RUTGERS
THE STATE UNIVERSITY OF NEW JERSEY

U.S. Wheat & Barley Scab Initiative

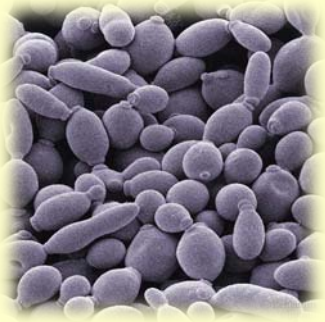
Trichothecene mycotoxins inhibit mitochondrial translation – Implications for the mechanism of toxicity

Anwar Bin-Umer
Tumer Laboratory

SCAB USA 2011 Meeting
December 5th, 2011

Rationale

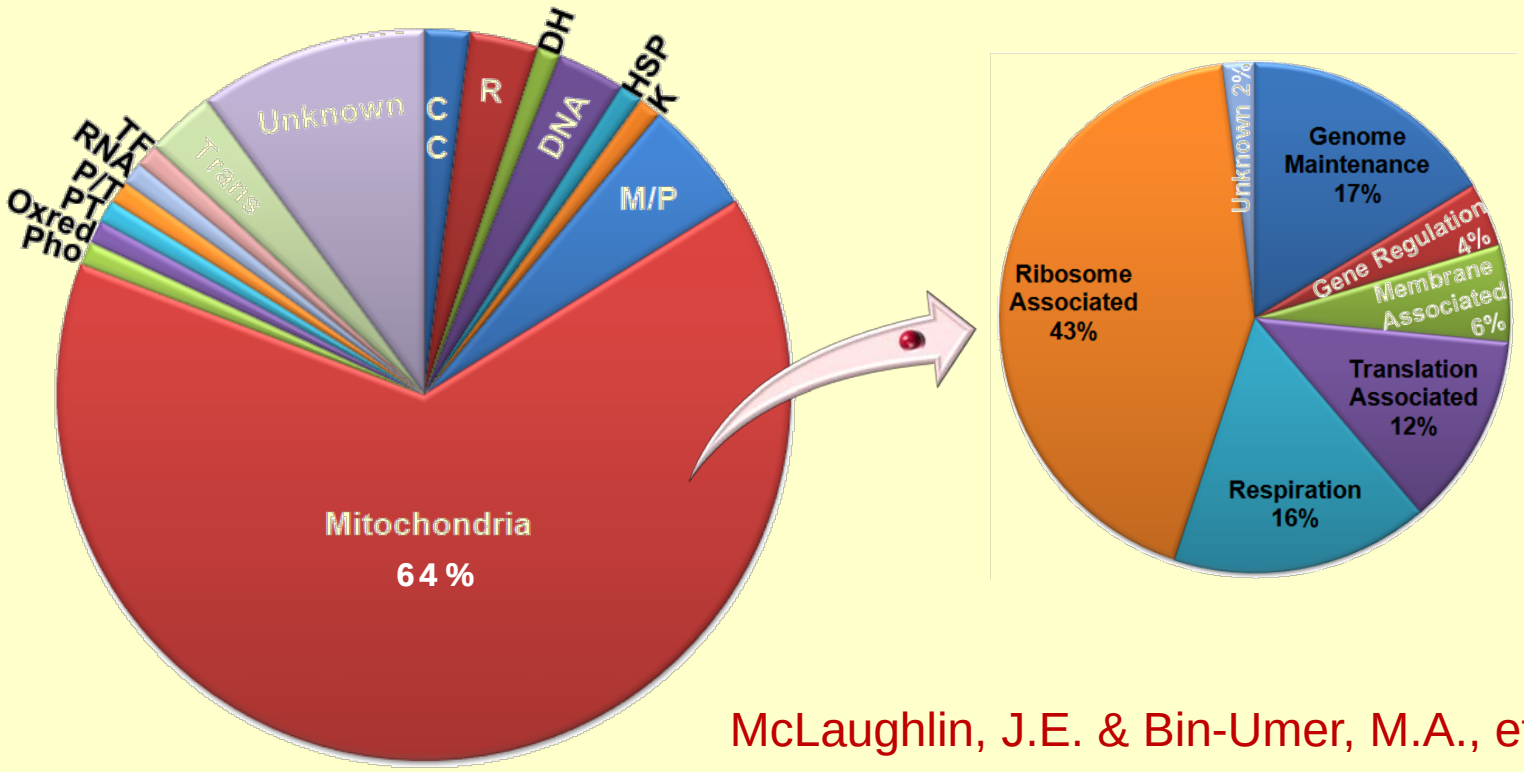
- Trichothecenes contribute to the virulence of the pathogen.
- Toxicity is not simply due to cytosolic translation inhibition.
  - Mechanism of action is not yet fully understood.
- Identify molecular mechanisms underlying trichothecene toxicity → Identify genes conferring resistance to *Fusarium*-related diseases.
- "Model" the mechanism of toxicity and identify key players in it using a genome wide approach in yeast.
- Identify targets in *Arabidopsis thaliana* and then in wheat & barley.



Gene deletions affecting mitochondrial function is the largest group resistant to Tcin

Yeast non-essential Gene deletion library (~5000 mutants) → **Mutants resistant to Tcin (138)** → **Deletions affecting mitochondrial functions (88)**

- 43% associated with **mitochondrial translation**.
- Mitochondrial morphology related mutants were all defective in **membrane fusion**.



