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# Lipid transfer proteins confer resistance to trichothecenes

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**Tumer Laboratory**
**National Fusarium Head Blight Forum**
**December 6th, 2012**

U.S. Wheat & Barley Scab Initiative

**FY09-11:**

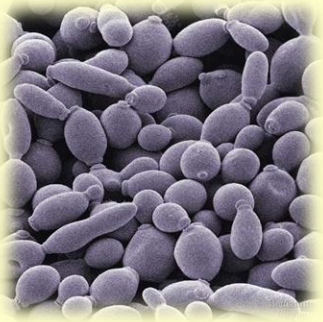
- **Identify trichothecene resistance genes by genome-wide screening in *Saccharomyces cerevisiae***
  - **Translation, mito morphology, lipid metabolism**

**FY11-13**

- **Identify plant genes for trichothecene resistance by activation tagging in *Arabidopsis thaliana***

**FY13-14**

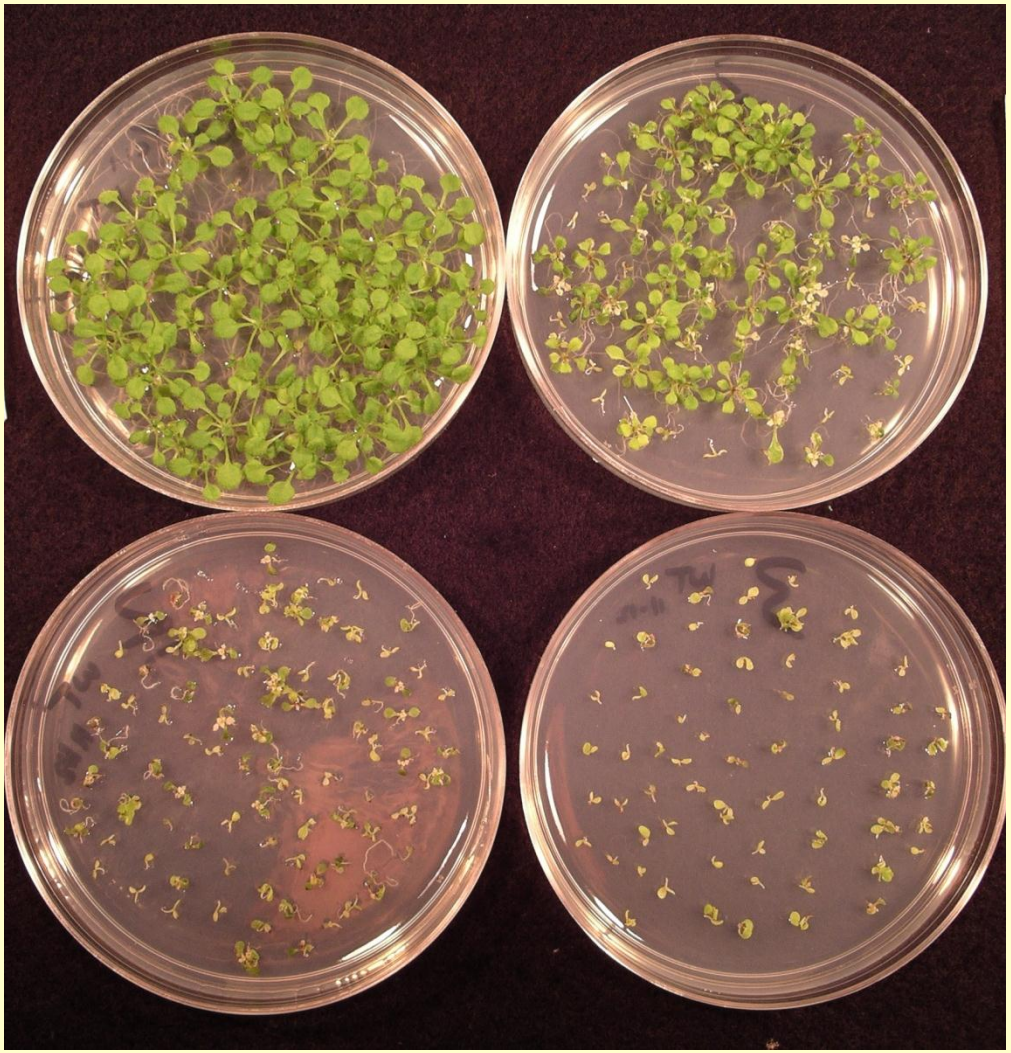
- **Demonstrate resistance in wheat**



# Screening Arabidopsis (Col-0) for Tcin sensitivity using a germination assay (14 dpg)

0 µM Tcin

1 µM Tcin



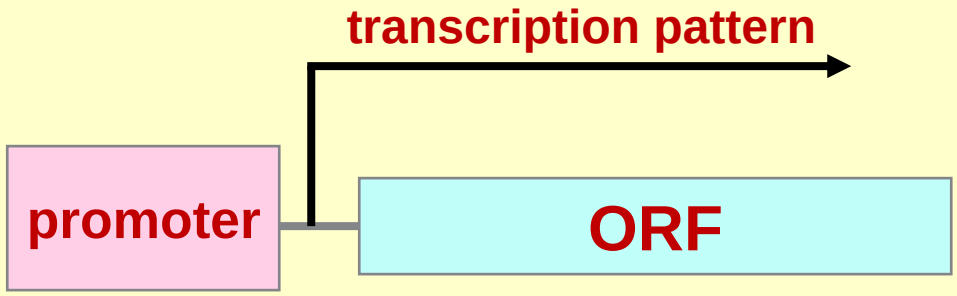
2 µM Tcin

3 µM Tcin

# Root/vegetative growth is severely inhibited at Tcin concentrations greater than 1 µM

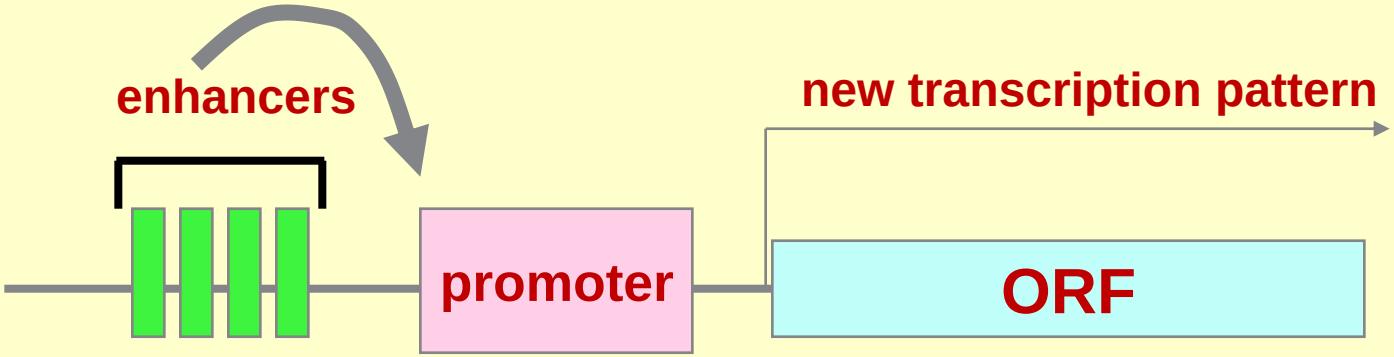
0 µM 1 µM 2 µM 3 µM

# Activation tagging: Dominant Gain-of-function




Susceptible to toxin

Insertion of activation tagged vector containing 4X enhancers




Resistance to toxin

# Activation Tagging in *Arabidopsis*

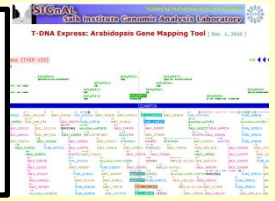
Transformation of Col-O with pSKI015 (floral dip)

Generate M1 and M2 populations

1º Screen Identify M2 plants which set root

2º Screen Confirmation of Phenotype

TAIL PCR To Identify Flanking Sequence





RNA isolation and qRT PCR to measure gene expression

?

Overexpression Detected

?

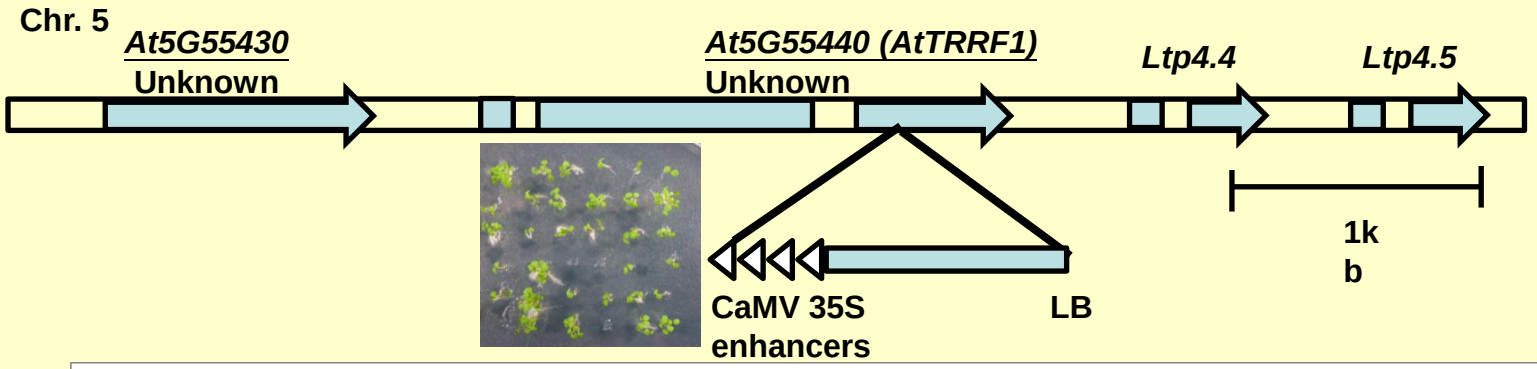
Test independently generated knockout (SALK)

