Aspergillus fumigatus signal transduction mechanisms for secondary metabolism production and self-protection

Gustavo H. Goldman

Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo, Brazil

Aspergillus: the ‘Dr Jekyll and Mr Hyde’ genus of fungi

Mr. Hyde personality:

<u>Opportunistic pathogens</u>:
Aspergillus fumigatus
Aspergillus flavus
Aspergillus sydowii

Dr. Jekyll side:

<u>Model system</u>:
Aspergillus nidulans
<u>Biotechnology</u>:
Aspergillus niger
Aspergillus oryzae
Aspergillus sojae
Aspergillus kawachii

185 recognized species of *Aspergillus*, 20 are known to cause infections in humans (aspergillosis). *Aspergillus fumigatus* causes about 65% of all invasive infections in humans and is the mostly encountered species in pulmonary infections

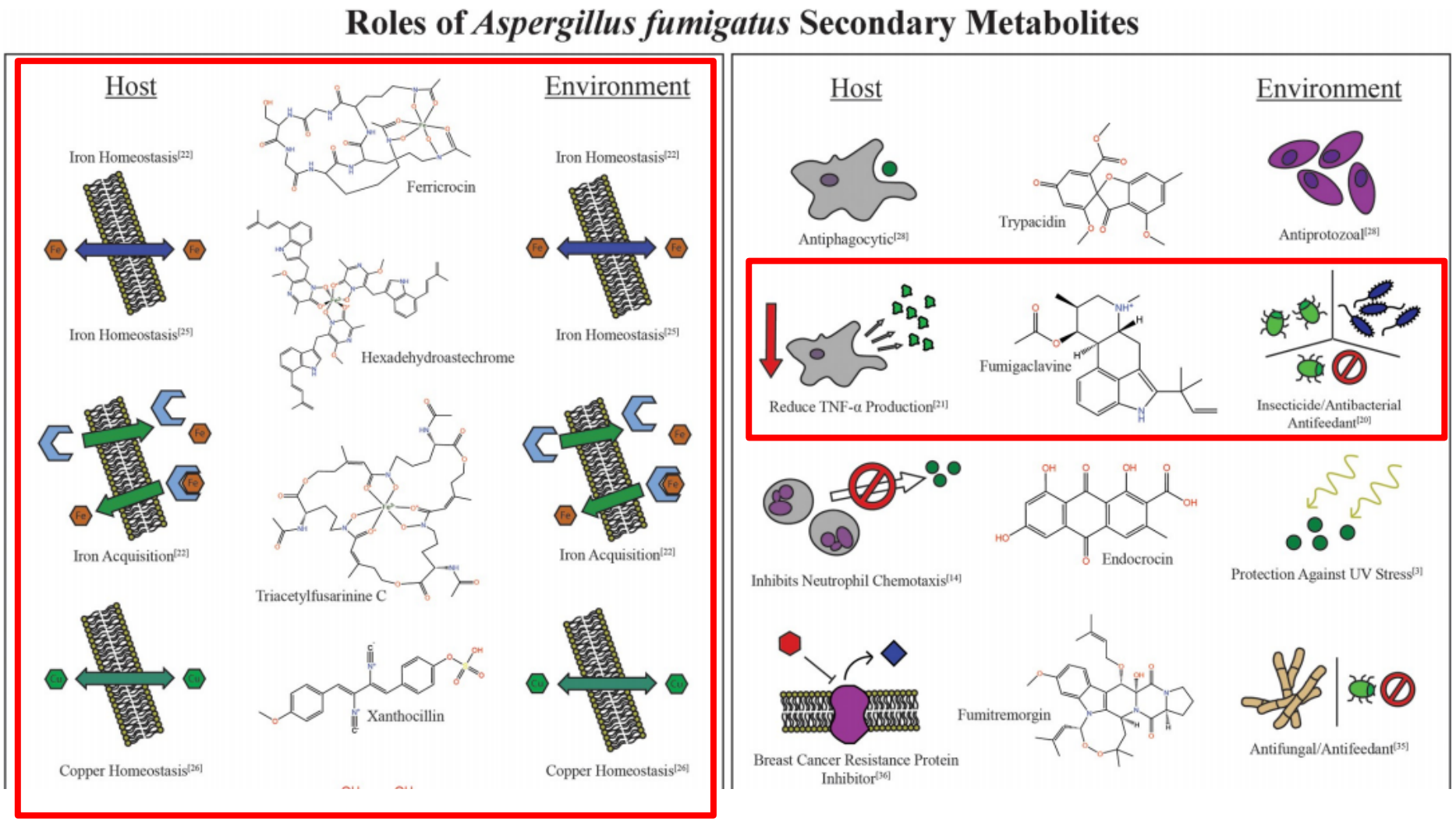
Gibbons, J.G. and Rokas, A. 2013. Trends in Microbiology, 21: 14–22

Aspergillosis (most of it due to *A. fumigatus*)

There are an estimated 3,000,000 cases of pulmonary aspergillosis annually and more than 200,000 cases of IA each year reaching a mortality rate of up to 90% in the most susceptible populations

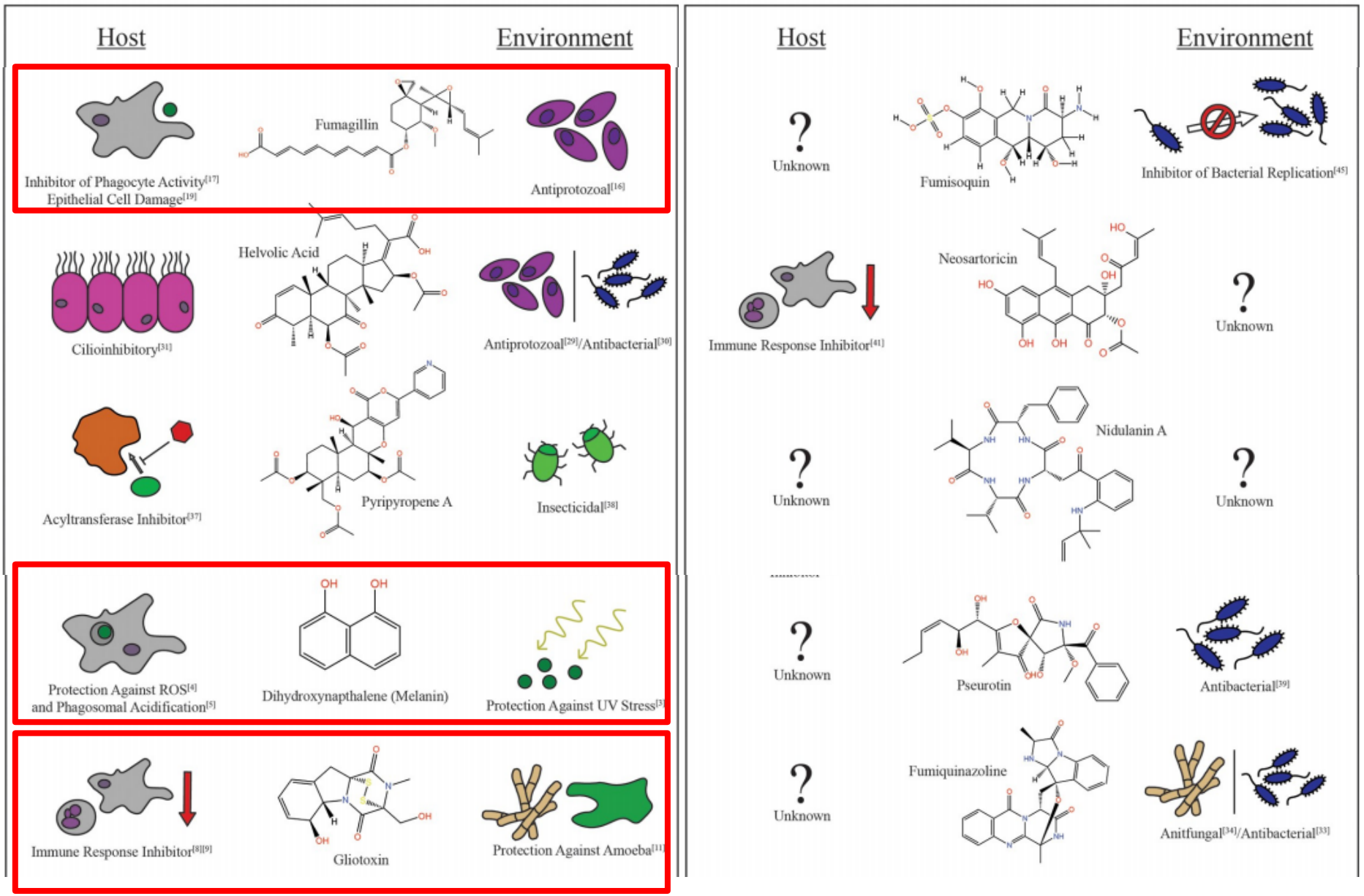
Interaction of *Aspergillus* with the host

One unique feature of this pathogen is its arsenal of small molecules that impact disease development



Raffa and Keller, 2019 PLoS Pathog. 15(4):e1007606

Roles of *Aspergillus fumigatus* Secondary Metabolites



Raffa and Keller, 2019 PLoS Pathog. 15(4):e1007606

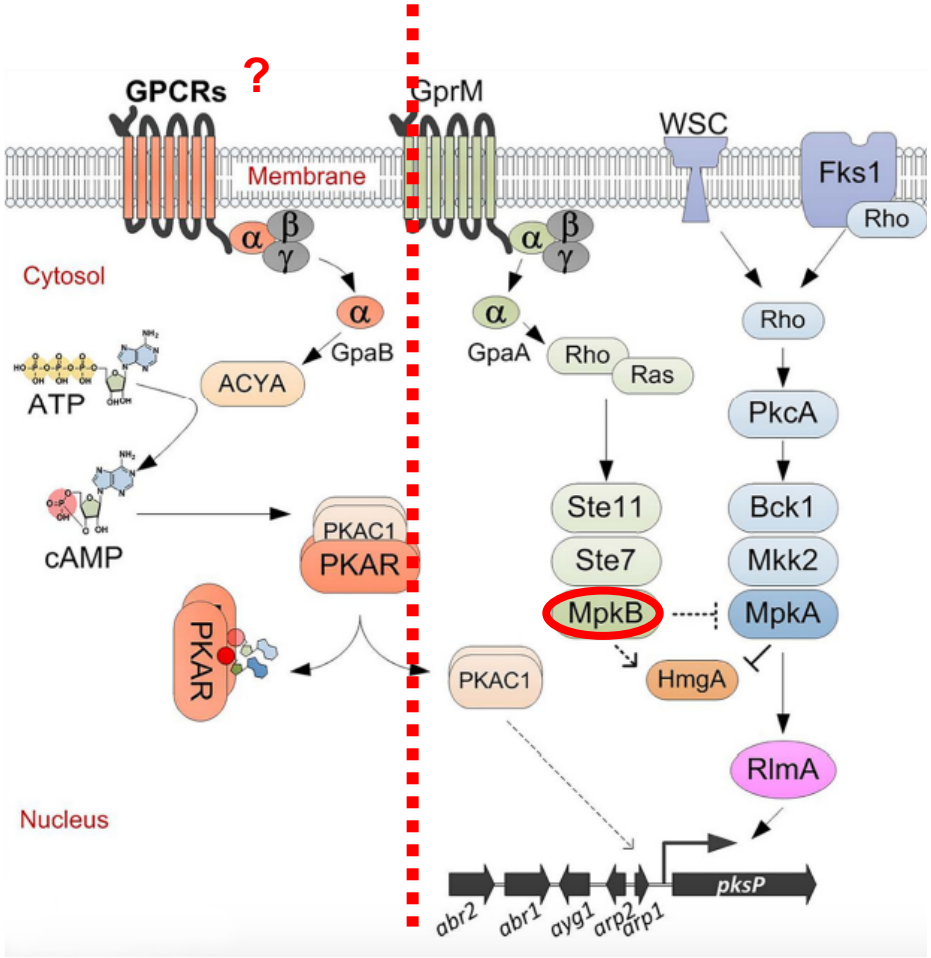
Evasion and modulation of the host immune system

