A. fumigatus mtla-1 gene as query sequence identified all of the known fungal HMG mating-type proteins as best hits, among them the MAT a-1 protein of N. crassa. In this analysis, the closest homologue appears to be the MAT1-2 protein of Mycosphaerella graminicola, showing 76 positives in an overlap of 129 amino acids.

Recently, the mating-locus of the homothallic A. nidulans, a close relative of A. fumigatus, was analyzed by Dyer (2002). The mating-type locus of A. nidulans contains only one single gene, which encodes a HMG domain protein. This is a unique situation among homothallic euascomycetes, since all other homothallic euascomycetes so far analyzed either contain only an  $\alpha$ 1-domain gene or both an  $\alpha$ 1-domain gene and a HMG gene in the mating-type locus (Pöggeler 2001). Similar to A. nidulans, a tblastn search of the A. fumigatus genome revealed no indication for the presence of a gene encoding an  $\alpha$ 1 domain matingtype protein. Within the flanking region of the putative mating-type gene of A. fumigatus, homology was found with genes encoding a putative component of the anaphase-promoting complex from Schizosaccharomyces pombe and a DNA (apurinic or apyrimidinic) lyase from S. pombe. Interestingly, similar genes were also identified in the flanking regions of the mating-type loci of M. graminicola and A. nidulans (Dyer 2002; Waalwijk et al. 2002). Therefore, it is that A. fumigatus is derived either from a homothallic ancestor or from a heterothallic mat a strain.

## Pheromone precursor genes

Similar to the yeast *Saccharomyces cerevisiae*, two different classes of pheromones are believed to be involved in cell recognition and mating in both heterothallic and homothallic filamentous ascomycetes. One class of genes

A. f	umigat	us	
Af1	HITP	WCHLPGOGC	YMLKE
Af2	POSP	WCHLPGOGC	AKAKR
AL2	- 201	Neilli Ggoe	mon
s. m	acrosp	oora	
Sm1	EAEA	QWCRIHGQSCW	KVKR
Sm2	EAEA	QWCRIHGQSCW	KKAKR
Sm3	EAEA	QWCRIHGQSCW	<b>k</b> kr
Sm4	EANP	QWCRIHGQSCW	KAKR
Sm5	EADP	QWCRIHGQSCW	KR
			_
N. C	rassa		
Nc1	EAEA	QWCRIHGQSCW	KVKR
Nc2	EAEA	QWCRIHGQSCW	<b>KKA</b> KR
Nc3	EAEA	QWCRIHGQSCW	KR
Nc4	EAEP	QWCRIHGQSCW	<b>k</b> kr
Nc5	EANP	QWCRIHGQSCW	KAKR
MO	risea		
Ma1	LEAR	OWCPRRGOPCW	KVKE
Mg2	LEAR	OWCPRRGOPCW	KR
Mas	T.AKP	OWCPERCOPCW	KP
Mg4	LTKP	OWCRINCOSCW	K P
Mgi	TI TIME	Quertingopen	IXIX
C. p	arasit	ica	1000
Cp1	EADP	WCLFHGEGCW	KR
Cp2	EADP	WCLFHGEGCW	KR
Cp3	DPEA	WCLFHGEGCW	KEKR
Cp4	EADP	WCLFHGEGCW	KEKR
Cp5	DPEA	WCLFHGEGCW	KVKR
Cp6	DAEP	WCLFHGEGCW	KVKR
Cp7	VAAR	WCLFHGEGCW	KVKR

**Fig. 2** Sequence comparison of predicted  $\alpha$ -factor-like pheromones from the filamentous ascomycetes *A. fumigatus, Sordaria macrospora* (Pöggeler 2000), *N. crassa* (Bobrowicz et al. 2002), *Magnaporthe grisea* (Shen et al. 1999), and *Cryphonectria parasitica* (Zhang et al. 1998). Repeats are shown in white on black, Kex2processing sites (*KR*) are *indicated in white on gray*, and STE13 processing sites are *boxed in gray* 

encodes  $pro-\alpha$ -factor-like pheromone precursors that contain multiple copies of the mature peptides flanked by protease cleavage sites, whilst the other class of pheromone genes encodes small pro-a-factor-like proteins with a CAAX motif at the carboxy-terminus. This motif is expected to produce a mature lipopeptide pheromone with a C-terminal carboxy methyl isoprenylated cysteine (Zhang et al. 1998; Shen et al. 1999; Pöggeler 2000; Bobrowicz et al. 2002). In order to identify the putative pheromone precursor encoded by the A. fumigatus genome, amino acid sequences of the α-factor-like pheromone precursor PPG1 and the pro-afactor-like precursor PPG2 of Sordaria macrospora (Pöggeler 2000) were used to carry out a tblastn search. Contig 1,320 revealed the presence of a putative A. fumigatus gene encoding a 102-amino-acid a-factorlike pheromone precursor. A reciprocal blastp search using the A. fumigatus ORF as a query sequence identified the pheromone precursor PPG1 of S. macrospora and N. crassa, and the pheromone precursor MF1-1 of Cryphonectria parasitica as best hits.