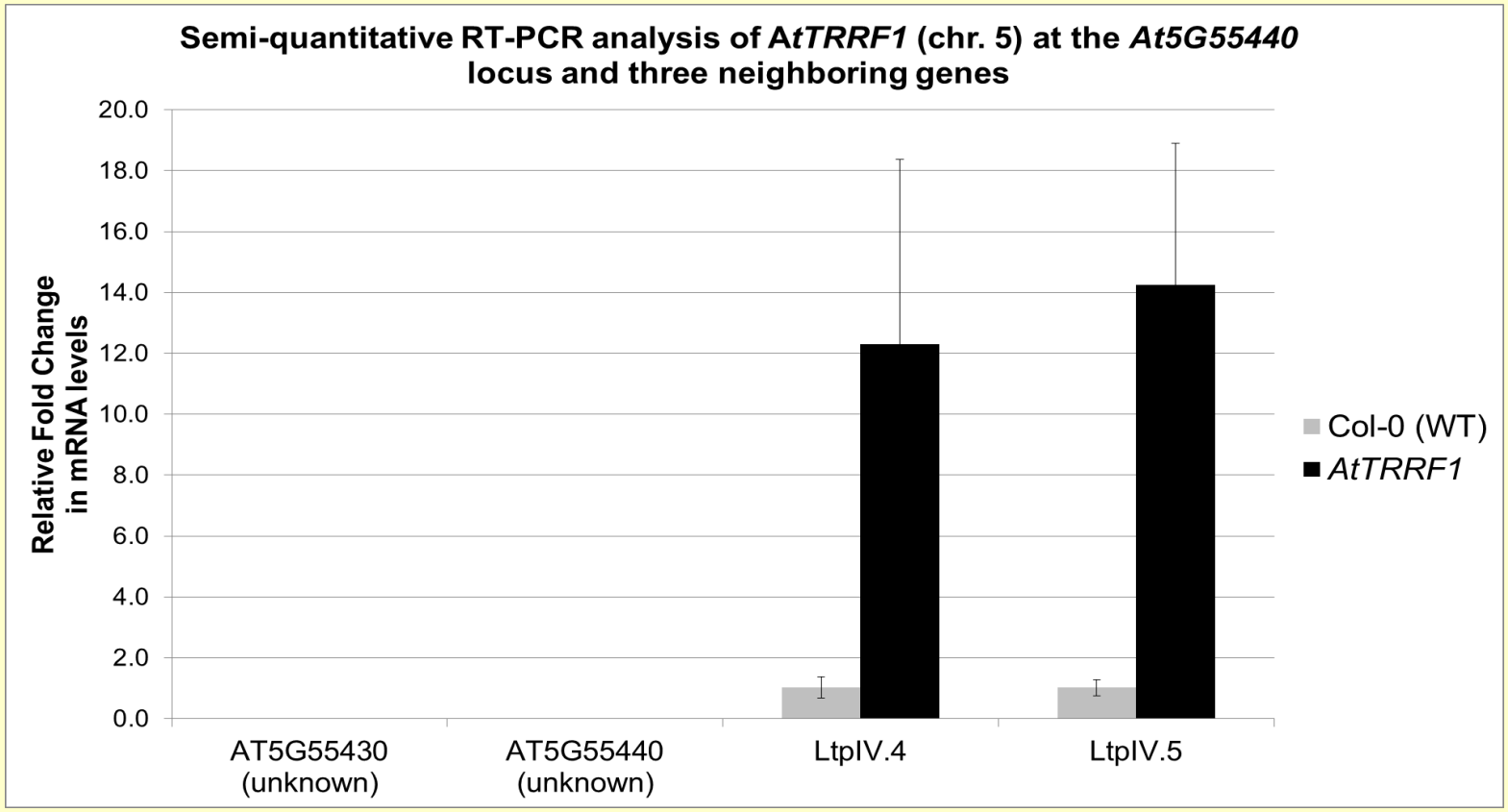
Transform Col-0 With overexpression gene vector

Specific controlling gene

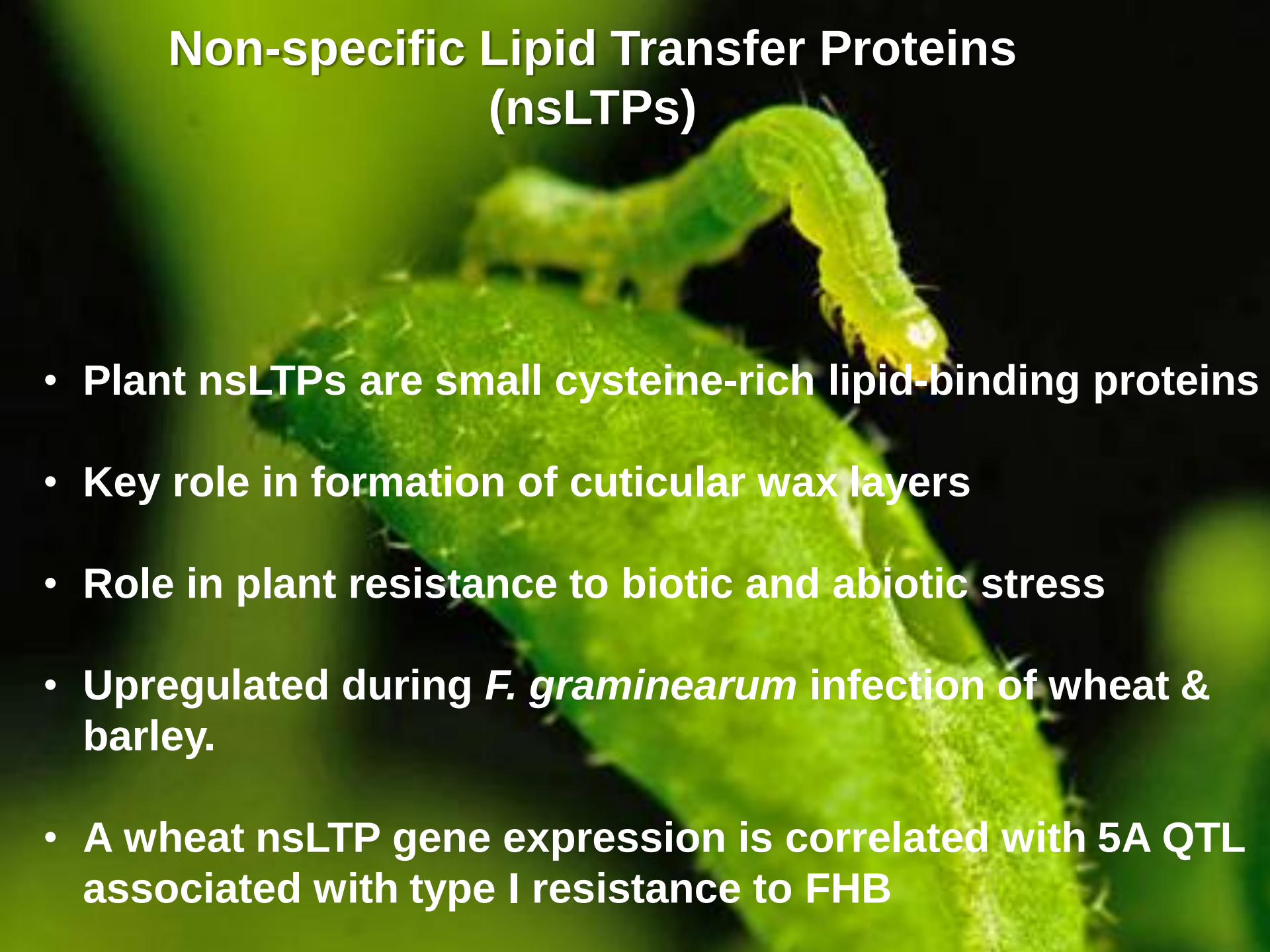
Introduce gene into crop (or knockout)

# RUTGERS 250,000 activation tagged lines were screened



# Non-specific Lipid Transfer Proteins (nsLTPs)

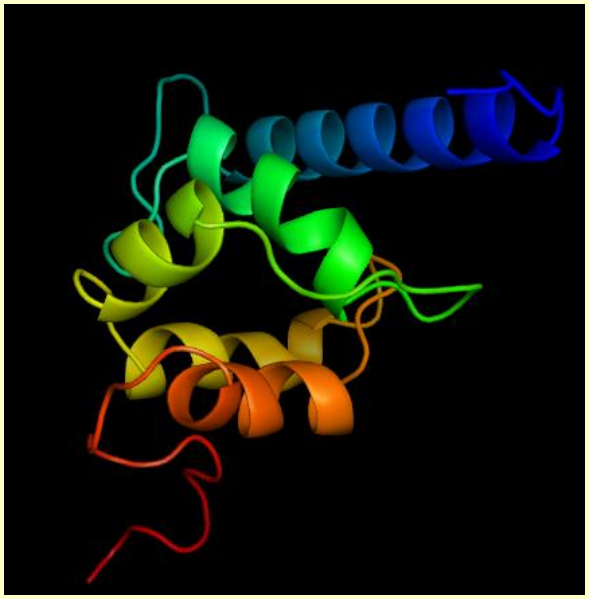


- Plant nsLTPs are small cysteine-rich lipid-binding proteins
- Key role in formation of cuticular wax layers
- Role in plant resistance to biotic and abiotic stress
- Upregulated during *F. graminearum* infection of wheat & barley.
- A wheat nsLTP gene expression is correlated with 5A QTL associated with type I resistance to FHB

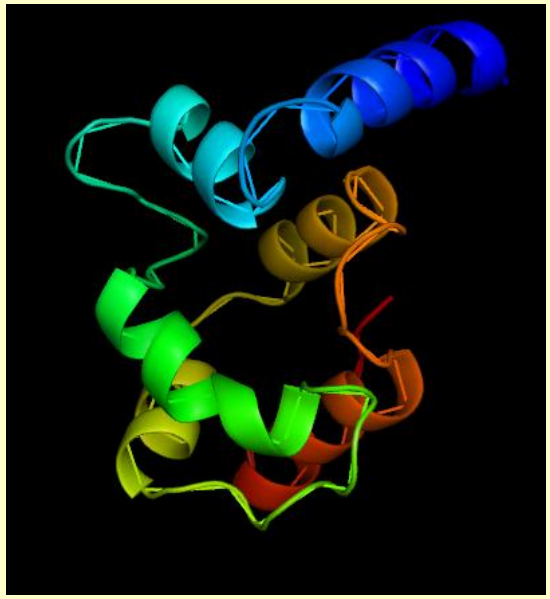
# Plant nsLTP functions

- **LTPs bind to and transfer phospholipids between membranes *in vitro***
- **Plant nsLTPs contain an internal hydrophobic pocket, which can accommodate a lipid**
- **Plant nsLTPs contain signal peptides, which target them to cell wall/apoplast**
- **Some nsLTPs are upregulated in response to infection and exhibit antibacterial and antifungal activity**

