Antifungal strategies and mechanisms of resistance

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Current status of antifungals

Azoles:

- fluconazole
- itraconazole
- posaconazole
- voriconazole
- ravuconazole

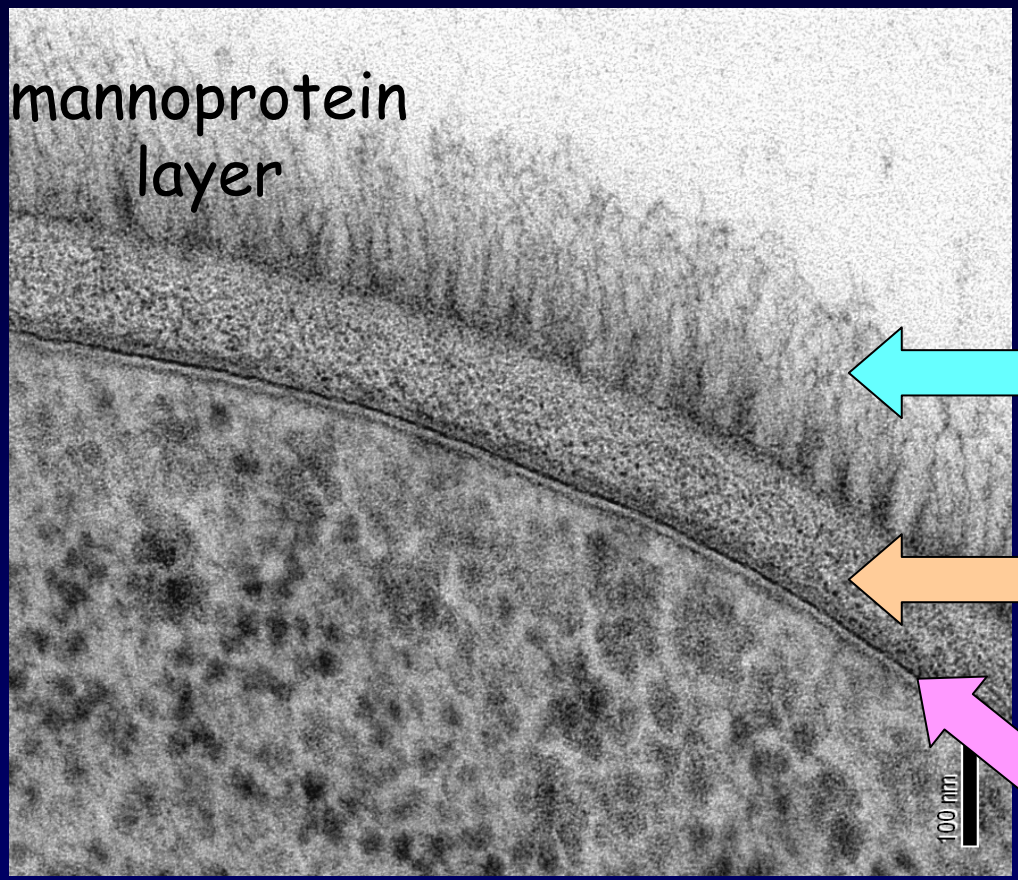
Polyenes:

- Amphotericin B
- (lipid formulations)

Echinocandins:

- caspofungin
- micafungin
- anidulafungin

Fungal cell envelope is an attractive target for antifungal therapies



Jean Paul Latgé
Antonio Cassone

Vaccine targets/
cell wall proteins

Echinocandins/vaccine
β(1,3)-glucan

Azoles/ergosterol
Polyenes/ergosterol

HPF-TEM of *C. albicans* yeast cell wall – Louise Walker, Aberdeen

Why do we need more?

Resistance and cross-resistance

High mortality rates – IFI or underlying condition?

Clinic – shorter treatment/better compliance

- site of infection - drugs that penetrate to all infection sites including catheter associated biofilms
- fungal load at onset of treatment, intervention as early as possible
- broader spectrum – emerging pathogens

Improvement in immune status of patient

Primeval Series 3

Killer Fungus

The Fungus Creature "eats" its victims, but retains a vaguely human shape, making it both sinister and frighteningly dangerous.

The team initially think the answer is to burn the organism but that causes the fungus to reproduce or sporulate.

Eventually they discover that cold is the answer and they succeed in trapping it freezing it to death.

Spectrums of activity

	Azoles			Candins	Polyenes
	Flu	Vori	Posa		
C. albicans	✓	✓	✓	✓	✓
C. neoformans	✓	✓	✓	X	✓
A. fumigatus	X	✓	✓	✓	✓
Zygomycetes (*Rhizopus, Absidia, Cunninghamella*)	X	X	✓	X	✓
Hyaline moulds (*Fusarium, Scedosporium*)	X	✓	✓	X	X

