Structure based Functional Distinction between Cln1 and Cln2 Depends on the Ubiquitin-Proteasome Pathway

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Abstract

Cln1 and Cln2, G1/S cyclins of the ascomycetous budding yeast Saccharomyces cerevisiae (S. cerevisiae), oscillate during the cell cycle, rising in late G1 and falling in early S phase. We have tried to elucidate the structure basis of the functional distinction between Cln1 and Cln2. Here we performed in silico simulations: construction and evaluation of three dimensional structures of Cln1-Cdc28 and Cln2-Cdc28 complexes. Our in silico simulations suggested that the interaction of Cln1 and Cln2 with Cdc28 were in the two distinct situations, designated as flip and flop conformation, at the extra amino acid region in the cyclin box of Cln1 and Cln2. We speculated the trigger of this flip-flop conversion of the extra amino acid region in the cyclin box of Cln1-Cdc28 and Cln2-Cdc28 might be regulated by the ubiquitination of the sequences rich in Pro (P), Glu (E), Ser (S) and Thr (T), so-called PEST motifs, in Cln1 and Cln2. Furthermore, we presumed that the functional superiority between Cln1 and Cln2 in the G1/S phase of S. cerevisiae might be controlled by flip-flop conversion and ubiquitin-proteasome pathway.

Keywords: Cln1; Cln2; Cdc28; Ubiquitination; PEST; In silico simulation

Abbreviations: S. cerevisiae: Saccharomyces cerevisiae; Cdk: Cyclin-Dependent Kinase; Cdc28: Cyclin-dependent kinase of Saccharomyces cerevisiae; Cln3: G1 cyclin of Saccharomyces cerevisiae; Cln1, Cln2: G1/S cyclin of Saccharomyces cerevisiae; SBF: Swi4/6-dependent cell cycle Box Binding Factor; MBF: MluI-binding factor; PEST motif: Sequences rich in Pro (P), Glu (E), Ser (S) and Thr (T); 3D: Three Dimension; MD: Molecular Dynamics; IE: Interaction Energy; EC: Electrostatic Complementarity

Introduction

The control system of cell cycle is the regulatory network that governs the order and timing of cell cycle events. The key components of the cell cycle regulation system are the cyclin-dependent kinase (Cdk) enzymes and their regulators of cyclins, which are assembled into a robust and versatile regulatory network that is responsive to a variety of intracellular and extracellular information [1-3]. Cyclins are highly conserved proteins that activate CDKs to regulate the cell cycle, transcription and other cellular processes. These different cyclin types are produced at different cell cycle stage to accumulate at specific times during the cell cycle, leading to waves of activation of distinct cyclin/Cdk complexes.

In the ascomycetous budding yeast Saccharomyces cerevisiae (S. cerevisiae), a single Cdk (Cdc28), forms complexes with nine cyclins: G1 cyclin Cln3, G1/S cyclins Cln1 and Cln2, S cyclins Clb5 and Clb6, G2/M cyclins Clb1, Clb2, Clb3, and Clb4 [4,5].

As summarized in Figure 1, after S. cerevisiae cell is born, Cdc28-kinase complexes promote cell-cycle commitment once the cell reaches a critical size during late G1 phase; this commitment phase of the cell cycle has been termed ‘START’. The timing of START, a major control point at the G1/S transition, in S. cerevisiae cells are controlled principally by G1 cyclin Cln3 and G1/S cyclins Cln1 and Cln2 [6]. Each of these three cyclins contains a sequence motif conserved among all of them, which is thought to mediate their association with the cell cycle regulatory kinase Cdc28 [7]. However, Cln3 is distantly related to Cln1 and Cln2, it differs markedly from them in its transcriptional regulation and biochemical activity [8]. Moreover, Cln3, its protein and kinase activity levels are no oscillation throughout the cell cycle, activates a transcription program mediated by the transcriptional factors SBF and MBF that regulate the rate of G1 progression in S. cerevisiae (Figure 1) [9-12].

