

Overview of Fungal Drug Resistance



COLUMBIA UNIVERSITY

*College of Physicians
and Surgeons*

Department of Microbiology

Center for Interdisciplinary Research
on Antimicrobial Resistance

Aaron P. Mitchell

HHSC 906

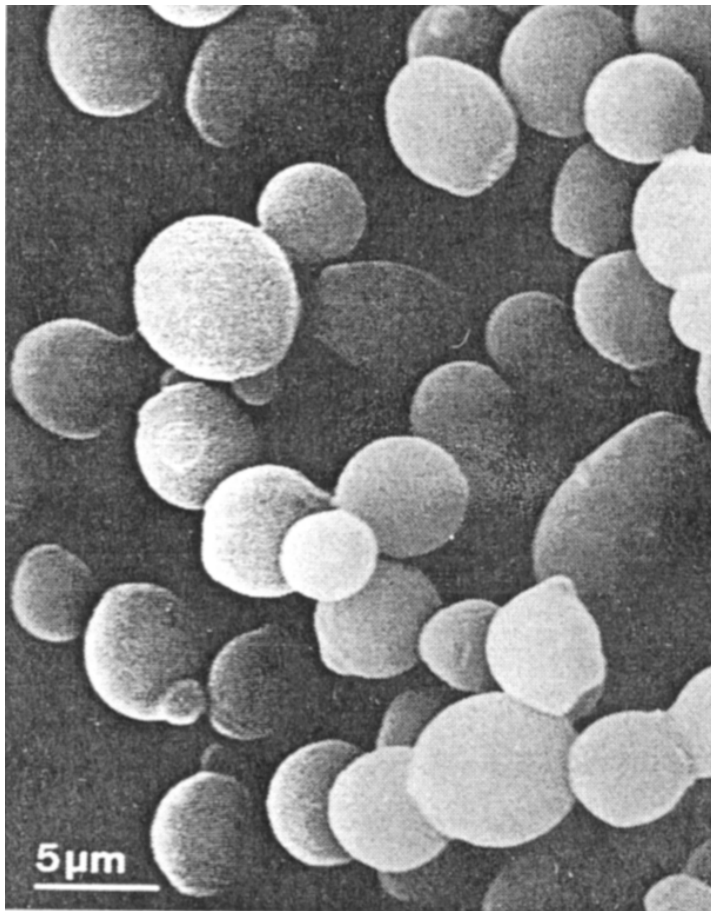
apm4@columbia.edu

What are fungi?

- Fungi are one of the five kingdoms of life.
- Definition: nonmotile eukaryotes that lack flagella and develop from spores.
- Fungi include yeasts, molds, and mushrooms.
- For fungi that cause infectious disease, the two main morphological forms are yeasts and molds.

Yeasts:

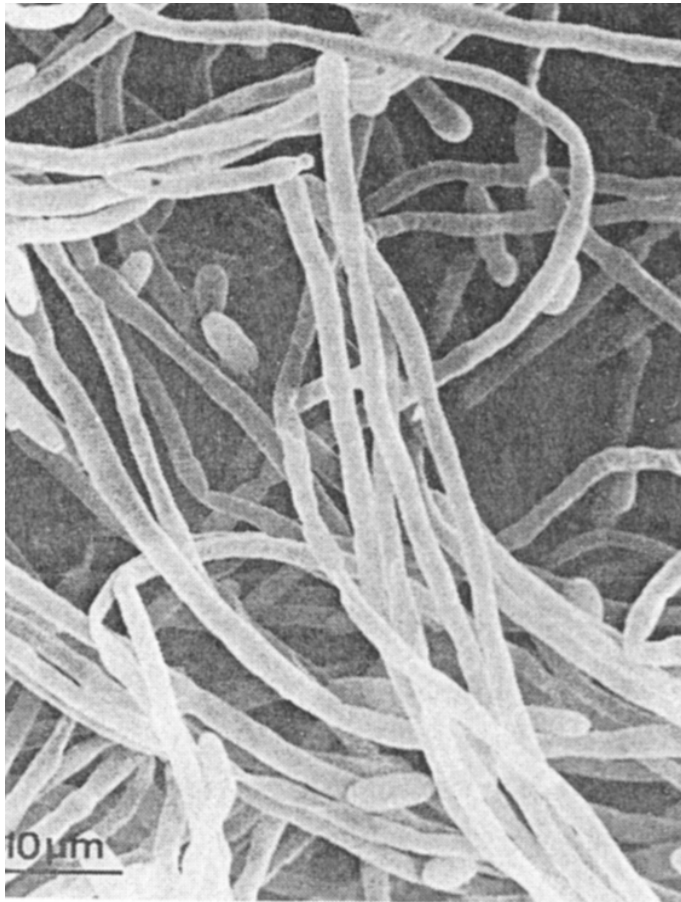
- individual, separated cells
- 5 -20 μ diameter
- divide by budding or by fission



Colonies and individual cells of *C. albicans* yeast form

Molds:

- long filamentous chains of cells
- hyphae or pseudohyphae - individual chains
- mycelium - a mass of hyphae or pseudohyphae

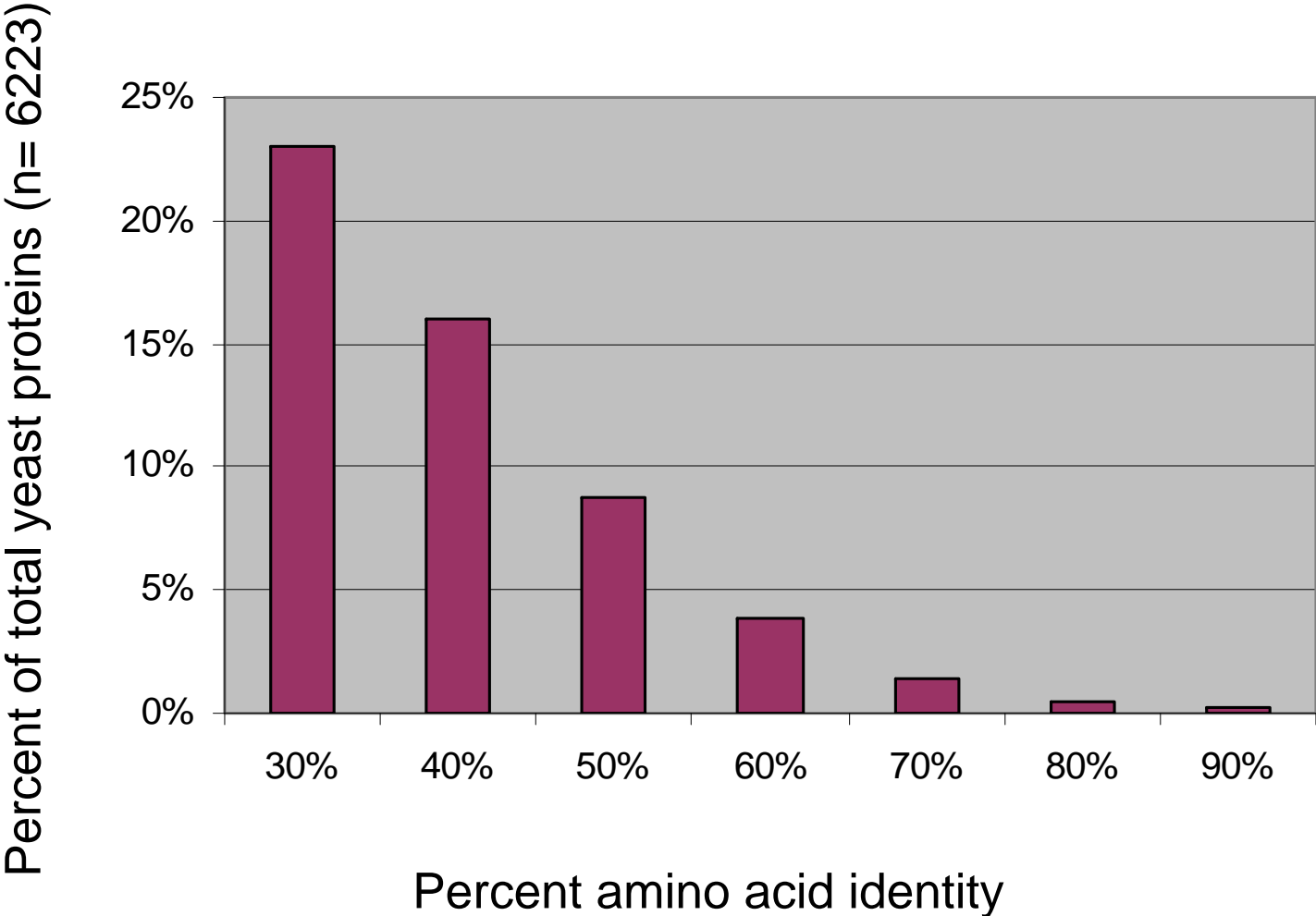


A colony and individual hyphae of *H. capsulatum* mold form

Fungal cells have general features of eukaryotes like us:

- intracellular membrane-bound organelles
- nuclear genome packaged into chromatin
- genome divided among linear chromosomes
- division that occurs via mitotic cell cycle
- high degree of protein sequence conservation and functional conservation between fungi and humans

Similarity between proteins of baker's yeast and humans



Nobel Prizes that recognize yeast models of human cell & molecular biology and physiology



Sir Paul Nurse
The Nobel Prize in Physiology or Medicine 2001

Nobel Lecture Controlling the Cell Cycle



Sir Paul Nurse held his Nobel Lecture December 9, 2001, at hall Aula Magna, Stockholm University. He was presented by Professor Anita Aperia.



Roger D. Kornberg
The Nobel Prize in Chemistry 2006

Nobel Lecture The Molecular Basis of Eukaryotic Transcription



Roger Kornberg delivered his Nobel Lecture on 8 Dec at Aula Magna, Stockholm University. He was introduced by Professor Håkan Wennerström, Chairman of the Nobel Committee for Chemistry.



Leland H. Hartwell
The Nobel Prize in Physiology or Medicine 2001

Nobel Lecture Yeast and Cancer



Leland H. Hartwell held his Nobel Lecture December 9, 2001, at hall Aula Magna, Stockholm University. He was presented by Professor Anita Aperia.



Otto Meyerhof
The Nobel Prize in Physiology or Medicine 1922



“Yeast and Muscle”

Fungal cells also have unique features:

- cell wall consisting of glucan, chitin, and mannoprotein
- ergosterol is the main membrane sterol, rather than the cholesterol found in mammals
- Diverse metabolite biosynthetic capacity

These unique features provide pathogen-specific drug targets to be exploited in development of antifungal drugs.