Flu = fluconazole
Vori = voriconazole
Posa = posaconazole

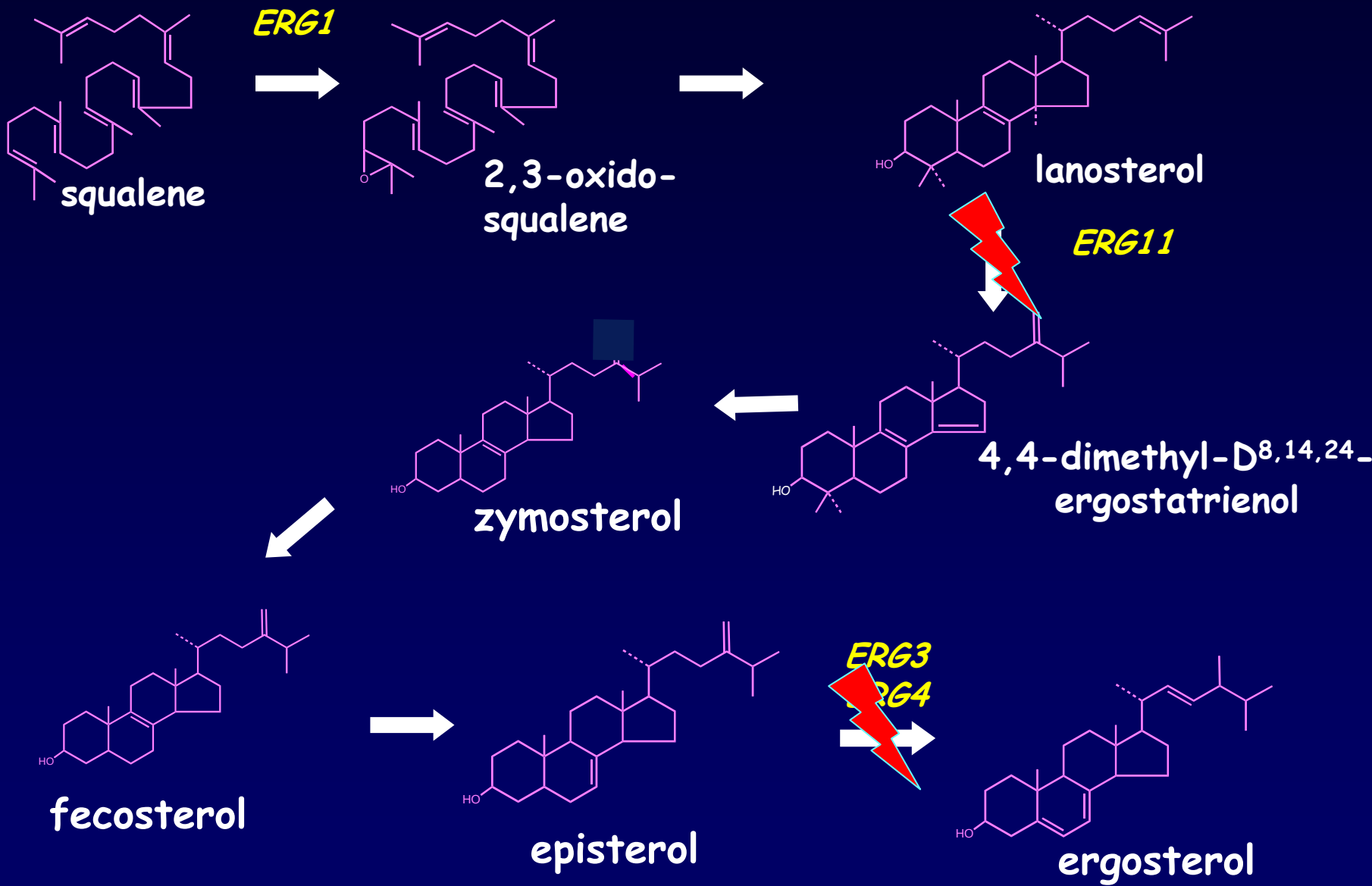
Aspergillus tereus Ampho B^R

Antifungal resistance mechanisms

Agent	Alter target structure	Keep drug out	Alter target levels
amphotericin B	*	no	lower ergosterol
triazoles	yes	efflux pumps	increase Erg11
caspofungin	yes	??	not known

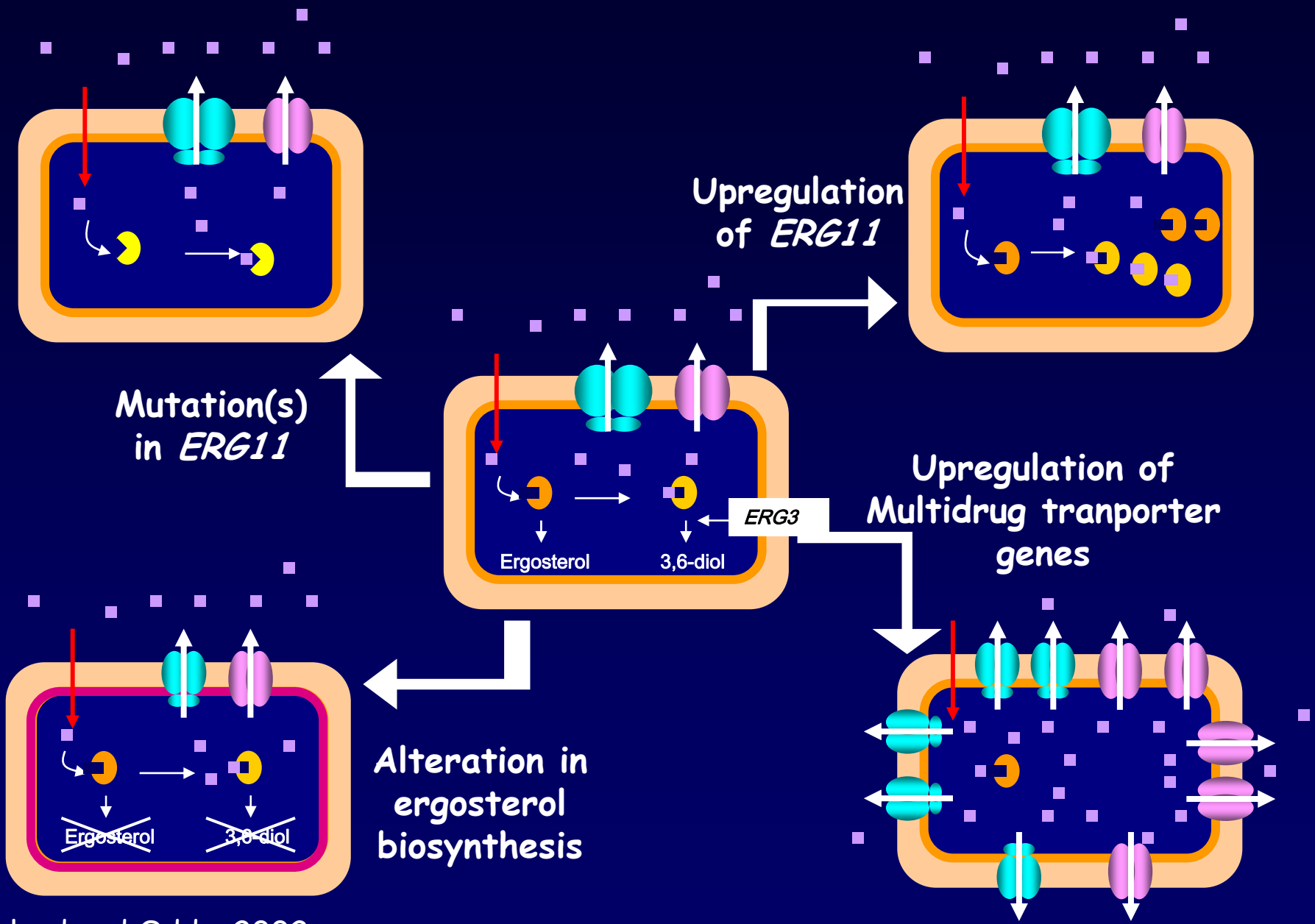
*fungal membranes containing sterols other than ergosterol tend to be less sensitive to amphotericin B

