

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

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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*.

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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 (yA1 pabaA1) 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 GGOKORI. 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, 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 μ l 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 $[\alpha^{-32}P]$ -

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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 μ g/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 10⁴ *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 abcA-D.

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	Walker A	ABC signature Walker B		
A. nidulans A	ygfgssgcgkttvisll	lsggqrqriaiaralirdpelllfdeatsald		
A. nidulans B	alvgpsgcgksttiall	lsggqkqrigs		
A. nidulans C	alvgpsgagkstiisIv	fsggqkqri		
A. nidulans D	alvgpsgcgksttiall	lsggqkqrvaiarallrdpkillldeatsald		
A. fumigatus MDR1	alvgpsgcgksttiall	lsggqkqrvaiarallrdpkvllldeatsald		
A. flavus MDR1	alvgasgsgksttiall	lsggqkqriaiaralirnpkillldeatsald		
S. pombe PMD1	afvgssgcgksttigli	lsggqkqriaiaralirnpkillldeatsald		
CneMDR1	alvgpsgcgksttiqml	lsggqkqriaiaralirnpkvllldeatsald		
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 = HindIII and X = XhoI).

Molecular structure of the abcD 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

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-552 -492 -432 -372 -312 -252 -192 -192 -132 -72 -12 49 gaacctgctgagacttcaacgacggaggagcaagcttctacacccacacgctgcggacgag E P λ E T S T T E E Q λ S T P H λ λ D E 109 asgammatcetengegaceteteggeteentetagtaetaengeaaceeegeaaang K K I L S D L S A P S S T T A T P A D K 169 gagcaccgtcctaaatcgtcgtccagcaataatgcggtctcggtcaacgaagtcgatgcg 229 cttattgcgcacctgccagaagacgaggcaggtcttgaagacgcagctggaggagatc L I A H L P E D E R Q V L K T Q L E E I 289 aaagtaaacatctccttcttcggtctctggcggtatgcaacaaagatggatatacttatc K V N I S F F G L W R Y A T K M D I L I 116 349 atggtaatcagtacaatctgtgccattgctgccggtgctgcattgcctctattcacggca Μ V I S T I C A I A A G A A L P L F T --- 135 409 cgtatttgttcctgcaggttttcagaagctcgcgggctaacttgtccagatcctcttcggt -----ILFG 135 469 tcgctagcgtcgactttccagaggataatgttatatcaaatctcgtacgacgagttctat S L A S T F Q R I M L Y Q I S Y D B F Y 155 529 gatgaattgaccaagaacgtactgtacttcgtatacctcggtatcggcgagtttgtcact D E L T K N V L Y F V Y L G I G B F V T 175 589 gtctatgttagtactgttggcttcatctataccggagaacacgccacgcagaagatccgc V Y V S T V G F I Y T G E H A T Q K I R 649 gagtattaccttgagtctatcctgcgccagaacattggctattttgataaactcggtgcc EYYLESILRQNIGYFDKLGA215 