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Molecular characterization of ABC transporter-encoding genes in *Aspergillus nidulans*

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ABSTRACT. As a preliminary step towards characterizing genes encoding ATP-binding cassette (ABC) transporters that confer pleiotropic drug resistance in *Aspergillus*, we used a PCR-based approach to isolate four DNA fragments corresponding to different ABC type transporter genes. DNA sequencing and Southern blot analysis confirmed that they were distinct genes, which were designated *abc*A-D. One of these genes, *abc*D, was cloned and characterized. It was found to have a predicted 1,452-amino acid translation product with a calculated molecular mass of 147,467 kDa. The *abc*D gene specifies a single transcript of approximately 5.0 kb; there was a two- to six-fold enhancement of mRNA levels following exposure to miconazole, camptothecin, methotrexate, and ethidium bromide.

Key words: ATP-binding cassette transporters, Fungal infections, *Aspergillus nidulans*, Multidrug resistance

INTRODUCTION

The incidence of fungal infections has dramatically increased in recent decades. *Candida albicans* is the predominant cause of fungal infections in hospital patients, although in immunocompromised individuals, invasive aspergillosis is an increasingly common disease of mortality. *Aspergillus fumigatus* and *A. flavus* are two of the most prevalent opportunistic pathogens involved in human aspergillosis. Mortality due to this disease has remained excessively high despite treatment with antifungal agents (Denning and Stevens, 1990). Recent failures in the drug treatment of fungal infections and improvements in the performance and standardization of antifungal-susceptibility testing have drawn attention to the problem of antifungal resistance. Although extremely rare ten years ago, resistance to antifungal drugs is quickly becoming a major problem in certain populations, especially in patients infected with HIV and drug-resistant yeasts that cause oropharyngeal candidiasis (for a review, see White et al., 1998). It is now clear that antifungal resistance presents clinical challenges that are analogous to those found with antibiotic-resistant bacteria (Vanden Bossche et al., 1994, 1998; Rex et al., 1995; Albertson et al., 1996; Kelly et al., 1996; Denning et al., 1997a,b; Nolte et al., 1997; Joseph-Horne and Hollomon, 1997).

The typical determinants of multidrug resistance (MDR) in eukaryotic organisms, i.e., the development of resistance to a wide range of unrelated cytotoxic compounds, are transport proteins responsible for the efflux of toxic compounds. In this context, the P-glycoprotein family of transporters accounts for high-level resistance of tumor cells to anticancer drugs (for reviews, see Gottesman and Pastan, 1993; Gottesman et al., 1995). Overexpression of the human MDR1 gene produces a P-glycoprotein, an ATP-dependent membrane pump that results in an increased efflux of chemotherapeutic drugs (Gottesman and Pastan, 1993). These proteins require ATP hydrolysis to pump a substrate (or several substrates) across a cell membrane against a concentration gradient (Higgins, 1992). ATP-biding cassette (ABC) transporters have been identified in a wide variety of organisms, including mammals, yeast, filamentous fungi, bacteria, insects, and protozoa (van Veen and Konings, 1998). Energy-dependent drug efflux mechanisms have been implicated in MDR in *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, *Candida* spp., and more recently in *Aspergillus nidulans*, *A. fumigatus*, *A. flavus*, and *Penicillium digitatum* (for reviews, see Balzi and Goffeau, 1991, 1994; Del Sorbo et al., 1997; Tobin et al., 1997; Kolaczkowski and Goffeau, 1997; Decottignies and Goffeau, 1997; White et al., 1998; Nakaune et al., 1998; de Souza et al., 1998; Angermayr et al., 1999). However, little work has been done on clinical drug resistance in pathogenic *Aspergillus* species. Denning et al. (1997a) reported the occurrence of itraconazole resistance in *A. fumigatus* and provided evidence for two different resistance mechanisms involving drug efflux and target modification.

A. nidulans is a nonpathogenic species with a well-developed genetic system that has been useful for studying the molecular genetics of microtubules, mitosis and development. It is an excellent model system for investigating different aspects of drug resistance in filamentous fungi. As a preliminary step towards characterizing genes encoding ABC transporters that confer pleiotropic drug resistance in *Aspergillus*, we used a PCR-based approach to isolate DNA fragments that correspond to ABC transporter-encoding genes. We discovered, cloned and partially characterized genes encoding MDR-like proteins in *A. nidulans*.

MATERIAL AND METHODS

Aspergillus nidulans strains and growth methods

All strains of *A. nidulans* are derived from a haploid nucleus and therefore are isogenic, except for differences induced by mutagenic treatments (Pontecorvo et al., 1953). The strain R21 (*y*A1 *paba*A1) was used throughout this work. A complete medium was used (YAG: 2% glucose, 0.5% yeast extract, 2% agar, and trace elements). Additional trace elements, vitamins and nitrate salts are described in Kafer (1977).

