Antifungal resistance mechanisms in pathogenic fungi

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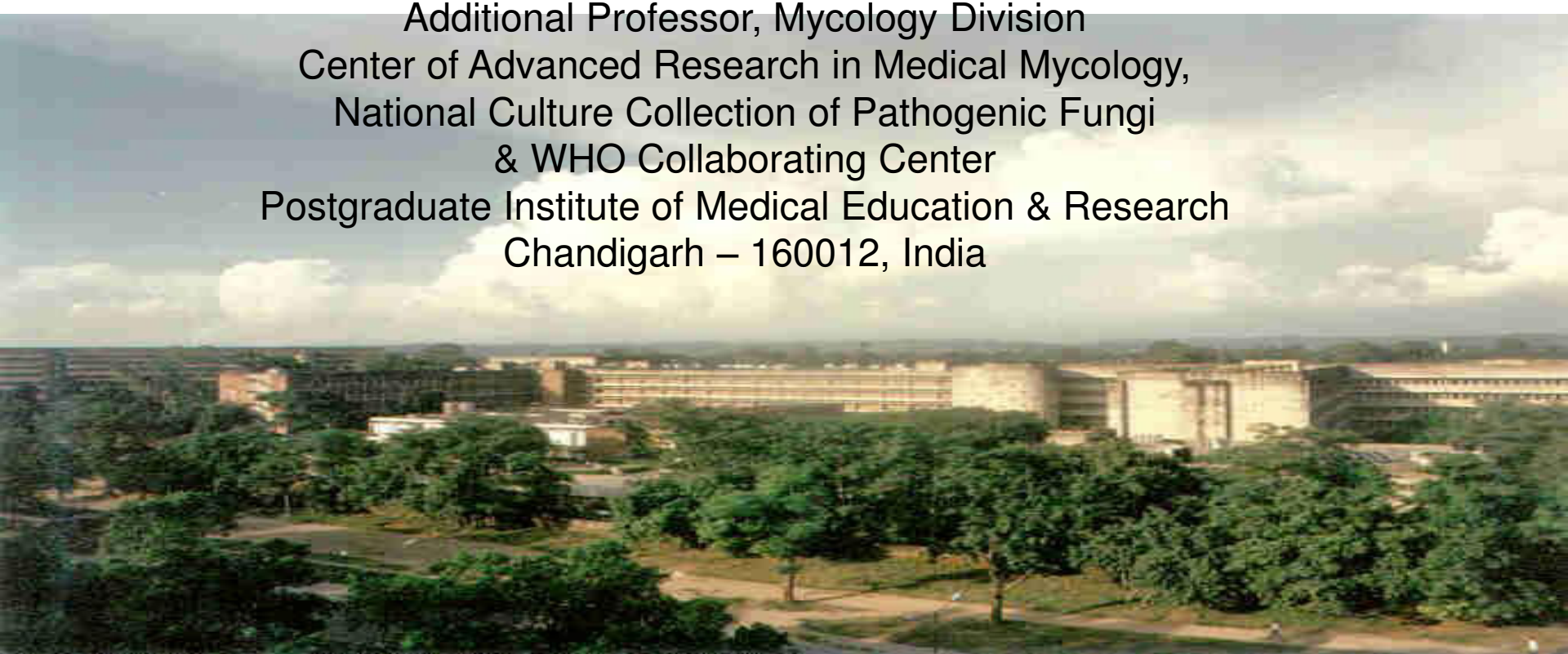
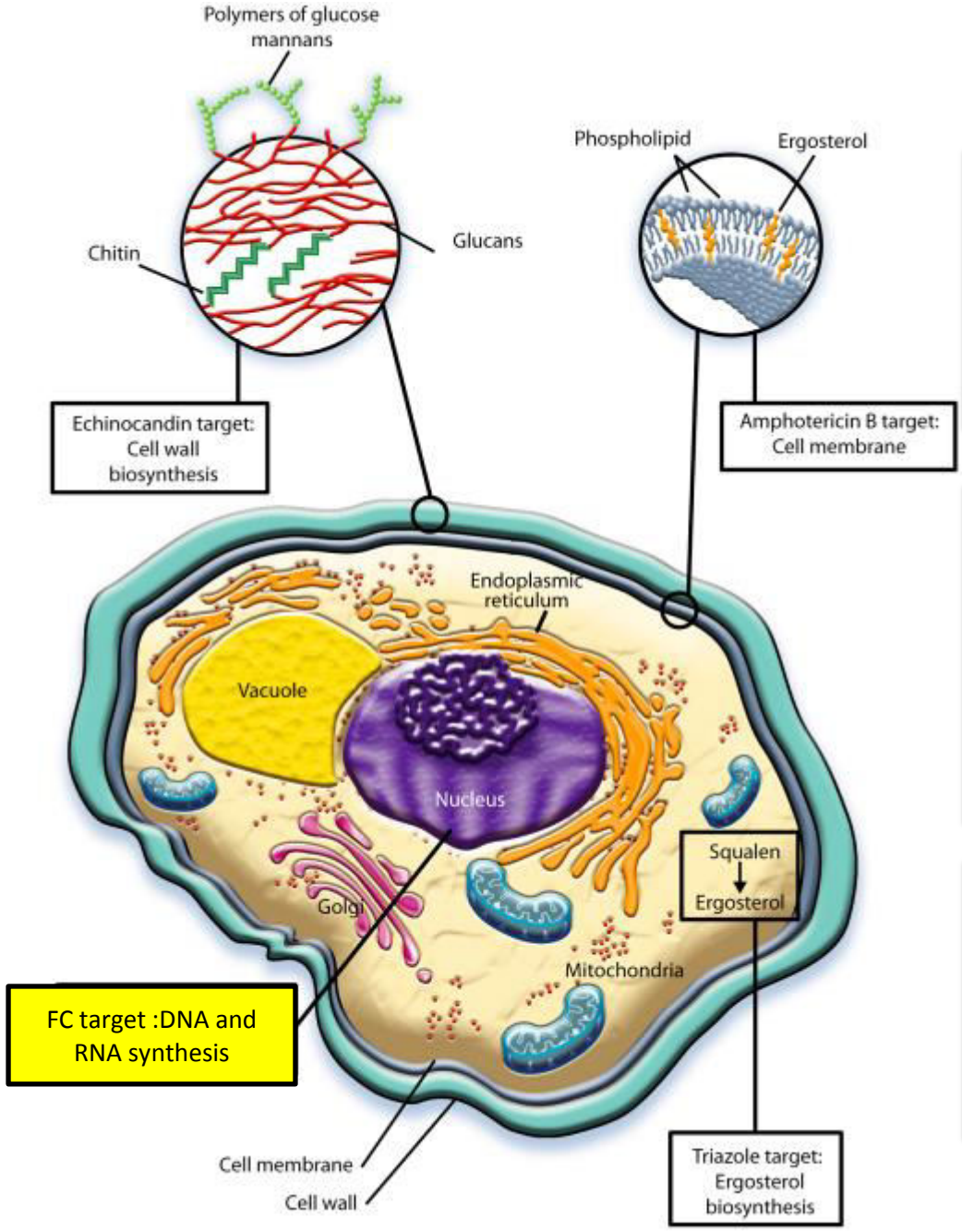


Antifungal drugs
-by structure

- **POLYENES**
  Amphotericin B, nystatin
- **AZOLES**
  Imidazoles: Ketoconazole..
  Triazoles: Fluconazole, itraconazole, voriconazole, posaconazole, ravuconazole
- **ALLYLAMINES**
  Terbinafine, butenafine
- **MORPHOLINE**
  Amorolfine
- **FLUORINATED PYRIMIDINE**
  Flucytosine
- **ECHINOCANDINS**
  Caspofungin, anidulafungin, micafungin
- **PEPTIDE-NUCLEOSIDE**
  Nikkomycin Z
- **TETRAHYDROFURAN DERIVATIVES**
  Sordarins, azasordarins
- **OTHER**
  Griseofulvin



Cell membrane

Fungi use principally Ergosterol instead of cholesterol

DNA Synthesis

Some compounds may be selectively activated by fungi, arresting DNA synthesis.