709 ggggaagtgaccacccgtataacagccgatacaaaccttatccaggatggcatttcggag G E V T T R I T A D T N L I Q D G I S E 239 769 aaggteggteteaetttgactgeeetggegacattegtgacageatteattategeetae K V G L T L T A L A T F V T A F I I A Y 829 gicaaatactggaagttggctctaatttgcagctcaacaattgtggccctcgttctcacc V K Y W K L A L I C S S T I V A L V L T 889 atgggcggtggtteteagttateateaagtacageaaaagtegettgaeagetaeggt M G G G S Q F I I K Y S K K S L D S Y G 949 gcaggcggcactgttgcggaagaggtcatcagctccatcagaaatgccacagcgtttggc A G G T V A E E V I S S I R N A T A F G 319 1009 gacaagcttgcgaagcagtatgaggtccacttagacgaagctgagaaatgggga D K L A K Q Y E V H L D E A E K W G 333 1069 cagattgtcatgggtttcatgattggcgccatgtttggccttatgtactcg Q I V M G F M I G A M F G L M Y S 355 1129 aactacggtettggettetggatgggttetegtteetggtagatggtgeagtegatgtg N Y G L G F W M G S R F L V D G A V D V 37 1189 1249 atgeteaageatttacaaacgetgtggeegeggeegeaaagatatttggaaeg N A Q A F T N A V A A A A K I F G T 411 1309 cagtccccattagatccatattcgaacgaagggaagacgctcgaccatttt Q S P L D P Y S N E G K T L D H F 1369 gagggccacattgagttacgcaatgtcaagcatatttacccatctagacccgaggtcacc E G H I E L R N V K H I Y P S R P E V T 45 1429 gtcatggaggatgtttctctgtcaatgcccgctggaaaaacaaccgctttagtcggcccc V N E D V S L S N P A G K T T A L V G P 479 1489 tetggetetggaaaaagtacggtggteggettggttgagegattetacatgeetgttege S G S G K S T V V G L V E R F Y M P V R 499 1549 ggtacggttttgctggatggccatgacatcaaggacctcaatctccgctggcttcgccaa G T V L L D G H D I K D L N L R W L R Q 519 1609 cagatetetttggttagecaggageetgttettttggcacgaegatttataagaatatt Q I S L V S Q B P V L P G T T I Y K N I 533 1669 acggtetcateggeacaaagtaegagaatgaateegaggataaggteegggaaete H G L I G T K Y E N E S E D K V R E L 559 1729 cgcggcaaaaatggcgaatgctcatgacttattactgccttgcctgaaggt A A K M A N A H D F I T A L P E G 579 1789 aatgttgggcagcgtggctttctcctttcaggtggccagaaacagcgcat N V G Q R G F L L S G G Q K Q R I 1849 gcaatcgcccgtgccgttgttagtgacccaaaaatcctgctcctggatgaagctacttcg A I A R A V V S D P K I L L D E A T S 619 1909 gcettggacacaaaateegaaggegtggtteaageagetttggagagggcagetgaagge A L D T K S E G V V Q A A L E R A A E G 639 1969 cgaactactattgtgatcgctcatcgcctttccacgatcaaaacggcgcacaacattgtg R T T I V I A H R L S T I K T A H N I V 659 2029 atggcaaaattgctgaacaaggaactcacgatgaattggttgaccgcgga N G K I A E Q G T H D E L V D R G 2089 ggegettategeaaaettgtggaggeteaaegtateaatgaaeaggaagetgaegee G A Y R K L V E A Q R I N E Q K E A D A 699 2149 cgccgacgctgaggatctcacgaatgcagatattgccaaaatcaaaactgcg A D A E D L T N A D I A K I K T A 719 2209 tcaagegeatcatecgatetegaeggaaaacceaeaaceattgaeegegeaeceae S S A S S D L D G K P T T I D R T G T H 739 2269 aagtetgtttecagegegattetttetaaaagaeeeeeggaaacaaeteegaaataetea K S V S S A I L S K R P P B T T P K Y S 759

