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RESEARCH ARTICLE

Stefanie Pöggeler

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 α-factor of the yeast *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 α-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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S. Pöggeler
Lehrstuhl für Allgemeine und Molekulare Botanik,
Ruhr-Universität Bochum, 44780 Bochum, Germany
E-mail: stefanie.poeggeler@ruhr-uni-bochum.de

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

(HMG) boxes and α1 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 pheromone-signaling 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 Putative pheromone-processing enzymes encoded by the *Aspergillus fumigatus* genome, as shown by a BLAST search (http://blast.wustl.edu) of the *A. fumigatus* unfinished genome (http://tigrblast.tigr.org/ufmg/) In Ste14p 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 α-factor processing	616:a_fumigatus (4,858–8,318)/844 aa	2.8e–132/48%; 64% in 553 aa
Ste13p (NP 014862)	Dipeptidyl aminopeptidase α-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

S. cerevisiae 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
Ste11p (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 CLUSTAL 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 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 *m*ating-*t*ype-*l*ike). 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

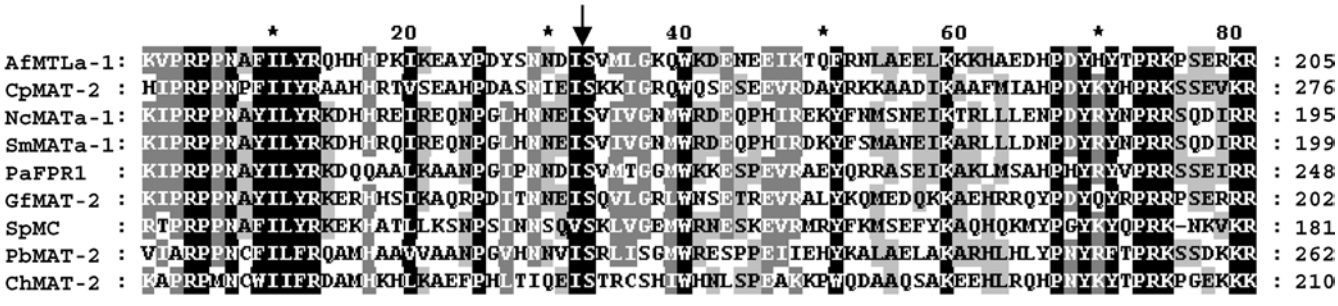

Fig. 1 Highly conserved high-mobility group domain found in fungal *Aspergillus fumigatus* MTLa-1 homologues: AfMTLa-1 (*A. fumigatus* MTLa-1), CpMAT-2 (*Cryphonectria parasitica* MAT1-2-1, Acc. No. AF380364), NcMATa-1 (*Neurospora crassa* MAT a-1, Acc. No. P36981), SmMATa-1 (*Sordaria macrospora* SMT 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

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 α1-domain gene or both an α1-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 α1 domain mating-type 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, itmight be 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

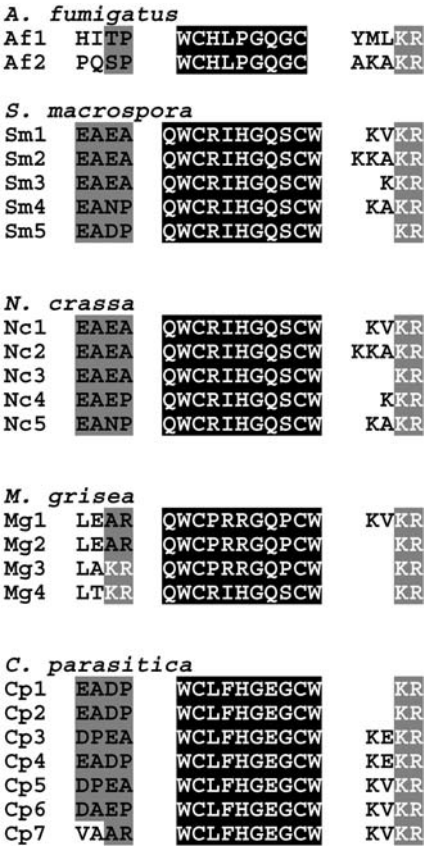

Fig. 2 Sequence comparison of predicted α-factor-like pheromones from the filamentous ascomycetes *A. fumigatus*, *Sordaria macrospora* (Pöggeler 2000), *N. crassa* (Bobrowicz et al. 2002), *Magnaporthe grisea* (Shen et al. 1999), and *Cryphonectria parasitica* (Zhang et al. 1998). Repeats are *shown in white on black*, Kex2-processing sites (*KR*) are *indicated in white on gray*, and STE13 processing sites are *boxed in gray*

encodes pro-α-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-a-factor-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 α-factor-like 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 α-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 amino-acid positions 18 and 19, could be detected with the program SignalP V1.1 (Nielsen et al. 1997) in the N-terminus of the *A. fumigatus* PPGA. Therefore, the putative α-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 α-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 *Ste14*, *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 α-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