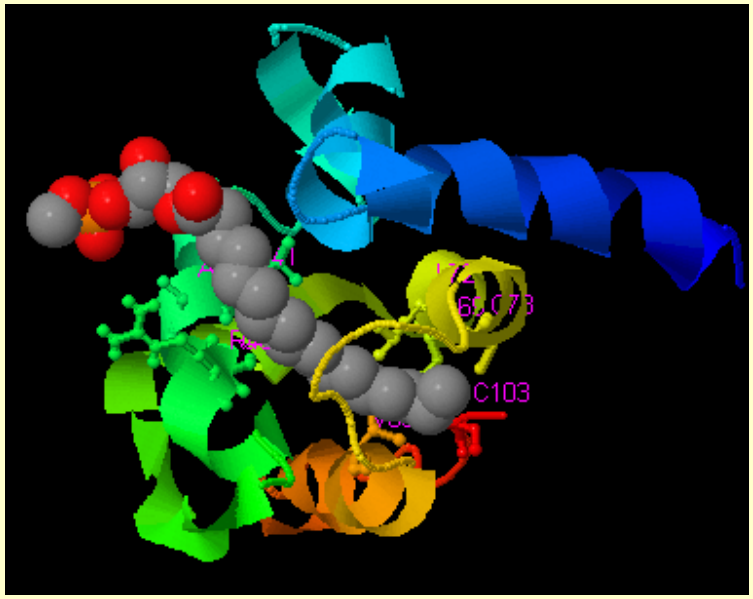
LTP4.4



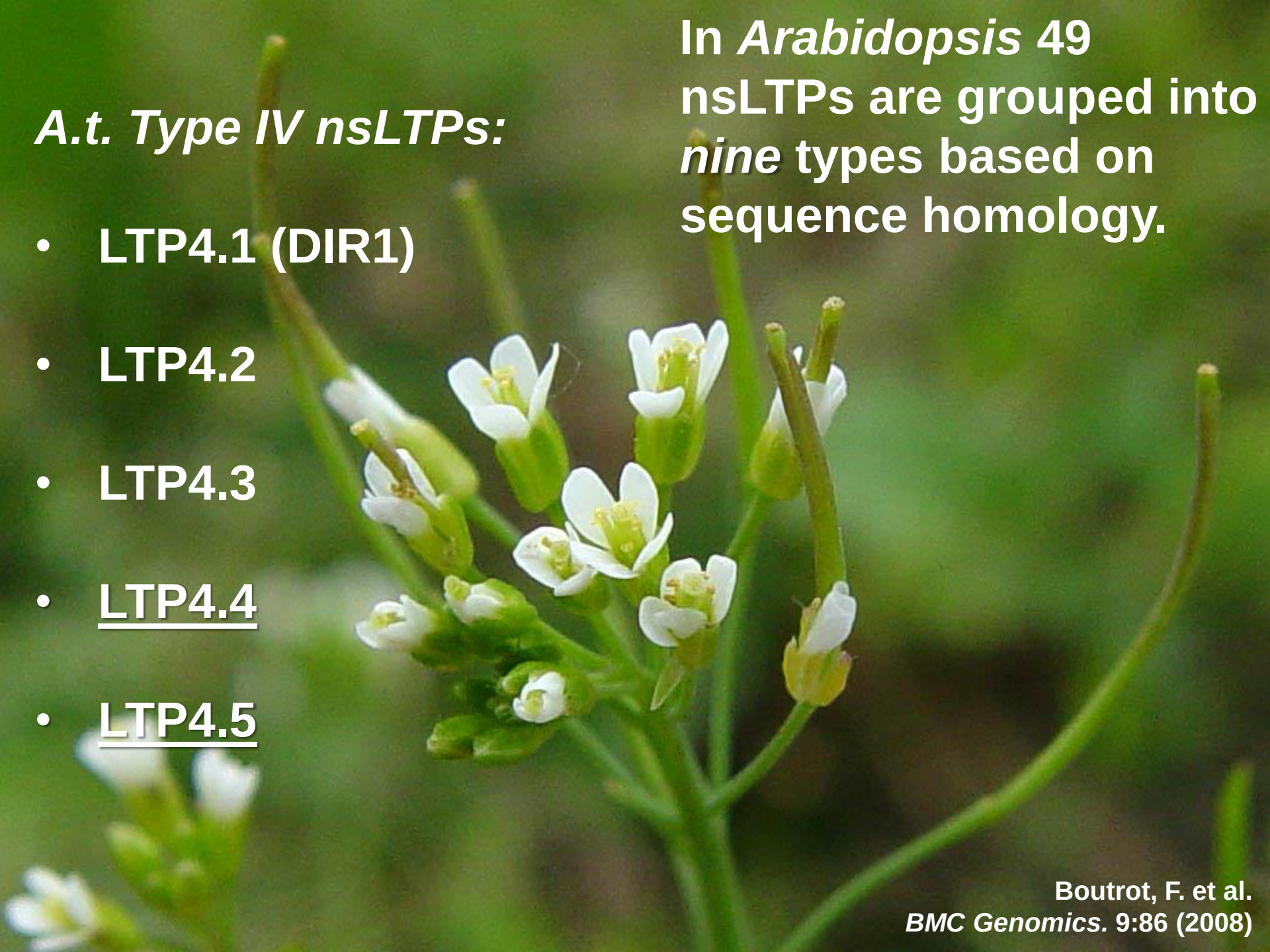
LTP4.5



LTP4.1




**Predicted Structures for LTP4.4 and LTP4.5 compared to the X-ray crystal structure of DIR1 (Defective in induced resistance) from *Arabidopsis* (Type 4.1 LTP) bound to a lipid**



In *Arabidopsis* 49 nsLTPs are grouped into *nine* types based on sequence homology.

*A.t. Type IV nsLTPs:*

- LTP4.1 (DIR1)
- LTP4.2
- LTP4.3
- LTP4.4
- LTP4.5

Boutrot, F. et al.
*BMC Genomics.* 9:86 (2008)

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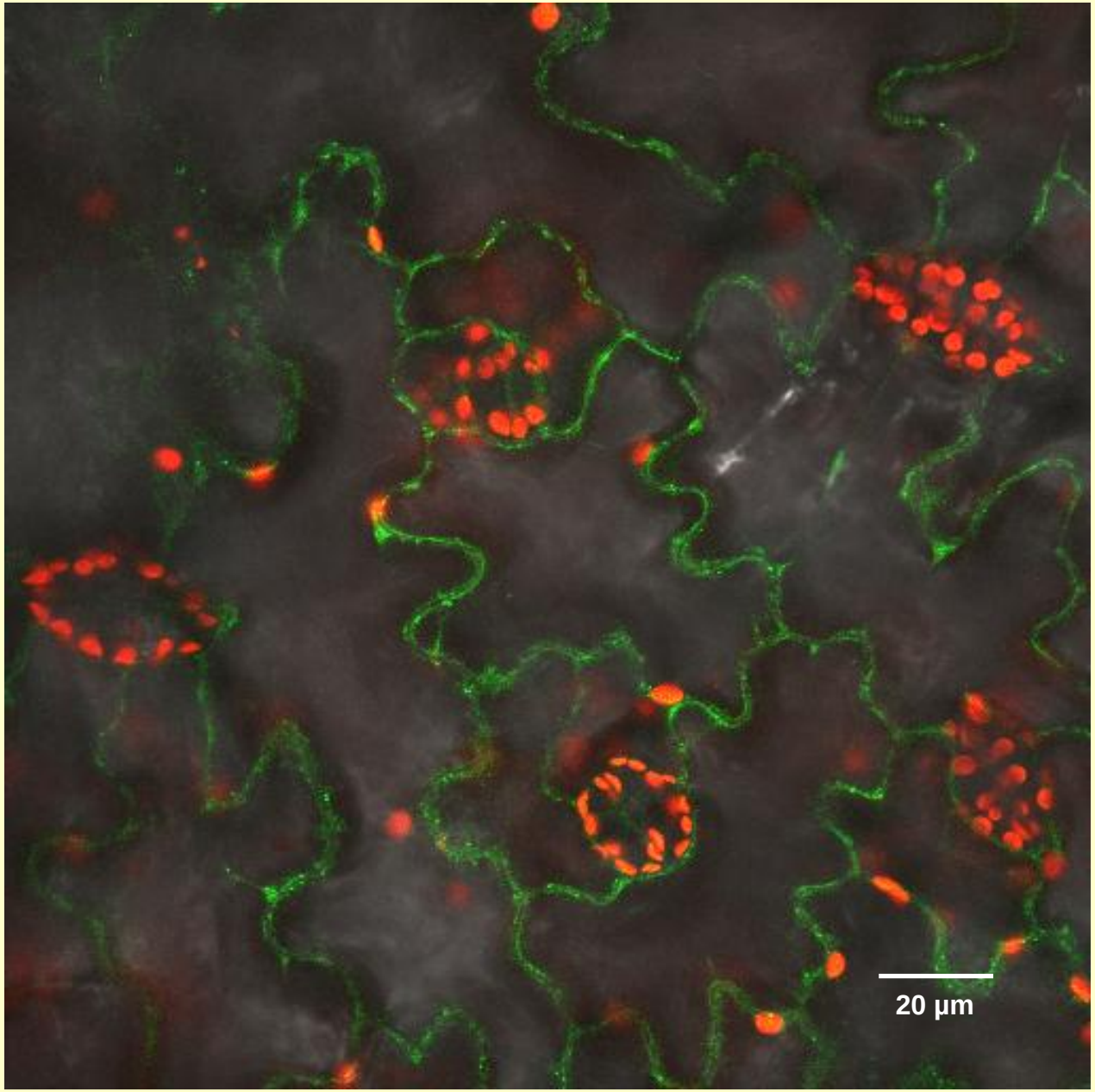
# LTP4.4:GFP is localized in the cell wall



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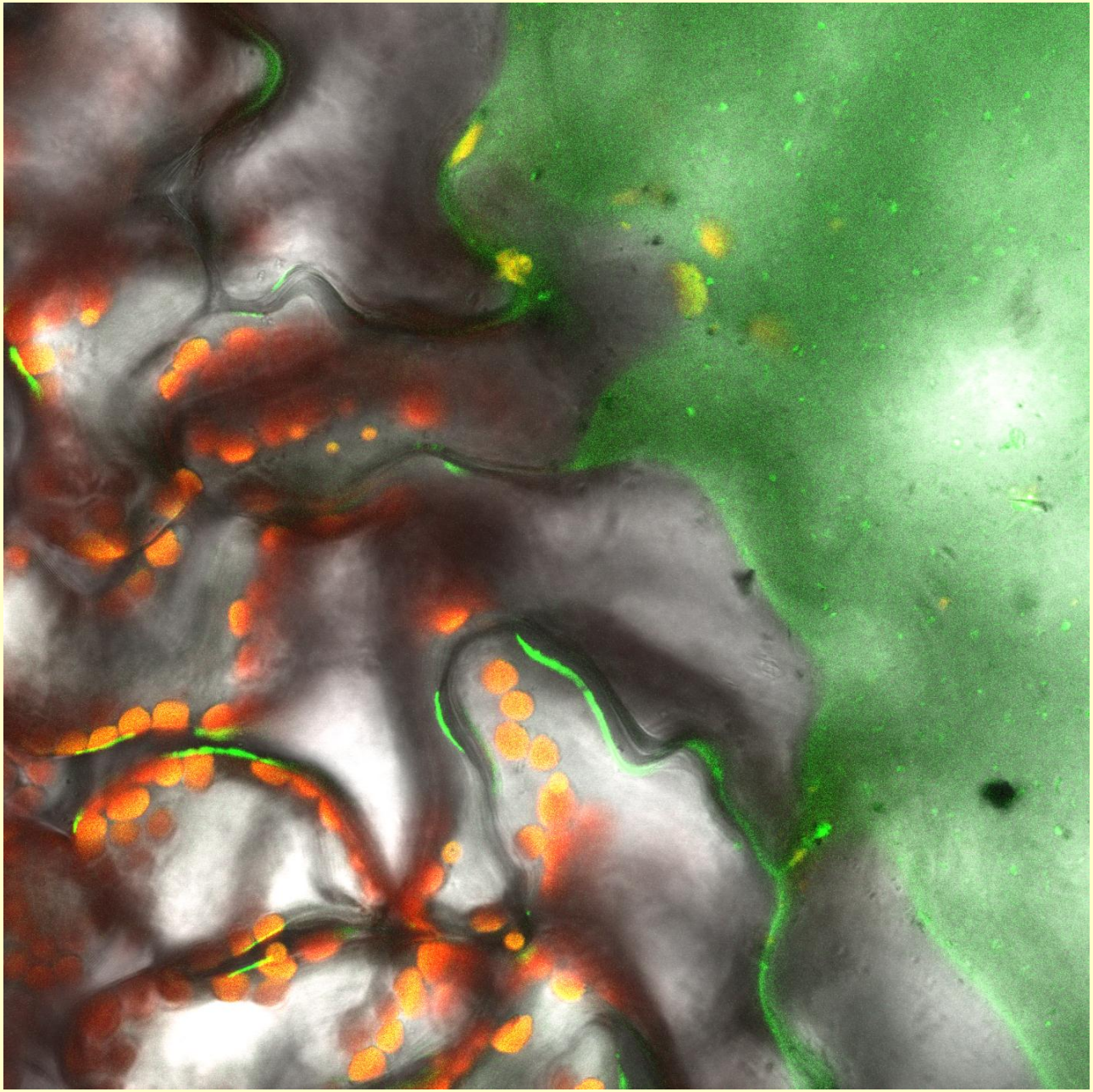
# LTP4.5:GFP Localization in *Arabidopsis*



