Available online at www.sciencedirect.com
SCIENCE DIRECT®
Fungal Genetics and Biology 41 (2004) 148–156
FUNGAL GENETICS AND BIOLOGY
www.elsevier.com/locate/yfgbi

A putative high affinity hexose transporter, *hxtA*, of *Aspergillus nidulans* is induced in vegetative hyphae upon starvation and in ascogenous hyphae during cleistothecium formation

Huijun Wei,[a,1] Kay Vienken,[a] Robert Weber,[a] Sam Bunting,[a] Natalia Requena,[b] and Reinhard Fischer[a,*]

[a] *Department of Microbiology, University of Marburg and Max-Planck-Institute for Terrestrial Microbiology, Karl-von-Frisch-Strasse, Marburg D-35043, Germany*
[b] *Department of Botany—Ecophysiology, University of Tübingen, Auf der Morgenstelle 1, Tübingen D-72076, Germany*

Received 1 September 2003; accepted 22 October 2003

Abstract

Fungi employ different carbohydrate uptake systems to adapt to certain environmental conditions and to different carbon source concentrations. The hydrolysis of polymeric carbohydrates and the subsequent uptake of monomeric forms may also play a role in development. *Aspergillus nidulans* accumulates cell wall components during vegetative growth and degrades them during sexual development. We have identified the *hxtA* (high affinity hexose transporter) gene in a differential library, which was enriched for sexual-specific genes. The *hxtA* gene is disrupted by 6 introns and predicted to encode a 531 amino acid protein with high similarity to major facilitator superfamily members including the high affinity hexose transporter Gtt1 from *Trichoderma harzianum*. *A. nidulans* HxtA contains the 12 predicted transmembrane domains characteristic for this family. Deletion of *hxtA* did not impair growth of *A. nidulans* on a variety of carbon sources nor did it inhibit sexual development suggesting redundant sugar uptake systems. We found at least 17 putative hexose transporters in the genome of *A. nidulans*. Despite the high similarity of HxtA to fungal high affinity glucose transporters, the *hxtA* gene did not restore growth on glucose of a *Saccharomyces cerevisiae* mutant, in which all hexose transporters were deleted. Northern blot analysis revealed that the *A. nidulans hxtA* gene was repressed under high glucose conditions and expressed in vegetative hyphae upon carbon starvation and during sexual development. We found *hxtA(p)::sgfp* expression in developing cleistothecia specifically in ascogenous hyphae and propose that HxtA is a high affinity glucose transporter involved in sugar metabolism during sexual development.

Keywords: Spore formation; Hexose transporter; Fungal development; Fruiting body (cleistothecium)

1. Introduction

The first step of carbohydrate metabolism is the uptake of the molecules into the cell. To achieve this, microorganisms employ a variety of different membrane-bound transport proteins of the major facilitator superfamily. The unicellular yeast *Saccharomyces cerevisiae* has a set of 20 different hexose transporters, which are characterized by their substrate specificities as well as their kinetic parameters (Boles and Hollenberg, 1997; Ozcan and Johnston, 1999). The expression of several of these genes is under transcriptional control depending on the source and the amount of carbon available. In contrast to the situation in *S. cerevisiae* and other yeasts (*Kluyveromyces*, *Candida*, and *Pichia*), very little is known about sugar transporters in filamentous fungi. Several hexose transporter homologues as well as sugar sensors were identified as a result of fungal sequencing projects, differential screenings or in targeted approaches. However, there are very few studies regarding their functions in the corresponding organisms. In two biotrophic plant-interacting fungi two monosaccharide transporters involved in the

* Corresponding author. Fax: +49-6421-178-309.
E-mail address: FischerR@staff.uni-marburg.de (R. Fischer).
URL: http://www.uni-marburg.de/mpi/research.html.
[1] Present address: Department of Molecular Medicine, College of Veterinary Medicine, Cornell University, Ithaca, NY 14853, USA.

1087-1845/$ - see front matter © 2003 Elsevier Inc. All rights reserved.
doi:10.1016/j.fgb.2003.10.006

uptake of sugars from the host plant were characterized. In the basidiomycete rust fungus *Uromyces fabae* a glucose transporter is specifically expressed in haustoria, the structure where nutrient exchange between the plant and the fungus occurs. Complementation in yeast and determination of glucose transport rates revealed a K_M value of 360 μM for D-glucose and 1 mM for D-fructose (Voegele et al., 2001). In the basidiomycete ectomycorrhizal fungus *Amanita muscaria* a monosaccharide transporter was isolated using a targeted approach (Nehls et al., 1998). The protein could restore growth of a corresponding monosaccharide transport mutant of *Schizosaccharomyces pombe*. Although the expression of the mycorrhizal transporter was constitutive, it appeared to be upregulated in the presence of high glucose concentrations. In both fungi, *Uromyces* and *Amanita*, a functional analysis has yet to be done, since deletion experiments are difficult to achieve in these species. In the saprophytic fungus *Neurospora crassa* several putative hexose transporters were discovered in the genome (Galagan, 2003). Functional analysis was done before for *rco-3* (Madi et al., 1997). This gene was discovered in a mutant screening. The corresponding strain sporulated in liquid culture without carbon limitation. Glucose transport activity was altered and it appeared that Rco3 acts as a glucose sensor. Recently, a high affinity glucose transporter was characterized in *Trichoderma harzianum* (Delgado-Jarana et al., 2003). The gene, *gtt1*, was identified in a differential display experiment. It was repressed in the presence of high glucose concentrations and fully induced under low carbon conditions. *gtt1* could not complement a hexose transporter deficient *S. cerevisiae* strain and deletion of the gene in *T. harzianum* has yet to be done. However, overexpression of Gtt1 in *T. harzianum* caused a threefold increase of glucose transport velocity and the K_M value for the transport was determined to 12 μM.

