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Increased mRNA Levels of *ERG16*, *CDR*, and *MDR1* Correlate with Increases in Azole Resistance in *Candida albicans* Isolates from a Patient Infected with Human Immunodeficiency Virus

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Resistance to antifungal drugs, specifically azoles such as fluconazole, in the opportunistic yeast *Candida albicans* has become an increasing problem in human immunodeficiency virus (HIV)-infected individuals. The molecular mechanisms responsible for this resistance have only recently become apparent and can include alterations in the target enzyme of the azole drugs (lanosterol 14α demethylase [14DM]), or in various efflux pumps from both the ABC transporter and major facilitator gene families. To determine which of these possible mechanisms was associated with the development of drug resistance in a particular case, mRNA levels have been studied in a series of 17 clinical isolates taken from a single HIV-infected patient over 2 years, during which time the levels of fluconazole resistance of the strain increased over 200-fold. Using Northern blot analysis of steady-state levels of total RNA from these isolates, we observed increased mRNA levels of *ERG16* (the 14DM-encoding gene), *CDR1* (an ABC transporter), and *MDR1* (a major facilitator) in this series. The timing of the increase in mRNA levels of each of these genes correlated with increases in fluconazole resistance of the isolates. Increased mRNA levels were not observed for three other ABC transporters, two other genes in the ergosterol biosynthetic pathway, or the NADPH–cytochrome P-450 oxidoreductase gene that transfers electrons from NADPH to 14DM. Increases in mRNA levels of *ERG16* and *CDR1* correlated with increased cross-resistance to ketoconazole and itraconazole but not to amphotericin B. A compilation of the genetic alterations identified in this series suggests that resistance develops gradually and is the sum of several different changes, all of which contribute to the final resistant phenotype.

Oral candidiasis is recognized as one of the earliest and most frequent opportunistic infections associated with AIDS (10). Candidiasis is caused by the opportunistic yeast *Candida albicans* and related yeasts (25). Oral candidiasis has been controlled largely by use of the systemic azole drug fluconazole and topical azole and polyene drugs. However, the use and occasional overuse of fluconazole has resulted in the emergence of azole-resistant strains of *Candida* (34). One recent estimate suggests that up to 33% of AIDS patients have an oral commensal strain of *Candida* that is azole resistant (20).

The azole drugs are fungistatic and work by competitive inhibition of lanosterol 14α demethylase (14DM), part of the biosynthetic pathway of ergosterol, a major and necessary membrane sterol (18, 25). *ERG16*, the gene coding for 14DM from *C. albicans*, has been cloned and sequenced (15, 19), as have two other genes in the ergosterol biosynthetic pathway, those encoding squalene epoxidase (*ERG1*) (17, 42) and oxidosqualene cyclase (*ERG7*) (13, 36), and the gene coding for NADPH–cytochrome P-450 oxidoreductase that transfers electrons from NADPH to 14DM (*PDR1*) (38). Biochemical analysis of azole-resistant strains of *C. albicans* has demonstrated that alterations in the ergosterol biosynthetic pathway are associated with azole resistance (11).

Recently, evidence has been presented that efflux of the azole drugs from the cells is associated with azole resistance (5, 26, 27, 40, 41). Sanglard and coworkers (41) have shown increased mRNA levels of the *CDR1* and *MDR1* genes in azole-resistant clinical isolates of *Candida*. *CDR1* is a member of the ABC transporter family of efflux pumps and *MDR1* is a member of the major facilitator family of pumps. Recently, this group has shown that genetic knockouts of these pumps in *Candida* result in hypersensitivity to antifungal drugs as well as other metabolic inhibitors (40).

The ABC transporters are a gene family of efflux pumps associated with the movement of small molecules across the plasma membrane (24). Four families of ABC transporters, *MDR*, *CFTR*, *YEF*, and *PDR*, have been identified in *Saccharomyces cerevisiae* based on gene homologies (24). To date, eight genes for ABC transporters have been identified in *Candida*. These genes include five *CDR* genes (*CDR1* to *CDR5*) (3, 38, 39) that are members of the *PDR* gene family, *HST6* from the *MDR* family (31), *YCF1* from the *CFTR* family (16), and *ELF1* from the *YEF* family (43).

The major facilitators are a related family of genes coding for efflux pumps (23). Family members include many transport molecules of prokaryotic cells, including the tetracycline transporter. The *MDR1* gene is the only major facilitator that has been cloned from *Candida* (2, 7, 9).