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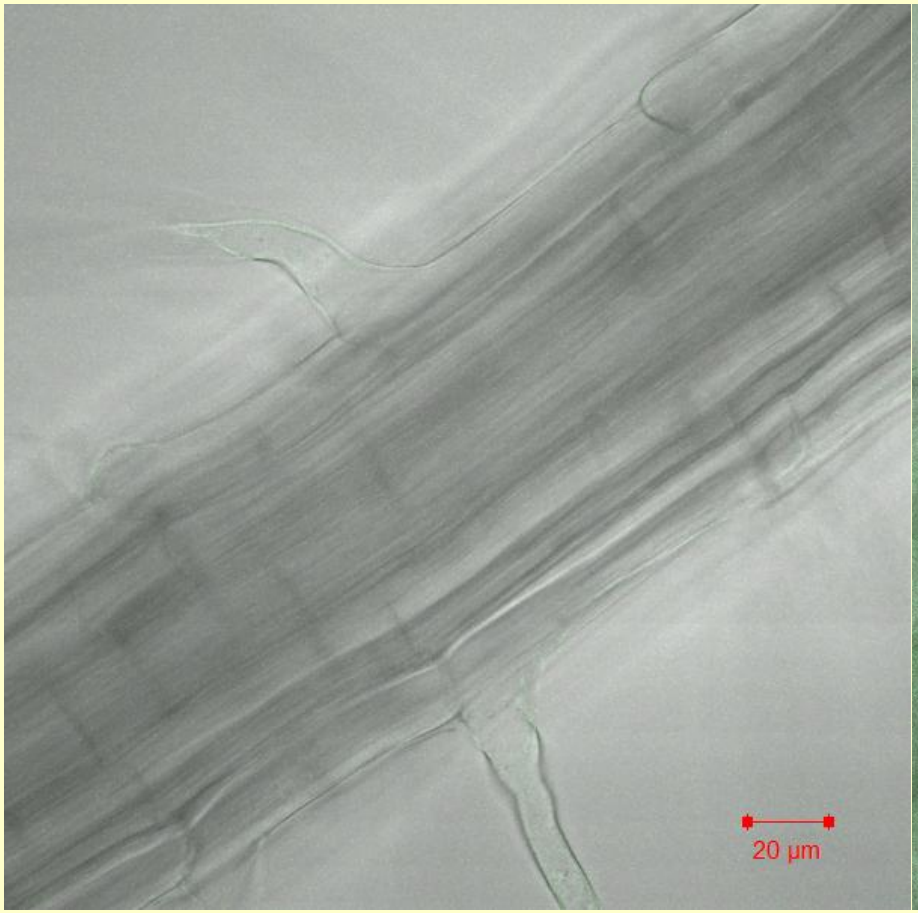
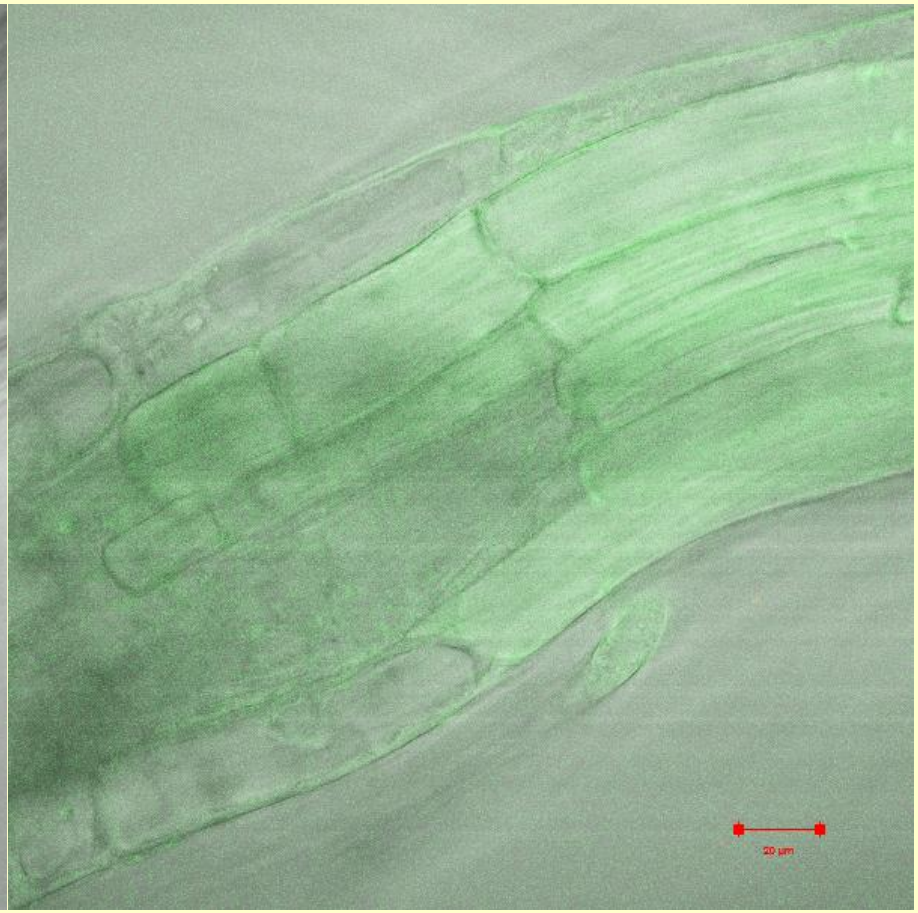
# LTP4.4:GFP is expressed in the roots

## Col-0




## LTP4.4 (Line 13)



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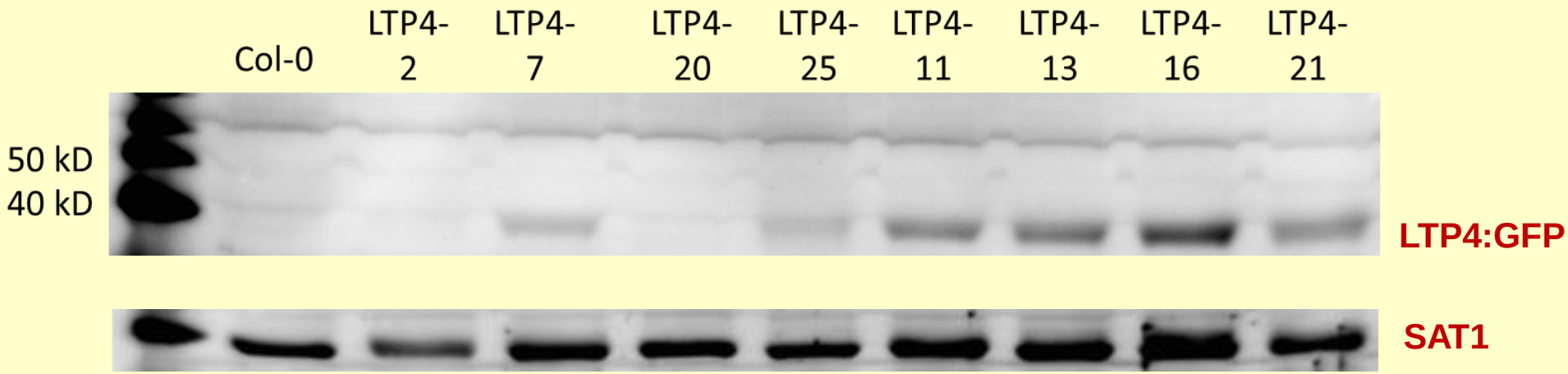
# LTP4.4 protein expression in Arabidopsis




