Figure S1. Phylogenetic tree of Cdc13 proteins from the sub-phylum Saccharomycotina, constructed based upon M-coffee alignment of sequences and visualized with NJPlot; species in the *Saccharomyces*, *Kluveromyces* and *Candida* are indicated in red, green and blue, respectively. Although the tree recapitulates the currently accepted organismal phylogeny, it lacks an outgroup species, due to the inability to identify Cdc13 orthologs outside Saccharomycotina; the root of the tree was placed based on published organismal phylogenies.

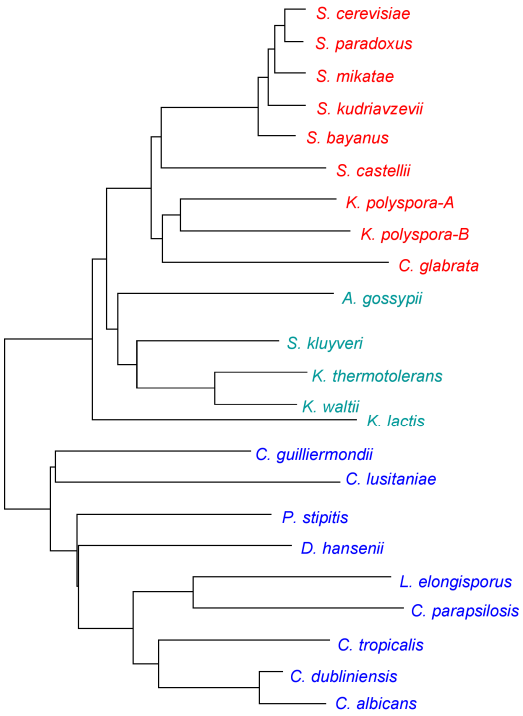

Table S1. Sequence of oligonucleotides tested for single-stranded DNA binding.

Species		Oligos tested (5' to 3')	Template (3' to 5') [1]
S. cerevisiae		n.a.	TGGTGTCTGGGTG
C. glabrata		(TGGGGTCTGGGTG)$_2$	CTGTGGGGTCTGGGTG
C. albicans	Ca T1	(TGGTGTACGGATG)$_2$	TCTAACTTCTTGGTGTACGGATG
	Ca T2	TGGTGTACGGATG	
	Ca T3	(GTGTACGGATGTCTAACTTCTTG)$_2$	
C. parapilosis		(TGAGGTCCGGATG)$_2$	TTGATTATACTGAGGTCCGGATG
	R1	AGTACTAGGATATATCAGACTATGAC	
	R2 [2]	(CAAGAAGTTAGACATCCGTACAC)$_2$	
	R3	CATCGCGACTAATGGCAGTATCTCGA	

[1] The 13 bases of the telomerase template which are conserved among the Saccharomycotina sub-phylum (1) are indicated in blue, with sequence variations relative to the *S. cerevisiae* sequence indicated in aqua; additional regions of the template which are incorporated into telomeric repeats are in grey.

[2] This oligomer is the complement to Ca T3.

EXPERIMENTAL METHODS

Identification of Cdc13 proteins from the *Candida* clade: Cdc13 proteins from the *Saccharomyces* and *Kluvermyces* clades were readily recovered by standard BLAST searches of the non-redundant protein database from NCBI. Candidate proteins from the *Candida* clade were subsequently identified using iterative PSI-BLAST searches, using either the full length *S. cerevisiae* Cdc13 protein or just the DNA binding domain (a.a. 497–694). Using the full length *S. cerevisiae* protein, a potential homolog in *C. tropicalis* was recovered on the second iteration with an *E*-value of 1 x 10^{-4}. Two additional iterations of the PSI-BLAST search revealed a family of proteins within the *Candida* clade. PSI-BLAST searches with just the *S. cerevisiae* DBD identified a potential homolog in the *Candida* clade of budding yeast (*Pichia stipitis*, 4 x 10^{-3}); the second (*D. hansenii*, *C. tropicalis, C. guilliermondii*) and third iterations (*C. dubliniensis* and *C. albicans*) identified further potential homologs in the clade. A reciprocal PSI-BLAST search with

