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# Towards understanding cell cycle control in *Cryptococcus neoformans*: Structure–function relationship of G1 and G1/S cyclins homologue CnCln1

Eric V. Virtudazo [a,1], Akiko Suganami [b,1], Yutaka Tamura [b,*], Susumu Kawamoto [a,*]

[a] Division of Molecular Biology, Medical Mycology Research Center, Chiba University, Chiba 260-8673, Japan
[b] Department of Bioinformatics, Graduate School of Medicine, Chiba University, Chiba 260-8670, Japan

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ABSTRACT

We have previously reported that only a single Cdk1-related G1 and G1/S cyclin homologue was found in the genome sequence of the pathogenic basidiomycetous yeast *Cryptococcus neoformans* (*C. neoformans*) and designated it CnCln1. Surprisingly, CnCln1 was not only able to complement the function of the G1 cyclins of the ascomycetous budding yeast *Saccharomyces cerevisiae* (*S. cerevisiae*), such as ScCln3, but also the G1/S cyclins of *S. cerevisiae*, such as ScCln1 and ScCln2. In this study, we investigated how CnCln1 cooperates with the cyclin-dependent kinases of *S. cerevisiae* (ScCdk1) and substitutes the function of G1 and G1/S cyclins of *S. cerevisia* from a point of view of their structure–function relationship. Our *in silico* analysis demonstrated that the CnCln1/ScCdk1 complex was more stable than any of the yeast cyclin and ScCdk1complexes. Thus, 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 cyclins of *S. cerevisiae*.



## 1. Introduction

The cell-cycle control system is composed of a series of biochemical switches that trigger the events of the cycle in the correct order [1]. The key components of this system are cyclin-dependent kinases (Cdks) and their regulators, which are assembled into a robust and versatile regulatory network that is responsive to a variety of intracellular and extracellular information [2]. The Cdks are a small family of enzymes that require cyclin subunits for their activity [3].

Usually, the main Cdk implicated for cell cycle control in the ascomycetous budding yeast *Saccharomyces cerevisiae* (*S. cerevisiae*) is Cdc28 (ScCdk1), which forms complexes with nine cyclins (ScCln1-3, ScClb1-6) [4,5]. With one exception (ScCln3), the *S. cerevisiae* cyclins are encoded by pairs of closely related genes (ScCln1, 2; ScClb1, 2; ScClb3, 4 and ScClb5, 6), which share partially redundant functions. Their products accumulate at specific times during the cell cycle, leading to waves of activation of distinct cyclin/ScCdk1 complexes. In general, cyclins can be divided into four classes based on their behavior in the cell cycle of vertebrate somatic cells and yeast cells: G1/S cyclins, S cyclins, M cyclins and G1 cyclins. G1 cyclins do not behave like the other cyclins, in that the concentrations increase gradually (with no oscillation), throughout the cell cycle based on cell growth and the external growth-regulatory signals. ScCln3 is an integrator for signals that regulate the rate of G1 progression in *S. cerevisiae* [5–7]. On the other hand, the G1/S cyclins, ScCln1 and ScCln2 in *S. cerevisiae*, oscillate during the cell cycle, rising in late G1 and falling in early S phase.

*Cryptococcus neoformans* (*C. neoformans*) is a basidiomycetous yeast that is an opportunistic pathogen of worldwide distribution and responsible for life-threatening infections among immune-compromised persons [8–10]. We have been studying the molecular mechanisms of the cell cycle control in *C. neoformans* and have reported that the cell cycle behavior of this yeast is different from the cell cycle control model exhibited by the common budding yeast, *S. cerevisiae* [11,12]. *C. neoformans* exhibits a delay in budding as the growth phase progresses into late log to early stationary phase, resulting in a tendency to accumulate unbudded cells with G2 rather than G1 DNA content [13]. This was found to be the organism's inherent response to stressful cultural conditions such as changes in oxygen availability, pH and temperature and was implicated as a possible additional virulence mechanism during survival within host tissues. In the light of the functional specialization of G1 and G1/S cyclins in *S. cerevisiae*, it was surprising to find that *C. neoformans*, a budding yeast very similar to *S. cerevisiae*, was found to have a single G1 and G1/S cyclin in the genome [14]. Thus, it is important to understand the mechanisms that govern this yeast's unique cell cycle behavior during G1-S phase transition and the role of this single cyclin in this unique stress response pathway.

* Corresponding authors. Fax: +81 43 226 2813 (Y. Tamura), +81 43 226 2486 (S. Kawamoto).
*E-mail addresses:* yutaka_tamura@faculty.chiba-u.jp (Y. Tamura), kawamoto@faculty.chiba-u.jp (S. Kawamoto).
[1] These authors have equal contribution.

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In this study, we investigated the cell cycle control mechanism of CnCln1 by comparing its activity with G1 and G1/S cyclins of *S. cerevisiae* from a point of view of their structure–function relationship [15–17].

## 2. Materials and methods

### 2.1. Plasmid and strain construction and growth analysis

Cloning of the single *CLN1* gene of *C. neoformans* and complementation in *S. cerevisiae* has been previously described [14]. *ScCLN1*, *ScCLN2* and *ScCLN3* were similarly cloned into the yeast expression vector pYES2 (Invitrogen, CA, USA). These four plasmids were transformed into an *S. cerevisiae* G1 and G1/S cyclin triple mutant strain (TK-209/K3413: *MAT a*, *ade2-1*, *trp1-1.can1-100*, *leu2-3112*, *his3-11,15*, *ura3*, *SSD1*, *cln1*::*hisG*, *cln2*::Δ, *cln3*::*LEU2*, *CLA1*, containing YIpLac204-$P_{MET}$-*Cln2* [18,19]) using lithium acetate/PEG method [20]. The pYES2 expression vector carries the *URA3* marker and places expression of G1 and G1/S cyclin genes under the *GAL* promoter. The *S. cerevisiae* strain carries the *CLN2* gene under the *MET* promoter and does not grow in medium supplemented with methionine. Transformants were selected for growth in uracil deficient medium and tested for growth in medium supplemented with methionine to suppress *S. cerevisiae CLN2* expression and with galactose as carbon source, to induce expression of each of G1 and G1/S cyclins from pYES2. Growth curves and doubling times were written and analyzed using ORIGIN (version 8.5, OriginLab Corporation, MA, USA).