On the other hands, Cln1 and Cln2 oscillate during the cell cycle, rising in late G1 and falling in early S phase [6,13-17]. Cln1 and Cln2, which are synthesized as a consequence of SBF activation wave, control the trigger of post-START processes such as budding, spindle pole body duplication, and the initiation of DNA replication (Figure 1) [18]. Finally, degradation of Cln1 and Cln2 is performed by the ubiquitination of the sequences rich in Pro (P), Glu (E), Ser (S) and Thr (T), so-called PEST motifs [19]. Thus, the extensive work carried out on G1/S cyclins has revealed an extraordinary similarity between Cln1 and Cln2. In addition to the numerous reports outlining the similarity between Cln1 and Cln2, some differences have been previously described [20,21].

In this study, we investigated the cell cycle control mechanism between Cln1 and Cln2 from a point of view of their structure-function relationship. We focused on the role of the extra amino acid region in the cyclin box of Cln1 and Cln2, which is typically observed in G1/S cyclins (Cln1 and Cln2) but not in G1 cyclin (Cln3). Moreover, we investigated the allosterically regulation of the extra amino acid region in the cyclin box of Cln1 and Cln2 by the ubiquitin-proteasome pathway. We also provide a structural mechanisms contributing to the functional distinction between Cln1 and Cln2.

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Materials and Methods

Calculation of sequence identity

Protein sequence of Cln 1, Cln2, and Cln3 were obtained from the National Center for Biotechnology Information advances science and health by providing access to biomedical and genomic information (NCBI). Accession number of Cln1, Cln 2, and Cln3 are P20437.2: Full=G1/S-specific cyclin CLN1 (Saccharomyces cerevisiae S288c), P20438.2: Full=G1/S-specific cyclin CLN2 (Saccharomyces cerevisiae S288c), and P13365.2: Full=G1/S-specific cyclin CLN3 (Saccharomyces cerevisiae S288c), respectively. The amino acid sequence identity of the cyclin box among Cln1 (101-189), Cln2 (103-191), and Cln3 (137-202) were calculated with GENETYX (version 15, GENETYX CORPORATION, Tokyo, Japan)

Prediction of secondary structures

Protein secondary structure of the cyclin box of Cln1 (101-189), Cln2 (103-191) and Cln3 (137-202) were predicted with the protein secondary structure prediction server (Jpred3: http://www.compbio.dundee.ac.uk/www-jpred/).

Prediction of PEST motif

PEST motif of Cln1 and Cln2 were predicted by the calculation with GENETYX Software for PEST sequence (version 15, GENETYX CORPORATION, Tokyo, Japan) with the consideration of the report by Hadwiger et al. [6].

Molecular modeling and evaluations

The three dimensional structures of Cln1-Cdc28, Cln2-Cdc28, and Cln3-cdc28 were constructed with homology model in 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 using amber99 force field in MOE.

Molecular dynamics (MD) simulations were performed using the AMBER9 package with the modified TIP3P water potential (CONFLEX, Tokyo, Japan). The starting structures, the two distinct conformations (flip state and flop state) at the extra amino acid region in the cyclin box of Cln1 and Cln2, were placed in a pre-equilibrated solvent box consisting of water and sodium ions, where the number of sodium ions was adjusted to neutralize the systems. The solvent box was extended 10 Å beyond the Cln1-cdc28 and Cln2-cdc28 atoms. MD simulations were performed on the resulting systems for 1000 ps at 1 atm and 300 K using the isobaric-isothermal (NPT) ensemble. The integration time step was 2 fs, and the SHAKE algorithm was used to constrain all covalent bonds involving hydrogen atoms. The long-range electrostatic interactions beyond the cutoff distance were calculated using the particle mesh Ewald method.

To calculate interaction energy and electrostatic complementarities, we used MOE and MolFeat-EC (FiatLux, Tokyo, Japan) as previously described with some modifications [22-25]. The three-dimensional structures of these complexes were displayed using MolFeat (Ver. 4, FiatLux, Tokyo, Japan).