full length *C. albicans* Cdc13 identified *P. stipitis* Cdc13 with an *E*-value of 1 x 10^{-7} on the first iteration and *S. cerevisiae* Cdc13 on the second iteration with an *E*-value of 7 x 10^{-5}. The ability to recover homologs from *Saccharomyces* and *Candida* clades by reciprocal searches argues that the *Candida* proteins are *bona fide* orthologs of the *S. cerevisiae* Cdc13 protein. We were unable to identify potential homologs of Cdc13 in species outside Saccharomytina, which parallels the inability of others (2).

Structure predictions: Structural predictions of the putative *C. albicans* DNA binding domain were performed using a panel of structure prediction programs, as described previously (3, 4). Submission of an alignment of *Candida* proteins for the region encompassing the predicted DNA binding domain to the HHpred structure-prediction server identified similarity to the *S. cerevisiae* Cdc13 DBD structure with 100% confidence. Similarly, submission of the predicted *C. albicans* DBD to the SAM-T08 structure-prediction server also identified the *S. cerevisiae* Cdc13 DBD as the top structural template with an *E*-value of 4.9 x 10^{3}. Similar domain boundaries were used for the submission of the *C. albicans* protein to the I-TASSER server. The first I-TASSER model had an estimated TM score of 0.78 +/- 0.1, suggesting a model of correct topology. Furthermore, structural alignment of the top I-TASSER models with the HHpred or SAM predictions gave average TM scores of 0.88 and 0.69, respectively, demonstrating a strong agreement between all three methods. Based on analysis of previous CASP results, this high level of similarity between models strongly suggests that the models predicted are close to accurate and hence that the predicted DNA binding domain of the *C. albicans* proteins adopts an OB-fold which is structurally similar to the *S. cerevisiae* DBD.

Protein purification: The genes encoding Cdc13-DBD proteins were cloned into the pRSET.A plasmid (Invitrogen) containing an N-terminal hexahistidine tag and expressed in *E. coli* BL21 (DE3) cells. Protein expressed in the soluble fraction was purified by passage over a His-Select HF Ni affinity gel column (Sigma) followed by size exclusion chromatography (Superdex G200, GE Healthcare) to isolate the monomeric proteins. Single-stranded DNA oligos used for EMSA assays were ordered from Integrated DNA Technologies (IDT) with PAGE purification. 50 pmoles of each single-stranded DNA oligo to be used was 5' end-labeled with ^{32}P-(γ-ATP) using T4 PNK (NEB) and unincorporated ^{32}P-(γ-ATP) was removed using a G-25 spin column (GE Healthcare**).** 5% native gels (1X TBE, 5% glycerol, 4 mM $MgCl_2$) were run in 1X TBE, 5% glycerol, 2.5 mM $MgCl_2$ at 4°C for 30 minutes at 107 volts. Binding assays proceeded as follows: ^{32}P-ssDNA oligos to be tested were incubated at 95°C for 20 minutes then cooled on ice until use. Protein dilutions were prepared in protein dilution buffer (10 mM Hepes pH 7.8, 75 mM KCl, 1 mM DTT). ^{32}P-ssDNA dilutions (400 pM) were prepared using sterile DNA binding buffer (10 mM Hepes pH 7.8, 75 mM KCl, 5 mM $MgCl_2$, 1 mM DTT, 20% glycerol, 1 mg BSA (NEB), 0.1 mM EDTA). 5 μL of each protein dilution or protein dilution buffer was mixed with 5 μL of ^{32}P-ssDNA and incubated on ice for 1 hour. 5 μL of each reaction was run on a native gel, dried down on Whatman paper and exposed to phosphorimaging plates. ImageQuant 5.1 (GE Healthcare) software was used to quantify the ^{32}P-ssDNA signal from each gel. KaleidaGraph software was used to fit and plot the data. Three independent experiments were performed and were globally fit using nonlinear curve regression to a standard two-state binding model to calculate the K_d, as described previously (5). Errors in the reported K_d values are the standard error of the mean extracted from the global fits.

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