MOLECULAR ANALYSIS OF CALCINEURIN IN HUMAN PATHOGENIC FUNGI

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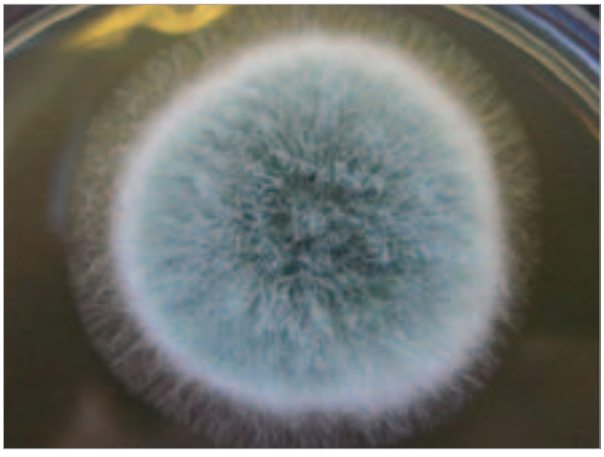

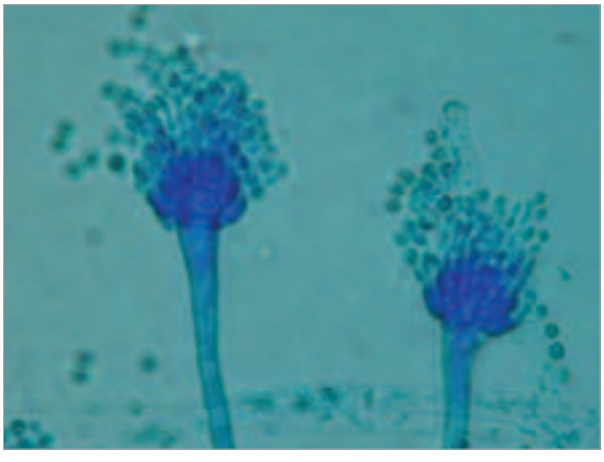

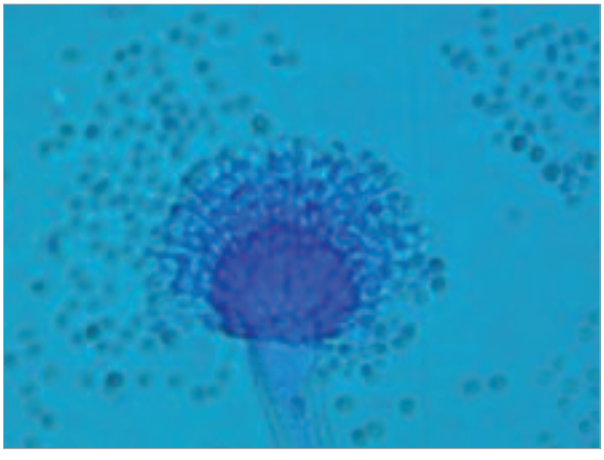

(A) A four day A. fumigatus *culture on malt extract agar (above). Light microscopy pictures are taken at 1000x, stained with lacto-phenol cotton blue (right). (B) and (C) show details of the asexual structure named conidiophore*