In the life cycle of *A. nidulans* the availability of nutrients and their metabolism is linked to the differentiation of the fungus (Zonneveld, 1977). *A. nidulans* is able to reproduce with mitotically derived conidiospores and by meiotic ascospores (Adams et al., 1998; Fischer and Kües, 2003). Both spore forms are generated at or in morphologically differentiated structures called conidiophores and cleistothecia, respectively (Fischer, 2002). Cleistothecia are embedded into a layer of thick walled Hülle cells, which may play a role in nutrient supply for development. Whereas the developmental program for conidiophore formation is well studied, cleistothecium differentiation is only poorly understood. Conidiophore production occurs after 20 h of vegetative growth at a water–air interphase (on agar plates). However, when the mycelium is starved for carbon or nitrogen the induction of the developmental program is possible in liquid culture and hence some conidiophores are generated under these conditions (Skromne et al., 1995). A link between the nutritional status of the mycelium and development is more evident in the case of sexual reproduction. This process is initiated after several days of vegetative growth on agar plates, when external glucose is depleted (Zonneveld, 1972a,b, 1973). Since sexual development requires massive cell proliferation, energy and carbon for these processes has to be provided from sources other than glucose. It was suggested that α-1,3 glucan serves as the main reserve material and is accumulated during vegetative growth in the cell wall. Thus, after glucose exhaustion, α-1,3 glucanase is secreted to release monosaccharides. These are then taken up by the mycelium and metabolized (Zonneveld, 1972a,b, 1973, 1974). Surprisingly, we showed that deletion of the *mutA* gene encoding an α-1,3 glucanase (mutanase), did not impair sexual development although its expression was specific in Hülle cells (Wei et al., 2001). This suggested that other structural polymers (e.g., α-1,6 glucan) as well as other hydrolases were supplying the required carbon at that developmental stage. In any case, after successive degradation of the cell wall polymers the developing mycelium requires an efficient uptake system to acquire the released monosaccharides.

In this paper we identified a putative hexose transporter, HxtA, in a differential screening for sexual development-specific genes, and 16 other putative hexose transporter encoding genes in the *A. nidulans* genome sequence. The functional analysis of *hxtA* revealed that it is specifically expressed in ascogenous hyphae and in vegetative hyphae after starvation. However, it is dispensable for vegetative growth and sexual development.

2. Materials and methods

2.1. Strains, culture conditions, and plasmids

Supplemented minimal and complete media for *A. nidulans* were prepared as described, and standard strain construction procedures were used (Käfer, 1977). We used the following *A. nidulans* strains: FGSC4 *veA*$^+$ (Fungal Genetics Stock Centre, Kansas, USA; SRF200 (*pyrG89*; *ΔargB::trpCΔB*; *pyroA4*; *veA1*) (Karos and Fischer, 1999); RMS011 (*pabaA1*, *yA2*; *ΔargB::trpCΔB*; *trpC801*; *veA1*) (Stringer et al., 1991); SHWH11 (SRF200 transformed with pHHarg11); SWHH7 (cross between SHWH11 and RMS011; *pabaA1*, *yA2*; *hxtA::argB*). SWHHgfp (SRF200 transformed with pHHgfp4). Standard laboratory *E. coli* strains (XL-1 blue, Top 10 F′) were used. Standard DNA transformation procedures were used for *A. nidulans* (Yelton et al., 1984) and *E. coli* (Sambrook and Russel, 1999). For PCR experiments standard protocols were applied using a capillary Rapid Cycler (Idaho Technology, Idaho Falls, USA) for the reaction cycles. DNA sequencing was done commercially (MWG Biotech, Ebersberg). Genomic DNA was extracted from the fungus with the DNeasy Plant Mini Kit

(Qiagen, Hilden, Germany). RNA was isolated with TRIzol from Invitrogen according to the manufacturer protocol (Karlsruhe, Germany). DNA and RNA analyses (Southern and northern hybridizations) were performed as described (Sambrook and Russel, 1999).

2.2. Plasmids

2.2.1. Cloning of the hxtA gene, a corresponding cDNA and sequencing

The partial sequence obtained from the SSH (suppressive subtractive hybridization) library was used to design primers for amplification of a specific *hxtA* fragment (HexA5: 5′-GTTTCGACATCTCGTCGATG-3′; HexA3: 5′-CCAGCGGTCCTTGCTCG-3′). With this fragment we isolated a cosmid (pH38E7) carrying the entire gene. We subcloned two overlapping restriction fragments, a 4.0 kb *Hin*dIII (pHHH1) and a 3.0 kb *Sal*I–*Pst*I into pBluescript KS- (Invitrogen) (pHHPS4). The restriction fragments were sequenced on both strands and the sequence information assembled with the genome data from Cereon Genomics LLC (Cambridge, USA). *hxtA* cDNA was obtained by RT-PCR using RNA isolated from the sexual developmental stage and the following primer combinations: (HexR4: 5′-ATGCTTTT CTTCATTGATTAGTG-3′; Hex-ex2: 5′-AATGGA CTCGAGATTACACCGCCTTCTCCGGT-3′). cDNA was cloned into pBluescript KS- into *Eco*RV–*Xho*I (*Xho*I site included in the primer, underlined) and subsequently sequenced.

2.2.2. Cloning of the hxtA disruption construct (pHHRarg11)

We substituted a 0.6 kb *Pst*I–*Eco*RI fragment (see Fig. 2) in pHHH1 by *argB*. pHHRarg11 was linearized by *Xba*I and transformed into *A. nidulans* SRF200.