Cell Wall

Unlike mammalian cells, fungi have a cell wall

Resistance terminology

Types of antifungal resistance

- Intrinsic and acquired resistance
- Microbiological and clinical resistance

Intrinsic resistance and Acquired resistance

- Intrinsic resistance

— Primary resistance

— resistance present before exposure to the drug

— intrinsically resistant and hypersusceptible strains – extremes of a MIC distribution of a random collection of isolates

- Acquired resistance/ secondary resistance

— Develop. of resistance after exposure to drug

— Molecular basis of resistance can be explored

Microbiological resistance and Clinical resistance

- Clinical resistance

  - infection persists despite treatment with an antifungal drug

  - infecting fungus could show normal susceptibility to the agent in vitro

  - antifungal fails to reach the infected site in sufficient quantity

  - Immune system is unable to clear the fungus inhibited in growth by the antifungal

- Microbiological resistance
  - infecting fungus shows *in vitro* resistance
  - patient responds clinically to the treatment
  - Patients own immune system clears the infection
  - agent reaches the target site in unusually high quantity
  - agent interacts synergistically with other molecules at the site of infection

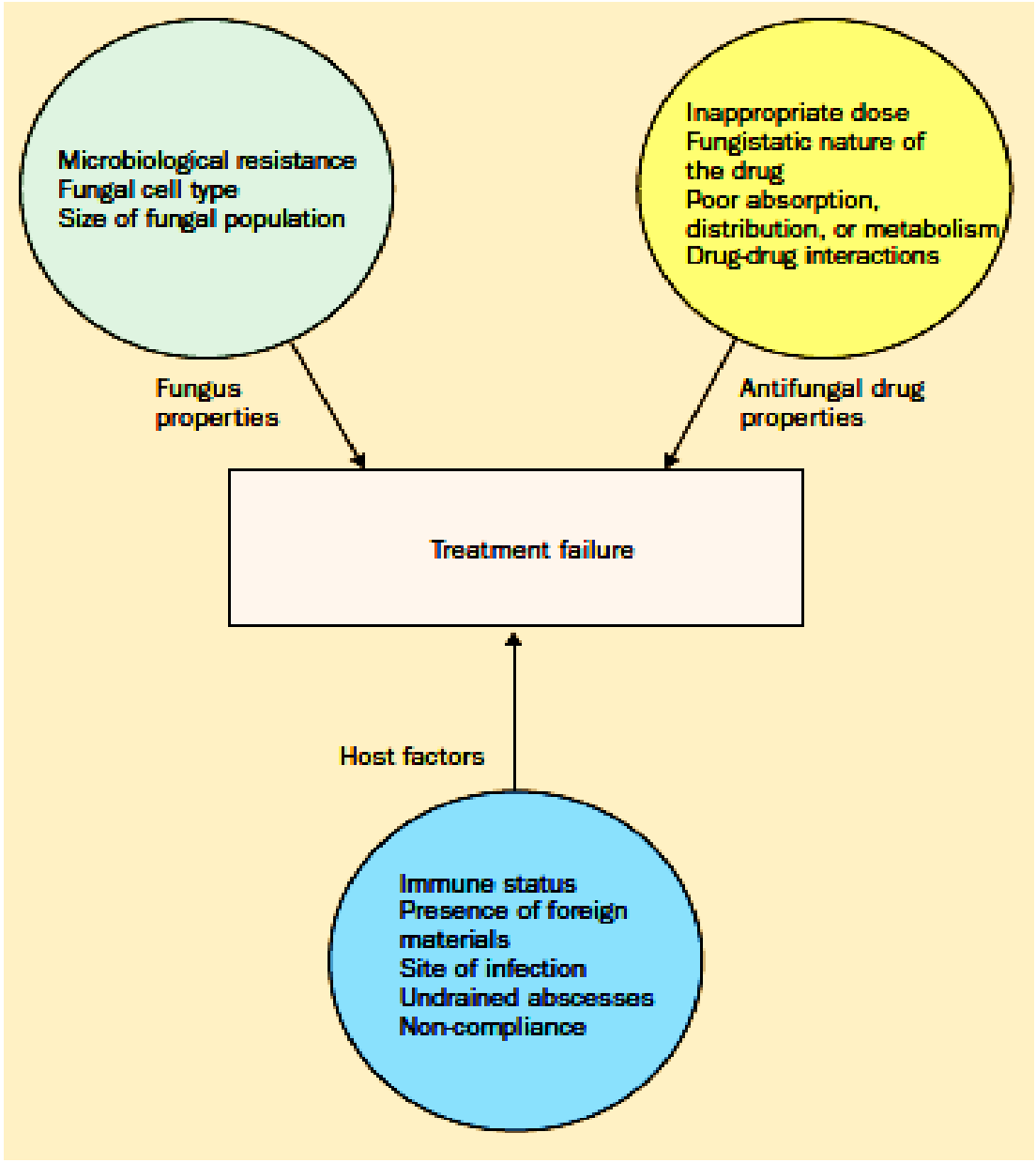
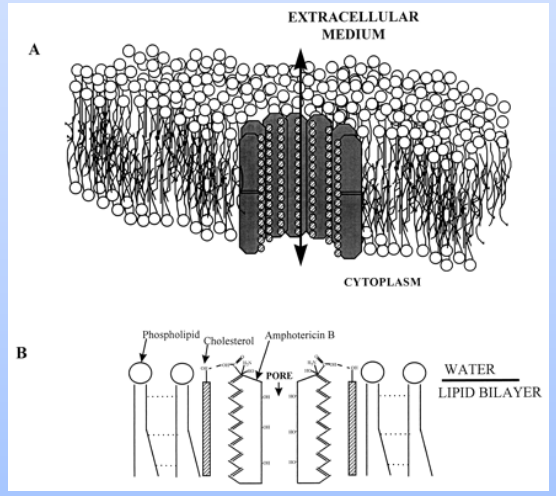


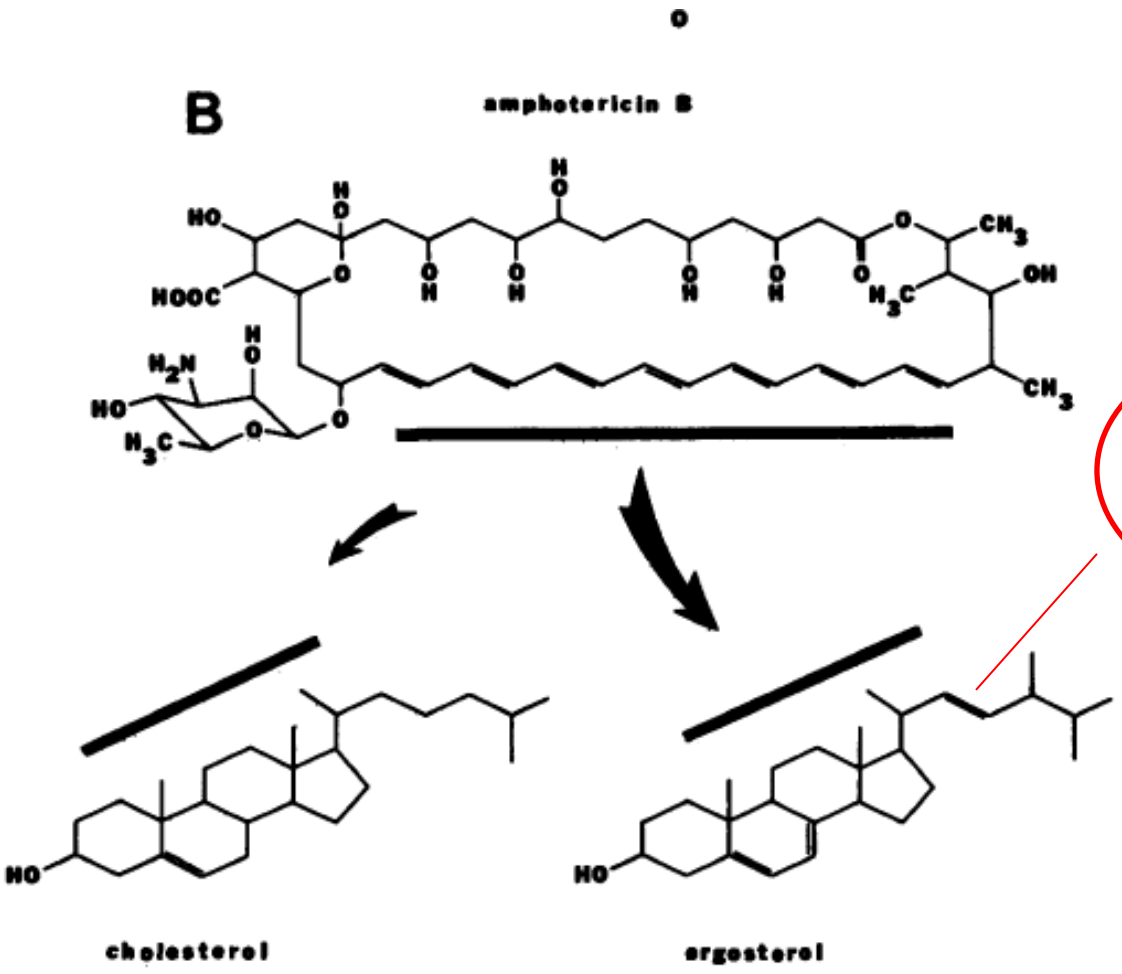
Figure 1. Principal causes of antifungal treatment failure.