Ergosterol biosynthetic pathway in *C. albicans*



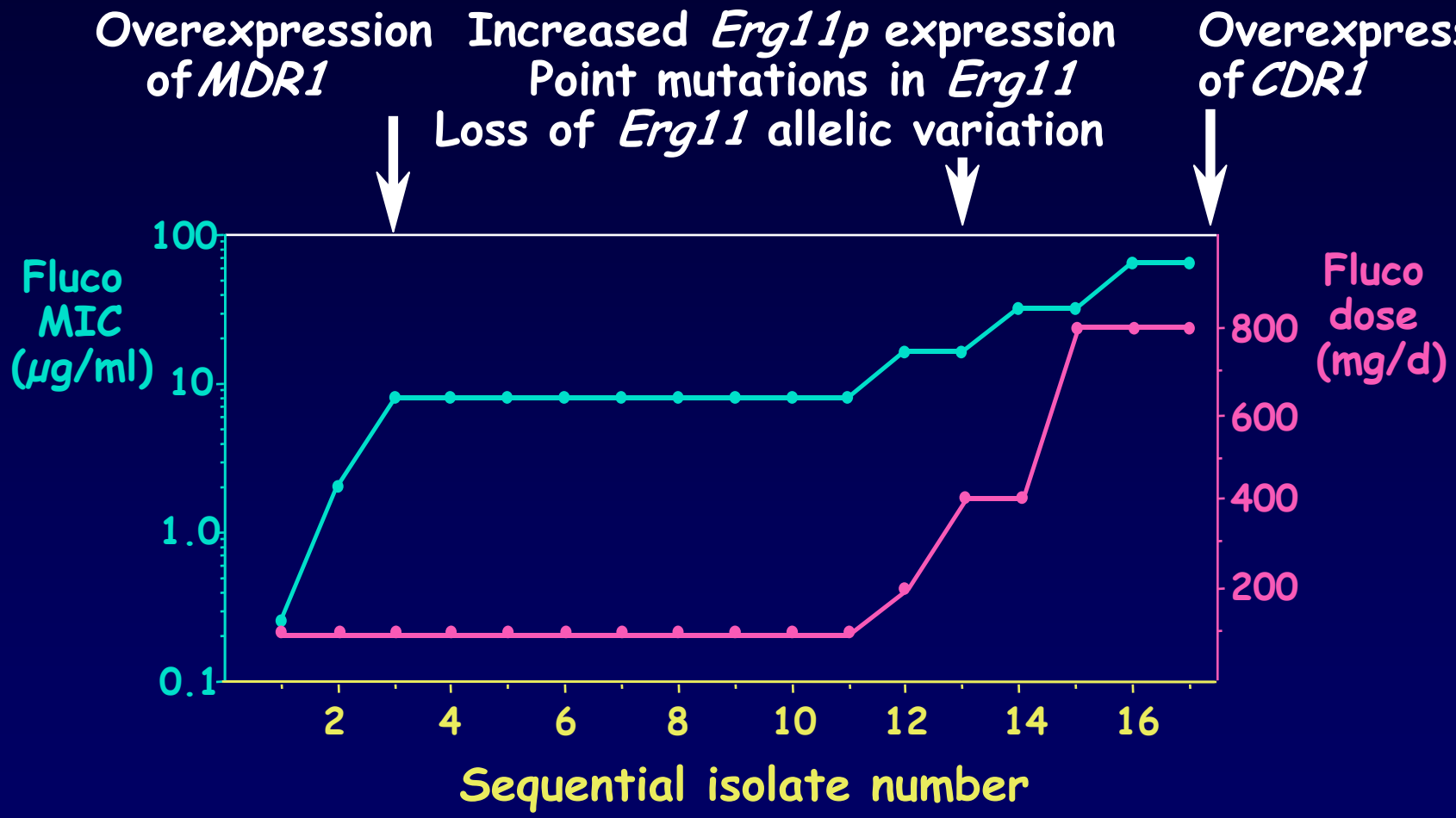
Adapted from Sanglard *et al.*, 2003

Possible Mechanisms of azole resistance in *C. albicans*



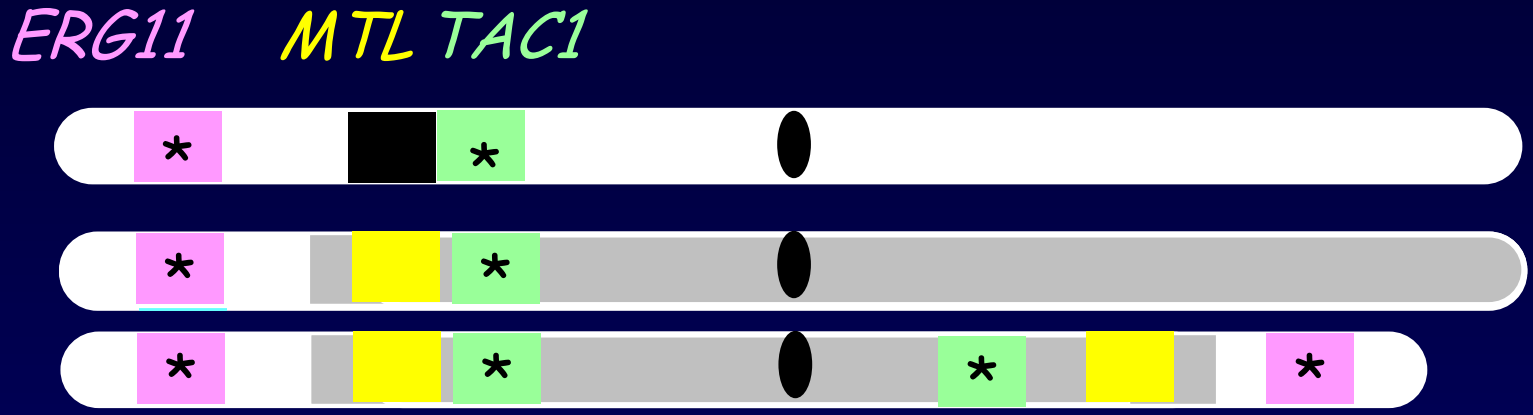
Sanglard and Odds, 2002

Azole resistance — case study (oropharyngeal *C. albicans* in AIDS)



White *et al.*, 1998

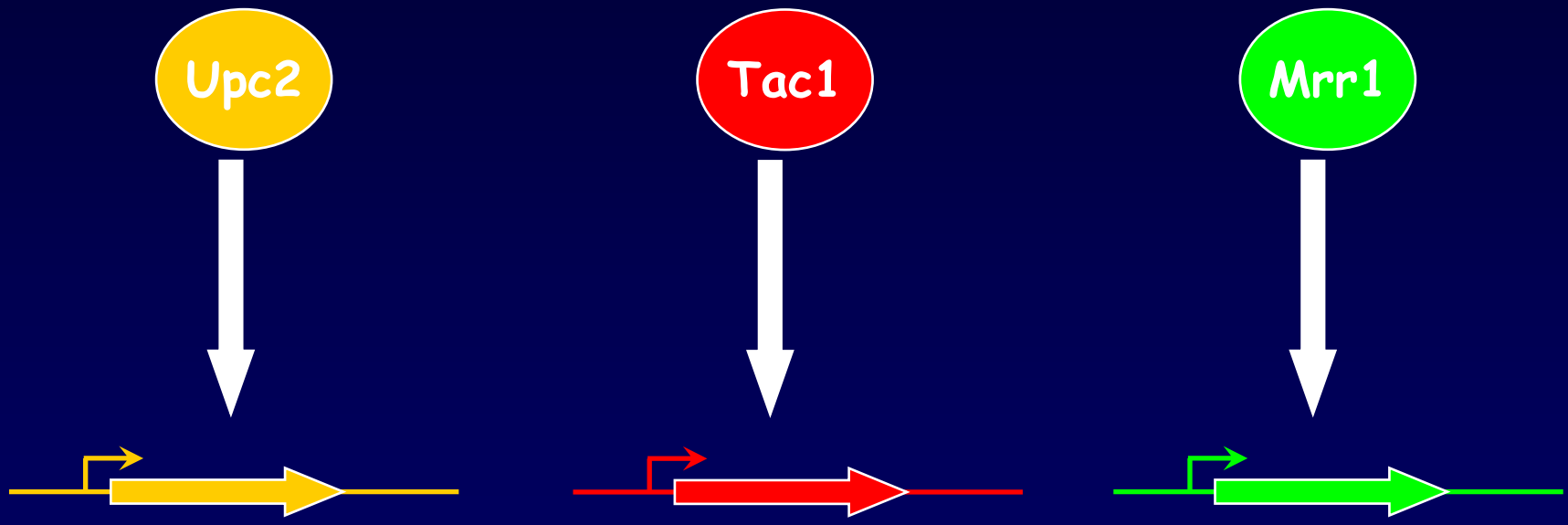
Isochromosome 5 formation and azole resistance