2.2.3. Expression of hxtA in S. cerevisiae

The cDNA of the *hxtA* open reading frame was amplified with HexR2 (5′-TGAAGATGGCTTTCAAG AAGTCCT-3′) and Hex-ex2 using Pfu DNA polymerase with proofreading function (Promega, Madison, USA). The cDNA clone was introduced into the yeast expression vector pDR196 under the control of the constitutive promoter PMA1 (H^+-ATPase). The vector was kindly provided by D. Rentsch (Neuchatel, Switzerland). The plasmid was transformed into the yeast strain EWY.4000 lacking all 20 hexose transporters (Wieczorke et al., 1999). As a positive control we used the high affinity yeast hexose transporter gene *HXT2*.

2.2.4. Construction of the hxtA::sgfp transcriptional fusion (pHHgfp4)

The putative *hxtA* promoter region (2.5 kb) was amplified by PCR with KS-Rev1 (5′-GCCAAGCGCGCAA TTAACCCTCACT-3′) and Hex–*Not*I (5′-CGCGGCCGC CATCTTCAAAGGCGGTGCT-3′) using Pfu polymerase and pHHPS4 as template. The PCR fragment was cloned blunt end into pBluescript *Eco*RV. The Hex-*Not*I primer introduced a *Not*I restriction site (underlined) at the start codon of *hxtA*. This *Not*I site and the one in the pBluescript polylinker were used to insert sGFP as a *Not*I fragment obtained from pRF917 (Requena et al., 2001). Translation of the construct will be terminated by a stop codon within the polylinker after the sGFP.

2.3. Fluorescence microscopy

sGFP-expressing strains were observed with a Zeiss Axiophot microscope and appropriate filter combinations. For the documentation we used a OrcaII ER camera (Hamamatsu, Munich).

3. Results and discussion

3.1. hxtA encodes a putative high affinity hexose transporter

In order to isolate sexual development-specific genes, we constructed a SSH library (Wei et al., 2001). One partial sequence obtained in the library encoded a peptide with high homology to high affinity hexose transporters. The partial cDNA sequence was used to isolate a cosmid (pH38E7) from the pUI library (kindly provided by B. Miller, Idaho, USA). Sequences from subclones of the cosmid (pHHH1 and pHHPS4) were assembled together with partial sequences obtained from the genomic DNA database at Cereon LLC (Cambridge, USA). According to the genomic DNA sequence meanwhile available at the Whitehead Institute (http://www-genome.wi.mit.edu/annotation/fungi/aspergillus/) the *hxtA* gene is located on chromosome I. In order to deduce a putative hexose transporter protein sequence, we analyzed a corresponding cDNA generated by RT-PCR (see Section 2). Comparison of genomic DNA with the cDNA sequences revealed the presence of 6 short introns, between 44 and 67 bp in length. Four are located at the 5′-end of the gene, one in the middle and one close to the end of the coding region (Fig. 1). A putative promoter was identified using a promoter prediction program (http://genes.mit.edu/McPromoter.html) which determined the start of transcription 68 bp upstream of the start of translation. After removal of the intron sequences, an open reading frame of 531 amino acids could be deduced, which encodes a protein with a calculated molecular mass of 59 kDa and an isoelectric point of 8.44. Sequence analysis (http://www.cbs.dtu.dk/services/TMHMM) revealed the presence of 12 transmembrane domains and a larger cytoplasmic loop between the sixth and seventh transmembrane helix. These features are characteristic for members of the major facilitator

