ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, May 1990, p. 831–836
0066-4804/90/050831-06$02.00/0

Vol. 34, No. 5

Azole Susceptibility and Hyphal Formation in a Cytochrome P-450-Deficient Mutant of *Candida albicans*

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Received 11 October 1989/Accepted 7 February 1990

A cytochrome P-450-deficient mutant of *Candida albicans*, strain D10, was employed to study the mode of action of imidazole antifungal agents. This mutant accumulates exclusively 14-α-methylsterols, resulting in a sterol profile which mimics that of azole-treated wild-type strains. Since the widely accepted primary effect of imidazoles is the inhibition of cytochrome P-450-mediated demethylation of the ergosterol precursor lanosterol, strain D10 and its wild-type revertant, strain D10R, were grown in the presence of concentrations of clotrimazole, miconazole, and ketoconazole known to inhibit demethylation. The growth of strain D10 was unaffected by these antifungal agents, while that of strain D10R was significantly reduced. At higher azole concentrations (which are known to exert a direct, disruptive action on the cell membrane), the growth of both strains was immediately and completely inhibited by clotrimazole and miconazole. Ketoconazole was membrane disruptive only for strain D10; this is the first report of a direct membrane effect for this drug. Because hyphal formation has been implicated in the pathogenesis of *C. albicans* and because it has been shown to be inhibited by azoles, the hypha-forming capability of strain D10 was examined. Strain D10 was shown to be seriously defective in hyphal formation, suggesting that this function may be dependent on the 14-α-demethylation of lanosterol. The results of this study suggest that inhibition of lanosterol demethylation per se is neither fungicidal nor fungistatic, although the growth rate is reduced. In addition, the substitution of 14-α-methylsterols for ergosterol results in defective hyphal formation and in a cell that is more susceptible to membrane-active agents such as ketoconazole.

Candida albicans is an imperfect, dimorphic yeast which is found as a commensal inhabitant of the human body. This organism is the most important and prevalent human fungal pathogen causing superficial as well as potentially life-threatening systemic mycoses (12, 18). The major groups of currently available antifungal compounds include the polyenes and the azoles. Amphotericin B, a polyene, is the major antibiotic employed in systemic infections. It has a history of effective use spanning three decades (26). This drug acts by binding to ergosterol, the major fungal membrane sterol, and causing lethal changes in membrane structure and function (24, 32, 38). However, amphotericin B has several undesirable side effects which restrict its use.

The azole (imidazole and triazole) antifungal agents have been reported to have a variety of deleterious effects on fungi. The most thoroughly investigated antifungal effect of the azoles is the specific inhibition of the cytochrome P-450-dependent demethylation of the ergosterol precursor lanosterol (13, 14, 35, 37, 38). The detailed mechanism by which this inhibition occurs has been described previously (38). It occurs at low drug concentrations and results in the accumulation of 14-α-methylsterols which replace ergosterol in the membrane. It has been suggested (38) that the presence of 14-α-methylsterols in the cell membrane results in the cessation of cell growth (fungistatic effect). This conclusion is based on two observations. The first is the fact that the substitution of lanosterol for ergosterol in fungal membranes induces a variety of functional alterations such as permeability changes and leakiness that result in growth inhibition. The second observation is based on studies using *Saccharomyces cerevisiae* which indicate that hormonal amounts of the end product sterol, ergosterol, are required for the G1-to-S transition in the cell cycle ("sparking" function; 9). Since the azole inhibition of lanosterol demethylation would be predicted to result in both the accumulation of lanosterol and the absence of ergosterol, it was concluded that the efficacy of the azoles was based on growth inhibition. The sparking sterol requirement in *S. cerevisiae* may be different from that of *C. albicans* since all demethylase mutants of the former are either leaky for ergosterol production or are accompanied by a second mutation. Several demethylase mutants of *C. albicans* have been reported (1, 15, 29), and one of these (1) has been shown to be a single-lesion mutant that is devoid of detectable levels of cytochrome P-450 and ergosterol.