Identification of DNA fragments that correspond to ABC transporter-encoding genes

Identification and isolation of *A. nidulans* genomic DNA sequences homologous to other genes encoding ABC transporter proteins was accomplished using the polymerase chain reaction (PCR) technique. The primers used for amplification were designed on the basis of consensus sequences derived from an alignment of the most highly conserved segments, the so-called Walker motifs (Walker et al., 1982), in the ATP-binding domains of more than 30 presumptive eukaryotic ABC-type transporters. The oligonucleotide primers synthesized also reflected the codon usage bias of *A. nidulans* (Lloyd and Sharp, 1991). The primer Asp1 (5'-GCYCTCGTYGGICCCTCIGG-3') or Asp3 (5'-GCYCTCGTYGGICCCAGYGG-3'), encoding the amino acid sequence ALVGPSG, was used in combination with Asp2 (5'-GATRCGYTGCTTYTGICCICC-3'), the complementary strand to that encoding GGQKQRI. The primer Asp4 (5'-GTYGGTTCHTCHGGHTGYGGWAA-3'), encoding the amino acid sequence VGSSGCGK was used in combination with Asp5 (5'-RTCYAAAGCDGADGTDGCYTCATC-3'), the complementary strand to that encoding the amino acid sequence DEATSALD. PCR analysis was performed in a reaction mixture consisting of 50 mM KCl, 1.5 mM $MgCl_2$, 10 mM Tris-HCl, pH 8.8, 50 µM (each) dATP, dCTP, dGTP, and dTTP (Boehringer), 1 µg of primer, 0.5 U of Taq DNA polymerase (Perkin-Elmer), and 50 ng of template DNA. Amplification was performed in a PTC-100 Programmable Thermal Controller (MJ Research, Inc.). All manipulations were carried out with dedicated DNA-free pipettes in a sterile field to minimize the risk of contamination. All reagents were added together except for the Taq polymerase. The reaction mixture was overlaid with 50 µl of mineral oil and was incubated in the DNA thermalcycler. The DNA amplification was through 30 cycles, as follows: 94°C for 2 min, 94°C for 45 s, a touchdown in the annealing temperature from 45 to 40°C for 30 s (Asp4 x Asp5) and from 55 to 50°C for 30 s (Asp1 x Asp2 and Asp2 x Asp3), 72°C for 1 min and 30 s. The reaction mixture was held at 4°C until required. The amplified products were resolved by electrophoresis on a 1% agarose gel TBE buffer. The PCR fragments were subcloned using a pMOS kit (Amersham-Pharmacia).

Genomic library and screening

Colonies of a chromosome specific library developed from *A. nidulans* (Fungal Genetics Stock Center) were transferred onto Hybond-N membranes (Amersham) and hybridized with an approximately 400-bp PCR fragment that corresponds to the *abc*D gene from *A. nidulans*. This fragment was radioactively labeled by random primer reaction (Boehringer) using [α-^{32}P]-

dCTP (Amersham). Hybridization was carried out at 65°C in 2X standard saline citrate (SSC), 0.25% milk powder, 0.1% sodium dodecyl sulfate (SDS) solution, and 40 µg/ml salmon-sperm DNA. The filters were washed at 65°C twice for 15 min in 2X SSC and 0.05% SDS. The filters were exposed on Kodak XAR-5 X-ray film at -70°C using intensifying screens. The complete sequence of the *abc*D gene was determined by the dideoxy-chain termination method from both strands, using synthetic oligonucleotide primers with the Big-Dye Terminator kit (Perkin-Elmer).

DNA/RNA manipulations

Restriction enzyme digests and DNA ligations were performed in accordance with the suppliers' (Boehringer/Amersham) recommendations. Plasmid DNA isolation from *E. coli* and Southern blotting were performed using standard procedures (Sambrook et al., 1989). DNA probes were made using a random primer system according to the manufacturer's instructions (Boehringer).

Northern analysis material was prepared by inoculating 5.0 x 10^4 *A. nidulans* conidiospores per ml of complete medium. The cultures were incubated in a reciprocal shaker at 37°C for 12 h and then the mycelia were aseptically transferred to fresh YG medium where the different drugs were added. Twenty micrograms of RNA from each treatment was then fractionated in 2.2 M formaldehyde, 1% agarose gel, and then transferred to Hybond-N+ membranes (Amersham) with a vacuum, in 0.05 N NaOH. Prehybridization and hybridization were performed according to Sambrook et al. (1989). In all the Northern analysis experiments, the RNA concentration was normalized by densitometric analysis of the ribosomal RNAs using the program Molecular Analysis (BioRad).