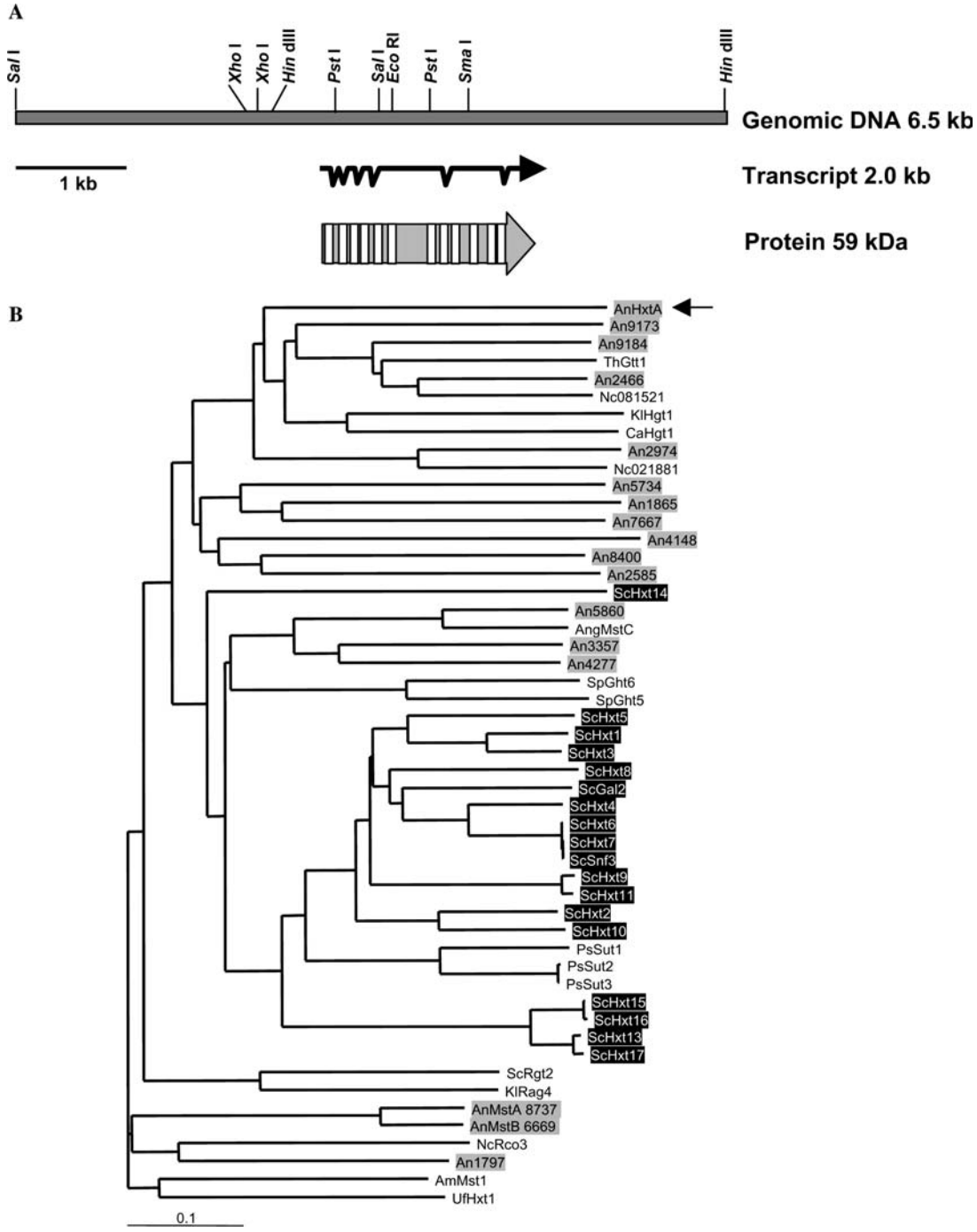

Fig. 1. (A) Scheme of the *hxtA* locus. (B) Phylogram of 51 hexose transporter-related proteins from *A. nidulans* and other fungi. Besides *A. nidulans* HxtA (arrow) and MstA, the other putative *A. nidulans* proteins are predicted primary structures. The sequence of *A. nidulans hxtA* is available under the Accession No. AJ535663. The other *A. nidulans* protein sequences were obtained from: http://www.genome.wi.mit.edu/ftp/pub/annotation/aspergillus/assembly1/aspergillus_nidulans_1_proteins.fasta.gz. The phylogram was constructed with the neighbor-joining algorithm implemented on the program ClustalW. A tree was derived from the primary data using the program TreeView. *Abbreviations*: Sc, *S. cerevisiae*; An, *A. nidulans*; Th, *T. harzianum*; Nc, *N. crassa*; Kl, *Kluyveromyces lactis*; Ca, *Candida albicans*; Ang, *A. niger*; Sp, *Schizosaccharomyces pombe*; Ps, *Pichia stipitis*; Am, *Amanita muscaria*; Uf, *Uromyces fabae*.

superfamily (Marger and Saier, 1993). The putative HxtA protein shared similarities between 30 and 43% to a predicted protein of *N. crassa* (NCU08152.1), to the high affinity glucose transporter Gtt1 from *T. harzianum* (Delgado-Jarana et al., 2003) and several other fungal high affinity hexose transporters (Fig. 1). The sequence of *A. nidulans hxtA* is available at the EMBL database under the Accession No. AJ535663.

To address the question how many hexose transporters might exist in *A. nidulans* besides HxtA, we analyzed the *A. nidulans* genomic DNA sequence at the Whitehead Institute (http://www-genome.wi.mit.edu/annotation/fungi/aspergillus) for putative candidates. The genome of *S. cerevisiae* contains 20 hexose transporter-related proteins, Hxt1-17, Gal2, and two glucose sensors Snf3 and Rgt2 (Wieczorke et al., 1999). However, Hxt12 appears to be a pseudogene and the role in hexose uptake is rather questionable (Boles, Frankfurt, personal communication). Therefore, we used the 19 yeast transporters to search in the *A. nidulans* database and analyzed the first 10 hits of the blast search, the first 10 hits from a blast search with *A. nidulans* HxtA as well as several other fungal hexose transporters obtained in a blast search of the NCBI database for the construction of an unrooted phylogram (Fig. 1). The analysis showed that *A. nidulans* contains at least 17 putative hexose transporters with amino acid numbers between 477 and 584. Candidate genes encoding homologues to the glucose sensors Rgt2 and Snf3 in *S. cerevisiae*, which are about 200 and 300 amino acids longer than the other transporters, are not obvious in *A. nidulans* (Fig. 2). However, the sensor of *N. crassa* Rco-3 consists of 594 amino acids and the C-terminal extension has no similarity to the extensions of Snf3 or Rgt2. The identities between the 17 proteins of *A. nidulans* range from 20 to 62% (Fig. 2). Two proteins MstA and MstB are even 84% identical. MstA was deposited in the NCBI database but two sequences in the Whitehead database were annotated with MstA. To distinguish the two, we named the second Whitehead Sequence MstB. The contig covering *mstA* was annotated on linkage group III whereas *mstB* derived from linkage group I. Several important amino acids are conserved in all 17 *A. nidulans* sequences. Since the sequence of most of the genes has not been confirmed yet and especially the exon–intron borders were not determined experimentally, some sequences still might contain some sequence mistakes. Therefore, we also highlighted amino acid residues in Fig. 2 when only more than 8 were identical. Since the genome of *A. nidulans* contains more than 100 candidates with similarity to major facilitator proteins it is likely that the total number of hexose transporters is even higher. In *Candida albicans* 20 putative hexose transporters were identified (Fan et al., 2002).