All figures from the MD simulations of Cln1-cdc28, Cln2-cdc28, and Cln3-cdc28 complexes were produced using VMD (http://www.ks.uiuc.edu/Research/vmd/).

Ligand Interaction of PEST motifs of Cln1-Cdc28 and Cln2-Cdc28 were calculated and displayed with MOE.

Results and Discussion

The cell cycle is controlled by a series of biochemical switches that triggers the events of the cycle in the correct order [1]. Cln1 and Cln2, G1/S cyclins of the ascomycete budding yeast Saccharomyces cerevisiae, contribute to the control of new cell-cycle entry in response to growth or extracellular factors [6]. To clarify the structure-function relationship of G1/S cyclins of S. cerevisiae, we performed in silico simulations [26]; construction of three dimensional (3D) structure by homology modeling [27,28], optimization of 3D structure by molecular mechanics calculations and molecular dynamics (MD) simulations [29], evaluation of interaction energy (IE) [30] and electrostatic complementarity (EC) [22] of Cln1-Cdc28 and Cln2-Cdc28 complexes. Additionally, we also performed in silico simulations for Cln3-Cdc28 to elucidate the structural differences between G1 and G1/S cyclins (Table 1).

Construction of 3D structures of Cln1-Cdc28 and Cln2-Cdc28

To sufficiently analyze the structural properties of G1/S cyclins of S. cerevisiae, we constructed the 3D structure of Cln1-Cdc28 and Cln2-Cdc28.

Prior to perform the construction of the 3D structure of them, we checked the sequence alignment and the secondary structure of G1/S cyclins Cln1 and Cln2. They have an identity of 57% at sequence level. However, the N-terminal regions of them, which contain the cyclin box (about 100 a.a.), are retained high similarity (74% identity). Furthermore, as shown in Figure 2a, Cln1 and Cln2 characteristically possessed the extra amino acid region (21 a.a.) in the cyclin box region among the G1 and G1/S cyclins of fungi, such as A. fumigatus Cln1, C. neoformans Cln1, S. pombe Puc1, etc. [31]. On the other hand, conservation is relaxed in their C-terminal regions (45% identity). This

| Interaction Energy (kcal/mol) | EC |
|------------------------------|----|
| Cln1 (flip) - Cdc28          | -745.2 | 0.50 |
| Cln1 (flop) - Cdc28          | -836.1 | 0.54 |
| Cln2 (flip) - Cdc28          | -672.8 | 0.51 |
| Cln2 (flop) - Cdc28          | -875.7 | 0.54 |

Table 1: Interaction Energy and Electrostatistic Complementarity of Cln1-Cdc28, and Cln2-Cdc28.
would suggest that the elements responsible for the distinction between both cyclins (for instance, specific protein-interacting domains) could be located in the C-terminal portions of the proteins.

Subsequently, we predicted the secondary structure of the cyclin box of these cyclins. Figure 2b shows that the extra amino acid region in the cyclin box of Cln1 and Cln2 are proposed to be a linear structure, partially beta-sheet was included in Cln2 [32].

Taken together these information, we have constructed the 3D structure of Cln1-Cdc28 and Cln2-Cdc28 by using protein homology modeling applications in MOE: 1) homology sequence analysis, 2) multiple-structure alignment and superposition, 3) structural homologue identification, and 4) 3D model building.

Notably, the resulted 3D structure of Cln1-Cdc28 and Cln2-Cdc28 were demonstrating the two distinct conformations at the extra amino acid region in the cyclin box of Cln1 and Cln2.