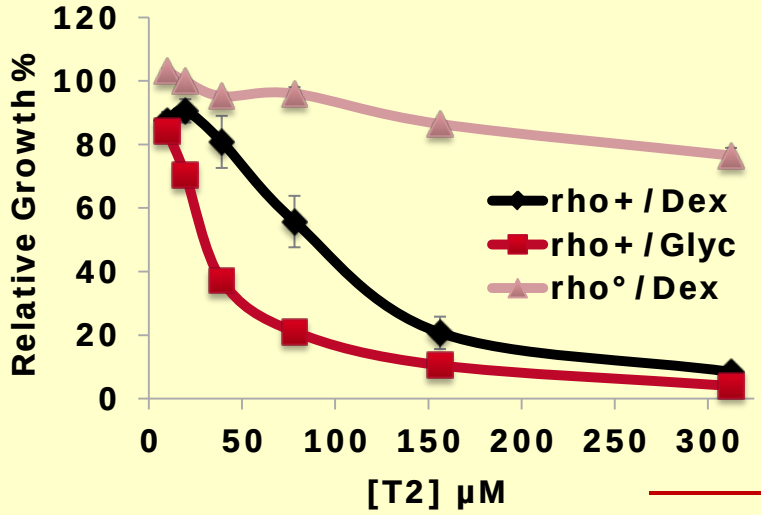
McLaughlin, J.E. & Bin-Umer, M.A., et al. PNAS 2009

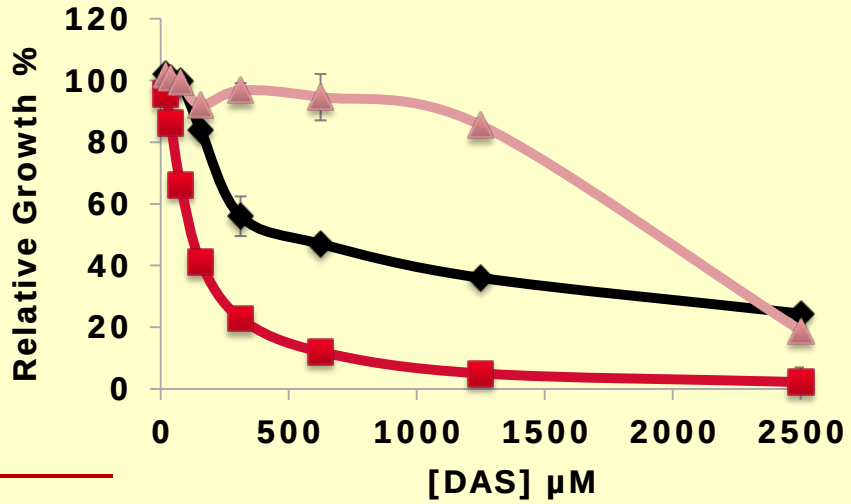
How critical is mitochondria in trichothecene toxicity?

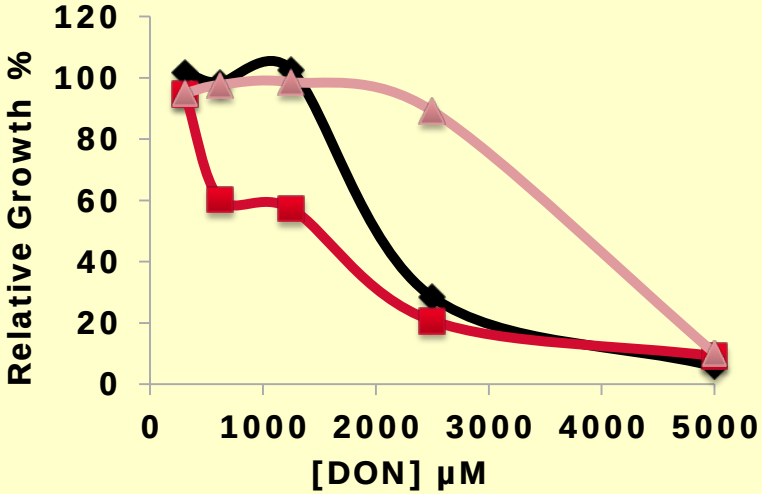
- 64% of the gene deletions conferring resistance to Tcin were associated with mitochondria.

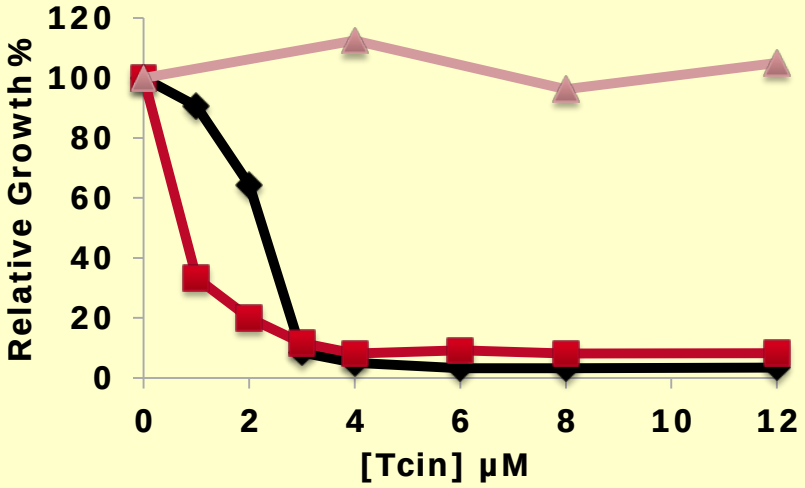
Functional mitochondria are necessary for survival when energy source is *non fermentable* glycerol (Glyc)