The identified *A. fumigatus* gene was named *ppgA*. Within the polypeptide encoded by this gene, two

identical repeats of a putative nonapeptide sequence (WCHLPGQGC) pheromone are present, which bear significant similarity to pheromones from other filamentous ascomycetes (Fig. 2). The two repeats are flanked by maturation signals similar to those of the  $\alpha$ -factor precursors of Saccharomyces cerevisiae. The repeats are preceded by the dipeptide XP, which is a substrate for the Ste13 dipeptidyl aminopeptidase in S. cerevisiae (Julius et al. 1983). The basic dipeptide motif KR, a processing site for the Kex2 protease, is present C-terminal to each of the two pheromone repeats. The two repeats end with either YMLKR or AAKAKR. It is possible that these peptide extensions are processed by a Kex1 homologue or, alternatively, the two repeats produce pheromones with different C-termini. A hydrophobic signal sequence, which is predicted to be cleaved between aminoacid positions 18 and 19, could be detected with the program Signal V1.1 (Nielsen et al. 1997) in the N-terminus of the A. fumigatus PPGA. Therefore, the putative

Fig. 3 Comparison of the *A. fumigatus* PREA with pheromone receptors of yeast and filamentous ascomycetes. Identical residues in a column are indicated *in white on black*, four out of five identical residues per column are indicated *in white on gray*, conserved changes are boxed in gray, and gaps are indicated as dashes. *Black bars* indicate potential transmembrane helices (*I–VII*) predicted by the TMAP program (http://www.mbb.ki.se/tmap) on the basis of the multiple sequence alignment. *Ncprel N. crassa* PRE1 (Acc. No. AJ313528), *Smprel Sordaria macrospora* PRE1 (Acc. No. AJ344137), *Mgprel M. grisea* PRE1 (contig 1,1804, http:// www.fungalgenomics.ncsu.edu), *AfpreA A. fumigatus* PREA, *ScSte3 Saccharomyces cerevisiae* Ste3p (Acc. No 224734)

 $\alpha$ -factor-like pheromone is most likely secreted from the cell via the classic secretion pathway. As indicated in Table 1, genes involved in pheromone processing of the  $\alpha$ -factor precursor in *S. cerevisiae*, such as *KEX1*, *KEX2*, and *Ste13*, have homologues in the *A. fumigatus* genome.

Extensive tblastn analysis failed to identify a *ppg2* homologue encoding an a-factor like pheromone in *A. fumigatus*. The reason for this might be that a-factor-like pheromone-encoding genes are very short, in most cases encoding small polypeptides of about 20–30 amino acids. However, homologues of genes encoding a-factor-like pheromone-processing enzymes and pheromone transporters in *S. cerevisiae*, such as *Stel4*, *Ste23*, *RAM1*, *RAM2*, *STE24*, *RCE1*, and *STE6* (Davey et al. 1998), are present in the *A. fumigatus* genome (Table 1). These data suggest that *A. fumigatus* may have preserved the ability to produce not only an  $\alpha$ -factor-like but also an a-factor-like pheromone.

#### Pheromone-receptor genes

The detection of a pheromone gene in *A. fumigatus* implicates the presence of pheromone-receptor genes, which were recently detected in filamentous ascomycetes (Pöggeler and Kück 2001). Database search analyses with the BLAST program tblastn were done with the Ste3p and Ste2p pheromone receptors of the yeast *S. cerevisiae*, which are functionally well characterized and are considered as a model system for studying the seven-transmembrane segments (7-TM) receptor family