Amphotericin B

- Binds ergosterol in fungal cell membrane
- Creates transmembrane channel and electrolyte leakage.



Amphotericin B and ergosterol specificity



Double bond at C-22 in ergosterol

Amphotericin B - Clinical Uses

The drug of choice for:

- Cryptococcal meningitis
- Mucormycosis (zygomycosis)
- Aspergillosis
- Invasive fungal infection, not responding to other therapy

AmpB resistance

- Intrinsic resistance
  - *Scedosporium spp.*
  - *A. tereus*
  - *Fusarium spp.*
  - *C. lusitanie*
  - *Trichosporon beigelli*
- Secondary resistance
  - rare
  - Occ. seen in isolates from heavily IC patients

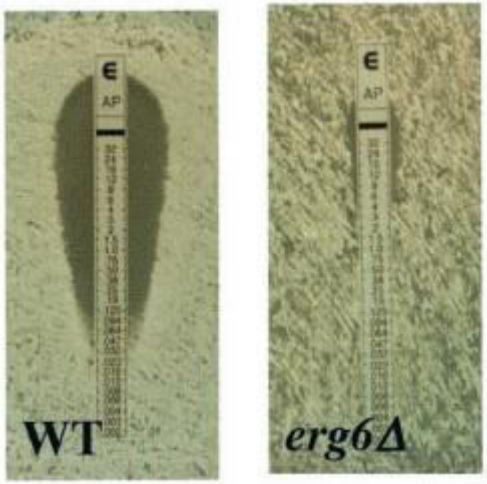
Mechanisms of amphotericin B resistance

Amphotericin B resistant fungi

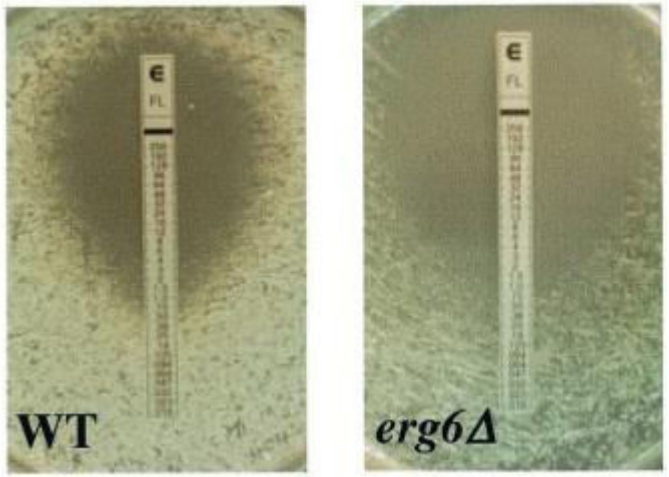
- Quantitative or qualitative alterations in the lipid composition of cell membrane
- ↓or ↑ ergosterol content limiting the binding of drug

AMB resistance in *C. lusitanie*

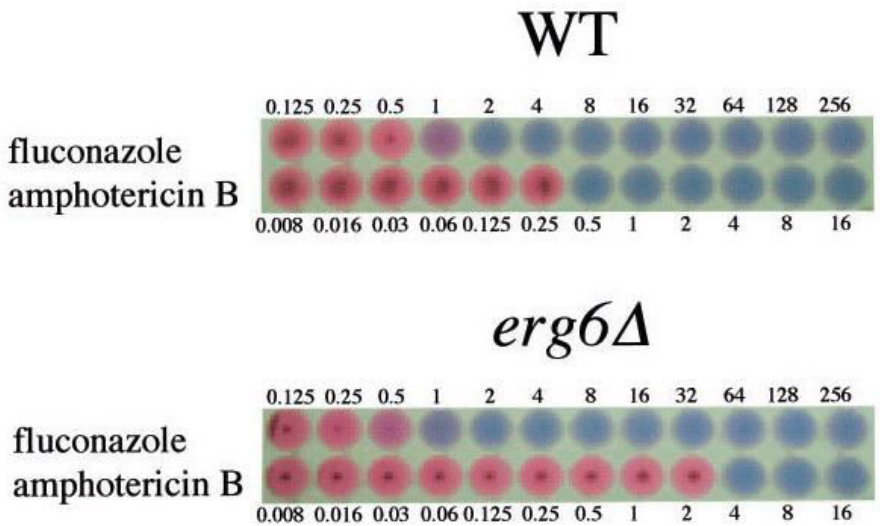
- Disruption of ergosterol biosynthesis
- *ERG*6 a non-essential gene
- Mutation in *ERG*6(Sterol methyltransferase)
- *erg6* mutant strains
- resistance to Polyenes
- decreased ergosterol content



amphotericin B



fluconazole



Flucytosine – Clinical uses

Monotherapy
- now limited

- Candidiasis
- Cryptococcosis
- Chromoblastomycosis

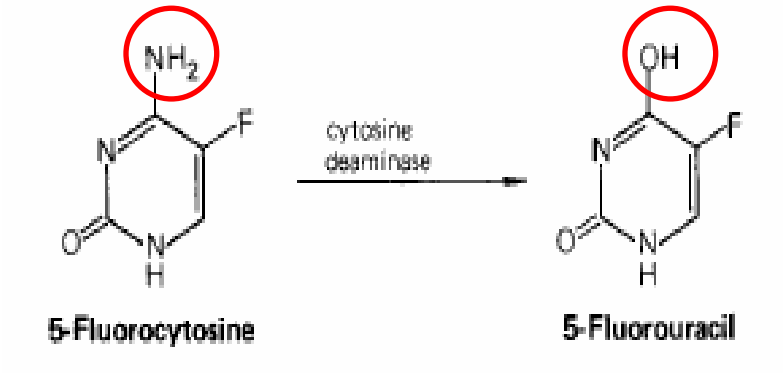
} In combination with amphotericin B or fluconazole.

Flucytosine

- Acquired Resistance:
  - result of monotherapy
  - rapid onset
  - decreased uptake (loss of cytosine permease activity e.g., *C. glabrata*
  - altered 5-FC metabolism (loss of cytosine deaminase or UMP pyrophosphorylase activity)

