



8th Advances Against Aspergillosis

Lisboa Congress Centre, Lisbon, Portugal

Mechanisms of non-CYP51 azole resistance

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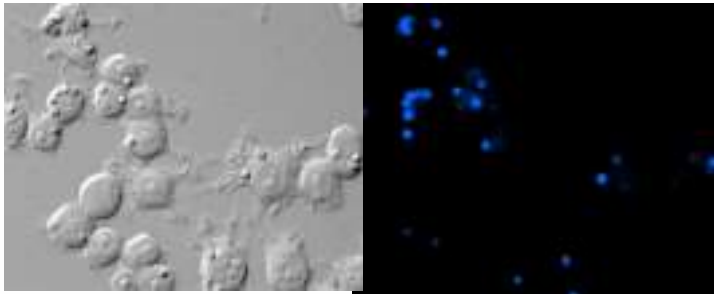
Current Research Interests

Part I: Morphological development in *A. nidulans*

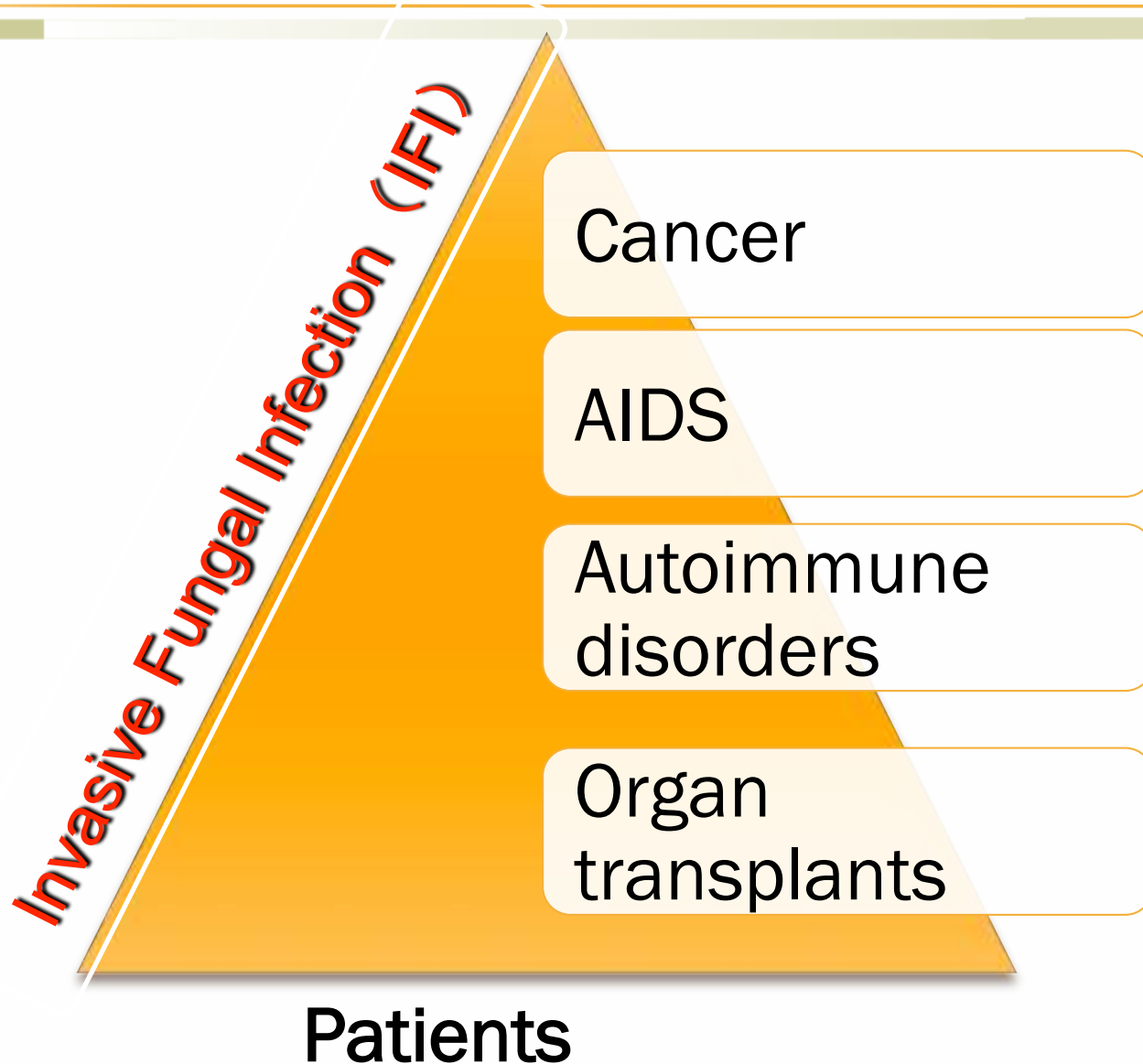
Part II: Drug-resistance and virulence of *A. fumigatus*