	*		20	*	40	*	60	*	80	*	100	*		
Ncprel :	MNNTDSWASV	ANITTPY	TTPEERLG	P-PAPYTDI	OU OVNLEFR	VELGILGIL	LVLAR LITIN	IGE SATVHCM	STUT	VVNSL IHRDNN	VKKWMAGYC	CDFHT	:	109
Smorel :	MNN	TFY	TGPOERNG	P-PPPYTDI	GLOVNLFFR	VFLGLLGIL	LV AK L TN	IGE F GATVHCL	STUA	VVNSLIWRDNN	VKKWMAGYC	CDFHT	:	98
Moprel :		MS	STDPDIOYG	LLAAELASP	GLVVDLVLR	VSUATUATIAT	V FR L RS	GE ALA LAA	NILITRFIT	IIQAL IWRDDA	ALDGERCOC	CDVQA	:	94
AfpreA :				MDSATT	RSPOAVVL	PVILSSILAW/	IPTII H KN	RN PATALIG	WFULNTEN	TINALIMPTON	VD SHADCHC	LCDVET	:	81
ScSte3 :				MSYKSA	IIG	UCLUAVILLI	APPLAWHSHT	KNIPAILII	WLUTURLTC	IVDAAIWSDDI	FLTRIDGKG	CDIVI	:	73
					-		-				_			
						1			11					
	120		*	140	*	160	*	180	*	200	*	220		
Ncprel :	<b>WFFAVETVF</b>	TILFDI	LGANKIG	NPRVTSSP	KEKKRKORI	SAL-HIFGNEL	LOVLLTWFIII	TORYDVFTLA	GCNAVEDPN	GVFFVFF IUPS	SPVF TVGAAG	LAGUCE	:	218
Smprel :	TIFFAVETIS	TILFDI	LGUANKIG	NPRVTSSP	KEKKRKO RI	SAL-IIFGN <mark>PV</mark>	VQVLLTYFVI	LORYNYSTLA	GCNPVFDPN	GVFLVFFILPS	SPIF TVGAAGI	LAGVCF	:	207
Mgprel :	YINIPLSTMY	ATSHEAV	OHVSAOVS	LRRATG	AEKRRRRL	QAL-IIFPV <mark>RI</mark>	IQVALSYPVH	AYRYSITTL	GCDGGFEGN	LUTFFFFLUPI	EPVYVI SV	ПТН	:	198
AfpreA :	KUMIASYVGI	PGINVCIO	RSUASVILD	TRSAMLVPS	IGQRWRDRD	MDFSFCVIIPV	INIGTHILYO	RSRYLILTIS	GOVNNDOS	WISLILAFIWE	PHICLLAAF	YCALTL	:	191
ScSte3 :	K OVGANIGI:	SCAUTIN	YNCHTICK	ADSVLPDLS	SWTKIN	KDVISLFT	WIGESTLL	VERYGUARYN	GCONLLSPT	HITTVLYTYM	LI SFVG V	YATIVL	:	180
							,	_			V			
	III					n n	/				v			
	*	:	240	*	260	*	280	*	300	*	320	*		
Ncprel :	KRQLEKLT	REVLPSD	DSIRTARQK	RLRRKLYFL	TLSINVLVV	PHACALLAL	IOG^PWTL	PFDLHRIHAN	IINFVSFTT-	ERMQVSTVLTI	IYVPVVSSVA	ICITEG	:	325
Smprel :	YK RQLEKLT	REALIPSN	<b>DSIRTARQK</b>	RLRRKUVFL	TLSIUVLVV	P VCVFFAFRI	AUG <sup>+</sup> PWSL	PFDLDRIHA	IIIFVSFTTT	EKMQVTEVLTI	IYIPV VNAVA	ICITEG	:	315
Maprel :	QRKLSTGS	-STHQQN	GAAARSN	RARRKLYLM	TVSILAPY	PIQIAFLVIRD	MNT   G^FRL Y	SWEDFQSQP1	LR GUMRVP S	WEASWISTRY	YHAH SAIP	INVEFG	:	307
AfpreA :	<b>YRLHKYRSQF</b>	GDILINSA	SHLNKS	RFLR-LFFL	ACVANATIO	PIQAYVVYRRID	MYNLPHPYS	WSRLHGPHSE	WSTILKIP-	MHGEVFFDI	<b>MIPLAAGCT</b>	LFILFG	:	295
ScSte3 :	<b>W</b> YKKRKDV	RDILHCT	SGLNLT	RFAR-ULIF	CFINLV	PFSVYTFVQDL	Q TVEGHY1	FKNTHSS-T]	WTIIKFD-	PGRP YN	WLYVDMSYL	VILLIFG	:	280
					VI						V			
					VI					100	v			
	340	-	*	360	· · · ·	380	*	400	*	420	*	440		
Ncprel :	TTVEAYNQYR	RLVLVFL	GL GKIWPKL	YQEYDPDES	EPPSELS	ISSOSMASSI	MAKNGKRGAV	TRYETHKPH	IITIT				:	402
Smprel :	TTVEAYNOYR	-LVLVF	<b>FECKTABK</b>	YQEYDPDDS	EPPSELS	SSKSWWSSI	MAKNSKRGAS	SARYV TRELS	ITIT				:	391
Mgpre1 :	TTKDAVNTAR	-GYCLAL	GL GKIFPRI	KEEYDPD	RPRGTQN	I-LQSWGTGSI	VSATDRKASV	AR					:	370
AfpreA :	CCHDALRLYQ	-SAFRL	BIGSCLSG	RTLS	QGTSASF	ASRARLLS	-SRKQSPG10						:	349
ScSte3 :	LGSDALHMYS	-KFLRS	K GFVLDMW	KRFIKKE	KRAGITTNK	LSCREESENPE	STDSENVIST	CTENYSPCVG	TPISQAHEY	VDYRIPDDPRI	KSQNKSKKYL	FADKET	:	389
	2						500		500					
	*	•	460	*	480		500		520					
Ncprel :														
Smprel :														
Mgprel :										-				
AfpreA :		BCDUTD	mococerr	ETCL COPCY	UTI DUCEUT	INCACOMERCE	CI CYCDACVE	ENCOMENCO	FNTACD	470				
ScSte3 :	DDILDEIDLK	ESBUILDA	<b>ALŐGŐZEDD</b>	ETSP66E2K	VILDISEKL	UN2W22NLEGE:	SLUISPASKE	EUSSSNEUSS	SERIAGP :	470				