A series of 17 isolates collected during a 2-year period from a single AIDS patient has been studied extensively, because resistance to fluconazole developed gradually in this series and is correlated with the doses that were given to the patient (29, 32, 48). The isolates represent a single strain of *C. albicans* (29, 32, 48), although Ca3 typing indicates that one substrain is replaced by a closely related substrain between isolates 1 and 2 in the series (48). The phenotype of the resistant isolate is stable for at least 600 generations (48).

This study continues the characterization of the resistance mechanisms present in this series of isolates. mRNA levels of *ERG16*, *MDR1*, and *CDR1* as well as other genes that might be

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TABLE 1. Probes used in Northern blot analysis

Gene	DNA[a]	Sequence coordinates		GenBank sequence accession no.	Reference(s)
		Forward primer[b]	Reverse primer[c]		
ACT1	Oligonucleotide		2428–2527	X16377	21
CDR1	PCR	1210–1230	1999–2016	X77589	30
ELF1	Plasmid			Unpublished	43
ERG1	PCR	1–18	417–435	Unpublished[d]	42, 17
ERG7	Plasmid			L04305	36
ERG16	PCR	164–188	1564–1589	X13296	19
HST6	PCR	717–736	2100–2119	U13193	31
MDR1	PCR	2885–2904	3735–3754	X53823	7
PRD1	PCR	1–19	332–349	Unpublished[d]	38
YCF1	Plasmid			Unpublished[d]	16, 47

[a] Type of DNA used. Plasmids contain an insert from the gene. The *ACT1* probe is an oligonucleotide spanning the sequence coordinates listed under reverse primer, since the oligonucleotide is synthesized to hybridize with the coding strand. PCR DNA refers to gene fragments amplified by PCR. All probes were labeled by random priming with the exception of the *ACT1* probe, which was labeled with polynucleotide kinase (see Materials and Methods).
[b] The sequence coordinates are listed for the forward primers for all PCR fragments.
[c] The sequence coordinates are listed for the reverse primers for all PCR fragments as well as the ACT1 oligonucleotide probes.
[d] These unpublished sequences can be accessed on the *Candida* web page (42).

involved in resistance were investigated. By using the entire series of isolates, the timing of these changes has been resolved. In addition, MICs of other azoles (ketoconazole and itraconazole) and of amphotericin B have been monitored in this series.

MATERIALS AND METHODS

Strains and growth of cultures. The *C. albicans* isolates used in this study were obtained from single colonies and include 16 of the series of 17 oral isolates from a single patient. The remaining isolate in the series, isolate 10, was not available for analysis. The control strain was laboratory strain 3153A (ATCC 28367). Isolates were maintained at 25°C on YEPD agar plates (10 g of yeast extract, 20 g of peptone, 20 g of dextrose, and 15 g of Bacto Agar per liter) and subcultured weekly or were stored at −70°C in YEPD containing 10% glycerol. Cell cultures were inoculated with a single colony grown on a YEPD agar plate. Culture media components were obtained from Fisher Scientific Co. (Pittsburgh, Pa.). MICs of ketoconazole, itraconazole, and amphotericin B were determined using E-test strips (AB Biodisk North America Inc., Piscataway, N.J.) following the manufacturer's instructions. All E-test results were confirmed by the National Committee for Clinical Laboratory Standards broth macrodilution method (8).

RNA manipulations and Northern blots. All RNAs were prepared from logarithmically growing cells in the absence of azole drugs. RNA preparation and gel electrophoresis, Northern blotting, PCR amplification, labeling with polynucleotide kinase, and random priming for radioactive probe preparation were performed according to standard published methods (1, 22, 37).

The 10 probes that were used for Northern blots were prepared in several ways. Three different methods were used to prepare probes, because three different types of DNA were available to the author. Six probes were synthesized by PCR amplification using the oligonucleotide sets. Plasmids containing gene inserts were used for three probes, and a long oligonucleotide (50 nucleotides) was used for the final probe. Table 1 presents the 10 probes, the types of DNA used, the types of radioactive labeling used, the positions within the sequence from which the oligonucleotides were designed, and the GenBank reference numbers of the sequences. The choice of probe labeling was dictated by the type of DNA available. All of the probes were within the coding regions of the genes.