RESULTS

Identification of ATP-binding cassettes by PCR

To detect ABC transporter-encoding genes in *A. nidulans*, we performed PCR on genomic DNA, using degenerate oligonucleotide primers corresponding to the sequences of the Walker A and B motifs in the ATP-binding domains (Walker et al., 1982). Agarose gel electrophoresis of PCR products revealed three strong bands at the expected size of ~400 bp for all the combinations of primer mixtures. These bands were excised from the gel and DNA fragments were isolated and cloned. Sequencing of inserts of plasmids from about 100 transformant colonies produced four different sequences (one for the combination Asp1 x Asp2, one for Asp2 x Asp3, and two for Asp4 x Asp5; see Material and Methods). All four fragments contained typical ATP-binding boxes and ABC signature sequences and were thus identified as ABC fragments, designated A-D (Figure 1). The putative protein sequence of fragment A was identical with the previously published ATRC transporter from *A. nidulans* (Angermayr et al., 1999). Since eukaryotic ABC transporters generally contain two ABC, Southern blot analysis was performed to investigate whether three of the four identified cassettes belonged to the same gene. The four different fragments were radiolabeled and hybridized to restriction-digested *A. nidulans* genomic DNA. The four different fragments produced different hybridization patterns (Figure 2), strongly indicating that they are part of distinct genes, which were designated *abc*A-D.

	Walker A	ABC signature	Walker B
A. nidulans A	ygfgssgcgkttvisll	lsggqrqriaiaralirdpelllfdeatsald	
A. nidulans B	alvgpsgcgksttiall	lsggqkqrigs----------	
A. nidulans C	alvgpsgagkstiislv	fsggqkqri----------	
A. nidulans D	alvgpsgcgksttiall	lsggqkqrvaiarallrdpkillldeatsald	
A. fumigatus MDR1	alvgpsgcgksttiall	lsggqkqrvaiarallrdpkvlldeatsald	
A. flavus MDR1	alvgasgsgksttiall	lsggqkqriaiaralirnpkillldeatsald	
S. pombe PMD1	afvgssgcgksttigli	lsggqkqriaiaralirnpkillldeatsald	
CneMDR1	alvgpsgcgksttiqml	lsggqkqriaiaralirnpkvlldeatsald	
G. gallus CMDR1	alvgssgcgkstvvqll	lsggqkqriaiaralirkpqillldeatsald	
X. laevis MDR	alvgssgcgksttvsll	lsggqkqriaiaralirkpkillldeatsald	

Figure 1. Alignment of PCR fragments A-D that correspond to ABC transporter genes in *Aspergillus nidulans* with the Walker A, B, and ABC signature of ABC transporters. These fragments were aligned with the corresponding regions from different ABC transporters: *A. fumigatus* MDR1 (U62933; Tobin et al., 1997), *A. flavus* MDR1 (U62931; Tobin et al., 1997), *Schizosaccharomyces pombe* PMD1 (P36619; Nishi et al., 1992), CneMDR1 from *Cryptococcus neoformans* (U62929; Thornewell et al., 1997), *Gallus gallus* CMDR1 (AJ009799; Edelmann et al., 1999), and *Xenopus laevis* MDR (U17608; Castillo et al., 1995).

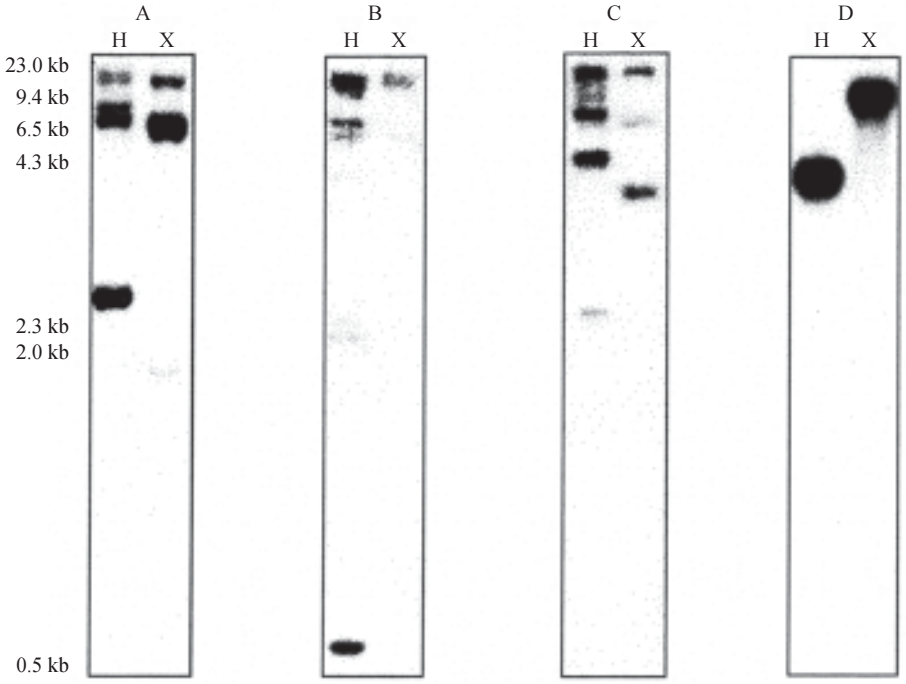


Figure 2. Southern blot analysis of the PCR fragments that correspond to genes encoding ABC transporters in *Aspergillus nidulans*. Panels A-D show the Southern blots hybridized with PCR fragments A-D, respectively (H = *Hin*dIII and X = *Xho*I).