Main classes of antifungal drugs

Plasma Membrane

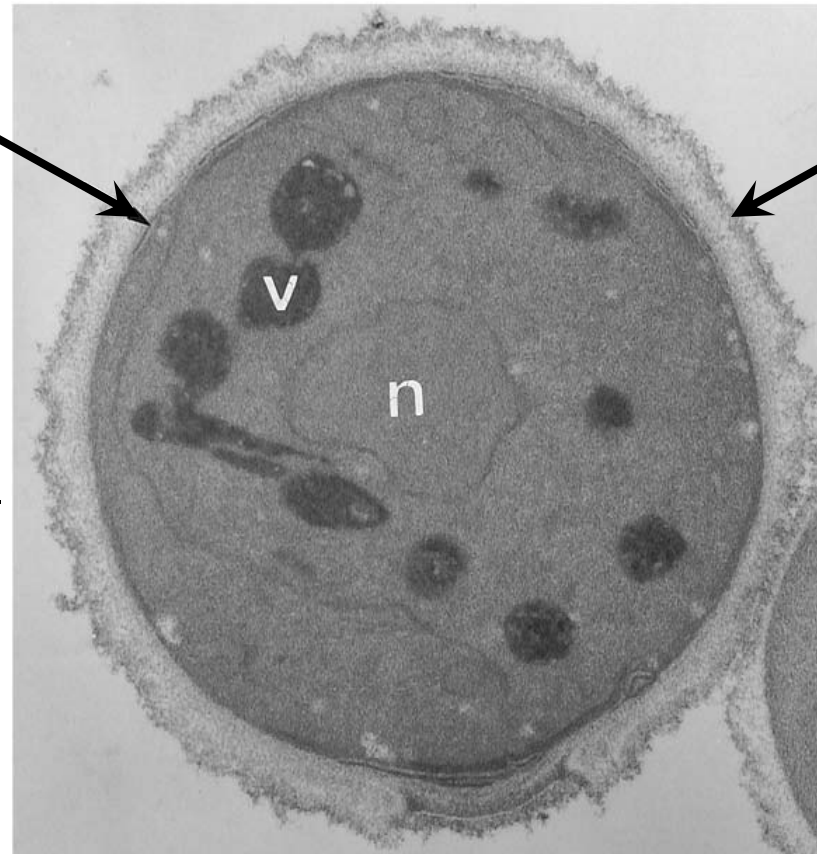
Polyenes

(Amphotericin B) –

Disrupt ergosterol-containing membranes

Azoles (Fluconazole) –

Block ergosterol synthesis



Cell Wall

Echinocandins

(Caspofungin) –

Block β -glucan synthesis

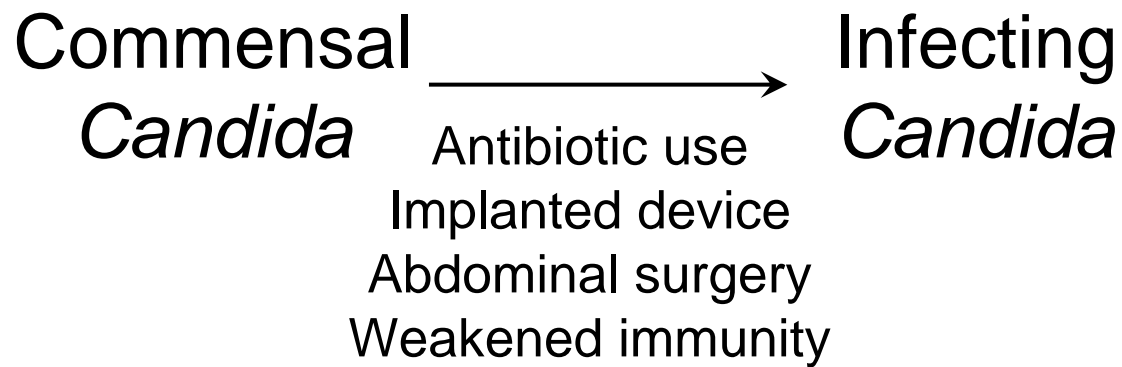
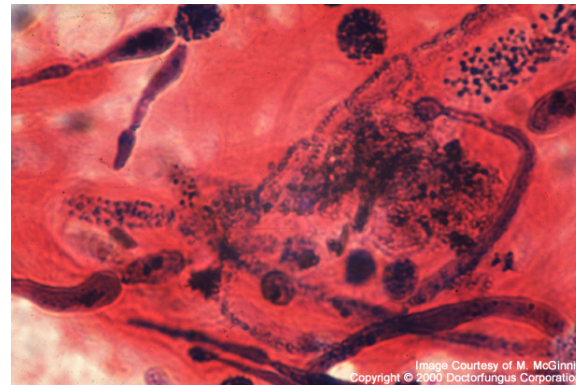
Image of a fungal cell in the electron microscope

Our focus: *Candida* and Azoles

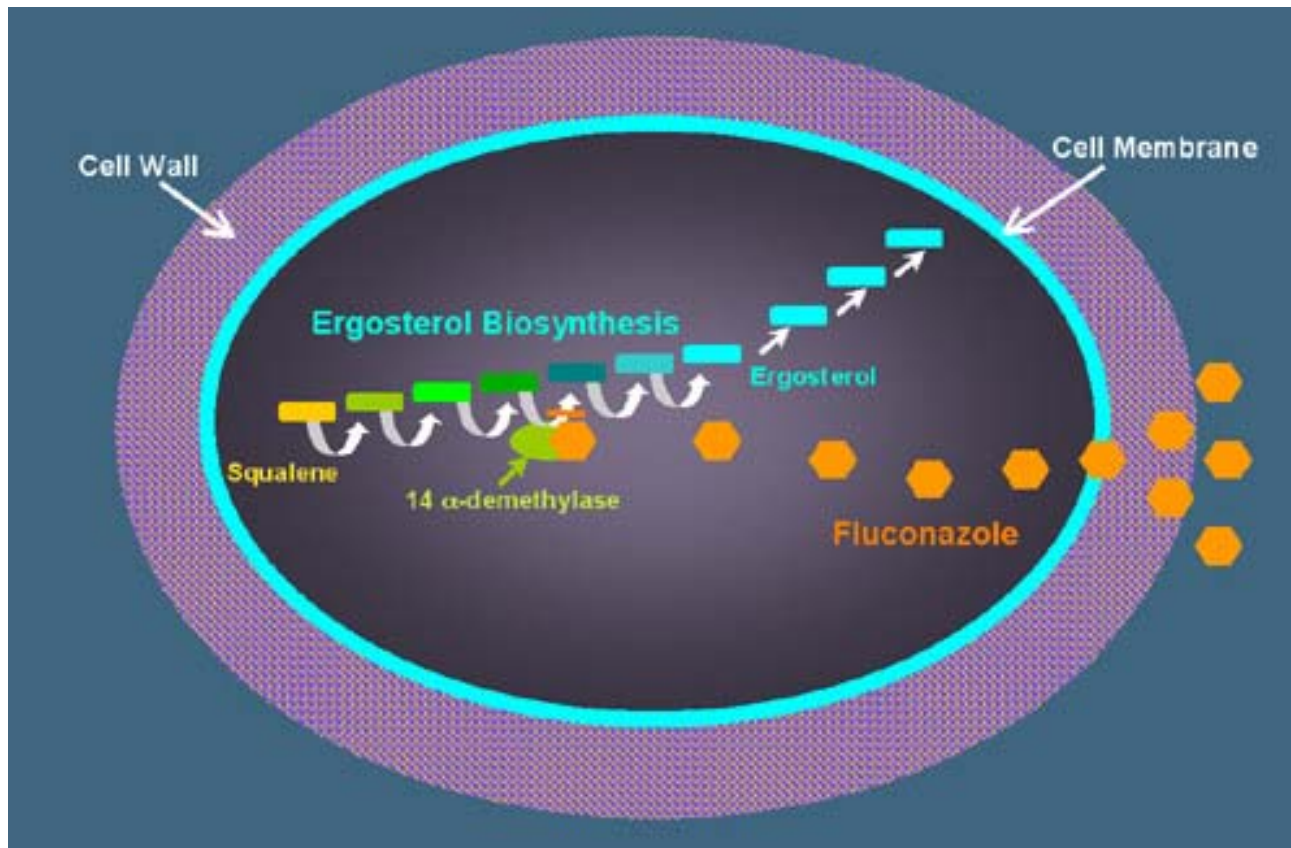
Mucosal Infection



Invasive Infection

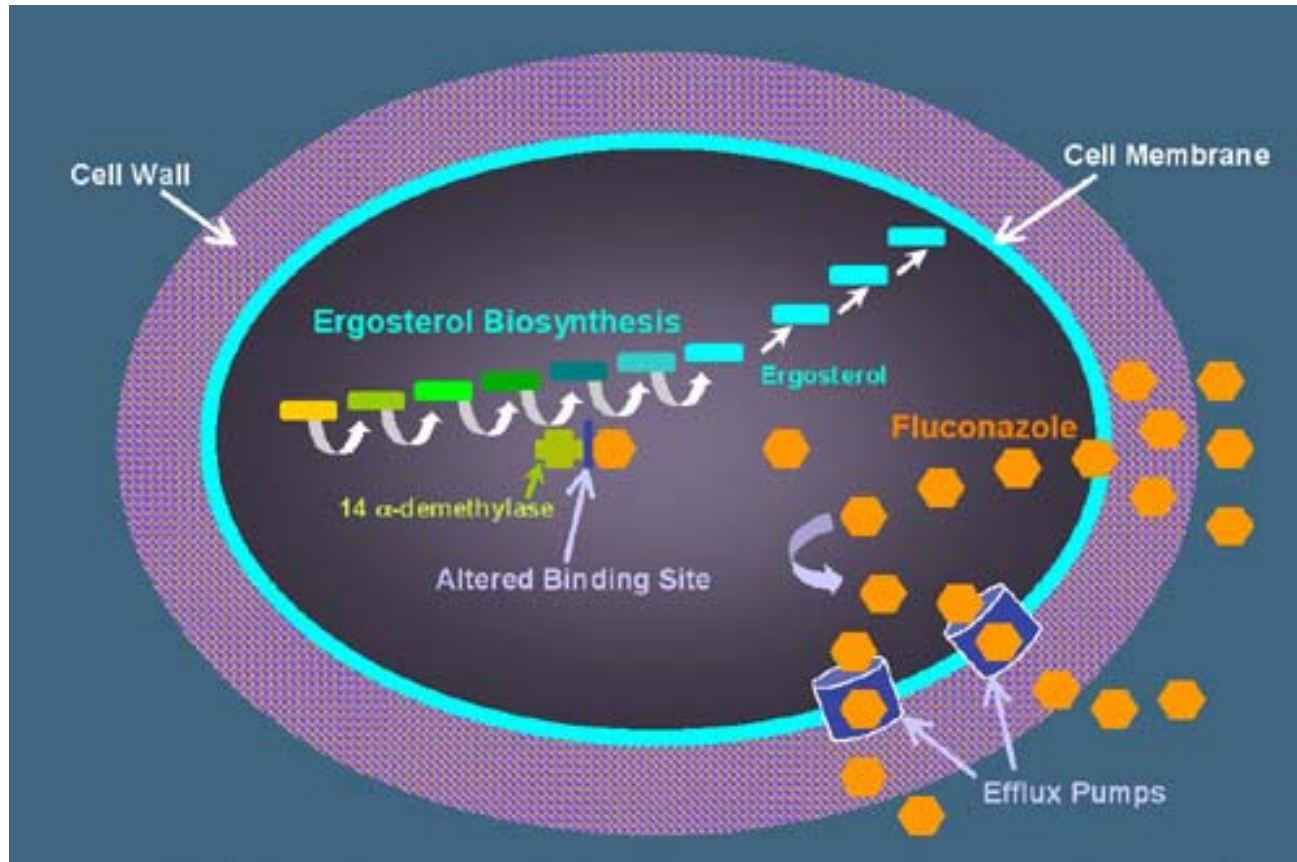


Azoles – mechanism of action



- Azoles inhibit a demethylase (Erg11) required for ergosterol synthesis
- Lack of ergosterol and buildup of intermediates are both deleterious