Macrophages

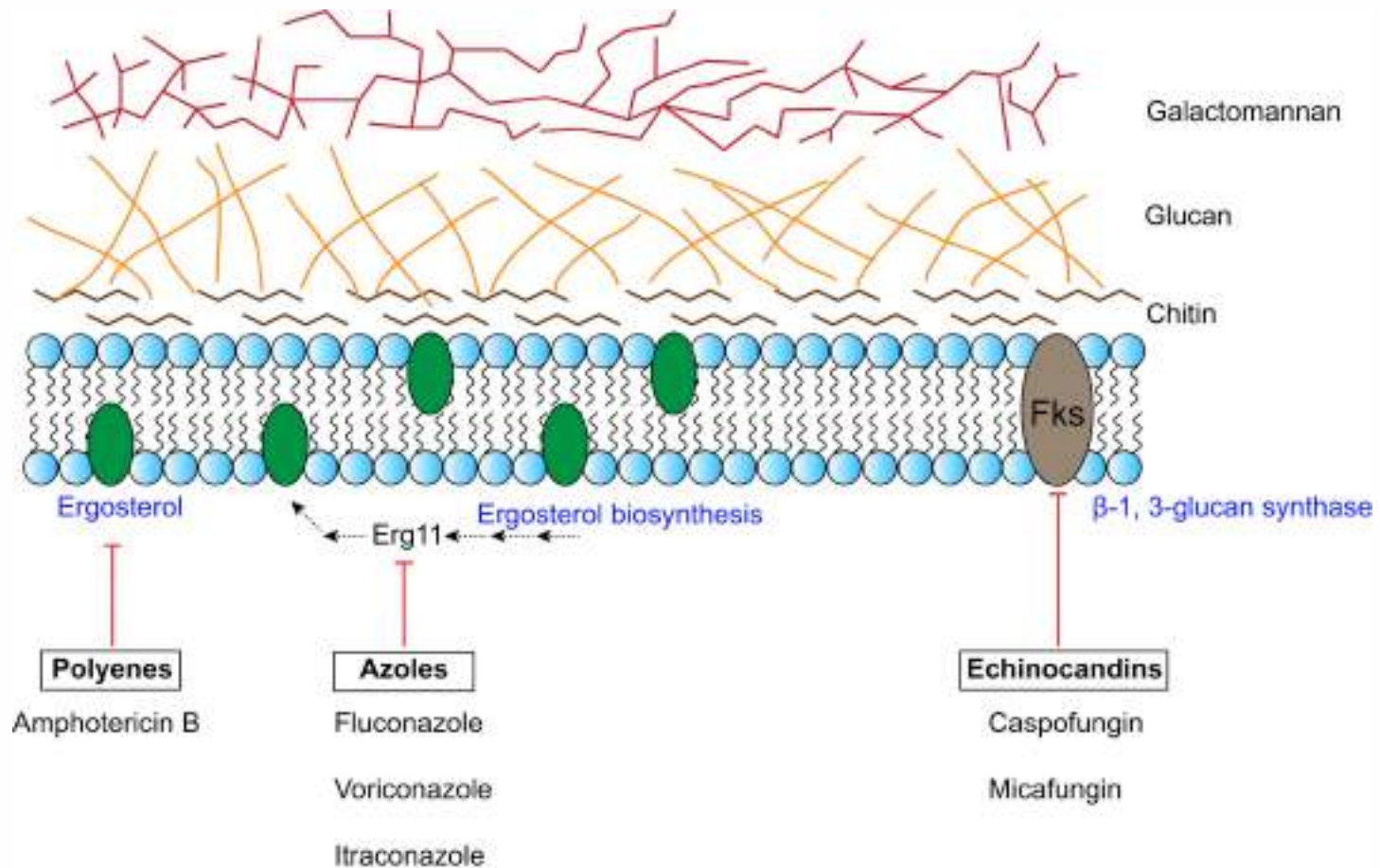
Animal Models



Fungal diseases with high mortality and morbidity



There are currently three classes of antifungal agents



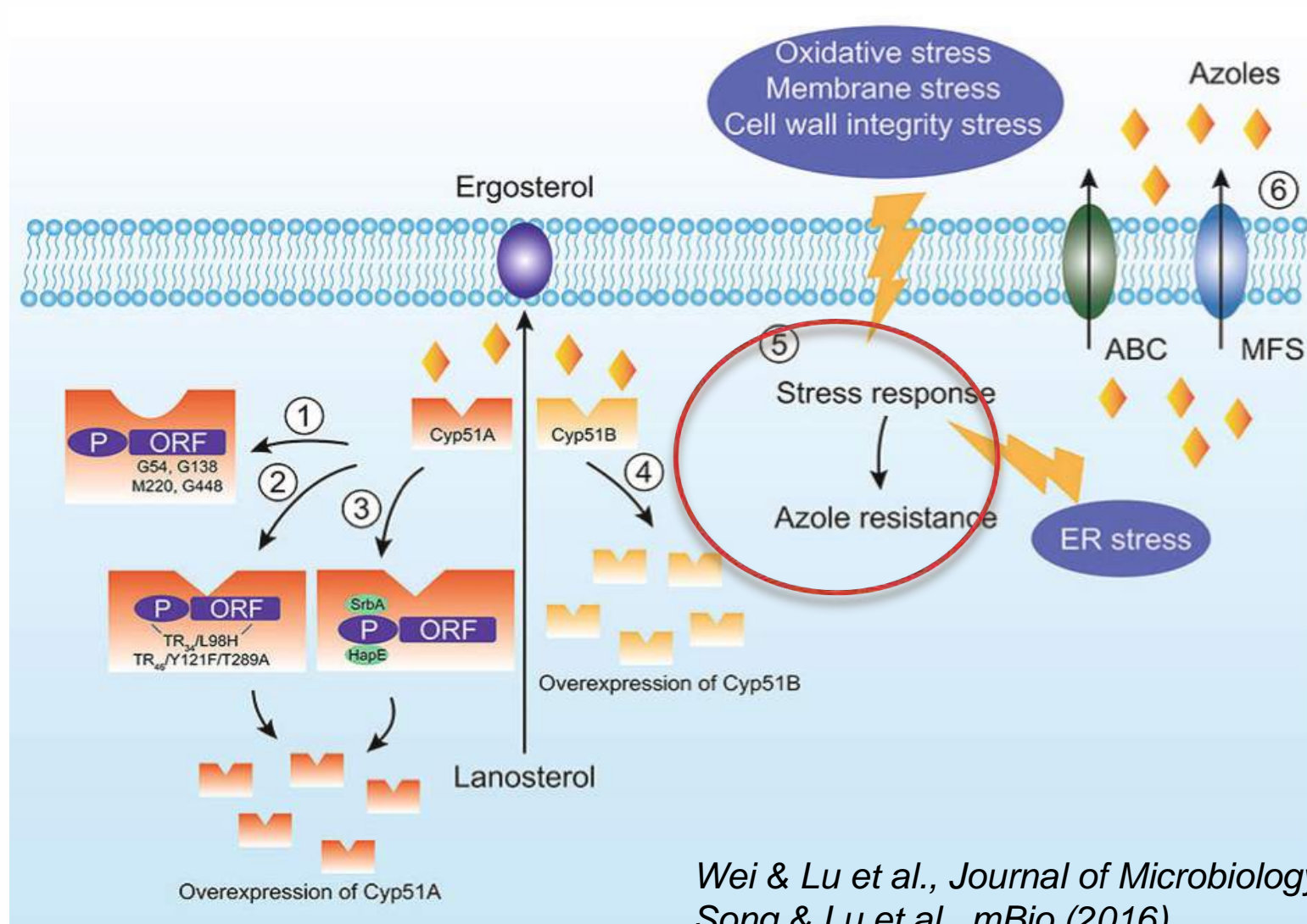
Amphotericin B is severe **toxicity**

Azoles are safer, though emergence of **resistance**

Treatment strategies to combat these infections and resistance

- **Verify drug-resistance mechanism in fungal pathogens**
- **Screening the antifungal agents with a novel mode of action and new drug targets**

Azole resistance mechanisms: Changes of the drug target Cyp51; Activation of the drug efflux pump; Induction of cellular stress responses.