3.2. *hxtA is dispensable for vegetative growth, asexual and sexual development*

In order to ascribe a biochemical and physiological function to HxtA, we deleted the corresponding gene. A *hxtA* wild type strain (SRF200) was transformed with the knock-out construct pHHRarg11 and arginine prototrophic strains were selected. From 20 transformants only one presented a homologous integration event, which deleted a 0.6 kb *Pst*I–*Eco*RI fragment from the *hxtA* gene and disrupted the coding region with the *argB* gene as shown by Southern blot analysis (Fig. 3). Genomic DNA was digested with *Bam*HI and hybridized to the probe indicated in the figure. In the wild type a ca. 17.5 kb band appeared while the deletion strain (SHWH11) showed two bands of 14.5 and 4 kb. Additional restriction digests with *Hin*dIII and *Xho*I confirmed the deletion (not shown). To exclude the possibility that the deletion strain still contained some wild type nuclei in a heterokaryon, we crossed the strain SHWH11 to the *hxtA* wild type strain RMS011 and selected a *ΔhxtA* strain (SWHH7), which only required *p*-aminobenzoic acid as supplement. This strain was used for phenotypic analysis (Fig. 3). The strain grew on minimal media supplemented with *p*-aminobenzoic acid and different glucose amounts (0.02–100 mM) like wild type. No abnormality with regards to hyphal extension, asexual development or fruiting body formation was obvious (data not shown). Since the expression pattern suggested a role in sexual development (see below), we studied this process in more detail but could not find any indication for a delay in fruiting body or ascospore development or the viability of ascospores. There was also no difference in vegetative growth with respect to a wild type on other carbon sources such as fructose, sorbose, mannose, galactose, galacturonic acid, xylose, ribose, arabinose, saccharose, lactose, maltose, cellobiose, melibiose, and raffinose. These results suggest that either we have not found the carbon molecule transported by HxtA or that the substrate spectrum of HxtA overlaps with that from other *A. nidulans* hexose transporters. This is also the case in *S. cerevisiae*, in which the *GAL2* gene (encoding the galactose permease) had to be deleted in addition to other 17 transporters (*HEX1-17*) in order to obtain a strain unable to use glucose (Wieczorke et al., 1999). However, evidence against an overlapping function of HxtA with other hexose transporters was provided by a complementation experiment in a yeast strain (EWY.4000) in which the 18 hexose transporter genes (*HEX1-17*, *GAL2*) were deleted (Wieczorke et al., 1999). There, *hxtA* cDNA was cloned into an expression vector under the control of the constitutive H^+–ATPase promoter. As a positive control we used the high affinity glucose transporter, Hxt2, from *S. cerevisiae*. Whereas the transfection of the *S. cerevisiae* gene restored growth of EWY.4000 on 2% glucose, introduction of *A. nidulans hxtA* did not. The transformed strain was also unable to grow on other sugars such as fructose, galactose, mannose and xylose. This result indicates that either HxtA is transporting a different carbon source or that the lack of complementation is due to, e.g., protein misfolding or improper insertion into the membrane. Another possibility is that the *A. nidulans* gene is not expressed in *S. cerevisiae* despite the fact that the expression system worked well with *HXT2*. A similar lack of heterologous complementation was recently described for the high