A second, well-documented effect of the azole antifungal agents is their direct, disruptive interaction with the nonsterol, lipid component of the fungal membrane (8, 13, 15, 31, 35, 36). The mechanism by which membrane disruption occurs has not been elucidated, although several parameters of this interaction have been characterized (3, 4). Since the drug concentrations required to induce direct membrane disruption are higher than could be reached in vivo, this mechanism of action is thought to be important only in topical applications of these compounds. In addition to the inhibition of lanosterol demethylation and direct membrane disruption, azoles have been shown to interfere with respiratory metabolism (28, 34), increase membrane permeability

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(35), alter fatty acid synthesis (6, 35), inhibit the formation of hyphae (10, 16, 23, 27), and interfere with the activity of membrane-bound enzymes (32, 38).

This multiplicity of effects plus the results presented here showing that some 14-α-methylsterols permit the growth of *C. albicans* raise the question as to the precise mode of action of the azole antifungal agents. The absence of this information becomes an important issue in understanding the physiology and pathogenicity of parasitic fungi as well as in the design of new antifungal compounds. In this study, a previously described cytochrome P-450-deficient mutant, strain D10, of *C. albicans* (1) is characterized with regard to susceptibility to azoles and to the ability to form hyphae. Strain D10 permits us to uncouple the inhibition of demethylation from the fungistatic and fungicidal activities of the azoles. Because the effect of the mutation mimics the effects of azole antifungal agents on sterol synthesis, D10 provides an excellent model system in which to pursue the effects of methylsterol accumulation on the physiological properties of *C. albicans* that are related to its pathogenesis.

MATERIALS AND METHODS

Organisms and growth conditions. Nystatin-resistant *C. albicans* D10 was obtained from the American Type Culture Collection, Rockville, Md. (ATCC 38247). Strain D10R, a wild-type revertant, was selected as a fast-growing colony on a complete medium plate containing a lawn of strain D10 and has been previously shown to have a wild-type sterol profile, with ergosterol constituting 70% of the total membrane sterol (1). Both strains were grown on a complete medium composed of 1% yeast extract (Difco Laboratories), 2% Bacto-Peptone (Difco), and 2% dextrose (YEPD). Solid medium was supplemented with 2% Bacto-Agar (Difco). Strain D10 was maintained on YEPD agar medium plus 40 μg of nystatin (Sigma Chemical Co.) per ml. Nystatin stocks (4 mg/ml) were prepared in dimethylformamide and stored frozen. All growth was at 30°C and was monitored in liquid cultures by using a Klett-Summerson colorimeter with a no. 66 red filter.

Azole susceptibility studies. Cells from overnight YEPD or YEPD plus nystatin plates were suspended in 0.9% saline and inoculated into 250-ml nephelometer flasks (Bellco Glass, Inc.) containing fresh medium to yield initial concentrations of 8 to 15 Klett units. For studies using elevated azole concentrations that would be expected to have a direct effect on the fungal membrane (10 μM and higher), the flasks were incubated in a Gyrotory shaker (model G-76; New Brunswick Scientific Co., Inc.) at 120 rpm at 30°C until the cells entered the exponential growth phase. Portions of azole (clotrimazole, miconazole, and ketoconazole; Sigma) stock solutions (100 mM in 95% ethanol or dimethyl sulfoxide) were added, and growth was monitored during continued incubation. Klett units were adjusted to account for the turbidity conferred by some of the azoles at the highest concentrations. Neither ethanol nor dimethyl sulfoxide affected growth at the concentrations employed. Both solvents yielded identical data. Mean doubling times (mdt) were calculated, and percent inhibition was determined from each mdt by comparison to the appropriate control.

For studies using low azole concentrations (10 μM and lower), cells from overnight plates were inoculated into nephelometer flasks containing fresh medium as described above, and portions of azole stock solutions were immediately added. Growth was monitored, and percent inhibition was calculated as described above.

Hyphal formation. Hyphal formation was carried out as previously described (5) in Eagle minimal essential medium (Sigma) supplemented with 1% nonessential amino acids (Sigma)–10% fetal calf serum (GIBCO Laboratories)–1.2 g of sodium bicarbonate per liter. D10 and D10R cells from fresh overnight YEPD or YEPD plus nystatin plates were inoculated at a concentration of 8×10^4 cells per ml into tissue culture plates containing the supplemented Eagle medium. The plates were incubated at 37°C in air containing 5% CO_2. Wells were viewed on an inverted microscope at 100× magnification after 4 and 24 h of incubation. Cells were scored for the presence or absence of hyphae.