**Immunoblot Analysis of transgenic *Arabidopsis* lines expressing LTP4.4:GFP.**

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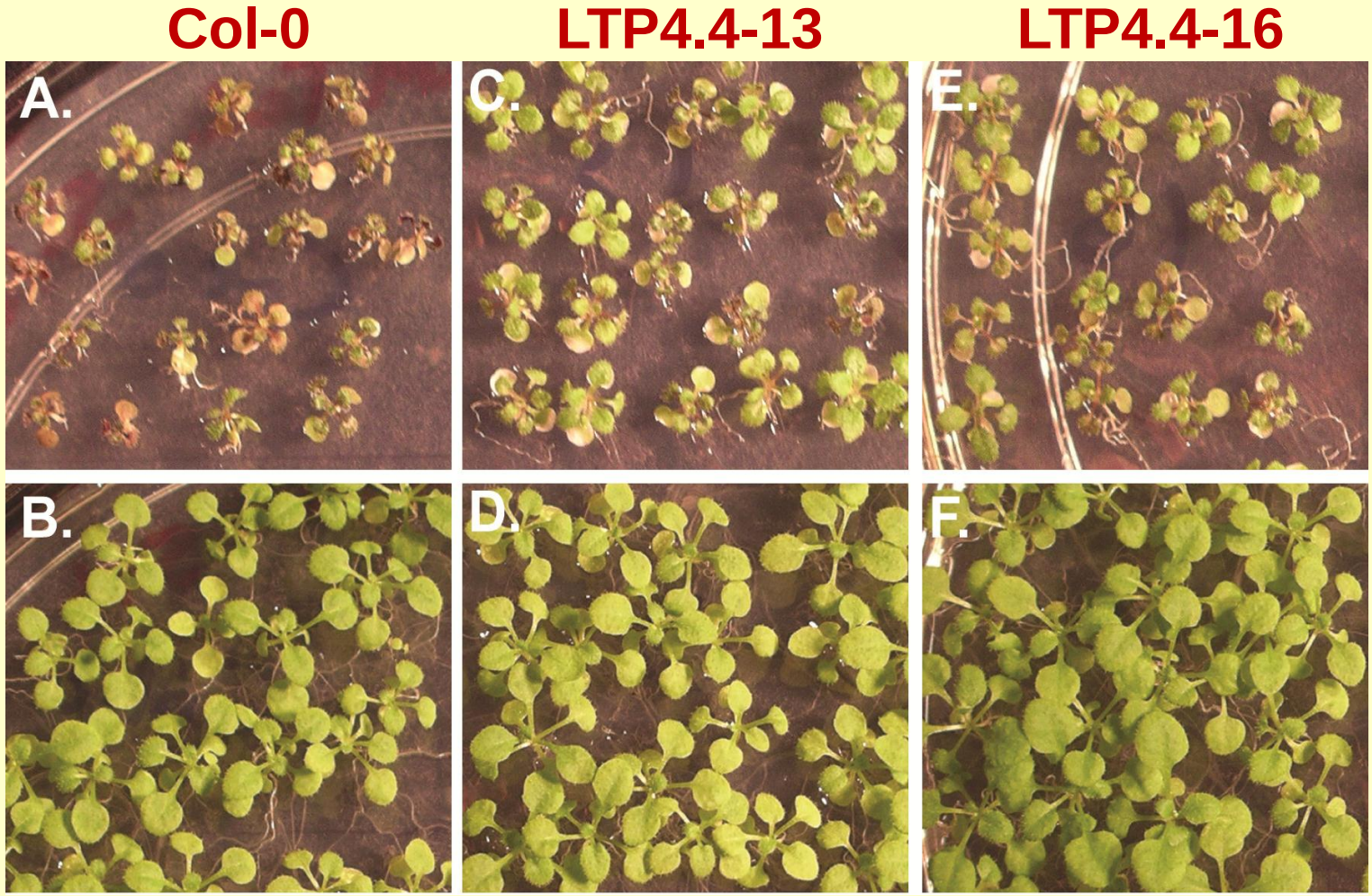
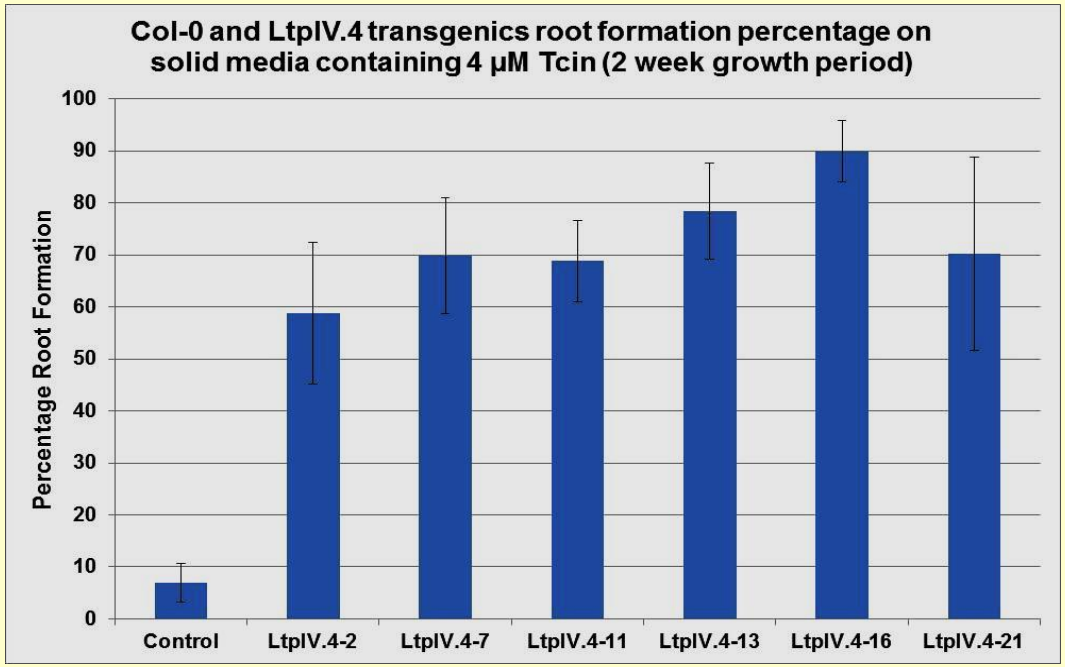
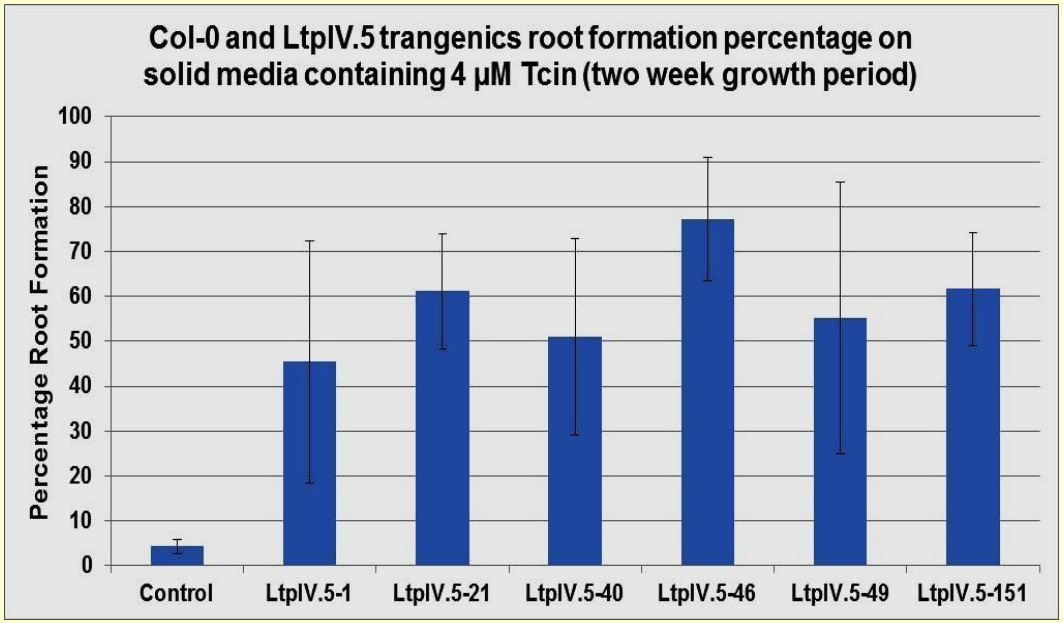
# Transgenic *Arabidopsis* overexpressing LTP4.4




**Transgenic *Arabidopsis* overexpressing LTP4.4 are phenotypically normal and show resistance to Tcin**




**Transgenic Arabidopsis lines overexpressing LTP4.4 and LTP4.5 are able to form roots on media containing 4 µM Tcin  better than wild type control plants**

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# Conclusions

- **Activation tagging in *Arabidopsis* identified a trichothecin resistant mutant containing a modified T-DNA vector, which enhanced expression of two nonspecific lipid transfer protein (nsLTP) genes.**

- **Overexpression of each nsLTP gene in Arabidopsis provided resistance to trichothecin, demonstrating that resistance in the T-DNA tagged line was due to overexpression of the nsLTP genes**

- **LTP4.4 and LTP4.5 localize to the cell wall/apoplast in the mesophyll, the epidermis, and root tissues.**

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# Ongoing and future work

- **Determine if expression of LTP4.4 and LTP4.5 confers resistance to DON and FHB in transgenic wheat and barley**
- **Lipid peroxidation is associated with trichothecene toxicity- Connection between LTPs and lipid damage?**
- **LTP genes may function in the production of a mobile signal for resistance, affect lipid composition of membranes/cuticle, or they may bind to trichothecenes**
  - **Isolate recombinant LTP4.4 and LTP4.5 proteins using the *Pichia* system**
  - **Binding of LTPs to trichothecenes using Surface plasmon resonance (SPR) analysis**

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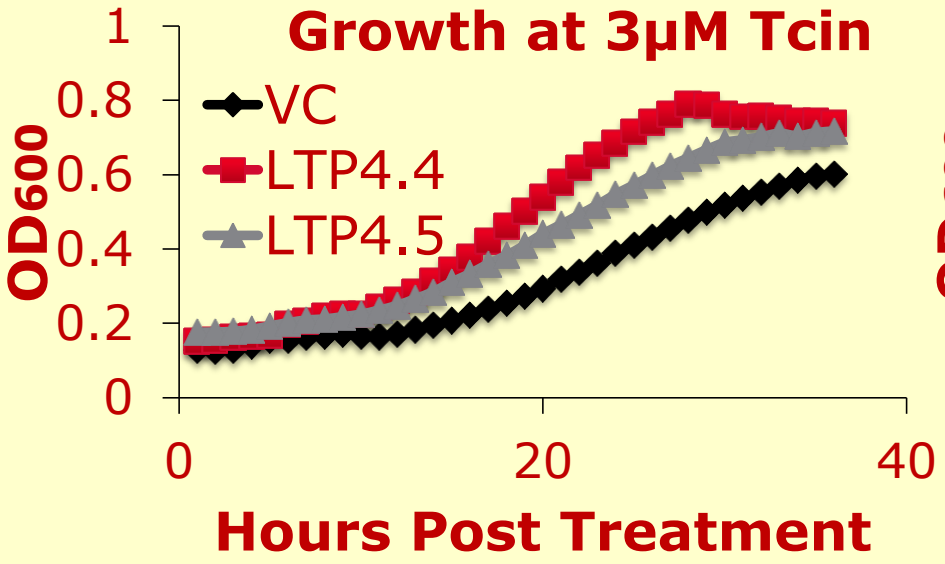
# Characterizing LTPs in yeast

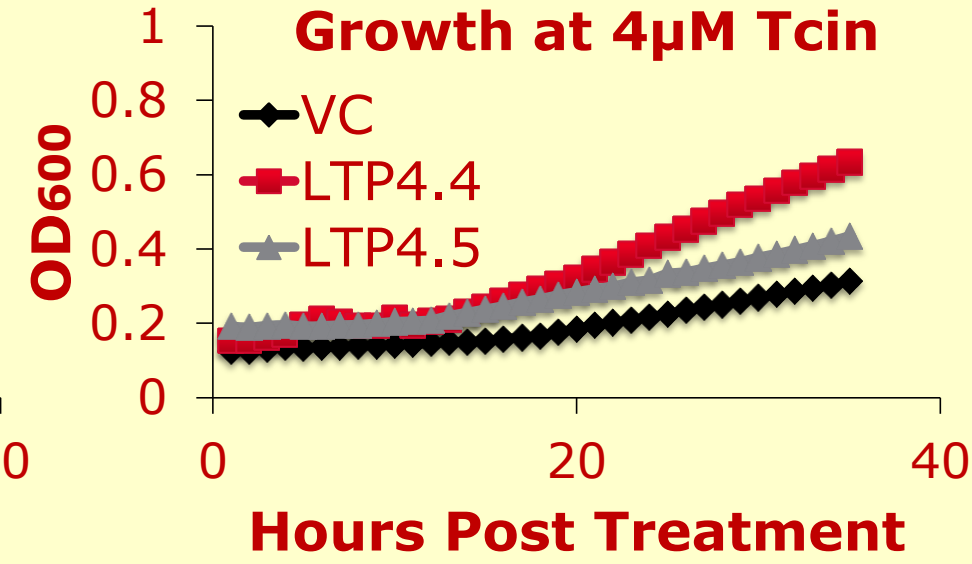
## Can At. LTP4.4 & At. LTP4.5 provide resistance to Tcin in yeast?