PCR fragments were purified from the oligonucleotide primers by spin column chromatography (28) and labeled by random priming with [α-^{32}P]dATP and the large subunit of DNA polymerase I (Klenow fragment). Plasmid probes were prepared by random priming of plasmids containing the gene. The ACT1 probe was synthesized as an oligonucleotide as previously described (46) and labeled with polynucleotide kinase and [γ-^{32}P]ATP. Unincorporated nucleotides were removed from each probe by spin column chromatography (28).

For quantitation, autoradiograms were scanned with a Sharp high-resolution color scanner, using the Adobe Photo Shop program, and quantitated by using the Molecular Dynamics ImageQuaNT program. Bands were compared after adjustment for differences in loading, based on the quantitation of actin bands, and for background.

RESULTS

Cross-resistance to other azoles and polyenes. MICs of fluconazole have previously been reported for this series, but MICs of other azole and polyene drugs have not been determined. The development of cross-resistance within the series might correlate with specific resistance mechanisms. MICs of the azoles itraconazole and ketoconazole and the polyene amphotericin B were determined (Fig. 1). The initial MICs of these drugs are within the range expected for susceptible strains of *Candida*. For both itraconazole and ketoconazole, some variation in the MICs is seen among the first 12 isolates, while clear increases in the MICs are observed in isolates 13 to 15 and large increases in MICs are observed in isolates 16 and

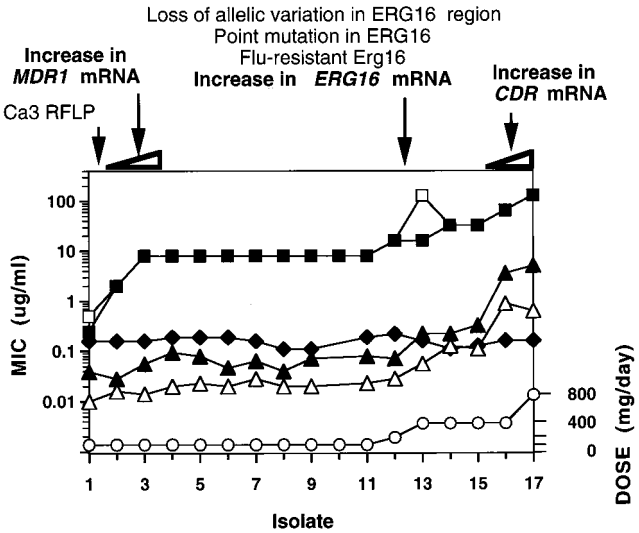

FIG. 1. Graph of fluconazole-resistant series. Isolates are shown on the *x* axis in the order in which they were obtained from the patient. The doses that was administered to the patient at the time of each isolate are shown (○) (48). Doses are given on a linear scale on the *y* axis to the right. MICs of fluconazole were determined by broth macrodilution (□) and broth microdilution (■) assays (48). Only two differences are observed between the two methods. MICs of itraconazole (▲), ketoconazole (△), and amphotericin B (◆) were determined by E-tests and confirmed by National Committee for Clinical Laboratory Standards broth macrodilution assays. MICs are given on a logarithmic scale on the *y* axis to the left. Genetic changes that were identified are summarized above the graph and are described in detail in the text. Alterations that have been previously described are in lightface text, including the Ca3 restriction fragment length polymorphism (RFLP) (48) as well as the changes in the *ERG16* gene and enzyme (44). Changes in mRNA levels are shown in boldface type and were first identified in this work.

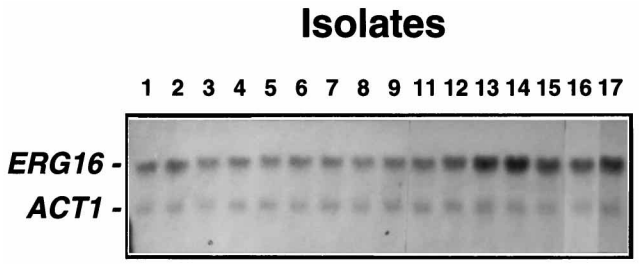

FIG. 2. Northern blots probed with *ERG16*. Total RNA (20 μg) from each isolate was electrophoresed through a formaldehyde agarose gel, blotted onto nitrocellulose, and probed with labeled DNA from *ERG16*. The blots were washed and exposed to X-ray film. Subsequently, the blots were rehybridized with a labeled probe for *ACT1*. RNA from isolate 16 was accidentally run between isolates 9 and 11 but has been moved to its appropriate location in the figure.