RESULTS

In this study, *C. albicans* D10, originally isolated by Pierce et al. (25) as a polyene-resistant, 14-α-demethylation-deficient strain, was employed to separate the effect of the inhibition of lanosterol demethylation from the direct membrane effects of azoles. We have previously shown (1) this strain to be devoid of detectable levels of cytochrome P-450 while containing levels of cytochromes *a*, *b*, and *c* equivalent to those found in the wild-type revertant, strain D10R. This latter observation, coupled with the capacity of strain D10 to grow on nonfermentable energy sources (M. C. Broughton, unpublished results) (indicating the presence of a functional respiratory chain), eliminated the possibility that the block in lanosterol demethylation was the result of a lesion in the heme biosynthetic pathway. Since this organism accumulated exclusively 14-α-methylsterols, it served as a mimic for an azole-treated cell but without the presence of the drug. Strain D10 accumulated 14-methylfecosterol (37%), 24-methylene-24,25-dihydrolanosterol (25.7%), 24,25-dihydrolanosterol (19.8%), obtusifoliol (13.4%), and lanosterol (4.1%) (1), which is similar to the sterol profiles for azole-treated cells (35).

Growth with low concentrations of imidazoles. The initial question posed was whether strain D10 was resistant to levels of azole that would be expected to inhibit lanosterol demethylation only. Figure 1A shows the growth response of strain D10 to clotrimazole at 1, 5, and 10 μM. A similar response was noted when miconazole was substituted for clotrimazole. The growth profile of strain D10 was unaffected at 1 and 5 μM, indicating that the absence of a functional demethylase enzyme results in a cell that is resistant to low levels of azoles. This suggests that lanosterol demethylation is a primary effect of azole treatment at these concentrations. At 10 μM, a decrease in growth rate was observed. This inhibition was likely due to a direct membrane effect, as has been reported to occur at concentrations of approximately 10 μM and higher (35). Ketoconazole had a lesser effect on the growth of strain D10 at all concentrations tested (Fig. 1B), which is consistent with reports (35, 36) that, because of its physical properties, this drug exhibits no direct membrane effects. In contrast, Fig. 1C shows the growth inhibition response of strain D10R with the same concentrations of clotrimazole. Similar inhibitory patterns were noted in the presence of miconazole and ketoconazole. The kinetic features of the inhibition showed a slow, gradual decrease in growth rate for a 3- to 4-h period followed by a constant, reduced growth rate. This decline in growth rate was likely due to the gradual substitution of 14-α-methylsterols for ergosterol. Once the sterol substitution was complete, the cells assumed a growth rate similar to that seen in untreated cultures of strain D10. Table 1 presents the growth data from two or three individual experiments using

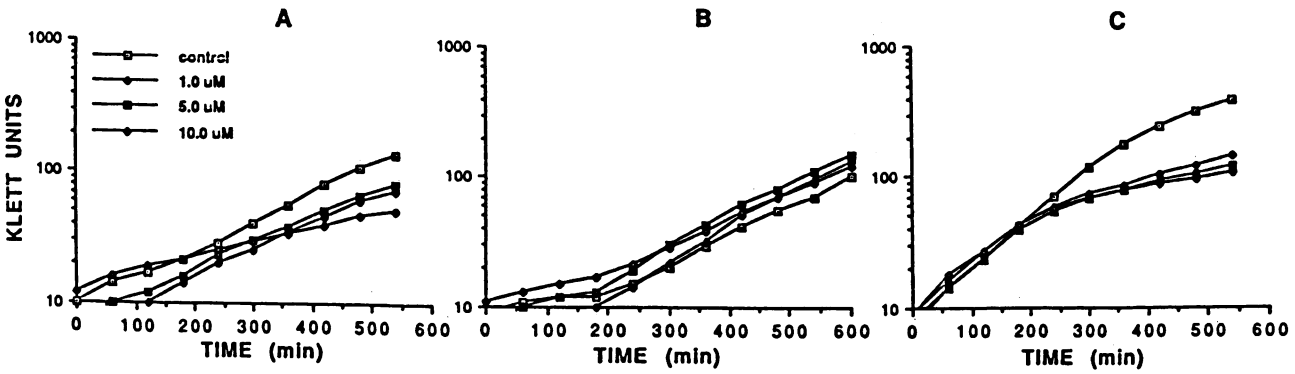

FIG. 1. Effects of low concentrations of azoles on the growth of *C. albicans* D10 and D10R. (A) D10 grown in the presence of clotrimazole; (B) D10 grown in the presence of ketoconazole; (C) D10R grown in the presence of clotrimazole.