Mitochondria are critical for trichothecene toxicity



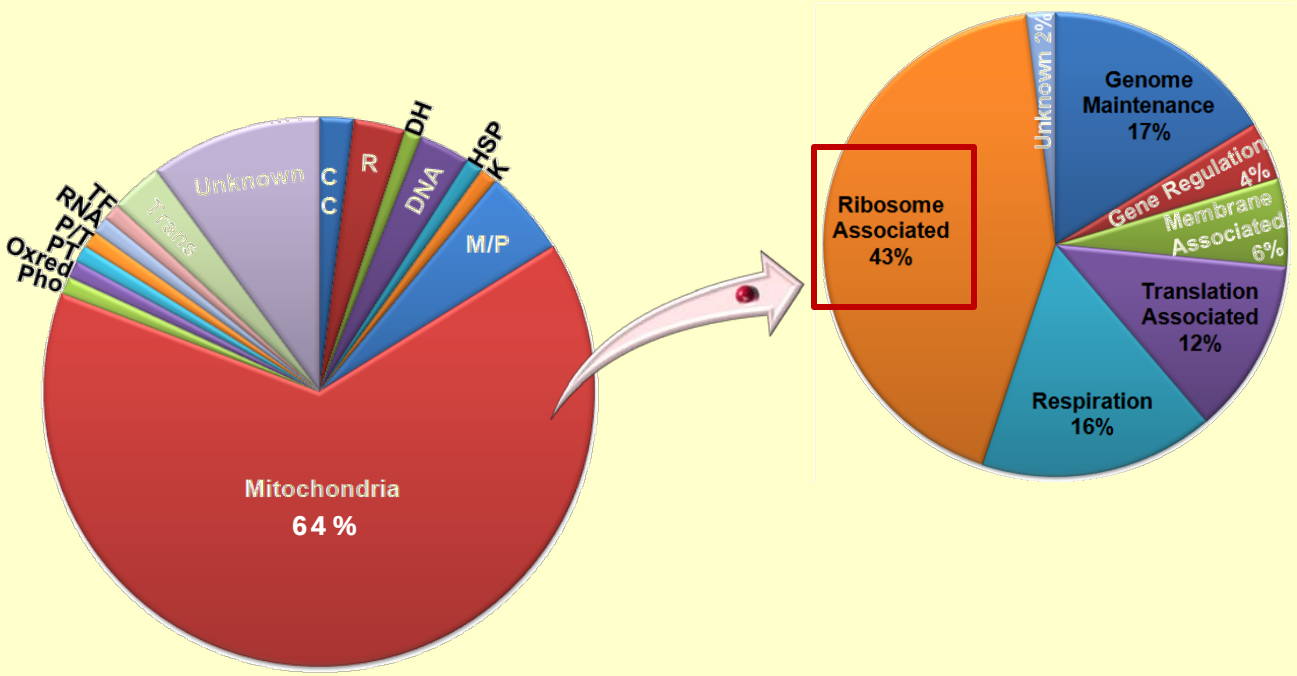






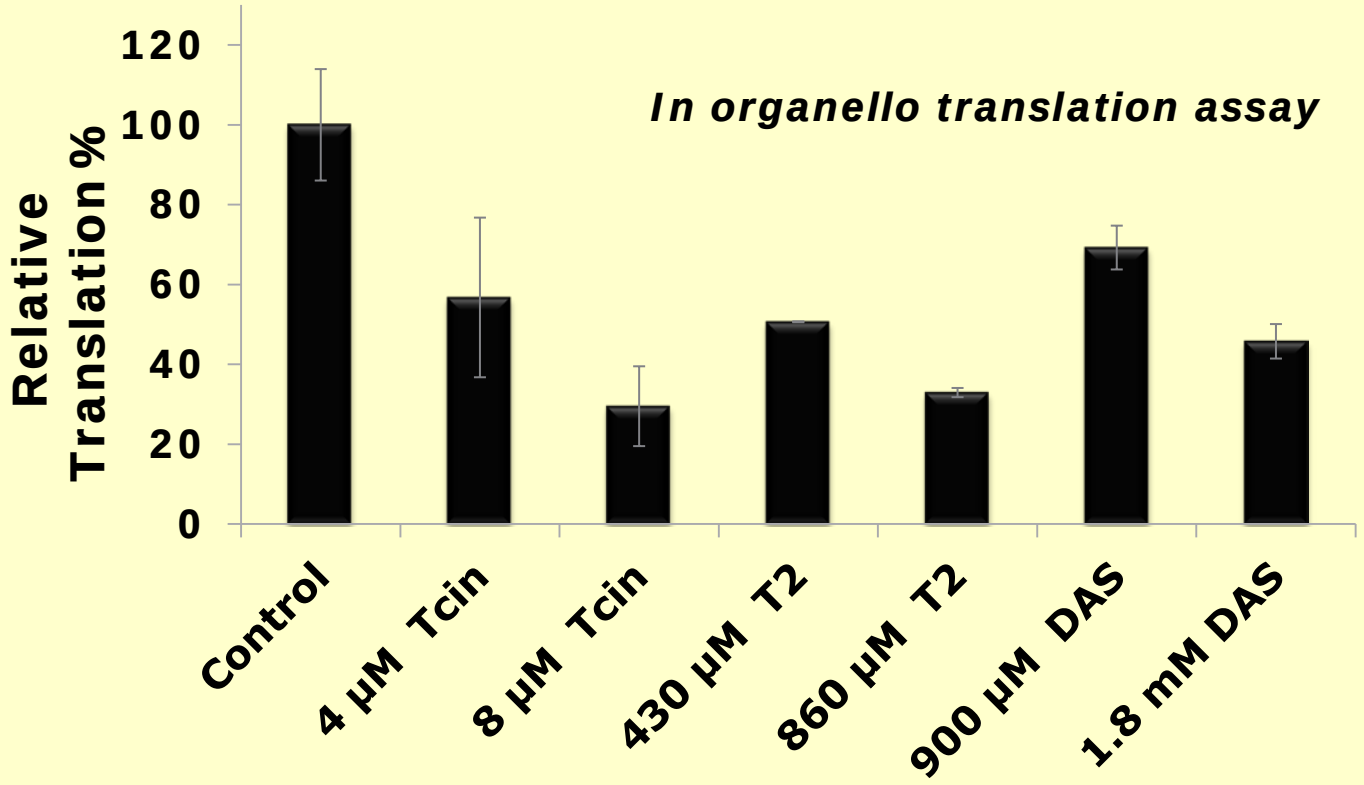
- Yeast cells are more sensitive to trichothecenes when carbon source is glycerol.
- Rho^0 (i.e. no functional mitochondria) cells are resistant to trichothecenes.