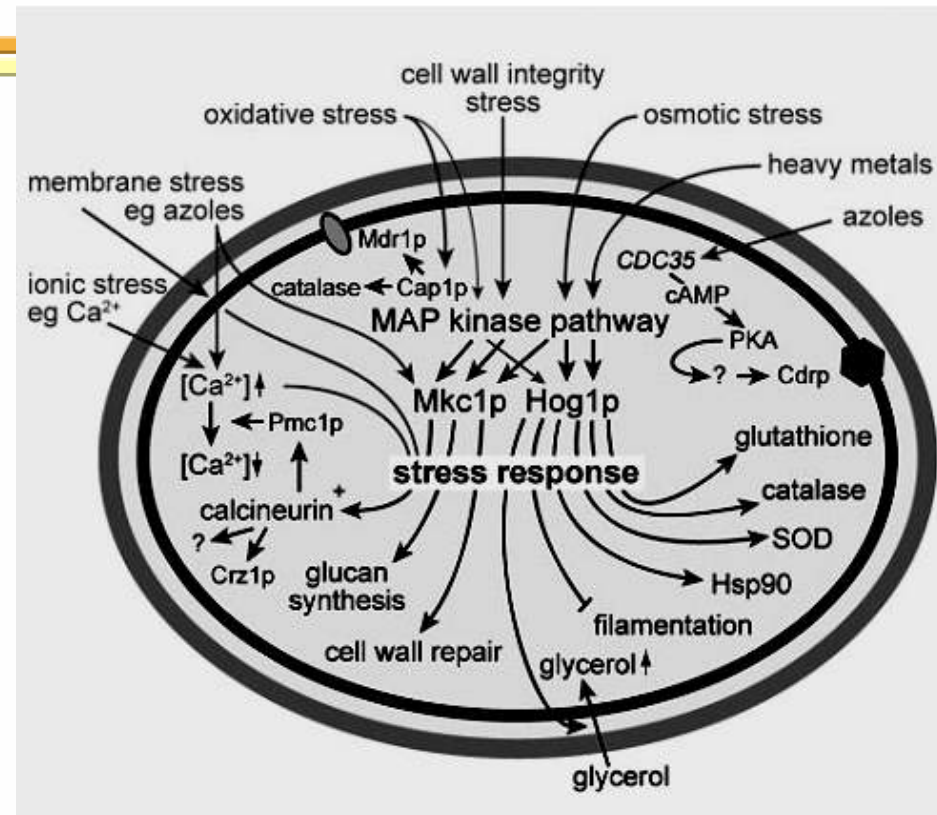
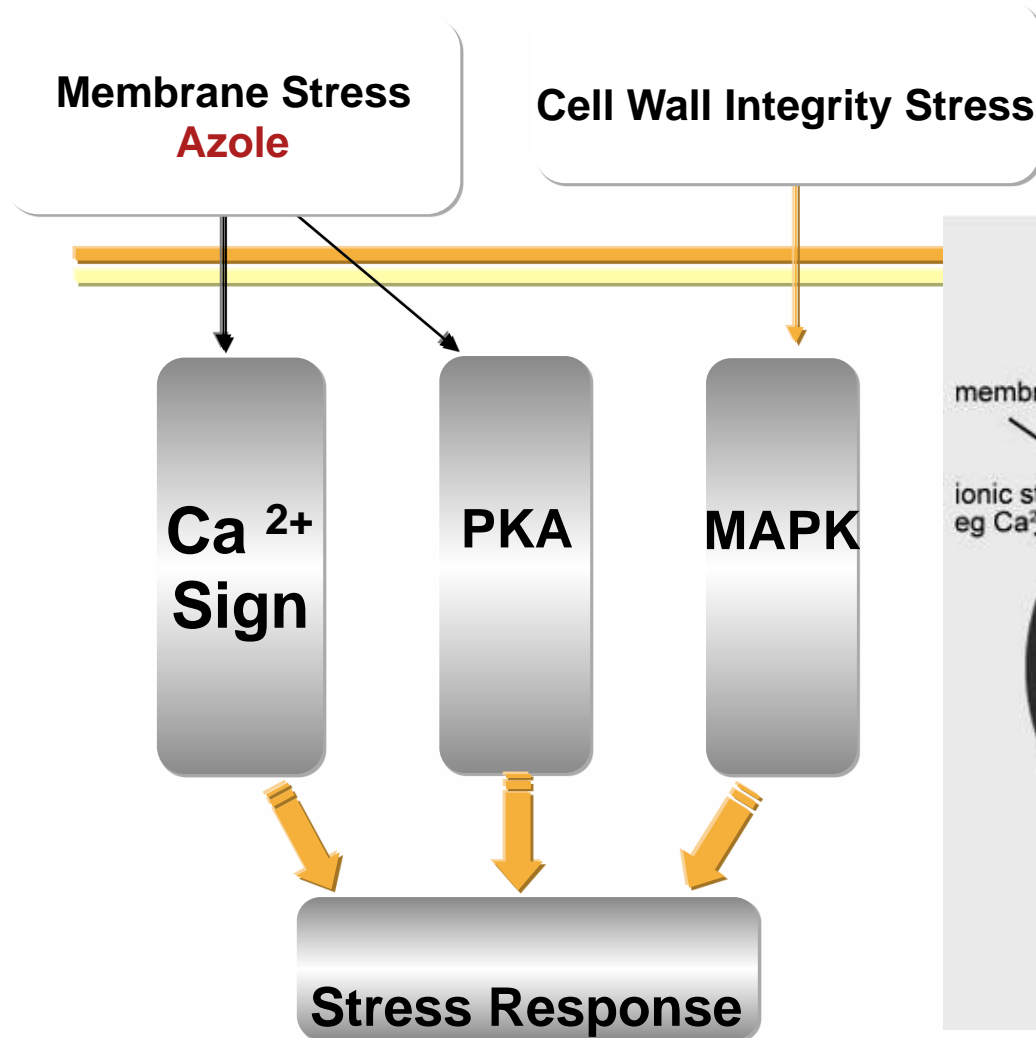


Wei & Lu et al., *Journal of Microbiology* (2015)
Song & Lu et al., *mBio* (2016)
Long & Lu et al., *AEM* (2017)

Questions

- ✧ **What signal transduction pathways are critical for fungi to survive under antifungal drug stress induced- conditions?**
- ✧ **How the stress-response pathway is involved in drug resistance?**

The calcium signaling pathway is required for the long-term survival of cells in stress conditions in model yeasts



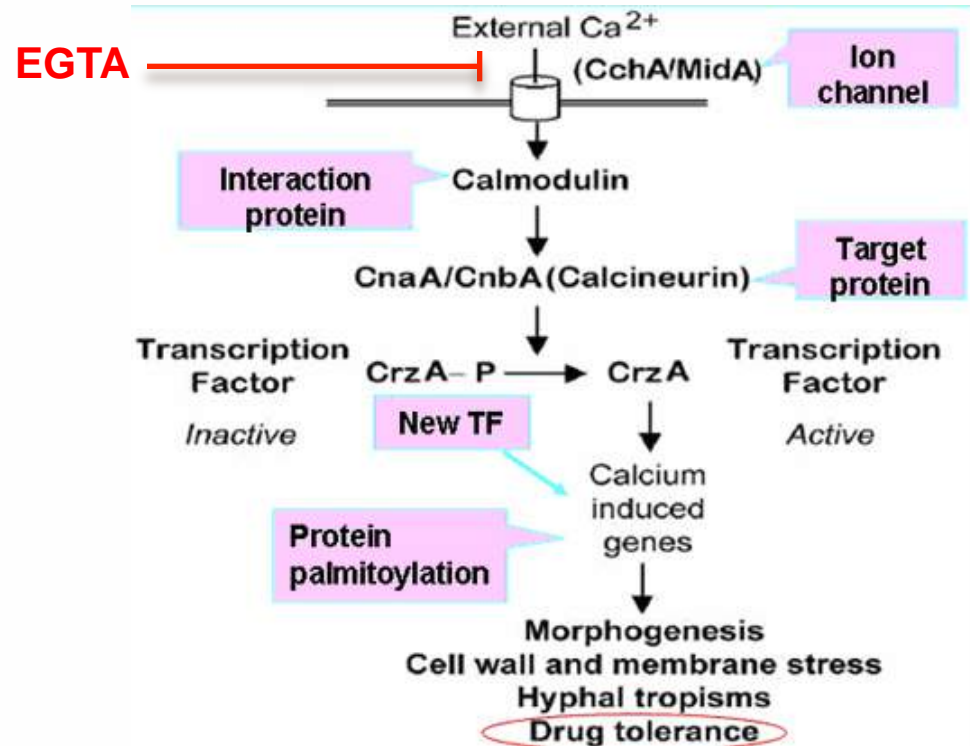
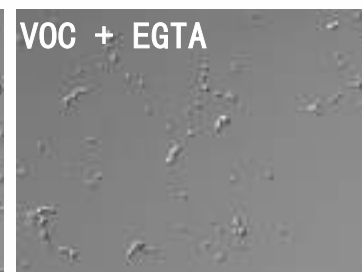
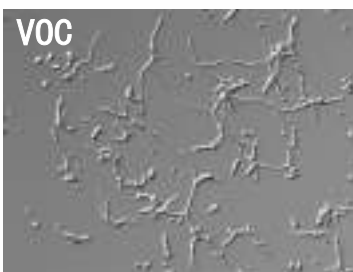
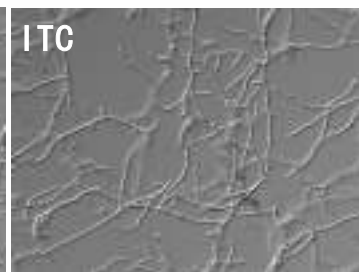
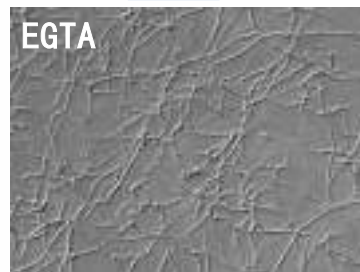
The calcium signaling pathway is required for azole tolerance in *A. fumigatus*