1. Mutations in *ERG11* and/or *TAC1*
2. Loss of Heterozygosity mechanisms
   - gene conversion
   - mitotic recombination
   - chromosome loss and duplication
3. Isochromosome 5 acquisition

Selmecki et al, 2006
Coste et al, 2007

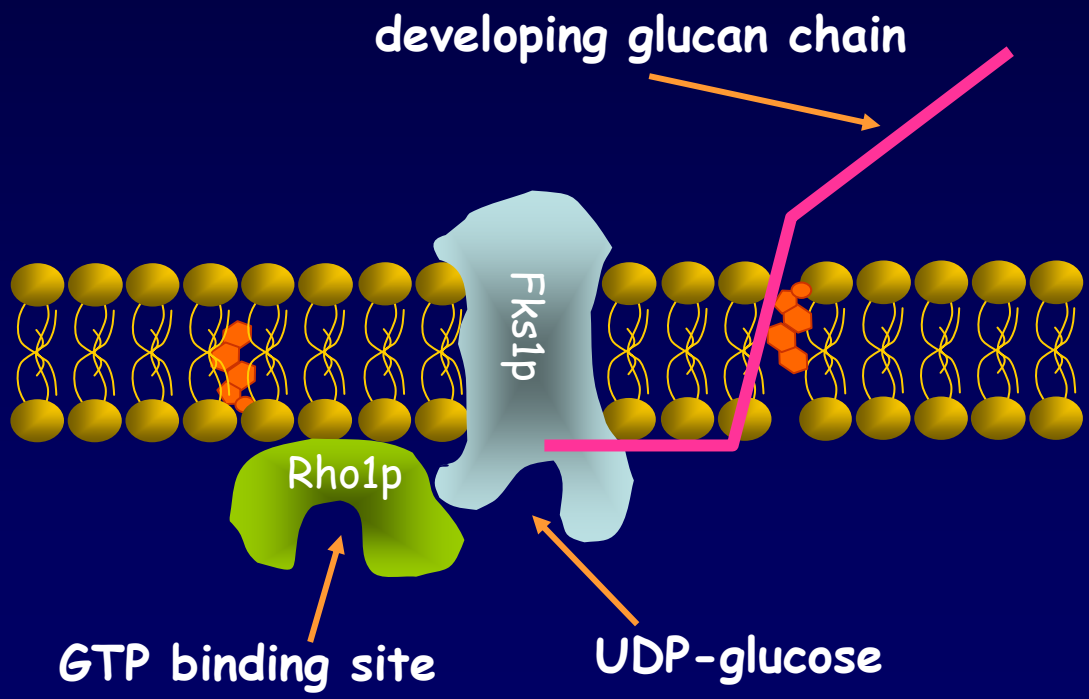
Transcription factors controlling azole drug resistance in *Candida albicans*