	100 *	
NCDre2 :MASSSSPPADIF GITOS INST-HATLIL TPPADR -HLENOVIFLEDNHEDLINUTTYDAFNN	MUSTINATOR :	79
STORE	MUSTAINNATUR	78
	LAFNTAUGUSTULE	69
MEDICAL STATES AND STA		110
Afpres : MPSKICIISPASTALEVS ALECUIDCKKEDVULLIVUL AND FIAOSFILDIL FESSMASIFUPH (A IL IS STIVSSLALADDILA	IIII REGINIGATIE : I	110
ScSte2 :GSTIMFDE GGEAN	ST TUALMAGARCE :	60
	12 A 12 12 12 12 12 12 12 12 12 12 12 12 12	
<u>120 * 140 * 160 * 180 * 200</u>	* 220	
NCpre2 : ATFUILAINLENT-PRRFKRUPTIISLIAICUNLERVVLLALFPPSHWTDFYVLYSGDW0FVPPGDM0ISVAATVLSIPVTALULSALMVQAWSD	MQLWTPLWCALV : 1	186
Smpre2 : ATFULLATILLATI-PRRFKRIPTITSLIGILLALTRVVLLSLEXPSH#TDFXVLYTGD+0FVPRSDMSISVAATVLSIPVTALLLSALMV(AWSD	MQLWTPLWRALV : 1	185
MODE 2 : ACTINE LVL THT-AVAREARIET UNTAAUVISUTE CT. LVTF TSTMEFYTT FSDDF SEVMEND IRRS VAA TVFAEL QLALVEAAUVI (AWAM	VELipRA VSG : 1	176
A FORCE : ACAVI FLYLLL I TRPEKTVS SV-FVLNV SALLANTIR LGCOL SVI STG-ARM ALLAGDES RUS RGAYAGO MASVEFTIVLI CVEASLVI (VOVV	CSNLLROWRIEL : 2	217
A DE STATE I MAALTE IWAWITSRSEKTPI-FLINOVSER III. HSA YFKALLSHYSSYTYALTEPPOTISRO WHYYGTNIIOVL WASHETSLYFOLKYT	FTGDN KRIG-LM : 1	166
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NCPTEZ VING STOLA STARCE WART I APPLES IL ARLIA TO TATI TETRO UTUTINGS FSTOLAR DU TINSIDI TO	GELDSA : 2	292
Smpre2 : ALVGE STALVV SFARCEFURENT Y-APPLPDYA RKLY A THE TATTET THE VARIATIES DESTROCKA DVI HIMAIDAT PL	G EGLD55 : 2	291
Mgpre2 : IAFSL ATV VAFKCASAAVTVKSA EPLDPRPYLA RUTD AFTAAVAAFCSL RVRLIAHVUNRS IPPTVKGLSP EVI MANGLID FEV	TAG YNGNFG : 2	283
AfpreB : LGAST AA WHIGVRLTYSVMNCHVTMAGTHDHLDA ESATNIA HVSHCHCAWSVVKIGIAHK-MSKRIG-VXKFGPIRVEFUNGCQTATHA	WWAICOMFSRI : 3	322
Sagton : TSISFT G A UT YEVSA KGMIVTYNDVSATODKYFNAST LIAS-SINMS VLWYA HALR-SRRFIG-GOODSFHUL MSCOSI US	SUIFI AUSLEPNQG : 2	273
DCDCe2		
IV V VI		
IV V VI 340 * 360 * 380 * 400 * 420	* _ 440	
IV      V      VI        340      *      360      *      380      *      400      *      420        Ncpre2      :      SGESGS TOTS WINDERGHANDSLEASSGENGSLELSN SFAGGGGGGGGGGGGGGGGIPP THN AATNFSSSIACSGI	* 440 (SCLPKVKRMTASSA : 4	402
IV      V      VI        340      *      360      *      380      *      400      *      420        Ncpre2      :      SGFESGSI TOTS VIVLETIG TUMORTATRGYMPD SLEASSGPNGSLPLSNISFAGGGGGGGSGGHK0KENGGGI IPP TNNTAATNFSSSIACSGI      Smpre2      :      SG ESGSI TOTS VIVLETIG TUMORTATRGYMPD SLEASSGPNGSLPLSNISFAGGGGGGGSGGHK0KENGGGI IPP TNNTAATNFSSSIACSGI        Smpre2      :      SG ESGSI TOTS VIVLETIG TUMORTATRGYMPD SLEASSGPN SMPLSD JULTGDDHHK0KRAEAGNINA PTN TTSFSSIAKSGV	* 440 (SCLPKVKRMTASSA : 4 (SCLPKAKRMTASSA : 3	402 392
IV      V      VI        340      *      360      *      380      *      400      *      420        Ncpre2      :      SGFESGSITOTSIVIVLPLGTUVAORIATRGYMPDSLEASSGPNGSLPLSNISFAGGGGGGGSGGHKDKENGGGIIPPTINN AATNESSSIACSGI      Smpre2      :      SGFESGSITOTSIVIVLPLGTUVAORIATRGLLPDS-EASGGPN-SMPLSDINLTGDDHHKDKRAEAGNINATPTNITTSFSSIAKSGV        Mgpre2      :      0      ESASITITSIVLVLPLGTUVAORIATRGLLPDS-EASGGPN-SMPLSDINLTGDDHHKDKRAEAGNINATPTNITTSFSSIAKSGV	* 440 (SCLPKVKRMTASSA : 4 /SCLPKAKRMTASSA : 3 	402 392 349
IV  V  VI    340  *  360  *  380  *  400  *  420    Ncpre2  :  SGESGS  TOTS VIVLPLG TVAORTATRGYMPD CLASSGPHOSLPLSN  SFACGGGGGGGGGGKKKENGGGI IPP TNN AATNESSSIACSGI    Smpre2  :  -0*ESSTTOTS VIVLPLG TVAORTATRGYMPD CLASSGPHOSLPLSN  SFACGGGGGGGGGKKKENGGGI IPP TNN AATNESSSIACSGI    Mgpre2  :  -0*EASTTOTS VIVLPLG TVAORTATRGLPDS  -SAGGPN -SMPLSD INITG	* 440 ISCLPKVKRMTASSA : 4 /SCLPKAKRMTASSA : 3 	402 392 349 378
IV      V      VI        340      *      360      *      380      *      400      *      420        Ncpre2      :      SG_ESGS_TOTS_VIVLPLG_IVAORIATRGYMPDS_LEASSGPNGSLPLSN_SFACGGGGGGGGGGGGGGGGGGGIPPT_TNN_AATNFSSSIACSGI        Smpre2      :      SG_ESGS_TOTS_VIVLPLG_IVAORIATRGYMPDS_LEASSGPNGSLPLSN_SFACGGGGGGGGGGGGGGGHKDKENGGGI IPP_TNN_AATNFSSSIACSGI        Mgpre2      :      -0 ESAS_TITS_VIVLPLG_IVAORIATRGLPDS_EASGGPN_SMPLSD_NLTGDKLAFLGDKLAFLGDKLAFLGDKLAFLGDKLAFLGDKLAFLGDKLAFLG	* 440 ISCLPKVKRMTASSA : 4 VSCLPKAKRMTASSA : 3 	402 392 349 378 337