2329	ttatggacgctgctcaaatttgttgcttccttcaaccgccctgaaatcccgtacatgctc L W T L L K F V A S F N R P E I P Y M L	779
2389	atcggtcttgtcttctcagtgttagctggtggtgggccaacccaacgcaagtgctatat I G L V P S V L A G G G Q P T Q A V L Y	799
2449	gctaaagccatcagcacactctcgctcccagaatcacaatatagcaagcttcgacatgat A K A I S T L S L P E S Q Y S K L R H D	819
2509	gcggattttggtcattgatgttttttcgtggttggtatcattcagttatcacgcagtca A D F W S L M F P V V G I I Q F I T Q S	839
2569	accaatggtgctgcatttgccgtatgctccgagagacttattcgtcgcgcgagaagcact T N G A A P A V C S E R L I R R A R S T	859
2629	gootttoggacgatactoogtcaagacattgotttotttgacaaggaagagaatagcacc A F R T I L R Q D I A F F D K E E N S T	879
2689	ggegetetgacetetteetgteeacegagaegaageateteteeggtgttageggtgt G A L T S F L S T E T K H L S G V S G V	899
2749	actctaggcacgatcttgatgacctccacgaccctaggagcggctatcattattgccctg Τ L G T I L M T S T T L G λ λ Ι Ι Ι λ L	919
2809	gcgattgggtggaaattggcettagttgtateteggttgtgeeggtteteetggcatgc A I G W K L A L V C I S V V P V L L A C	939
2869	ggtttttaccgatttatatgctagcccagtttcaatcacgctccaagcttgctt	959
2929	ggatetgcaaactttgcttgcgaggctacatcgtctatccgcacagttgcgtcattaacc G S A N F A C E A T S S I R T V A S L T	979
2989	cgggaaagggatgtttgggagatttaccatgcccagcttgacgcacaaggcaggaccagt R E R D V W E I Y H A Q L D A Q G R T S	999
3049	ctaatctctgtcttgaggtcatccctgttatatgcgtcgtcgcaggcacttgtttcttc L I S V L R S S L L Y A S S Q A L V P P	1019
3109	tgcgttgcgctcgggttttggtacggagggacacttcttggtcaccacgagtatgacatt C V λ L G P W Y G G T L L G H H E Y D I	1039
3169	tteegettettigttigttigtteeegagattetettiggigetaateegegggeacegie F R P F V C F S E I L F G A Q S A G T V	1059
3229	ttttcctttgcaccagacatgggcaaggagaagaatgcggccgccgaattccgacgactg F S F A P D M G K A K N A A A B F R R L	1079
3289	ttcgaccgaaagccacaattgataactggtctgaagagggggagaagctcgaaacggtg F D R K P Q I D N W S E E G E K L E T V	1099
3349	gaaggtgaaatcgaatttaggaacgtgcacttcagatacccgacccgcccagaacagcct E G E I E F R N V H F R Y P T R P E Q P	1119
3409	gtcctgcgcggcttggacctgaccgtgaagcctggacaatatgttgcgcttgtcggaccc V L R G L D L T V K P G Q Y V A L V G P	1139
3469	agggttgtgggcaagagtaccaccattgcattgcttgagcgcttttacgatgcgattgcc S G C G K S T T I A L L E R F Y D A I λ	1159
3529	gggtccatccttgttgatgggaaggacataagtaaactaaatatcaactcctaccgcagc G S I L V D G K D I S K L N I N S Y R S	1179
3589	tttctgtcactggtcagccaggagccgacactgtaccagggcaccatcaaggaaacatc F L S L V S Q E P T L Y Q G T I K E N I	1199
3649	ttacttggtattgtcgaagatgacgtaccggaagaattcttgattaaggcttgcaaggac L L G I V E D D V P E E F L I K A C K D	1219
3709	gctaatatctacgacttcatcatgtcgctcccgtaagttcatatttctgtccttcatcct A N I Y D F I M S L P	1230
3769 -	atacctggtccgctaacatgcaacaatagggaggggtttaatacagttgttggcagcaag E G P N T V V G S K	1240
3829	ggaggcatgttgtctggcggccaaaagcaacgtgtggccattgcccgagcccttcttcgg G G M L S G G Q K Q R V A I A R A L L R	1260
3889	gateccaaaateettettetegatgaagegaegteageetegaeteegagteagaaaag D P K I L L L D E A T S A L D S E S E K	1280
3949	gtcgtccaggcggctttggatgccgctgcccgaggccgaaccacaatcgccgttgcacac V V Q A A L D A A A R G R T T I A V A H	130 0
4009	cgactcagcacgattcaaaaggcggacgttatctatgttttcgaccaaggcagatcgtc R L S T I Q K A D V I Y V F D Q G K I V	1320
4069	gaaagcggaacgcaacagcgaactggtccagaaaaaggggccggtactacgagctggtcaac E S G T H S E L V Q K K G R Y Y E L V N	1340
4129	ttgcagagcttgggcaagggccattgatcgcatctcccctcataattatcttcccggct L Q S L G K G H -	1348
4189 4249 4309 4369 4429 4489 4549	actticttgeatatatatettggatatetecttgacagtetggtggtgetagea gutttecutacetgacttttggatagaatetegggetggtagecagttatgg Littlecuttetttatttgattgatagaatetettittatttgate Littlecuttetttatttgattgatgatettittattgatgagagaanagaa aangegageamacetteettateceaategecettitgatagatagaangaa aangegageamaceateetateteetaaeetaggtaeettatgatgatgaanagaa agetateetaaeeeteeta	