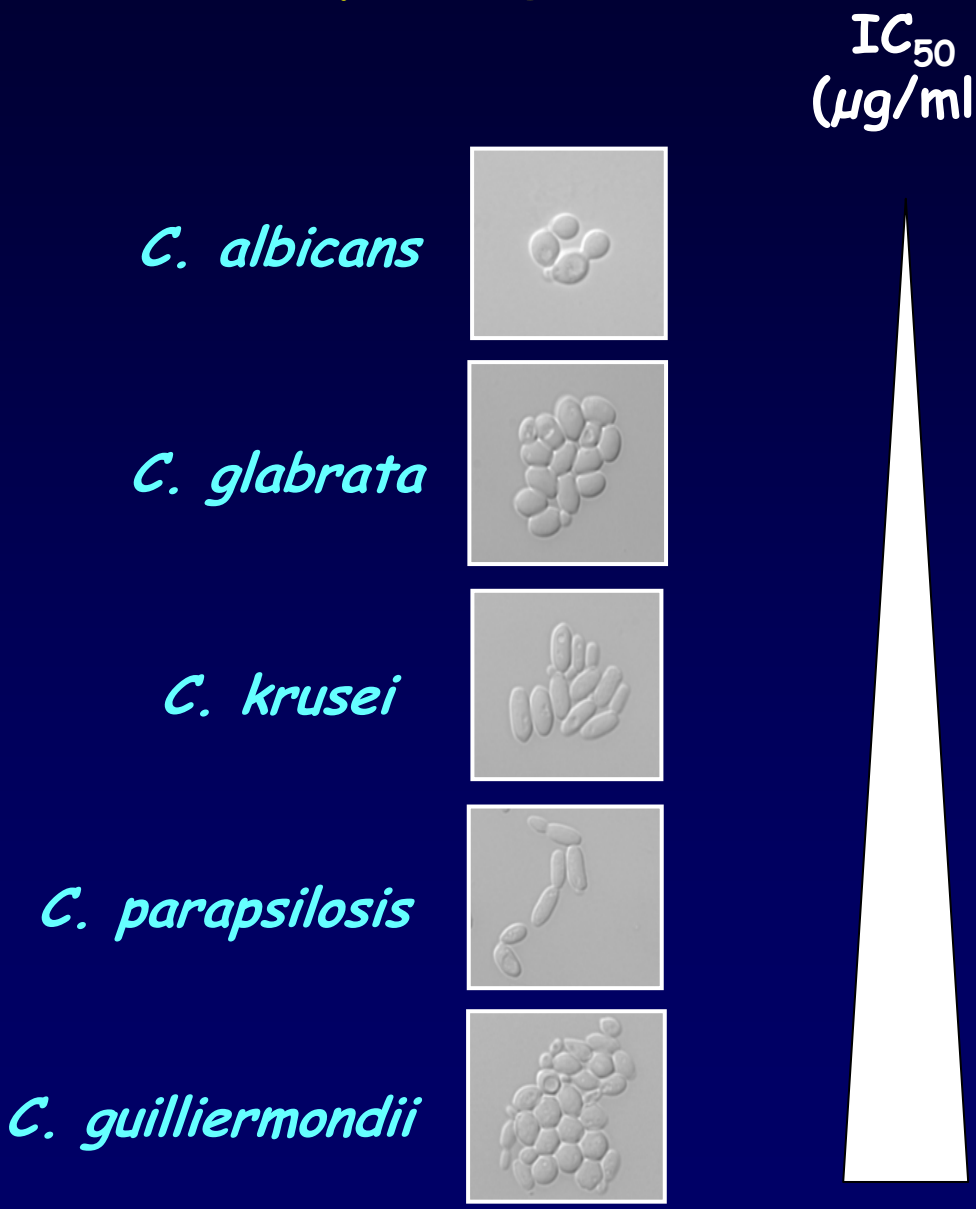
Joachim Morschhäuser

Echinocandins inhibit β-(1,3)-D glucan synthesis

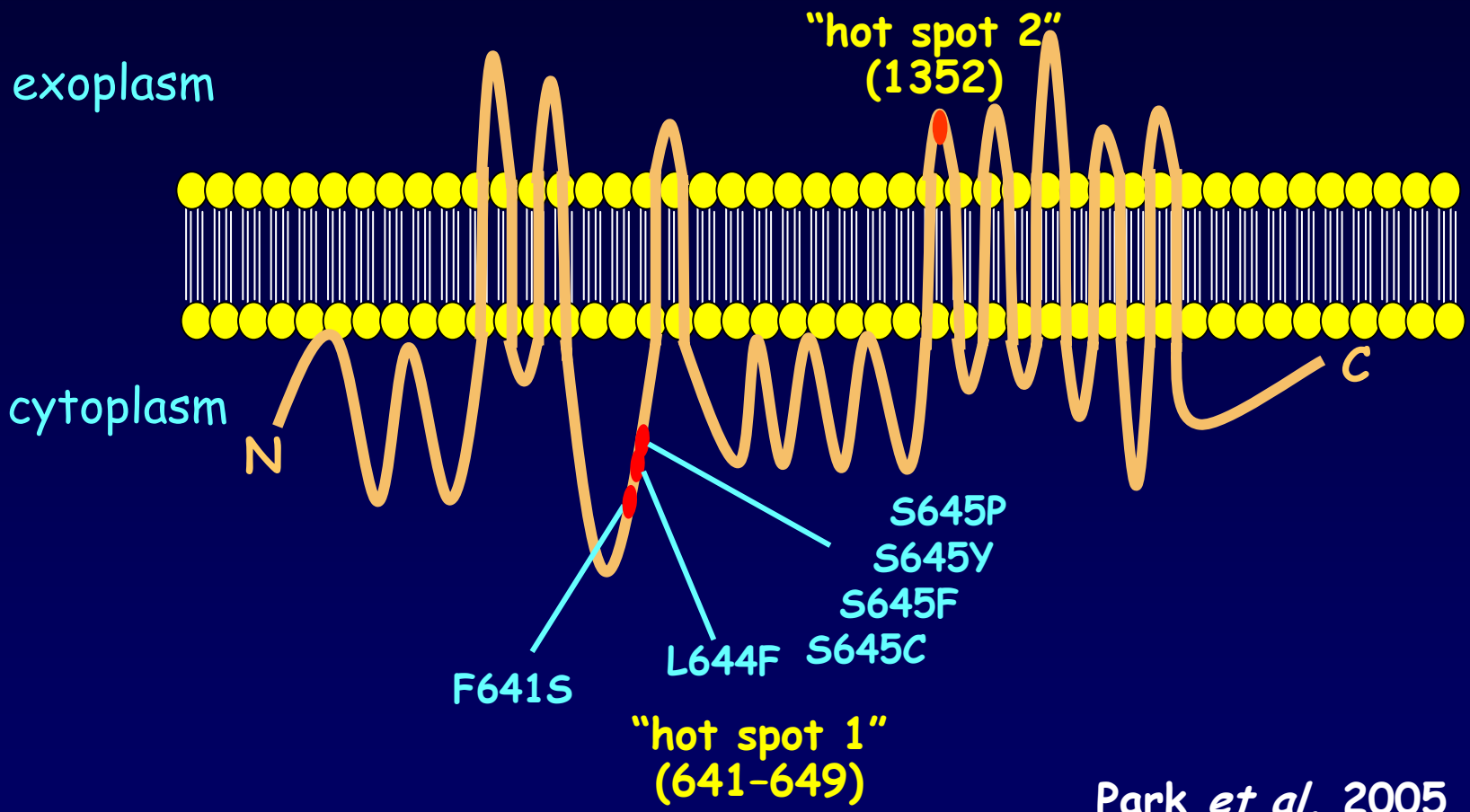
Target of echinocandins – Fks1 sub-unit of glucan synthase complex



Other *Candida* species are more resistant to caspofungin than *C. albicans*



C. albicans echinocandin-resistant clinical isolates have Fks1 mutations



Park *et al.* 2005
Balashov *et al.*, 2006
Perlin, 2007

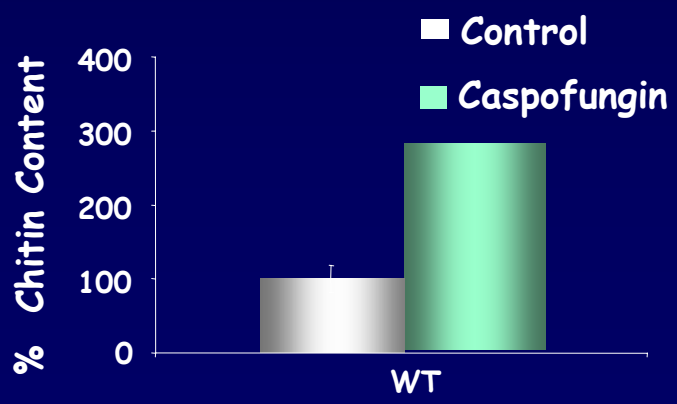
Breakthrough Infections

Clinical Setting	Pathogen	Caspofungin Therapy	Outcome and Resistance Data	
oesophagitis AIDS patient	*C. albicans*	+ micafungin 10 months	**Clinical failure: >33 fold increase in echinocandin MIC(S)**	Fks1 mutation
oesophagitis AIDS patient	*C. albicans*	2 courses increasing dose	**Clinical failure. Only one isolate tested for MIC = 8mg/L**	Fks1 mutation
candidaemia leukaemia patient	*C. krusei*	17 days	**throat isolate ≥ 4 fold higher MIC to 3 candins**	Fks1 mutation
prosthetic aortic valve endocarditis	*C. parapsilosis*	+ FLZ 6 wks	**Subsequent relapse: >8 fold increase in caspofungin, MIC**	Strain also FLZ^R
candidaemia becoming disseminated	*C. krusei*	15 days		**Pre-treatment blood isolate caspofungin MIC 2mg/L**
candidaemia	*C. glabrata*	136 days	**>8 fold increase in caspofungin, MIC**	**1 isolate had suscept to Ampho B**

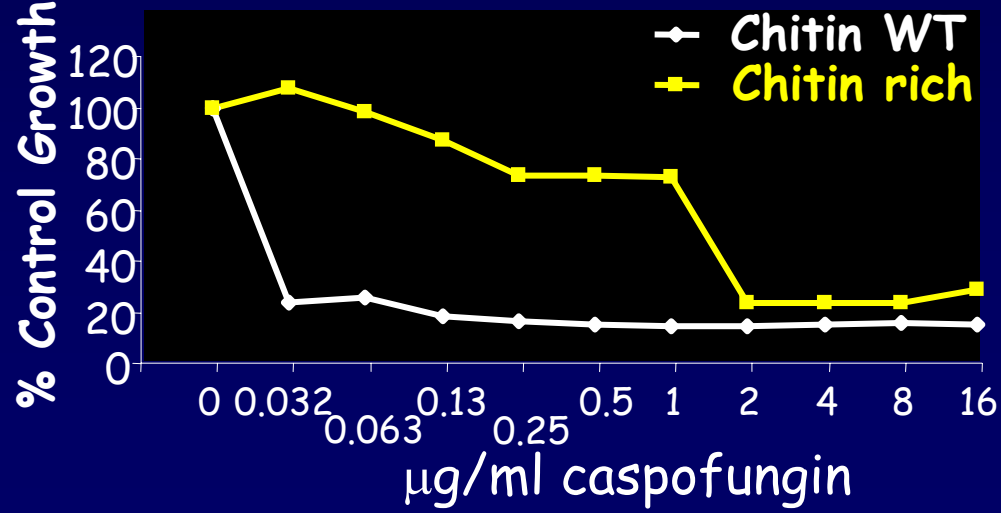
Cell wall salvage mechanisms are activated by the echinocandins