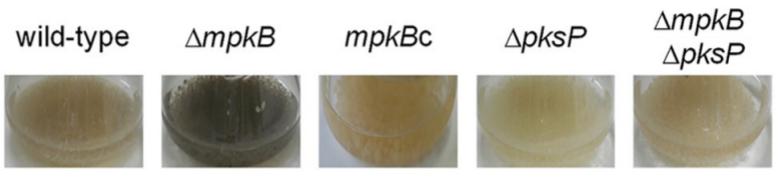
- Conidial **melanin**: 1. pyomelanin protects against ROS and cell wall stress; 2. **DHN melanin** possesses immune-modulatory properties

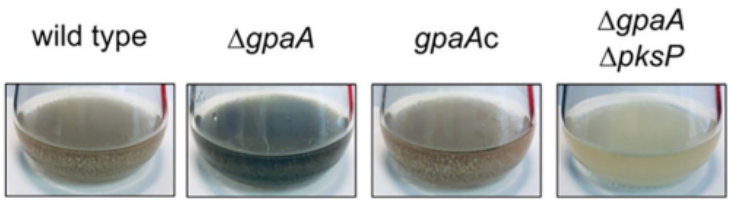
- **Fumagillin** prevents neutrophil degranulation and correct assembly of NADPH oxidase, it can cause epithelial cell damage

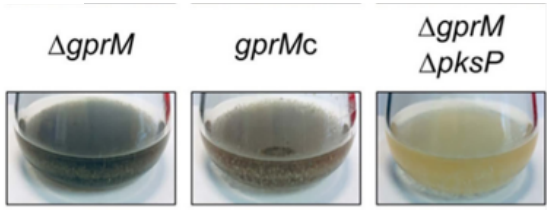
- **Gliotoxin** inhibits macrophage-mediated phagocytosis, NADPH oxidase assembly, subunit phosphorylation and translocation as well as inhibiting the biosynthesis of the neutrophil chemoattractant leukotriene

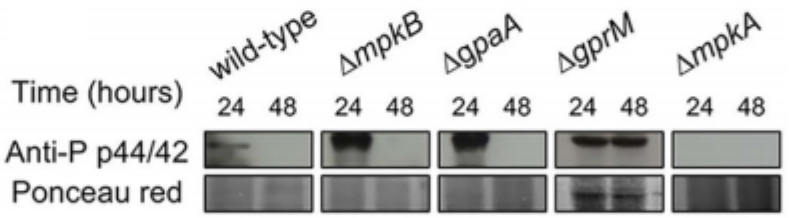
There is increased DHN-melanin production in the MAPK *mpkB*^{FUS3}, and GPCRs *gprM* and G-α protein *gpaA* null mutants



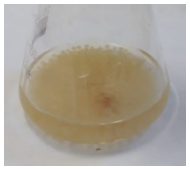




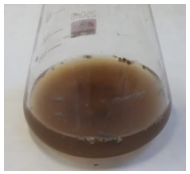




Wild-type

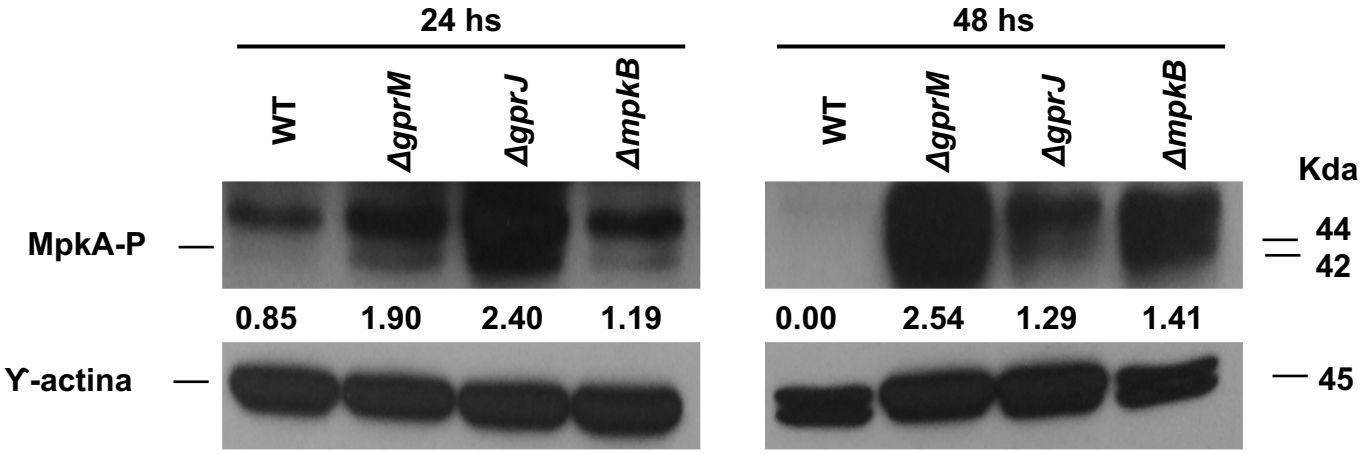


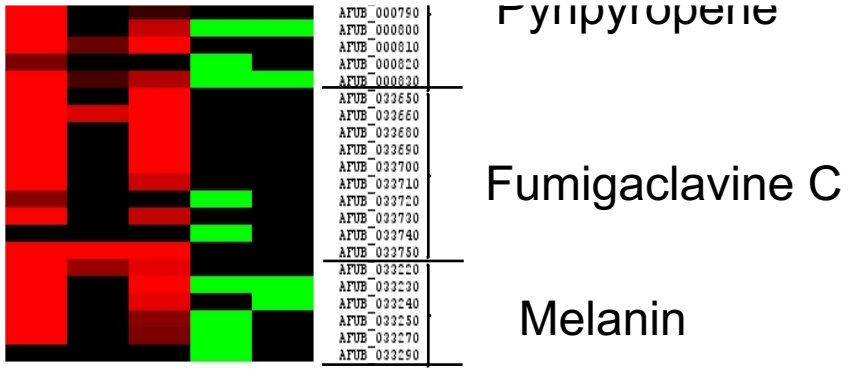
ΔgprJ



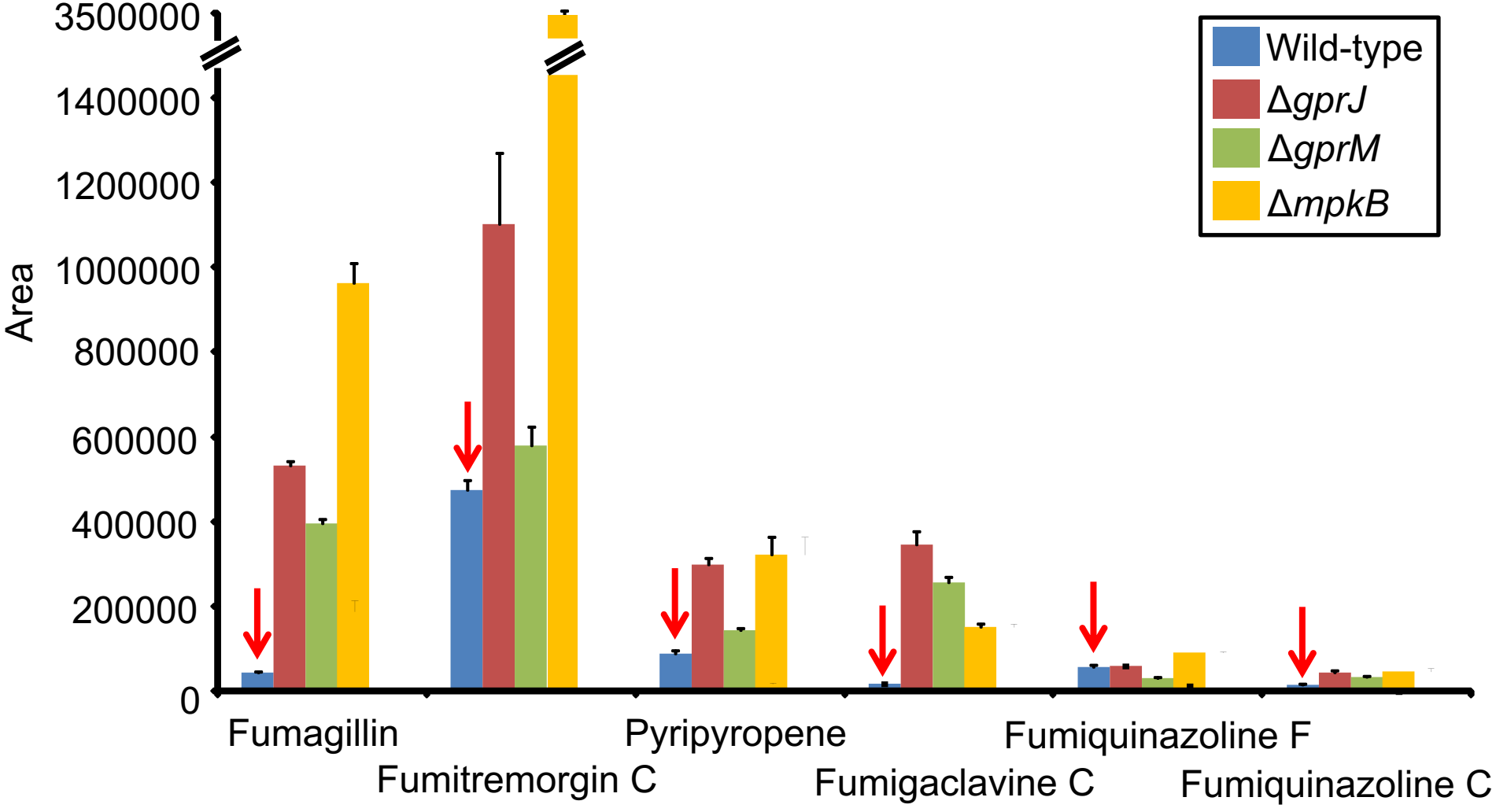
Manfiolli *et al.* (2019), mBio 10(2)

RNAseq shows that MpkB^{Fus3}, GprM, and GprJ also regulate the production of other secondary metabolites, such as fumagillin

