The Asexual Pathogen *Aspergillus fumigatus* Expresses Functional Determinants of *Aspergillus nidulans* Sexual Development

Verena Große and Sven Krappmann

Institute of Microbiology and Genetics, Georg-August-University Göttingen, Göttingen, Germany, and Research Center for Infectious Diseases, Julius-Maximilians-University Würzburg, Würzburg, Germany

Received 6 May 2008/Accepted 19 August 2008

The major fungal pathogen of humans, *Aspergillus fumigatus*, lacks a defined sexual cycle, although the presence of genes encoding putative mating type idiomorphs and regulators of *Aspergillus* sexual development heightens the potential for cryptic sexuality in this deuteromycete. To test the functionality of these genetic determinants, we transferred the alpha box-encoding *mat1*-1 idiomorph from an *A. fumigatus* isolate to the homothallic fertile species *Aspergillus nidulans*. Abundant formation of fruiting bodies (cleistothecia) containing viable ascospores establishes functionality of this mating type gene product in the transgenic strain. Using a similar approach, we also established that the conserved transcriptional regulator from *A. fumigatus*, the *nsdD* gene product, can act as a functional, positively acting factor for *A. nidulans* cleistothecium development; moreover, high-level expression of NsdD in the endogenous host *A. fumigatus* profoundly alters hyphal development by triggering the formation of coiled hyphae. Our findings demonstrate that the presumably asexual pathogen *A. fumigatus* encodes functional regulators of mating and sexual development, thereby potentiating the case for cryptic sexuality in this fungal pathogen.

In recent decades, pathogenic fungi, such as *Aspergillus* spp., have gained significance as severe threats to human health, particularly in the immunocompromised individual. Recent genomic advances (10, 12, 27) have provided the opportunity to undertake comparative studies of pathogenic and nonpathogenic *aspergilli*. In an evolutionary context, restricted access to sexuality may have driven acquisition of pathogenicity in *A. fumigatus* (13, 18, 26). The existence of cryptic sexuality in this species has been proposed by Paoletti and coworkers, based on the near-1:1 distribution of both mating type idiomorphs among a population of worldwide isolates (29). Moreover, phylogenetic analyses support the occurrence of genetic recombination within the *Aspergillus fumigatus* population (32). As deduced from in silico analyses, the genome of the *A. fumigatus* sequence reference strain AF293 contains many putative gene products that are orthologous to recognized determinants of fruiting body formation in the fertile species *Aspergillus nidulans* (10, 27). Among these is the GATA-type transcription factor NsdD, which has been characterized as a crucial regulator of sexual development (14). NsdD acts positively on early steps of sexual reproduction in *A. nidulans* without affecting asexual conidiation, as demonstrated by analysis of deletion mutants and overexpression studies (16).

Sexual propagation in fungi is widespread and comprises distinct developmental steps to eventually form recombiant progeny from meiotic spores. To support genetic variability, two nuclei must fuse in a process termed karyogamy. Karyogamy follows fusion of haploid ascomycete cells, resulting in diploid formation. Diploid cells eventually undergo meio-

* Corresponding author. Mailing address: Research Center for Infectious Diseases, Julius-Maximilians-University Würzburg, Young Investigator Group 2, Röntgenring 11, D-97070 Würzburg, Germany, Phone: 0049-931-312153, Fax: 0049-931-312578. E-mail: sven.krappmann@uni-wuerzburg.de.