all three drugs on both strains. mdt for azole-treated cultures of strain D10R were determined after ergosterol replacement by 14-α-methylsterols was complete and the growth rate was constant. Clotrimazole was the most efficient of all three azoles in inhibiting fungal growth. Strain D10R was significantly inhibited at all concentrations of the three azoles, whereas strain D10 showed essentially no inhibition at 1 μM concentrations and slight inhibition at 5 μM. The latter effect may be the result of direct membrane interaction, which occurred to a much greater extent at 10 μM concentrations. The appearance of low levels of inhibition of strain D10 by 10 μM ketoconazole was surprising since a direct membrane effect has not been previously reported for this drug. This observation may reflect a change in membrane structure which now permits a direct interaction with this azole.

Growth with high concentrations of azoles. To assess the effects of sterol substitution on membrane sensitivity to azoles, strains D10 and D10R were grown in the presence of concentrations of azoles that have been shown to exert a direct, disruptive membrane effect (8, 35). Since this direct membrane interaction has been reported to occur without delay (8), the growth protocol was modified. Freshly inoculated flasks were grown for 2 to 3 h to early exponential phase before the addition of azole. Figure 2A shows a growth profile seen for strain D10 with clotrimazole at concentrations of 10, 50, and 100 μM. Similar profiles were observed with strain D10 in the presence of miconazole and with strain D10R in the presence of clotrimazole or miconazole. Concentrations of 50 and 100 μM immediately and completely stopped growth. Viability of the cells was also rapidly lost, indicating a direct, lethal effect on the cells. Concentrations of 10 μM showed a partial inhibition of growth of both strains. The results with ketoconazole (Fig. 2B and C) indicated a different pattern of growth inhibition. Strain D10R (Fig. 2B) showed no evidence of a direct membrane effect. However, all three concentrations of the drug produced a gradual nonlinear slowing of growth similar to that shown in Fig. 1C, where low levels of ketoconazole were employed. A more interesting response was noted with strain D10 (Fig. 2C). Concentrations of 50 and 100 μM ketoconazole clearly showed an immediate, complete inhibition of growth identical to that noted with similar concentrations of clotrimazole and miconazole. This suggested that the membrane structural changes resulting from the substitution of 14-α-methylsterols for ergosterol produce a membrane that is subject to direct damage by ketoconazole. Table 2 shows the mean percent inhibitions obtained in two or three experiments (Fig. 2). The mdt calculated for strain D10R were determined within 2 to 3 h of drug addition and, therefore, did not take into account the full effects of

TABLE 1. Growth inhibition of *C. albicans* D10 and D10R with low concentrations of azoles

Azole (μM)	% Growth inhibition (mean ± SD) in[a]:	
	D10	D10R
Clotrimazole		
1	7.0 ± 7.0	68 ± 7.0
5	14.5 ± 10.6	69.7 ± 11.6
10	51 ± 5.7	78 ± 4.4
Miconazole		
1	−6.0 ± 8.5	50.3 ± 1.5
5	7.3 ± 7.5	58.3 ± 1.5
10	26.3 ± 16.7	63.3 ± 9.3
Ketoconazole		
1	−5.3 ± 11.0	51.3 ± 7.6
5	0 ± 7.0	58 ± 4.4
10	9.0 ± 2.0	59.7 ± 2.9

[a] All values were calculated from two or three independent experiments.