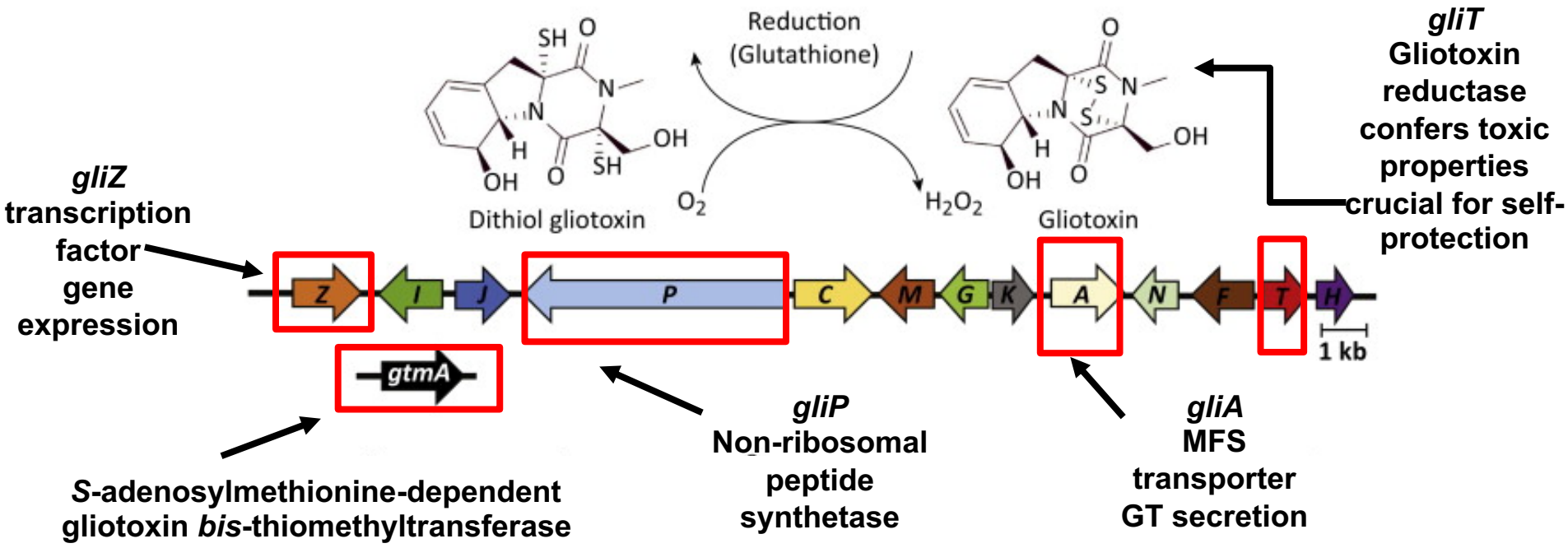




Mass spectrometry validates it for MAPK MpkB^{Fus3} and GPCRs GprM and GprJ



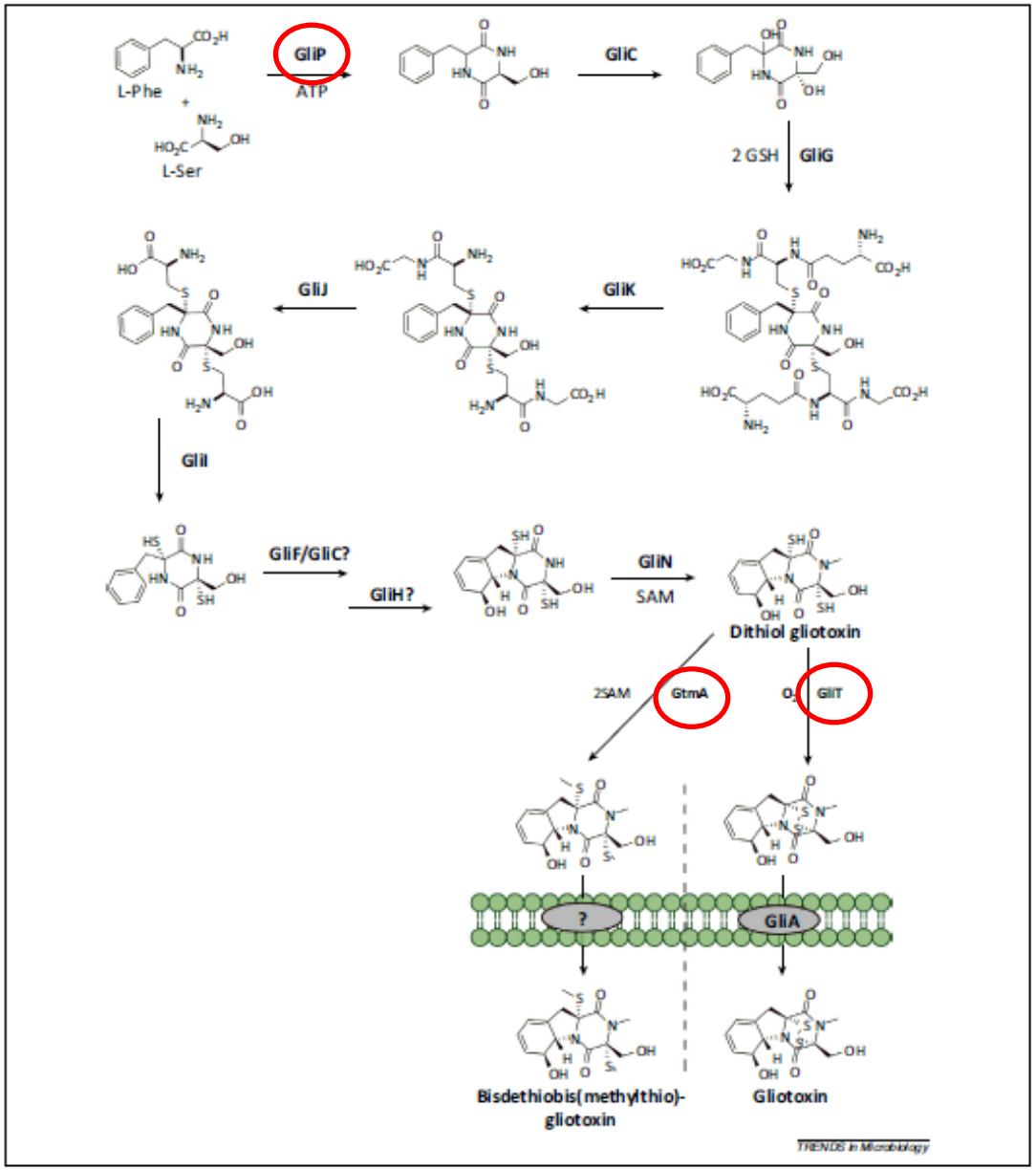
Gliotoxin biosynthesis, secretion, and regulation



- **GliZ does not regulate the expression of *gliT***
- Interconversion of gliotoxin between the reduced (dithiol) and oxidized (disulfide) forms
- **Transcription factor regulating *gliT* expression remains unknown**

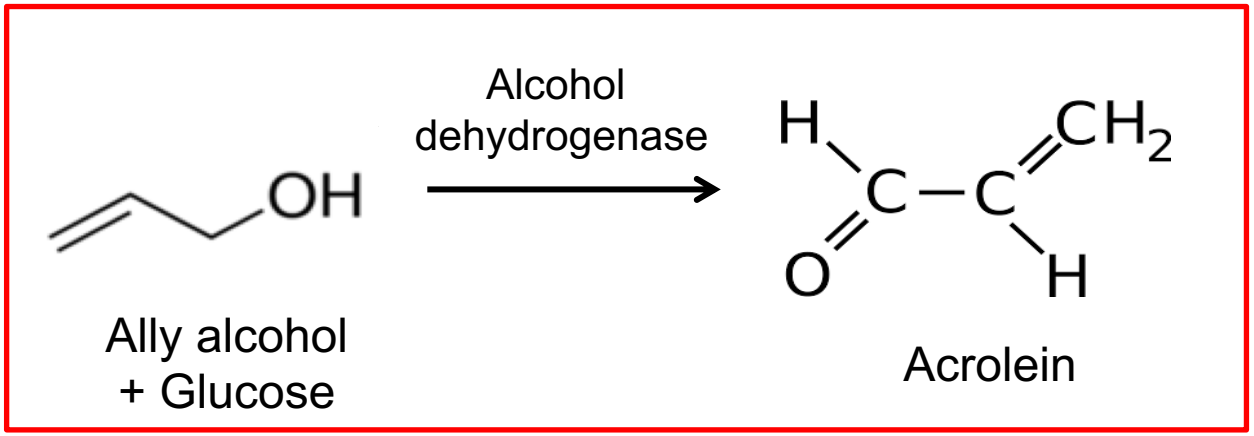
Dolan *et* al., 2015. Trends Microbiol. 23(7):419-28

Gliotoxin biosynthesis, secretion, and regulation

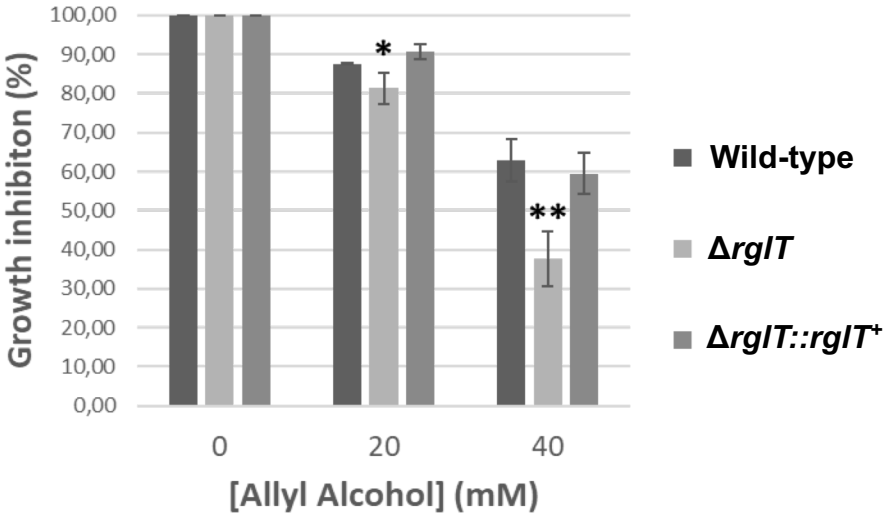


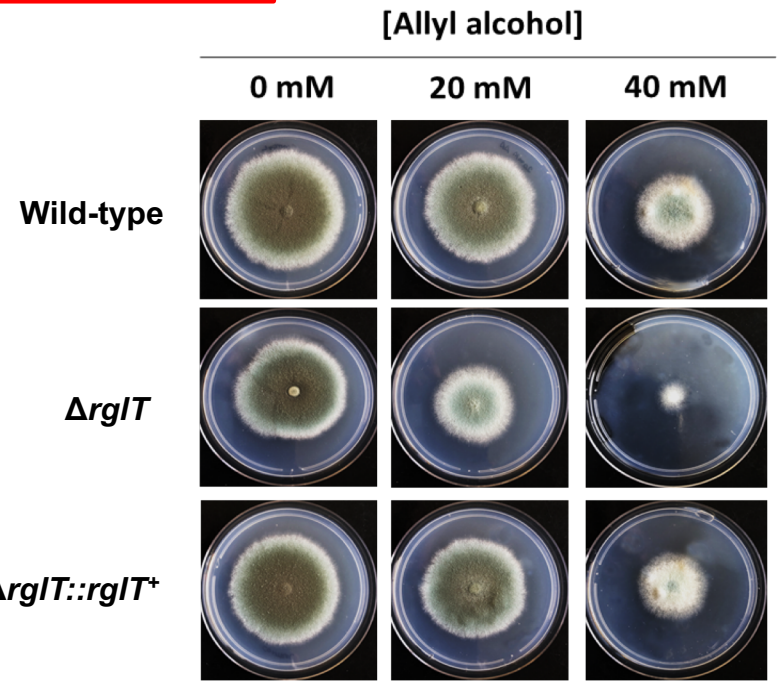
Dolan *et* al., 2015. Trends Microbiol. 23(7):419-28

Screening for allyl alcohol/acrolein/oxidative and stress sensitivity allowed us to identify RglT [Zinc binuclear (Zn(II)2Cys6) transcription factor]



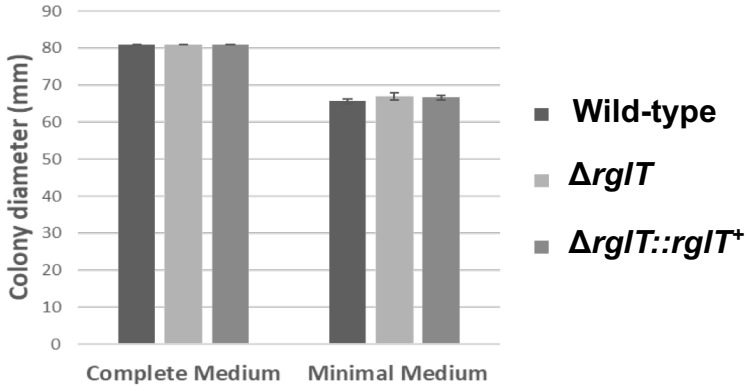
Screening of *A. fumigatus* 395 transcription factors null library 20 and 40 mM AA





High sensitivity of Δ*rglT* to AA is not due to defects in Carbon Catabolite Repression (CCR)

a



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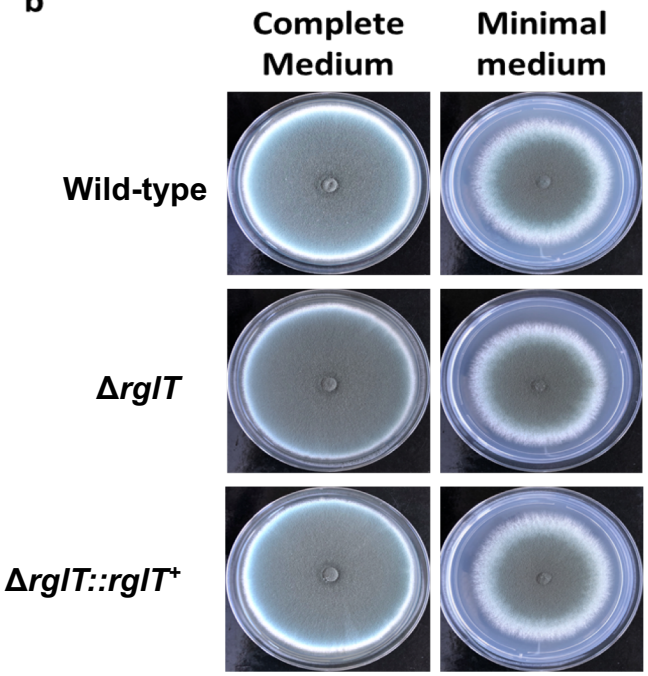