Is mitochondrial translation targeted by trichothecenes?



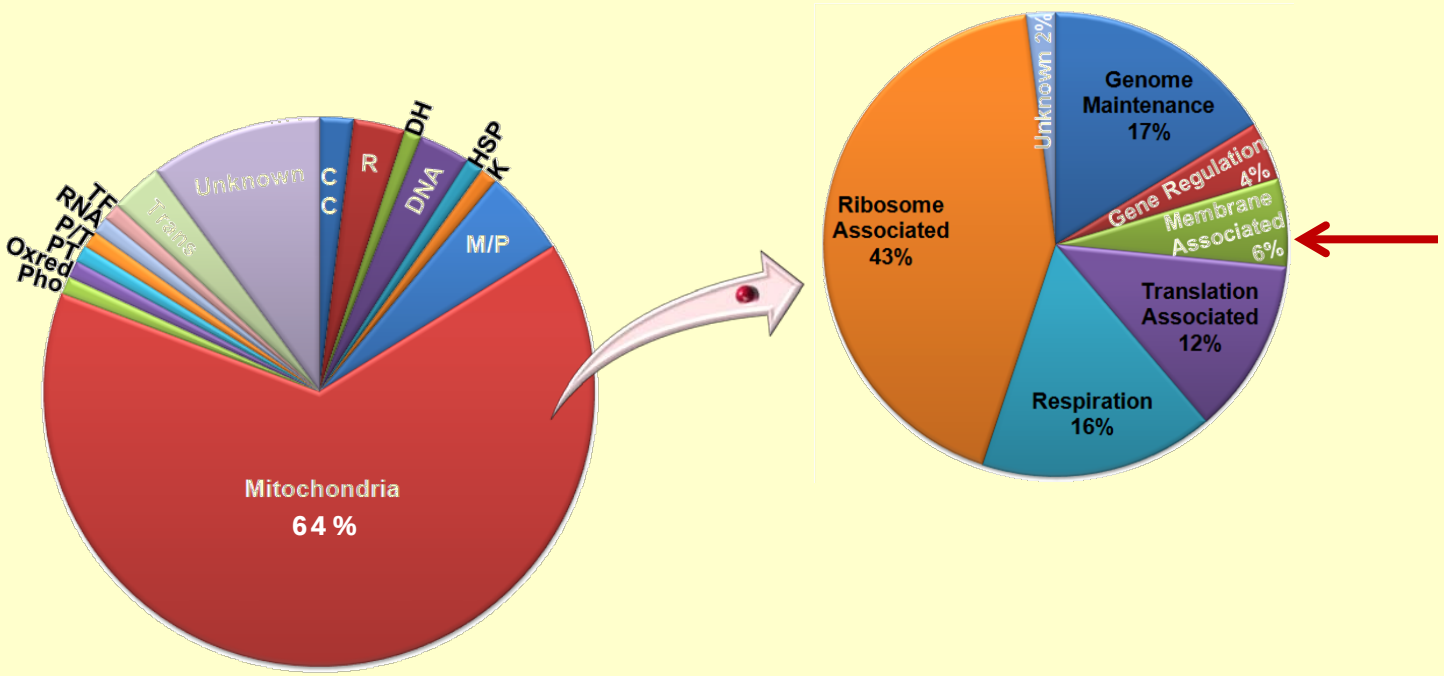
- 43% of the gene deletions associated with mitochondria were classified as translation-related.