ttatggacgctgctcaaatttgttgcttccttcaaccgccctgaaatcccgtacatgct

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

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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 abcD 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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50 ggmdr1p xxmdr1p abcD afumdr1p af1mdr1p -----MSPL ETNPLSPETA -----MPAP ETGASSREKS sppmd1p cnmdr1p MSASPGLTAA AAGPDHLQAR RDEKVIDSEK DALAHDAHAV NSGIPYPTAT 51 100 ggmdr1p xxmdr1p MHSEDE MEPARTSTT EEQASTPHAA DEKKILSDLS APSSTTATPA DKEHRPKSSS L.EDLQVATL EKGRSTSSSG ADNEKPHDHH SLSDTIMAPP D....GKKKD ab afumdr1p af1mdr1p -MKSDKDILM sppmd1p cnmdr1p APNVGAPTVP ISVGRVSSAA EGKISRSSIA ASSDTLENSP LEKPISNAF 101 150 101 KCMHTVDCNT YEIANSNQDQ DPEDEKKGKK KKGKRPCMVS PLALFRYSSC GSADIAVAIS DPNSNSKEK GFFSKFKKKK EKTEXPEKUG VFIMFRYSST SNNAVSVNEV DALIAHLPED ERQVLKTQLE EIKVN...IS FFGLMRYATK HGRAVDIAD. DSLFAHLQEH EKEVLKRQLD APSVK...VS FFTLYRIASR KPLPKSPOTG STTTGHSVSH AEEVLDRQLH TPVSQ...IG PFGITRYATR SESHVUDVVK DFFQUTTRE QEILYRQIND TPAKL..SG YFRILSYADK KSHPYKKSKF DPL&SRKKKE EEERKNKEKE KEASVLPPVS FFALFFAAP ggmdr1p xxmdr1p abcD afundrin aflmdr1r sppmd1p cnmdr1p 151 200 ggmdr1p xxmdr1p abcD afundr1p aflmdr1p sppmd1p cnmdr1p 250 201 SSADV.... FNKLEEEMIR YAYYYSAIAA AVLVAAYIOT SFWILAAGRQ SMINA..... SRELQQQMIT YAYYYSGLGF GVMLCAYIQI SFWILSAGRQYQIS YDEFYDELTK NVLIFVYLGI GEFVTYYYST VOFIYTGEHLGTMP THEFYHELIK NVVFYIGIA AEFVAIYLAT VOFIYTGEHLHRIT YDHFHHELTK NVVFYIGIA AEFVAIYLAT VOFIYTGHUGKA GASFGHTVDH FCLIFFIJIAI GVFGCSHJT VFFIIAGEHI PETSAALQAA KDDLKTQSGH NALYLMAIGI GMFLATMLYM FIMNYTGELN ggmdr1p xxmdr1p abcD abcD afumdr1p aflmdr1p sppmd1p cnmdr1p 251 300 300 VKKIREKPFH ALMRQEIGMF DVNDAGELNT RLIDDVSKIN BOIDDKIGFL IKKIRSNPFH AVL&QEIGMF DINDAGELNT RLIDDVSKIN BOIDDKIARL TQKIRBYYLS SILAQNIGYF DKLGAGEVTT RITADTNLIQ DGISEKVGLT TQKIRBNILS AIL&QMANF DKLGAGEVTT RITADTNLIQ DGISEKVGLX VQQIRVETFQ AIL&QHAFF DTLGAGETT RITADTNLIQ DGISEKVGLX SKRIRERYLA AVLRQEIAYF DDLGAGEVAT RIQTDCHLVQ EGTSEKVALV ggmedr1p xxmedr1p abcD afumdr1p aflmdr1p sppmd1p 301 301 IQSETTFUTG FIVGFIRGMK LTLVILAVSP VLGLSAALMA KILTAPTOKE LQSLTTLVTG FIIGFIRGMK LTLVILAVSP VLGLSAALMA KVLSAFTNKE LTALATFVTA FIIATVKYMK LALICSSTIV ALVLTMOGGS GPIIKISKKS LTALATFVTA FIIATVKYMK LALICISTIV