Cryptococcal Titan cell formation and the calcineurin signaling pathway: are they connected?

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Cryptococcus neoformans is the etiological agent of cryptococcosis, an infectious disease that affects mainly immunocompromised patients and causes 180,000 deaths worldwide each year. Once inside the host, cryptococcal cells undergo a variety of adaptative processes that are regulated by a network of transcription factors (TFs). One of these is the TF Pdr802, which has been already shown as an important regulator of *C. neoformans* pathogenicity. In our recent work, we demonstrated that the *PDR802* expression is highly induced under host-like conditions *in vitro* and its deletion impairs *C. neoformans* survival in a mammalian host, mouse serum, and tissue culture media. Two important cryptococcal virulence determinants are negatively regulated by Pdr802: the polysaccharide capsule and Titan cells (TC) production. Using ChIP-Seq and RNA-Seq, we identified direct targets of Pdr802, which include the calcineurin-regulated proteins Had1 and Crz1. The calcineurin pathway is important for *C. neoformans* growth at 37°C, cell wall remodeling, and virulence. Upon intracellular calcium influx, calcineurin dephosphorylates the transcription factor Crz1, which then translocates to the nucleus and regulates gene expression. We found that Pdr802 binds the *CRZ1* gene promoter and positively regulates its expression. However, in the tested conditions, Pdr802 was shown not to be the sole regulator of *CRZ1* expression, since the *pdr802*-null strain accumulates intracellular calcium, but less pronounced than the observed for *crz1* mutant. Since Pdr802 is the major negative regulator of cryptococcal titanisation and Crz1 the main effector of the calcineurin signaling pathway, we aim to describe the possible interaction between these TFs during TC formation. Our *in silico* analysis showed that Pdr802 binds in the promoter region of different calcineurin-signaling components, such as *CNA1*, *CAM1*, *CMR1*, *YCV1*, and *CBP1*. To understand the role of Pdr802 in gene expression regulation of calcineurin targets, *pdr802*-null mutant cells were subjected to TC induction conditions *in vitro* and total RNA was extracted and sequenced. In parallel, a screening of TC formation by mutants of the calcineurin-signaling pathway was conducted by flow cytometry, however no differences were found using the wild-type and *pdr802* strains as controls in one particular *in vitro* condition. Since some of those mutants are sensitive to high temperatures, alternative TC induction media are under analysis.

Keywords: *Cryptococcus neoformans*, Titan cells, calcineurin, Pdr802, Crz1.

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