

International Conference on Transcriptomics

July 27-29, 2015 Orlando, USA

The capacity of *Aspergillus niger* to sense and respond to cell wall stress requires at least three transcription factors: RlmA, MsnA and CrzA

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Background: Cell wall integrity, vesicle transport and protein secretion are key factors contributing to the vitality and productivity of filamentous fungal cell factories such as *Aspergillus niger*. In order to pioneer rational strain improvement programs, fundamental knowledge on the genetic basis of these processes is required.

Aim: The aim of the present study was thus to unravel survival strategies of *A. niger* when challenged with compounds interfering directly or indirectly with its cell wall integrity: Calcofluor white, caspofungin, aureobasidin A, FK506 and fenpropimorph.

Results: Transcriptomics signatures of *A. niger* and phenotypic analyses of selected null mutant strains were used to predict regulator proteins mediating the survival responses against these stressors. This integrated approach allowed us to reconstruct a model for the cell wall salvage gene network of *A. niger* that ensures survival of the fungus upon cell surface stress. The model predicts that (i) caspofungin and aureobasidin A induce the cell wall integrity pathway as a main compensatory response via induction of RhoB and RhoD, respectively, eventually activating the mitogen-activated protein kinase kinase MkkA and the transcription factor RlmA. (ii) RlmA is the main transcription factor required for the protection against calcofluor white but it cooperates with MsnA and CrzA to ensure survival of *A. niger* when challenged with caspofungin and aureobasidin A. (iii) Membrane stress provoked by aureobasidin A via disturbance of sphingolipidsynthesis induces cell wall stress, whereas fenpropimorph-induced disturbance of ergosterol synthesis does not.

Conclusion: The present work uncovered a sophisticated defence system of *A. niger* which employs at least three transcription factors - RlmA, MsnA and CrzA – to protect itself against cell wall stress. The transcriptomic data furthermore predicts a fourth transfactor, SrbA, which seems to be specifically important to survive fenpropimorph-induced cell membrane stress. Future studies will disclose how these regulators are interlocked indifferent signaling pathways to secure survival of *A. niger* under different cell wall stress conditions.

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