Molecular structure of the *abc*D gene of *Aspergillus nidulans*

The complete gene for *abc*D was isolated from an *A. nidulans* chromosome library as described in Material and Methods. The *abc*D gene is located on linkage group VIII. The 4,356 nucleotide-coding region of the *A. nidulans abc*D gene, together with the deduced protein sequence and the 5'- and 3'-flanking sequence, are shown in Figure 3. The location of the open-reading frame and the position of the two introns were predicted from the sequence similarity to the corresponding gene, *afumdr1*, of *A. fumigatus* (Tobin et al., 1997). The expected translation product was 1,452-amino acids long, with a calculated molecular mass of 147,467 kDa and a

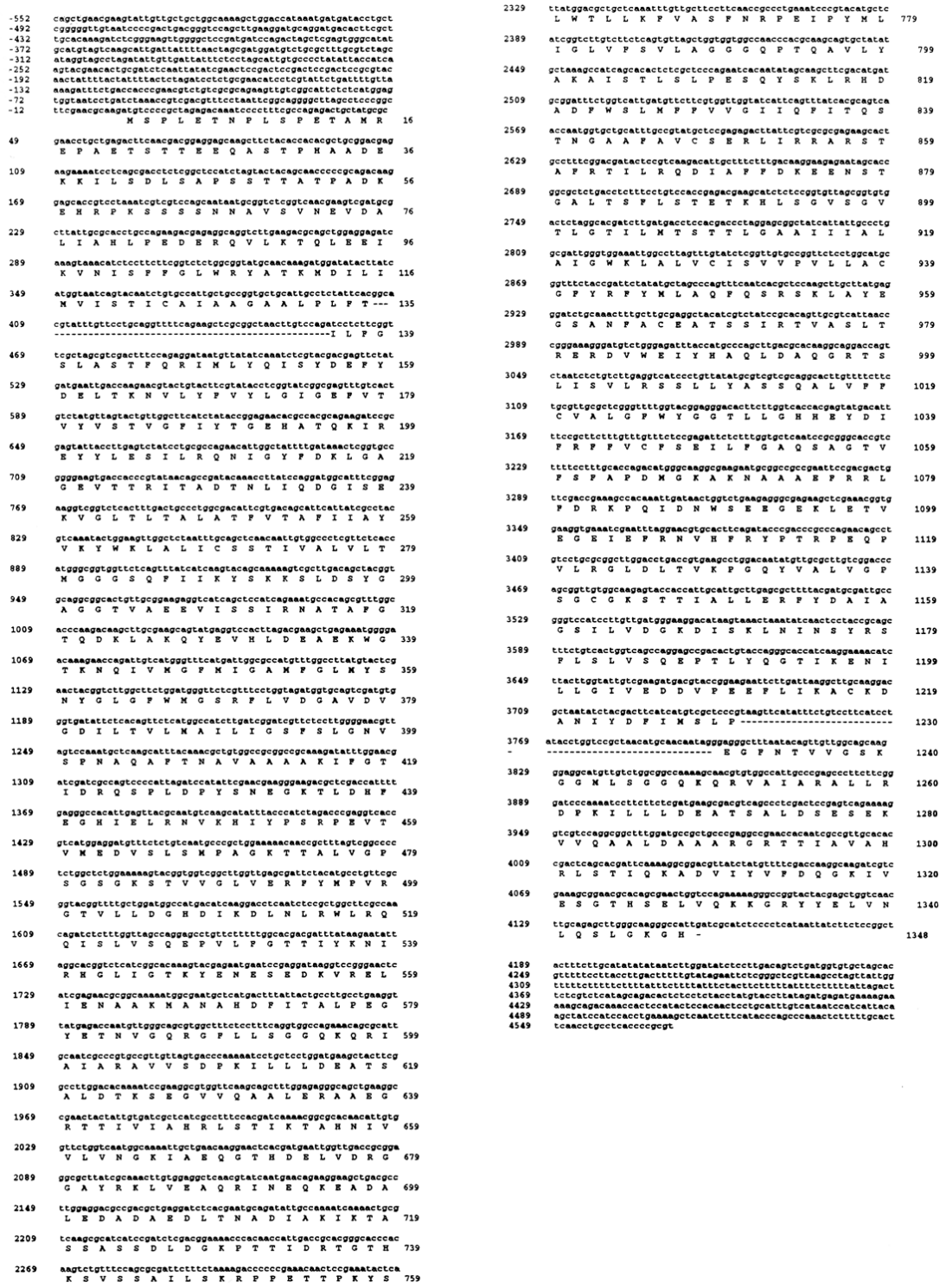


Figure 3. Nucleotide sequence and predicted amino acid sequence of the *Aspergillus nidulans abc*D gene. Conventional one-letter code is used for the amino acids (BankIt 284364).