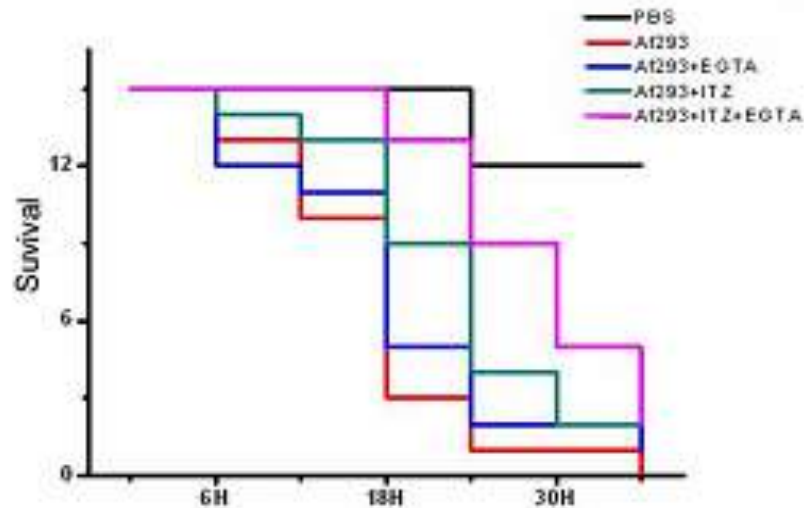
ITC



ITC+EGTA

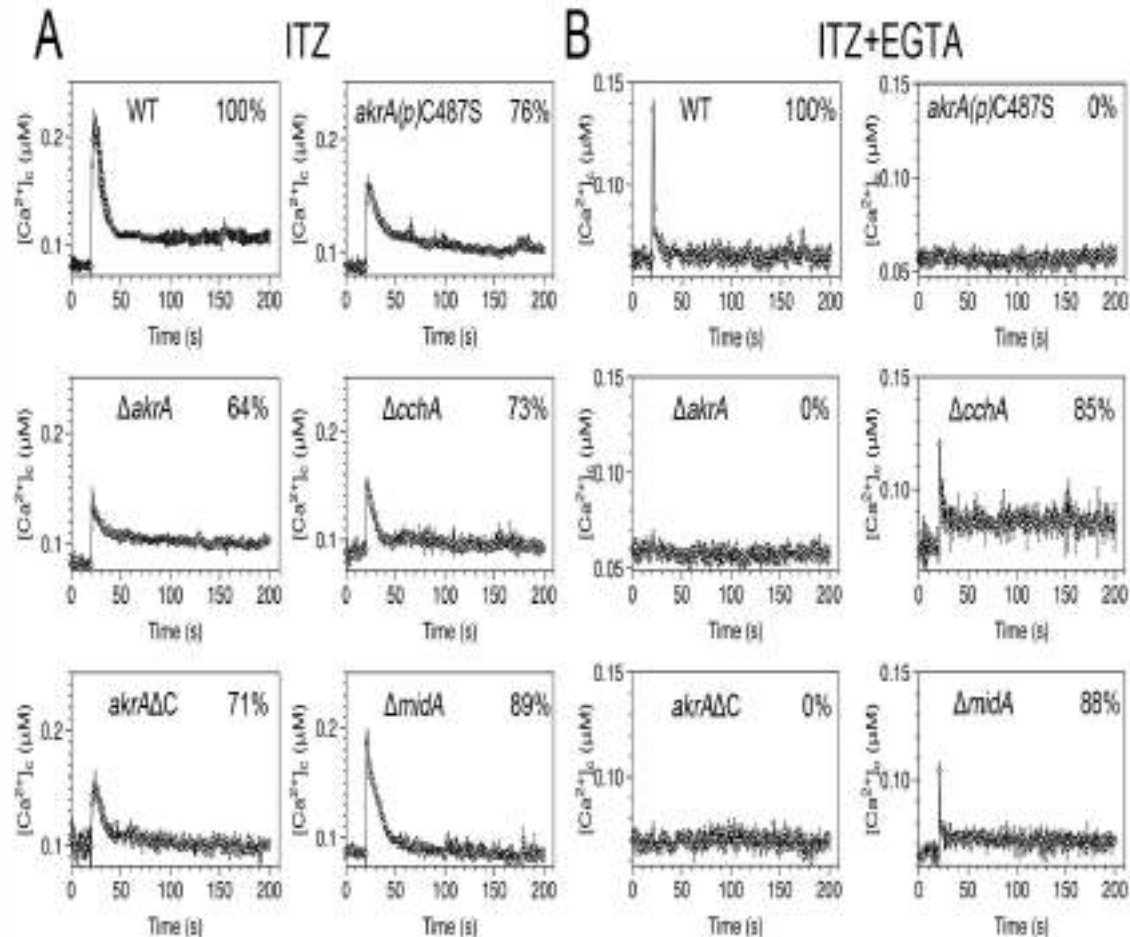
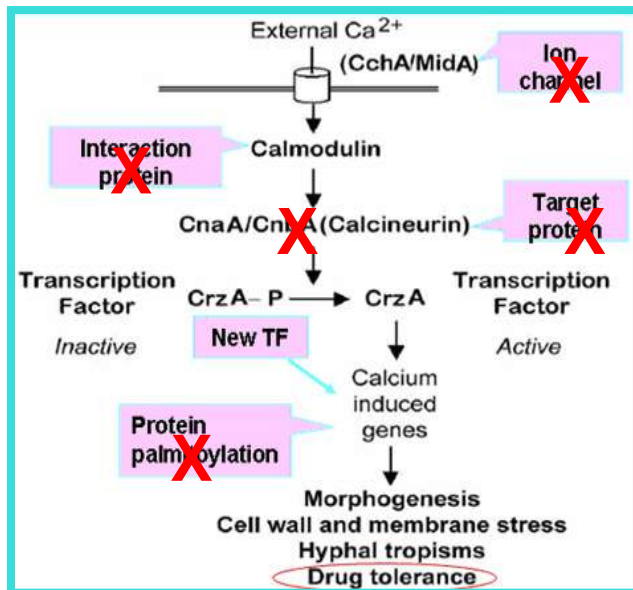
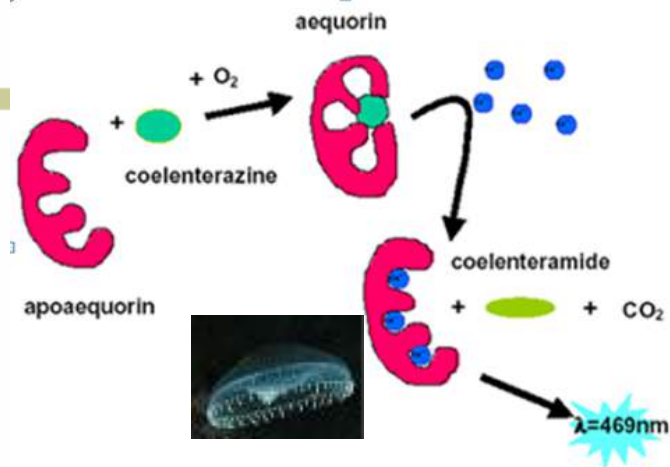


