

**TITLE:** THE CELL WALL INTEGRITY ASSOCIATED-TRANSCRIPTION FACTOR RlmA AND THE TRANSCRIPTION FACTOR SebA DIFFERENTIALLY REGULATE THE FUMIQUINAZOLINE C PRODUCTION IN *Aspergillus fumigatus*

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**ABSTRACT:**

Fungi remarkably produce a variety of secondary metabolites as a consequence of different environmental stimuli. These compounds can ultimately provide fitness attributes to the producing organism. Recently, we characterized two components of the *A. fumigatus* cell wall integrity pathway (CWI), *pkcA* and *rlmA* and observed that in addition to the cell wall related-phenotypes, the perturbation of the signaling circuit coordinated by the PkcA-MpkA-RlmA module impacts on the production of fumiquinazolines (Fq). Here we show that *pkcA*<sup>G579R</sup> and delta *rlmA* mutant strains produce lower FqC (24.7% and 27.9%, respectively) and that FqC concentrations were 10.5- fold lower in the delta *mpkA* strain. This decrease is accompanied by global down-regulation in mRNA expression of the Fq cluster genes during the asexual development. In addition, we showed by ChIP-qPCR that the transcription factor RlmA is able to activate specific genes related to fumiquinazolines production during the asexual development in *A. fumigatus* indicating that *rlmA* is a positive regulator of FqC production. Aiming to understand if other cell stresses could influence the production of FqC, we performed a screening using different mutant involved in cell wall integrity maintenance and heat shock adaptation and found that the deletion of the transcription factor SebA, overproduced FqC (about 4.5-fold increase) indicating that this transcription factor is a negative regulator of FqC. Likewise by using ChIP-qPCR we observed that promoter regions of the Fq cluster genes present several STRE motifs that are bind by SebA during the asexual development in this fungus. *A. fumigatus* is sensitive to FqC and this tolerance is decreased in the CWI pathway mutants and increased in the delta *sebA* strain. In addition, FqC can induce pore formation on the membrane of macrophages and highly stimulates the secretion the pro-inflammatory cytokine TNF- $\alpha$  and Il-6 by this cell type. We also used the soil amoeba *Dictyostelium discoideum* to study the phagocytic interaction of this organism with conidia from the delta *sebA* strain. Interestingly, conidia of the delta *sebA* were significantly less phagocytized by *D. discoideum* and the opposite occurred when conidia from the CWI pathway mutants were tested. Our results suggest that Fq production is regulated at different levels in *A. fumigatus* and that FqC can serve as a defense compound against other microorganisms or soil predators.

**Keywords:** Cell Wall Integrity, *Aspergillus fumigatus*, Fumiquinazoline C

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