ALVMOMOGS RFIVKISKKS LTGLSTFVTA FIIATVKYMK LALICISSIL ALLITMOGCS TMLIISKKA LTGLSTFVTA FIIATVKYMK LALICISSIL ALLITMOGCS TMLIISKKA PALATFVSG FVLAFINKK FILLISSMEP AICOGIGLGY PFITKIFKGQ FQYAGTFVCG FVLAFVRSPR LAGALVSILP VIMLCGGIMM TAMAKYGTAA ggmdr1p xxmdr1p abcD a... fundr1p afundrip aflmdrip sppmdip cnmdrip 400 QANARAGAV AEEVLSAVRT VIAFOGQEKE IKRYHENLED AKRIGIRKAI LKANARAGAV AEEVLSSIRT VRAFOGQEKE IHRYENNLED AKRIGIKAI LDSYGAGGTV AEEVISSIRN ATAFOTODKL AKQISVHLDE AEKMOTINGI IESYGAGGTV AEEVISSIRN ATAFOTODKL AKQISVHLAE AEKMOTINGI LEYGGRGASM AEDILDSIRT VAAFNAQETL ARKYESHKAD ASDFORKSKU IAVVAESSIF VEEVISNIRN AFAFOTOLI ALKINENLIT AQRIGINKAI LDHIAKAGSL AEEVIGSIRT VQAPGKEKIL GDKFADHIEQ SKIVGRKGSI ggmdr1p xxmdr1p abcD afumdr1p aflmdr1p sppmd1p lr1p 401 401 TSNISNGAAF LLIYASYALA FWYGTTLILA NEYSI.GNV LTVYFSVLG TANVSIGPAF LMIYAAYSLA FWGGTTLID GGTTI.GSV LTVYFAVIG VNGFMIGAMF GLMYSNYGLG FWMGSRFLVG GAVDV.GDU LTVIMAILIG ILAMHIGAM GIMYSNYGLG FMMGSRFLVG KENNV.GV LTVIMAILIG IFAINVGALL CINTUNYGLG FMGSRFLVG GISNIKAGDV LTIMANILIG ANGLANGMF FVAJGYJGLA FMEGGRLIAR GDLDV.SKL IGCFFAVLIA FEGFGLSIMF FVIYAAYALA FFYGGILVSN GQAD.SGVV INVFMSILIG ggmedr1p xxmedr1p abcd afumdr1p aflmdr1p sppmd1p cnmdr1p 451 451 AFSIQQTAFS IEAFANARGA AYAIFNIIDN EPEIDSYSDA GHKPDHIKGN AFAVQQTSFN IEAFANARGA AYIIFNIIDN QFKIDSFSKE GLKPDKIKGD SFSLGNVSFN AQAFTNAVAA AAKIFGTIDR QSPLDPYSDE GKVLDHFEGH SFSLGNVAFN QQAFINVVA AAKIFGTIDR QSPLDALSDQ GKVLDHFEGH SYSLANISFN MQSFVGSAS AKKIFGTIDR VSFINAFFPT GUVVRDIKGE SFSMMLAFE LAAVTKARGA AAKLFATIDR VPAIDSASEE GFKPDGLRGE ggmdr1p xxmdr1p abcD afundr1p aflmdr1p sppmd1p cnmdr1p 501 501 LEPCNYPNT PSRPDVELLK GLNLKVNOGQ TVALVQASGC GKSTTVQLLQ LEPCNYIPTT PSRPDVELLK GLNLKVNOGQ TVALVQASGC GKSTTVQLLQ IELRNKNITY PSRPSTVME DVSLSMPAGK TTALVQASGS GKSTVVGLVE IERNKNKIT PSRPSTVME DVSLSMPAGK TTALVQASGS GKSTVGLVE ILLKNIRTYY PRRPSTVAL DLSCYIPAGK TTALVQASGS GKSTIIGLVE IELRNIRTYY PRRPSTVLD NFSLVCPSGK ITALVQASGS GKSTIVSLE ISPENVKFHY PSRPSIPILK GFTTTFEAGK TFALVQASGS GKSTVVSLIE ggmdr1p xxmdr1p xxmdr1p abcD afumdr1p aflmdr1p sppmd1p cnmdr1p 531 RYIDEKEJTI TIDOODLKSL NVRYLREIIG VVNOEPVLFA TTILBIIRYG RYIDEKEJTI TIDOODLKSL NIRVLREIIG VVSOEPILFD TTILDIIRYG RYINEVRGYL LLDGHDIGL NIRVLRQIIS LVSOEPVLFG TTIFNIERG RYILPVGQV LLDGHDIQTL NIRVLRQIS LVSOEPVLFS TTIFNIERG ggmdr1p xxmdr1p