* Published ahead of print on 29 August 2008.
TABLE 1. Fungal strains

| Name            | Description                       | Reference            |
|-----------------|-----------------------------------|----------------------|
| FGSC A4         | Aspergillus nidulans Glasgow wild type | Fungal Genetics Stock Center |
| KHH52           | A. nidulans nsdDΔ deletion strain | 16                   |
| TNO2A3          | A. nidulans nkuΔ deletion strain; pyrG89 pyroA4 | 25                   |
| AnS22           | Complementation of KHH52 by forced expression of AnnsdD via pSK393 | This study |
| AnS23           | Complementation of KHH52 by forced expression of AnnsdD via pSK395 | This study |
| AnS24           | Overexpression of AnnsdD in FGSC A4 via pSK393 | This study |
| AnS25           | Overexpression of AnnsdD in FGSC A4 via pSK395 | This study |
| AnS30           | A. nidulans expressing A. fumigatus MAT1-1 ΔpyrG | This study |
| AnS31           | A. nidulans expressing A. fumigatus MAT1-1Δbox allele; ΔpyrG | This study |
| D141            | Aspergillus fumigatus wild-type strain (syn. NRRL 6585), clinical isolate [mat1-1] | 41 |
| Af293           | A. fumigatus genome sequence reference strain, clinical isolate [mat1-2] | 28 |
| AfS41           | pabaA::loxP; riboB::loxP derivative of D141 | This study |
| AfS45           | pabaA::loxP; pyroA::loxP derivative of D141 | This study |
| AfS53           | Overexpression of AnnsdD via pSK393 in AfS41 | This study |
| AfS54           | Overexpression of AnnsdD via pSK395 in AfS41 | This study |
| AfS55           | Overexpression of AnnsdD via pSK393 in AfS45 | This study |
| AfS56           | Overexpression of AnnsdD via pSK395 in AfS45 | This study |
| AfS60           | Overexpression of AnnsdD^{his2A} allele via pSK446 in AfS41 | This study |

* AnnsdD, A. fumigatus nsdD; AnnsdD, A. nidulans nsdD. Other genes are shown similarly.

TABLE 2. Plasmid constructs

| Name             | Description                       | Reference |
|------------------|-----------------------------------|-----------|
| pBluescript II KS| General cloning plasmid (bla, MCS) | Stratagene |
| pJE1             | Positive selection vector, pUC19 derivative (bla, MCS in eco47IR) | Fermentas |
| pSK432           | 5′matB::mat1-1::ΔpyrG;3′matB gene replacement cassette | This study |
| pSK433           | 5′matB::mat1-1Δbox::ΔpyrG;3′matB gene replacement cassette | This study |
| pSK490           | Aspergillus expression vector (An::niaA::niaD::Pmel-Adhis2A::ptrA) | This study |
| pSK493           | Construct for forced expression of A. fumigatus nsdD (niaD::AnnsdD::his2A; ptrA) | This study |
| pSK495           | Construct for forced expression of A. nidulans nsdD (niaD::AnnsdD::his2A; ptrA) | This study |
| pSK496           | Construct for forced expression of A. fumigatus nsdD^{his2A} loss-of-function allele | This study |

* ΔpyrG, A. fumigatus pyrG; An::niaA::niaD, A. nidulans niaAniad promoter; other genes are shown similarly. MCS, multiple cloning site.
A. fumigatus nsdD ORF at position 1363, which changes an alanine residue at position 455 in the NsdD primary structure to a proline.

**Light and electron microscopy.** Aspergillus colonies and fruiting bodies were inspected using a Nikon SMZ680 binocular microscope combined with a Coolpix 4500 digital camera. Fungal hyphae were examined with an Olympus SZX12 binocular or a Zeiss Axioslab microscope; photographs were taken with a Kappa digital camera using the Image Base software package or with a Xillix Microimager digital camera in combination with the Improvision Openlab software. For scanning electron microscopy, samples from strains grown on culture medium-agar coated glass slides were essentially prepared according to the procedure described by Sohn and Yoon (39).