Environmental sensing and the retrieval of essential nutrients from the environment are general metabolic traits that are associated with the growth of *P. brasiliensis* and *A. fumigatus* in an inhospitable environment such as its human host. Calcium is a ubiquitous second messenger that functions in signal transduction pathways in eukaryotic organisms. Calcium signaling is important for several cellular processes, including fertilization and development, exocytose, muscle contraction, motility, chemotaxis, cell division, differentiation, programmed cell death and chromatin remodeling in multicellular eukaryotes. Our project proposes to study the system of calcineurin regulation in two human pathogenic fungi, *P .brasiliensis* and *A. fumigatus*, and the relationship between this system and two essential aspects of pathogenicity in these organisms: 1) the mycelium-to-yeast transition in *P. brasiliensis* and 2) the sensing of the human host environment and the nutrient acquisition in *A. fumigatus*. In the last four years, our group has been actively working with both pathogenic fungi, developing a series of resources and molecular tools aiming to understand the mechanisms of virulence/pathogenicity in these organisms. Thus, the main objectives of this proposal are: 1) to analyze microarray gene expression of the *P. brasiliensis* mycelium-to-yeast transition blocked by cyclosporine; 2) by using yeast two-hybrid screening, to identify cDNAs that correspond to genes that encode *P. brasiliensis* proteins that interact with calcineurin during the mycelium-to-yeast transition; 3) construction of a vector for transformation and RNAi inhibition in *P. brasiliensis*; 3) molecular characterization of an *A. fumigatus* gene that encodes the homologue of the *Crz*1 (*CrzA*) transcription factor that is transported to the nucleus after dephosphorylation by calcineurin; 5) to identify genes that suppress by high copy number the absence of *CalA* e *CrzA* and microarray gene expression analysis of the wild type and *calA* e *crzA* mutants in different stressing conditions; 6) characterization of genes involved in the inorganic phosphate utilization and their relationship with *calA* and *crzA* mutant; and 7) molecular characterization of *A. fumigatus* genes that encode proteins with Ca^{2+}-binding domains (EF-hands).

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

Calcium is a ubiquitous second messenger that functions in signal transduction pathways in eukaryotic organisms. Our project proposes to study the system of calcineurin regulation in two human pathogenic fungi, *Aspergillus fumigatus* and *Paracoccidioides brasiliensis*. The protein phosphatase calcineurin is an important mediator connecting calcium-dependent signaling to various cellular responses in multiple organisms. In fungi calcineurin acts largely through regulating Crz1p-like transcription factors. We characterized an *Aspergillus fumigatus Crz1* homologue, *CrzA*, and demonstrated its mediation of cellular tolerance to increased concentrations of calcium and manganese. In addition to acute sensitivitiy to these ions, and decreased conidiation, the *crzA* null mutant suffered altered expression of calcium transporter mRNAs under high concentrations of calcium, and loss of virulence when compared to the corresponding complemented and wild type strains. We used multiple expression analyses to probe the transcriptional basis of *A. fumigatus* calcium tolerance by identifying several genes having *calA* and/or *crzA* dependent mRNA accumulation patterns. We also demonstrated that contrary to previous findings, the gene encoding the *A. nidulans* calcineurin subunit homologue, *CnaA*, is not essential, and that the *cnaA* deletion mutant shared the morphological phenotypes observed in the corresponding *A. fumigatus* mutant, *△calA*. By then exploiting the *A. nidulans* model system, we have linked calcineurin activity with asexual developmental induction, finding that CRZA supports appropriate developmental induction in a calcineurin and *BrlA*-dependent manner in both species.

Not only is our project related to calcium metabolism in *A. fumigatus*, but we are also using genomic tools in collaboration with several groups around the world trying to understand the biology of this organism. Recently, we presented the genome sequences of a new clinical isolate of *A. fumigatus*, A1163, and two closely related but rarely pathogenic species, *Neosartorya fischeri* NRRL181 and *A. clavatus* NRRL1. Cross-species comparison has revealed that 8.5%, 13.5% and 12.6%, respectively, of *A. fumigatus*, *N. fischeri* and *A. clavatus* genes are species-specific. These genes are significantly smaller in size than core genes, contain fewer exons and exhibit a subtelomeric bias. We extended these studies by identifying fungal attributes preferentially employed during adaptation to the mammalian host niche generating multiple genome-wide gene expression profiles from minute samplings of *A. fumigatus* germlings during initiation of murine infection. As functions of phylogenetic conservation and gene localisation, around 28 and 30 per cent, respectively, of the entire *A. fumigatus* subtelomeric and lineage-specific gene repertoire is induced relative to laboratory culture, and physically clustered genes including loci directing pseurotin, gliotoxin and siderophore biosyntheses are a prominent feature.

MAIN PUBLICATIONS

Soriani FM, Malavazi I, Ferreira MES, Savoldi M, Kress MR, Goldman MHS, Loss O, Bignell E, Goldman GH. 2008. Functional characterization of the *Aspergillus fumigatus CRZ1* homologue, *CrzA*. *Molecular Microbiology*. **67**:1274-1291.

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