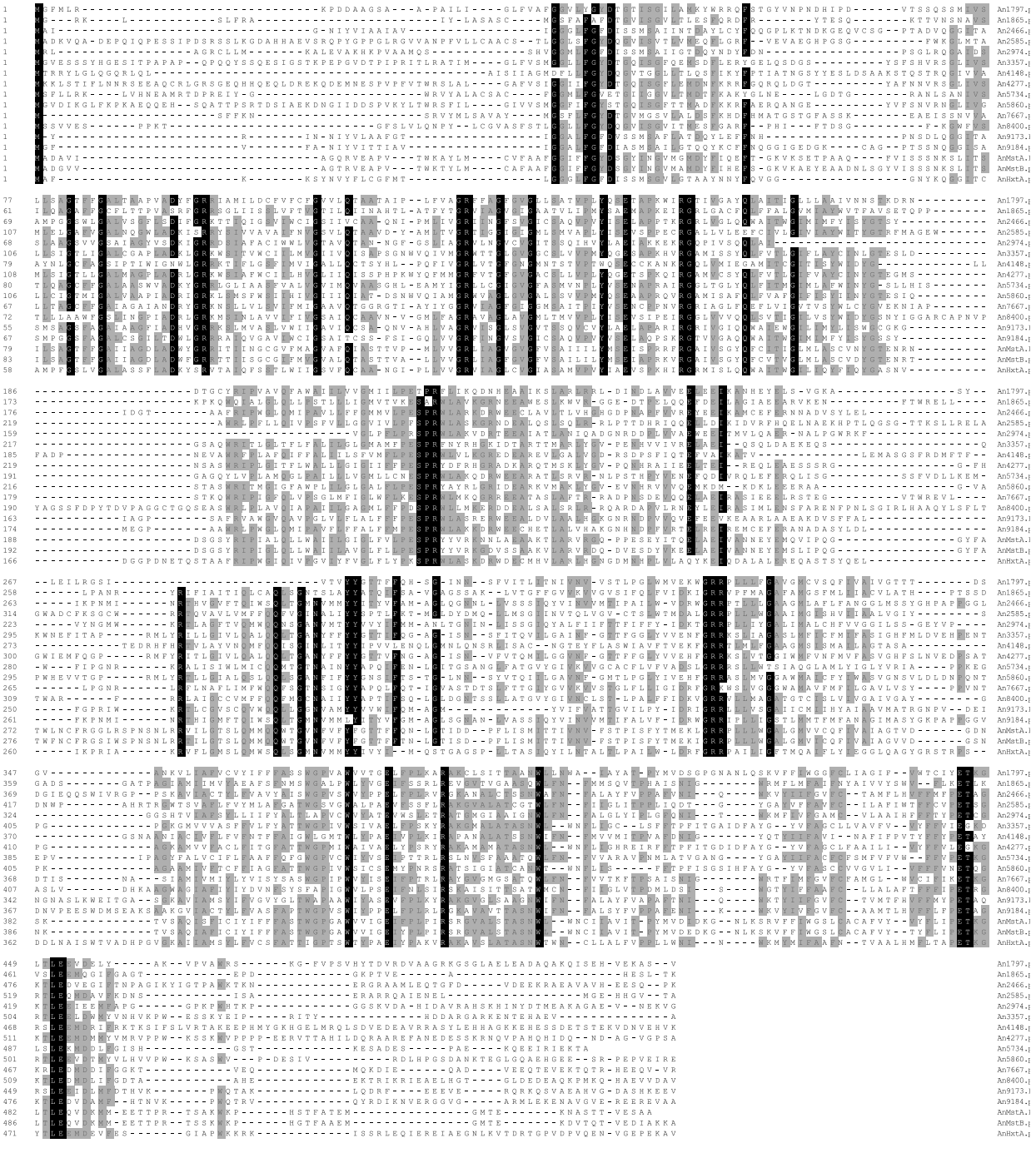

Fig. 2. Alignment of 17 putative hexose transporters from *A. nidulans*. The sequences were aligned with ClustalW from the software package DNAStar. If more than 8 amino acids were identical they were shaded in gray and if more than 15 amino acids were identical they were highlighted in black.

affinity glucose transporter Gtt1 from *T. harzianum* (Delgado-Jarana et al., 2003), which clustered very closely to HxtA in our phylogenetic analysis (Fig. 1).

3.3. hxtA is transcriptionally induced upon starvation and during sexual development

To study the expression of the putative hexose transporter, we analyzed the transcript levels at different physiological and developmental stages. *A. nidulans* (FGSCA4) was grown in liquid culture for 20 h, then the mycelium washed and incubated either in minimal medium with 100 mM glucose or in the absence of glucose. Mycelium was harvested and processed for northern blot analysis. The *hxtA* transcript was not detectable in the presence of glucose but was induced in the cultures without glucose indicating that starvation induces *hxtA* expression (data not shown). In addition to the northern

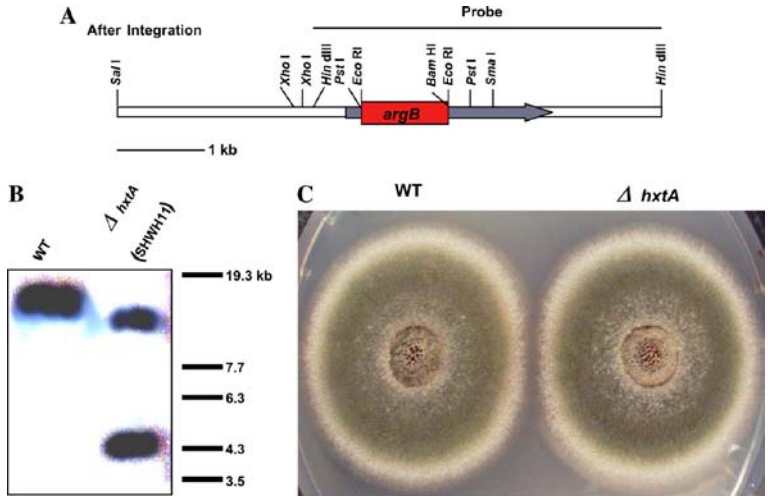

Fig. 3. Deletion of the *hxtA* gene. (A) Scheme of the *hxtA* locus after integration of the deletion construct. (B) Southern blot analysis of a *hxtA* deletion (SHWH11) and a wild type strain. Genomic DNA was restricted with *Bam*HI and after separation and blotting hybridized to the probe indicated in (A). (C) Colonies of the *hxtA* deletion (SWHH7) and a wild type strain after 3 days of growth at 37 °C.

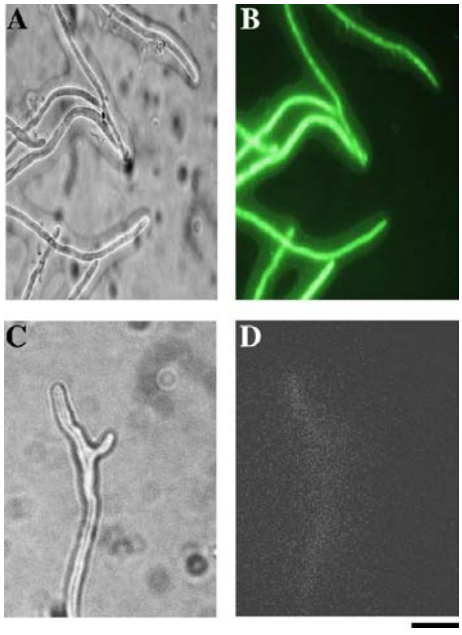

Fig. 4. Expression of *hxtA* in hyphae. The *hxtA* promoter was fused to *sgfp* and transformed into SRF200. Strain SWHHgfp was grown in minimal medium with 2% glucose for 12 h and then transferred to minimal medium without glucose (pictures A and B) or to minimal medium with 2% glucose (pictures C and D). Pictures were taken after 2 h of incubation. Left, phase contrast; right, gfp. The scale bar represents 50 μm (A and B) and 20 μm (C and D).