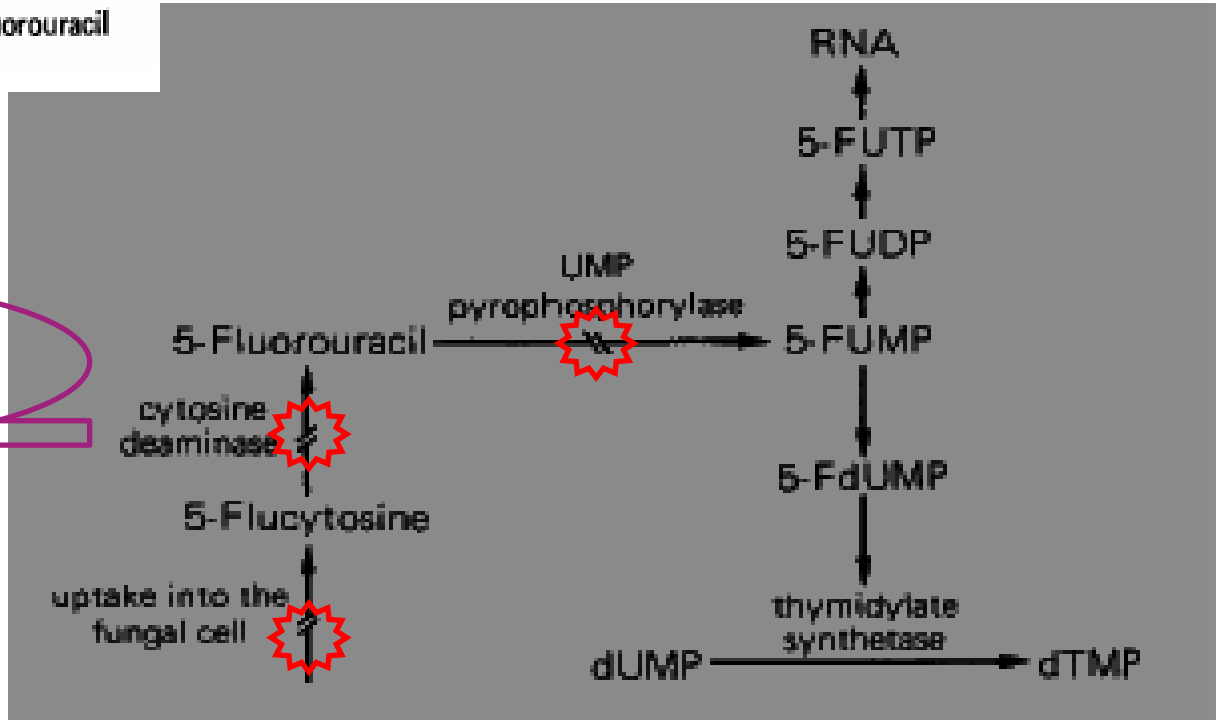
    e.g., *C. albicans* & *Cryptococcus neoformans*

Flucytosine



Fluorinated pyrimidine related to flurouracil.

Absent in mammals



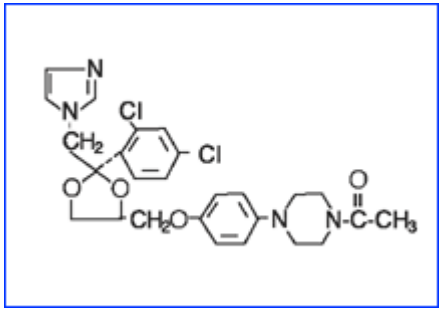
Ergosterol Biosynthesis Inhibitors(EBIs)

- Azoles
- Morpholines
- Allylamines
- Thiocarbamates

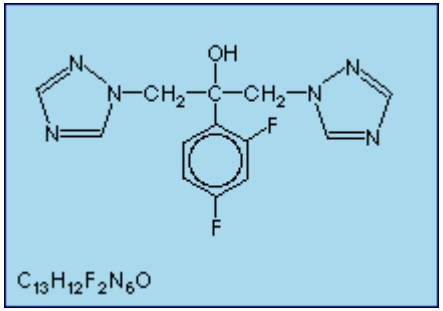
Azoles

- Most important EBIs
- Demethylation inhibitors(DMIs)

– Ketoconazole – Miconazole – Clotrimazole	Imidazoles
– Fluconazole – Itraconazole – Voriconazole – Posaconazole	Triazoles



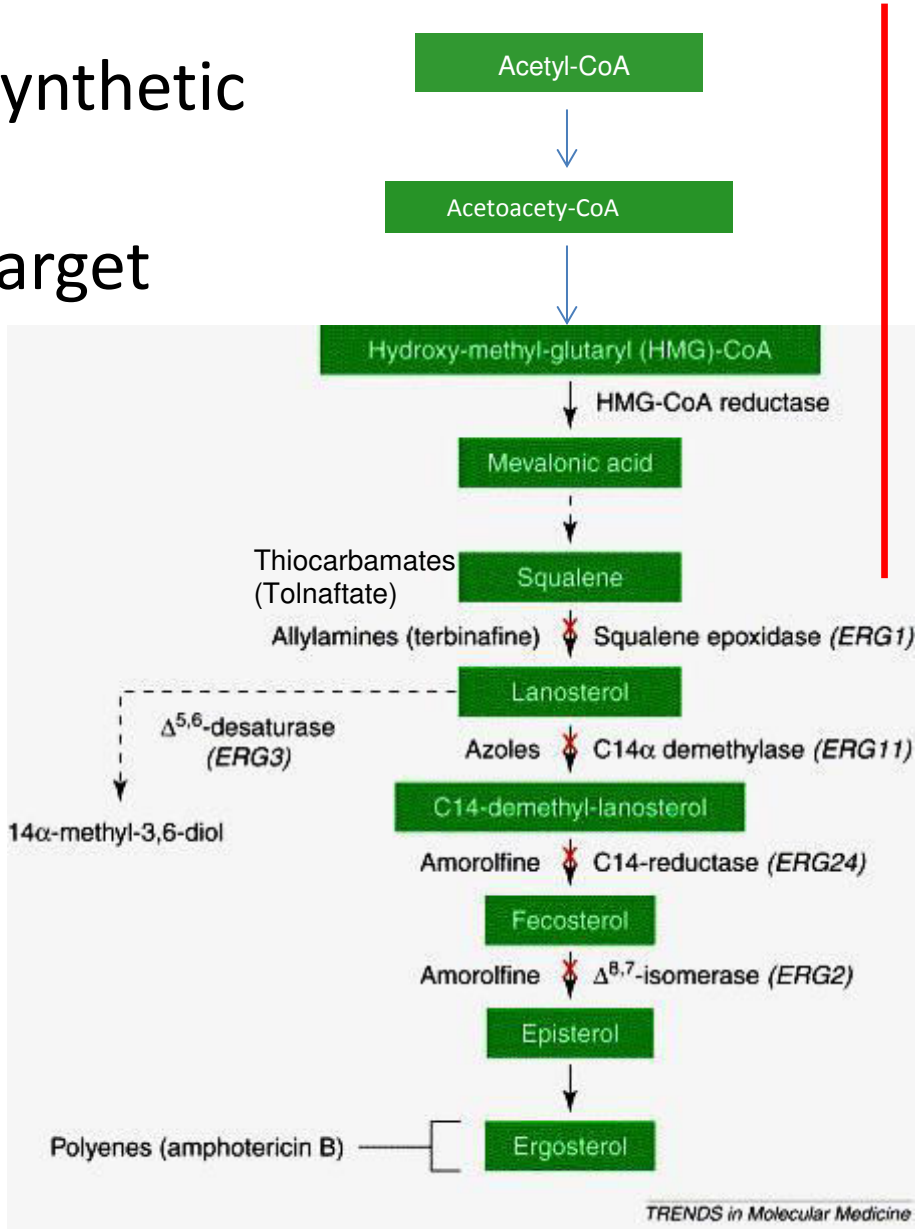
Ketoconazole



Fluconazole

Imidazoles:
- Limited use for treatment of systemic fungal infections
- localized surface infections