### RESULTS

**The A. fumigatus mating type gene mat1-1 codes for a functional orthologue of the A. nidulans α-box counterpart.** In contrast to the A. fumigatus genome sequence reference strain, A293, which encodes the mat1-2 idiomorph, the clinical isolate D141 (41) was identified as a mat1-1 strain encoding an α-box domain protein (data not shown). Recent studies suggest the existence of a cryptic sexual cycle of A. fumigatus, which would require functional DNA binding proteins expressed from the respective mating type loci. To put this to the test, the native matB ORF of the homothallic species A. nidulans was swapped for its A. fumigatus orthologue by a one-step procedure (Fig. 1A). After transformation of the NHEJ-deficient recipient TNO2A3 (25) with a suitable gene replacement cassette, several isolates were recovered for which correct gene replacement could be validated by Southern hybridization analyses (Fig. 1B). One representative (AnS30) was chosen for phenotypic characterization. After 8 days of incubation under conditions favorable for sexual differentiation, its ability to form fruiting bodies at densities similar to the ones displayed by the recipient strain and with cleistothecial dimensions comparable to those of its progenitor strain was observed (Fig. 1C). As estimated from crushed cleistothecia, fruiting bodies of either origin contained red-pigmented ascospores (Fig. 1D). However, the number of ascospores was reduced in fruiting bodies derived from the mat1-1 transgenic strain (data not shown), and analysis of cleistothecial contents revealed the presence of ascogenous hyphae and ascocarp cells, suggestive of slower maturation. Continued incubation for an additional 4 days eventually resulted in mature fruiting bodies accompanied by formation of fertile ascospores, which displayed similar viabilities to those harvested from the progenitor strain (data not shown). When the endogenous matB gene of TNO2A3 was replaced by an allele of *A. fumigatus mat1-1* in which the α-box had been deleted, cleistothecium formation was abolished in all (*n* = 8) validated transformants (Fig. 1C), which conclusively demonstrates the functionality of the *A. fumigatus* mat1-1-encoded DNA-binding protein for cleistothecial development.

**A. fumigatus expresses a functional NsdD orthologue.** Having demonstrated that the deuteromycete *A. fumigatus* expresses mating type gene products that are functional in an ascomycetous host, we extended our analysis to another regulator of *Aspergillus* sexual development. From a BLAST search at the CADRE database (www.cadre-genomes.org.uk) (24), an *A. fumigatus* locus (Afu3g13870) with the capacity to encode an *A. nidulans* NsdD transcription factor orthologue was identified. Alignment of the translation product sequences illustrates large regions of identity, particularly among the carboxy-terminal residues (Fig. 2A), where the conserved DNA-binding motif resides. This conserved feature is a GATA zinc finger domain of the IVb type (43), characterized by a C-X2-C-X15-C-X2-C sequence, which is known to be required for functionality of *A. nidulans* NsdD in triggering sexual development (16). The zinc finger domain is identically present in the *A. fumigatus* orthologue, which hints at a conserved cellular role among the NsdD factors from *A. fumigatus* and *A. nidulans*.

We next tested functionality of the *A. fumigatus* NsdD protein by cross-complementation studies. To transfer the *nsdD* coding sequence from *A. fumigatus* to the heterologous host *A. nidulans*, a suitable expression vector was constructed by inserting a cDNA fragment into the plasmid pSK390. The *A. fumigatus* *nsdD* cDNA could be amplified from mycelial as well as sporulating samples, which indicates that the *nsdD* gene is indeed expressed in this assexual fungus. To assess whether forced expression of the *A. fumigatus* *nsdD* gene is able to trigger sexual development in a wild-type genetic background, the expression construct pSK393 was transformed into the *A. nidulans* strain FGSC A4. Overexpression of either native or *A. fumigatus* NsdD resulted in formation of numerous cleistothecia (Fig. 2B). In stark contrast, untransformed FGSC A4 formed only a few fruiting bodies under these conditions of illumination and aeration, which favor asexual sporulation and the formation of conidiophores. Transforming the expression constructs into the *nsdD* deletion recipient KH5H2 revealed complementation capacities of the *A. fumigatus* *nsdD* coding sequence. The deletion recipient is characterized by retarded hyphal growth, earlier conidiophore formation, and most strikingly, the absence of any cellular structures of sexual development.

