Towards understanding the molecular link between cell cycle regulation and hypoxic adaptation in *Cryptococcus neoformans*

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Objectives:

The objective of this study is to understand cell cycle regulation and hypoxic adaptation in the pathogenic yeast *Cryptococcus neoformans* and the molecular link between them.

Methods:

We performed in silico simulations and analyses using GENETYX software (version 15).

Results:

We have reported that the cell cycle behavior of the pathogenic yeast *Cryptococcus neoformans* (*C. neoformans*) is different from the cell cycle control exhibited by the model yeast *Saccharomyces cerevisiae* (*S. cerevisiae*), and also have reported the molecular characterization and physiological roles of the two main eukaryotic cell cycle genes, *C. neoformans* cyclin dependent kinase 1 (CnCdK1) and cyclin homologues. Only a single Cdk1-related G1 and G1/S cyclin homologue was found in the genome sequence of *C. neoformans* and was designated CnClN1. Surprisingly, CnClN1 was not only able to complement the function of the G1 cyclins of *S. cerevisiae*, such as ScClN3, but also the G1/S cyclins of *S. cerevisiae*, such as ScClN1 and ScClN2. Our in silico analysis demonstrated that the CnClN1/ScCdK1 complex was more stable than any of *S. cerevisiae* cyclins (ScClN1, ScClN2, ScClN3) and ScCdK1 complexes. These results are consistent with in vitro analysis that has revealed the flexible functional capacity of CnClN1 as a Cdk1-related G1 and G1/S cyclin of *S. cerevisiae*. On the other hand, in *S. cerevisiae*, Cln1 and Cln2, G1/S cyclins of *S. cerevisiae*, oscillate during the cell cycle, rising in late G1 and falling in early S phase. We have been trying to elucidate the structure basis of the functional distinction between Cln1 and Cln2.

We investigated the cell cycle control mechanism between Cln1 and Cln2 from a point of view of their structure-function relationships in *S. cerevisiae*.

In the obligate aerobic pathogenic yeast *C. neoformans*, limited aeration has been demonstrated to cause slowdown in proliferation and delayed budding, resulting eventually in a unique unbudded G2-arrest. The ability to adapt to decreased oxygen levels during pathogenesis has been identified as a virulence factor in *C. neoformans*. We have identified and characterized the gene that is necessary for the proliferation slowdown and G2-arrest caused by limited aeration. This gene was also identified in a parallel studies as homologous both to calcineurin responsive (Crz1) and PKC1-dependent (SP1-like) transcription factors. We have confirmed the role of the cryptococcal homologue of CRZ1/SP1-like transcription factor in cell integrity, and newly demonstrated its role in slowdown of proliferation and survival under reduced aeration, in biofilm formation and in susceptibility to fluconazole. Our data also demonstrate a tight molecular link between slowdown of proliferation during hypoxic adaptation and maintenance of cell integrity in *C. neoformans* and present a role for the CRZ1 family of transcription factors in fungi.

Conclusion:

Our study revealed the flexible functional capacity of CnClN1 (only a single cyclin in *C. neoformans*) as a Cdk1-related G1 and G1/S cyclin (ScClN1, ScClN2, and ScClN3) of *S. cerevisiae*, and also demonstrate a tight molecular link between cell cycle regulation and hypoxic adaptation in *C. neoformans*.

3 Interaction Energy and Electrostatic Complementarity (EC)

	Interaction Energy (kcal/mol)	EC
CnClN1 / ScCdK1	-730.1	0.73
ScClN1 / ScCdK1	-730.9	0.53
ScClN2 / ScCdK1	-727.7	0.56
ScClN3 / ScCdK1	-653.6	0.59