# LTP expression leads to Tcin resistance in yeast

**Cloned LTPs into a yeast expression system and transformed them into yeast.**

**Grew yeast in selective media +/- Tcin**



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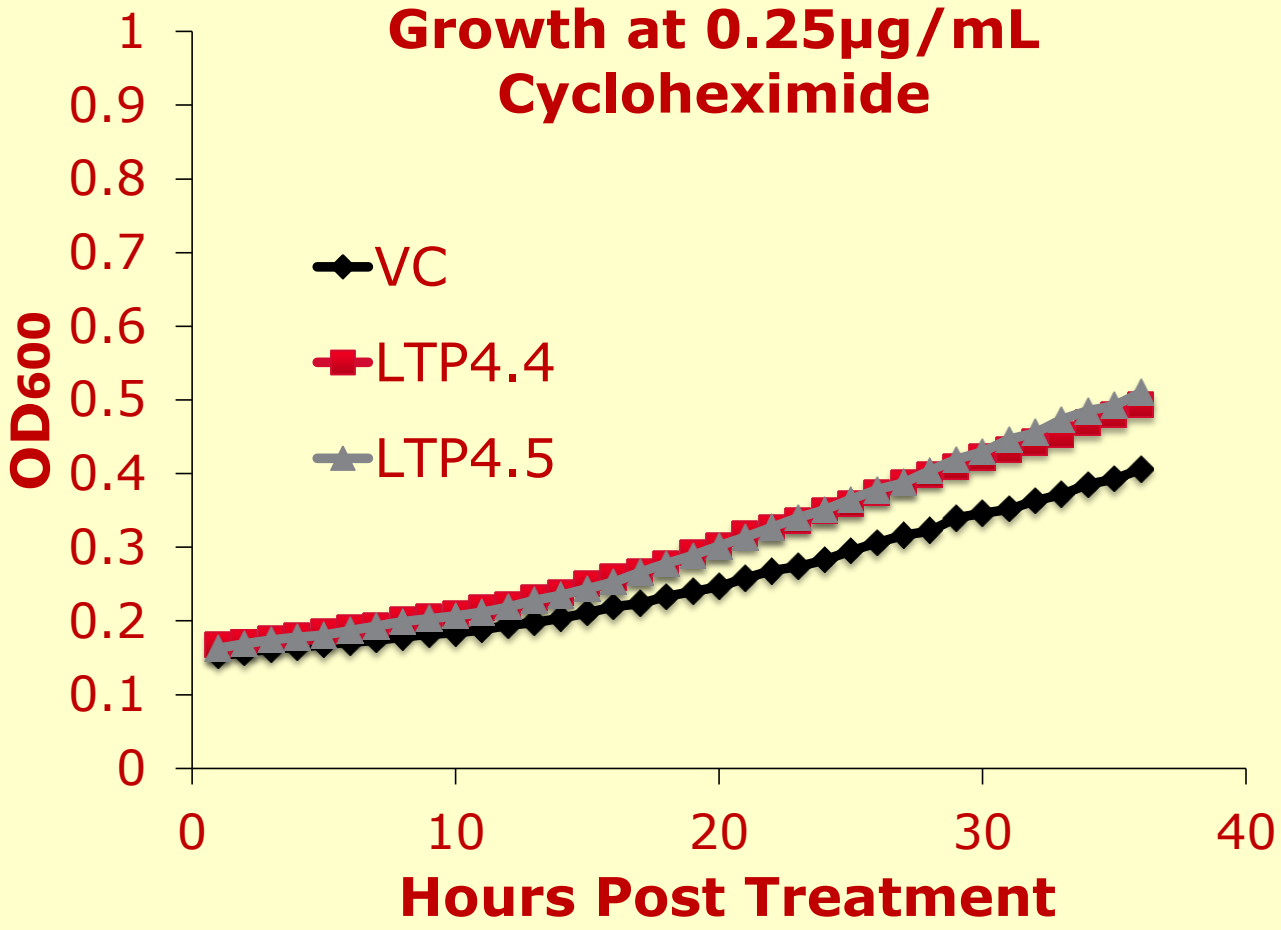
# Is LTP-mediated resistance a general response?

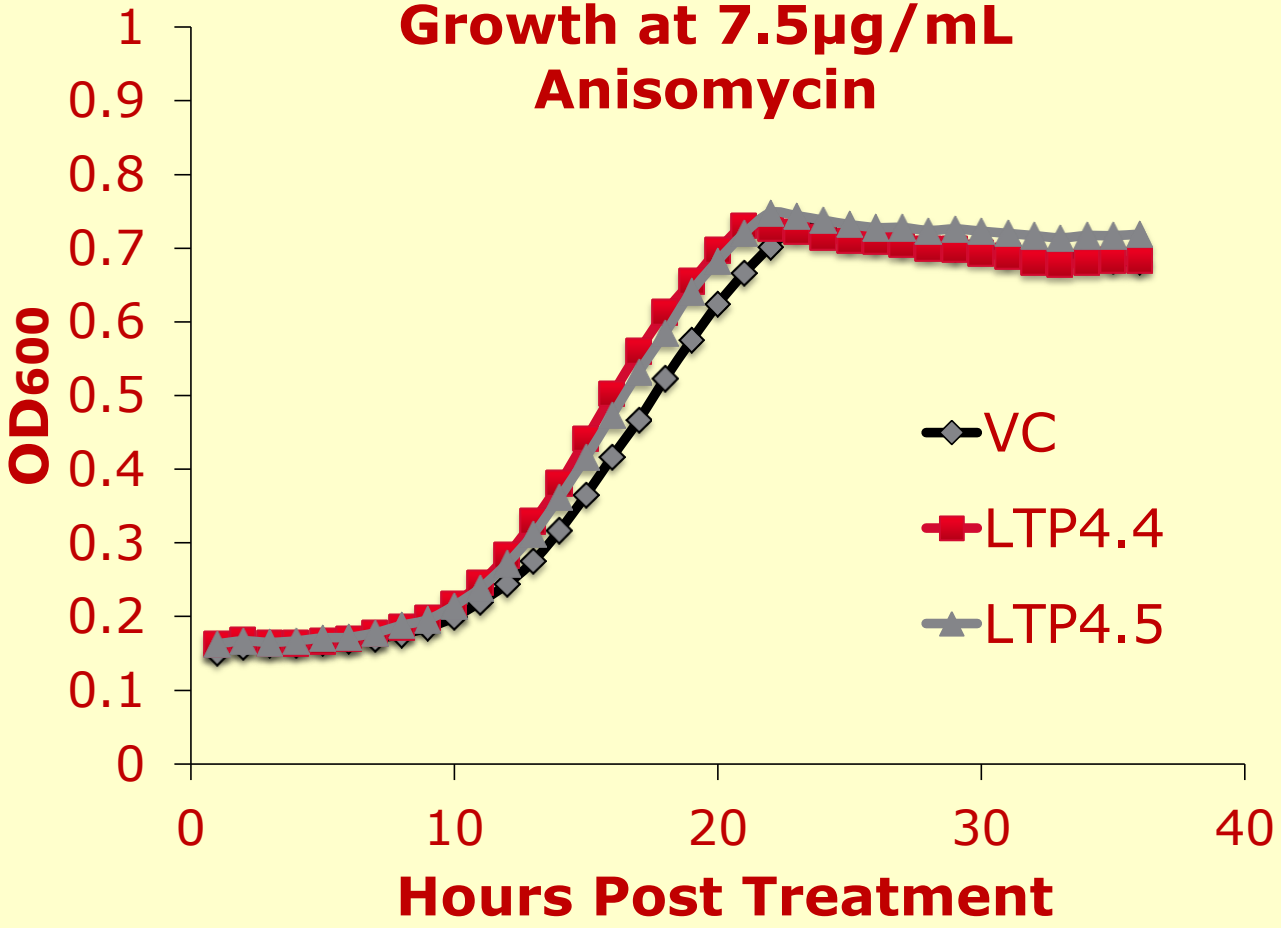
# LTP-mediated resistance is specific to Tcin

## Inhibitors of yeast growth & translation: *Cycloheximide*



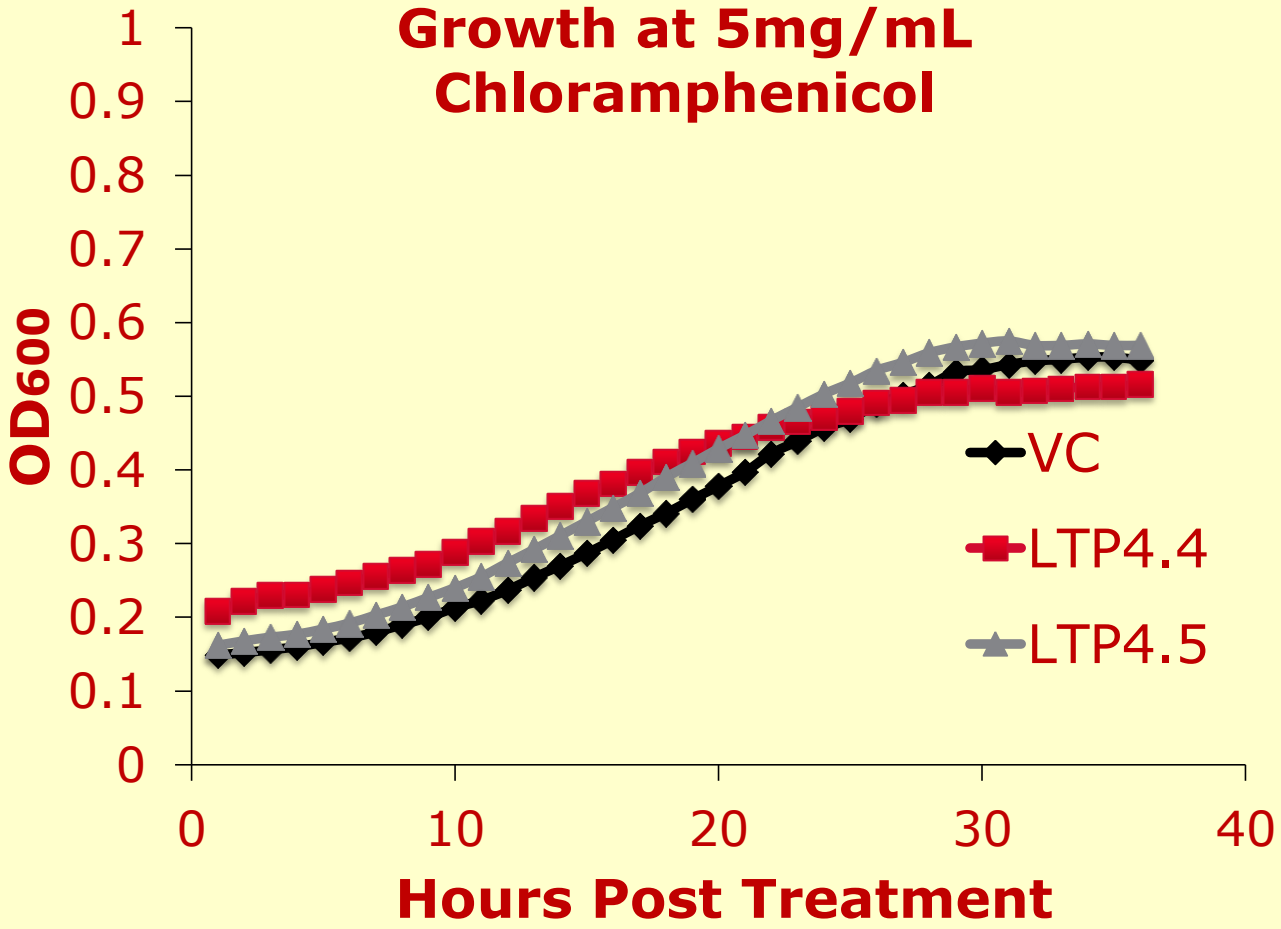
# LTP-mediated resistance is specific to Tcin