### TABLE 3. Oligonucleotides

| Name        | Sequence                                      |
|-------------|------------------------------------------------|
| AFM1 5′-CCT GTA CGC GAT GGG GTG G-3′ |                                                          |
| AFM2 5′-CGG TCC TCA TCA GAA CCA CTC G-3′ |                                                          |
| AFM3 5′-CGG AAT CCT GAT GTT GCC AGG C-3′ |                                                          |
| Sv135 5′-ATG GGA TCA TTA GAG GCA ACA CAT AG-3′ |                                                          |
| Sv136 5′-CTA TCG GCT ACC GGG AGG CTT GGT GAC G-3′ |                                                          |
| Sv207 5′-ATG GGA TCA TTA GAG GCA ACA CAT AG-3′ |                                                          |
| Sv406 5′-TTA ATG ACT CCT CGG TGA CAC C-3′ |                                                          |
| Sv491 5′-TTC GAC GTT CTC ACT CTT AAA GC-3′ |                                                          |
| Sv492 5′-GAT TGC AGC TTC CAT GAC GGC TGG GGG ATT GCC GTT GAT CTA TCT TGG-3′ |                                                          |
| Sv493 5′-AAC GGC ACC GCT ATG GAA GCC GCT ACC TCG GCT GCT C-3′ |                                                          |
| Sv494 5′-AAG AGG GTA AAG AGC TCA GAC GAT GTT GTA TTG ATC AAT GTC-3′ |                                                          |
| Sv495 5′-TAC ATC AAC GTC TGA GCT CTT ACC CCT CTT CGC GGG TCT GAA ATA CC-3′ |                                                          |
| Sv496 5′-CTG GAA CCA ACC AGC ACC TGT AGA GGA GGC ACT GCT GAT GAT GGG-3′ |                                                          |
| Sv497 5′-CTC TCA TCA GAC ACG TGT CCG GTT CCA GGC AAC TAC GAT GCT CTA G-3′ |                                                          |
| Sv498 5′-AGT ACT ACG GAT TAG TCG GGG TGG C-3′ |                                                          |
| Sv511 5′-ATG AAA GAT ATA TAC AAC AAG G-3′ |                                                          |
| Sv512 5′-ATC TCA GAA CCA AAC TGT ACC-3′ |                                                          |
| Sv515 5′-GAC GAC CAT GAA AGC GAG G-3′ |                                                          |
| Sv516 5′-TTC CTT TCT TCC TCC TGT GCT GC-3′ |                                                          |
| Sv549 5′-ATT GGT CAA CAC CCC CTG ACC ACA ATA CTA TG-3′ |                                                          |
| Sv550 5′-TAG GCC ACA GGG ATT GCA CAA TGT CCG AGC-3′ |                                                          |
ment, such as Hüle cells or cleistothecia. In contrast, the majority (6 out of 8 for pSK393 and 11 out of 14 for pSK395) of the resulting transformants formed fruiting bodies on inducing medium (Fig. 2C). As a positive control, KHH52 was transformed with the expression construct harboring the \emph{A. nidulans} \emph{nsdD} coding sequence to display profound sexual development. Cleistothecia harvested from either transformant contained numerous ascospores that were able to germinate when transferred to culture medium. We therefore conclude that the \emph{A. fumigatus} \emph{NsdD} factor is able to replace its \emph{A. nidulans} counterpart in a functional manner.

**Forced expression of NsdD results in circular growing hyphae.**

Given the pronounced phenotype of \emph{nsdD} overexpression in fertile \emph{A.nidulans}, we were interested in whether forced expression of this transcriptional activator in the asexual species \emph{A. fumigatus} would result in any related phenotype. The expression constructs for the \emph{A. fumigatus} and \emph{A. nidulans} transcription factors were transformed into \emph{A. fumigatus} strain AfS41, which is a descendant of the wild-type isolate D141, or strain AfS45, which stems from the reference strain Af293. To confirm proper and inducible transcription of each \emph{nsdD} coding sequence, RNA preparations from selected transformants were subjected to Northern analysis (Fig. 3A). As expected, strong hybridization signals were obtained for samples from nitrate-containing cultures, whereas from medium with ammonium as the sole source of nitrogen, weaker expression of each