The calcium signaling pathway is required for azole tolerance in *A. fumigatus*

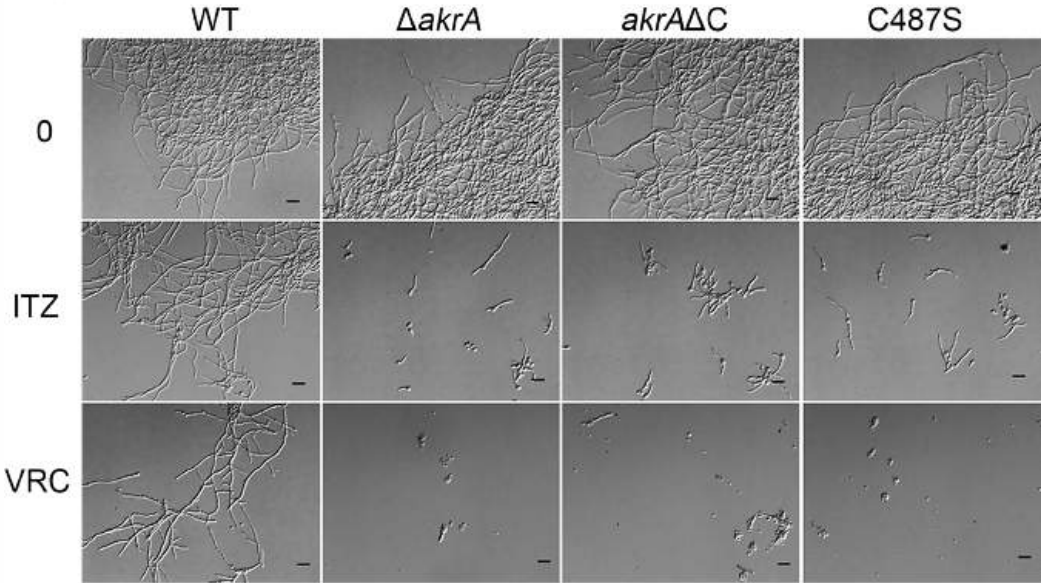
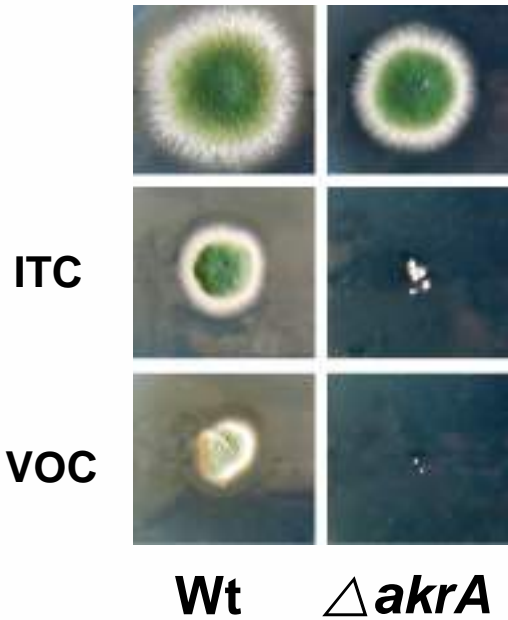
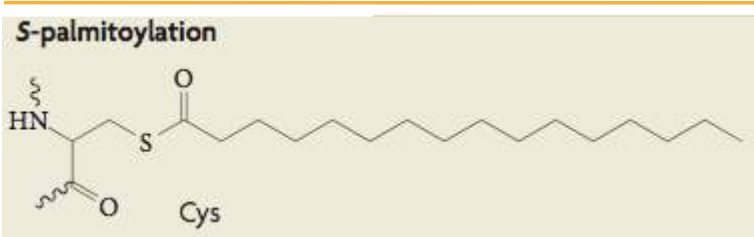
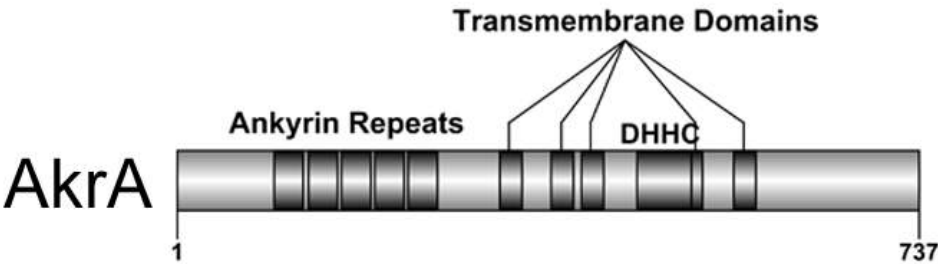


Liu & Lu et al., *Fungal Genetics & Biology*, 2015, 81, 182-90

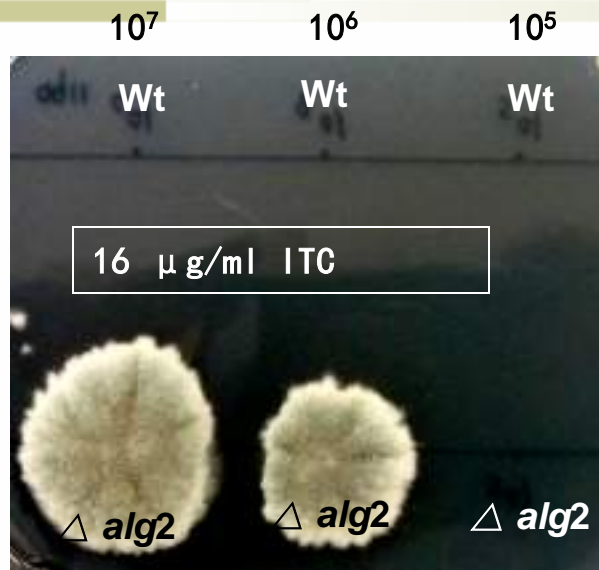
Calcium signaling components involved- $[Ca^{2+}]_c$ transient is induced by antifungal azole treatment



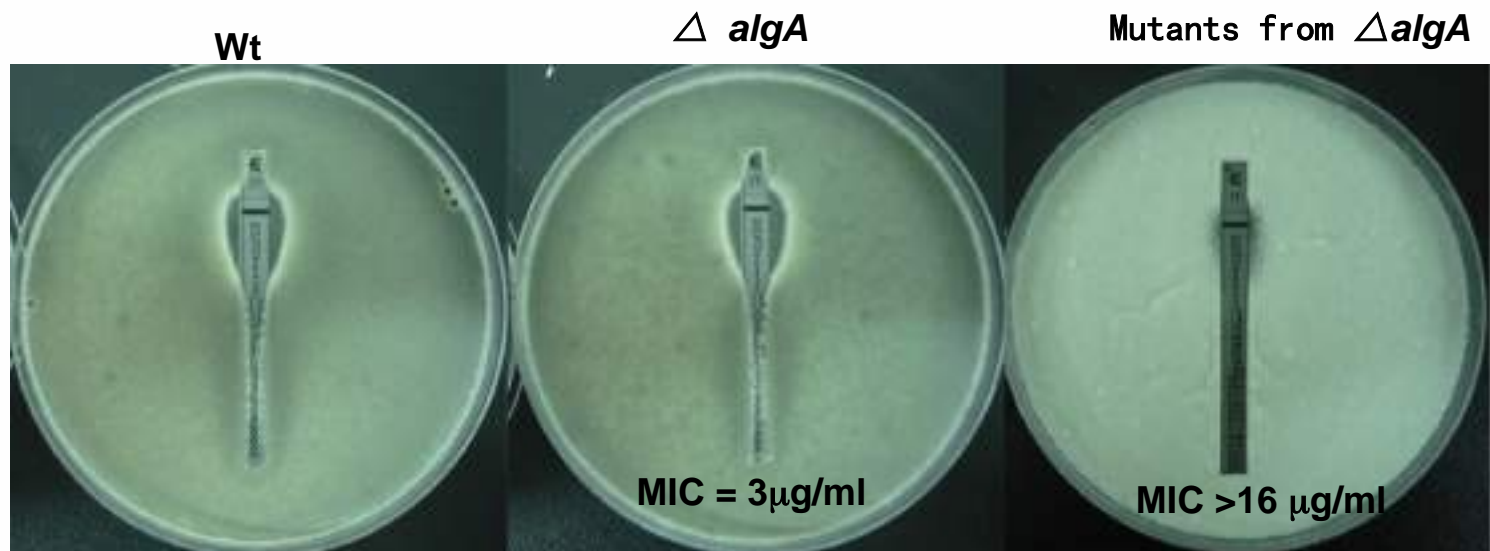
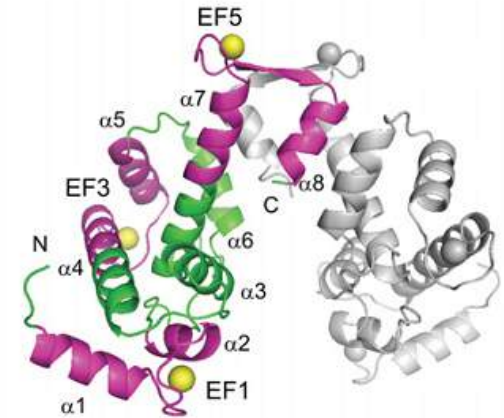
The palmitoyl transferase AkrA, a member of the high-affinity calcium uptake system, contributes to [Ca²⁺]_i transient and is required azole tolerance