TABLE 2. Growth inhibition of *C. albicans* D10 and D10R with high concentrations of azoles

Azole (μM)	% Growth inhibition (mean ± SD) in[a]:	
	D10	D10R
Clotrimazole		
10	48.5 ± 3.5	25.5 ± 9.2
50	81.5 ± 7.8	87 ± 18.3
100	100 ± 0.0	100 ± 0.0
Miconazole		
10	24.5 ± 7.8	34 ± 9.9
50	100 ± 0.0	92 ± 11.3
100	100 ± 0.0	100 ± 0.0
Ketoconazole		
10	22.7 ± 4.5	14 ± 13.5
50	85.3 ± 1.5	35 ± 10.2
100	100 ± 0.0	33.7 ± 17.0

[a] All values were calculated from two or three independent experiments.

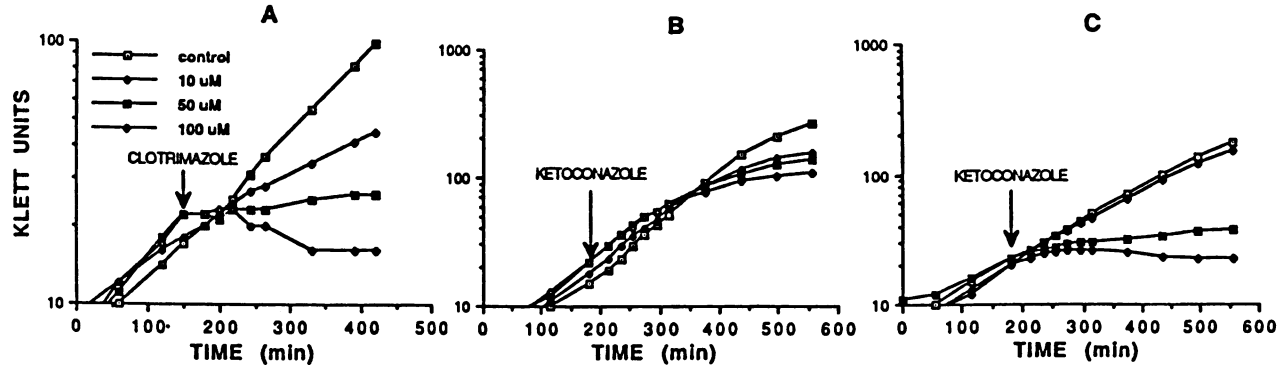

FIG. 2. Effects of high concentrations of azoles on the growth of *C. albicans* D10 and D10R. (A) D10 grown in the presence of clotrimazole; (B) D10R grown in the presence of ketoconazole; (C) D10 grown in the presence of ketoconazole.

ergosterol replacement. Thus, the percent inhibitions were not directly comparable to those presented in Table 1. The ketoconazole-induced complete and immediate inhibition of growth of strain D10 was equal to the inhibition levels shown for clotrimazole and miconazole.

Hyphal formation. Since hyphal formation has been implicated in the pathogenesis of *C. albicans* (29, 30) and antifungal agents including the azoles have been shown (10, 16, 23, 27) to inhibit hyphal formation, it was of interest to assess the hypha-forming capabilities of strain D10. Shimokawa et al. (29) reported that a 14-α-methylsterol-accumulating, polyene-resistant mutant (strain KD4700) of *C. albicans* was defective in hyphal formation. Although the sterol intermediates produced were neither completely identified or quantitated, nor was the absolute absence of ergosterol verified, strain KD4700 exhibited a reduced growth rate similar to that seen here for strain D10. Revertants of the sterol biosynthetic lesion in this strain were shown to have a wild-type sterol profile as well as normal hypha-forming capacity. Table 3 presents the results of an established procedure (5) for assessing hyphal formation in *C. albicans*. Since strain D10 grew more slowly than strain D10R, observations of hyphal formation were made at two different time intervals to eliminate the possibility that decreased hyphal formation was merely the result of a time factor proportional to growth rate. The results clearly indicate that strain D10 has a significantly reduced capability to form hyphae after 4 h of incubation (Table 3). Prolonged incubation (24 h) did not enhance the extent of hyphal formation.

TABLE 3. Percent hyphal formation in *C. albicans* D10 and D10R

Strain (incubation time)	% Hyphal formation	
	Individual expt (mean ± SD)[a]	All cells observed[b]
D10		
4 h	6.0 ± 4.6	4.1
24 h	4.8 ± 3.2	3.9
D10R		
4 h	100 ± 0.0	100
24 h	100 ± 0.0	100

[a] Values represent the means ± standard deviations of seven individual experiments.