Known azole resistance mechanisms

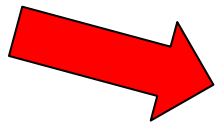


Resistance can arise through:

- Altered Erg11 structure
- Increased Erg11 expression
- Increased expression of efflux pumps (Cdr1, Cdr2, Mdr1)

Azole resistance mechanisms

Types of resistance problems:



1. Intrinsic
2. Epigenetic
3. Acquired

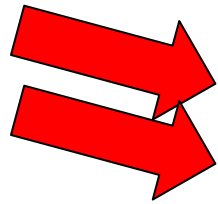
Intrinsic azole resistance

Fluconazole Minimum
Inhibitory Concentration
for 50%/90% of isolates

Species
(no. of isolates
tested)

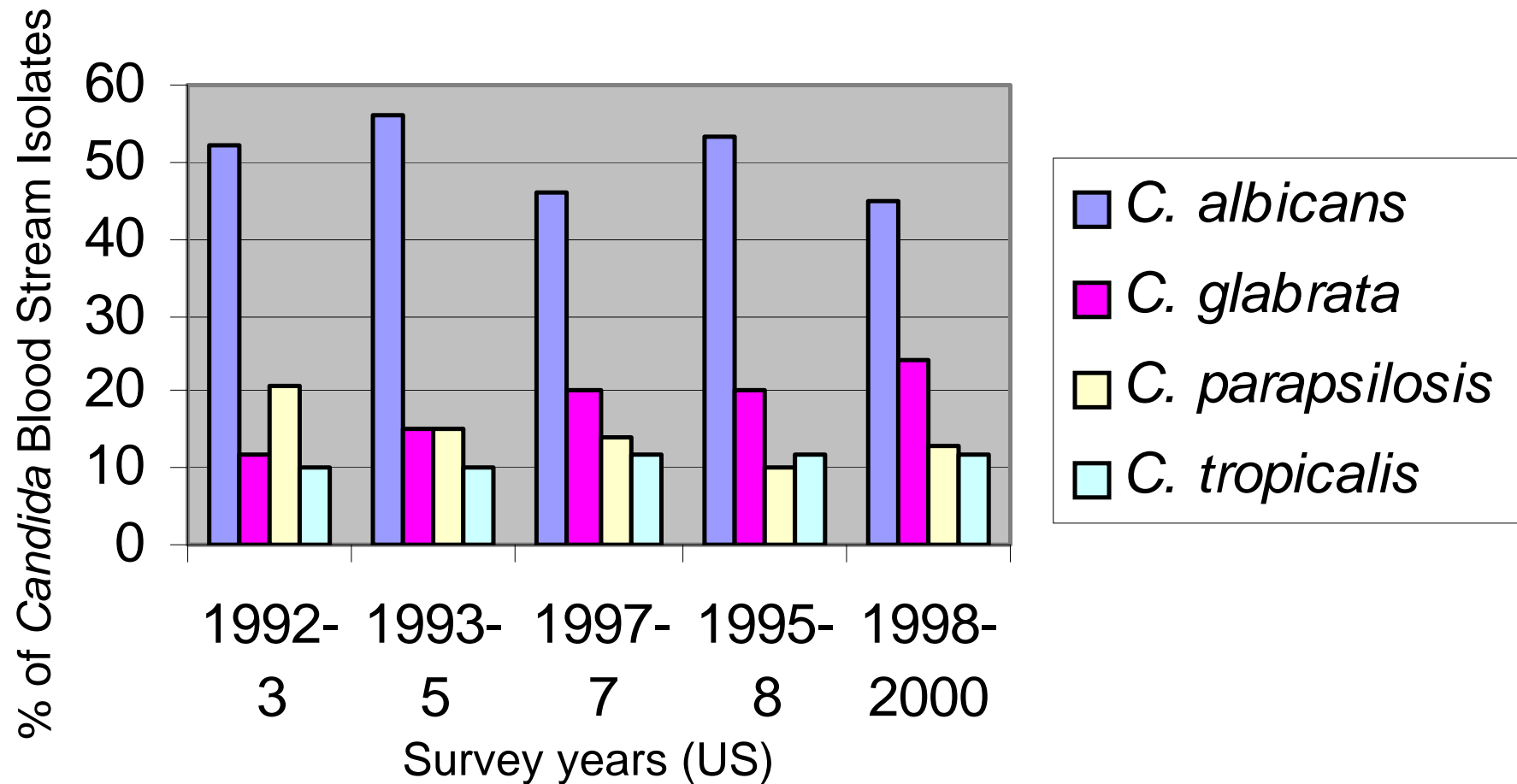
MIC₅₀
(μ g/ml)

MIC₉₀
(μ g/ml)



<i>C. albicans</i> (129)	0.25	1
<i>C. glabrata</i> (31)	16	32
<i>C. tropicalis</i> (40)	1	>64
<i>C. parapsilosis</i> (23)	1	4
<i>C. krusei</i> (5)	32	32
<i>C. lusitanae</i> (3)	2	4
<i>C. lipolytica</i> (1)	64	64

Consequence of intrinsic azole resistance

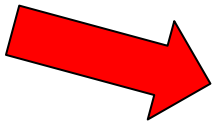


C. glabrata has gone from rare to commonplace (now $\geq 30\%$ of *Candida* BSI)

Azole resistance mechanisms

Types of resistance problems:

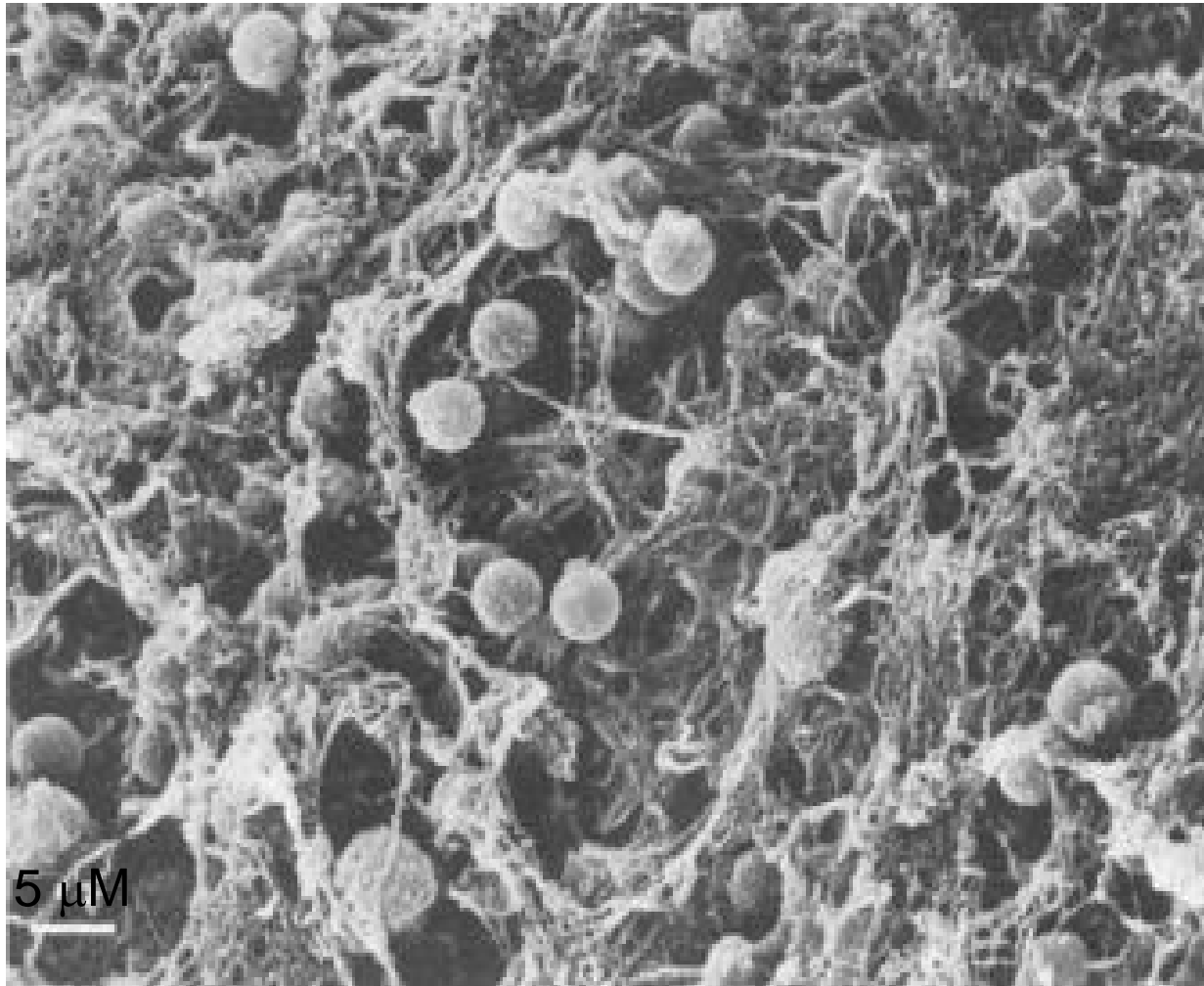
1. Intrinsic – We see increased frequency of infection by intrinsically resistant species
2. Epigenetic
3. Acquired