## Inhibitors of yeast growth & translation: *Anisomycin*



# LTP-mediated resistance is specific to Tcin

## Inhibitors of yeast growth & translation: *Chloramphenicol*



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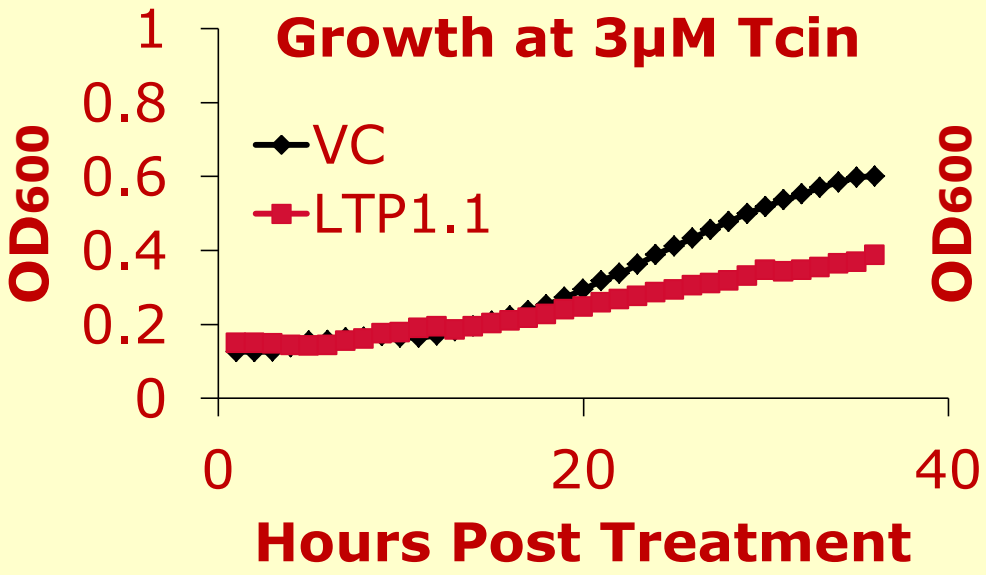
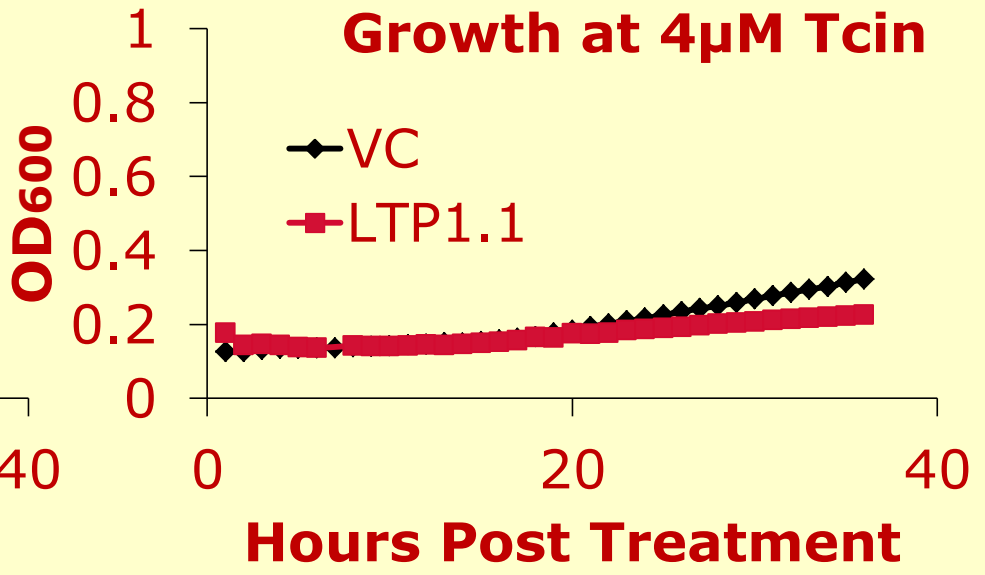
# Do all LTPs provide resistance to trichothecenes?

# Not all LTPs mediate resistance to trichothecenes

**At. LTP1.1 done not confer resistance but rather increases susceptibility**



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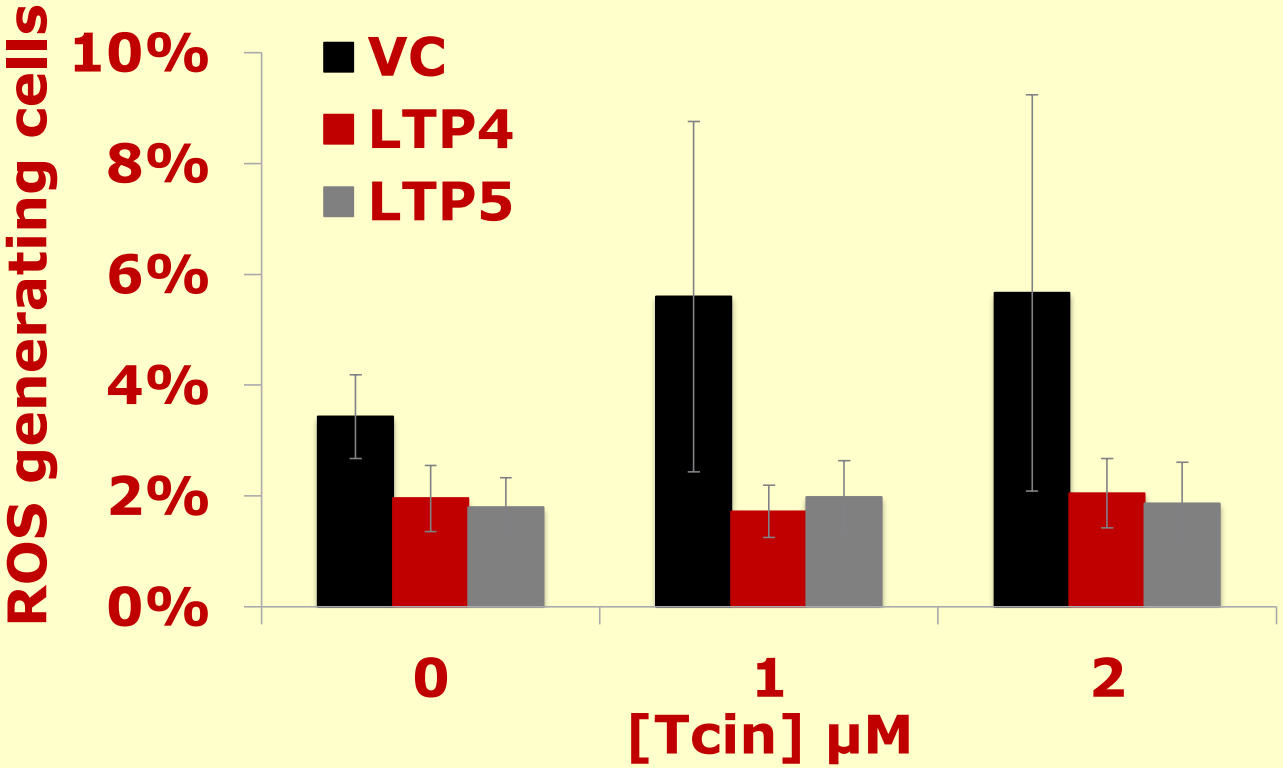
# Are early and late time point events due to Tcin affected by LTP overexpression?

# LTP overexpression lowers ROS levels

**Reactive oxygen species (ROS) levels were detected by DCFH-DA staining and quantified by flow cytometry**



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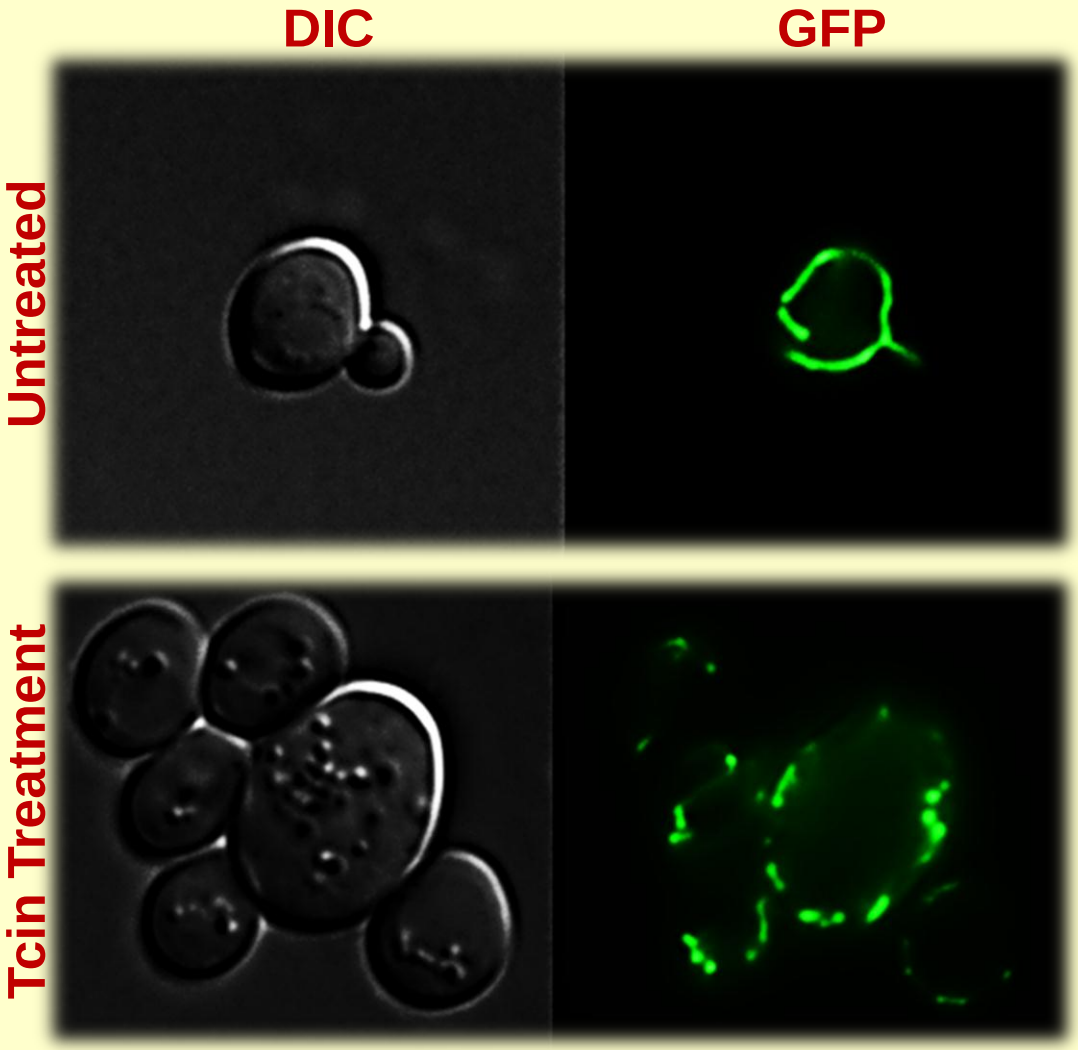
# Tcin causes mitochondrial fragmentation in yeast