As shown in Figure 3a-1 and 3b-1, the one conformation represented the extra amino acid region in the cyclin box of Cln1 and Cln2 did not bind with Cdc28, designated as the flip state. On the other hand, the other conformation represented the extra amino acid region of them bind with Cdc28, designated as the flop state (Figure 3a-2 and 3b-2). We intuited that these two distinct conformations might be playing an important role to carry out the function of G1/S cyclins function of S. cerevisiae. Therefore, we performed MD simulations for the flip and flop conformation of Cln1-Cdc28 and Cln2-Cdc28 to observe the state of the structural change, respectively.

In fact, prior to MD simulations, we presumed that the extra amino acid region in the cyclin box were in the equilibrium between flip and flop conformation. However, the results of MD simulations were deviated from our expectation. We did not observe any typical flip and flop conformation. However, the results of MD simulations indicated that the role of flop conformation is to stabilize Cln1-Cdc28 and Cln2-Cdc28 complexes for carrying out G1/S cyclins function. Furthermore, it is consistent with in vitro results that Cln2-Cdc28 plays a major role as G1/S cyclins of S. cerevisiae than Cln1-Cdc28 [6].

In addition to calculation of IE, we investigated EC for quantitative evaluation of 3D structures of Cln1-Cdc28 and Cln2-Cdc28. To better understand the flip-flop conversion of the extra amino acid region in the cyclin box of Cln1 and Cln2, we checked the interaction energy (IE) and electrostatic complementarity (EC) of the complex as a set of folding doors presentation (image of open book). In accordance with our observation, the EC value of Cln1-Cdc28 in flip and flop state was 0.50 and 0.54, respectively. On the other hand, that of Cln2-Cdc28 was 0.51 and 0.54, respectively. Indeed, the symmetricalness of the electrostatic potential (blue; positive charge, red; negative charge) on the protein-protein interface domain of G1/S cyclins indicated that the role of flop conformation is to stabilize Cln1-Cdc28 and Cln2-Cdc28 complexes higher than that of flip state. We presumed that these gap of the electrostatic potential between flip and flop conformation were induced by the high EC of the extra amino acid region in the cyclin box of Cln1 (0.68) and Cln2 (0.63).

Taken together these results, it seems difficult to induce the flip-flop conversion on Cln1-Cdc28 and Cln2-Cdc28 without any driving forces.


evaluation of 3D structures of Cln1-Cdc28 and Cln2-Cdc28

To better understand the flip-flop conversion of the extra amino acid region in the cyclin box of Cln1 and Cln2, we checked the interaction energy (IE) and electrostatic complementarity (EC) of the most stable complex structure of Cln1-Cdc28 and Cln2-Cdc28 during MD simulations.

The IE values of Cln1-Cdc28 in flip and flop conformation was -745.2 kcal/mol and -836.1 kcal/mol, respectively. On the other hand, that of Cln2-Cdc28 was -672.8 kcal/mol and -875.7 kcal/mol respectively. Moreover, the energy gap in IE values between flip and flop conformation of Cln1-Cdc28, ΔE(Cln1-Cdc28), and Cln2-Cdc28, ΔE(Cln2-Cdc28) were 90.9 kcal/mol and 202.9 kcal/mol, respectively. Therefore, we suspected that the flip-flop conversion is easily induced on Cln1-Cdc28, ΔE(Cln1-Cdc28): 90.9 kcal/mol, rather than Cln2-Cdc28, ΔE(Cln2-Cdc28): 202.9 kcal/mol. These results presumably indicated that the role of flop conformation is to stabilize Cln1-Cdc28 and Cln2-Cdc28 complexes for carrying out G1/S cyclins function. Furthermore, it is consistent with in vitro results that Cln2-Cdc28 plays a major role as G1/S cyclins of S. cerevisiae than Cln1-Cdc28 [6].