Epigenetic azole resistance & Device-associated infection

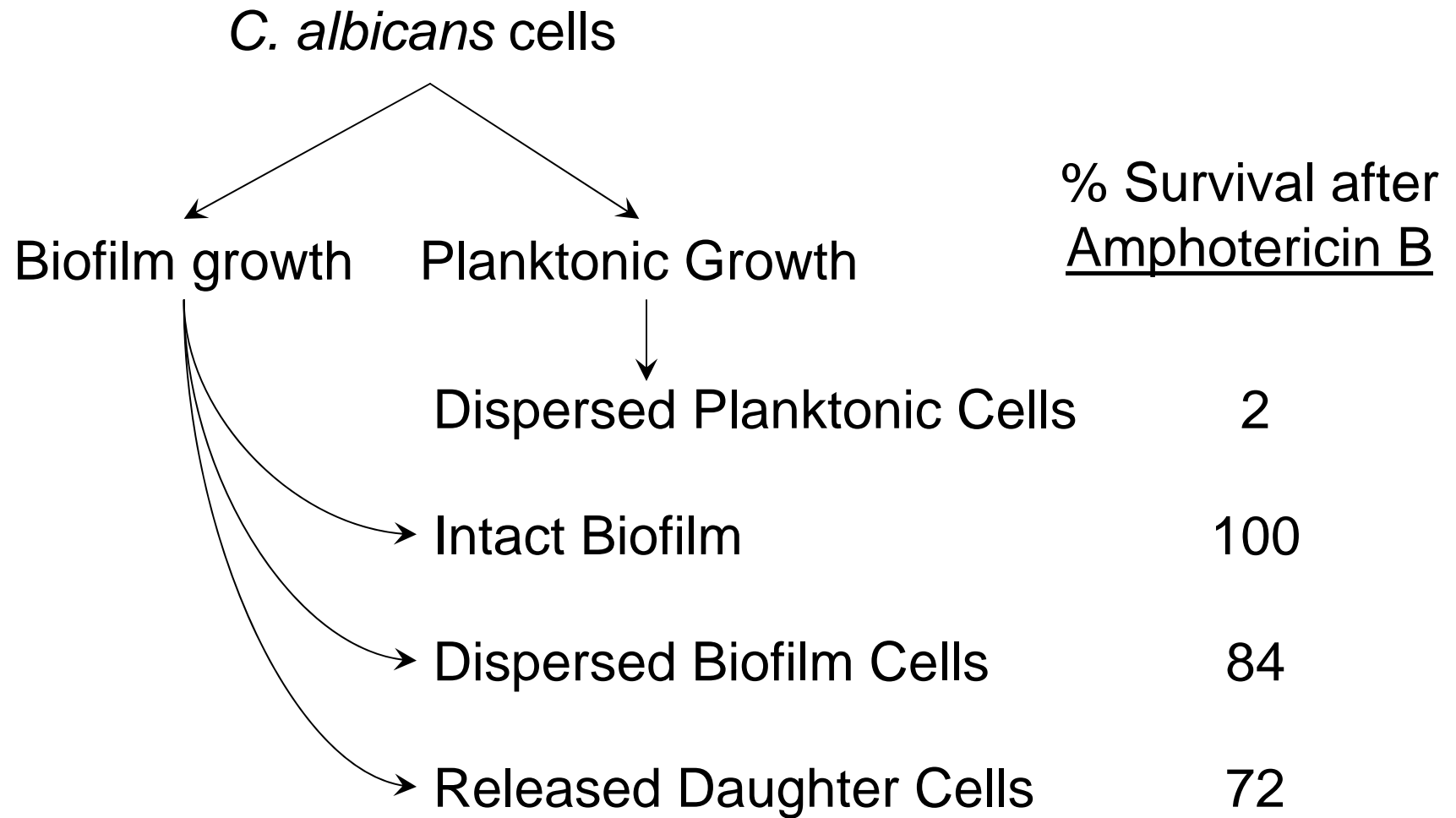
Device	Annual use (USA)	% Infection
Vascular catheters	5×10^6	3-8
Joint prostheses	6×10^5	1-3
Prosthetic cardiac valves	8.5×10^4	2.9
Urinary catheters	3×10^7	10-30

The basis of device-associated infection: Biofilm formation

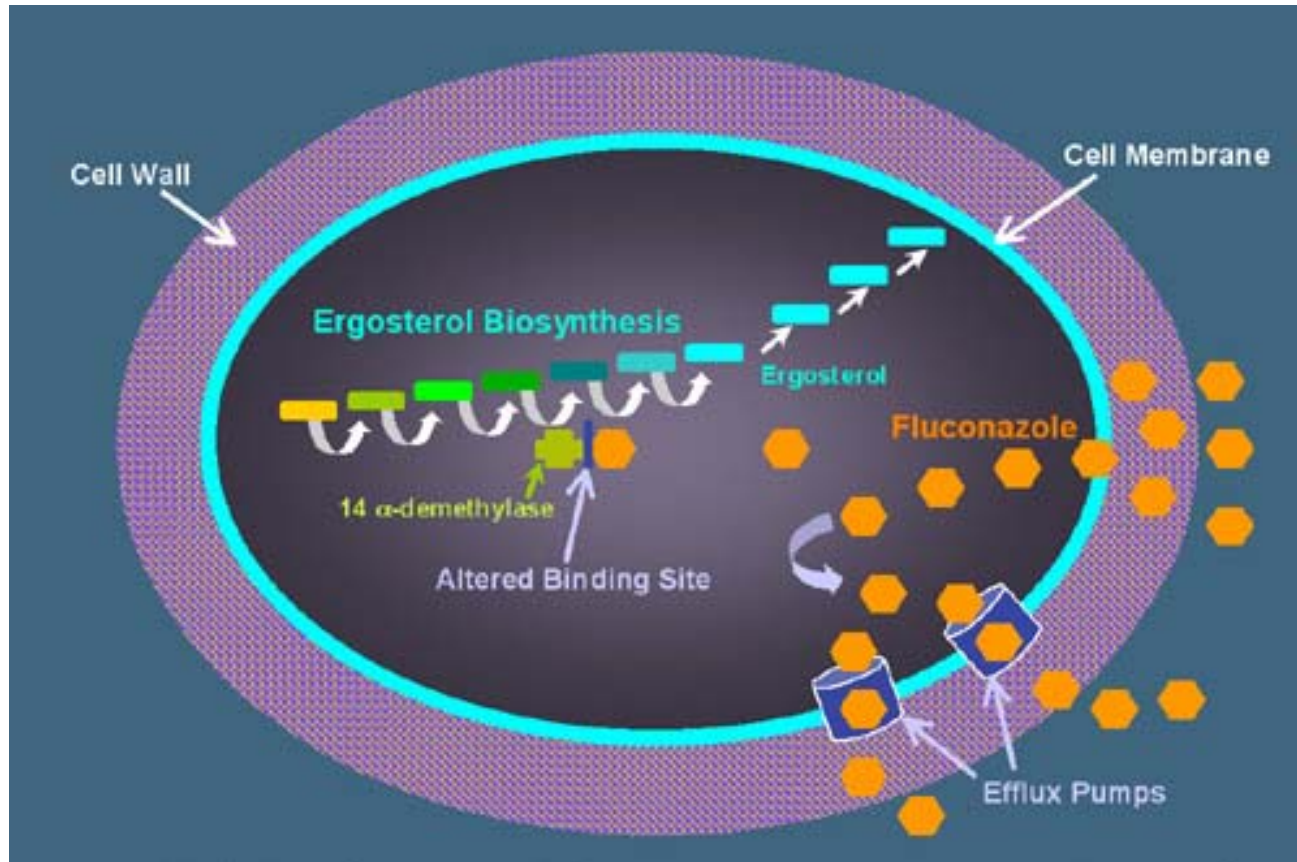


Scanning electron micrograph of *Candida* biofilm on patient intravenous catheter
Marrie TJ, Costerton JW. J Clin Microbiol. 1984 May;19(5):687-93.

Epigenetic azole resistance
Does biofilm growth alter drug susceptibility?



Known azole resistance mechanisms



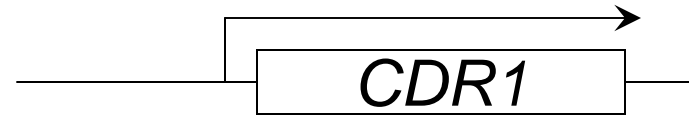
Resistance can arise through:

- Altered Erg11 structure
- Increased Erg11 expression
- Increased expression of efflux pumps (Cdr1, Cdr2, Mdr1)

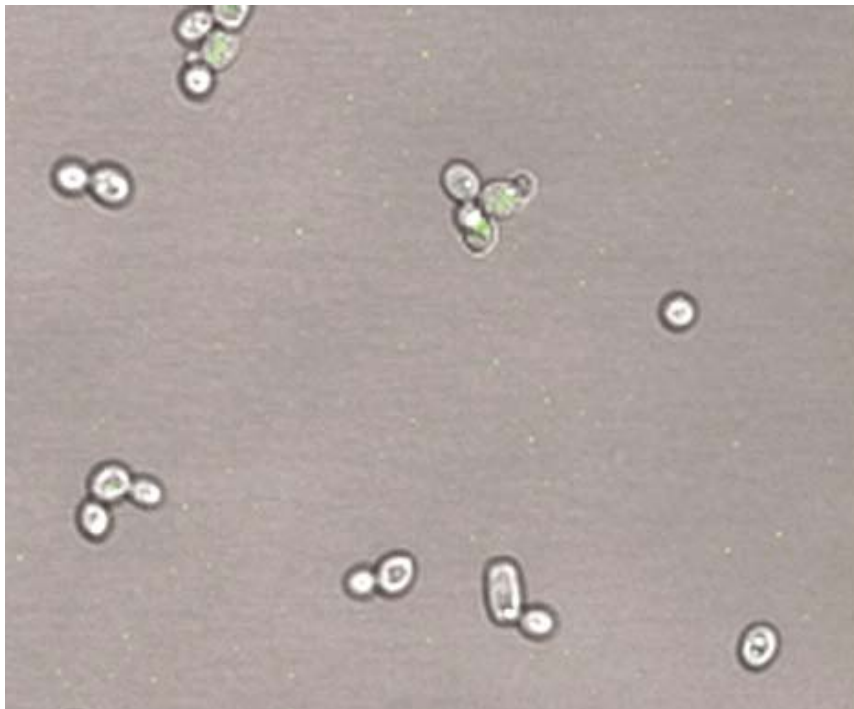
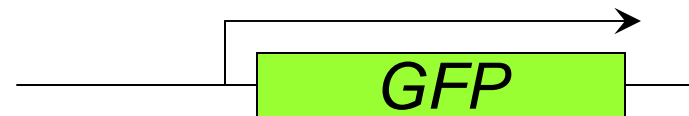
This is one factor
in epigenetic
resistance