IV      V      VI        340      *      360      *      380      *      400      *      420        Ncpre2      :      SGEESGSI TOTS VIVLETE TIVAORIATRGY OPD SLEASSGENGSLELSNI SFAGGGGGGGSGGHKOKENGGGI IPP TNNTAANF SSSIACSGI      Smpre2      :      SGEESGSI TOTS VIVLETE TIVAORIATRGLIPP SEASGEPH SMPLSDI NLTG	* 440 ISCLPKVKRITASSA : 4 /SCLPKAKRITASSA : 3 FAGSSA : 3 ATRDKP : 3 TNNDAK : 3	402 392 349 378 337
IV  V    340  * 360  * 380  * 400  * 420    Ncpre2  :  SGFESGSIT0TS: VIVLPLGTUVAORIA TRGYMPDSLEASSGPNGSLPLSNISFAGGGGGGGSGGHKDKENGGGIIPPTTNN AATNFSSSIACSGI    Smpre2  :  :  :  :  :  :    Mgpre2  :  -  :  :  :  :    AfpreB  :  :  :  :  :  :    ScSte2  :  :  :  :  :  :    VI  :  :  :  :  :  :	* 440 ISCLPKVKRHTASSA : 4 VSCLPKAKRHTASSA : 3 PAGSSA : 3 ATRDKP : 3 TNNDAK : 3	402 392 349 378 337
IV  V    340  * 360  * 380  * 400  * 420    Ncpre2  :  SG ES GS I 0TS VIVEPEGTIVAORIA TRGYMPD LEASSGPHOSLPLSN SFACGGGGGSGGHKDKENGGGEI IPP TNN AATNESSSIACSGI    Smpre2  :  -0 ES SI 10TS VIVEPEGTIVAORIA TRGYMPD CEASGGPN - SMPLSD UNITG DDHHKDKRAFAGNINAIPTNITTS FSSIAKSGV    Mgpre2  :  -0 ES SI 10TS VIVEPEGTIVAORIA TRGLIPD - EASGGPN - SMPLSD UNITG DDHHKDKRAFAGNINAIPTNITTS FSSIAKSGV    Mgpre3  :  -0 ES SI 10TS VIVEPEGTIVAORIA VNNTVAG - SANTDMD DK IAFLG NAITVTSSAAG	* 440 ISCLPKVKRMTASSA : 4 /SCLPKAKRMTASSA : 3 FAGSSA : 3 ATRDKP : 3 ATRDKP : 3	402 392 349 378 337
IV  V    340  *    360  *    380  *    400  *    380	* 440 ISCLPKVKRHTASSA : 4 VSCLPKAKRHTASSA : 3 FAGSSA : 3 ATRDKP : 3 TRNDAK : 3 540 *	402 392 349 378 337
IV    V    VI      340    * 360    * 380    * 400    * 420      Ncpre2    :    SGESGS: NOTS; VIVLPECTIVAORIATRGYIPP LEASSGPHGSLPSN: SFAGGGGGGSGGHKDKENGGGI IPP TNN AATNFSSSIACSGI      Smpre2    :    SGESGS: NOTS; VIVLPECTIVAORIATRGLIPP; -EASGGPH -SMPLSD: NLTG	* 440 ISCLPKVKRITASSA : 4 SSCLPKAKRITASSA : 3 PAGSSA : 3 ATRDKP : 3 ATRDKP : 3 TNNDAK : 3 540 * NDDE	402 392 349 378 337 504
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IV    V    VI      340    * 360    * 380    * 400    * 420      Ncpre2    : SG ES GS IN OTS VIVEPEGTUAORI TRGYMPD SEASSGPHOSLPLSN SFAGGGGGGSGGHKDKENGGGEI IPP THN AATNESSSIACSGI      Smpre2    : SG ES GS IN OTS VIVEPEGTUAORI TRGYMPD SEASGPHOSLPLSN SFAGGGGGGSGGHKDKENGGGEI IPP THN AATNESSSIACSGI      Mgpre2    : -0 ES AS IT ITS VIVEPEGTUAORI TRGLEPD SEASGGPNSMPLSD UNLTGDDHHKDKRAEAGNINA TPTN TTSFSSIAKSGV      Mgpre2    : -0 ES AS IT ITS VIVEPEGTUAORI AVNITVAG SANTAMDDK AFEG      AfpreB    PE SHNVITLVITSEPES IWAGFALDQANSTAR-STESRHHLWNI SSDG      ScSte2    : TDVL TVALL-AVLSEPI SSTWAFAANNASKTN ITSDFTTSTDRFYPG      ScSte2    : TDVL TVALL-AVLSEPI SSTWAT-ANNASKTN ITSDFTTSTDRFYPG      VII    *      460    * 480      *    500      *    520      *    460      *    500      *    520      *    460      *    500      *    520      *    460      *    500      *    520      *    460      *    500      *    520      *<	* 440 ISCLPKVKRMTASSA : 4 VSCLPKAKRMTASSA : 3 FAGSSA : 3 ATRDKP : 3 ATRDKP : 3 INNDAK : 3 540 * IDDEDDGY : 5 IDDEADDLDDDDSY : 4 IDDEDDGY : 3	402 392 349 378 337 504 493 392
IV    V    VI      340    * 360    * 380    * 400    * 420      Ncpre2    :    SGFESGS: TOTS VIVLETG TVAORTATRGHIPD CLEASSGEPHOSLEN.SNESAGGGGGGGGGGGGGGGGGHKKENGGGEI IPP TINITAANTPSSSIACSGEI      Smpre2    :    SGFESGS: TOTS VIVLETG TVAORTATRGHIPD CLEASSGEPH SMPLSD NLTGDHHKKKRAEAGNINA PTN TTSFSSIAKSGV      Mgpre2    :    -0°E ASI TITS VIVLETG TVAORTATRGHIPD CLEASSGEPH SMPLSD NLTG	* 440 ISCLPKVKRHTASSA : 4 VSCLPKAKRHTASSA : 3 FAGSSA : 3 ATRDKP : 3 ATRDKP : 3 	402 392 349 378 337 504 493 392 421