To better understand the flip-flop conversion of the extra amino acid region in the cyclin box of Cln1 and Cln2, we checked the interaction energy (IE) and electrostatic complementarity (EC) of the complex as a set of folding doors presentation (image of open book). In accordance with our observation, the EC value of Cln1-Cdc28 in flip and flop state was 0.50 and 0.54, respectively. On the other hand, that of Cln2-Cdc28 was 0.51 and 0.54, respectively. Indeed, the symmetricalness of the electrostatic potential (blue; positive charge, red; negative charge) on the protein-protein interface domain of flip state of Cln1-Cdc28 and Cln2-Cdc28 were higher than that of flip state. We presumed that these gap of the electrostatic potential between flip and flop conformation were induced by the high EC of the extra amino acid region in the cyclin box of Cln1 (0.68) and Cln2 (0.63). Taken together these results, it seems difficult to induce the flip-flop conversion on Cln1-Cdc28 and Cln2-Cdc28 without any driving forces.
Additionally, the IE and EC value of Cln3-Cdc28 are -653.6 kcal/mol and 0.59, respectively (Supplementary Figure 2). IE value of Cln3-Cdc28 (-635.6 kcal/mol) is lower than that of Cln1(flop)-Cdc28 (-836.1 kcal/mol) and Cln2(flop)-Cdc28 (-875.7 kcal/mol). On the other hand, EC value of of Cln3-Cdc28 (0.59) is also lower than that of Cln1-Cdc28 (0.68: the extra amino acid region) and Cln2-Cdc28 (0.63: the extra amino acid region). The difference of these values among them is presumably due to the existence of the extra amino acid region in the cyclin box which is possessed in Cln1 and Cln2. Moreover, these results showed coincidence with the functional difference of among them that Cln3 is a very-low-abundance protein with a much weaker associated kinase activity relative to Cln1 and Cln2 [9].

**Conversion and Degradation of Cln1-Cdc28 and Cln2-Cdc28**

It has been proposed that the oscillation of G1/S cyclins of *S. cerevisiae* Cln1 and Cln2 during the cell cycle is controlled by the rapid degradation of them through the ubiquitination of the sequences rich in Pro (P), Glu (E), Ser (S) and Thr (T), so-called PEST motifs [6]. Here, we hypothesized that the flip-flop conversion of Cln1-Cdc28 and Cln2-Cdc28 might be regulated allosterically through the ubiquitination of G1/S cyclins of *S. cerevisiae* Cln1 and Cln2 [19,33-35].

To sufficiently characterize the structure-function relationship of the PEST motifs of G1/S cyclins of *S. cerevisiae* Cln1 and Cln2, we predicted the location of them on the 3D structure of Cln1 (35-546, flop conformation)-Cdc28 and Cln2 (37-534, flop conformation)-Cdc28. As shown in Figure 5a, two PEST motifs were predicted in Cln1. One is located near the Cdc28 binding domain in the cyclin box that is close to the N-terminal domain (233-274) and the other is located on the C-terminal domain (398-425). On the other hand, as shown in Figure...
S. cerevisiae. It is inferred from these observations that the flip-flop conversion by the ubiquitination of the PEST motif plays a dominant role in oscillation of Cln1-Cdc28 and produce the modest degradation [33]. These results might be consistent with a single PEST motif of Cln2 [35].

Figure 5: Schematic models and the flip state molecular interaction models of (a) Cln1 (35-546)-Cdc28 (5-298) and (b) Cln2 (37-534)-Cdc28 (5-298). Ribbon: Cln1/2, Spacellfilling: Cdc28. Gray region in schematic models is the cyclin box (Cln1; 101-189, Cln2; 103-191), blue and red in both schematic models and the flip state molecular interaction models is the extra amino acid region and the PEST motif, respectively.

Figure 6: Model of the flip-flop regulation mechanism of Cln1 and Cln2 during late G1/S phase Cln1/2 in blue, Cdc28 in brown, and the PEST motif in red.

Conclusion

In conclusion, our research may be deciphering the role of the extra amino region in the cyclin box of G1/S cyclins of S. cerevisiae, which, until now, to our knowledge, has not been assigned significance in cyclins functions and structural analysis.