aflmdrip RFYDPVAGTI MLDGHDIQTL NLRWLRQQMS LVSQEPRLFA TTIAENIRYG sppmdip RFYDPIGOQV FLOGKDLKTL NVASLANDIS LVQQEFVLFA TTVFENITYG nmddrip RFYDFVSGVV KLDGRDIRSL NLNWLRQQIG LVSQEPTLFG TTVRGNVEHG 601 650 ggmdr1p xxmdr1p abcD afumdr1p aflmdr1p sppmd1p cnmdr1p 651 700 700 GOQKQRIAIA RALVHNPKIL LLDEATSALD TESESVVQAA LDKAREGRTT GOQKQRIAIA RALVKNPKIL LLDEATSALD TESESVVQAA LDKAREGRTT GOQKQRIAIA RAVVSDPKIL LLDEATSALD TKSEGVVQAA LDKAAEGRTT GOQKQRIAIA RAVISDPKIL LLDEATSALD TKSEKLVQAA LDKAAEGRTT GOQKQRIAIA RAVISDPKIL LLDEATSALD SKSEVLVQAA LDKAASGRTT GOQKQRIAIA RAVISDPKIL LLDEATSALD SKSEVLVQAA LDKAASGRTT ggmdr1p xxmdr1p abcD afumdr1p aflmdrin sppmd1p cnmdr1p 701 ggmdr1p xxmdr1p abcD afumdr1p aflmdr1p sppmd1p 701 VVVAHRLSTV RNADLIAVFE SGVITEQGNH SQLI.EKKGI YYKUVNAQTI IVVAHRLSTI RNANAIAGPD NGVIVEQGSH KELM.ERGGV YNLVTLQTV IVIAHRLSTI KYANNIVVLV NGKIAEQGTH DELV.DRGGA YKNUVEAQR. IVIAHRLSTI KYANNIVLAN GGKIAEQGTH DELV.DRKGT YYKUVEAQR. IVIAHRLSTI RAXNIVVAN GGKIVEQGSH EELL.DLANGA YARUVEAQKL IVIAHRLSTI RNADNIVVN GGKVEQGSH NELLLANENGP YAQLVNNQKL cnmdr1p 751 800 abcD afumdr1p af1mdr1p sppmd1p cnmdr1p 801 850 ggmdr1p xxmdr1p abcD afundr1p af1mdr1p sppmd1p cnmdr1p 851 ggmdr1p xxmdr1p abcD afumdr1p aflmdr1p sppmd1p cnmdr1p 900 ELPPVSFLKL KKL.....N KNEMFYFVAG TFCALVNGAL OPAFSVIFSE GPEPVSFFKV KKL.....N KPEMFYFVAG VICAMINGAT OPAFAIIFSR TTFKYSLMTL LKFVASFNRP..EIGYNLIG LYTSVLAGG OPTDAVLTAK OFEKTSLMTL VKFIGAFNRP..EIGYNLIG LYTSVLAGG OPTDAVLTAK KEEAISFMTL FKFLASFNRP..EMFFLLG LCASILAGG OPSDAVLTAK NNHEINSLT LMFHISFYRT MIEIICLIG ILASILGGAN YFVGAVFAR SFGLYARLLR MNSADKF.....IYIIA FIAAICAGMV YPSLAILFGK 901 950 IIGIFSETDO KV..LREKSN LYSLLFLALG IISFFTFFVQ GPAFGKAGEI ggmdrip xxmdrip abcD afumdrip afimdrip IIGITSETD IV...KEKKAN LISLFIALG IISFFFFFQ GFTFGAGE IIGVTAGVS Q...MSESS MYSLFIALG GVSTIFFQ GFTFGAGE AISTLSLPES QYSKLREDAD FMSLMFFVG ILQFITQSTN GAAFAVCSER AISTLSLPES MHKLREDAN FMSLMFFVG IAQFISLSIN GTAFANCSER AVSTLSLPFL EFFKLREDAN FMCLMFLMIG