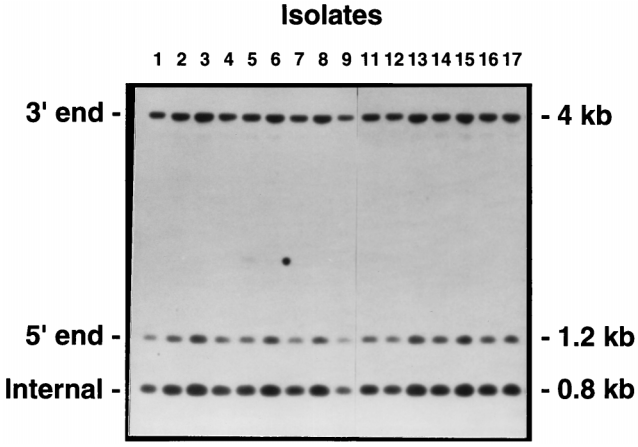

FIG. 3. Southern blots probed with *ERG16*. Genomic DNA (10 μg) was digested with *Hinc*II, electrophoresed through an agarose gel, blotted onto nitrocellulose, and probed with labeled DNA from *ERG16*. The blots were washed and exposed to X-ray film. Slight variations in the intensity of each lane correspond with slight variations in the amount of DNA that was loaded in the gel (45).

17. The MICs of both drugs are increased by >1 log between the first and last isolates. The MICs of amphotericin B are unchanged in the series.

Increased mRNA levels of *ERG16* without gene amplification. Increased expression of the target enzyme is a common phenomenon in eukaryotic drug resistance and has rarely been described previously for *Candida*. Total RNA was prepared from the 17 isolates grown in the absence of drug. Northern blots of these RNAs were probed with a gene fragment from *ERG16* and with an oligonucleotide for actin (*ACT1*) as a control (Fig. 2). Although some variability in signal was observed, densitometric analysis of the Northern blot in Fig. 2 indicated that the *ERG16* signal did not increase significantly until isolate 13. mRNA levels of *ERG16* are increased in isolates 13 to 17 approximately four- to fivefold compared to those in isolates 1 to 12. This increase coincides with a recently identified point mutation in *ERG16* and with the loss of allelic variation in the *ERG16* genomic region (44).

Overexpression of a gene in eukaryotic drug resistance is usually associated with gene amplification. To test for this possibility, Southern blots of genomic DNA from the 17 isolates digested with *Hinc*II were hybridized with a gene probe to *ERG16* (Fig. 3). The *ERG16* gene is not appreciably amplified in the series. In addition, *Hinc*II divides the *ERG16* gene into three fragments encoding the 5′ end of the gene, an internal fragment, and the 3′ end of the gene. The constant pattern of these fragments in the genomic Southern blots (Fig. 3) suggests that large-scale rearrangements have not occurred in the region surrounding the *ERG16* gene.

Increased mRNA levels of *MDR1* and *CDR1*. Sanglard and coworkers have shown that mRNA levels of *MDR1* and *CDR1* are increased in several fluconazole-resistant isolates of *C. albicans* (41). Northern blots of total RNA from the series of 17 isolates were hybridized with an *MDR1* gene probe (Fig. 4) and with a *CDR1* gene probe (Fig. 5). mRNA levels of *MDR1* are not detectable in isolate 1 and are dramatically increased in isolates 2 and 3 (Fig. 4). The signal from isolate 2 is at least 12-fold greater than that from isolate 1, and that from isolate 3 is approximately 25-fold greater than the signal from isolate 1. The high levels of mRNA for *MDR1* seen in isolate 3 continue for the rest of the series. RNA from isolate 13 was slightly degraded in this particular gel, but the decrease in signal intensity for the *ACT1* control was comparable to the decrease in the *MDR1* signal. This observation was confirmed by phosphorimager analysis and repeated with other RNA samples. The mRNA for *CDR1* is present at constant levels in isolates 1 to 15 and is dramatically increased in isolates 16 and 17, suggesting that *CDR1* contributes to resistance only at the end of this series (Fig. 5). The background hybridization in lanes 16 and 17 was more intense because the Northern blot was first hybridized to *CDR* and then to *ACT1*. The intense signal for *CDR* in lanes 16 and 17 could not be completely removed before hybridization with the *ACT1* probe. Therefore, the background hybridization was more intense. Phosphorimaging was used to analyze the amount of overexpression. The *CDR* band was quantitated based on the *ACT1* band only. Quantitation suggests that *CDR1* is increased approximately fivefold in isolates 16 and 17.