1. Activation of PKC cell wall integrity pathway

2. Cell wall remodelling and activation of chitin synthesis

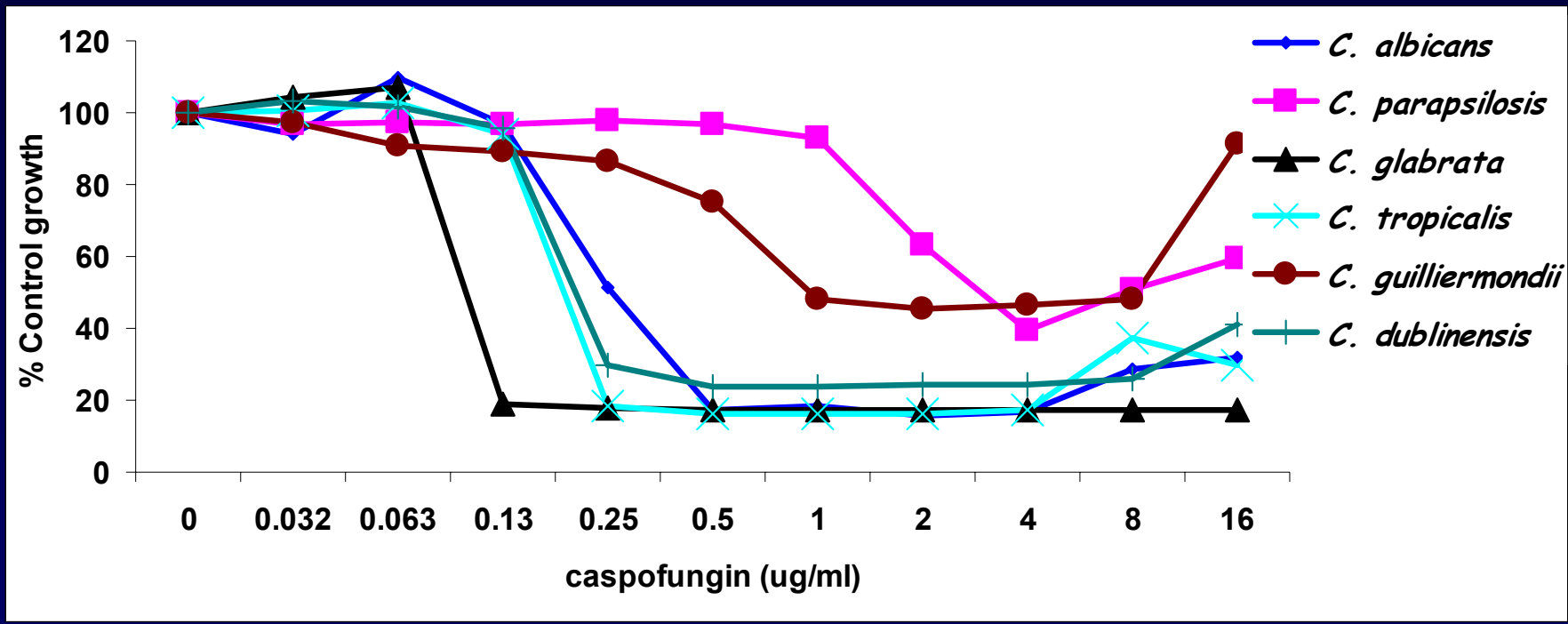


3. Chitin-rich cells are less susceptible to echinocandins



Paradoxical growth at supra-MIC echinocandins

Observed in some clinical isolates of *C. albicans*, *C. dubliniensis*, *C. tropicalis* and *C. parapsilosis* and *C. krusei*, (? *C. glabrata*) with all 3 candins



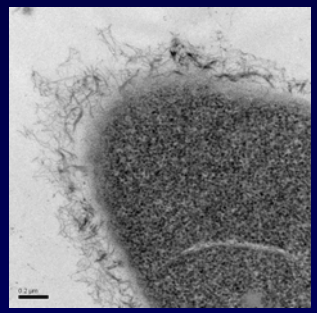
Stevens DA, et al 2006 showed in a clinical isolate that paradoxical effect accomplished by increased cell wall chitin AAC 50(9):3160-1.

Challenges facing the development of new therapies:

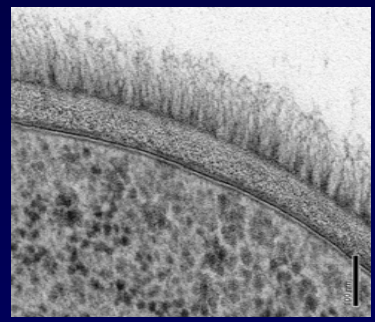
1. Broad spectrum

   Different fungus – different cell envelope

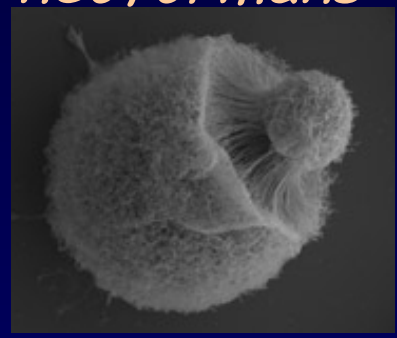
A. fumigatus



C. albicans



Cryptococcus neoformans



Common features: chitin

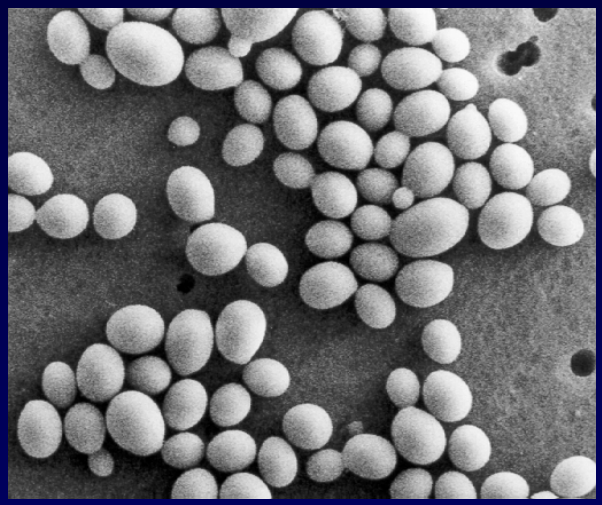
β(1-3)-glucan

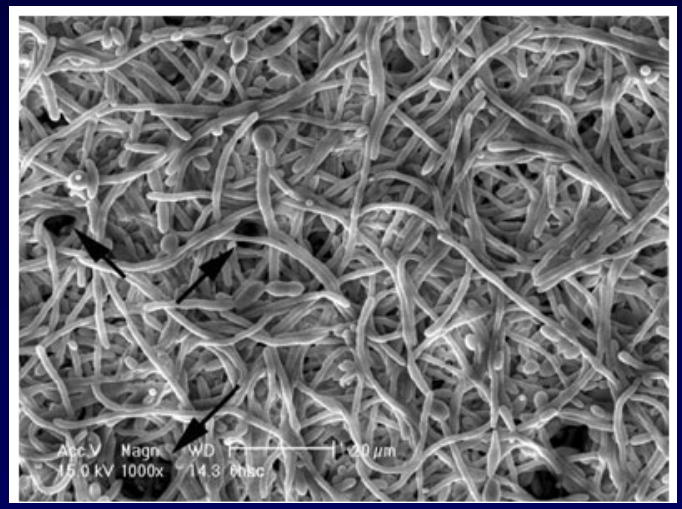
ergosterol

2. **Fungicidal vs fungistatic**

Terry Roemer

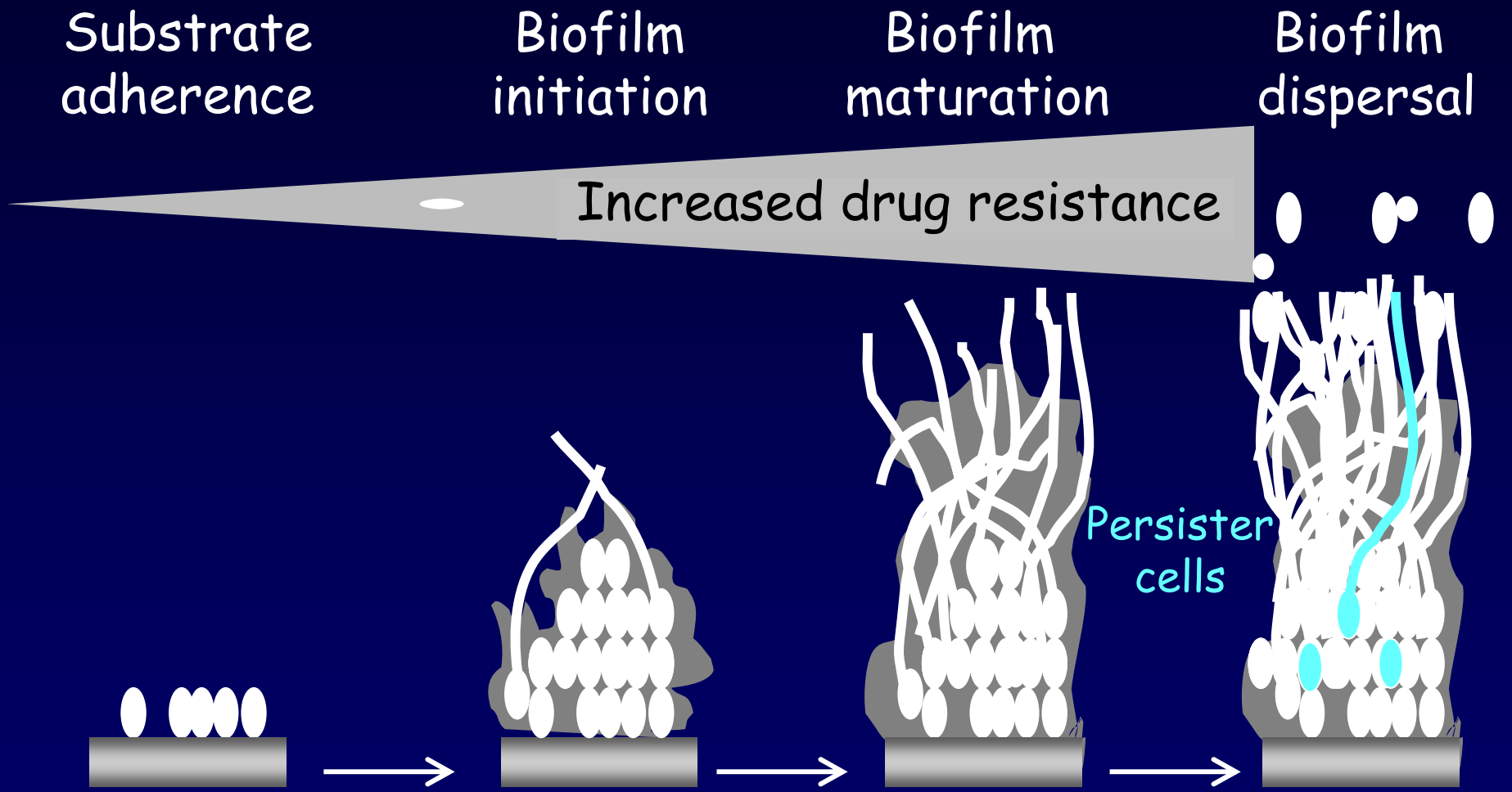
3. Planktonic vs biofilm formation





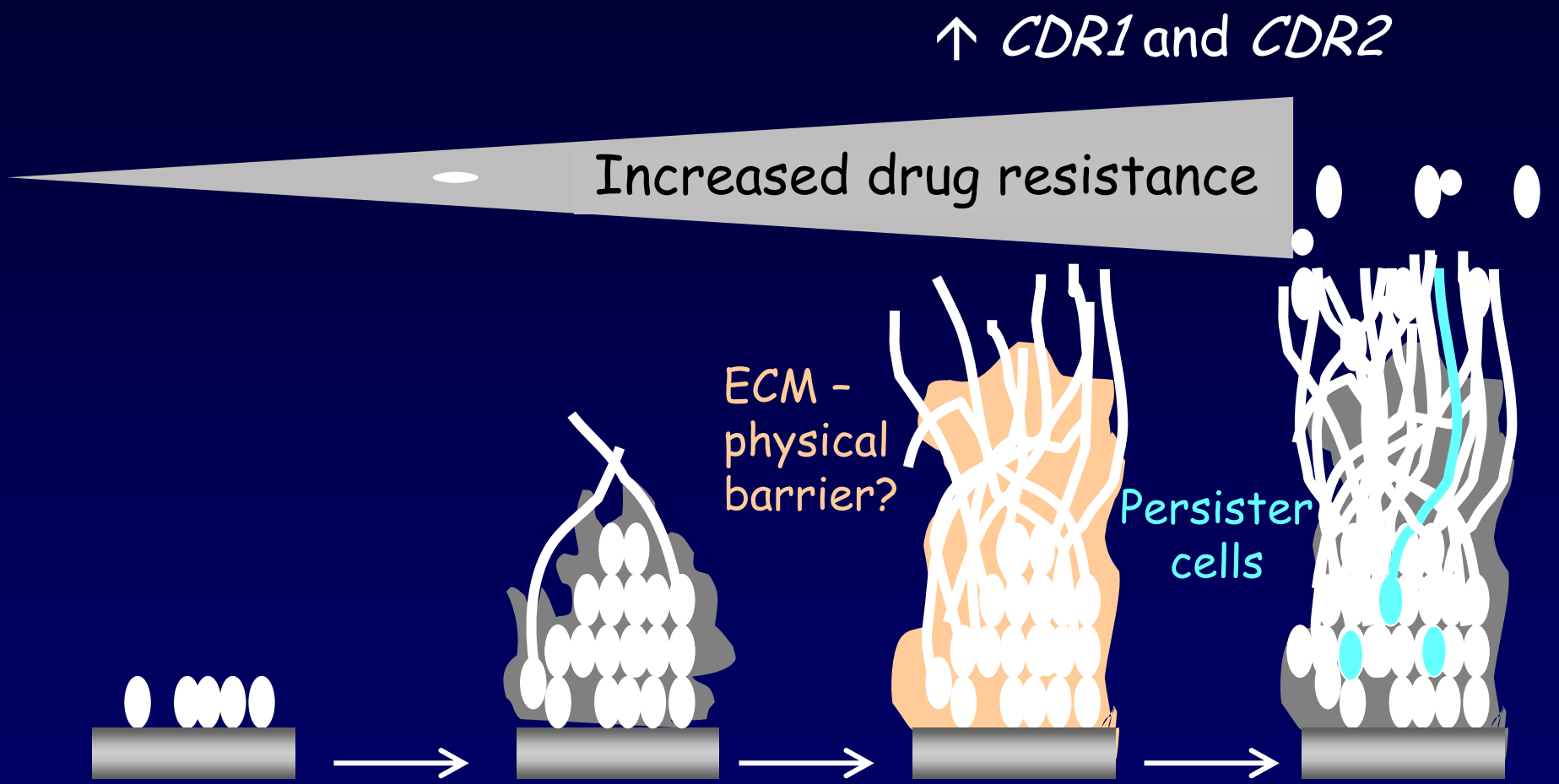
4. Immunomodulation of the host?

Biofilm formation and dissemination



Blankenship JR, Mitchell AP. How to build a biofilm: a fungal perspective. Curr Opin Microbiol. 2006 Dec;9(6):588-94.

Biofilm formation and dissemination



Aaron Mitchell

Potential for combination therapies?

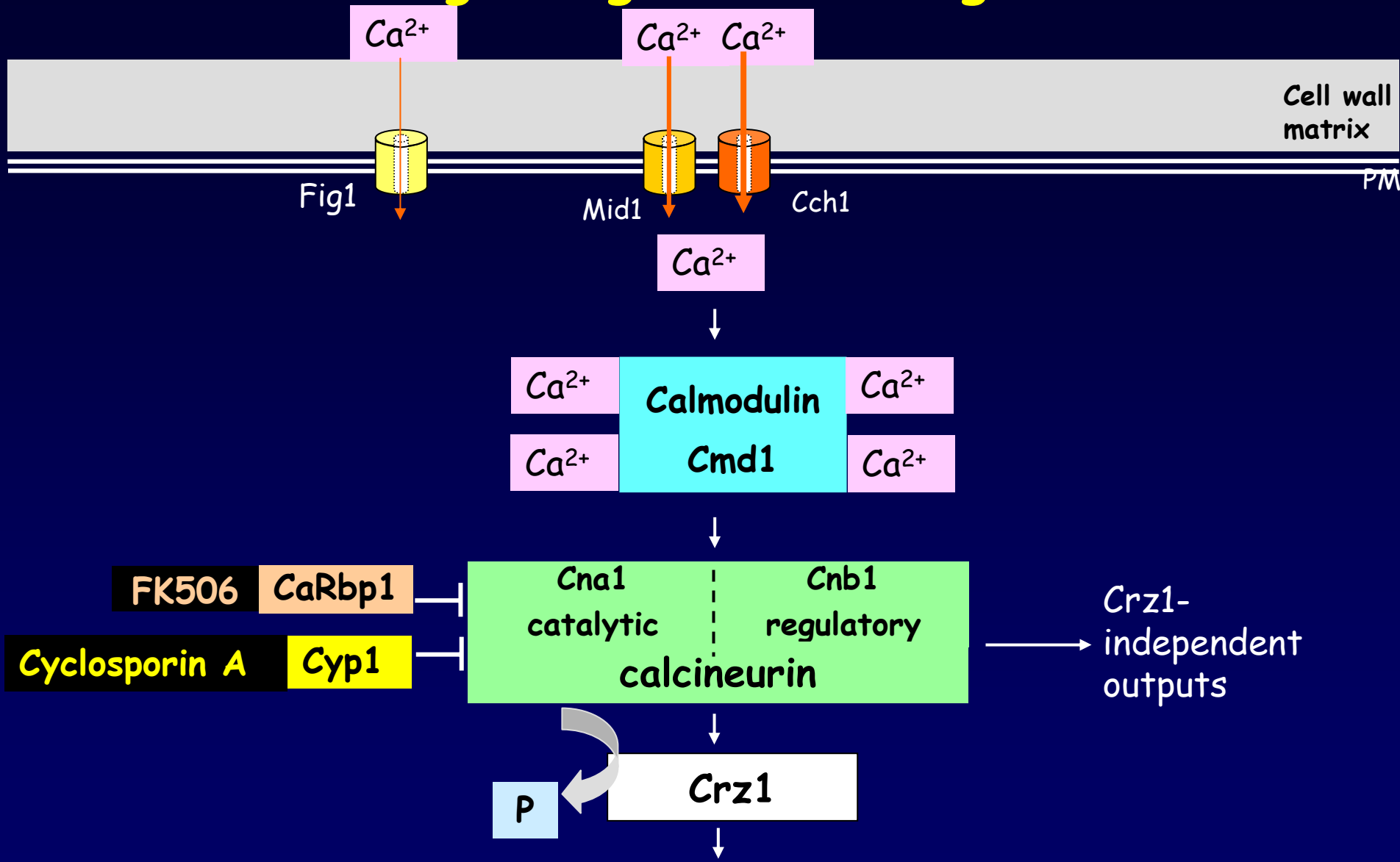
Amphotericin B + echinocandin

Amphotericin B + azole

Azole or echinocandin + calcineurin inhibitor

Chitin synthase inhibitor + echinocandin

Calcium signalling and antifungals



antifungal resistance and tolerance

Session 3 overview

9.20 **Joachim Morschhäuser**, Würzburg Universität, Germany

Transcriptional control of drug resistance in *Candida albicans*

9.50 **Jean-Paul Latgé**, Institut Pasteur, Paris, France

The cell wall of *A. fumigatus*

10.20 **Coffee break**

10.40 **Aaron Mitchell**, Carnegie Mallon University, Pittsburgh, USA

Biofilm matrix regulation by *Candida albicans* Zap1

11.10 **Antonio Cassone**, Istituto Superior di sanita, Roma, Italy

Fungal vaccines: a critical view

11.40 **Terry Roemer**, Merck Frosst Canada, Montreal, Canada

Fungal genomics and natural product discovery: in search of novel antifungal agents