calculated pI value of 5.82. The coding sequence of the *abc*D gene is interrupted by two introns with 51 and 56 nucleotides at nucleotide positions 405-456 and 3734-3790. Each intron contained the splicing donation and accepting consensus sequences 5'-GT and 3'-AG, respectively, which are observed in fungal genes (Balance, 1991). Hydrophobicity and homology analyses of the deduced amino acid sequence of the encoded protein (ABCD) suggested the presence of 12 transmembrane domains and two nucleotide-binding sites, arranged in two homologous halves. Each half of ABCD consisted of a hydrophobic region with six transmembrane domains and one nucleotide-binding site (Figure 4). The deduced amino acid sequence comparisons

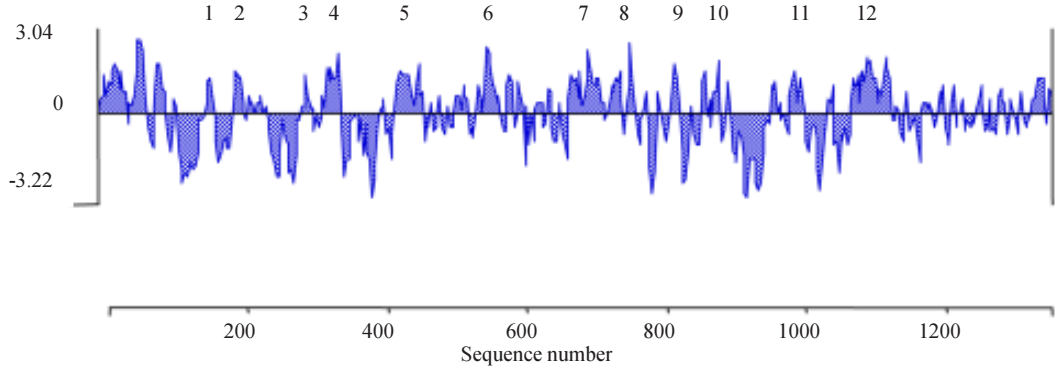


Figure 4. Hydropathy profile of the protein encoded by *abc*D. Plots were derived according to the algorithm of Kyte and Doolittle (1982), using a window size of 9 amino acid residues. Putative transmembrane regions are indicated by numbers.

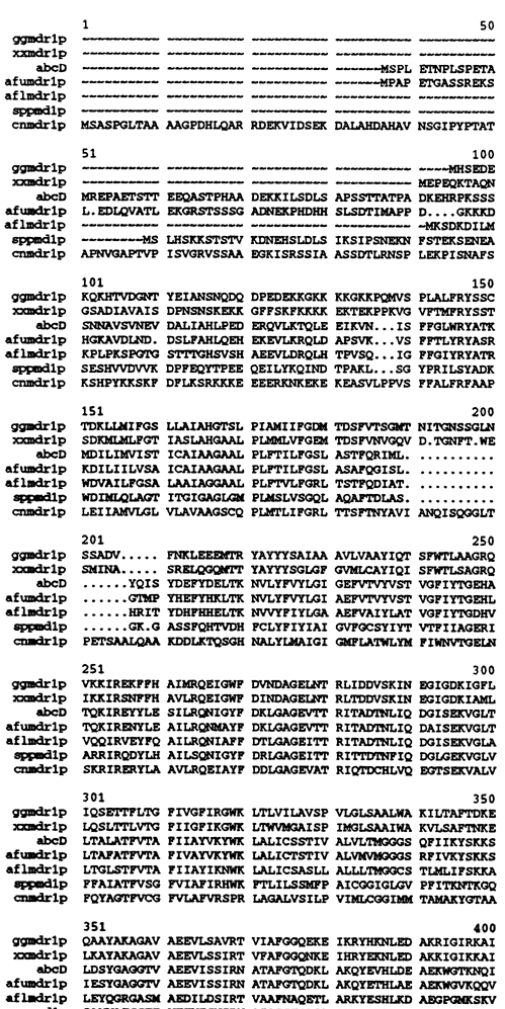
showed a high homology with ABC transporter genes from other species: 77% identity with AfuMDR1 from *A. fumigatus*, 59% identity with AfuMDR1 from *A. flavus*, 46% identity with leptomycin B resistance protein, 43% identity with MDR protein from *Filobasidiella neoformans*, 40% identity with ABC transporter protein from *Gallus gallus*, 40% identity with P-glycoprotein from *Xenopus laevis*, and 39% identity with *Cricetulus* sp. (Figure 5).