Epigenetic azole resistance

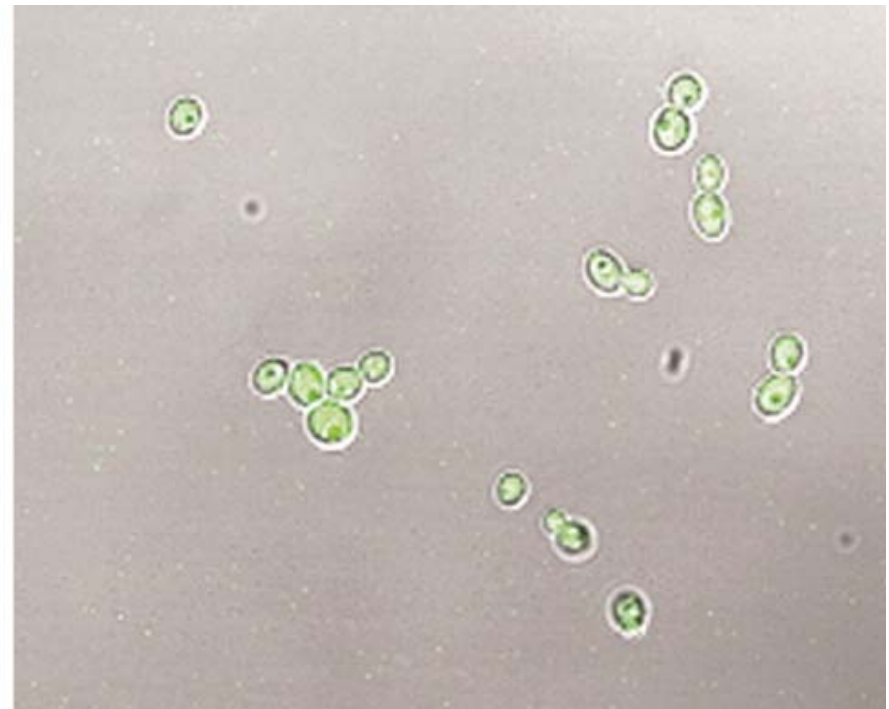
Wild-type *C. albicans* strain



Engineered *C. albicans* strain



Engineered strain – planktonic

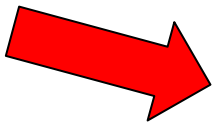


Engineered strain – 10' after surface contact

Azole resistance mechanisms

Types of resistance problems:

1. Intrinsic – We see increased frequency of infection by intrinsically resistant species
2. Epigenetic – Basis is complex; consequence is difficulty in treating device-associated infections
3. Acquired



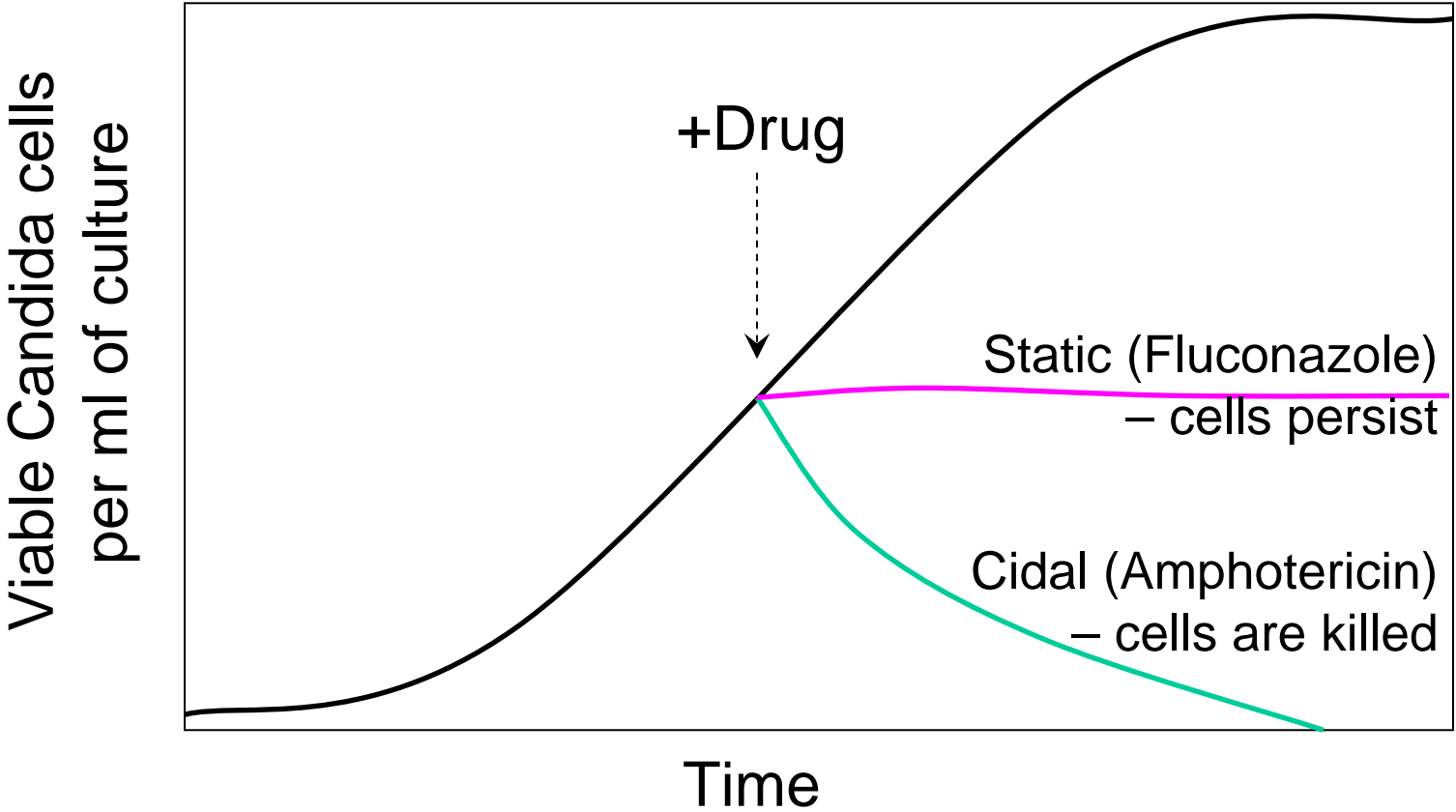
Acquired azole resistance

(The Perfect Storm? Or just another blizzard?)

1. Azoles are static
2. Resistance mechanisms are diverse and cumulative
3. An evolutionary accident (?) links diverse resistance genes

Acquired azole resistance

1. Azoles are static



Acquired azole resistance

1. Azoles are static - Thus sterilization of infected tissue after azole treatment requires some clearance capability.
2. Resistance mechanisms are diverse and cumulative
3. An evolutionary accident (?) links diverse resistance genes

Acquired azole resistance

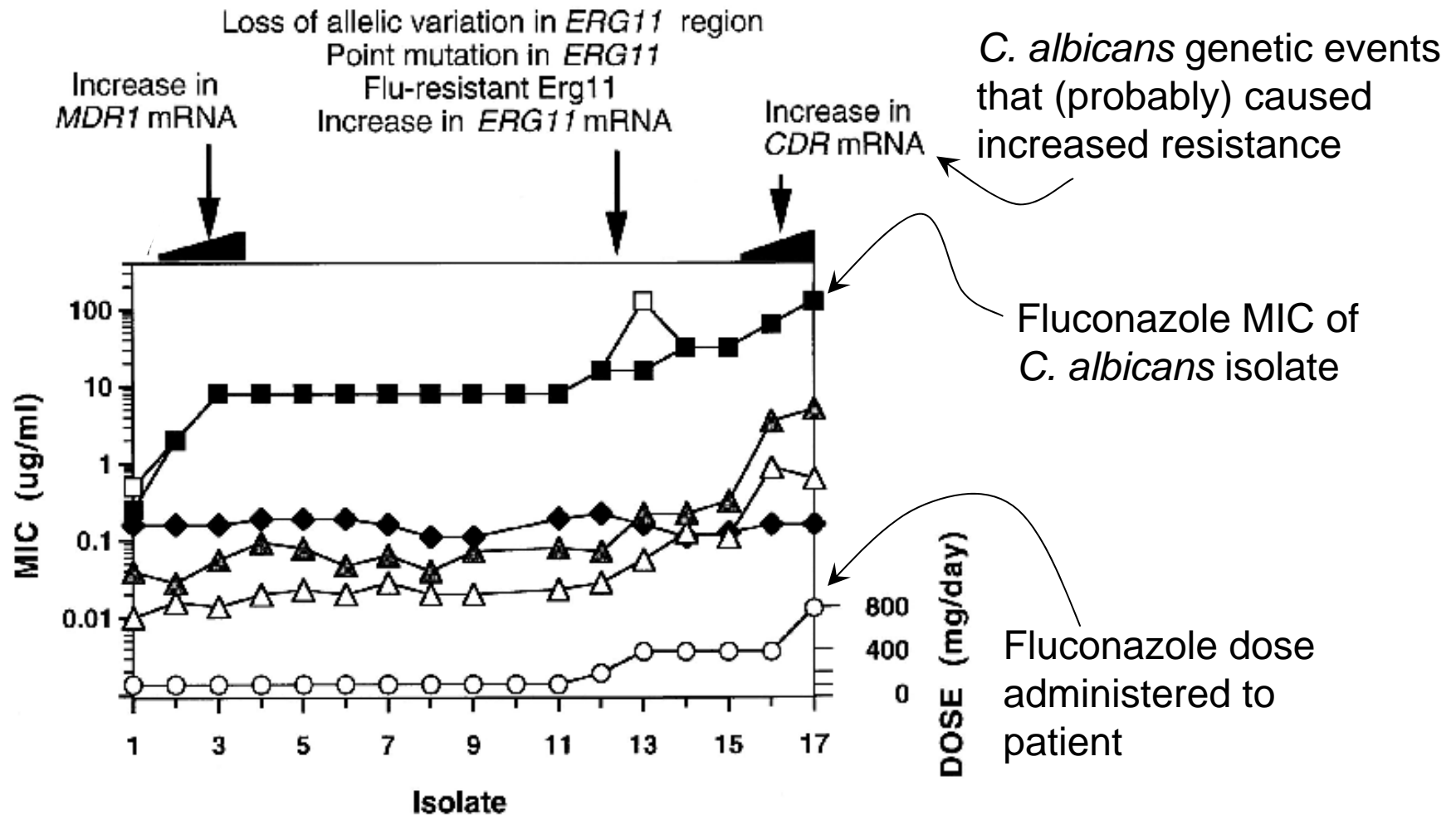
2. Resistance mechanisms are diverse and cumulative