IVSLVLYSVQ GTLFAYSSEK finift..dl setdfibkvn vfavymlia ivgffayais nfastysmea ALSDFEIDD ...ASLRMALS RALMFITA LAARFVIFFQ SAGTSRGMD sppmd1p cnmdr1p 951 1000 1000 LINKLEFMAF KAMLRQDHAM FDDFKNSTGA LITTRLANDAS QVKGATOVEL LITRALEASF KSMLROBIGM FDDSKNSTGA LITTRLATDAS QVKGATOVEL LIRRARSTAF RTILRQDIAF FDKEENSTGA LITSFLSTETK HLSGVSGVTL LIRRARSGAF RSILRQDISF FDREENSTGA LITSFLSTETK NLSGVSGVTL MVTRARSGAF RSILRQDISF FDREENSTGA LITSFLSTETK USLSGSFTL LNGVLRKKLF TATLRHDIEM FDEERNSTGA VTSNLADOPQ KVQGLFGPTL ggmdr1p xxmdr1p abcD afumdr1p aflmdr1p sppmd1p cnmdr1p 1001 1050 1001 ALIAQBIANL GTGIIISLVY GWQLTLLLA VVPIIAVAGM IBMUNLAGHA ALLAQBIVANL GTAIIISPIY GWQLTLLILA IVPVIAAGL VENDHAGHA GTILMESTLI GAAHIIALAI GWKLALVCIS VVPILLACGF YRYNLAOPQ GTILMESTLI GAAHIIALAI GWKLALVCIS VVPILLACGF IRYNMLAERQ GTILVSVNL VASLGVALVI GWKLALVCIS AVPALLMCGF VKVMLERQ GTFPQILINI ISVTILSLAT GWKLALVLS SSPUITAGY YKVAALDQVQ GTVVQSCATL IGGCIIGLCY GPLLALIGIA CIPILVSOGY IRLKVVVLKD ggmdr1p xxmdr1p abcD afumdrip aflmdrip sppmd1p cnmdr1p 1051 KKOKIELEAA GKIATEAIEN IRTVASLITRE KRFELMYGEH LLVPYRNSYK KKOKKELEKA GKISTDAVLN IRTVVSLITRE RKFEAMYEKS LØGPYRNSIK SRSKLAYØGS ANFACEATSS IRTVASLITRE RDVMEIYHAQ LØRGGRKSLI QRSKSAYØGS ASYACEAATSA IRTVVSLITRE TEALOSYOAQ LRRQLKSDIL EKLSAAYRES AAFACESTA IRTVVSLITRE ENVFARTOS LIKORGESAI QRMKKLHAAS AHLASEAAGA VKTVASLITRE KDVRRIYSEA LKAPMKLMPR ggmdr1p xxmdr1p abcD afumdr1p af1mdr1p sppmd1p cnmdr1p 1101 1150 1101 1150 gomdrip KAHIGGCFS LSQAMMFFTY AGCFR.FGAY LVVNGHISYK TYUVFSAVV xxmdrip KAHIGGUTYG LSQAMMVJCL CWVFSVLAAY LVVNGHISYK TYUVFSAVV abcD SVLRSSLLYA SSQALVFFCV ALGP.WYGGT LLGHHETGIF RFFVCFSEL afumdrip SVLRSSLLYA SSQALVFFCV ALGP.WYGGS LLGHHETGIF RFFVCFSEL afimdrip FYXFSSLLYA SSQALVFFCV ALGP.WYGGS LLGHHETGIF RFFVCFSEL afimdrip TXXSSLLYA SQALVFFCV ALGP.WYGGS LLGHHETGIF QFYCFSSVI cnmdrip TXIKSQCLFA ASQCITFLIA ALTP.WYGST LMRRGFTNIV QFYTCFIAIV 1151 1200 gomdrip FGANALGOTS SFAPDYAKAK ISAAHLFVLF NRVPPIDSYR EDGRKPE. K