Recent work by several researchers has identified four other *CDR* genes that may be involved in drug resistance. Preliminary work shows that these genes cross-hybridize to each other under the conditions used (3, 38, 39, 45). Therefore, the increased mRNA levels seen in isolates 16 and 17 can be attributed to one of the *CDR* genes, but which of the genes it should be attributed to cannot be determined by this analysis.

Constant mRNA levels of other ABC transporters. As discussed in the introduction, a gene from each of four families of the ABC transporters has been identified in *C. albicans*. The *CDR* genes described above are members of the *PDR* gene

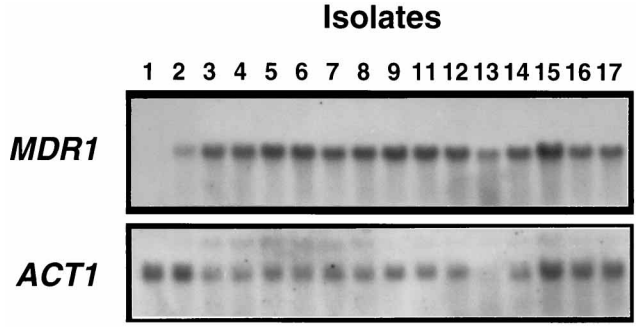

FIG. 4. Northern blots probed with *MDR1*. Total RNA (20 μg) from each isolate was electrophoresed through a formaldehyde agarose gel, blotted onto nitrocellulose, and probed with labeled DNA from *MDR1*. The blots were washed and exposed to X-ray film. Subsequently, the blots were rehybridized with a labeled probe for *ACT1*.

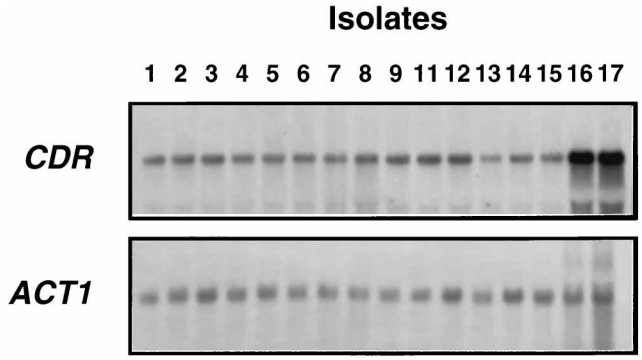

FIG. 5. Northern blots probed with *CDR1*. Total RNA (20 μg) from each isolate was electrophoresed through a formaldehyde agarose gel, blotted onto nitrocellulose, and probed with labeled DNA from *CDR1*. The blots were washed and exposed to X-ray film. Subsequently, the blots were rehybridized with a labeled probe for *ACT1*. The strong smearing in lanes 16 and 17 when the isolates were probed with *ACT1* is due to the *CDR1* signal that could not be removed before probing with *ACT1*.

family. To survey the other three families of ABC transporters, Northern blots of the entire series were hybridized with the gene probes listed in Table 1. Since no changes were observed in the series, Fig. 6 shows only Northern blot analyses from the first two isolates (1 and 2) and the last two isolates (16 and 17). For *ELF1* and *YCF1*, Northern blots from these four isolates illustrate the constancy of mRNA levels in the series for each probe. No change in mRNA levels is detected throughout the series for either of these two ABC transporter genes, members of the *YEF* and *CFTR* gene families.

Northern blot analysis (45) was also performed on *HST6*, a member of the *MDR* gene family that appears to be the *Candida* homolog of the *STE6* gene of *Saccharomyces*. No *HST6* mRNA was detected in any of the isolates from this series. Control blots of genomic DNA indicate that the hybridizations were working correctly, and hybridization of the Northern filters with the *ACT1* probe indicated that the filters were functional. This suggests that *HST6* is not expressed at detectable levels in the isolates under these growth conditions.