### 2.2. Calculation of sequence identity

Sequence identity of CnCln1, ScCln1, ScCln2 and ScCln3 were calculated with GENETYX (version 15, Genetyx Corporation, Tokyo, Japan).

### 2.3. Molecular modeling and calculation of binding energy

The three dimensional structures of CnCln1/ScCdk1, ScCln1/ScCdk1, ScCln2/ScCdk1 and ScCln3/ScCdk1 were constructed with MOE (version 2009, CCG Inc., Montreal, Canada) according to the Brookhaven Protein Databank 1E9H.

The molecular mechanics calculations were performed to obtain the local minimum structure and calculate the interaction energy of these complexes using amber99 force field in MOE. To calculate electrostatic complementarities of these complexes, we used MolFeat-EC (Ver. 1, FiatLux, Tokyo, Japan) as previously described [21–23] with some modifications. The three-dimensional structures of these complexes were displayed using MolFeat (Ver. 4, FiatLux, Tokyo, Japan).

## 3. Results

### 3.1. In vitro analysis

In *S. cerevisiae*, the cyclin-dependent kinase 1 of *S. cerevisiae* (ScCdk1) forms complexes with nine cyclins (ScCln1-3, ScClb1-6) and accumulate at specific times during the cell cycle, leading to waves of activation of distinct cyclin/ScCdk1 complexes [3,4]. The G1 cyclins, such as ScCln3, and the G1/S cyclins, such as ScCln1 and ScCln2, of *S. cerevisiae* contribute to the control of new cell-cycle entry in response to growth or extracellular factors [6]. Previously, we reported that the G1 cyclin of *C. neoformans*, namely CnCln1, could replace G1 and G1/S cyclins functions of *S. cerevisiae* by complementation analysis in *S. cerevisiae* [14]. To compare the abilities of these four cyclins (CnCln1, ScCln1, ScCln2 and ScCln3) in supporting the growth of *S. cerevisiae* when present singly in the cell, we cloned these genes, *CnCLN1* and *ScCLN1-3*, into an *S. cerevisiae* expression vector pYES2. Then, we compared the abilities of these four cyclins to support growth of *S. cerevisiae* by expressing singly in the ScCln1-3 triple-mutant strain. As shown in Fig. 1, CnCln1 could support the growth of *S. cerevisiae* at the highest level

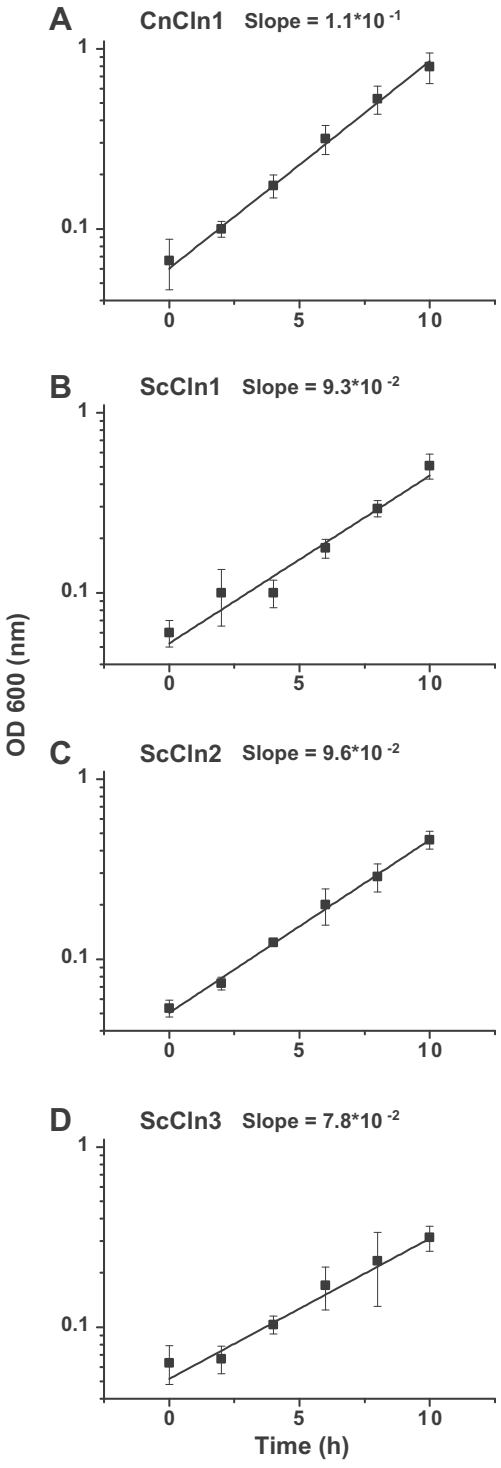


**Fig. 1.** Comparison of the growth abilities of (A) CnCln1, (B) ScCln1, (C) ScCln2 and (D) ScCln3 in the ScCln1-3 triple-mutant strain of *S. cerevisiae*.