Trichothecenes directly inhibit mitochondrial translation



- Mitochondria isolated from yeast cells were treated with trichothecenes for 10 minutes.

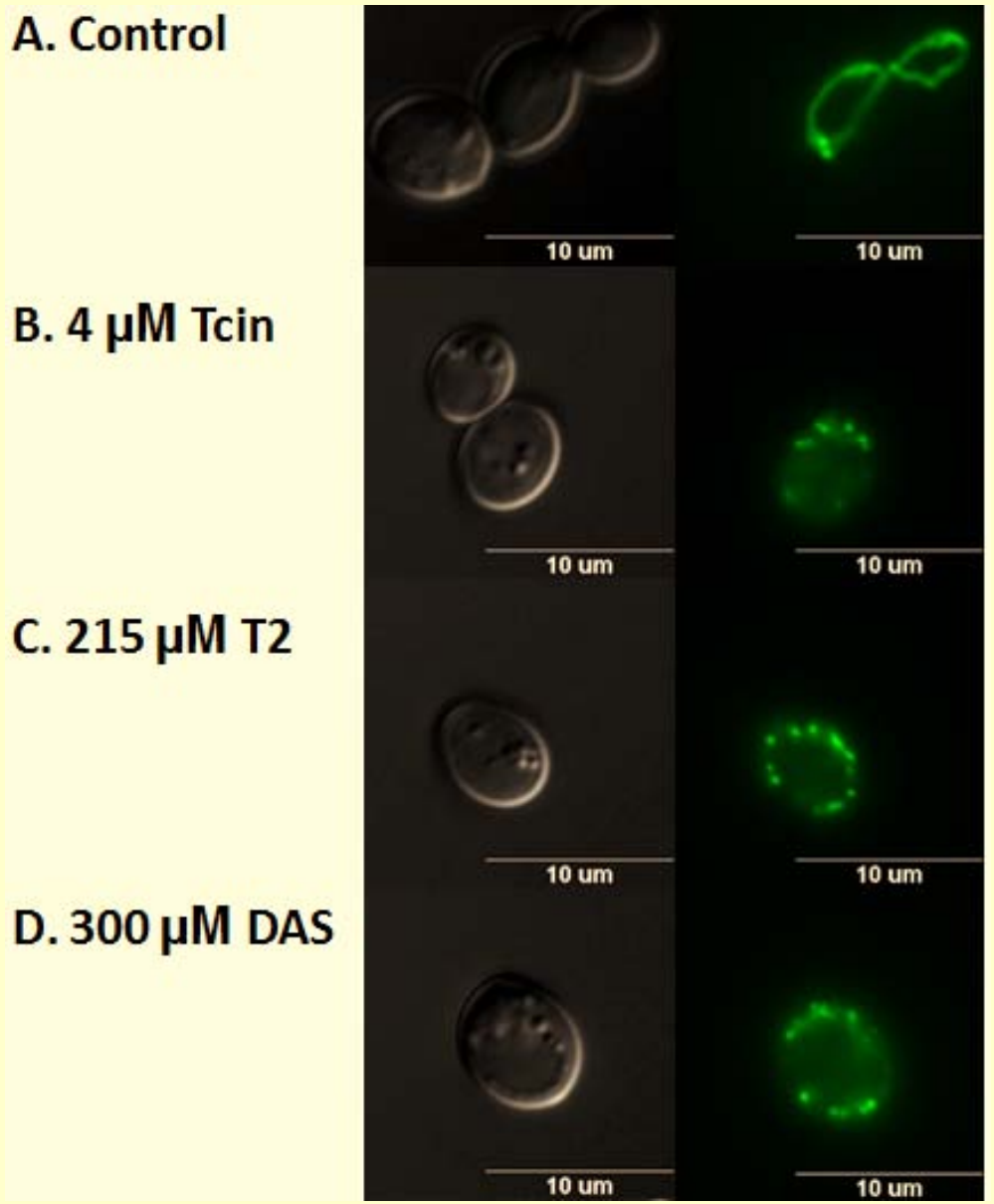
Is mitochondrial membrane integrity damaged by trichothecenes?



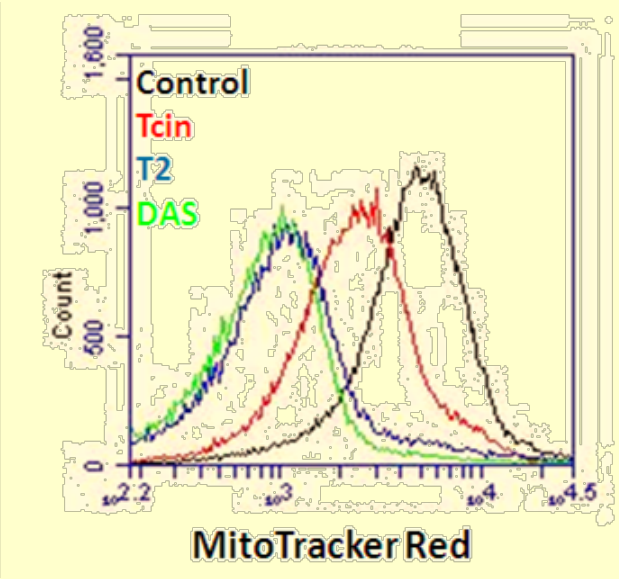
- All mitochondrial morphology related mutants resistant to Tcin were defective in membrane fusion.

