

**TITLE:** THE *ASPERGILLUS FUMIGATUS* TRANSCRIPTION FACTOR RglT IS CRUCIAL FOR OXIDATIVE STRESS RESISTANCE, GLIOTOXIN BIOSYNTHESIS AND VIRULENCE

**AUTHORS:** RIES, L.N.A.; PARDESHI, L.; ARAUJO, L.R.; CORREA DOS SANTO, R. A.; ALMEIDA, F.; RODRIGUEZ HERNANDEZ, D.C.; PUPO, M.T.; LOURES, F.V.; WONG, C.K.H.; GOLDMAN, G.H.

**INSTITUTION:** FACULDADE DE MEDICINA DE RIBEIRAO PRETO DA UNIVERSIDADE DE SÃO PAULO (FMRP-USP), RIBEIRAO PRETO, SP (AVENIDA BANDEIRANTES, 3900 – VILA MONTE ALEGRE, CEP 14049-900, RIBEIRAO PRETO – SP, BRAZIL)

**ABSTRACT:**

*Aspergillus fumigatus* is an opportunistic fungal pathogen that causes aspergillosis in immunocompromised individuals, a term collectively referring to a spectrum of lung diseases, whose severity depends on the type of underlying disturbance in the immune system. *A. fumigatus* expresses and secretes an array of immune-evasive and –modulatory determinants, including secondary metabolites (SMs), that contribute to enhancing fungal fitness and growth within the mammalian host. Gliotoxin (GT) is a SM that induces defects in the function and recruitment of neutrophils, a type of white blood cell that is crucial in fighting *A. fumigatus* infections. One of the strategies employed by neutrophils, aiming at getting rid of invading *A. fumigatus*, is through the production of reactive oxygen species (ROS) that induce oxidative stress to the fungal cells. The aim of this work was to uncover previously uncharacterised transcription factors (TFs) that are essential for conferring resistance against oxidative stress in order to further elucidate *A. fumigatus* oxidative stress resistance mechanisms. An *A. fumigatus* transcription factor (TF) deletion library was therefore screened for growth on allyl alcohol (AA)-induced oxidative stress, which led to the identification of one strain that was highly sensitive to increasing concentrations of AA and additional oxidative stress-inducing compounds. Furthermore, this strain was attenuated in virulence in an immunocompetent murine model of pulmonary aspergillosis. RNA-sequencing in the presence of AA showed a significant down-regulation of *gliT*, encoding an enzyme required for GT biosynthesis and self-protection, in the TF deletion strain. Binding of this TF in the *gliT* promoter region in the presence of AA and in GT-inducing conditions was confirmed by ChIP-seq and ChIP-qPCR respectively, and the TF was re-named RglT (regulator of *gliT*). GT biosynthesis was abolished in the  $\Delta$ *rglT* strain and this strain also lost all ability to protect itself from exogenously added GT. In addition, increased recruitment of neutrophils was observed at the site of infection of the  $\Delta$ *rglT* strain. Functional conservation of RglT was also observed in other non-pathogenic *Aspergillus spp.*, highlighting the importance of this TF for *Aspergillus* biology. In conclusion, this work identified a TF that is essential for gliotoxin biosynthesis, subsequent modulation of the murine immune system and *in vivo* virulence.

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**Keywords:** *Aspergillus fumigatus*, transcription factor, oxidative stress, gliotoxin biosynthesis, virulence

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