Ergosterol synthetic Pathway - antifungal target



Early pathway

Late pathway

Fluconazole - spectrum

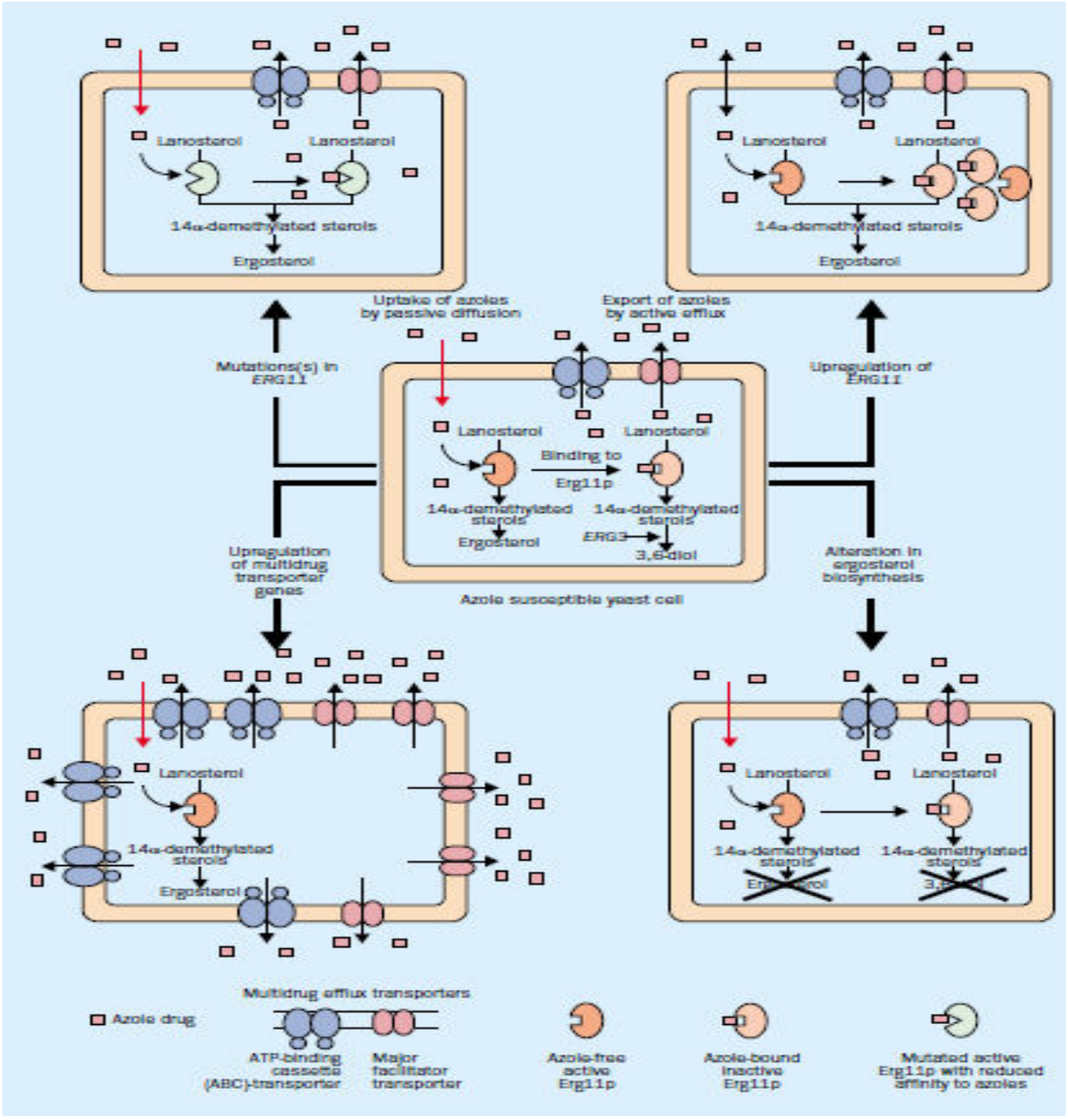
- Good activity against *C. albicans* and *Cryptococcus neoformans*
- Non-*albicans Candida* species more likely to exhibit primary resistance

Always resistant → **Sometimes resistant**

C. krusei > *C. glabrata* > *C. parapsilosis*
C. tropicalis
C. kefyr

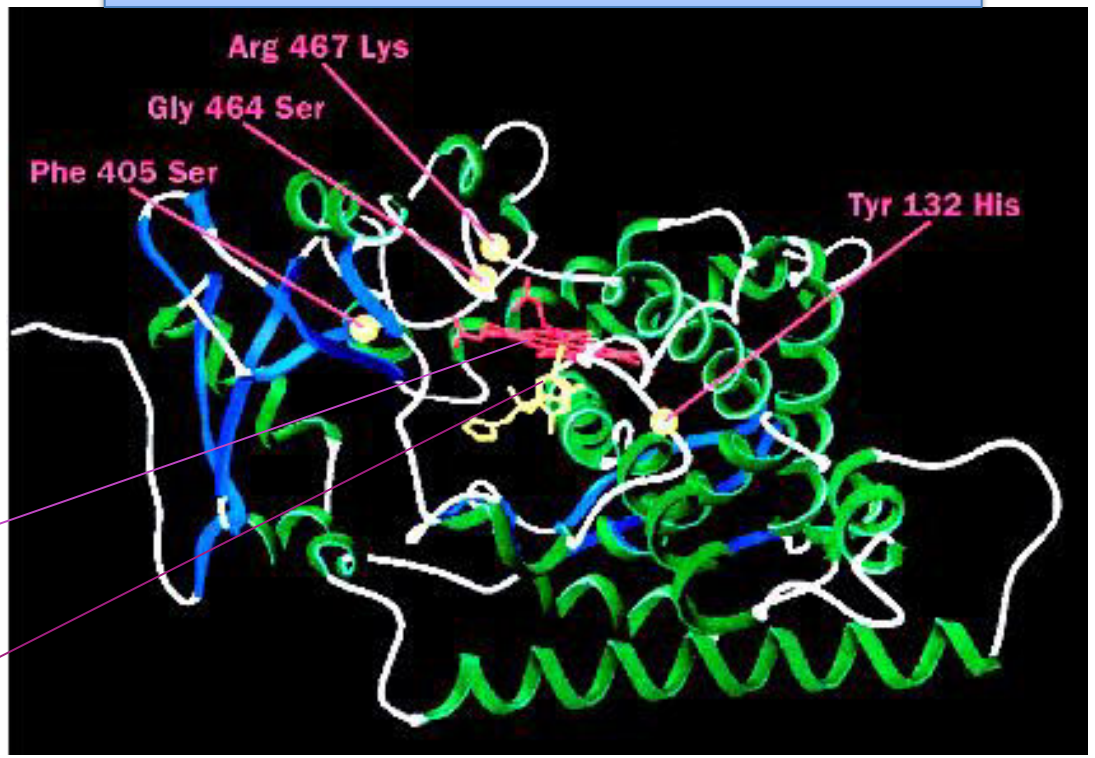
Mechanisms of azole resistance

- Enhanced efflux of agent
- Alteration of target enzyme
- Overexpression of target enzyme
- Alteration of Ergosterol pathway



Intrinsic resistance to fluconazole in *C. krusei* Is due to reduced affinity of Erg11p to the drug as a result of point mutations in it.

Crystal structure of Erg11p (CytP450) depicting Heme - Flu interaction



Drug efflux transporters

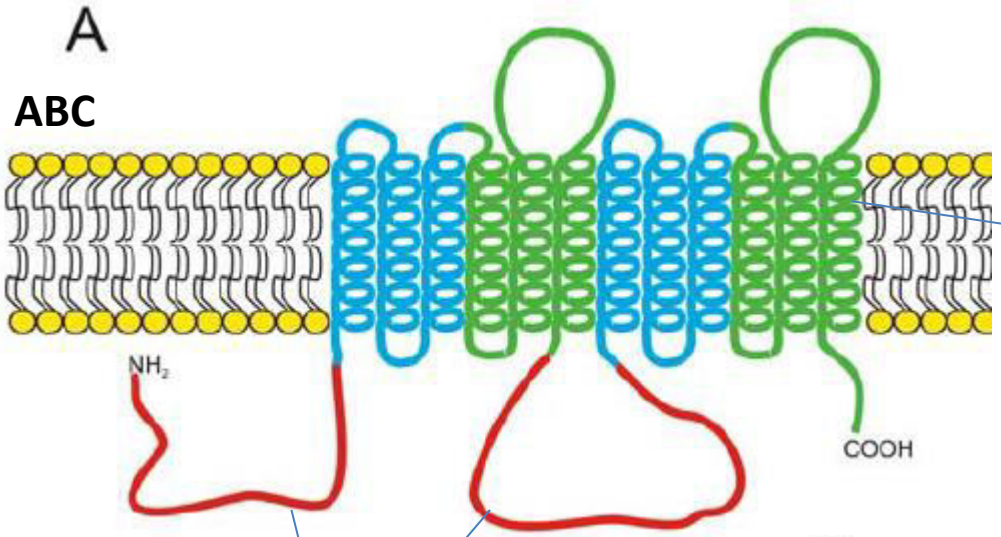
- Fungi overcome intracellular toxin accumulation by efflux pumps

— ABC transporters(ATP driven)

— MFS transporters(Proton gradient)