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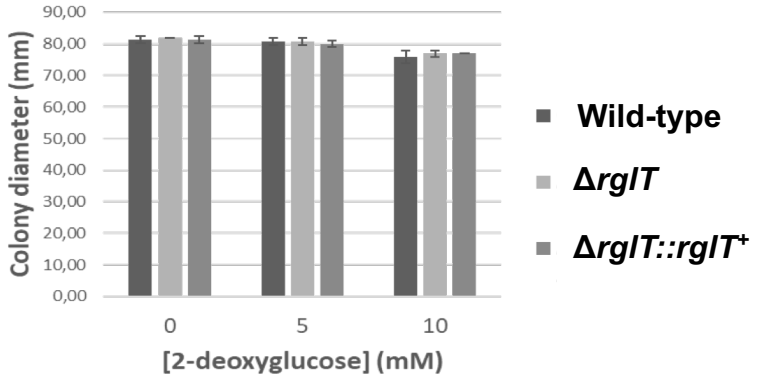
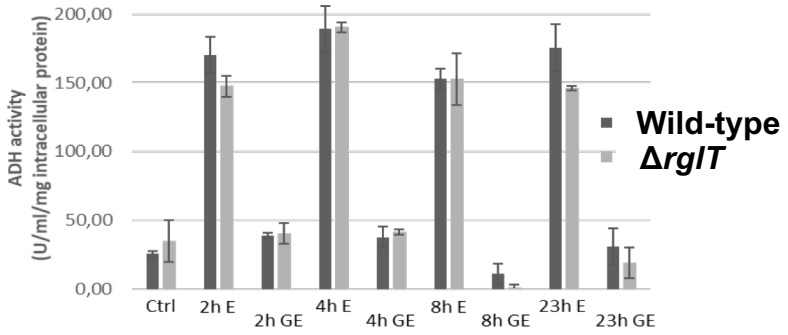
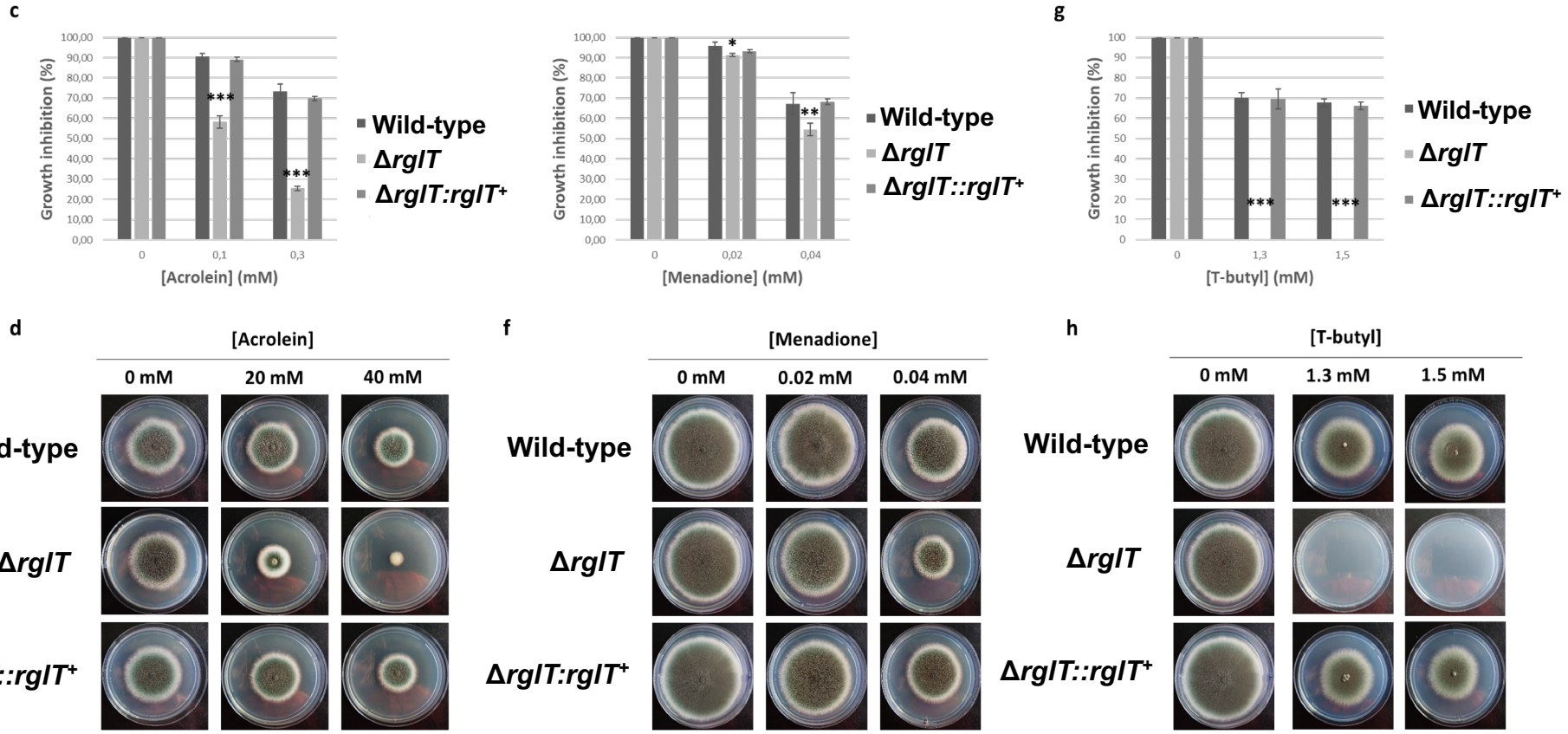


d



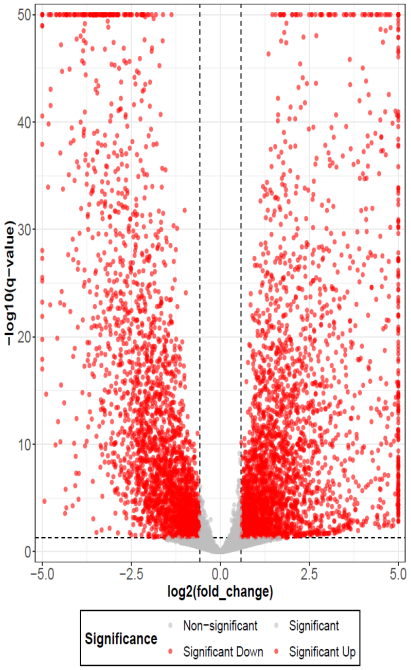
The Δ*rglT* is sensitive to acrolein and oxidative stressing agents



RNA-sequencing aiming to identify possible modulated genes

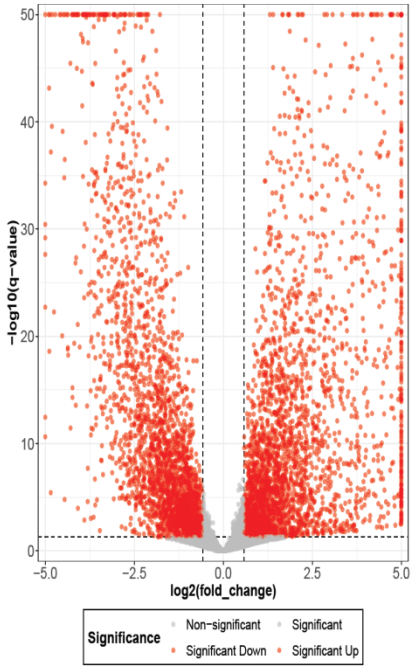
Down – 2,666 genes
Up – 2,932 genes

wild-type AA 10 mM/30 min
x
wild-type Ct



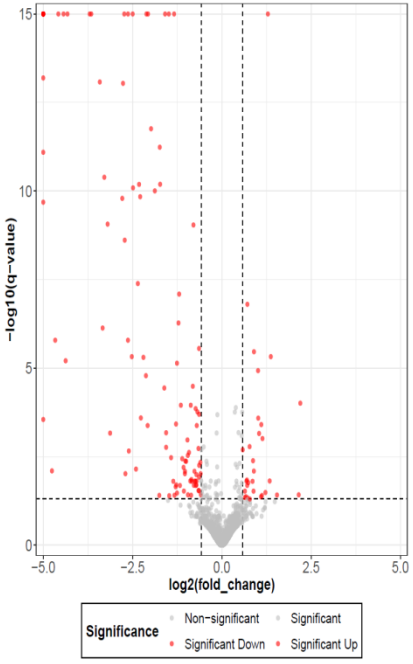
Down – 2,690 genes
Up – 2,859 genes

Δ*rglT* AA 10 mM/30 min
x
Δ*rgl* Ct



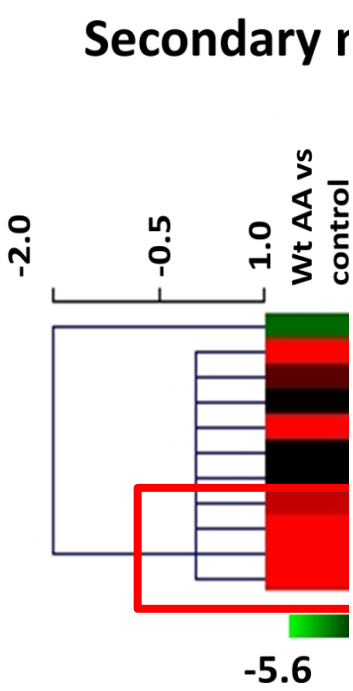
Down – 98 genes
Up – 17 genes

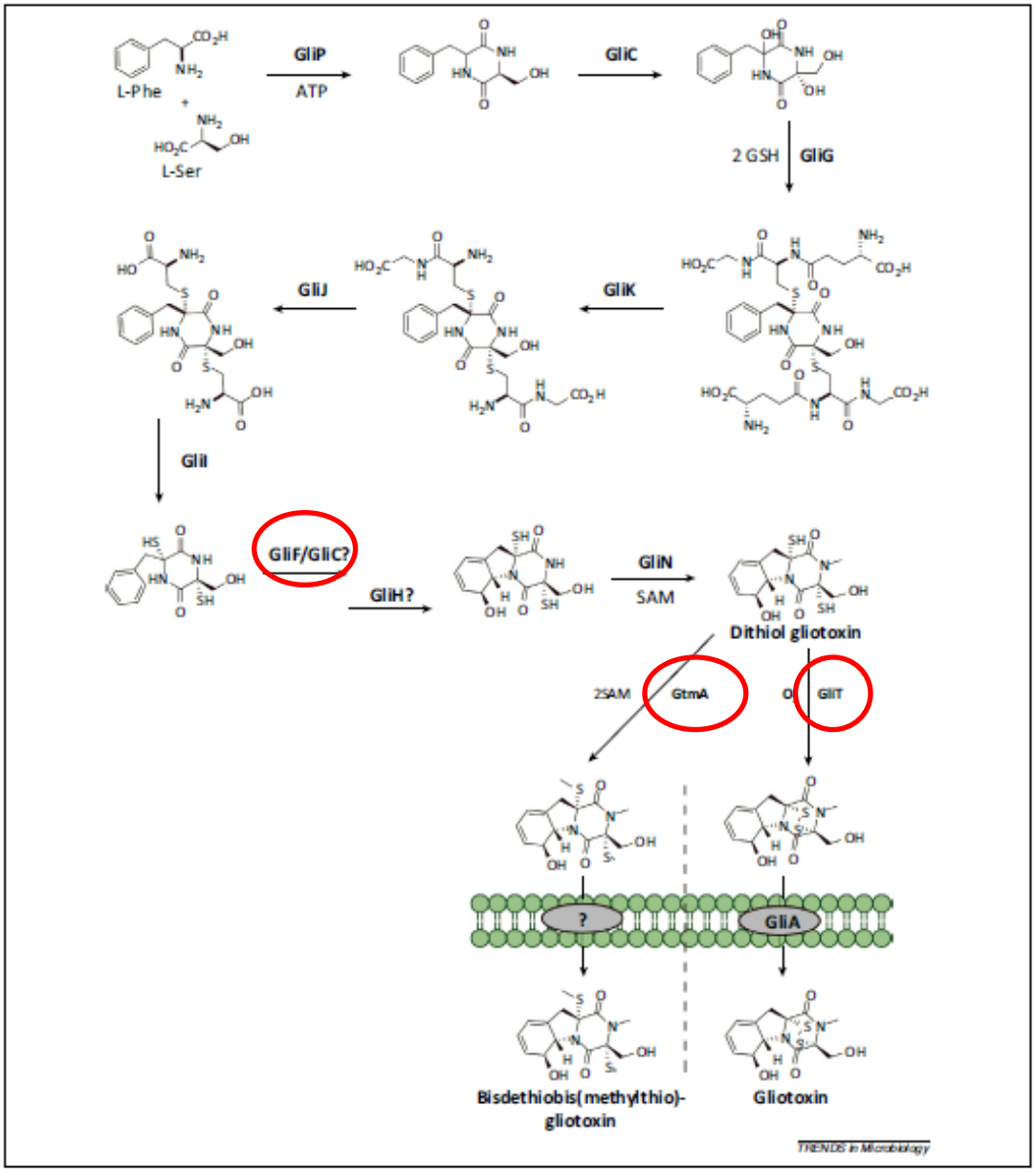
Δ*rglT* AA 10 mM/30 min
x
wild-type AA 10 mM/30 min