We insinuated that the flip-flop conversion of the extra amino region might be one of the key characteristics functions of the G1/S cyclins of S. cerevisiae, which controls the oscillation during cell cycle in G1/S cyclins (Cln1 and Cln2) but not in G1 cyclin (Cln3).

Furthermore, we speculate that the functional superiority between Cln1 and Cln2 in the G1/S phase of S. cerevisiae might be regulated by the flip-flop conversion by the ubiquitination of PEST motifs. However, Cln1 and Cln2 share an overlapping role in execution of G1/S function, Cln2 could be a major regulatory element of G1/S phase control because of the higher flip-flop conversion energy and the modest degradation through the ubiquitin-proteasome pathway [6,21].

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References

1. Wittenberg C (2005) Cell cycle: cyclin guides the way. Nature 434: 34-35.
2. Pines J (1995) Cyclins and cyclin-dependent kinases: a biochemical view. Biochem J 308 : 697-711.
3. Enserink JM, Kolodner RD (2010) An overview of Cdc1-controlled targets and processes. Cell Div 5: 11.
4. Küntzel H, Schulz A, Ehbrecht IM (1996) Cell cycle control and initiation of DNA replication in Saccharomyces cerevisiae. Biol Chem 377: 481-487.
5. Wittenberg C, Reed SI (2005) Cell cycle-dependent transcription in yeast: promoters, transcription factors, and transcriptomes. Oncogene 24: 2746-2755.
6. Hadwiger JA, Wittenberg C, Richardson HE, de Barros Lopes M, Reed SI (1989) A family of cyclin homologs that control the G1 phase in yeast. Proc Natl Acad Sci U S A 86: 6255-6259.
7. Yaglom J, Linskens MH, Sadis S, Rubin DM, Futcher B, et al. (1995) p34Cdc2-mediated control of Cln3 cyclin degradation. Mol Cell Biol 15: 731-741.
8. Andrews B, Meaday V (1998) The cyclin family of budding yeast: abundant use of a good idea. Trends Genet 14: 66-72.
9. Tyers M, Tokiwa G, Futcher B (1993) Comparison of the Saccharomyces cerevisiae G1 cyclins: Cln3 may be an upstream activator of Cln2 and other cyclins. EMBO J 12: 1955-1968.
10. de Bruin RA, McDonald VH, Kalashnikova TI, Yates J 3rd, Wittenberg C (2004) Cln3 activates G1-specific transcription via phosphorylation of the SBF bound repressor Whi5. Cell 117: 887-898.
11. Dirick L, Böhm T, Nasmyth K (1995) Roles and regulation of Cln-Cdc28 kinases...
11. Suganami A, Takase N, Sugiyama H, Virtudazo EV, Kawamoto S, et al. (2014) Structure based Functional Distinction between Cln1 and Cln2 Depends on the Ubiquitin-Proteasome Pathway. J Proteomics Bioinform 7: 102-107. doi:10.4172/jpb.1000309

12. Stuart D, Wittenberg C (1995) CLN3, not positive feedback, determines the timing of CLN2 transcription in cycling cells. Genes Dev 9: 2780-2794.

13. Nasmyth K, Dirick L (1991) The role of SW4 and SW6 in the activity of G1 cyclins in yeast. Cell 66: 995-1013.

14. Ogas J, Andrews BJ, Herskowitz I (1991) Transcriptional activation of CLN, CLN2, and a putative new G1 cyclin (HC526) by SW4, a positive regulator of G1-specific transcription. Cell 66: 1015-1026.

15. Barral Y, Jentsch S, Mann C (1995) G1 cyclin turnover and nutrient uptake are controlled by a common pathway in yeast. Genes Dev 9: 399-409.

16. Willems AR, Lanier S, Patton EE, Craig KL, Nason TF, et al. (1996) Cdc53 targets phosphorylated G1 cyclins for degradation by the ubiquitin proteolytic pathway. Cell 86: 129-140.