The expression of the *abc*D gene in *Aspergillus nidulans*

Transcription of the *abc*D gene in the presence of different drugs was investigated in the wild type strain. The *abc*D gene specifies a single transcript of about 5.0 kb (Figure 6). Northern analysis exhibited enhanced mRNA levels of *abc*D after exposure to miconazole (six-fold), camptothecin (three-fold), methotrexate (three-fold), and ethidium bromide (two-fold). However, no significant differences between untreated controls and RNAs from mycelia exposed to kanamycin, adriblastin, actinomycin, itraconazole, geneticin, and brefeldin were found. The *abc*D gene was constitutively transcribed at low levels (Figure 6).

DISCUSSION

Resistance to structurally unrelated drugs is a general phenomenon observed in both prokaryotes and eukaryotes (Higgins, 1992; Lewis, 1994). It is referred to as MDR. MDR can be caused by an increased ATP-dependent efflux of toxic compounds from the cytoplasm and plasma membrane that is mediated by the membrane-bound ATP-dependent transporters of the ABC superfamily (see reviews by Higgins, 1992, 1995; van Veen and Konings, 1998). In



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 xxmdr1p  LGAMALGQTS SFAPDYTKAM ISAAHIFSLL ERVPQIDSYS DQGEKPK..N
    abcD  FGAQSAGTVF SFAPDMGKAK NAAAEFRRLF DRKPQIDNWS EEGEKLETVE
afumdr1p  FGAQSAGTVF SFAPDMGKAK NAAAQFKKLF DSKPTIDIWS DEGEKLESME
aflmdr1p  FGAQAAGTVF SHAPDMGKAK HAAREFKRLF .SSDTMHASR SKGVPVTSMR
 sppmdl1p  FGIQQAGQFF GYSADVTKAK AAAGEIKYLS ESKPKIDTWS TEGKKVESLQ
 cnmdr1p  FASIQAGNVF TFVPDASKAN SSAASIFRSI DNEPAINAES NEGKVLDHKH

          1201                                                1250
 ggmdr1p  FGGNTRIKDV KFNYPNRPEV KILQGLNLAV EKGETLALVG SSGCGKSTVV
 xxmdr1p  CSGNVVFKGV NFNYPTRPDI TVLQGLDISV KQGETLALVG SSGCGKSTTV
    abcD  ..GEIEFRNV HFRYPTRPEQ PVLRGLDLTV KPGQYVALVG PSGCGKSTTI
afumdr1p  ..GEIEFRDV HFRYPTRPEQ PVLRGLNLSV KPGQYIALVG PSGCGKSTTI
aflmdr1p  ..GLVEFRDV SFRYPSRLEQ PILRHLNLTI KPGQFVALVG ASGSGKSTTI
 sppmdl1p  .SAAIEFRQV EFSYPTRRHI KVLRGLNLTV KPGQFVAFVG SSGCGKSTTI
 cnmdr1p  VVGHVRIEGV HFRYPTRPGV RVLRNLTIDV PAGTYVALVG PSGCGKSTTI

          1251                                                1300
 ggmdr1p  QLLERFYDPL SGEIVFDDID AKTLNIQWLR SHIGIVSQEP ILFDFTIAEN
 xxmdr1p  SLLERFYDPF EGEVLVDGLS VRNLNIQWVR AQMGIVSQEP ILFDCSIGDN
    abcD  ALLERFYDAI AGSILVDGKD ISKLNINSYR SFLSLVSQEP TLYQGTIKEN
afumdr1p  ALLERFYDAL AGGVFVDGKD ITKLNVNSYR SFLSLVSQEP TLYQGTIKEN
aflmdr1p  ALLERFYDPL KGGVYVDGKN IITLEMSSYR SHLALISQEP TLFQGTIREN
 sppmdl1p  GLIERFYDCD NGAVLVDGVN VRDYNINDYR KQIALVSQEP TLYQGTVREN
 cnmdr1p  QMLERFYDPL AGRVTLDGID IKELNLASYR SQISLVSQEP TLYAGTIRFN

          1301                                                1350
 ggmdr1p  IAYGDNS..R EVSHEEIISA AKAASIHSFI DSLPEKYNTR VGDKGTQLSG
 xxmdr1p  IAYGDNN..R KVTQEEIETA AKEANIHSFI ESLTDKYNTR VGDKGTQLSG
    abcD  ILLGIVED.. DVPEEFLIKA CKDANIYDFI MSLPEGFNTV VGSKGGMLSG
afumdr1p  ILLGVDKD.. DVSEETLIKV CKDANIYDFV MSLPEGFDTV VGSKGGMLSG
aflmdr1p  ILLGSNTP.. HVTDDFLVKA CKDANIYDFI LSLPQGFNTI VGNKGGMLSG
 sppmdl1p  IVLGASK... DVSEEEMIEA CKKANIHEFI LGLPNGYNTL CGQKGSSLSG
 cnmdr1p  ILLGANKPIE EVTQDEIDAA CKDANIYDFI VSLPDGFDTE VGGKGSQLSG