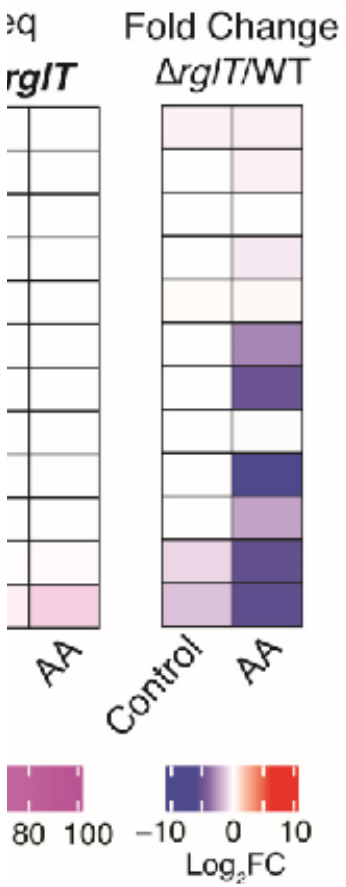


Allyl Alcohol induced a dramatic transcriptional response

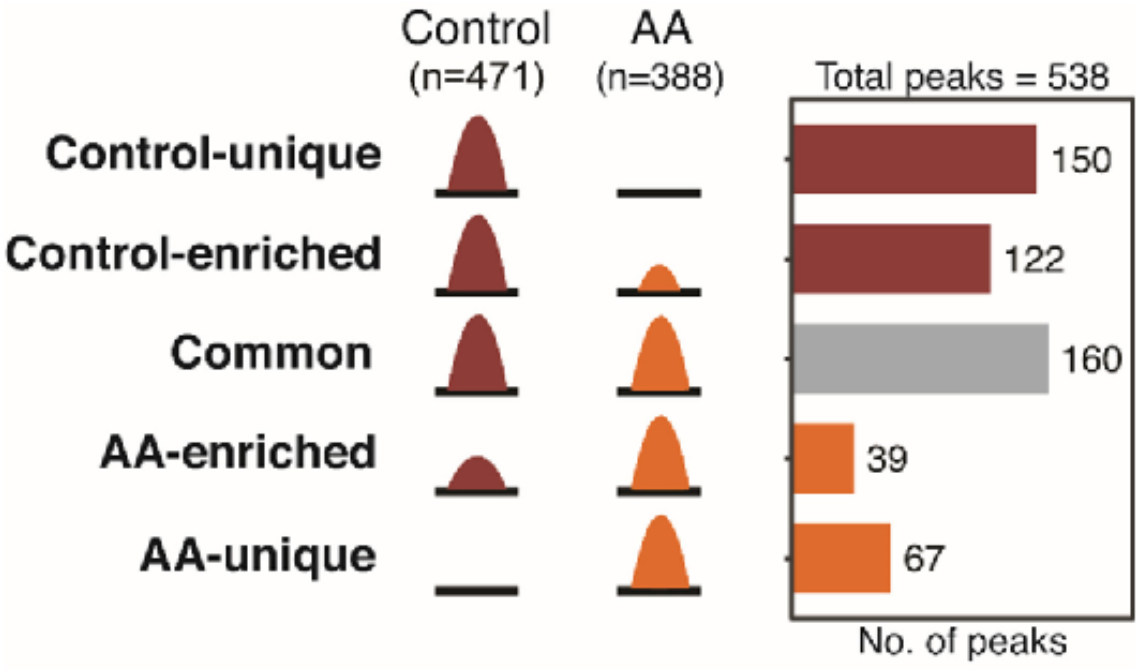
RNA-sequencing identifies *gliT* and some

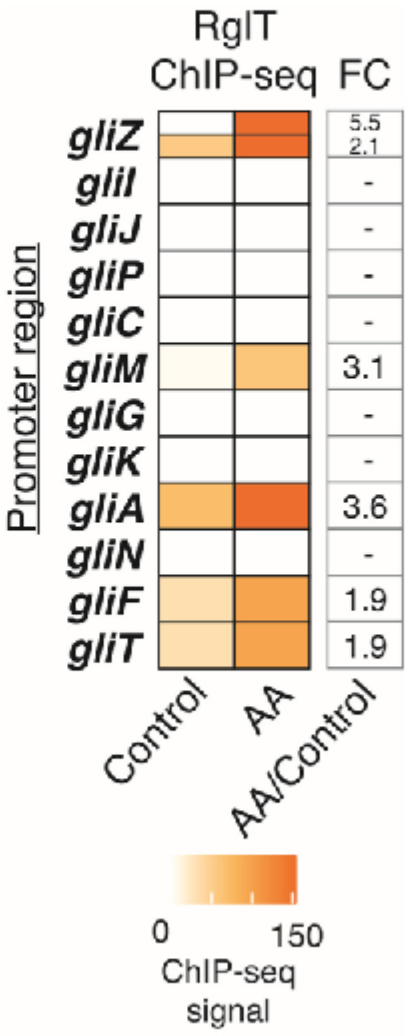






ChIP (chromatin immunoprecipitation) sequencing to identify RglT-binding promoter regions





RglT also binds directly to *gtmA* but not to *egtA* !!!!!

ChIp PCR also shows direct binding of RglT to the promoter regions of *gliZ*, *gliT*, and *gliF* upon gliotoxin inducing conditions

gliZ

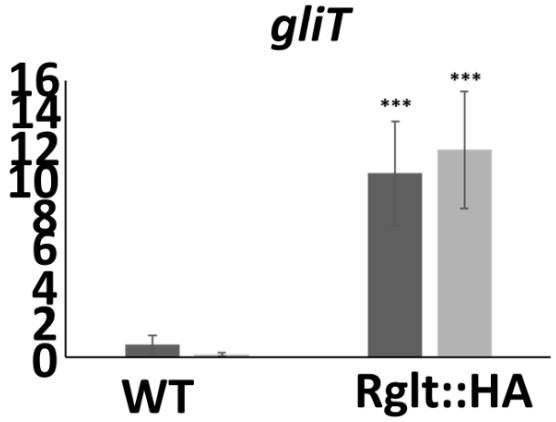
Sequence	Upstream from ATG (bp)
GGCGGGG	-641

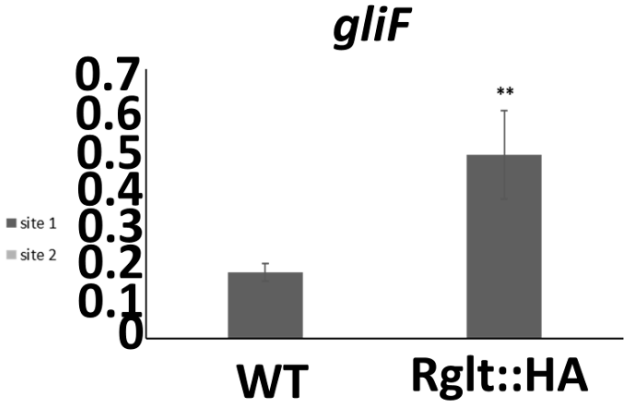
gliT

Sequence	Upstream from ATG (bp)
CAGGCCG	-368
CCGCACG	-335
CCCGACG	-81

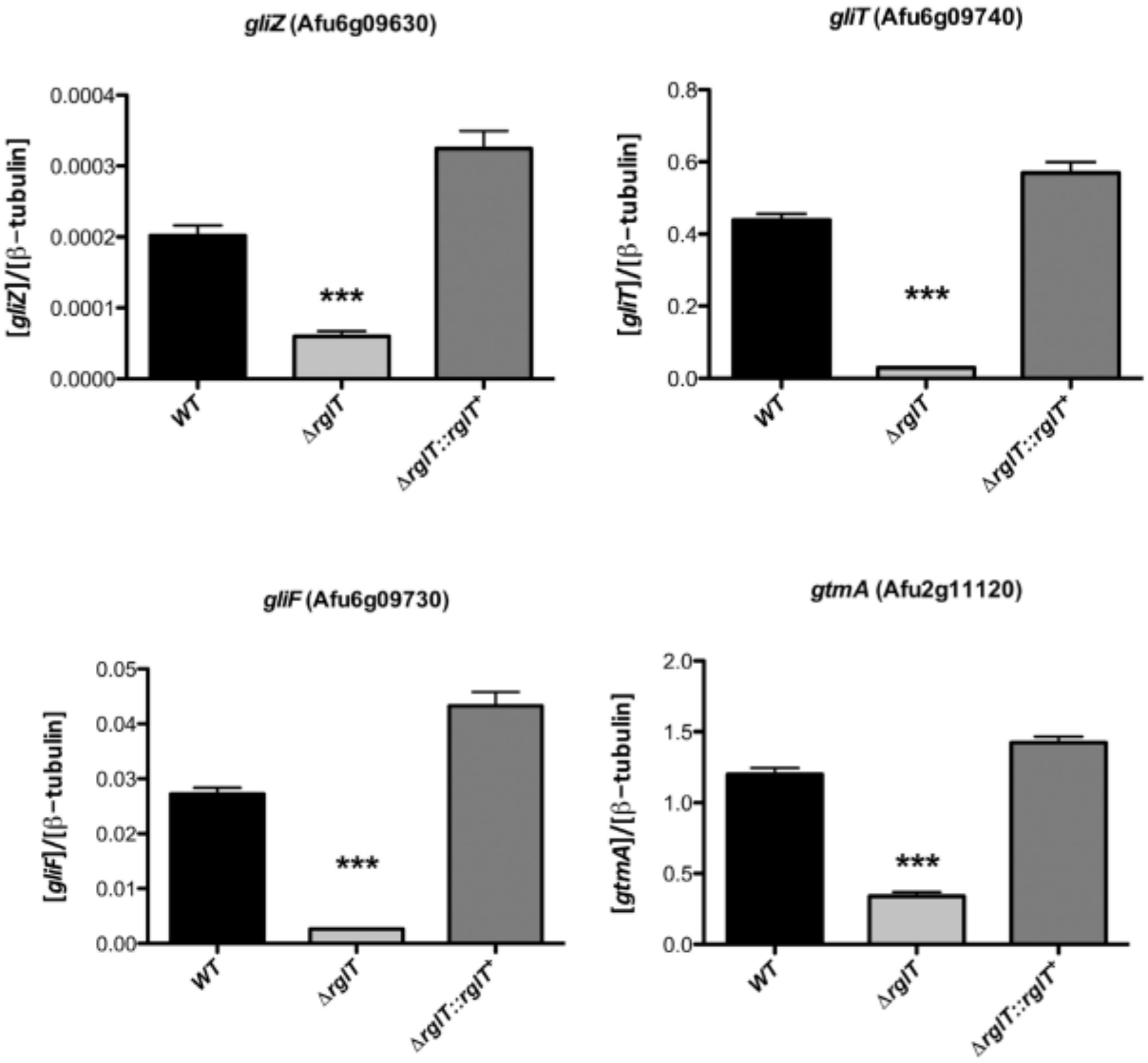
gliF

Sequence	Upstream from ATG (bp)
CAGGCCG	-398

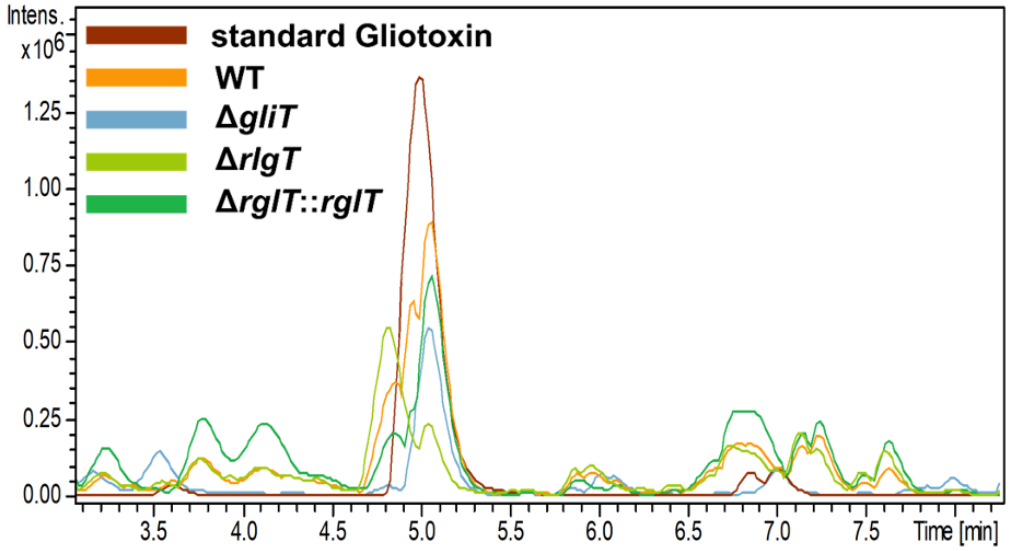


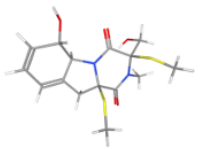
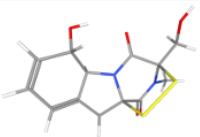