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xxmdr1p			ISAAHIFSLL		
abcD			NAAAEFRRLF		
afumdr1p	FGAQSAGTVF	SFAPDMGKAK	NAAAQFKKLF	DSKPTIDIWS	DEGEKLESME
aflmdr1p	FGAQAAGTVF	SHAPDMGKAK	HAAREFKRLF	. SSDTMHASR	SKGVPVTSMR
sppmd1p	FGIQQAGQFF	GYSADVTKAK	AAAGEIKYLS	ESKPKIDTWS	TEGKKVESLQ
cnmdr1p	FASIOAGNVF	TFVPDASKAN	SSAASIFRSI	DNEPAINAES	NEGKVLDHKH
	1201				1250
agmedr1p	FGGNTRIKDV	K FNY PNR PEV	KILOGLNLAV	EKGETLALVG	SSGCGKSTVV
xxmdr1p			TVLQGLDISV		
abcD			PVLRGLDLTV		
afundr1p			PVLRGLNLSV		
aflmdr1p			PILRHLNLTI		
sppmdlp			KVLRGLNLTV		
cnmdr1p			RVLRNLTIDV		
cumor 1b	**GHVK123*	III KIII IKI GV	KT LAUTOT TOT	1791114019	1000000111
	1251				1300
ggmdrlp		CONTRACTO	AKTLNIQWLR	CUTOTUCOPE	
			VRNLNIQWUR		
xxmdr1p abcD			ISKLNINSYR		
			ITKLNVNSYR		
afundr1p			LITLEMSSYR		
aflmdr1p					
sppmd1p			VRDYNINDYR		
cnmdr1p	QMLERFYDPL	AGRVTLDGID	IKELNLASYR	SQISLVSQEP	TLYAGTIRFN
	1301				1350
ggmdr1p			AKAASIHSFI		
xxmdr1p			AKEANIHSFI		
abcD			CKDANIYDFI		
afundr1p			CKDANIYDFV		
aflmdr1p	ILLGSNTP	HVTDDFLVKA	CKDANIYDFI	LSLPOGFNTI	VGNKGGMLSG
sppmdlp	IVLGASK	DVSEEEMIEA	CKKANIHEFI	LGLPNGYNTL	CGOKGSSLSG
cnmdr1p	ILLGANKPIE	EVTODEIDAA	CKDANIYDFI	VSLPDGFDTE	VGGKGSQLSG
	1351				1400
ggmdr1p	GOKORIAIAR	ALIRKPOILL	LDEATSALDT	ESEKIVQEAL	DKAREGRTCI
xxmdr1p	GOKORIAIAR	ALIRKPKILL	LDEATSALDT	ESEKVVQEAL	DKARMGRTCI
abcD			LDEATSALDS		
afumdr1p			LDEATSALDS		
aflmdr1p			LDEATSALDS		
sppmd1p			LDEATSALDS		
cnmdr1p			LDEATSALDS		
cimer rp	ogngninini,	ADDIAL COLOR	20111011200	goatti t gana	Diventin
	1401				1450
ggmdr1p		NADKTAVION	GKVIEQGTHQ	OLLAEKGEVY	
xxmadr1p			GKVVEQGTHQ		
abed			GKIVESGTHS		
afundr1p					ELVNLOSLGK
aflmdr1p					ELVHLONPDA
					ELVVEQGLNK
sppmd1p cnmdr1p					ELVOMONLSR
cimer 1p	ATARKUSSIQ	NOURITIFSE	GRVALIGTHO	EDUNKOGII	PRAMMARK
	1451				
a mada 1 -	1451				
ggmdrlp	NM				
xxmdr1p	~~~~				
abcD	GH				
afundr1p	TH				
aflmdrlp	TGTK				
sppmd1p					
cnmdr1p	Q				

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*, afundr1 (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

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Figure 6. Northern blot analysis of *Aspergillus nidulans abcD* 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.

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ABC transporters in Aspergillus nidulans

We have described the cloning and characterization of one of these ABC transporterencoding genes, *abcD* (renamed *atrD*). 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 atrD 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 atrA and atrB 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 *imaA* and/or *imaB* mutations that confer resistance to the azole fungicide imazalil. atrB is constitutively transcribed at a low level in the wild type and in strains carrying *imaA* or *imaB* mutations. Imazalil treatment enhances transcription of *atrB* to a similar extent in all strains tested. atrA, unlike, atrB, displays a relatively high level of constitutive expression in strains carrying the imaB mutation. Imazalil enhances transcription of *atrA* more strongly in imaB mutants, suggesting that the *imaB* locus regulates *atrA*. 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 atrC 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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