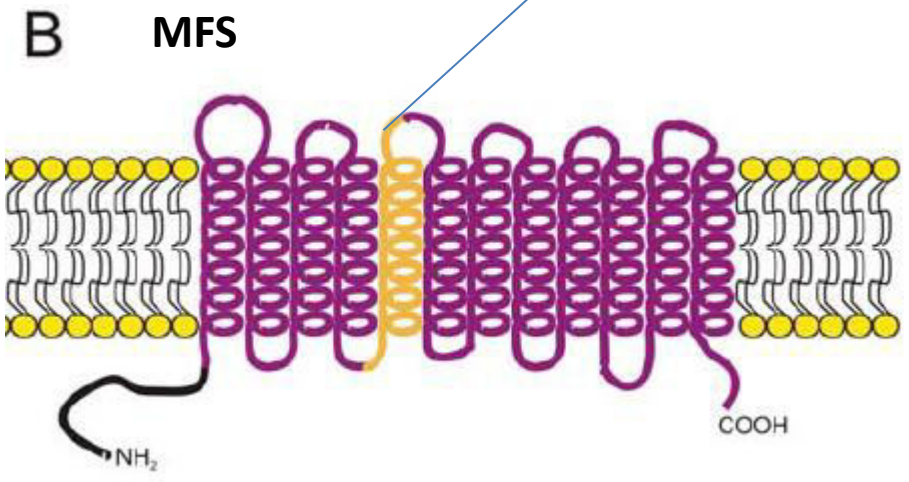
- Fungi – 10 to 30 genes encoding transporters per 10^6 bp of genome
- MFS transporters – most common in all sequenced fungal genomes

Structure of ABC and MFS transporters

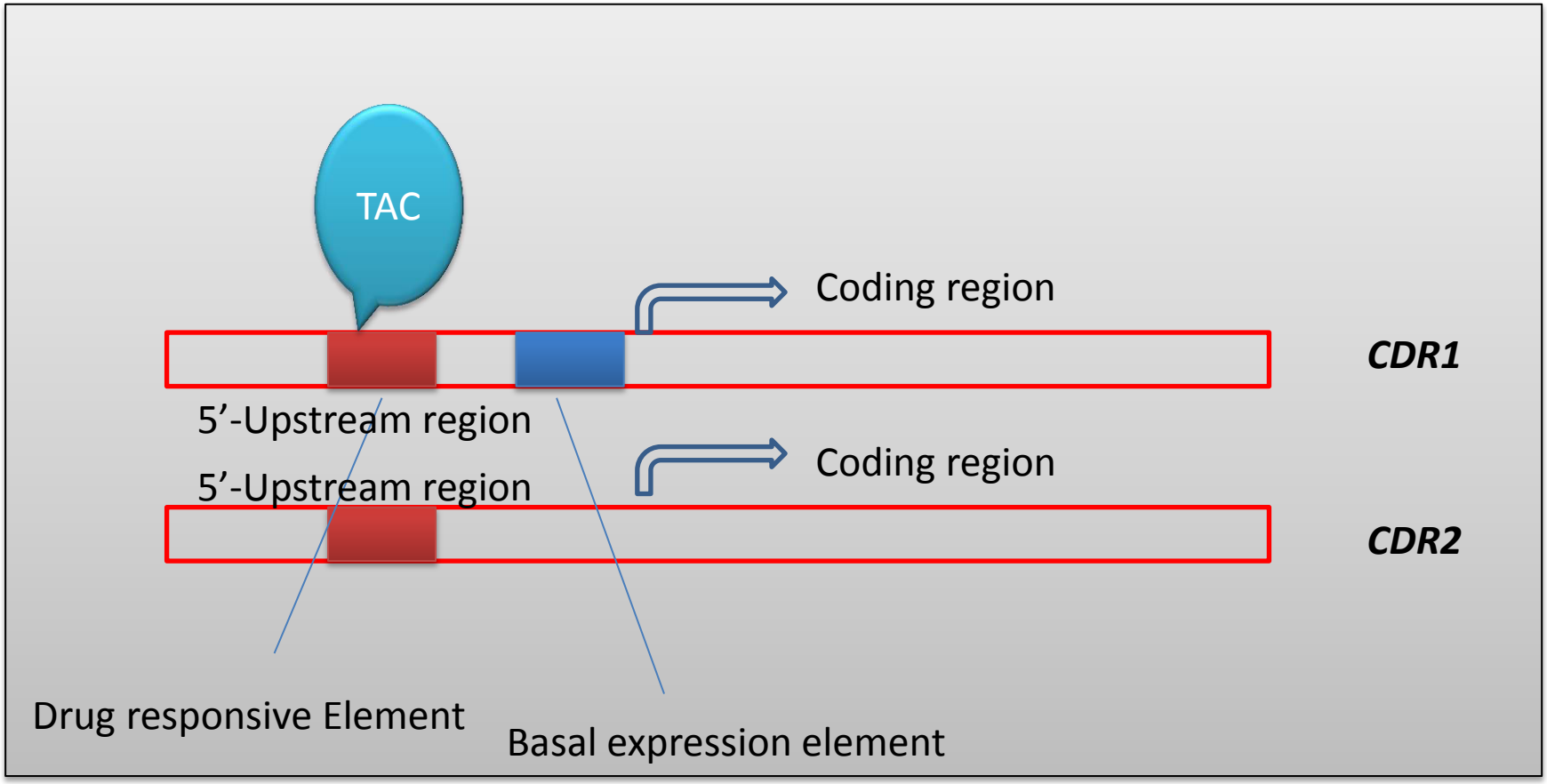


Transmembrane domains for substrate specificity

Nucleotide binding domains



Efflux pump transporters in azole resistance in *Candida* spp.



- *CDR1 and CDR2*

— General phospholipid translocators

— Fluconazole resistance

— Most common mechanism of azole resistance in clinical strains of *C. albicans*

- Increased transcriptional activation of *CDR*1 and increased mRNA stability in fluconazole resistant isolates of *C. albicans*

Raman *et al*, Antimicrob agents Chemother, 2008

Smirti *et al*, Yeast 2002

Type of resistance	*Candida* species	Author and date	Name of Journal	principle mechanisms of resistance
Azole resistance	*C .tropicalis(n=52)*	Jiang et al,2013	J antimicrob Chemother	• High *ERG* 11 expression(n= 31) • Miss-sense mutations in ERG11 (12)
Azole resistance	*C .tropicalis* (n=1)	Vandeputte et al 2005	Antimicrob agents Chemother	Overexpression of Ct*ERG*11
Azole resistance	*Candida* spp. (n=4)	Henry et al, 2000	Antimicrob agents Chemother	• Global ERG upregulation

Type of resistance	*Candida* species	Author and date	Name of Journal	principle mechanisms of resistance
Azole Resistance	C.parapsilosis(n=3)	Silva et al,2011	Antimicrob agents Chemother	Overexpession of MDR1,an MFS transporter
Fluconazole resistance	C.albicans(n=1)	Yan et al,2008	Acta Biochim Biophys sin	Mutation in TAC TF resulting in Cdr1 and Cdr2
Azole resistance	C.albicans(n=2)	Morio et al,2012	J antimicrob Chemother	Sterol $\Delta^{5,6}$ desaturase(*ERG*3)
Azole resistance	C.albicans	Martel et al,2010	Antimicrob agents Chemother	Mutations in Erg3 (mainly) and Erg11