Constant mRNA levels of genes involved in ergosterol biosynthesis. The increased mRNA levels of *ERG16* may be specific to the gene, or common to several genes in the ergosterol biosynthetic pathway. In addition to *ERG16*, two other genes in the same pathway, *ERG1* and *ERG7*, have been cloned and sequenced. Probes to these genes were used against Northern blots of total RNA from the series (Fig. 6). The mRNA levels of these genes are not altered in the series. Similarly, a probe for *PRD1*, the reductase associated with *ERG16*, was used against Northern blots of total RNA from the series (Fig. 6); mRNAs levels for *PRD1* are not altered in the series. Relative decreases in band intensity observed for *YCF1*, *ERG1*, *ERG7*, and *PDR1* are comparable to the relative decreases for *ACT1*. Phosphorimager analysis confirmed that the relative changes were proportional. In addition, Fig. 6 presents only 4 of the 17 isolates. In order to minimize the size of the figure, the entire series is not presented. Inspection of the entire series for each gene confirms the constant expression of the genes throughout the series (45). This suggests that increased mRNA levels are restricted to *ERG16* and are not a general characteristic of the *ERG* biosynthetic pathway or genes associated with the pathway.

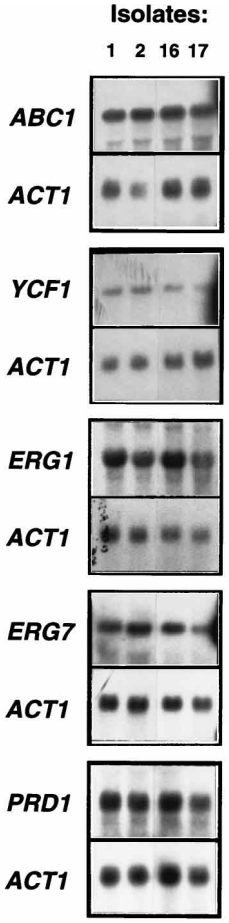

FIG. 6. Northern blots of total RNA from isolates 1, 2, 16, and 17 probed with *ABC1* (renamed *ELF1*), *YCF1*, *ERG1*, *ERG7*, and *PRD1*. Total RNA (20 μg) from each isolate in the series was electrophoresed through a formaldehyde agarose gel, blotted onto nitrocellulose, and probed with labeled DNAs as indicated. The blots were washed and exposed to X-ray film. Subsequently, the blots were reprobed with a labeled oligonucleotide for *ACT1*. Since no change is observed in the series for each probe, only the first two isolates and the last two isolates in the series are presented in the figure.

DISCUSSION

The results presented above (summarized in Fig. 1) continue an analysis of the series of 17 isolates from a single AIDS patient. From this and previous analyses (29, 32, 44, 48), it is clear that several genetic alterations are involved in the development of resistance in this series, including the selection of a substrain of *C. albicans*, isolate 2, demonstrated by a shift in Ca3 pattern (48), increased mRNA levels of *MDR1* (for isolate 1 compared to isolates 2 and 3 and beyond), genetic alterations in *ERG16*, including an Erg16 enzyme with increased resistance to fluconazole, a point mutation within *ERG16* and a loss of allelic variation in the *ERG16* region (44), increased mRNA levels of *ERG16* (all occur between isolate 12 and 13), and increased mRNA levels of *CDR* (in isolates 16 and 17).

The MICs in Fig. 1 suggest that changes in *ERG16* and overexpression of *CDR* are associated with increased cross-resistance to itraconazole and ketoconazole, consistent with previous observations (40). These changes in MICs are associated with clinical breakpoints. For fluconazole, isolates 12 to 15 show intermediate levels of susceptibility (16 to 32 μg/ml), while isolates 16 and 17 show resistant levels of susceptibility (≥ 64 μg/ml) (33). Similarly, MICs of itraconazole are inter-

mediate in resistance for isolates 13 to 15 (0.25 to 0.5 μg/ml) and resistant for isolates 16 and 17 (≥1 μg/ml) (33). MICs of ketoconazole are close to the resistant breakpoint for isolates 13 to 15 (0.06 to 0.12 μg/ml) and highly resistant for isolates 16 and 17 (≥0.25 μg/ml) (35). None of the changes shown in Fig. 1 appears to affect the level of amphotericin B susceptibility (Fig. 1), which is consistently clinically sensitive (<1 μg/mL). Since resistance in this series of isolates is an accumulation of several mechanisms, generalizations about specific mechanisms and clinical resistance to the azoles should not be drawn.