There is loss of transcriptional induction of *gliT*, *gliF*, *gtmA, and gliZ* upon gliotoxin inducing conditions



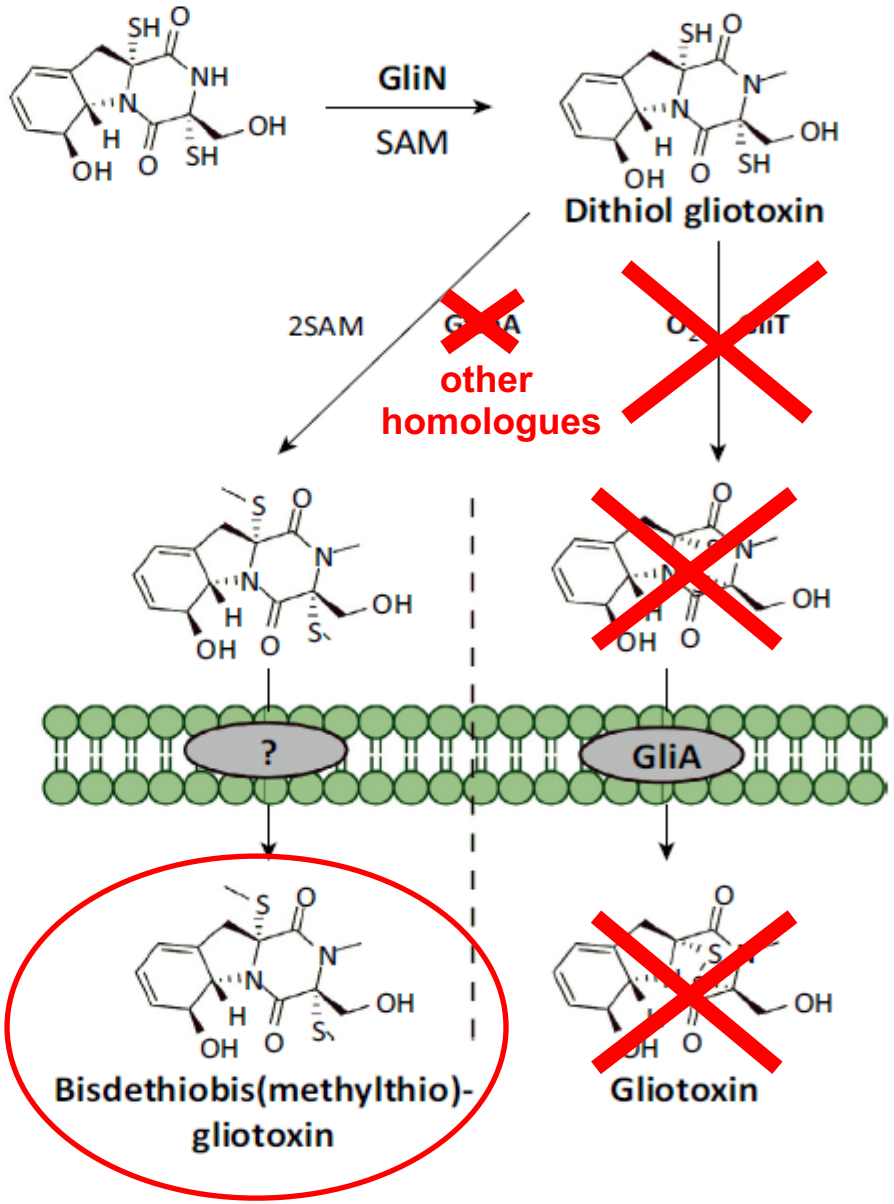
In gliotoxin-inducing conditions Δ*rglT* does not produce GT but rather BmGT



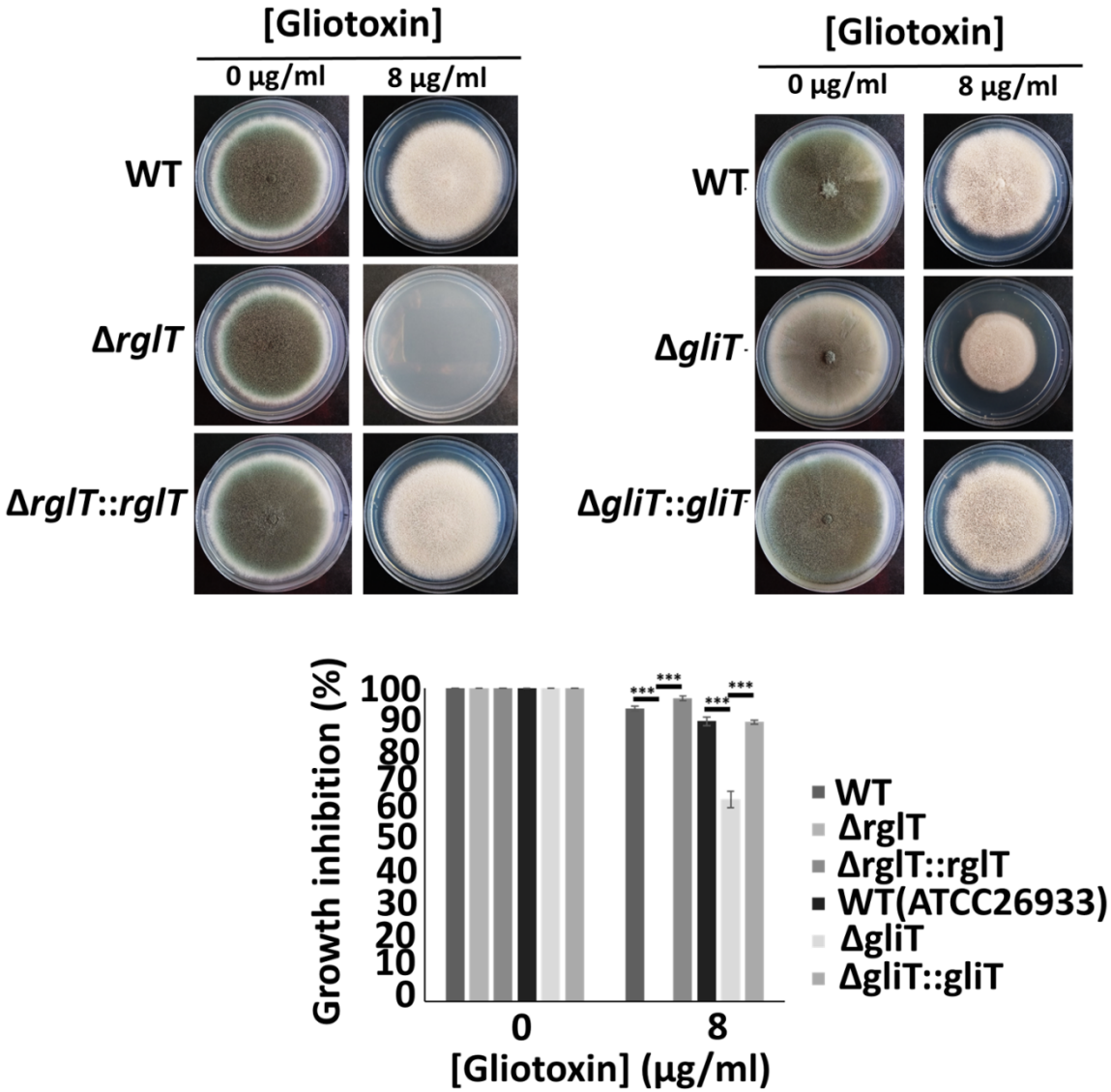
	ΔrlgT	ΔrglT::rglT	ΔgliT	WT
 bisdethiobis(methylthio) gliotoxin	●	●	●	●
 Gliotoxin	●	●	●	●

● Detected ● Not detected

Δ*rglT* does not produce GT but rather BmGT



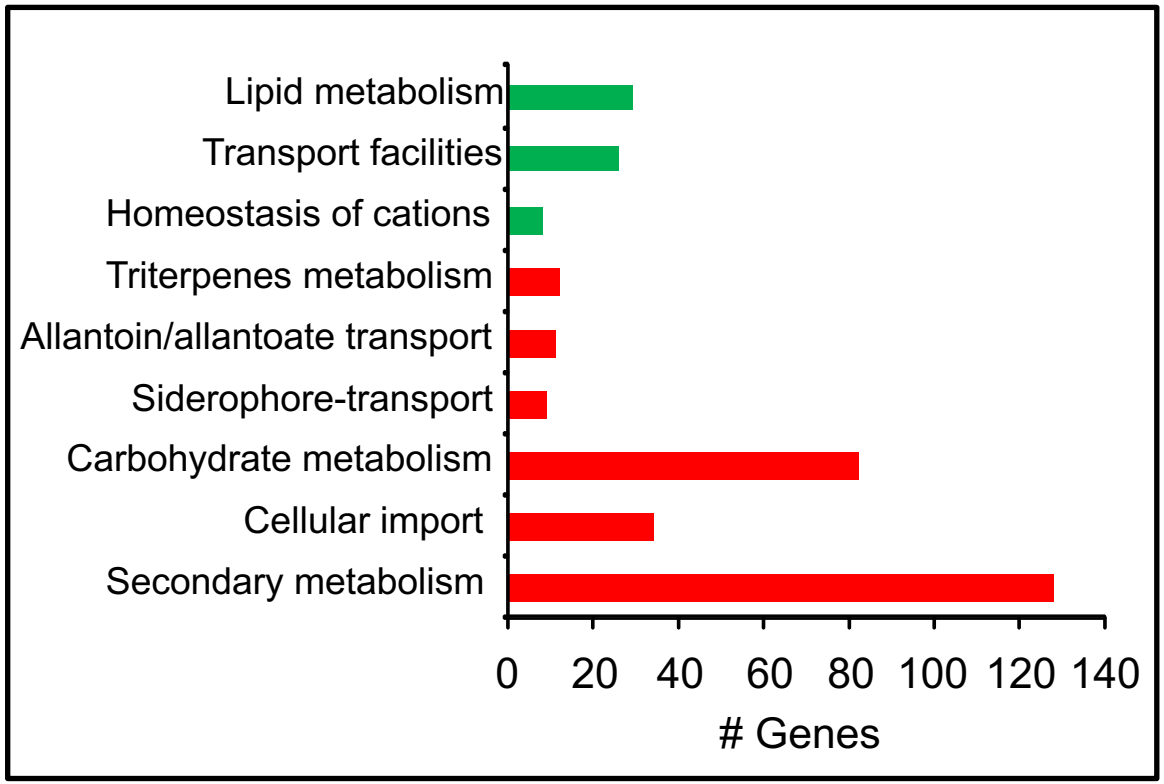
Deletion of *rglT* results in the loss of self-protection from gliotoxin