Loss of a component in calcium signaling pathway-AlgA causes an increased frequency of azole-resistant isolates

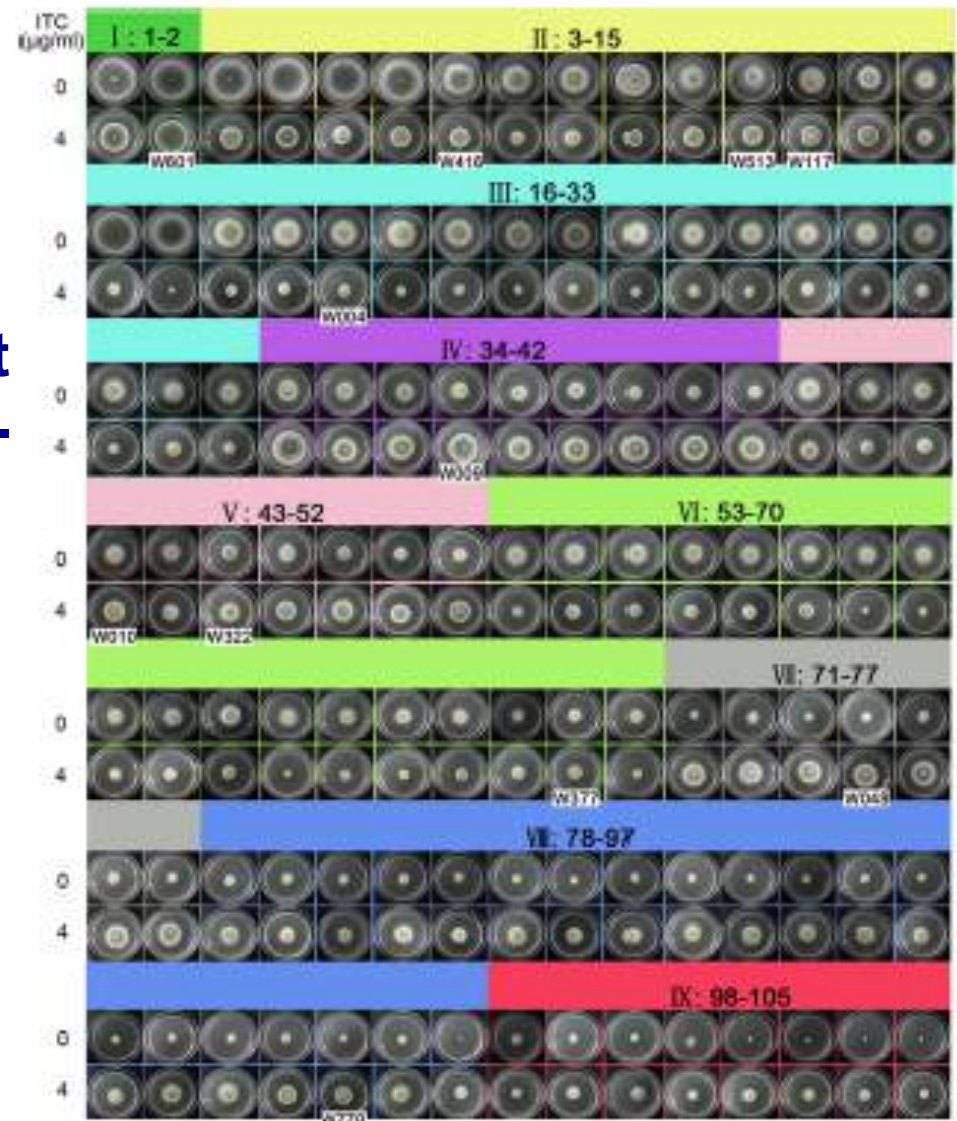


AlgA



Many unexplored non-*cyp51A* mutations that contribute to azole resistance in *A. fumigatus*

104/105 isolated resistant colonies retain the wild-type *cyp51A* gene.



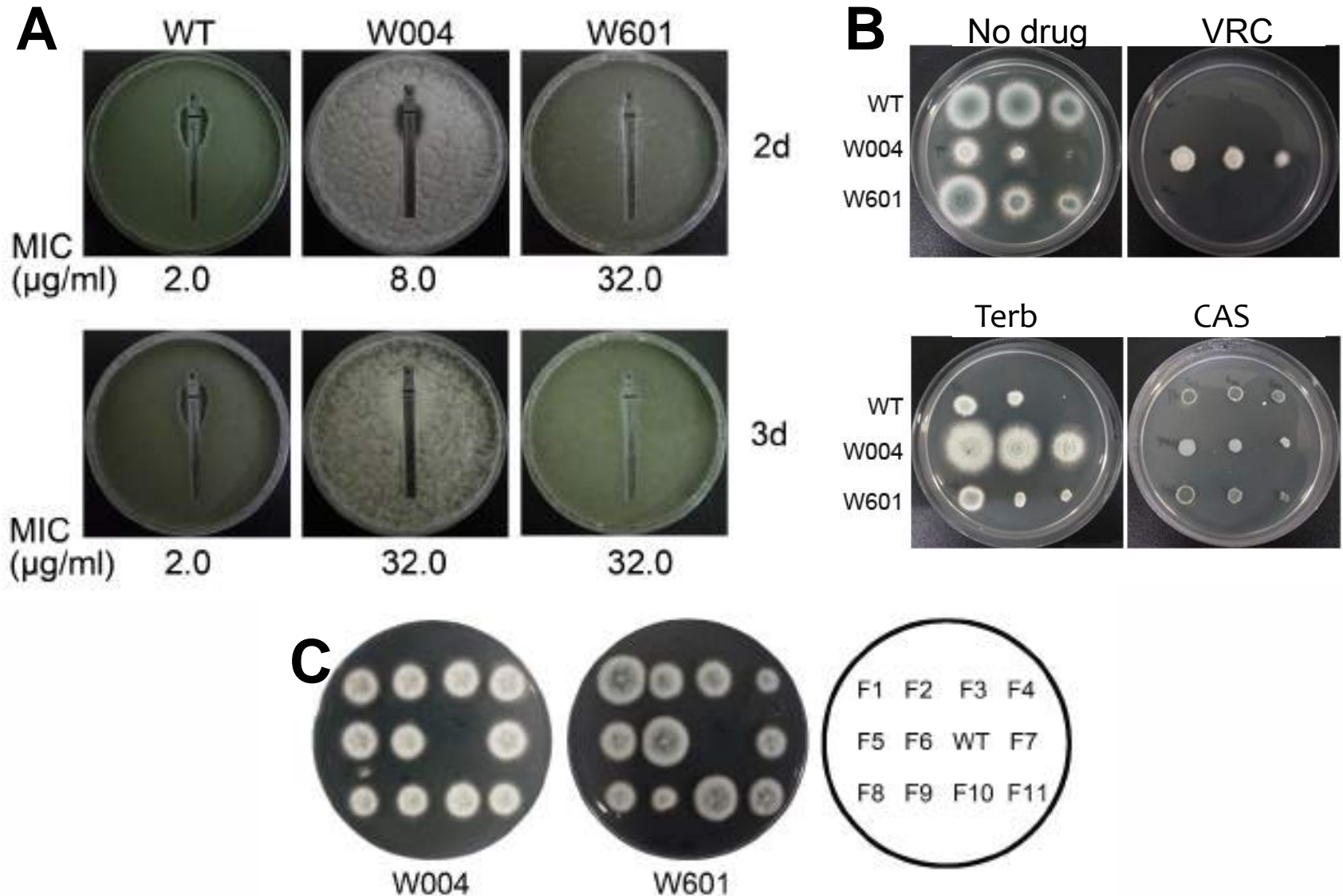
Evolved lineages (isolates) may acquire azole resistance independently