It is important to stress that changes in mRNA levels are not necessarily the result of an increase in transcription. Changes in mRNA levels can be the result of alterations in capping and polyadenylation, in mRNA transport from the nucleus, in translation rates of the mRNA, and in mRNA degradation, all of which can occur by several pathways. The results for this series, and for the other clinical isolates that have been described (41), clearly indicate that increased mRNA levels are associated with resistance. Further analysis is needed to determine the process by which mRNA levels for *ERG16*, *CDR*, and *MDR1* were altered in these isolates. It is also important to stress that increased mRNA levels do not necessarily lead to increases in protein levels or enzymatic activity. Further analysis of the Erg16 enzyme is currently in progress.

Changes in the transcript levels of *ERG16* (Fig. 2) have been described previously for *Candida*, although those changes were not associated with resistance (41). Increased expression of the 14DM gene in *S. cerevisiae* has been described previously (4, 6, 12, 14). In each case, increases in mRNA levels were associated with small increases in resistance. The increases in mRNA levels in *Candida* are not the result of gene amplification, as determined by Southern blot analysis (Fig. 3). Increased mRNA levels may be due to a loss of allelic variation in the *ERG16* gene region (44). This loss of variation was most likely the result of gene conversion or mitotic recombination. If the donor allele in the gene alteration encoded a stronger promoter, the alteration would result in increased mRNA levels as documented here.

Increased mRNA levels for *CDR* and for *MDR1* have been previously described for other clinical isolates (41). However, this is the first clinical isolate in which both genes are expressed simultaneously. In addition, a series of isolates was not previously available to define the timing of the increased levels or to correlate those levels with levels of resistance as has been done with this series of 17 isolates. For both *MDR1* and *CDR*, stepwise increases are associated with increased resistance (Fig. 1). For *MDR1*, the increase builds from no detectable mRNA in isolate 1 to high levels of mRNA in isolate 3. For *CDR*, a large increase in mRNA levels is detected in both isolates 16 and 17. In describing the *CDR* increases, we have been unable to determine which of the five *CDR* genes is responsible for the mRNA increase because of cross-hybridization of the probes (45).

The *CDR* genes are all members of the *PDR* family of ABC transporters. mRNA levels were also examined for members of three other families, *MDR*, *CFTR*, and *YEF*. Transcripts are not detected for *HST6*, a member of the *MDR* family. Transcripts are detected for *ELF1*, a member of the *YEF* family and *YCF1*, a member of the *CFTR* family, although the mRNA levels are the same throughout the series (Fig. 6). This suggests that in this series, increased mRNA levels are restricted to the *PDR* family and are not a general phenomenon of all ABC transporters. These findings are consistent with those for the ABC transporter families in *Saccharomyces*, for which the *PDR* family is most frequently associated with drug resistance. Further conclusions await the identification of other ABC transporters. At this time, *MDR1* is the only major facilitator that has been identified in *Candida*.

Increased levels of *ERG16* mRNA may also be correlated with transcriptional regulation of the entire ergosterol biosynthetic pathway. To test this, mRNA levels in the series were monitored for two other genes from the pathway, *ERG1* and *ERG7*, and for *PRD1*, the gene encoding the NADPH reductase associated with *ERG16* (Fig. 6). The transcripts from these genes are not altered in the series, suggesting that the transcriptional control of *ERG16* mRNAs is not a general effect on every gene in the ergosterol pathway. The *PRD1* gene has previously been monitored for expression in resistant clinical isolates (41) for which no change in expression was identified.

The summary of results in Fig. 1 emphasizes that resistance develops gradually and that a highly resistant isolate can be the result of several different genetic alterations. The order of these changes as defined by this series of isolates is probably not important because there is no evidence to suggest that increased levels of *MDR1* must precede changes in *ERG16*. However, it is noteworthy that under selective pressure from an antifungal agent, *Candida* cells can generate a resistant phenotype by using a variety of different genetic changes. As each genetic alteration occurs, cells with that mutation can overgrow the more susceptible cells in the population. Under continued selective pressure from an antifungal agent, several rounds of genetic alteration and overgrowth can occur, and the final result is a highly resistant strain containing a collection of mutations, each of which contributes to the phenotype. These results have serious clinical implications for the prevention and treatment of antifungal resistance, since several unrelated mechanisms can contribute to the resistant phenotype in a strain of *Candida*.

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