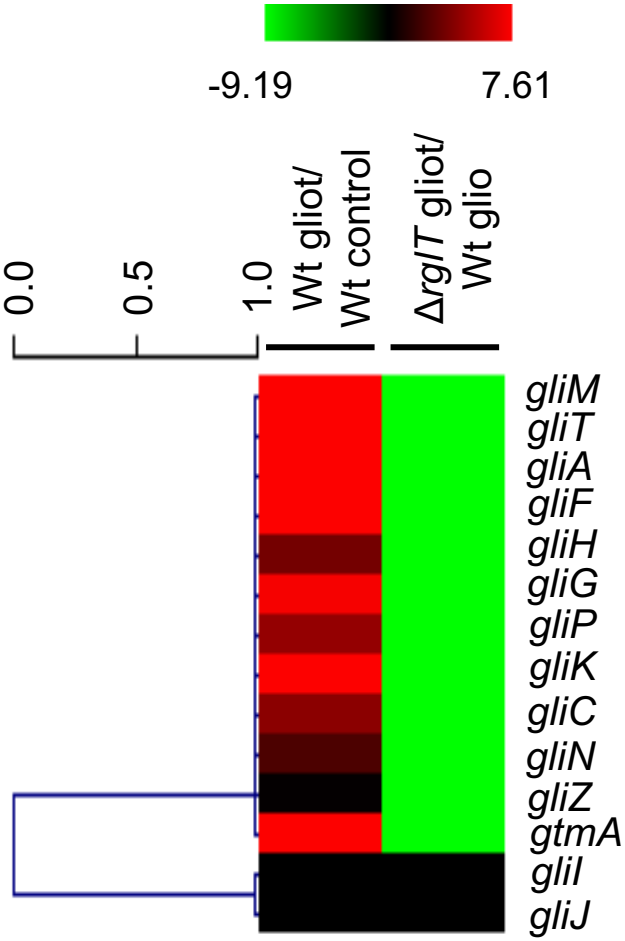


RNA seq for gliotoxin sensitivity (5 µg/ml for 3 h) revealed RglT (*R*egulator of *gl*iotoxin *T*) controls most of the *gli* genes

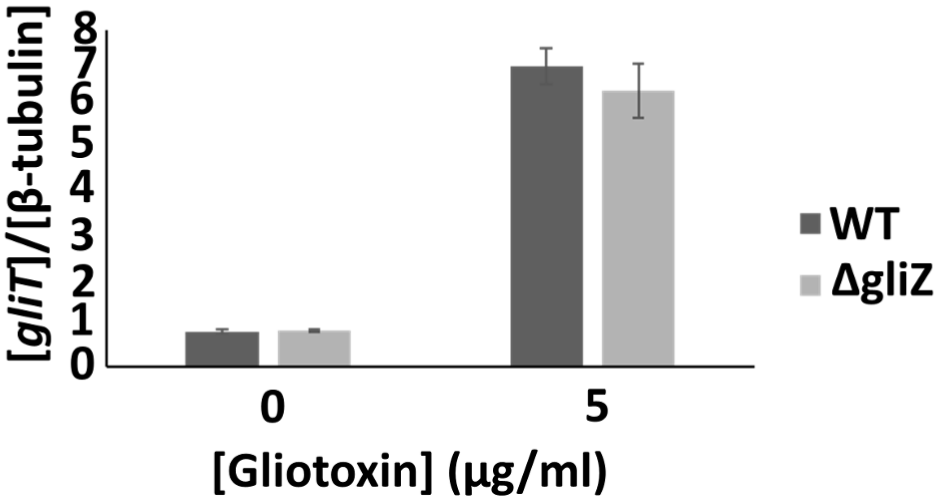
Δ*rglT* gliotoxin 5 µg/ml for 3 h
x
wild-type gliotoxin 5 µg/ml for 3 h

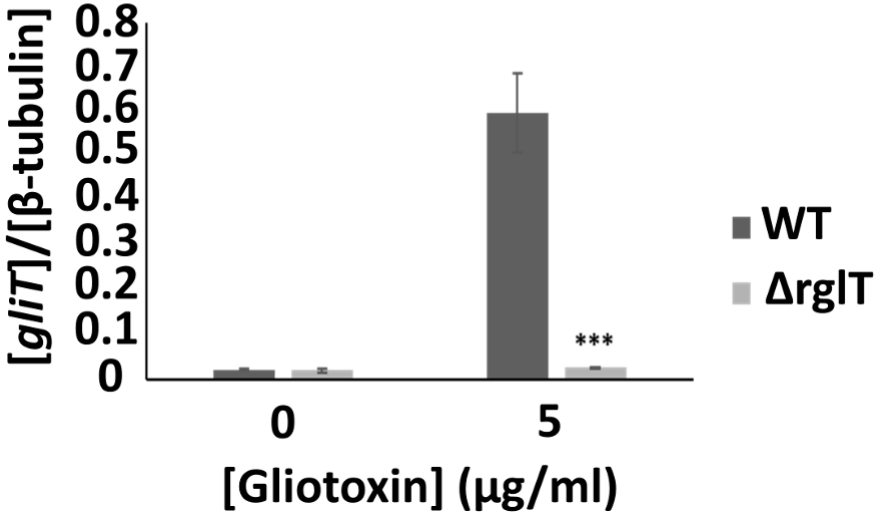
Down - 694 genes
Up - 270 genes

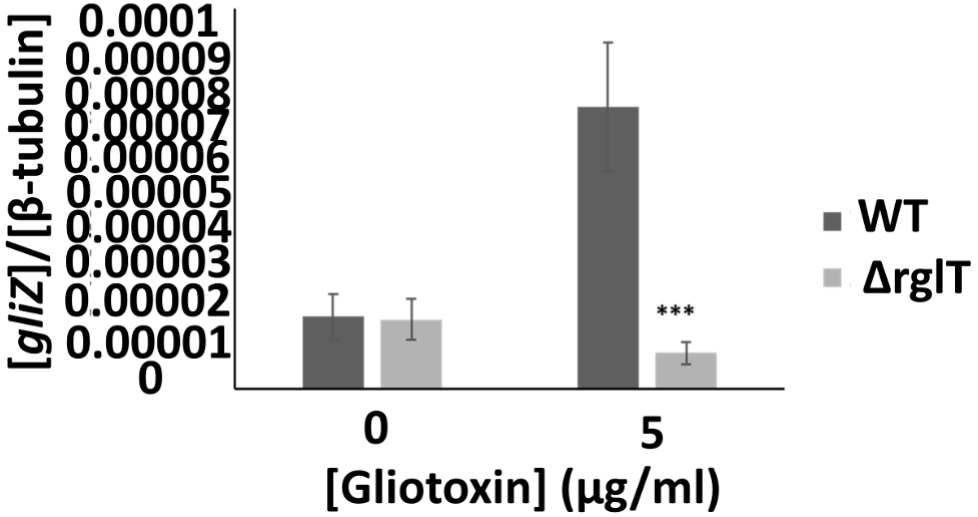


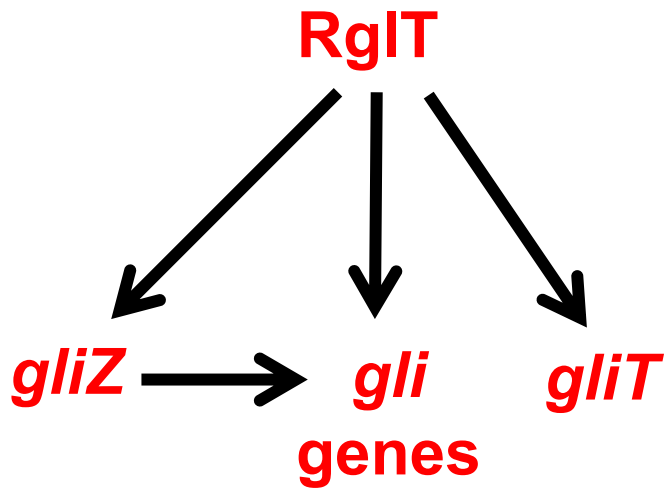


The regulation of self-protection by RglT: the *gliT* mRNA accumulation is dependent on RglT but not GliZ



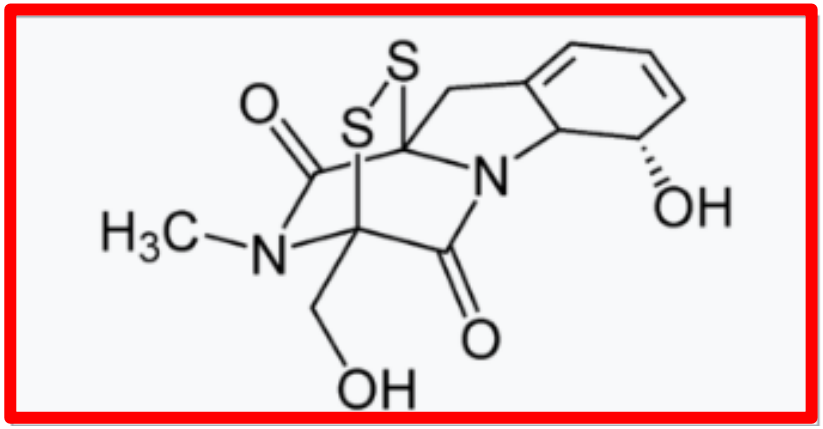






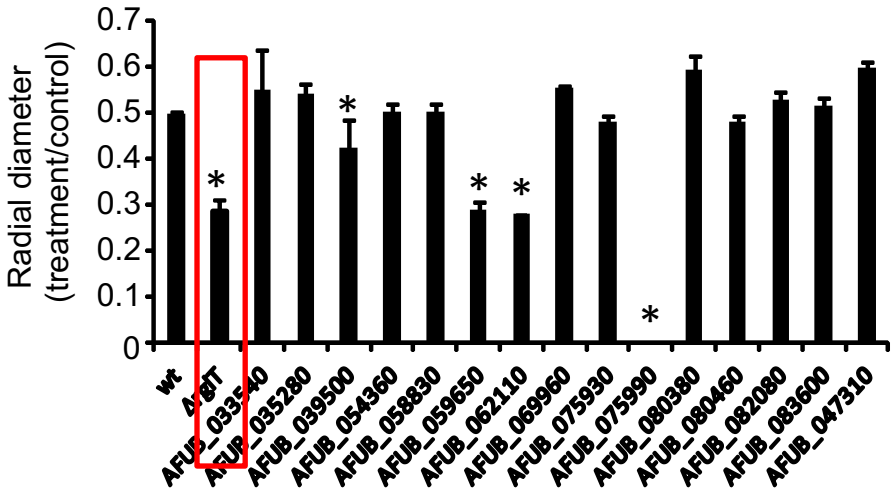
Screening for gliotoxin sensitivity revealed 16 TF null mutants: the Δ*rglT* mutant was identified again !!!