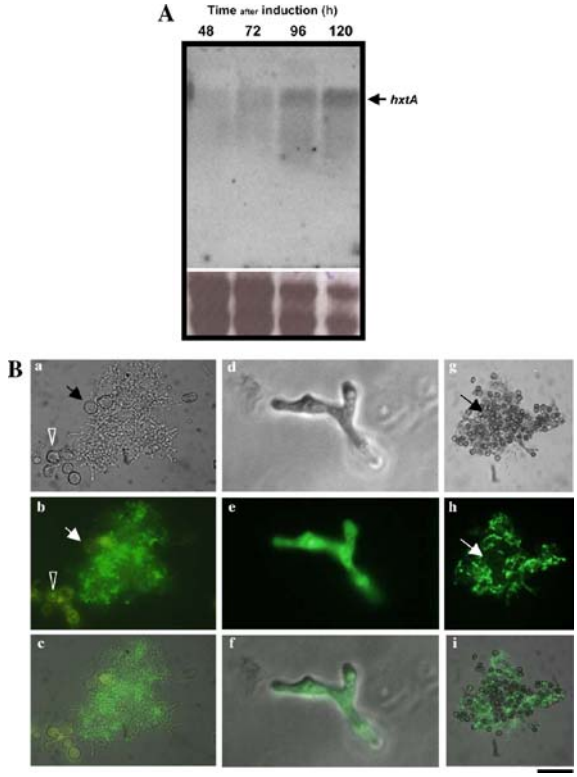

Fig. 5. Expression of *hxtA* during sexual development. (A) RNA of FGSCA4 was isolated from mycelia grown on an agar surface for the times indicated above the lanes and hybridized to a *hxtA*-specific probe in a Northern blot. (B) Strain SWHHgfp was induced for sexual development and cleistothecia were inspected for GFP fluorescence. Upper panels, phase contrast; middle, GFP channel; lower panels, overlay. Pictures a–c show hyphae from inside the cleistothecium. The arrow points to a Hülle cell which is not fluorescent, the open arrow head to a fluorescent Hülle cell. d–f ascogenous hypha. g–i ascogenous hyphae and ascospores (arrow). The scale bar represents 50 μm in (a–c and g–i) and 20 μm in (d–f).

blot analysis, we studied the expression using GFP as a reporter. The 2.5 kb of the putative *hxtA* promoter region were fused to the *gfp* gene. This transcriptional reporter construct was transformed into RMS011 and 20 strains were isolated. Growth of these strains in medium with glucose concentrations above 0.5% did not

induce any fluorescence. However, when these strains were grown on concentrations below 0.5% glucose or when glucose was omitted from the medium a strong GFP signal was observed in the cytoplasm (Fig. 4). Weak signals appeared already after 20 min of starvation. These results support again the hypothesis that HxtA, similarly to Gtt1 from *T. harzianum*, is likely to be a high affinity glucose transporter.

To study the expression during development, we grew *A. nidulans* FGSC4 on filters on agar plates. Time-course mRNA analysis showed that after about 40 h of growth, when Hülle cells appeared in the mycelial mat, no *hxtA* transcript was detectable yet. However, after 48 h, when cleistothecia started to develop, the *hxtA* transcript appeared and its level increased steadily up to 120 h when large amounts of cleistothecia were present (Fig. 5).

Since sexual development is induced when the external glucose supply decreases, the appearance of the *hxtA* transcript in developing cultures could be simply due to carbon starvation (Zonneveld, 1972a, 1974). To address this question we studied the spatial expression of the *hxtA gfp* fusion. Two strains were induced for sexual development and GFP fluorescence was analyzed in the aging mycelium and developing cleistothecia. Whereas in the mycelium only weak fluorescence was detected, we found strong signals within the hyphae inside young cleistothecia (Fig. 5). In particular, ascogenous hyphae showed strong fluorescence. Similarly, in *S. pombe* a putative hexose transporter was identified in a screening for meiosis-specific genes and was shown to be dependent on the transcription factor Mei4, which is required for the progression of meiosis (Watanabe et al., 2001). In contrast to the expression pattern of *hxtA*, low in Hülle cells and high in ascogenous hyphae, we found that the mutanase encoding gene *mutA* was highly expressed in Hülle cells, in which the young cleistothecia are embedded, and not in cleistothecia (Wei et al., 2001). Interestingly, a similar localization pattern to HxtA in *A. nidulans* was found for the product of the *asd1* gene in *N. crassa*. It encodes a putative rhamnogalacturonase and was detected in croziers and young asci, but only very weak in mature asci and developing ascospores (Nelson et al., 1997). Those and our results suggest a complex regulation of cell wall turnover and carbon supply of developing tissues in fungi. Our hypothesis is that in *A. nidulans*, Hülle cell secretion of cell wall lytic enzymes releases monosaccharides from the reserve material (cell walls) and that turns on the expression of high affinity glucose transporters to nurse the developing cleistothecia, indicating a task distribution between different cell types during sexual development.

Acknowledgments

We thank Dr. E. Boles (University of Frankfurt) for the permission to use the yeast strain EWY.4000. We thank A. Leyva and A. Ocon (University of Tübingen) for their help with the yeast transformation experiments. Sam Bunting was a student of the Erasmus program between the University of Marburg and the University of Oxford. This work was supported by the SFB 395, the Fonds der Chemischen Industrie, the Deutsche Forschungsgemeinschaft (DFG) and the Max-Planck-Institute for terrestrial Microbiology.

References

Adams, T.H., Wieser, J.K., Yu, J.-H., 1998. Asexual sporulation in *Aspergillus nidulans*. Microbiol. Mol. Biol. Rev. 62, 35–54.

Boles, E., Hollenberg, C.P., 1997. The molecular genetics of hexose transport in yeasts. FEMS Microbiol. Lett. 21, 85–111.

Delgado-Jarana, J., Moreno-Matios, M.A., Benitez, T., 2003. Glucose uptake in *Trichoderma harzianum*: role of the *Gtt1* gene in glucose transport. Eukaryot. Cell 2, 708–717.