          1351                                                1400
 ggmdr1p  GQKQRIAIAR ALIRKPQILL LDEATSALDT ESEKIVQEAL DKAREGRTCI
 xxmdr1p  GQKQRIAIAR ALIRKPKILL LDEATSALDT ESEKVVQEAL DKARMGRTCI
    abcD  GQKQRVAIAR ALLRDPKILL LDEATSALDS ESEKVVQAAL DAAARGRTTI
afumdr1p  GQKQRVAIAR ALLRDPKVLL LDEATSALDS ESEKVVQAAL DAAARGRTTI
aflmdr1p  GQKQRIAIAR ALIRNPKILL LDEATSALDS ESEKVVQAAL DAAARGRTTI
 sppmdl1p  GQKQRIAIAR ALIRNPKILL LDEATSALDS HSEKVVQEAL NAASQGRTTV
 cnmdr1p  GQKQRIAIAR ALIRNPKVLL LDEATSALDS QSEKVVQEAL DKAAKGRTTI

          1401                                                1450
 ggmdr1p  VIAHRLSTIQ NADKIAVIQN GKVIEQGTHQ QLLAEKGFYY SLVNVQSGSC
 xxmdr1p  VIAHRLSTIQ NADKIAVIQN GKVVEQGTHQ QLLQLKGVYF SLVTIQLGHS
    abcd  AVAHRLSTIQ KADVIYVFDQ GKIVESGTHS ELVQKKGRYY ELVNLQSLGK
afumdr1p  AVAHRLSTIQ NADIIYVFDQ GKIVESGTHH ELIRNKGRYY ELVNLQSLGK
aflmdr1p  AVAHRLSTIQ RADLIYVLDQ GEVVESGTHR ELLRKKGRYY ELVHLQNPDA
 sppmdl1p  AIAHRLSSIQ DADCIFVFDG GVIAEAGTHA ELVKQRGRYY ELVVEQGLNK
 cnmdr1p  AIAHRLSSIQ HSDRIYYFSE GRVAEHGTHQ ELLAKKGGYY ELVQMQNLSR

          1451
 ggmdr1p  NM--
 xxmdr1p  ----
    abcD  GH--
afumdr1p  TH--
aflmdr1p  TGTK
 sppmdl1p  ----
 cnmdr1p  Q---
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Figure 5. Comparison of the amino acid sequence deduced for the *Aspergillus nidulans* ABCD protein (abcD) with the corresponding sequence from other ABC transporters: *A. fumigatus*, afumdr1 (U62933; Tobin et al., 1997); *A. flavus*, aflmdr1 (U62931; Tobin et al., 1997); *Schizosaccharomyces pombe* sspmdr1 (P36619; Nishi et al., 1992), *Cryptococcus neoformans*, cnmdr1p (U62929; Thornewell et al., 1997), *Gallus gallus*, ggmdr1p (AJ009799; Edelmann et al., 1999), and *Xenopus laevis*, xxmdr1p (U17608; Castillo et al., 1995).

general, the ABC transporters are transmembrane proteins that couple the energy of ATP hydrolysis to the selective transfer of substrates across biological membranes (Higgins, 1995). ABC transporters can be localized in the plasma membrane as well as in the membranes of intracellular organelles (endoplasmic reticulum, vacuoles, peroxisomes or mitochondria). Over 100 ABC transporters have been identified in diverse organisms including bacteria, yeast, filamentous fungi and bacteria (for reviews, see Higgins, 1995 and van Veen and Konings, 1998). Analysis of the complete yeast genome predicts the existence of 29 genes encoding putative ABC transporters in *S. cerevisiae* (Decottignies and Goffeau, 1997). Some of them (e.g., YCF1, PDR5, SNQ2, or YOR1) have been demonstrated to confer an MDR phenotype (for reviews, see Balzi and Goffeau, 1991, 1994). We have initiated a search for genes that encode ABC transporters in the filamentous fungus *A. nidulans*. We identified four genes encoding different ABC transporters by a PCR-based approach with degenerate oligonucleotide