[b] Over 700 D10 and 1,000 D10R cells were observed. Values represent the percent hyphal formation in all cells observed.

DISCUSSION

This report clarifies some aspects of the mechanism by which azoles are able to eliminate fungal infections. It is apparent that the efficacy of this class of drugs does not involve a simple, straightforward mode of action. The inhibition of lanosterol demethylation at low drug concentrations is clearly involved in the mechanism of action, although it cannot be directly or possibly exclusively responsible for the elimination of fungal infections since it is evident that genetic removal of the demethylation reaction is neither fungistatic nor fungicidal in *C. albicans*. It is clear from these analyses that the sterol composition of strain D10 permits reasonable growth (mdt, 105 min on YEPD) compared with that of the wild-type revertant (mdt, 70 min on YEPD). Similar reductions in growth rate have been reported for two other *C. albicans* strains, although the precise nature of the genetic lesions in these strains was not determined (15, 29).

The results presented here and those in other reports indicate several characteristics regarding the antifungal activity of the azoles. First, the inhibition of lanosterol demethylation results in a slower-growing cell but does not completely inhibit growth (fungistatic effect). Second, cells which accumulate 14-α-methylsterols are seriously defective in hyphal formation. Shimokawa et al. (29) reported that among 75 polyene-resistant (presumably ergosterol) mutants of *C. albicans*, only strain KD4700 accumulated 14-α-methylsterols and was defective in hyphal formation. This indicates that the presence of 14-α-methylsterols, rather than the absence of ergosterol, plays an important role in the inhibition of hyphal formation. The association of a specific membrane sterol composition with decreased growth rate and the inability to normally generate hyphae has important implications regarding the mechanism involved in hyphal formation as well as the mechanism of azole efficacy. Third, the substitution of 14-α-methylsterols for ergosterol results in a membrane with altered properties. Shimokawa et al. (30) have since reported increased susceptibility of strain KD4700 to a variety of compounds, indicating that membrane permeability properties were altered and the sensitivity of the membrane to certain membrane-active agents was elevated. In contrast, Hitchcock et al. (15) have reported that an azole-resistant demethylase mutant, strain 6 · 4, of *C. albicans* was less permeable to azole. The authors concluded that this may be the basis of resistance. Our results support those of Shimokawa et al., as we have recently reported (21) that the membrane fluidity in strain D10 is

significantly decreased throughout the lipid bilayer as a result of sterol substitution. Previous studies of *S. cerevisiae* have shown that increased membrane rigidity as the result of sterol substitution (19, 20) is related to increased permeability (2, 17), decreased growth rate (22, 24, 33), decreased respiratory function (22), increased sensitivity to membrane-active agents (22), and altered activity of membrane-bound enzymes (7, 11). These physiological characteristics are also among those reported for azole-treated cells (28, 32, 34, 35, 38). We have also determined (M. C. Broughton, unpublished results) that the growth of strain D10 is more susceptible to detergents compared with that of strain D10R. The results presented here also indicate that strain D10 shows a susceptibility to a direct membrane interaction with ketoconazole, an azole which has been previously reported not to have such an effect (35, 36). The observation that strain D10 and, therefore, an azole-treated cell have sensitized and potentially vulnerable membranes may be important in the development of membrane-active agents that could target this altered membrane. Such a therapy could be especially critical for treating fungal infections in immunocompromised individuals in whom the infection could be arrested by azole treatment, but viable cells would remain.

ACKNOWLEDGMENTS

This work was supported in part by Public Health Service grant 1R15 GM 37449-01 to M.B. from the National Institutes of Health.

We thank R. O. McCracken for his helpful suggestions in the preparation of the manuscript.