IV    V    VI      340    * 360    * 380    * 400    * 420      Ncpre2    :    SGESGSI TOTS VIVIPLET TVAORTATRESHIPD SLEASSEPHESH SFAGGEGEGEGEKEKKENEGEGI IPP TNN AATNESSSIACSEI      Smpre2    :    SGESGSI TOTS VIVIPLET TVAORTATRESHIPD SLEASSEPHESH SHILTGDHHKOKRAEAGNINA PTN TTSFSSIAKSEV      Mgpre2    :    -0 E SASI TITS VIVIPLET TVAORTATRELLPD S-EASGEPH SMPLSDI NLTGDHHKOKRAEAGNINA PTN TTSFSSIAKSEV      Mgpre2    :    -0 E SASI TITS VIVIPLET TVAORTATRELLPD S-EASGEPH	* 440 ISCLPKVKRITASSA : 4 SCLPKVKRITASSA : 3 FAGSSA : 3 ATRDKP : 3 ATRDKP : 3 DDGY : 4 DDEDDGY : 5 DDEADDLDDDSY : 4 DDEDDD- : 3 SVAHDISIH : 4	402 392 349 378 337 504 493 392 421 392
IV    V    VI      340    * 360    * 380    * 400    * 420      Ncpre2    :    SGESGSITOTS VIVIPLECTIVAORIT TRGYMPD LEASSGPROSLPLSH SFAGGGGGGSGGHKDKENGGGIIPP TNN AATNESSSIACSGI      Smpre2    :    SGESGSITOTS VIVIPLECTIVAORIT TRGYMPD CEASGEPN - SMPLSD INLTG	* 440 ISCLPKVKRITASSA : 4 VSCLPKAKRITASSA : 3 PAGSSA : 3 ATRDKP : 3 ATRDKP : 3 	402 392 349 378 337 504 493 392 421 392
IV    V    VI      340    * 360    * 380    * 400    * 420      Ncpre2    :    SG ES GS TOTS VIVLETG TVAORTATRGYMPD SLEASSGPNOSLELSN SFAGGGGGGSGGHKOKENGGGI IPP TNN AANTPSSSIACSGI      Smpre2    :    SG ES GS TOTS VIVLETG TVAORTATRGYMPD SLEASSGPNOSLELSN SFAGGGGGGGSGGHKOKENGGGI IPP TNN AANTPSSSIACSGI      Mgpre2    :    -0 ES AST TITS VIVLETG TVAORTATRGLIPD S-EASGGPN -SMPLSD NLTG	* 440 (SCLPKVKRHTASSA : 4 (SCLPKAKRHTASSA : 3 FAGSSA : 3 FAGSSA : 3 FAGSSA : 3 	402 392 349 378 337 504 493 392 421 392
IV    V    VI      340    * 360    * 380    * 400    * 420      Ncpre2    :    SG ES GS: NOTS: VIVLETG TUAORTATRESHIPD CLASSGEPHOSLEN: SN SEAGGGGGGGSGGHKOKENGGGI IPP TINN AATINFSSSIACSGI      Smpre2    :    SG ES GS: NOTS: VIVLETG TUAORTATRECHED S-EASGEPH SMELSD NLTG	* 440 ISCLPKVKRHTASSA : 4 VSCLPKAKRHTASSA : 3 PAGSSA : 3 ATRDKP : 3 ATRDKP : 3 	402 392 349 378 337 504 493 392 421 392
IV    V    VI      340    * 360    * 380    * 400    * 420      Ncpre2    :    SGESGES flots: VIVIPEG IVAORI TRGYIPP LEASSGPRSLPLSN SFAGGGGGGSGGHKDKENGGGI IPP TNN AATNESSSIACSGI      Smpre2    :    SGESGES flots: VIVIPEG IVAORI TRGLIPP - EASGGPN - SMPLSD NLTG	* 440 ISCLPKVKRITASSA : 4 SCLPKAKRITASSA : 3 PAGSSA : 3 ATRDKP : 3 DTRDKP : 3 DTRDKP : 3 DDGY : 4 DDEDDGY : 4 DDEDDGY : 3 SVAHDISIH : 4 PPTPTSS-KNTRIG : 3	402 392 349 378 337 504 493 392 421 392
IV    V    VI      340    * 360    * 380    * 400    * 420      Ncpre2    :    SGESGS TOTS VIVIPLE TIVAORT TREYMPD LEAS SEPRESLEN SFAGGGGGSGGKHKDKENGGGI IPP TNN AATNESSSIACSGI Smpre2    :    SGESGS TOTS VIVIPLE TIVAORT TREYMPD LEAS GEPRESLEN SFAGGGGGSGGKHKDKENGGGI IPP TNN AATNESSSIACSGI Mgpre2    :    -0    EAST TITS VIVIPLE TIVAORT TREILPD SEASGEPN SMPLSD INLTG	* 440 ISCLPKVKRITASSA : 4 SCLPKAKRITASSA : 3 FAGSSA : 3 ATRDKP : 3 DINDAK : 3 540 * DDEDDDGY : 5 DDEADDLDDDDSY : 4 DDEDDD- : 3 DDEDISIN : 4 DDEDISIN : 4	402 392 378 378 337 504 493 392 421 392
IV    V    VI      340    * 360    * 380    * 400    * 420      Ncpre2    : SG ESGS TOTS VIVIPLE TWORT TREYMPD LEASSEPHOSLELSN SFACGGGGSGGHKDKENGGGI IPP TNN AATMFSSSIACSGI      Smpre2    : SG ESGS TOTS VIVIPLE TWORT TREYMPD LEASSEPHOSLELSN SFACGGGGSGGHKDKENGGGI IPP TNN AATMFSSSIACSGI      Smpre2    : O ESAS TITS VIVIPLE TWORT TREULPD - EASGGPN - SMPLSD MLTG	* 440 (SCLPKVKRHTASSA : 4 (SCLPKAKRHTASSA : 3 FAGSSA : 3 FAGSSA : 3 FAGSSA : 3 	402 392 349 378 337 504 493 392 421 392
IV    V    VI      340    * 360    * 380    * 400    * 420      Ncpre2    SG ESGSTOTS VIVELEG IVAQRI TREVIND SEASGEPHISELSEN SEAGGGGGGGGGKBKENKENGGGI IPP TENE AATHESSSTACSGI      Smpre2    SG ESGSTOTS VIVELEG IVAQRI TREVIND SEASGEPHISED IN TG	* 440 ISCLPKVKRHTASSA : 4 VSCLPKAKRHTASSA : 3 PAGSSA : 3 PAGSSA : 3 PAGSSA : 3 	402 392 349 378 337 504 493 392 421 392