AIDS patient

2 years of treatment; 17 cases of oral candidiasis treated
with increasing Fluconazole doses

Acquired azole resistance

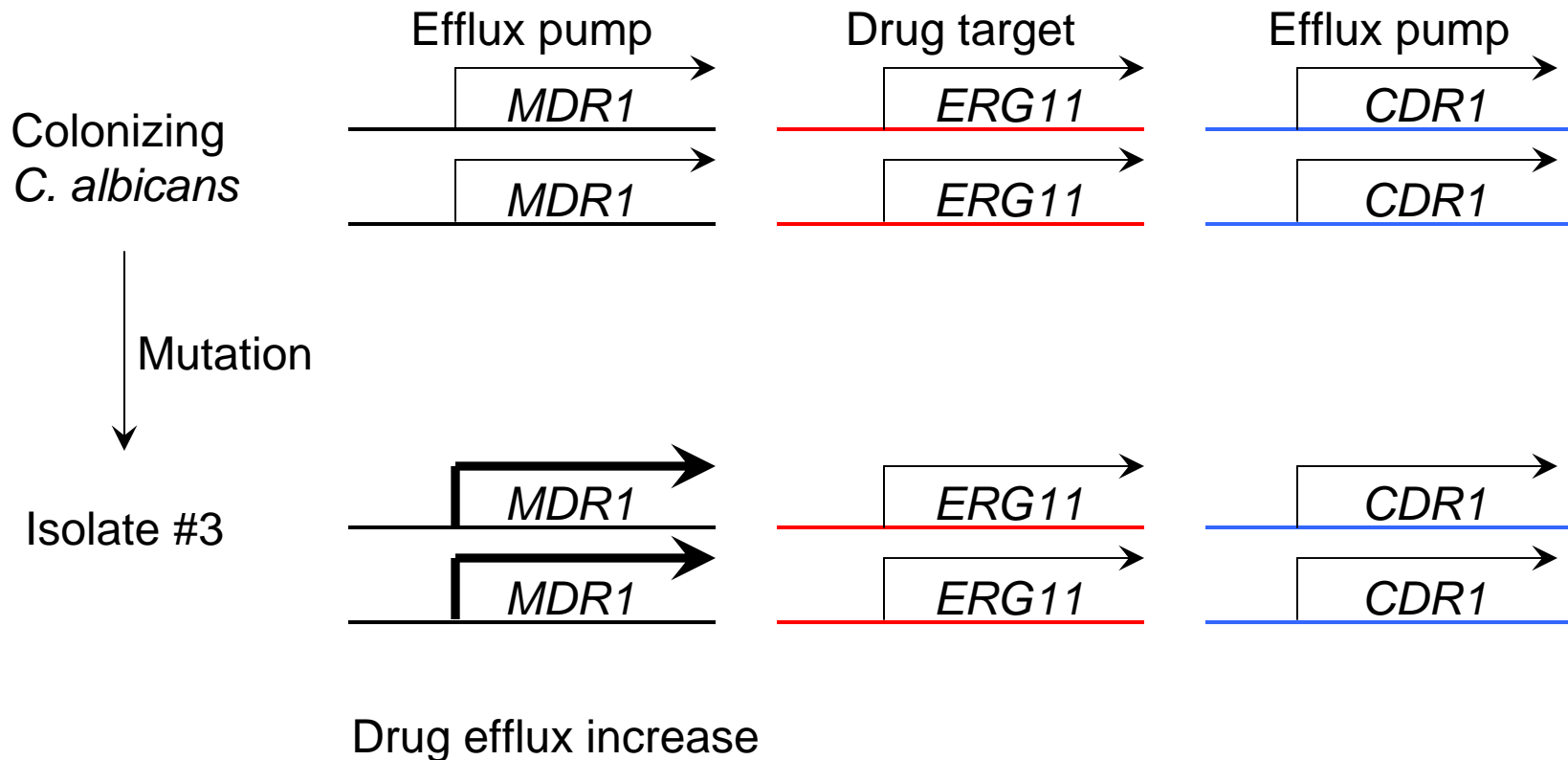
2. Resistance mechanisms are diverse and cumulative



Acquired azole resistance

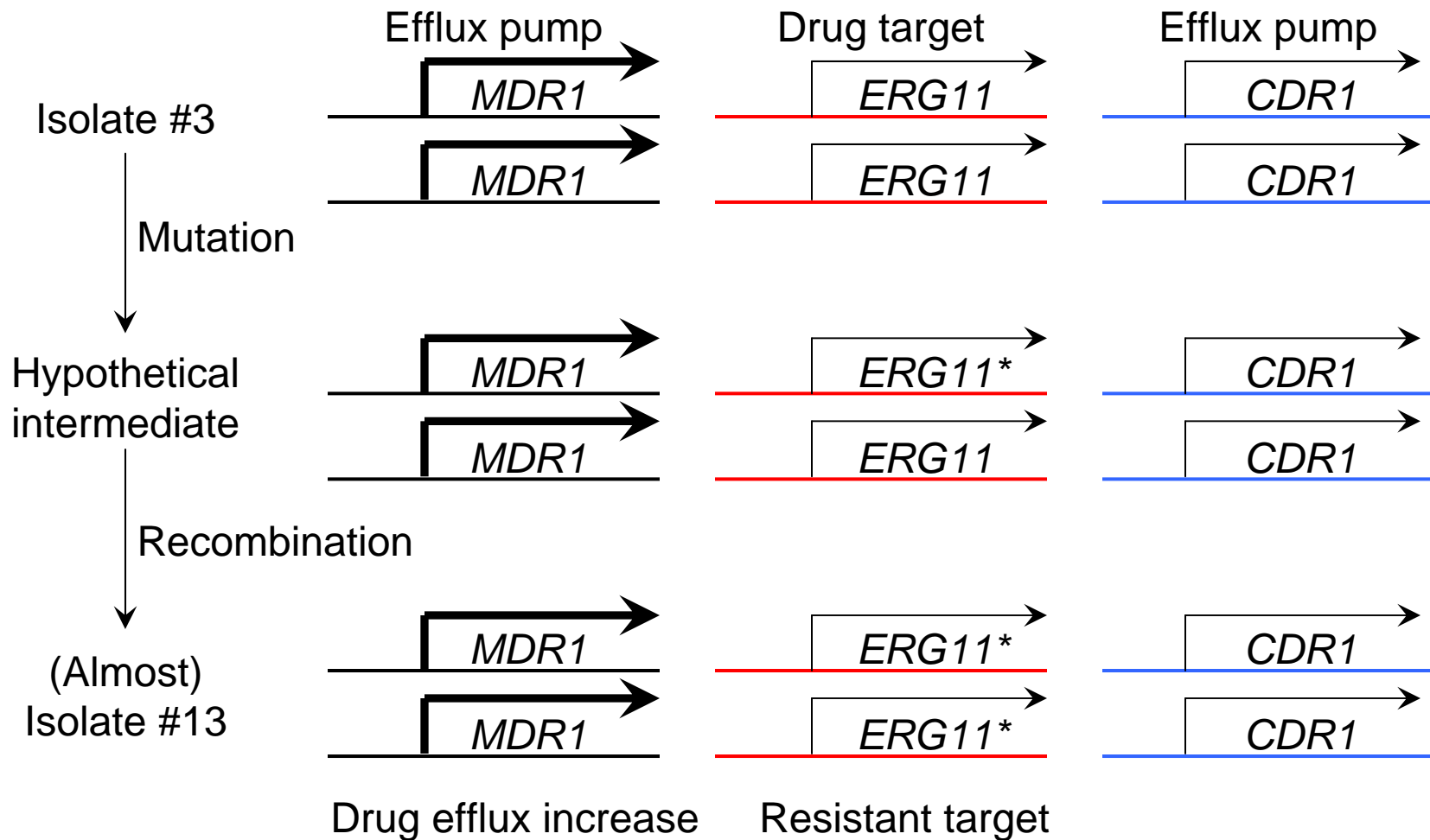
Multistep pathway to Fluconazole resistance

- three genes on different chromosomes:



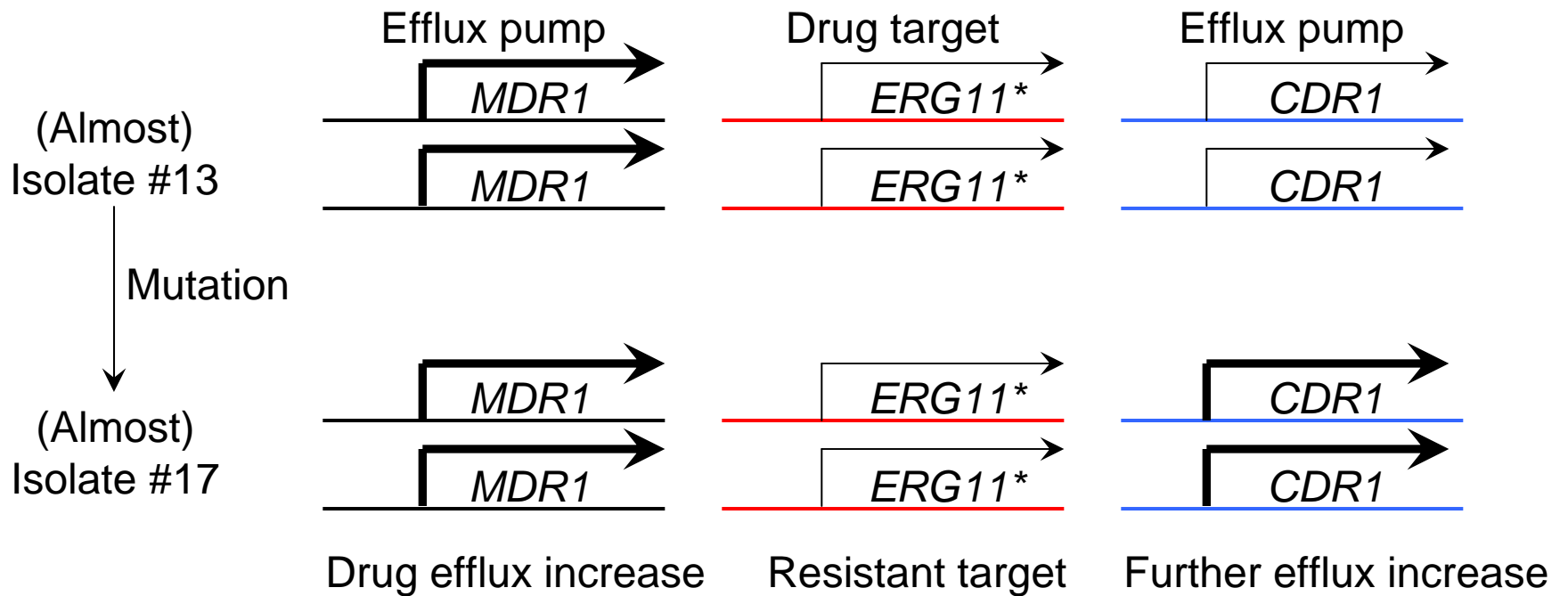
Acquired azole resistance

Multistep pathway to Fluconazole resistance



Acquired azole resistance

Multistep pathway to Fluconazole resistance

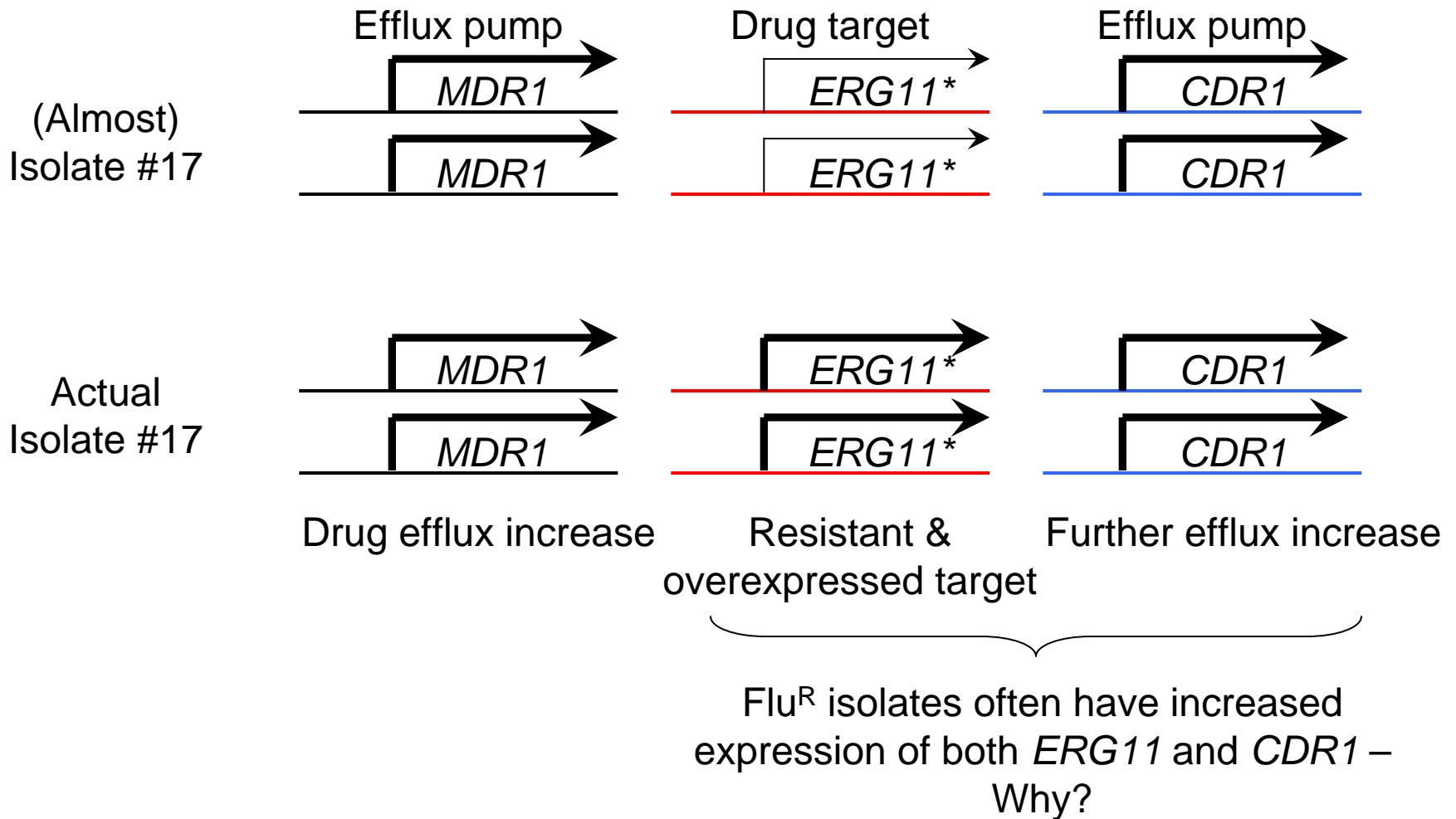


Acquired azole resistance

1. Azoles are static – Thus sterilization of infected tissue after azole treatment requires some clearance capability.
2. Resistance mechanisms are diverse and cumulative – Thus many different kinds of mutations can promote increased resistance; the static nature of azoles permits persistence of large microbial populations
3. An evolutionary accident (?) links diverse resistance genes