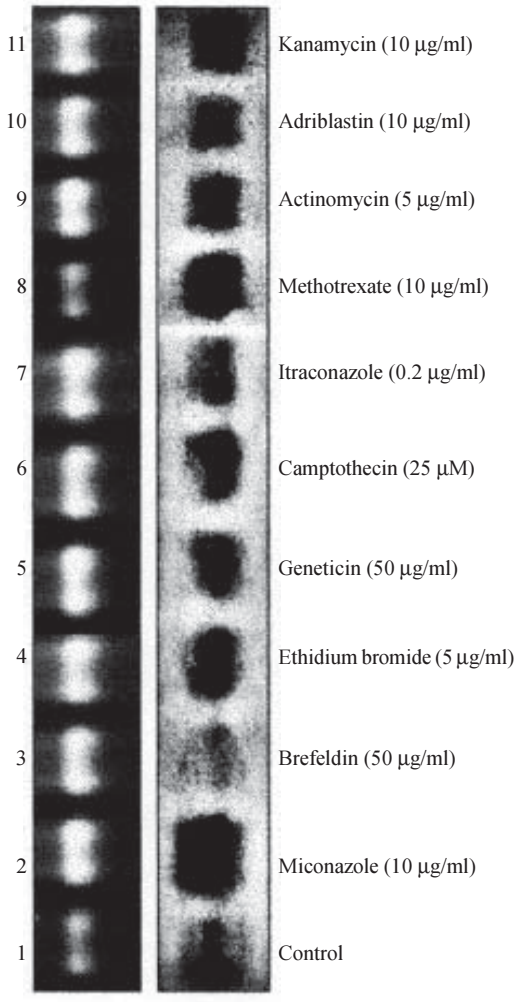

Figure 6. Northern blot analysis of *Aspergillus nidulans abc*D expression. From right to the left, *A. nidulans* grown on YG medium (lane 1, control) or YG medium supplemented with different drugs (lanes 2-11).

primers specific to highly conserved regions of these genes, which encode ATP-binding elements. This approach has already been used to identify members of the ATP transporter family in *S. cerevisiae*, *Leishmania donovani*, *Trypanosoma brucei*, *A. fumigatus*, and *A. flavus* (Kuchler et al., 1992; Henderson et al., 1992; Tobin et al., 1997; Maser and Kaminsky, 1998). In *A. nidulans*, two genes, *atr*A and *atr*B, encode ABC transporters (Del Sorbo et al., 1997). The PCR fragment that corresponds to the *abc*A gene was identified as identical to the recently isolated *atr*C gene (Angermayr et al., 1999). These authors pointed out that a homology search of the *A. nidulans* expressed sequence tag (EST) database (http://www.genome.ou.edu) revealed the presence of at least eight additional putative members of the ABC protein family, different from *atr*A-C. Therefore, the total number of putative ABC transporter-encoding genes in *A. nidulans* has been estimated to be at least 13 (eight from the EST database plus *atr*C, and *abc*B-D). Accordingly, we propose to rename the *abc*B-D described in this work as *atr*D-F. In addition, two ABC transporters have been identified in *A. fumigatus*, AfuMDR1 and AfuMDR2, and one, AflMDR1, in *A. flavus* (Tobin et al., 1997). All these genes are potential genetic determinants that can confer MDR or resistance to a specific drug.

We have described the cloning and characterization of one of these ABC transporter-encoding genes, *abc*D (renamed *atr*D). This gene shows high homology with the AfuMDR1 gene in *A. fumigatus*. The putative product of this gene closely resembles other members of the ABC transporter superfamily. The *atr*D encoded a so-called "full-length" MDR-like protein with 12 transmembrane regions and two nucleotide-binding sites. Northern blot experiments demonstrated that the *atr*D was induced by several unrelated drugs with different mechanisms of action, including miconazole, camptothecin, methotrexate, and ethidium bromide. The transcription of *atr*A and *atr*B in mycelia is strongly enhanced by treatment with azole fungicides and plant defense toxins. Transcription of the *atr* genes has been studied in a wild type and in a series of isogenic strains carrying the *ima*A and/or *ima*B mutations that confer resistance to the azole fungicide imazalil. *atr*B is constitutively transcribed at a low level in the wild type and in strains carrying *ima*A or *ima*B mutations. Imazalil treatment enhances transcription of *atr*B to a similar extent in all strains tested. *atr*A, unlike, *at*rB, displays a relatively high level of constitutive expression in strains carrying the imaB mutation. Imazalil enhances transcription of *atr*A more strongly in imaB mutants, suggesting that the *ima*B locus regulates *atr*A. Functional analysis demonstrated that the cDNA that corresponds to *atr*B can complement the drug hypersensitivity associated with PDR5 deficiency in *S. cerevisiae* (Del Sorbo et al., 1997). The *atr*C gene was shown by Northern analysis experiments to have its mRNA expression increased 10-fold in response to cycloheximide (Angermayr et al., 1999). In addition, expression of the AfuMDR1 gene in *S. cerevisiae* conferred increased resistance to the antifungal agent cilofungin (LY121019), an echinocandin B analog (Tobin et al., 1997). All these data taken together indicate that some of the ABC transporter-encoding genes described in *Aspergillus* spp. could mediate MDR and are regulated at the transcriptional level by drugs.

A. nidulans provides a convenient model system for studying MDR in filamentous fungi because this species is suitable for both classical and molecular genetics. The understanding of the genetic networks that operate on drug efflux by ABC transporters will surely be beneficial for the comprehension of multidrug clinical resistance of facultative pathogenic species of *Aspergillus* that can potentially cause life-threatening diseases in immunocompromised patients. The identification of ABC transporter-encoding genes in this species should be an initial step towards determining the contribution of these potentially detoxifying proteins to the basic mechanisms of antifungal resistance, and MDR in general.

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