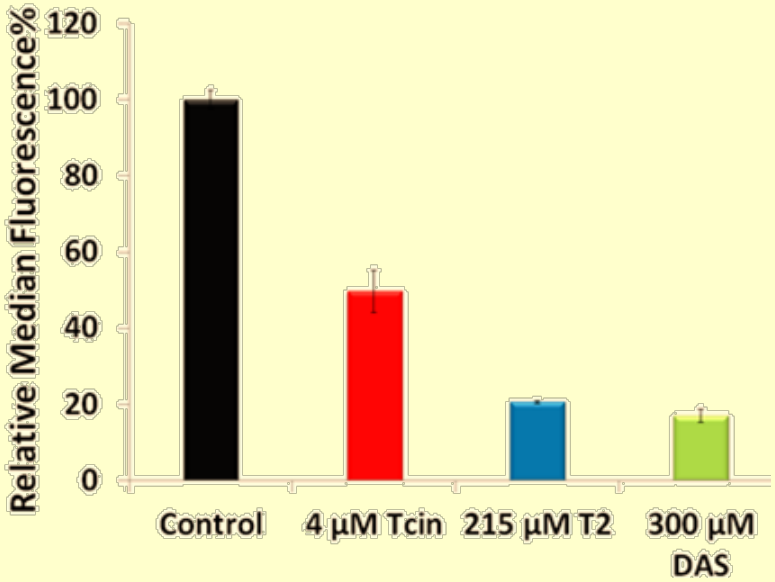
Trichothecenes alter mitochondrial morphology

- To visualize mitochondrial morphology → Yeast cells transformed with mtGFP
- Untreated → tubular network.
- 6 h post treatment → fragmented mitochondria.



Trichothecenes cause mitochondrial membrane depolarization





- **Mitochondrial Membrane Potential** (ψ_{mito}) : Biomaker for membrane integrity.
- **Membrane depolarization** → fragmentation.
- **MitoTracker Red**: Only enters actively respiring mitochondria.

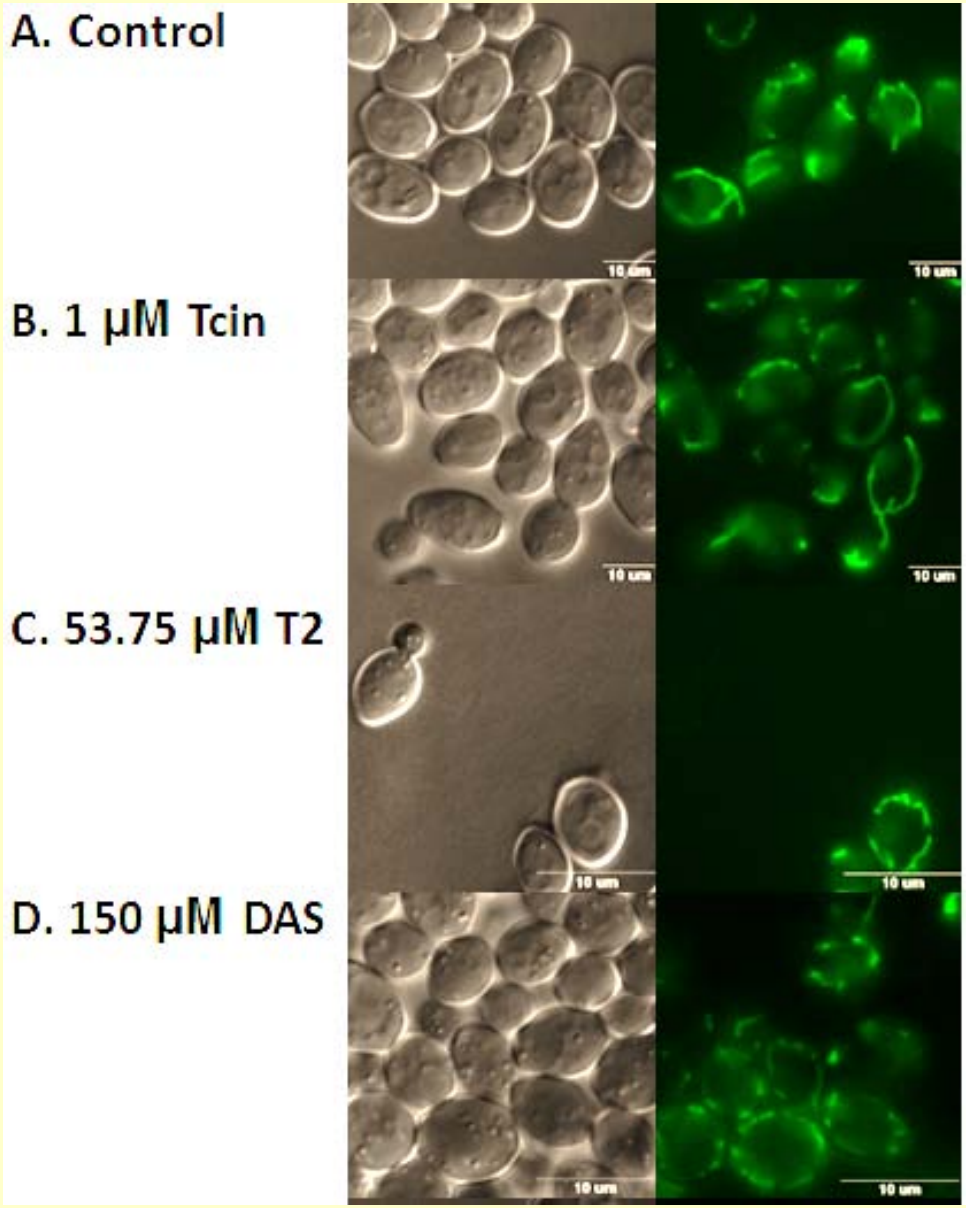
- Untreated → High fluorescence → ψ_{mito} ↑ → actively respiring mitochondria
- 6 h treatment → Low fluorescence → ψ_{mito} ↓ → dysfunctional mitochondria

Is mitochondrial translation inhibition due to membrane damage?