LITERATURE CITED

1. **Bard, M., N. D. Lees, R. J. Barbuch, and D. Sanglard.** 1987. Characterization of a cytochrome P450 deficient mutant of *Candida albicans*. Biochem. Biophys. Res. Commun. **147:** 794–800.
2. **Bard, M., N. D. Lees, L. S. Burrows, and F. W. Kleinhans.** 1978. Differences in crystal violet uptake and cation-induced death among yeast sterol mutants. J. Bacteriol. **135:**1146–1148.
3. **Beggs, W. H.** 1988. Requirement for nonprotonated drug molecules in the direct lethal action of miconazole against *Candida albicans*. Mycopathologia **103:**91–94.
4. **Beggs, W. H.** 1988. Regulation of the direct fungicidal action of miconazole against *Candida albicans* ATCC 26790. Mycoses **31:**266–228.
5. **Borgers, M., M. De Brabender, H. Van den Bossche, and J. Van Cutsem.** 1979. Promotion of pseudomycelium formation of *Candida albicans* in culture: a morphological study of the effects of miconazole and ketoconazole. Postgrad. Med. J. **55:**687–691.
6. **Borgers, M., and H. Van den Bossche.** 1982. The mode of action of antifungal drugs, p. 25–47. *In* H. B. Levine (ed.), Ketoconazole in the management of fungal disease. Adis Press, New York.
7. **Cobon, G. S., and J. M. Haslam.** 1973. The effect of altered membrane sterol composition on the temperature dependence of yeast mitochondrial ATPase. Biochem. Biophys. Res. Commun. **52:**320–326.
8. **Cope, J. E.** 1980. Mode of action of miconazole on *Candida albicans*: effect on growth, viability, and K^+ release. J. Gen. Microbiol. **119:**245–251.
9. **Dahl, C., H.-P. Biemann, and J. Dahl.** 1987. A protein kinase antigenically related to $pp60^{v\text{-}src}$ possibly involved in yeast cell control. Proc. Natl. Acad. Sci. USA **84:**4012–4016.
10. **Davies, A. R., and M. S. Marriot.** 1981. Inhibitory effects of imidazole antifungals on the yeast mycelial transformation in *Candida albicans*. Microbios Lett. **17:**155–158.
11. **Demel, R. A., and B. DeKruyff.** 1976. The function of sterols in membranes. Biochim. Biophys. Acta **457:**109–132.
12. **Georgopapadakou, N. H., B. A. Dix, S. A. Smith, J. Freudenberger, and P. T. Funke.** 1987. Effect of antifungal agents on lipid biosynthesis and membrane integrity in *Candida albicans*. Antimicrob. Agents Chemother. **31:**46–51.
13. **Henry, M. J., and H. D. Sisler.** 1979. Effects of miconazole and dodecyliminidazole on sterol biosynthesis in *Ustilago maydis*. Antimicrob. Agents Chemother. **15:**603–607.
14. **Henry, M. J., and H. D. Sisler.** 1984. Effects of sterol biosynthesis-inhibiting (sbi) fungicides on cytochrome P-450 oxygenations in fungi. Pestic. Biochem. Physiol. **22:**262–275.
15. **Hitchcock, C. A., K. J. Barrett-Bee, and N. J. Russell.** 1987. The lipid composition and permeability to azole of an azole- and polyene-resistant mutant of *Candida albicans*. J. Med. Vet. Mycol. **25:**29–37.
16. **Hitchcock, C. A., K. J. Barrett-Bee, and N. J. Russell.** 1989. The lipid composition and permeability to the triazole antifungal antibiotic ICI 153066 of serum-grown mycelial cultures of *Candida albicans*. J. Gen. Microbiol. **135:**1949–1955.
17. **Kleinhans, F. W., N. D. Lees, M. Bard, R. A. Haak, and R. A. Woods.** 1979. ESR determination of membrane permeability in a yeast sterol mutant. Chem. Phys. Lipids **23:**143–154.
18. **Kurtz, M. B., D. R. Kirsch, and R. Kelly.** 1988. The molecular genetics of *Candida albicans*. Microbiol. Sci. **5:**58–63.
19. **Lees, N. D., M. Bard, M. D. Kemple, R. A. Haak, and F. W. Kleinhans.** 1979. ESR determination of membrane order parameter in yeast sterol mutants. Biochim. Biophys. Acta **553:** 469–475.