---

**FIG. 1.** The \emph{A. fumigatus mat1-1} ORF supports sexual development in \emph{A. nidulans}. (A) Schematic presentation of \emph{matB/mat1-1} ORF replacement procedure showing the native \emph{matB} locus from \emph{A. nidulans} and the genome architecture after replacement with the \emph{A. fumigatus} \emph{α-box} protein-encoding \emph{mat1-1} ORF from isolate D141. Homologous regions are depicted by rhombic gray areas; BglII (B) and HindIII (H) recognition sites are indicated. The black bar represents the hybridizing probe in Southern analyses (B) of recipient strain TNO2A3 and one representative carrying the \emph{A. fumigatus mat1-1} gene after digestion of chromosomal DNA with BglII or HindIII; M, DNA molecular weight marker. (C) Colony appearance and cleistothecium (Cl) formation of strains AnS30 (\emph{mat1-1}) and AnS31 (\emph{mat1-1}^\emph{α-box}), expressing a functioning and malfunctioning allele of the \emph{A. fumigatus mat1-1} ORF, respectively; mature fruiting bodies (arrowheads) were formed only when functional MatB or Mat1-1 proteins were expressed from the endogenous \emph{matB} locus in \emph{A. nidulans}, and as estimated from cleistothecial densities, the \emph{A. fumigatus mat1-1} ORF supported fruiting body formation to an extent similar to that for its \emph{A. nidulans} orthologue (scale bar, 500 μm; n. a., not applicable; Cl densities were determined from five different 0.5-cm² areas of two independently inoculated culture plates). (D) Crushed cleistothecia from 8-day-old fruiting bodies of strain TNO2A3 (left) or AnS30 (right): ascosporogenesis of the \emph{mat1-1} strain appeared delayed, as indicated by the presence of unripe asci and ascogenous hyphae (cw, cleistothecial wall; as, ascospores). Yet after prolonged incubation, equal amounts of fertile ascospores were formed by the two strains.
transcript was evident. Transcription of the endogenous nsdD gene appeared to be repressed by forced expression of the A. nidulans gene, which supports previous data suggestive of autoregulation of NsdD expression (15). Validated transformants were inspected on inducing medium to find two distinct irregularities: the sporulation zone of colonies from transgenic A. fumigatus strains that expressed the nsdD genes at high levels was reduced (Fig. 3B), and moreover, transformants of both genetic backgrounds reproducibly formed coiled hyphal structures in the growth zone of mycelial extension (Fig. 3C). Whereas the majority of hyphal tip segments appeared normal and regularly shaped, some of them developed a curved and intertwining assembly, resulting in curls of approximately 20 μm in diameter. To exclude that these structures are due to expression of a foreign gene per se, a presumptive loss-of-function allele of the A. fumigatus nsdD gene encoding a proline instead of an alanine residue at position 455, which maps to the GATA zinc finger domain (37, 46), was expressed. The corresponding construct was unable to complement the nsdD deletion in strain KHH152 (data not shown) and did not result in hyphal coil formation in A. fumigatus (Fig. 3C).

To examine whether A. nidulans expressing any nsdD gene at high levels would form similar structures, strains AnS24 and AnS25 were inspected microscopically. In comparison to their progenitor, which formed hyphal coils very rarely, both strains displayed coiled hyphal tips at significantly higher frequencies on culture medium inducing expression of the nsdD gene (Fig. 3D). Accordingly, formation of these coils could be attributed specifically to the forced expression of either of the two nsdD transcriptional activators in two Aspergillus species, which further supports a conserved cellular function of this regulatory protein. By microscopic inspection, we were unable to track any further developmental fate of these hyphal coils or to correlate their appearance to cleistothecium formation.

In order to characterize the A. fumigatus hyphal coils further, microscopic inspection after nuclear and cell wall staining was carried out, revealing normal nuclear distribution within these hyphal segments and typical profuse apical cell wall synthesis (Fig. 4A). In this respect, the curled hyphae do not appear distinct from vegetative growing ones, which is also evident from scanning electron microscopy images (Fig. 4B). The fate of these structures within the vegetative mycelium could not be pursued since subsequent intertwining of growing hyphae superposed by asexual sporulation structures hampered microscopic inspection.

**DISCUSSION**

The data presented in this study demonstrate that the presumably asexual human pathogen A. fumigatus encodes functional determinants of sexual development, as determined by functional complementation in A. nidulans, a homothallic species where sexual development is closely linked to mating, since strains with any of the two mating types deleted are infertile and do not form ascospores (30). In agreement with this is the noncomplementation phenotype observed for the A. fumigatus matI-1 ORF, however, supports the A. nidulans sexual cycle accompanied by Hülle cell formation, cleistothecium development, and ascosporangogenesis. This finding complements a recent study (34) in which the A. nidulans matA ORF was swapped for its A. fumigatus counterpart: MatI-2 supported cleistothecial differentiation in the transgenic strain, which produced viable ascospores. According to this study, however, substitution by the A. fumigatus matI-2 ORF resulted in the delayed formation of hypertrophic fruiting bodies; we did not find this to be the case in our matI-1 complementation experiments, which hints at subtle differences in the functional conservation of each mating type factor. In summary, we can now conclude that both mating type idiomorphs of A. fumigatus encode products capable of supporting sexual differentiation and fruiting body formation (although to differing degrees) in a fertile, homothallic species of Aspergillus.