**Wild type cells:**
**Tubular network**

**6h post treatment → fragmented mitochondria**



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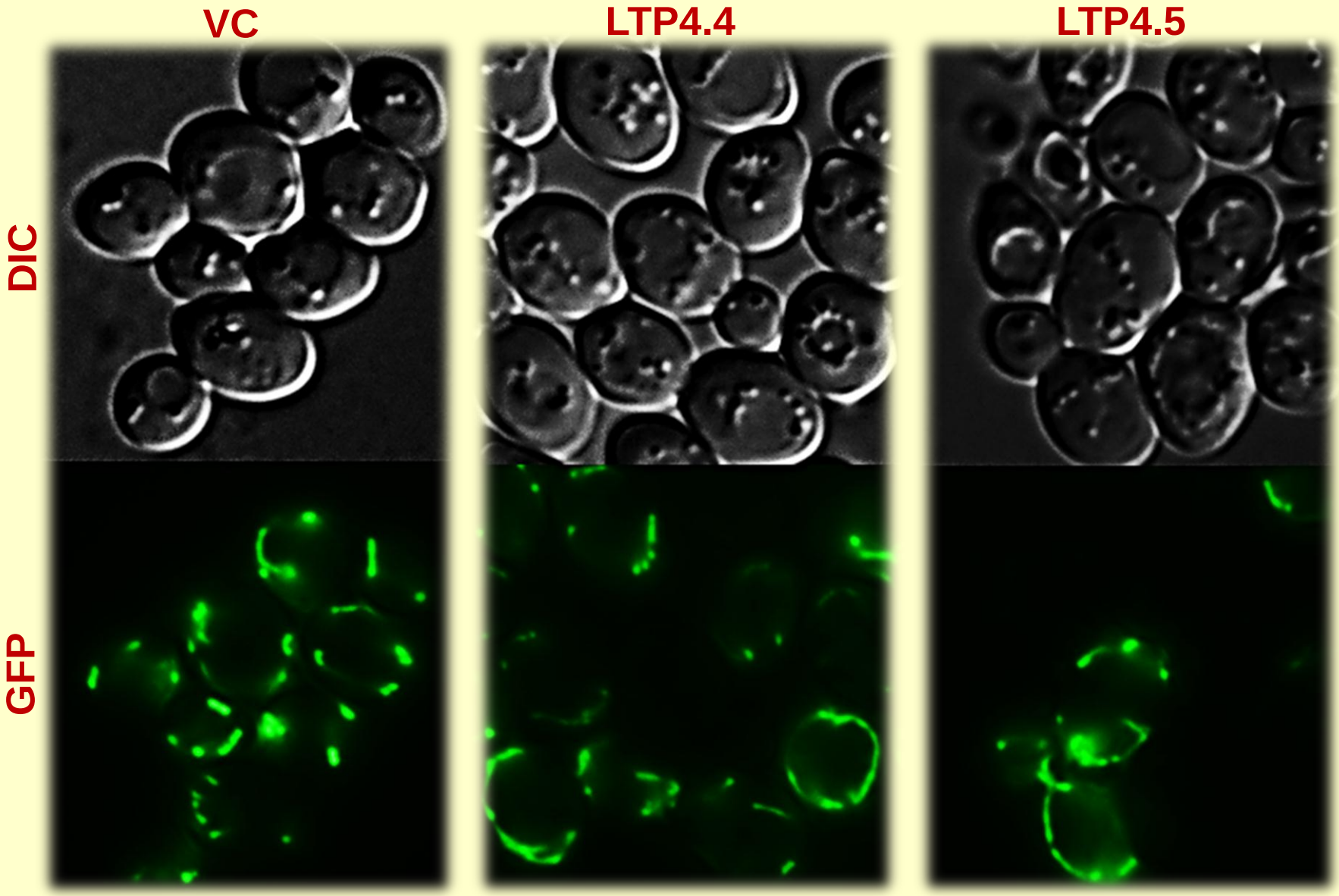
# LTP overexpression minimize mitochondrial fragmentation



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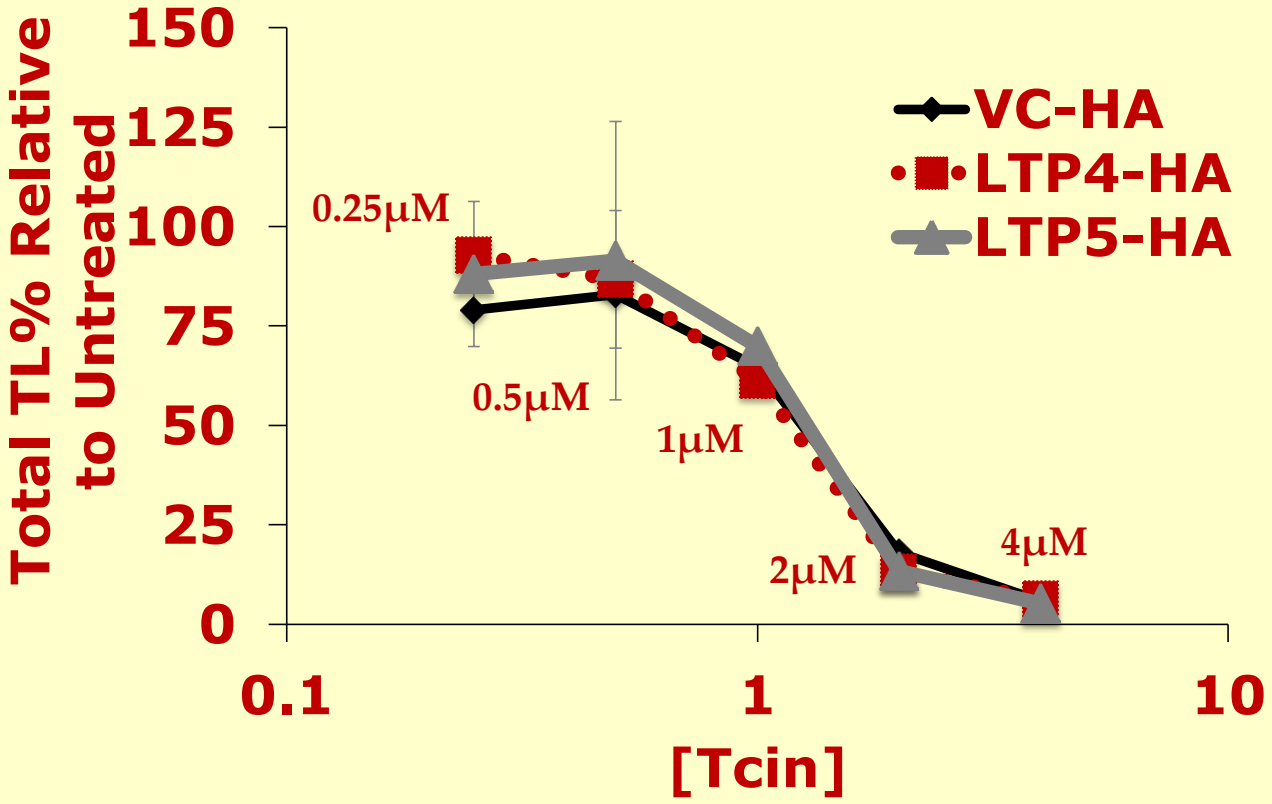
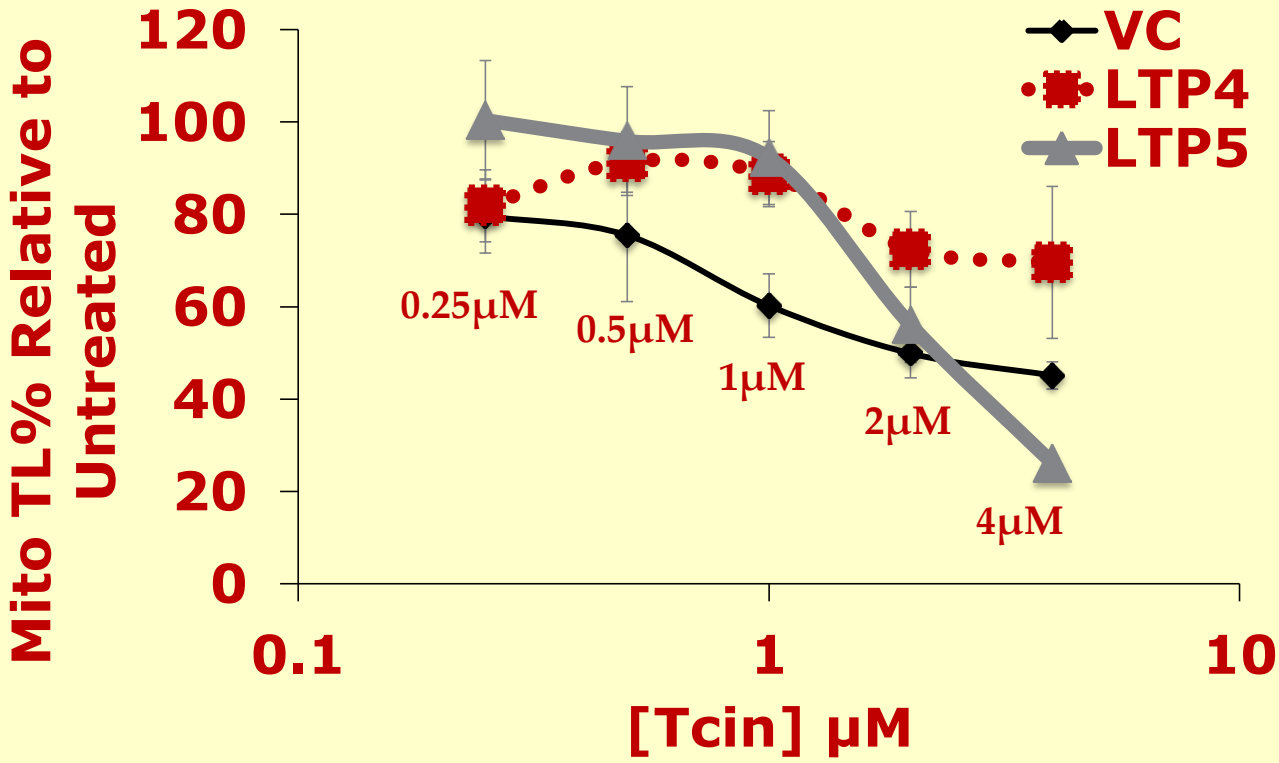
# Is inhibition of total & mitochondrial translation affected by LTP overexpression?

# Total translation is inhibited in LTP overexpressing cells



# Mitochondrial translation is *not* significantly inhibited in LTP overexpressing cells



# To summarize…

**Yeast serves as an ideal tool to identify plant LTPs that confer trichothecene resistance at the single cell level.**

**At. LTPs participate directly in resistance to Tcin.**

**LTP-mediated resistance is specific to trichothecenes and is not part of a general response against other translation inhibitors.**

**Mitochondria plays a role in LTP-mediated resistance to Tcin.**

# ACKNOWLEDGMENTS


Tumer Lab

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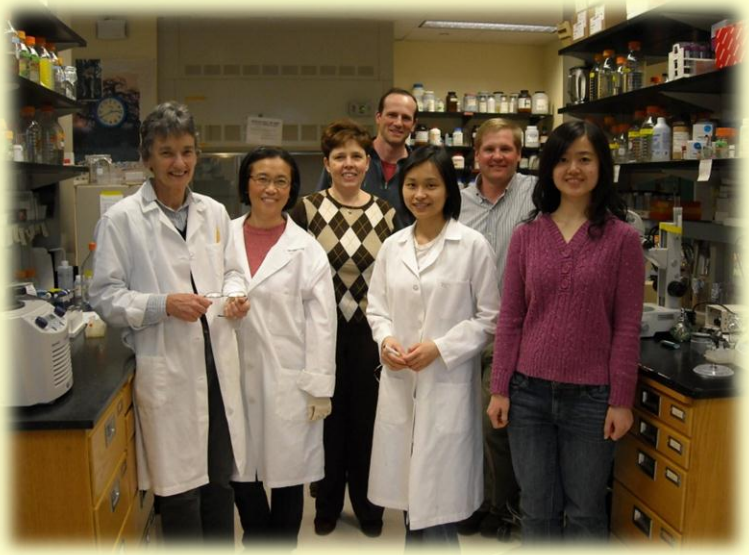
- Dr. Mike Pierce
- Dr. Kerrie May
- Qing Yan
- Debaleena Basu



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