Fig. 4 Comparison of the *A. fumigatus* PREB with pheromone receptors of yeast and filamentous ascomycetes. Identical residues in a column are indicated in *white on black*, four out of five identical residues per column are indicated *in white on gray*, conserved changes are *boxed in gray*, and gaps are *indicated as dashes. Black bars* mark potential transmembrane helices (*I–VII*) predicted by the TMAP program (http://www.mbb.ki.se/tmap) on the basis of the multiple sequence alignment. *Ncpre2 N. crassa* PRE2 (Acc. No. AJ314529), *Smpre2 Sordaria macrospora* PRE2 (Acc. No. AJ344136), *Mgpre2 M. grisea* PRE2 (contig 1,2019, http:// www.fungalgenomics.ncsu.edu), *AfpreB A. fumigatus* PREB, *ScSte2 Saccharomyces cerevisiae* Ste2p (Acc. No. S56228)

(Jeansonne 1994; Dohlman 2002). The BLAST search revealed a striking similarity between the a-factor receptor Ste3p of *S. cerevisiae* and a sequence contained in contig 221 of the *A. fumigatus* genome library. A putative ORF of contig 221 has 31% amino acid identity in a 162-amino-acid overlap. Further sequence analysis of contig 221 detected an ORF of 1,102 bp. One intron of 52 bp showing typical fungal splice sites was identified (Edelman and Staben 1994; Pöggeler 1997). The ORF, named *preA*, encodes a predicted 349-amino-acid protein having significant similarity not only with Ste3p but also with pheromone receptors of other filamentous ascomycetes (Fig. 3) and basidiomycetes. A reciprocal blastp search using the *A. fumigatus* PREA protein as a query sequence identified fungal pheromone receptors of ascomycetes and basidiomycetes as best hits. The *S. cerevisiae* Ste3p pheromone receptor was identified as the closest homologue (146 positives within an overlap of 313 amino acids).