This finding is an extension of prior studies, in which the idiomorphs had been identified and demonstrated to be expressed in A. fumigatus (29). Moreover, based on a near-1:1 ratio of distribution for both mating types within a worldwide collection of A. fumigatus isolates, the existence of a sexual cycle has been proposed. Our study strongly supports this hypothesis by demonstrating that two key factors of A. nidulans sexual development appear to be functionally conserved. Of course, the possibility exists that the tested gene products, MatI-1 and NsdD, have been assigned to alternative biological roles in A. fumigatus. Characterization of A. fumigatus mutant strains may shed light on this prospect, and the generation of corresponding A. fumigatus deletants is a next logical step.

The NsdD-associated phenotype of hyphal coil formation in the fertile species A. nidulans and the deuteromycete A. fumigatus remains vague thus far, although some parallels with early-stage cleistothecial formation in A. nidulans can be drawn, since cleistothecial development is characterized by the formation of circular growing hyphae that surround the ascogenous system (39). Ascocarp formation in several Aspergillus species is initiated by an unbranched hyphal coil, the ascogonium, which may represent a precursor of the spherical cleistothecial wall (1). Moreover, hyphal coils are similar to structures observed in related species, such as Aspergillus ruber, for

**FIG. 2.** The NsdD orthologue of A. fumigatus is functional in A. nidulans. (A) Pairwise alignment of NsdD proteins from A. fumigatus and A. nidulans. The alignment was created based on accession number sequences Afu3g13870 (A. fumigatus) and AAF16914 (A. nidulans); identical residues are shown in black and nonconserved ones in gray, and the GATA zinc finger domain is boxed with the Ala455 residue of A. fumigatus NsdD highlighted. (B) Colony appearance of A. nidulans FGSC A4 derivatives expressing A. fumigatus (Af) or A. nidulans (An) nsdD from the nitrate-inducible niaA promoter under repressing and inducing conditions: overexpression of either nsdD gene results in repression of asexual sporulation and formation of numerous nests and fruiting bodies. (C) Complementation of deletant KHH152: the recipient strain is unable to form cleistothecia but develops mature fruiting bodies (arrows) that harbor fertile ascospores when either nsdD gene is expressed. Scale bar, 300 μm (upper panels) or 50 μm (lower panel).
FIG. 3. Forced expression of nsdD in the deuteromycete *A. fumigatus* results in the formation of coiled hyphal structures. (A) Validation of forced nsdD overexpression in the *A. fumigatus* host strain AfS45 by Northern analysis. Autoradiographs derived from Northern analysis of RNA samples obtained under repressing (NH$_4^+$) or inducing (NO$_3^-$) conditions to demonstrate nsdD steady-state transcript levels expressed from the endogenous locus or the niiA promoter, respectively, are shown. Ethidium bromide-stained rRNA serves as a loading control. (B) Colony appearance of strains expressing the nsdD gene from *A. fumigatus* or *A. nidulans*. Expression constructs were introduced in the recipient strain AfS45, and confirmed transformants were inoculated on ammonium- or nitrate-containing medium. Reduced sporulation and altered hyphal extension at the colony periphery are characteristic for the transgenic strains expressing either nsdD gene at high levels. (C) Hyphal morphology resulting from forced expression of nsdD in *A. fumigatus*. The formation of coiled hyphal structures (arrows) resulting from curling tip extension is induced specifically by high nsdD expression, whereas expression of an nsdD loss-of-function allele does not support coil formation. (D) Coil formation in *A. nidulans* upon overexpression of nsdD. Strain FGSC A4 transformed with either construct for forced expression of the nsdD gene from *A. fumigatus* (AnS24) or *A. nidulans* (AnS25) displays fortified hyphal coil formation (arrowheads) on inducing medium. Scale bar, 50 μm.
which the ascogonial coil has been described as a fertilization structure (4, 6). In other fungal genera, the formation of tightly coiled hyphae has been attributed to sexual development and cleistothecium formation, exemplified by the pathogenic fungus *Histoplasma capsulatum* (22). We did not observe further development of the *A. nidulans* coils into primordial structures, and even after prolonged incubation of the *nsdD*-overexpressing *A. fumigatus* strains, cleistothecium-like structures were not formed. The presence of both mating types is likely to be a strict necessity, but preliminary data from crossing experiments indicate that NsdD overexpression in an *A. fumigatus* *mat1-1/
mat1-2* heterokaryon is not sufficient to drive further development (our unpublished results). Balanced regulation of mating type expression could be a prerequisite for the progression of a sexual cycle (38), or specific environmental conditions might have to be present that would trigger sexual differentiation.