Gliotoxin

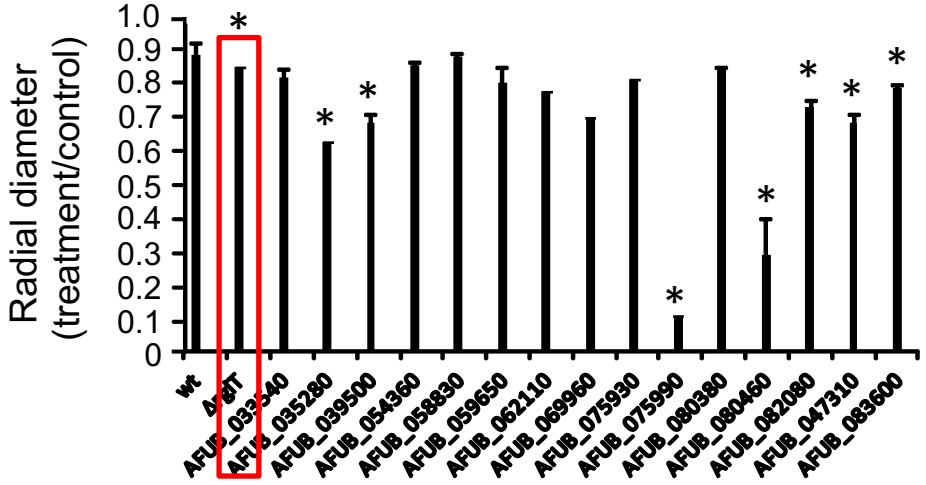


Screening of *A. fumigatus* 495 transcription factors null library
35 µg/ml (0.5 x MIC)
About 3 % of the TFs

Allyl alcohol 40 mM

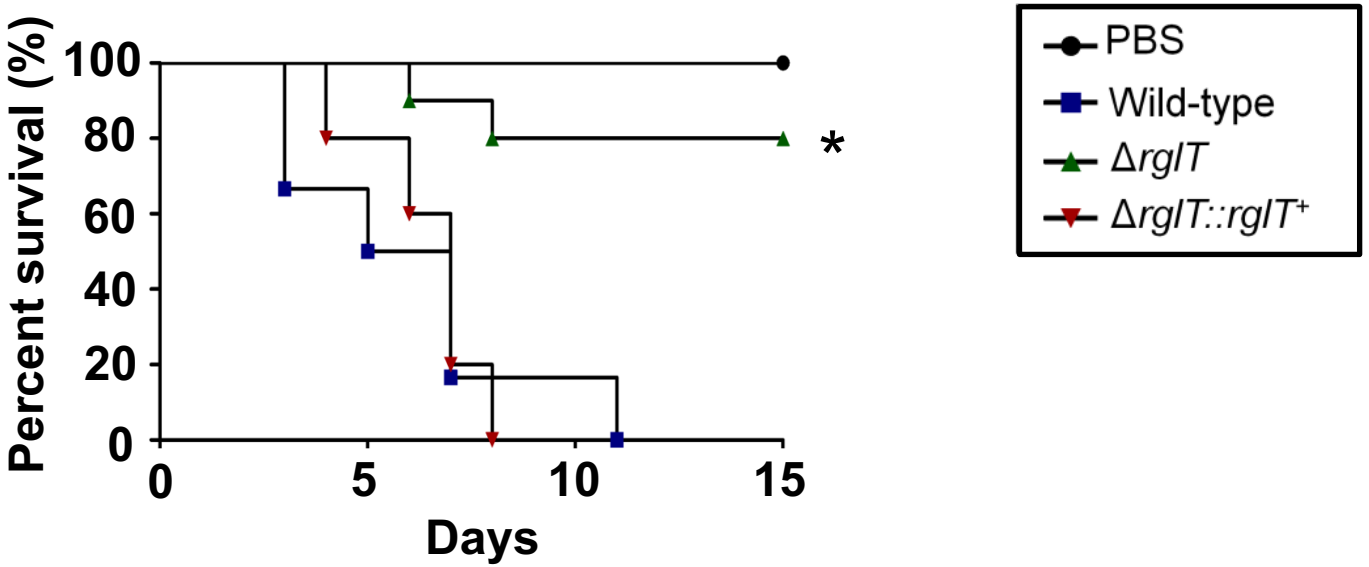


Menadione 0.01 mM

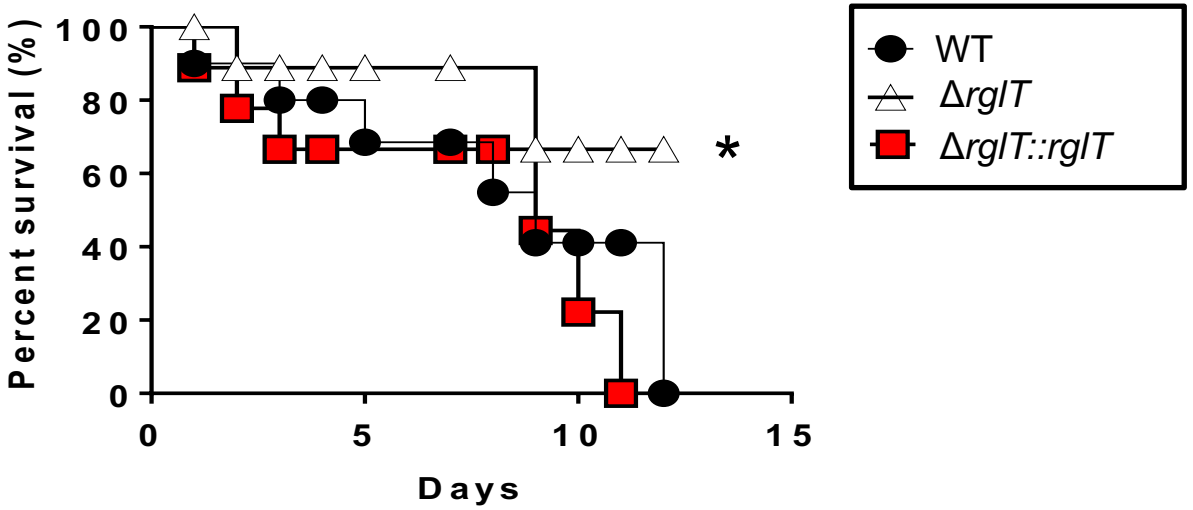


RglT is crucial for virulence in both neutropenic and immunocompetent murine models of invasive aspergillosis

Neutropenic mouse model



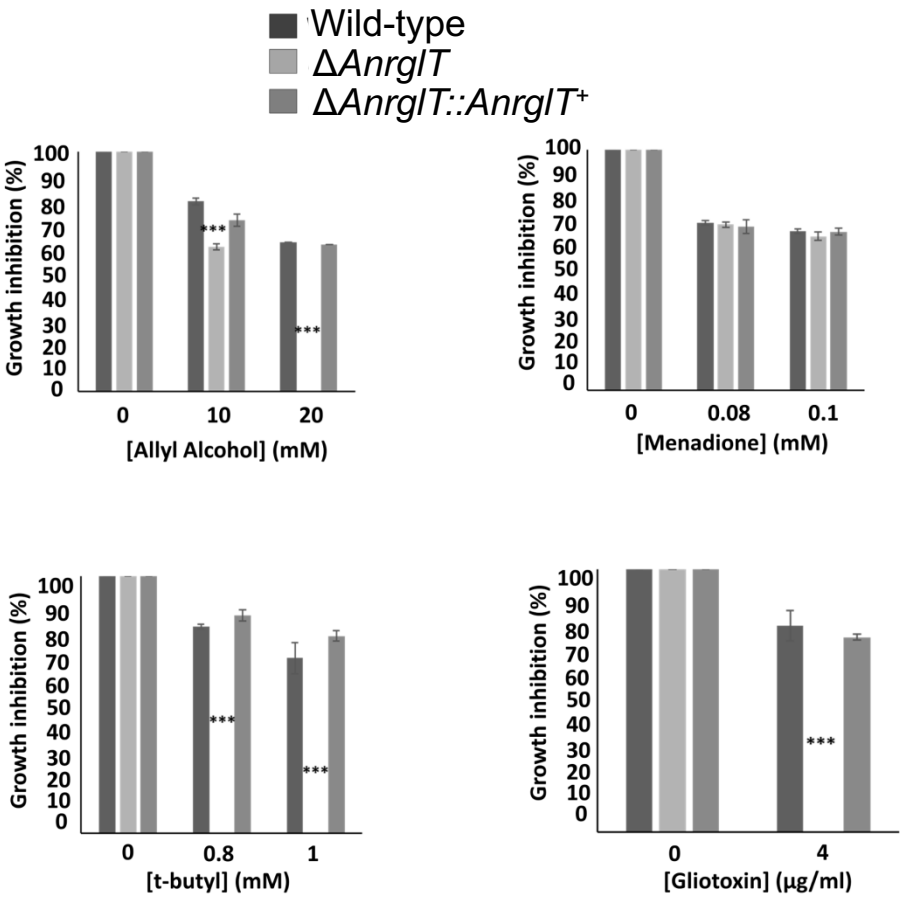
Immunocompetent mouse model

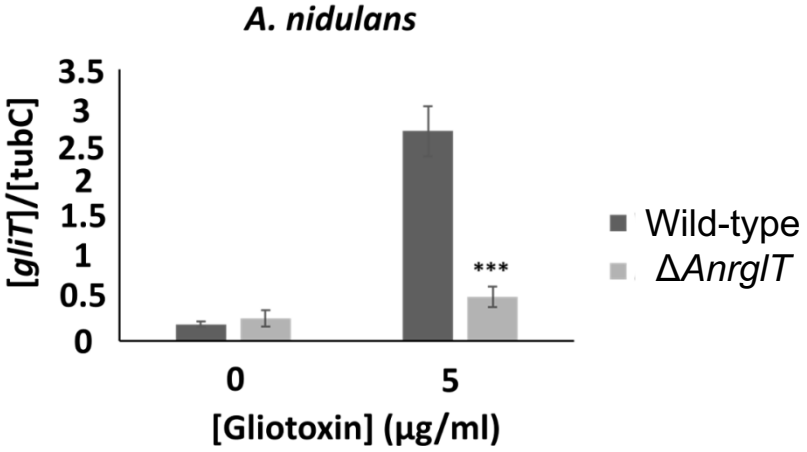


The taxonomic distribution of RglT, a homologous gliotoxin gene cluster, and *gliT* in 458 eurotiomycete and sordariomycete genomes

The pattern of occurrence of *RglT* is statistically dependent on the distribution of GliT but not vice versa

RglT is essential for GT self-protection and *gliT* regulation in the non-GT-producing fungus *A. nidulans*





Take home message

- We have identified two GPCR encoding genes as important for modulation of the production of several secondary metabolites. There is an important interplay between MAPK MpkB$^{Fus3\ and}$ MpkA^{Slt2} in the regulation of some secondary metabolites production

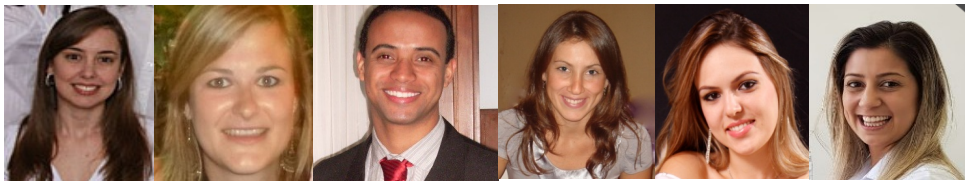
- Identification of RglT as the transcription factor that regulates the expression of *gliT* and of *gliZ* through direct binding to the gene promoter regions under oxidative stress, GT-inducing conditions and GT self-protection

- Loss of *rglT* results in a strain that cannot produce GT, cannot protect itself from GT and is avirulent in both neutropenic and immunocompetent mouse models

- Our phylogenetics analysis is consistent with an evolutionary scenario in which the GliT-based resistance mechanism is ancestral and the evolutionary recruitment of RglT regulation of GliT occurred subsequently.

- In addition to RglT, we identified 15 TFs that are important for gliotoxin self-protection

Universidade de São Paulo
Brasil



Patrícia Laure Leandro Ana Lilian Adriana

KU LEUVEN

MANCHESTER 1824
The University of Manchester

Patrick Van Dijck

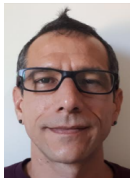
Michael Bromley

VANDERBILT UNIVERSITY

Antonis Rokas

Jacob Steenwyk

Mônica Pupo



Diego Hernández

Maynooth University
National University of Ireland Maynooth

Sean Doyle

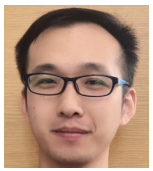
Rebecca Owens

UNIFESP

Flávio Loures

UNIVERSIDADE DE MACAU
UNIVERSITY OF MACAU

Lakhan



Zhiqiang

Chris Wong

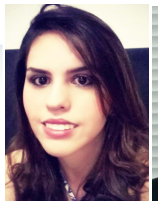
Leibniz Institute for Natural Product Research and Infection Biology
Hans Knöll Institute

Roberto Silva

Rafael Silva-Rocha

Fausto Almeida

ufscar



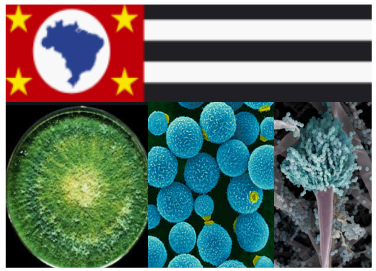
Marina Rocha

Iran Malavazi

Axel Brakhage

Vito Valiante

Sandra Höfgen



FAPESP

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Hans Fischer Senior Fellowship
Institute for Advanced Study (IAS)
Technische Universität München

São Paulo Fungal Group