20. **Lees, N. D., M. D. Kemple, R. J. Barbuch, M. A. Smith, and M. Bard.** 1984. Differences in membrane order parameter and antibiotic sensitivity in ergosterol-producing strains of *Saccharomyces cerevisiae*. Biochim. Biophys. Acta **776:**105–112.
21. **Lees, N. D., F. W. Kleinhans, M. C. Broughton, D. E. Pennington, V. A. Ricker, and M. Bard.** 1989. Membrane fluidity alterations in a cytochrome P-450-deficient mutant of *Candida albicans*. Steroids **53:**567–578.
22. **Lees, N. D., S. L. Lofton, R. A. Woods, and M. Bard.** 1980. The effects of varied energy source and detergent on the growth of sterol mutants of *Saccharomyces cerevisiae*. J. Gen. Microbiol. **118:**209–214.
23. **Odds, F. C., A. Cockayne, J. Hayward, and A. B. Abbott.** 1985. Effects of imidazole- and triazole-derivative antifungals on the yeast mycelial transformation in *Candida albicans*. J. Gen. Microbiol. **131:**2581–2589.
24. **Pesti, M., S. Paku, and E. K. Novak.** 1982. Some characteristics of nystatin-resistant sterol mutants of *Candida albicans*. Acta Microbiol. Hung. **29:**55–66.
25. **Pierce, A. M., H. D. Pierce, A. M. Unrau, and A. C. Oehlschlager.** 1978. Lipid composition and polyene antibiotic resistance of *Candida albicans* mutants. Can. J. Biochem. **56:** 135–142.
26. **Saag, M. S., and W. E. Dismukes.** 1988. Azole antifungal agents: emphasis on new triazoles. Antimicrob. Agents Chemother. **32:**1–8.
27. **Schaude, M., H. Ackerbauer, and H. Mieth.** 1987. Inhibitory effect of antifungal agents on germ tube formation in *Candida albicans*. Mykosen **30:**281–287.
28. **Shigematsu, M. L., J. Uno, and T. Arai.** 1982. Effect of ketoconazole on isolated mitochondria from *Candida albicans*. Antimicrob. Agents Chemother. **21:**919–924.
29. **Shimokawa, O., Y. Kato, and H. Nakayama.** 1986. Accumulation of 14-methyl sterols and defective hyphal growth in *Candida albicans*. J. Med. Vet. Mycol. **24:**327–336.
30. **Shimokawa, O., Y. Kato, and H. Nakayama.** 1986. Increased drug sensitivity in *Candida albicans* cells accumulating 14-methylated sterols. J. Med. Vet. Mycol. **24:**481–483.
31. **Sud, I. J., and D. S. Feingold.** 1981. Heterogeneity of action mechanisms among antimycotic imidazoles. Antimicrob. Agents Chemother. **20:**71–74.
32. **Surarit, R., and M. G. Shepherd.** 1987. The effects of azole and polyene antifungals on the plasma membrane enzymes of *Candida albicans*. J. Med. Vet. Mycol. **25:**403–413.
33. **Thompson, E. D., and L. W. Parks.** 1974. Effect of altered sterol composition on growth characteristics of *Saccharomyces cerevisiae*. J. Bacteriol. **120:**779–784.
34. **Uno, J., M. L. Shigematsu, and T. Arai.** 1982. Primary site of

action of ketoconazole on *Candida albicans*. Antimicrob. Agents Chemother. **21**:912–918.
35. **Van den Bossche, H., W. Lauwers, G. Willemsens, P. Marichal, F. Cornelissen, and W. Cools.** 1984. Molecular basis for antimycotic and antibacterial activity of N-substituted imidazoles and triazoles: the inhibition of isoprenoid biosynthesis. Pestic. Sci. **15**:188–198.
36. **Van den Bossche, H., J. M. Ruysschaert, F. Defrise-Quertain, G. Willemsens, F. Cornelissen, P. Marichal, W. Cools, and J. Van Cutsem.** 1982. The interaction of miconazole and ketoconazole with lipids. Biochem. Pharmacol. **31**:2609–2617.
37. **Van den Bossche, H., G. Willemsens, W. Cools, F. Cornelissen, W. F. Lauwers, and J. Van Cutsem.** 1980. In vitro and in vivo effects of the antimycotic drug ketoconazole on sterol synthesis. Antimicrob. Agents Chemother. **17**:922–928.
38. **Van den Bossche, H., G. Willemsens, and P. Marichal.** 1987. Anti-*Candida* drugs—the biochemical basis for their activity. Crit. Rev. Microbiol. **15**:57–72.