

ANTIFUNGAL *PISUM SATIVUM* DEFENSIN 1 INTERACTS WITH *NEUROSPORA CRASSA* CYCLIN F RELATED TO THE CELL CYCLE

Denise da Silveira Lobo¹, Iuri Bastos Pereira², Lucianne Fragel-Madeira², Luciano Neves², Luiz Mors², Jane Faria², Reinaldo Calixto¹, Rafael Linden² e Eleonora Kurtenbach³

¹PUC-Rio, ²Universidade Federal do Rio de Janeiro

Pisum sativum defensin 1 (*Psd1*) is a cysteine-rich antifungal peptide component of the plant innate immune system. It has been demonstrated that interaction of plant defensins with fungi membrane glucosylceramides are necessary but not sufficient for the inhibition of the fungus growth, suggesting that other targets may be involved in this mechanism. A GAL4-based yeast two-hybrid system was performed using *Psd1* as the bait and target proteins were screened within a conidial *Neurospora crassa* cDNA library. Nine out of 11 two-hybrid strong interaction candidates were nuclear proteins. One clone, detected with high frequency, presented sequence similarity to a cyclin-like protein related to the cell cycle control. *In vitro* GST pull-down assay corroborated this interaction. Fluorescence microscopy analysis of FITC-conjugated *Psd1* and DAPI-stained fungal nuclei showed *in vivo* co-localization of *Psd1* and the *Fusarium solani* nucleus. Analysis of the DNA content of *N. crassa* conidia using flow cytometry suggested that *Psd1* directed cell cycle impairment and caused conidia to undergo endoreplication. We also demonstrated that the antifungal peptide *Psd1* impaired the progression of the cell cycle, as measured by interkinetic nuclear migration in the retinal neuroblasts. Together, these results showed that the mechanism of action of the cationic antifungal plant peptide *Psd1* involves nuclear targets.