*A. fumigatus* reproduces by dispersal of clonal conidia; however, evidence for recent unlimited sexual recombination has been provided (29, 32). It is an open question to what extent either reproductive mode, sexual or clonal, directs *A. fumigatus* evolution. The apparent lack of sexuality of *A. fumigatus* is not based on the absence of functional mating type gene products; other reasons, such as inappropriate expression or the absence of up- or downstream components, are likely to account for this. Genome data mining has revealed the presence of a variety of *A. fumigatus* genes that have been characterized as being required for sexual development of *A. nidulans* (12), and, as exemplified for *nsdD*, their products might have retained functionality. From comparative analyses among closely related aspergilli, it is evident these genes are under purifying selection irrespective of the sexual nature of their host (10). Accordingly, their cellular roles in *A. fumigatus* remain to be scrutinized, which will yield further insight into cryptic sexuality of this human pathogen. This not only will enhance molecular biology for this fungus but might also reveal any impact of restricted sexuality on fungal pathogenicity; limiting genetic recombination and accordingly ensuring genome stability to a certain extent could support maintenance of virulence traits (18). In this respect, *A. fumigatus* behaves in line with at least two other fungal pathogens, the basidiomycete *Cryptococcus neoformans* and the commensal *Candida albicans*, which have maintained capacities for genetic recombination sexually via monokaryotic fruiting (23) or mitotically by parasexuality (19), respectively. For *A. fumigatus*, any sexual cycle awaits discovery; nevertheless, the data presented in this study are in favor of a cryptic sexuality in this saprophytic pathogen.

**ACKNOWLEDGMENTS**

We thank all former and present members of the departments for continuous support and inspiring discussions, especially Gerhard Braus and Joachim Morschhäuser and his group for help and advice. Michaela Dürrig is thanked for brilliant technical assistance, and we are indebted to Andreas Reimer from the Department of Geobiology, Center for Geosciences at the University of Göttingen, for assistance and for providing the scanning electron microscopy images. Kap-Hoon Han is thanked for the generous gift of strain KHH52 and Elaine Bignell for proofreading the manuscript.

Financial aid was received from the German Research Foundation, the Free State of Bavaria, and the University of Würzburg.

**REFERENCES**

1. Benjamin, C. R. 1955. Asccarps of *Aspergillus* and *Penicillium*. Mycologia 47:669–687.
2. Braus, G. H., S. Krappmann, and S. E. Eckert. 2002. Sexual development in ascomycetes—fruit body formation of *Aspergillus nidulans*, p. 215–244. In H. D. Osiewacz (ed.), Molecular biology of fungal development. Marcel Dekker, Inc., New York, NY.
3. Brown, T., and K. Mackey. 1997. Analysis of RNA by Northern and slot blot hybridization, p. 4.9.1–4.9.16. In F. M. Ausubel, R. Brent, R. E. Kingston, et al. (ed.), Current protocols in molecular biology. John Wiley & Sons Inc., Hoboken, NJ.
4. Bruggeman, J. 2003. Thesis. Wageningen University, Wageningen, The Netherlands.
5. Casselton, L. A. 2002. Mate recognition in fungi. Heredity 88:142–147.
6. Champe, S. P., and L. D. Simon. 1992. Cellular differentiation and tissue formation in the fungus *Aspergillus nidulans*, p. 63–91. In E. F. Rossomando and S. Alexander (ed.), Morphogenesisis: an analysis of the development of biological form. Dekker, New York, NY.