TABLE 1 MICs or MECs of itraconazole, voriconazole, terbinafine, and caspofungin for selected isolates^a

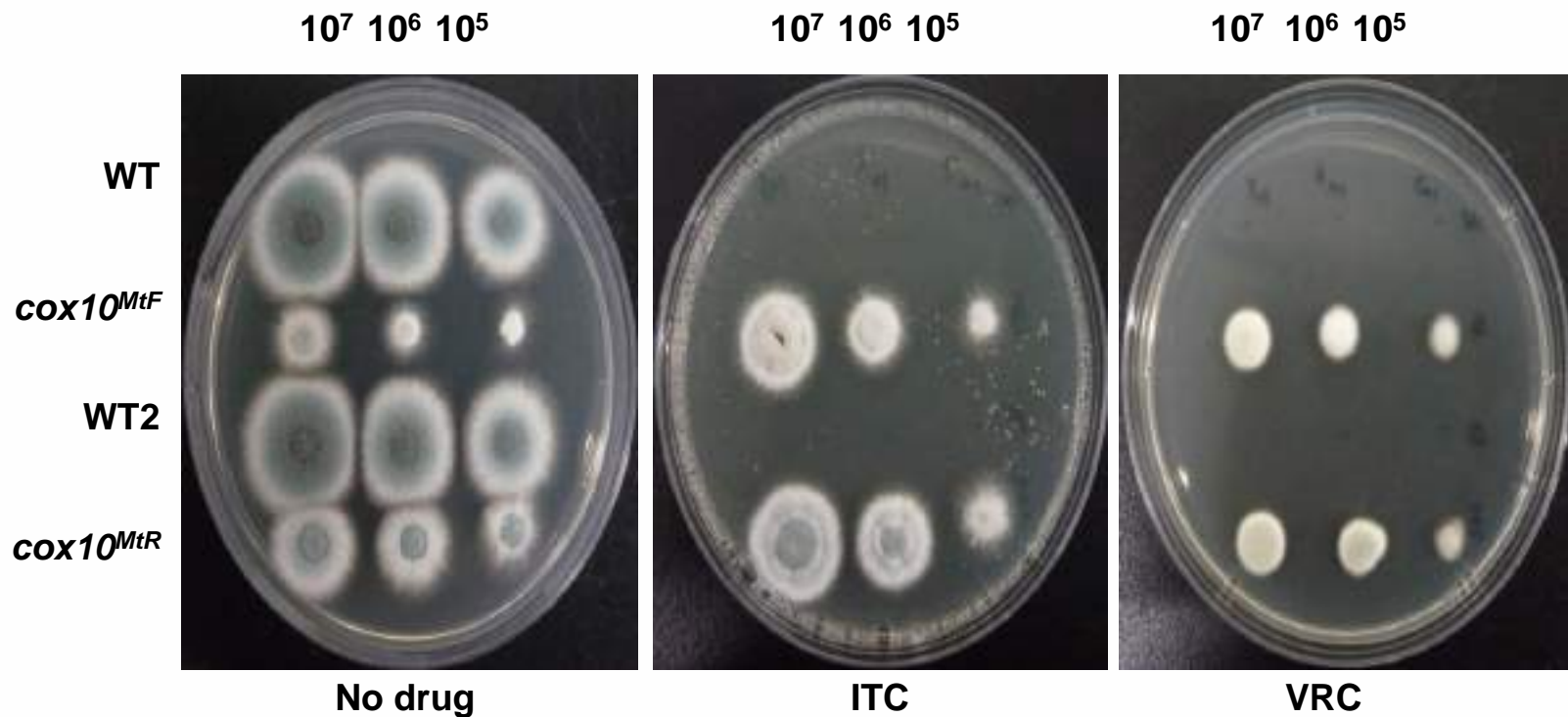
Strain	MIC or MEC ($\mu\text{g/ml}$)			
	ITC	VRC	Terb	CAS
A1160	1	0.5	8	0.25
W004	8	2	16	0.25
W009	8	2	16	0.06
W010	4	1	>16	>4
W048	8	2	>16	>4
W117	8	1	8	0.25
W322	4	1	16	0.06
W377	16	2	8	>4
W416	8	1	16	0.125
W513	16	1	16	0.125
W601	8	0.5	8	0.25
W770	4	1	16	0.25

^aITC, itraconazole; VRC, voriconazole; Terb, terbinafine; CAS, caspofungin.

Evolved lineages (isolates) may acquire azole resistance independently



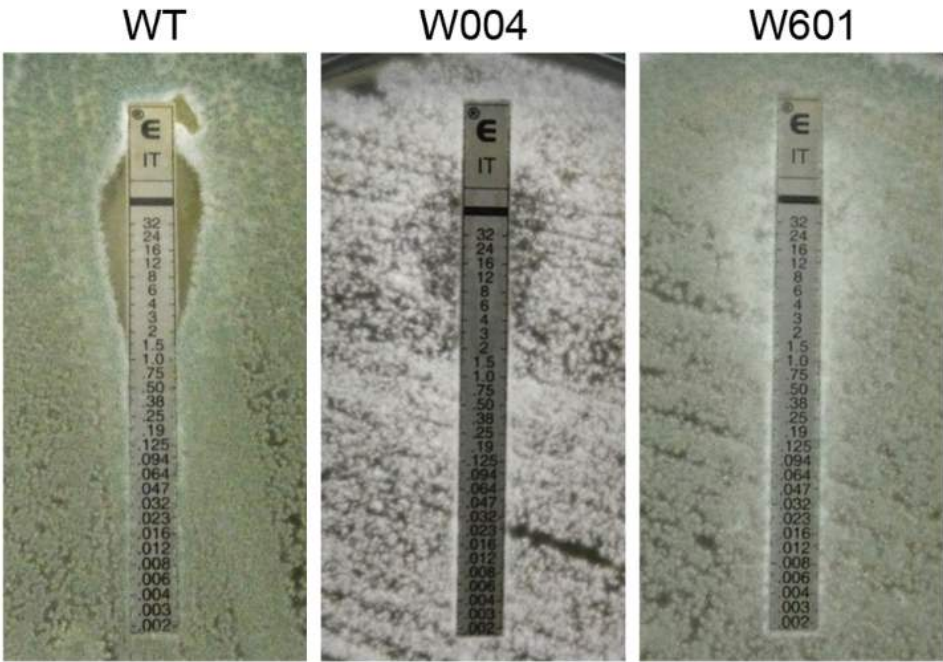
A new mutation (R243Q substitution) in W004 conferring azole resistance in the putative farnesyltransferase Cox10 (AFUB_065450)