17. Skowrya D, Craig KL, Tyers M, Elledge SJ, Harper JW (1997) F-box proteins are receptors that recruit phosphorylated substrates to the SCF ubiquitin-ligase complex. Cell 86: 209-219.

18. Di Como CJ, Chang H, Arndt KT (1995) Activation of CLN1 and CLN2 G1 cyclin expression by BCK2. Mol Cell Biol 15: 1835-1846.

19. Berzet C, Grici P, Tempel R, La Rue J, Wittenberg C, et al. (2002) Transferable domain in the G(1) cyclin Cln2 sufficient to switch degradation of Sic1 from the E3 ubiquitin ligase SCF(Cdc4) to SCF(Grr1). Mol Cell Biol 22: 4463-4476.

20. Gueralt E, Iglau JC (2004) Functional distinction between Cln1p and Cln2p cyclins in the control of the Saccharomyces cerevisiae mitotic cycle. Genetics 168: 129-140.

21. Quilis I, Iglau JC (2012) Molecular basis of the functional distinction between Cln1 and Cln2 cyclins. Cell Cycle 11: 3117-3131.

22. McCoy AJ, Chandana Epa V, Colman PM (1997) Electrostatic complementarity at protein/protein interfaces. J Mol Biol 268: 570-584.

23. Tonooka A, Kubo T, Ichimiya S, Tamura Y, Ilmarinen T, et al. (2009) Wild-type AIRE cooperates with p63 in HLA class II expression of medullary thymic stromal cells. Biochem Biophys Res Commun 379: 765-770.

24. Shimizu M, Nakamura H, Hirabayashi T, Suganami A, Tamura Y, et al. (2008) Ser515 phosphorylation-independent regulation of cytosolic phospholipase A2alpha (cPLA2alpha) by calmodulin-dependent protein kinase: possible interaction with catalytic domain A of cPLA2alpha. Cell Signal 20: 815-824.

25. Virtudazo EV, Suganami A, Tamura Y, Kawamoto S (2011) Towards understanding cell cycle control in Cryptococcus neoformans: structure-function relationship of G1 and G1/S cyclins homologue CnCln1. Biochem Biophys Res Commun 416: 217-221.

26. Jones S, Thornton JM (1998) Principles of protein-protein interactions. Proc Natl Acad Sci U S A 95: 13-20.

27. Sánchez R, Pieper U, Melo F, Eswar N, Martí-Renom MA, et al. (2000) Protein structure modeling for structural genomics. Nat Struct Biol 7 Suppl: 986-990.

28. Martí-Renom MA, Stuart AC, Fiser A, Sánchez R, Melo F, et al. (2000) Comparative protein structure modeling of genes and genomes. Annu Rev Biophys Biomol Struct 29: 321-325.

29. Wang J, Wolf RM, Caldwell JW, Kollman PA, Case DA (2004) Development and testing of a general amber force field. J Comput Chem 25: 1157-1174.

30. Halperin I, Ma B, Wolfson H, Nussinov R (2002) Principles of docking: An overview of search algorithms and a guide to scoring functions. Proteins 47: 409-443.

31. Castillo-Llueva S, Pérez-Martín J (2005) The induction of the mating program in the phytopathogen Ustilago maydis is controlled by a G1 cyclin. Plant Cell 17: 3544-3560.

32. Cole C, Barber JD, Barton JL (2008) The Jpred 3 secondary structure prediction server. Nucleic Acids Res 36: W197-201.

33. Salama SR, Hendrickx KB, Thormer J (1994) G1 cyclin degradation: the PEST motif of yeast Cln2 is necessary, but not sufficient, for rapid protein turnover. Mol Cell Biol 14: 7953-7966.

34. Giotzter M, Murray AW, Kirschner MW (1991) Cyclin is degraded by the ubiquitin pathway. Nature 349: 132-138.

35. Rechsteiner M, Rogers SW (1996) PEST sequences and regulation by proteolysis. Trends Biochem Sci 21: 267-271.