- Trichothecenes inhibited translation in isolated mitochondria.
- Trichothecenes → membrane depolarization → fragmentation.

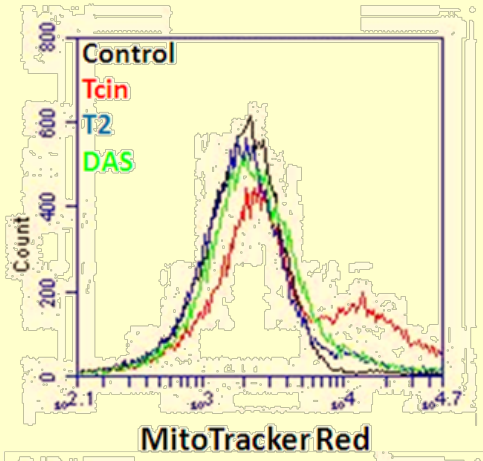
Trichothecenes do *not* alter mitochondrial morphology at low doses.

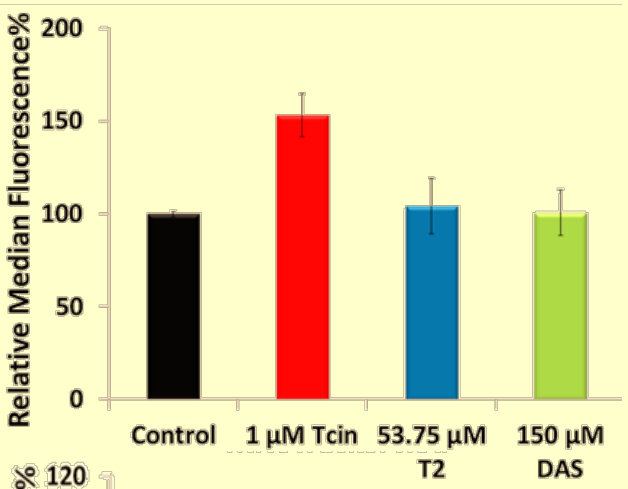
- Identified low doses → no membrane damage.
- To visualize mitochondrial morphology → Yeast cells transformed with mtGFP.
- 6 h post treatment → no severe fragmentation.



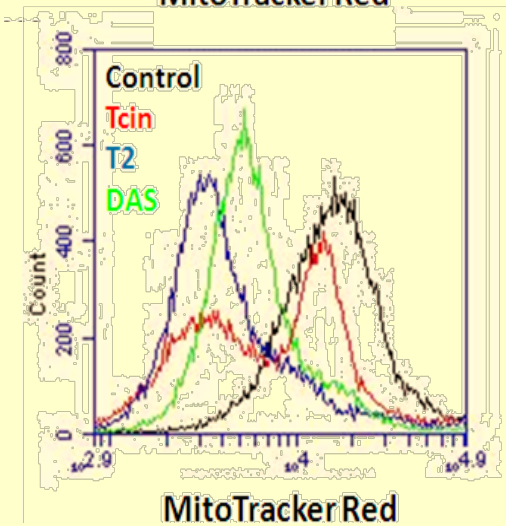
Mitochondrial membrane damage occurs late during trichothecene toxicity