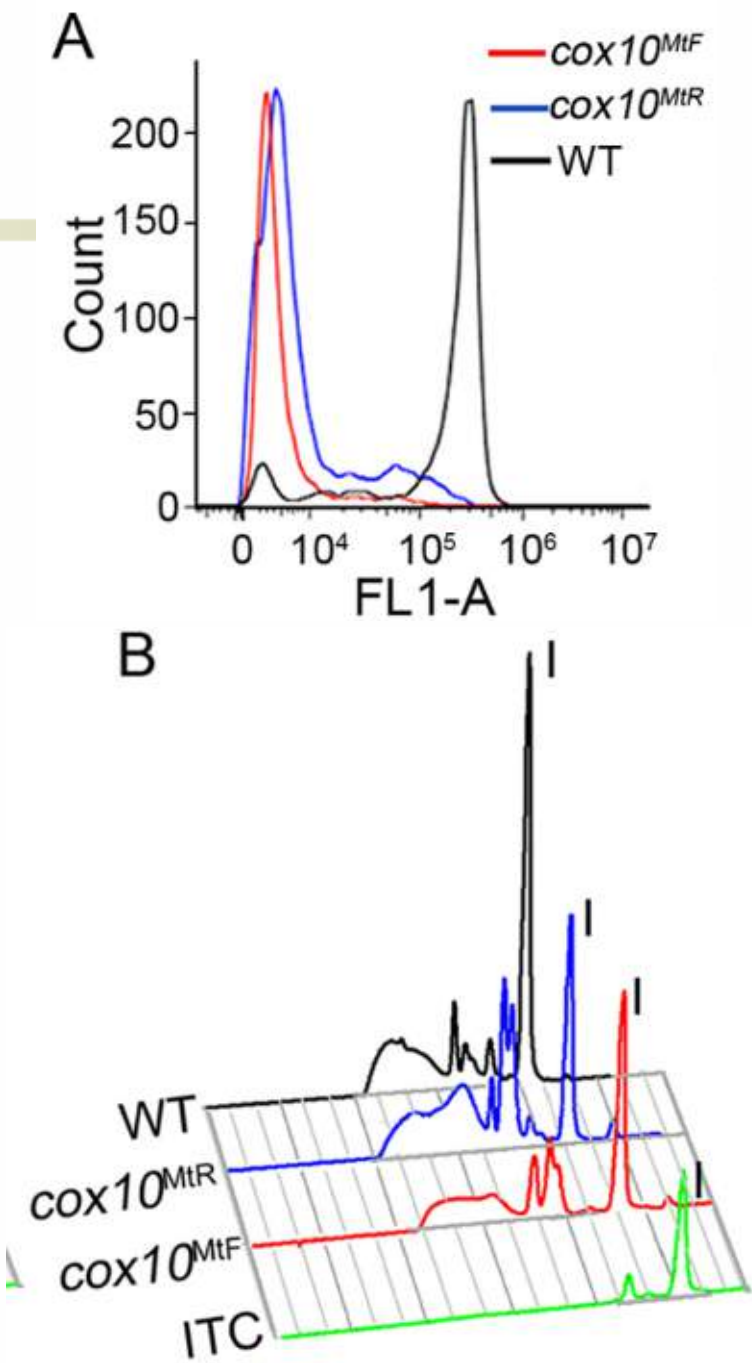


**What is the resistance mechanism in the putative
farnesyltransferase Cox10 mutants?**

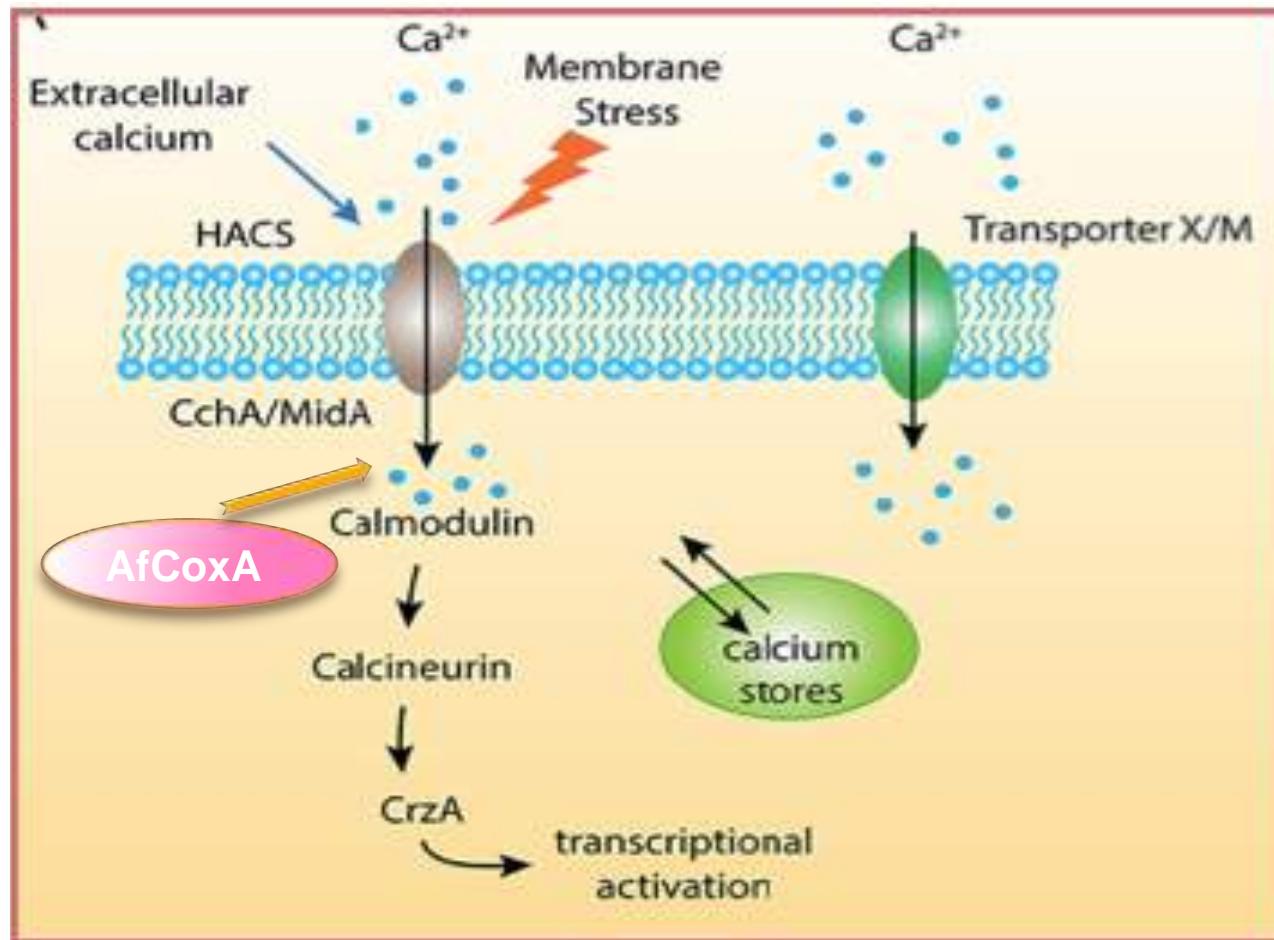
Reduced itraconazole contents in *Afcox10*



MIC (µg/ml)	WT	W004	W601
2 d	≈ 2	>32	>32



Cox10 defects cause persistent activation of calcium signaling



In Summary

- Induction of the cellular stress response confers azole tolerance or resistance
- The calcium signaling pathway is required for azole tolerance
- Loss of a component in calcium signaling pathway-AlgA causes an increased frequency of unexplored non-*cyp51A* mutations
- Farnesyltransferase *cox10* mutations are able to confer azole resistance

Finding Indications

- ◆ The calcium involved stress-response pathway could be used as new antifungal drug targets.
- ◆ $[Ca^{2+}]_c$ transient might be a useful biomarker to screen new antifungals for drug-resistance isolates.