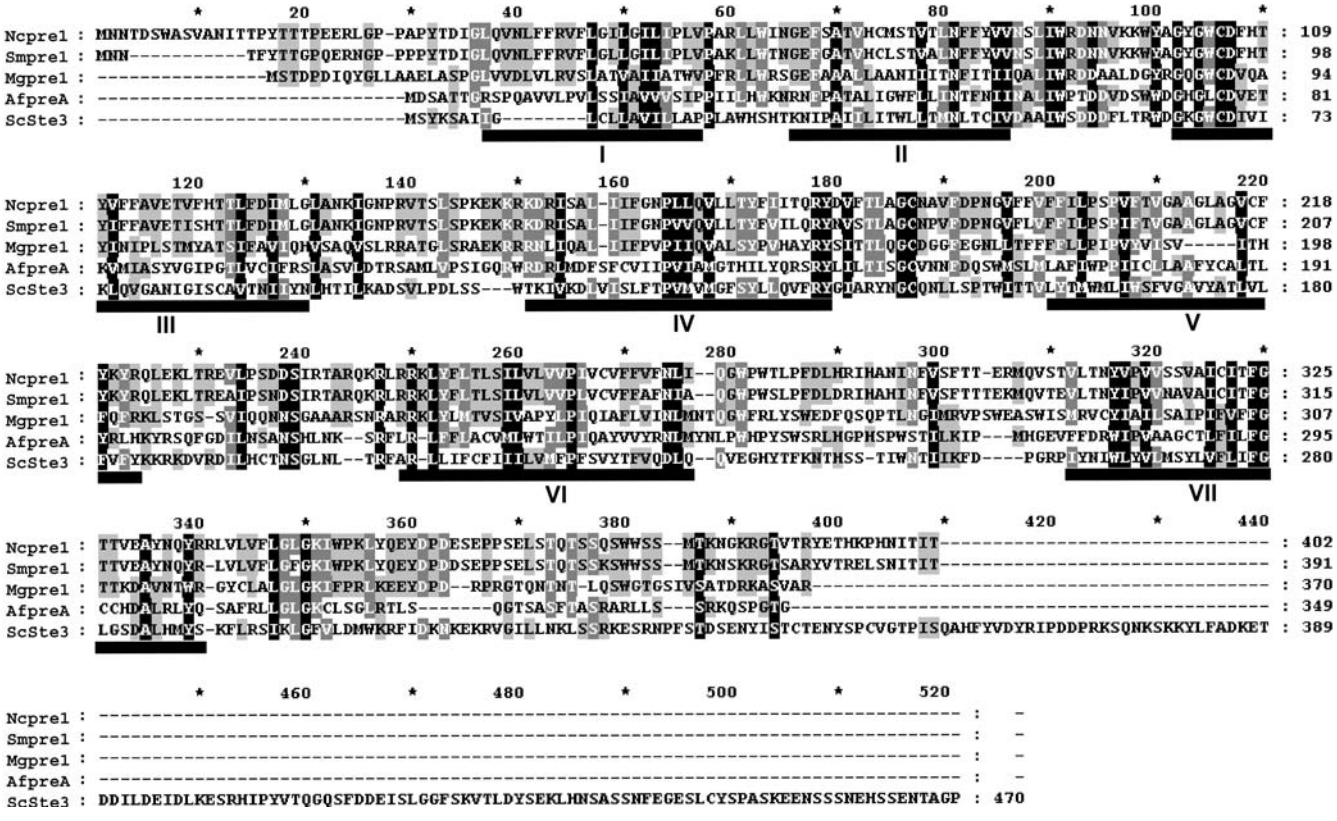

Fig. 3 Comparison of the *A. fumigatus* PREA with pheromone receptors of yeast and filamentous ascomycetes. Identical residues in a column are indicated *in white on black*, four out of five identical residues per column are indicated *in white on gray*, conserved changes are boxed in gray, and gaps 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. *Ncpre1 N. crassa* PRE1 (Acc. No. AJ313528), *Smpre1 Sordaria macrospora* PRE1 (Acc. No. AJ344137), *Mgpre1 M. grisea* PRE1 (contig 1,1804, http://www.fungalgenomics.ncsu.edu), *AfpreA A. fumigatus* PREA, *ScSte3 Saccharomyces cerevisiae* Ste3p (Acc. No 224734)