Fan, J., Chaturvedi, V., Shen, S.H., 2002. Identification and phylogenetic analysis of a glucose transporter gene family from the human pathogenic yeast *Candida albicans*. J. Mol. Evol. 55, 336–346.

Fischer, R., 2002. Conidiation in *Aspergillus nidulans*. In: Osiewacz, H.D. (Ed.), Molecular Biology of Fungal Development. Marcel Dekker, New York, pp. 59–86.

Fischer, R., Kües, U., 2003. Develomental processes in filamentous fungi. In: Prade, R.A., Bohnert, H.J. (Eds.), Genomics of Plants and Fungi. Marcel Dekker, New York, pp. 41–118.

Galagan, J.E. et al., 2003. The genome sequence of the filamentous fungus *Neurospora crassa*. Nature 422, 859–868.

Käfer, E., 1977. Meiotic and mitotic recombination in *Aspergillus* and its chromosomal aberrations. Adv. Genet. 19, 33–131.

Karos, M., Fischer, R., 1999. Molecular characterization of HymA, an evolutionarily highly conserved and highly expressed protein of *Aspergillus nidulans*. Mol. Genet. Genomics 260, 510–521.

Madi, L., McBride, S.A., Bailey, L.A., Ebbole, D.J., 1997. *rco-3*, a gene involved in glucose transport and conidiation in *Neurospora crassa*. Genetics 146, 499–508.

Marger, M.D., Saier, M.H., 1993. A major superfamily of transmembrane facilitators that catalyse uniport, symport and antiport. Trends Biochem. Sci. 18, 13–20.

Nehls, U., Wiese, J., Guttenberger, M., Hampp, R., 1998. Carbon allocation in ectomycorrhizas: identification and expression analysis of an *Amanita muscaria* monosaccharide transporter. Mol. Plant–Microbe Interact. 11, 167–176.

Nelson, M.A., Merino, S., Metzenberg, R.L., 1997. A putative rhamnogalacturonase required for sexual development of *Neurospora crassa*. Genetics 146, 531–540.

Ozcan, S., Johnston, M., 1999. Function and regulation of yeast hexose transporters. Microbiol. Mol. Biol. Rev. 63, 554–569.

Requena, N., Alberti-Segui, C., Winzenburg, E., Horn, C., Schliwa, M., Philippsen, P., Liese, R., Fischer, R., 2001. Genetic evidence for a microtubule-destabilizing effect of conventional kinesin and analysis of its consequences for the control of nuclear distribution in *Aspergillus nidulans*. Mol. Microbiol. 42, 121–132.

Sambrook, J., Russel, D.W., 1999. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

Skromne, I., Sánchez, O., Aguirre, J., 1995. Starvation stress modulates the expression of the *Aspergillus nidulans brlA* regulatory gene. Microbiology 141, 21–28.

Stringer, M.A., Dean, R.A., Sewall, T.C., Timberlake, W.E., 1991. *Rodletless*, a new *Aspergillus* developmental mutant induced by directed gene inactivation. Genes Dev. 5, 1161–1171.

Voegele, R.T., Struck, C., Hahn, M., Mendgen, K., 2001. The role of haustoria in sugar supply during infection of broad bean by the rust fungus *Uromyces fabae*. Proc. Natl. Acad. Sci. USA 98, 8133–8138.

Watanabe, T., Kiyashita, K., Saito, T.T., Yoneki, T., Kakihara, Y., Nabeshima, K., Kishi, Y.A., Shimoda, C., Nojima, H., 2001. Comprehensive isolation of meiosis-specific genes identifies novel proteins and unusual non-coding transcripts in *Schizosaccharomyces pombe*. Nucleic Acids Res. 29, 2327–2337.

Wei, H., Scherer, M., Singh, A., Liese, R., Fischer, R., 2001. *Aspergillus nidulans* alpha-1,3 glucanase (mutanase), mutA, is expressed during sexual development and mobilises mutan. Fungal Genet. Biol. 34, 217–227.

Wieczorke, R., Krampe, S., Weierstall, T., Freidel, K., Hollenberg, C.P., Boles, E., 1999. Concurrent knock-out of at least 20 transporter genes is required to block uptake of hexoses in *Saccharomyces cerevisiae*. FEBS Lett. 464, 123–128.

Yelton, M.M., Hamer, J.E., Timberlake, W.E., 1984. Transformation of *Aspergillus nidulans* by using a *trpC* plasmid. Proc. Natl. Acad. Sci. USA 81, 1470–1474.

Zonneveld, B.J.M., 1972a. Morphogenesis in *Aspergillus nidulans*. The significance of α-1,3 glucan of the cell wall and α-1,3 glucanase for cleistothecium development. Biochim. Biophys. Acta 273, 174–187.

Zonneveld, B.J.M., 1972b. A new type of enzyme, an exo-splitting α-1,3 glucanase from non-induced cultures of *Aspergillus nidulans*. Biochim. Biophys. Acta 258, 541–547.

Zonneveld, B.J.M., 1973. Inhibitory effect of 2-deoxy glucose on cell wall α-1,3-glucan synthesis and cleistothecium development in *Aspergillus nidulans*. Dev. Biol. 34, 1–8.

Zonneveld, B.J.M., 1974. α-1,3 glucan synthesis is correlated with α-1,3 glucanase synthesis, conidiation and fructification in morphogenetic mutants of *Aspergillus nidulans*. J. Gen. Microbiol. 81, 445–451.

Zonneveld, B.J.M., 1977. Biochemistry and ultrastructure of sexual development in *Aspergillus*. In: Smith, J.E., Pateman, J.A. (Eds.), Genetics and Physiology of *Aspergillus*. Academic Press, London, pp. 58–80.