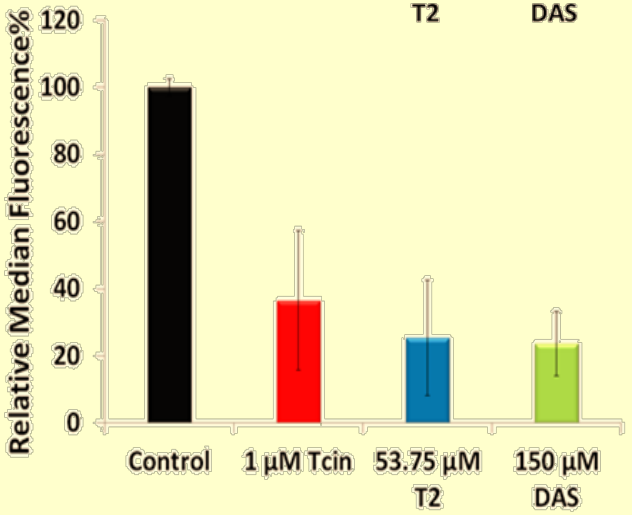
6h





18h



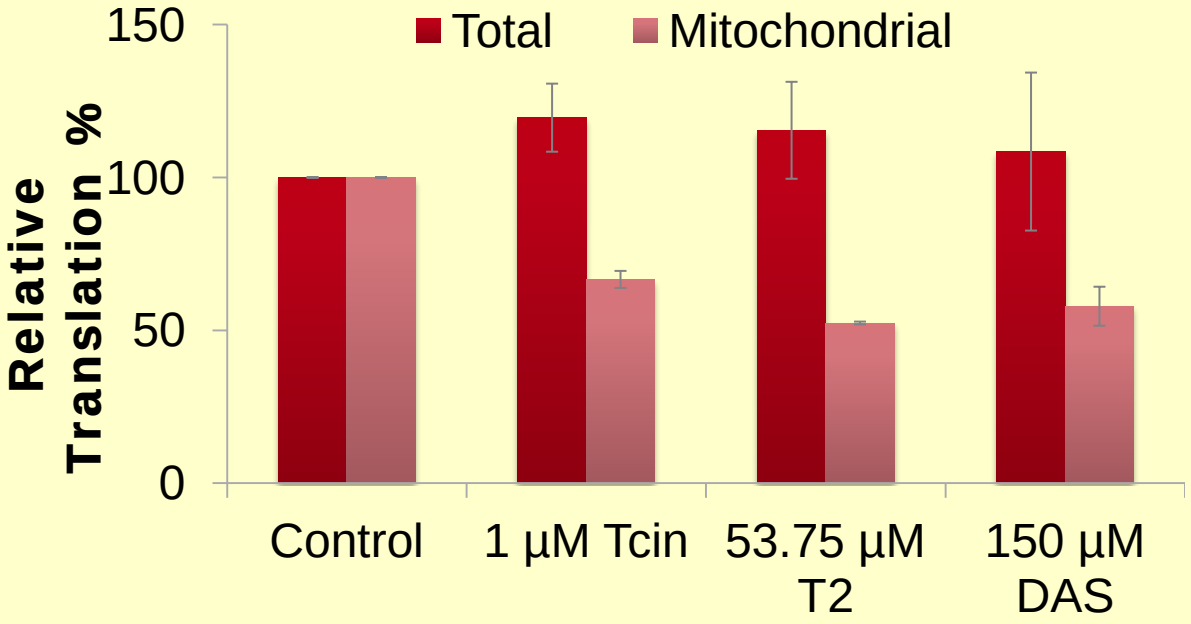


- 6 h treatment with low doses → no significant changes to ψ_{mito}
- 18 h treatment with low doses → drop in ψ_{mito} (membrane depolarization)

Is mitochondrial translation inhibited at these low doses?

- Trichothecenes, at low doses, damage mitochondrial membrane integrity in a time dependent manner.

Mitochondrial translation inhibition is not a secondary effect



- **6 h** post treatment with low doses:
  - Cytosolic translation is *not* inhibited
  - Mitochondrial membrane integrity is *not* damaged.
  - **Mitochondrial translation *is* inhibited.**
- Mitochondrial translation can be separated from cytosolic translation.

Conclusion

Effect of trichothecenes:	Low doses	High doses
Early Time Point	Late Time Point	
Mitochondrial Translation	**Membrane Damage**	**Cytosolic Translation**

- Mitochondrial translation is more sensitive to trichothecenes than cytosolic translation.
- During trichothecene toxicity mitochondrial translation is targeted before mitochondrial membranes and cytosolic translation.

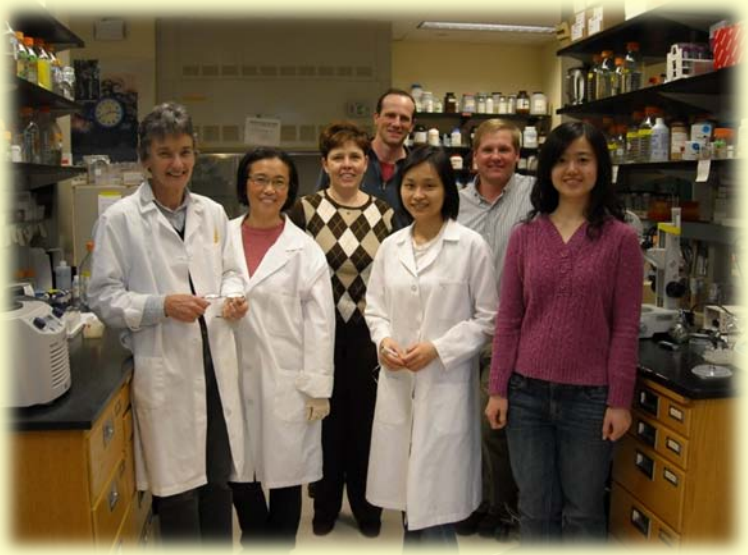
Implications

- Is mitochondrial translation inhibited in plant cells?
- Will protection against mitochondrial translation inhibition and membrane damage confer resistance?
- What is the role of chloroplast in trichothecene toxicity?
- How does the pathogen protect its own mitochondria?
- Mitochondria as potential target for engineering resistance against diseases like FHB.

Acknowledgments

- Tumer Lab
- Dr. Susan McCormick
- USDA (Project Funding)
- SCABUSA

- **Dr. Nilgun Tumer**
- Dr. John McLaughlin
- Dr. Xiao-Ping Li
- Dr. Jennifer Nielson Kahn
- Dr. Mike Pierce
- Dr. Kerrie May
- Qing Yan
- Debaleena Basu



Questions?