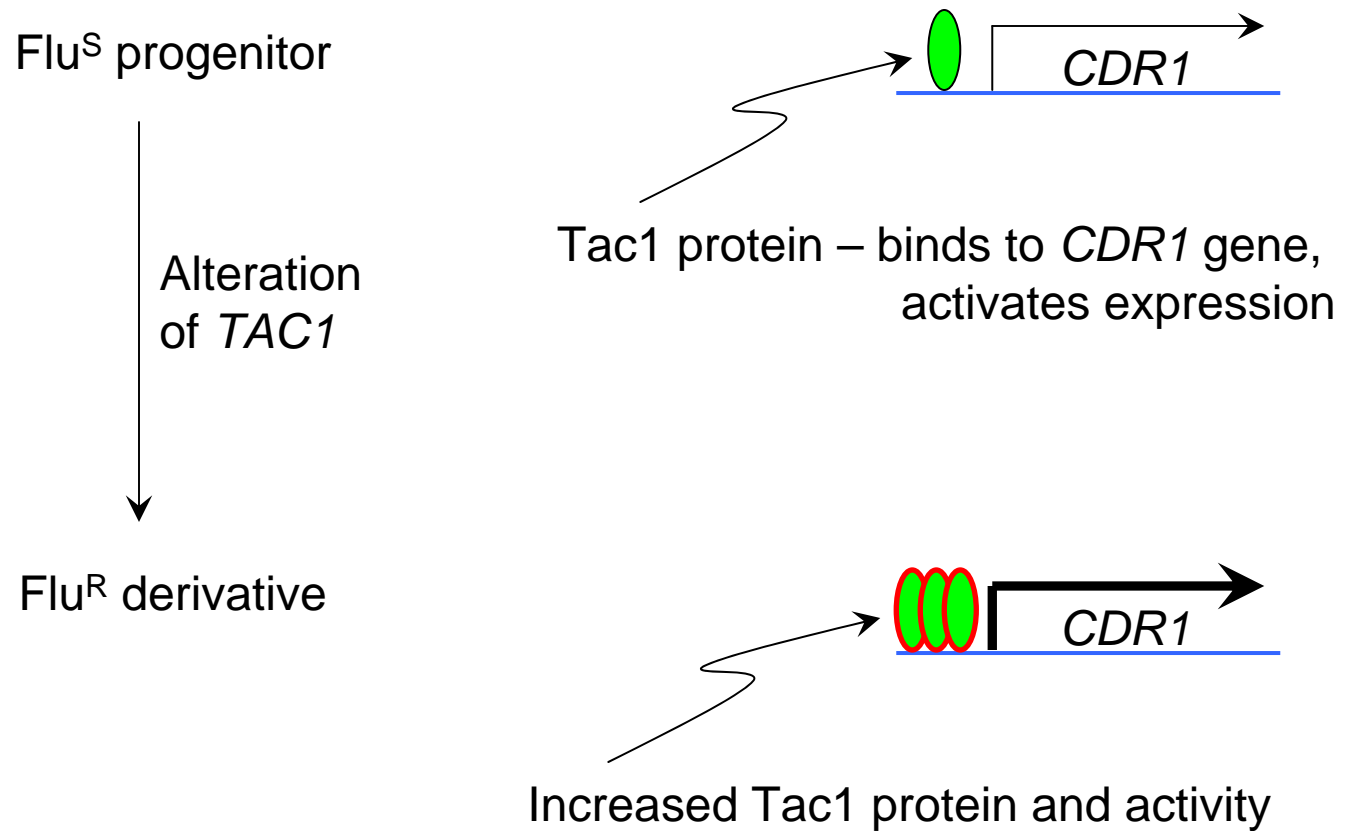
Acquired azole resistance

3. An evolutionary accident (?) links diverse resistance genes



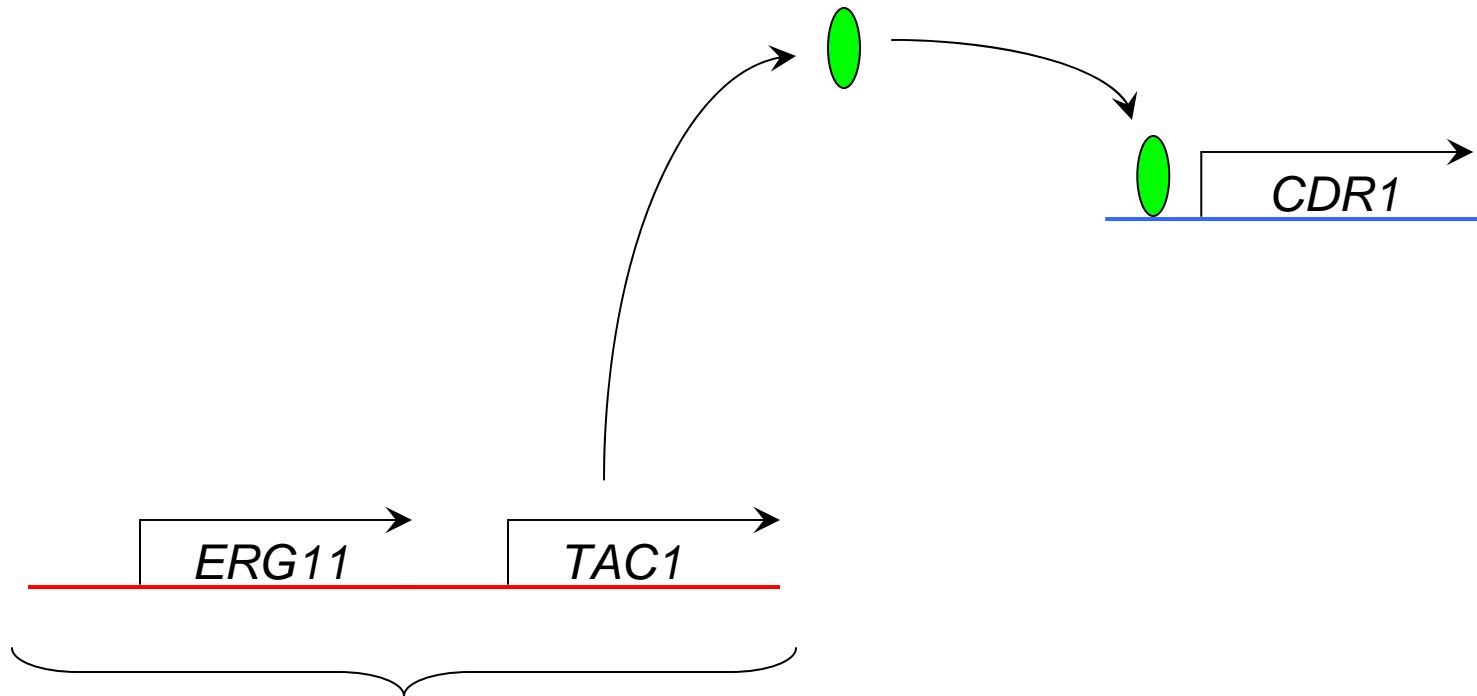
Acquired azole resistance

Mechanism of *CDR1* overexpression



Acquired azole resistance

Mechanism of *CDR1* overexpression



The regulator of *CDR1* is on the same chromosome as the target of fluconazole.
Hmmm.

Acquired azole resistance

Basis for increased *ERG11* and *TAC1* expression

Method: Competitive Genome Hybridization (CGH)

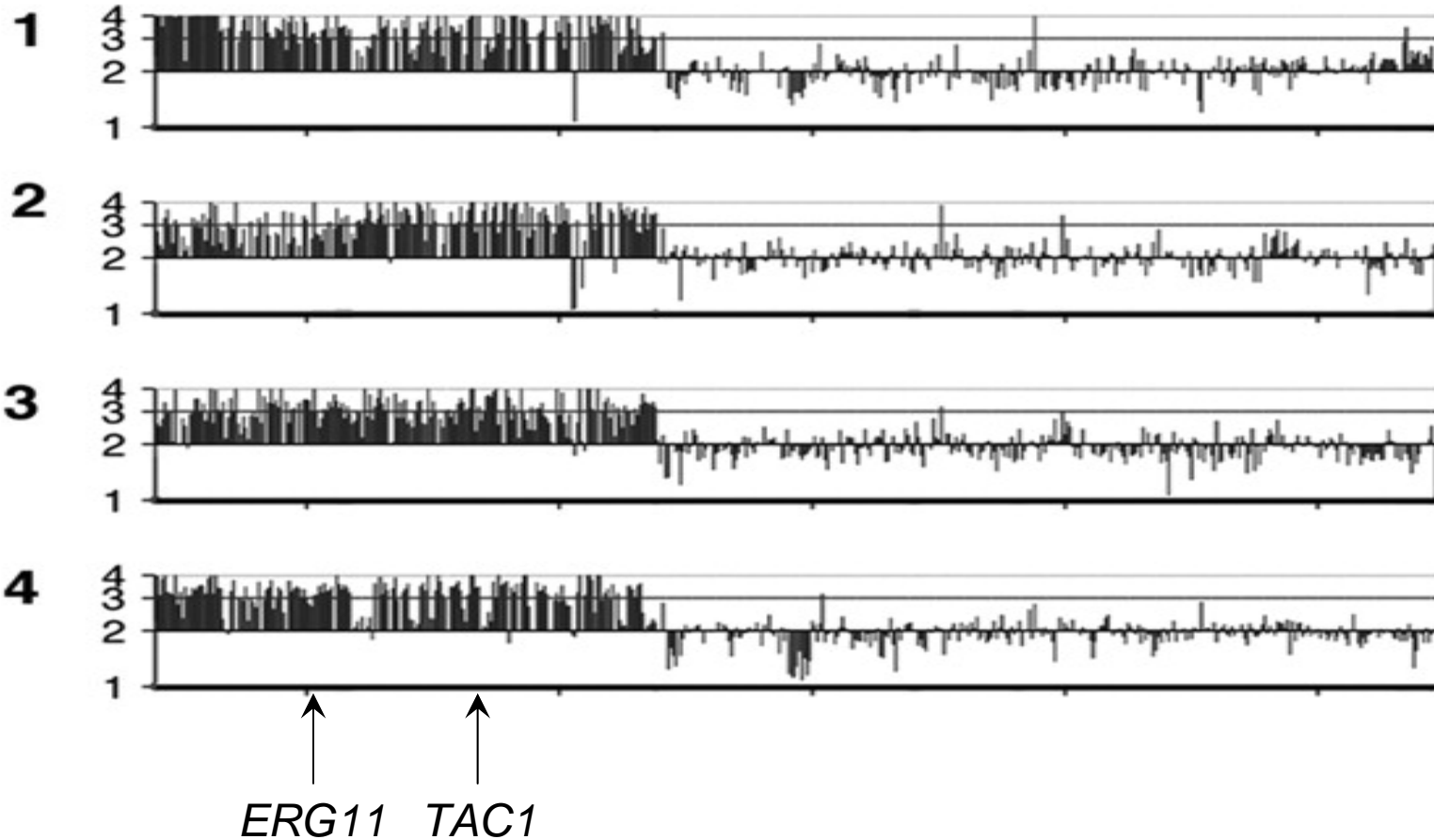
- Allows precise comparison of gene copy numbers
- (Has revealed much unanticipated human-to-human variation in gene copy number, not just gene sequence)

Acquired azole resistance

CGH of Fluconazole resistant isolates vs. index strain

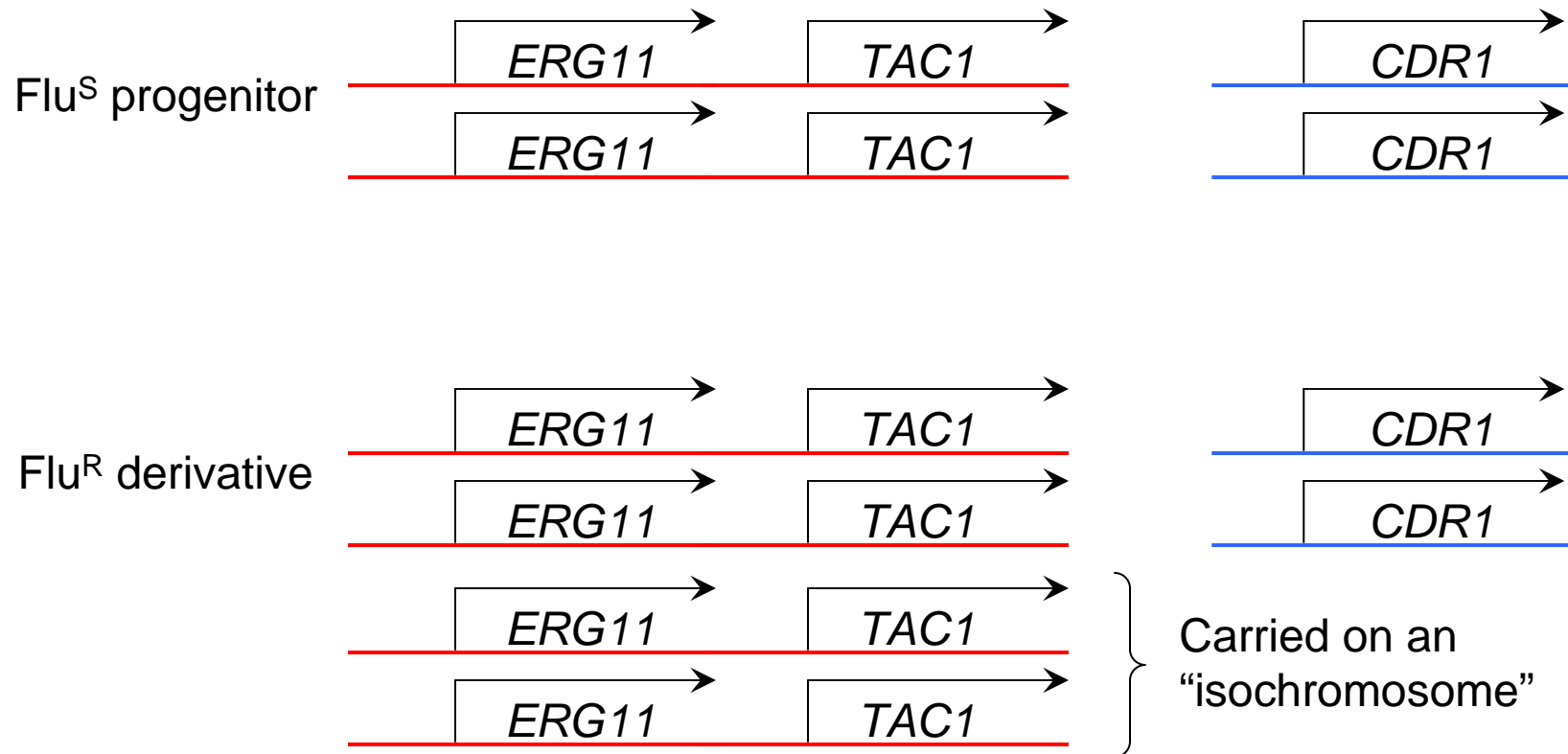
These signals reflect a consistent increase; copy number 2:1

These signals reflect typical noise; copy number 1:1



Acquired azole resistance

Basis for increased expression of both *ERG11* and *TAC1*



Acquired azole resistance

1. Azoles are static – Thus sterilization of infected tissue after azole treatment requires some clearance capability.
2. Resistance mechanisms are diverse and cumulative – Thus many different kinds of mutations can promote increased resistance; the static nature of azoles permits persistence of large microbial populations
3. An evolutionary accident (?) links diverse resistance genes – Thus a single (albeit unanticipated) event promotes dual modes of resistance.

Either way, we better start shoveling!

Azole resistance mechanisms - Summary

Types of resistance problems:

1. Intrinsic – We see increased frequency of infection by intrinsically resistant species
2. Epigenetic – Basis is complex; consequence is difficulty in treating device-associated infections
3. Acquired – Diverse mechanisms have cumulative effects; arises through familiar and unfamiliar genetic mechanisms.