Drug resistance in *Cryptococcus neoformans*

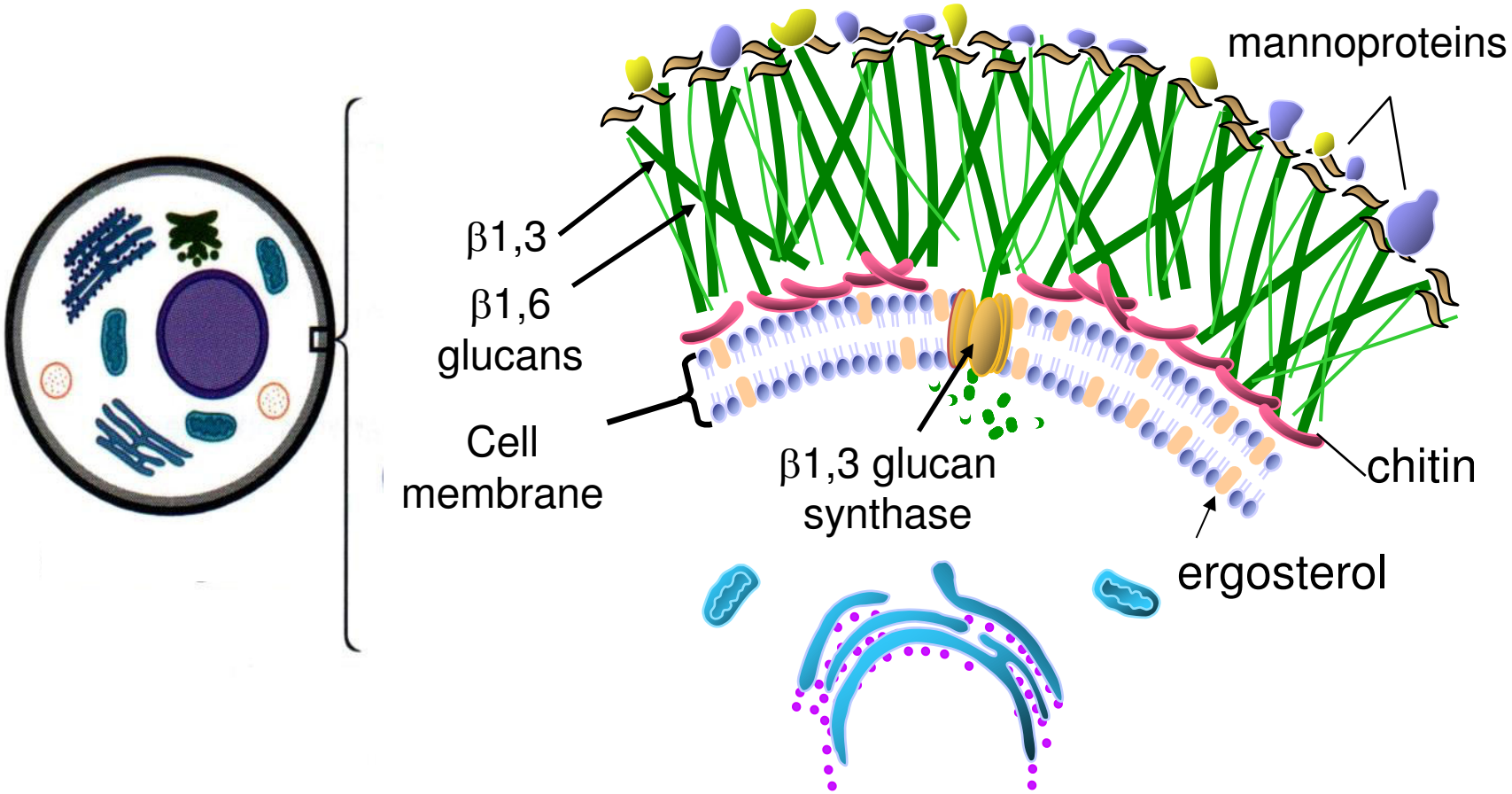
- Drug resistance in *Cryptococcus* is a complex problem
- Underlying disease of the patient
- Secondary complications- hydrocephalus, drug intolerance, poor drug compliance, PK issues & development of primary or secondary drug resistance

Drug resistance in *Cryptococcus neoformans*

- Ketoconazole
  - Limited penetration into CNS
  - ineffective in Cryptococcal meningitis
  - successfully used to treat extrameningial cases
- Fluconazole
  - Fails to eliminate infection from genito- urinary tract in disseminated cryptococcosis
  - in vitro resistance due to overexpression of ABC efflux pump Afr1

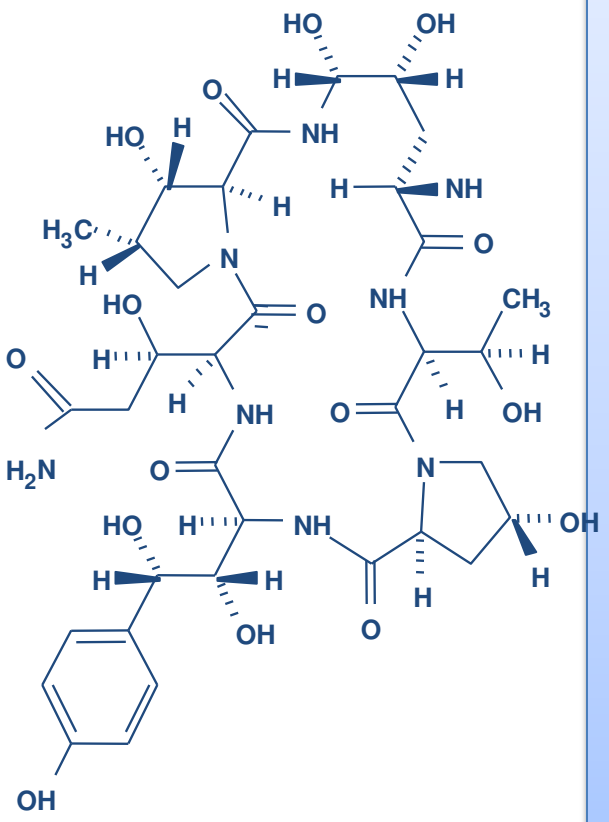
- Amphotericin B failure –
  - direct drug-related nephrotoxicity with the high doses required for treatment of meningitis.
- Innate resistance to echinocandins as *Cryptococcus* has little or no β(1,3)-D-glucan synthase enzyme

Echinocandins



Atlas of fungal Infections, Richard Diamond Ed. 1999
Introduction to Medical Mycology. Merck and Co. 2001

Echinocandins - Pharmacology



- Cyclic lipopeptide antibiotics that interfere with fungal cell wall synthesis by inhibition of ß-(1,3) D-glucan synthase
- Loss of cell wall glucan results in osmotic fragility

Spectrum:

- Candida species *including non-albicans* isolates resistant to fluconazole
- Aspergillus *spp. but not activity against other moulds (Fusarium,* Zygomycetes*)*
- No *coverage of Cryptococcus neoformans*

Azole resistance in the A. fumigatus

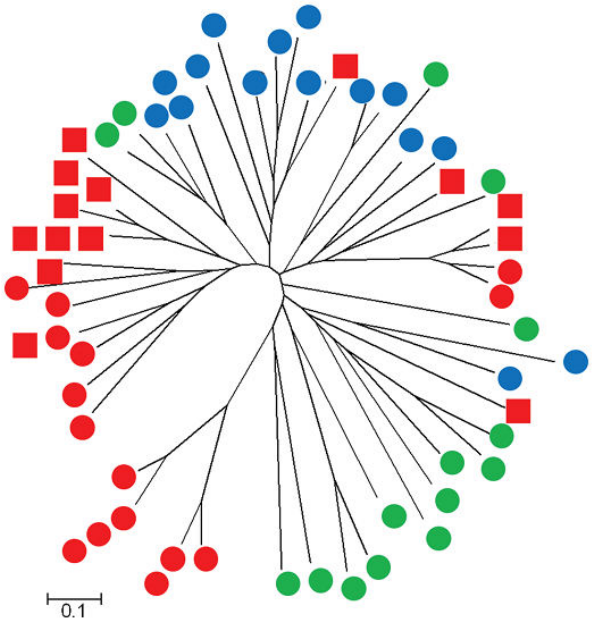
- DMIs have in vitro activity against A. fumigatus and their molecule structure are highly similar to clinically licensed triazoles.
- Azole resistant *A. fumigatus* isolates contain an alteration in the target protein sterol 14 α - demethylase (Cyp51) inhibiting drug binding.
- Changes are due to single nucleotide polymorphisms in the gene *(cyp51A)* encoding the protein leading to amino acid substitutions.