A tblastn search with the *S. cerevisiae*  $\alpha$ -factor receptor Ste2p resulted in the highest level of similarity, with 22% identical amino acids in a 246-amino-acid overlap with a putative ORF of 1,471 bp contained on contig 374. Two putative introns of 102 bp and 66 bp, showing typical fungal splice sites, were identified (Edelman and Staben 1994; Pöggeler 1997). The coding sequence for this gene, *preB*, can be translated into a protein of 431 amino acids, which shows significant similarity to the  $\alpha$ -factor receptor Ste2p of *S. cerevisiae* and pheromone receptors of other filamentous ascomycetes (Fig. 4). The pheromone receptor Ste2p of *S. cerevisiae* was identified as the reciprocal best hit.

As with the *S. cerevisiae* receptors Ste3p and Ste2p, PREA and PREB of *A. fumigatus* do not show sequence similarity to each other. However, both proteins are structurally very similar. The HMMTOP program (Tusnády and Simon 2001) predicted seven hydrophobic transmembrane-spanning helices (Figs. 3, 4), an extracellular N-terminal tail, three outer and three inner cytoplasmatic loops, and an inner C-terminal tail for PREA and PREB, respectively. Thus, both receptors belong to the 7-TM-type receptor family (Davis and Davey 1997). Mutational analysis of Ste3p and Ste2p from S. cerevisiae indicated that functional domains are organized in a manner similar to other G protein-coupled receptors (GPCRs). Transmembrane segments are thought to form a pocket that acts as a ligand-binding domain and the third intracellular loop is important for G-protein activation. The cytoplasmic C-terminus is a target for a receptor kinase that negatively regulates receptor signaling and functions in ligand-induced endocytosis (Dohlman and Thorner 2001). A conserved proline residue in transmembrane segment VI holds the critical role of governing the activity and trafficking of GPCRs (Konopka et al. 1996). As shown in Figs. 3, 4, the A. fumigatus PREA and the A. fumigatus PREB receptor contain a conserved Pro residue in transmembrane domain VI. In yeast, multiple Ser-Thr residues within the cytoplasmic C-terminus serve as phosphoryl acceptors. The level of phosphorylation increases upon exposure of the cells to the appropriate pheromone ligand (Feng and Davis 2000). Similar to the yeast pheromone receptors, the C-termini of both A. fumigatus proteins, PREA and PREB, carry multiple Ser-Thr residues, which can serve as phosphoryl acceptors. As predicted by the NetPhos program (http://www.cbs.dtu.dk), the C-terminus of the A. fumigatus PREA and PREB contain four and three putative phosphorylation sites, respectively.

In S. cerevisiae, after the binding of pheromones to a cell-type-specific receptor, the signal is transmitted by interaction of a hetrerotrimeric G protein composed of G $\alpha$  (Gpa1p), G $\beta$  (Ste4p), and G $\gamma$  (Ste18p) through a downstream mitogen-activated protein kinase cascade encoded by STE20, STE11, STE7, and FUS3. Homologues of all these genes have been identified in A. fumigatus (Table 2). Thus, it seems that, after the interaction of pheromones and pheromone receptors, A. fumigatus has the potential to trigger a G protein-linked signal transduction pathway.

Although sexual reproduction is absent in a large number of filamentous ascomycetes, mating-type sequences have been isolated from several asexual fungi (Pöggeler 2001). Moreover, in a population genetic study by Geiser et al. (1998), the asexual species A. flavus was shown to fall into two reproductively isolated groups. The lack of concordance among gene genealogies among isolates in one group was consistent with a history of recombination. It was speculated that, under some conditions in nature, sclerotia produced by A. flavus might harbor sexual reproduction as homologous structures do in the sexually reproducing relative, Petromyces alliaceus. Therefore, it seems likely that some fungi, which in the laboratory are known to reproduce exclusively asexually, display different modes of reproduction in nature.

Heterologous expression of mating-type genes from asexual species in their heterothallic relatives shows that mating-type genes from asexual fungi are

functional and, consequently, asexuality must be attributed to a property other than mating-type genes (Sharon et al. 1996; Arie et al. 2000; Yun et al. 2000). In addition to a mating-type gene, one putative pheromone gene and two putative pheromone-receptor genes were identified in the genome of the asexual A. fumigatus. Thus, the asexual ascomycete A. fumigatus has at least the genomic potential to accomplish a complete sexual life cycle. So far, there is no evidence for sexual reproduction in A. fumigatus. However, this might be because of the lack of an appropriate sexually compatible partner. A PCR-based analysis of natural A. fumigatus isolates using primers located in the flanking region of the *mtl a-1* gene would allow one to evaluate whether there exist strains with another mating-type allele or whether all strains have the *mtl a-1* gene of strain Af293. Natural isolates with another mating-type allele will be good candidates for mating partners of strain Af293.

The finding that the asexual *A. fumigatus* carries not only a mating-type gene, but also pheromone and pheromone receptor introduces the possibility to induce mating and sexual reproduction in *A. fumigatus* in the laboratory, as was recently shown for the classic "asexual" yeast, *Candida albicans* (Hull et al. 2000; Magee and Magee 2000).

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