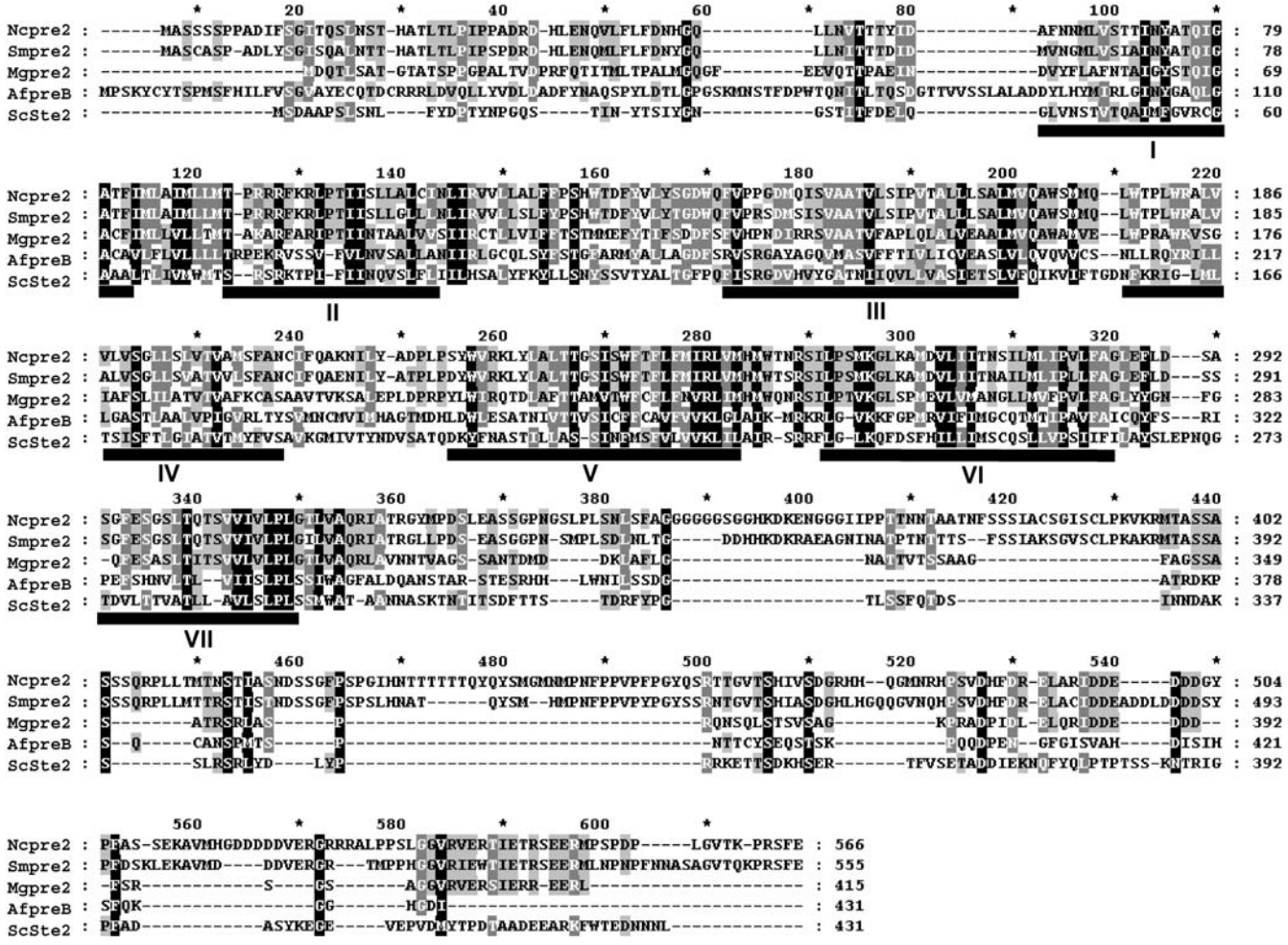

Fig. 4 Comparison of the *A. fumigatus* PREB with pheromone receptors of yeast and filamentous ascomycetes. Identical residues in a column are indicated in *white on black*, four out of five identical residues per column are indicated *in white on gray*, conserved changes are *boxed in gray*, and gaps 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. AJ313529), *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* α-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 α-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 Gα (Gpa1p), Gβ (Ste4p), and Gγ (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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