Aspergillosis due to Voriconazole Highly Resistant *Aspergillus fumigatus* and Recovery of Genetically Related Resistant Isolates From Domiciles

Jan W. M. van der Linden,[1,2,a] Simone M. T. Camps,[1,2,a] Greetje A. Kampinga,[3] Jan P. A. Arends,[3] Yvette J. Debets-Ossenkopp,[4] Pieter J. A. Haas,[5] Bart J. A. Rijnders,[6] Ed J. Kuijper,[7] Frank H. van Tiel,[8] János Varga,[9] Anna Karawajczyk,[10] J. Zoll,[1,2] Willem J. G. Melchers,[1,2] and Paul E. Verweij[1,2]

Clinical Infectious Diseases 2013;57(4):513–20

- Study period 1315 *A. fumigatus* isolates from 921 patients screened for resistance.
- Prevalence of azole resistance was 6.8% (63 of 921 patients)
- TR34/L98H - 74.6% (47/63)
- TR46/Y121F/T289A –**20.6%** (13 patients)
- No mutation in Cyp51A - 3 patients



- Microsatellite genotypes of the clinical and environmental resistant TR 46 /Y121F/T289A isolates, compared with TR 34/L98H and wild-type controls

Azole resistance in the *A. fumigatus*

- Alterations at codon 98 (L98H) of cyp51A is predominantly associated with resistance
  - 94% in The Netherlands, 53% in Spain
- Resistance is also associated with an increase in target concentration.
- Strains with L98H also contain a 34-base tandem repeat in the promoter region which causes an eight-fold increase in expression of the *cyp51A gene (TR34/L98H).*

TR34/L98H

- First emerged in 1998 - Dutch patients and endemic in Dutch Hospitals
- Now reported form other parts of Europe, China and India
- Seen both in azole –treated and azole- naïve patients
- Molecular typing studies

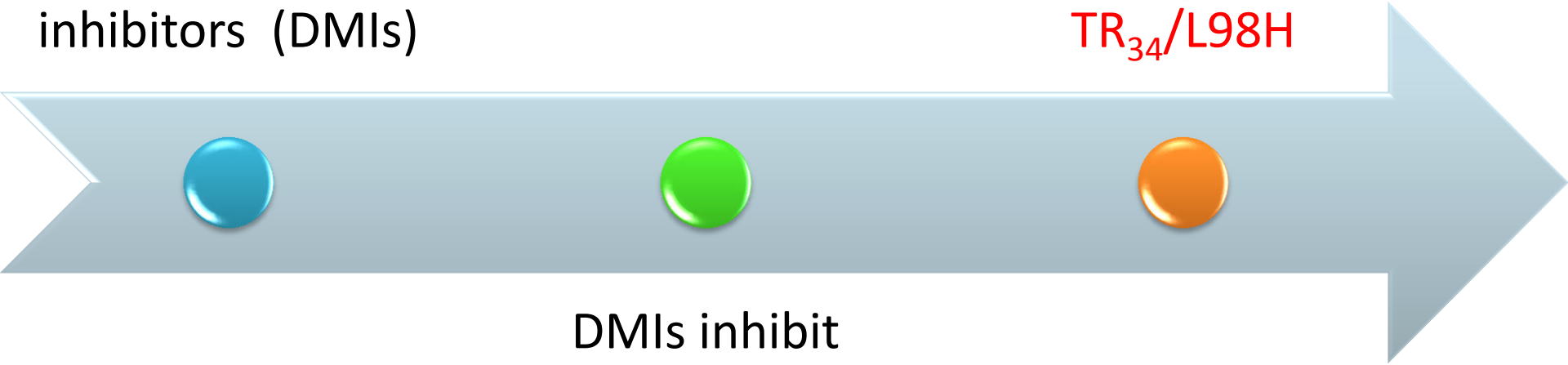
Fungicide driven route of resistance development carries high risk of geographical migration of this resistance trait

Mechanism of Resistance Development

Substitution at codon 98 in the *Cyp51A* gene
+
34 base-pair tandem repeat in the gene promoter

Environmental exposure to 14α - demethylase inhibitors (DMIs)

TR_{34}/L98H



DMIs inhibit fungal *Cyp51A* activity

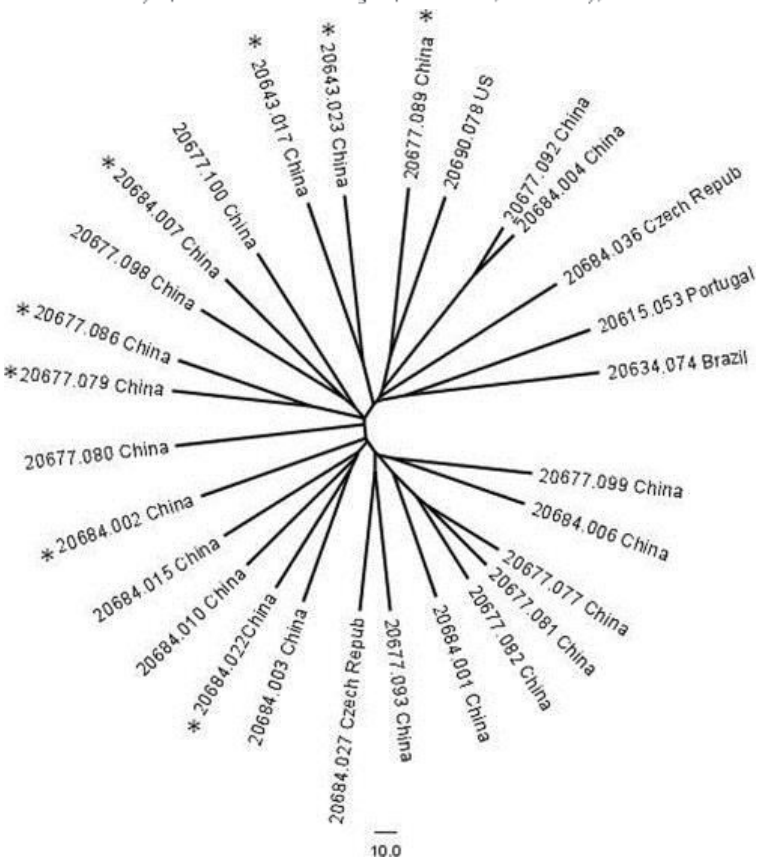
ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Sept. 2011, p. 4465–4468 Vol. 55, No. 9
0066-4804/11/$12.00 doi:10.1128/AAC.00185-11

Azole Resistance in *Aspergillus fumigatus* Isolates from the ARTEMIS Global Surveillance Study Is Primarily Due to the TR/L98H Mutation in the *cyp51A* Gene[▽]

Shawn R. Lockhart,[1]* João P. Frade,[1] Kizee A. Etienne,[1] Michael A. Pfaller,[2] Daniel J. Diekema,[2,3] and S. Arunmozhi Balajee[1]

Mycotic Diseases Branch, Division of Foodborne, Waterborne and Environmental Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia 30333,[1] and Department of Pathology[2] and Department of Internal Medicine,[3] University of Iowa Carver College of Medicine, Iowa City, Iowa 52242

28 isolates with high triazole MICs
From: Brazil, China, Czech Republic, Portugal, USA



Survey of 497 A. fumigatus
- Years 2008-2009
- Part of ARTEMIS global surveillance study

Dendrogram of all 28 isolates with elevated triazole MIC values. Isolates with the TR/L98H mutation are marked with an asterisk

Emergence of New Resistance Mechanism

- In Jan 2010 *A. fumigatus* isolated – Voriconazole well
- MIC of Vori >16mg/L, Itra -2mg/L and posa -0.5mg/L
- Sequencing analysis of Cyp51A – 2 mutation substitutions

  Y121F and T289A
- 46 bp tandem repeat in promoter gene promoter
- **TR46/Y121F/T289A**

Echinocandin resistance

- Manipulated or laboratory-selected strains with various degrees of caspofungin resistance have been described.
- They have mutations in the *ECM33 gene (AfuEcm33), encoding cell* wall proteins important for fungal cell wall organization.
- Laboratory engineered strains with mutations in the *FKS1 gene* encoding a subunit of the β -1,3-D-glucan synthase enzyme and decreased susceptibility to caspofungin have been generated (Gardiner et al *Med Mycol 2005)*

Summary of genetic mechanisms leading to antifungal resistance

		Genetic target	
	Candida		Aspergillus
Triazoles			
• Target site mutations	C. albicans	Erg11	Cyp51A
• Target site upregulation	C. albicans	Erg11	Cyp51A
• Drug efflux transporters			
ABC	C. albicans	Cdr1,Cdr2	Mdr1, Mdr4
	C. glabrata	Cdr1, Cdr2, Snq2	
MFS	C. albicans	Mdr1	Mdr3
• Transcription factors			
ABC	C. albicans	Tac1	
	C. glabrata	Pdr1	
MFS	C. albicans	Mrr1	
ERG	C. albicans	UpC2	
Chromosomal aneuploidy	C. albicans	Chromosome 5	
Echinocandins			
Target site mutations	C. albicans	Fks1	Fks1
	C. glabrata	Fks1, Fks2	