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

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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

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

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)
Yeast cells are more sensitive to trichothecenes when carbon source is glycerol.
Rho° (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.

In organello translation assay
Is mitochondrial membrane integrity damaged by trichothecenes?

- All mitochondrial morphology related mutants resistant to Tcin were defective in membrane fusion.
To visualize mitochondrial morphology → Yeast cells transformed with mtGFP

- Untreated → tubular network.

- 6 h post treatment → fragmented mitochondria.

Trichothecenes alter mitochondrial morphology
Mitochondrial Membrane Potential ($\psi_{mito}$): Biomarker for membrane integrity.

Membrane depolarization $\rightarrow$ fragmentation.

MitoTracker Red: Only enters actively respiring mitochondria.

Untreated $\rightarrow$ High fluorescence $\rightarrow$ $\psi_{mito}$ $\uparrow$ actively respiring mitochondria

6 h treatment $\rightarrow$ Low fluorescence $\rightarrow$ $\psi_{mito}$ $\downarrow$ dysfunctional mitochondria
Is mitochondrial translation inhibition due to membrane damage?

- Trichothecenes inhibited translation in isolated mitochondria.
- Trichothecenes $\rightarrow$ membrane depolarization $\rightarrow$ fragmentation.
Trichothecenes do not alter mitochondrial morphology at low doses.

- Identified low doses $\rightarrow$ no membrane damage.
- To visualize mitochondrial morphology $\rightarrow$ Yeast cells transformed with mtGFP.
- 6 h post treatment $\rightarrow$ no severe fragmentation.
Mitochondrial membrane damage occurs late during trichothecene toxicity

- 6 h treatment with low doses $\rightarrow$ no significant changes to $\psi_{mito}$
- 18 h treatment with low doses $\rightarrow$ drop in $\psi_{mito}$ (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.
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.
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Questions?