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Sequence-Specific Binding to Telomeric DNA Is Not a Conserved Property of the Cdc13 DNA Binding Domain

Edward K. Mandell,[†] Amy D. Gelinas,[‡] Deborah S. Wuttke,[‡] and Victoria Lundblad*[,†]

[†]Salk Institute for Biological Studies, La Jolla, California 92037, United States

[‡]Department of Chemistry and Biochemistry, University of Colorado, Boulder, Colorado 80309-0125, United States

Supporting Information

ABSTRACT: In the budding yeast *Saccharomyces cerevisiae*, chromosome end protection is provided by a heterotrimeric complex composed of Cdc13 in association with the RPA-like proteins Stn1 and Ten1. We report here that the high affinity and specificity of the *S. cerevisiae* Cdc13 DNA binding domain for single-stranded telomeric DNA are not widely shared by other fungal Cdc13 proteins, suggesting that restriction of this complex to telomeres may be limited to the *Saccharomyces* clade. We propose that the evolutionarily conserved task of Stn1 and Ten1 (and their associated large subunit) is a genome-wide role in DNA replication rather than a telomere-dedicated activity.

The single-stranded extension of the G-rich strand of chromosome ends is a highly conserved feature of eukaryotic telomere structure, as is the need to protect these natural DNA ends. Experiments in a number of organisms have demonstrated that unprotected telomeres are subjected to a variety of DNA processing insults, with resulting catastrophic consequences for genome integrity.[1]

In budding yeast, telomere integrity relies on a heterotrimeric complex composed of Cdc13, Stn1, and Ten1,[2] which is targeted to chromosome ends through the exceptionally high affinity and specificity that Cdc13 displays for G-rich telomeric single-stranded DNA (ssDNA).[3,4] Defects in any of these three proteins lead to the extensive loss of the telomeric C strand and cell cycle arrest,[5−7] showing that this complex protects telomeres from unregulated nucleolytic resection. Recent evidence indicates that these three proteins function as a telomere-dedicated RPA-like complex,[8−10] which we have called the t-RPA complex. While the canonical RPA complex binds to double-strand breaks and subsequently blocks cell cycle progression to coordinate DNA repair, the t-RPA complex is proposed to protect yeast telomeres from such events and thereby ensure cell cycle progression.

A potentially similar heterotrimeric complex has also been identified in human cells as an activity called AAF (α-accessory factor) that stimulates the DNA polymerase α−primase complex in vitro.[11,12] More recently, this complex has been proposed to perform a telomere capping function in parallel with the well-characterized shelterin complex.[13,14] However, the yeast and human complexes exhibit one key difference, which is that the human complex lacks telomere-specific DNA binding activity,[14] in sharp contrast to the specificity displayed by the *Saccharomyces cerevisiae* Cdc13 protein.

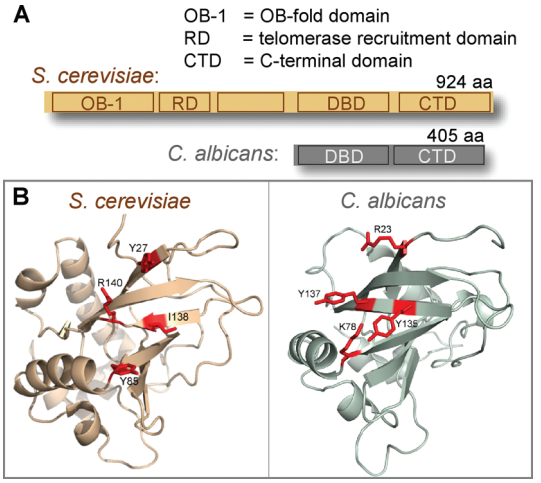

Figure 1. (A) Schematic comparison of the domain structure of Cdc13 and (B) the structure of the DBD, from *S. cerevisiae* and *C. albicans*, with residues that comprise the hot spot" for binding affinity colored red.

Because *S. cerevisiae* has played such a key role as a model organism for telomere biology, we asked whether the sequence-specific DNA binding behavior of Cdc13 is a conserved property. Specifically, we have analyzed Cdc13 proteins from the subphylum Saccharomycotina (budding yeast), which comprises the *Saccharomyces*, *Kluveromyces*, and *Candida* clades and represents an evolutionary distance equivalent to that from humans to tunicates.[15] Alignment of Cdc13 proteins from the *Saccharomyces* and *Kluveromyces* clades revealed the presence of five distinct domains (Figure 1A).[16] In contrast, Cdc13 homologues from the *Candida* clade are composed of only the DNA binding domain (DBD) and the C-terminal domain (Figure 1A).[10,17] The absence of the three N-terminal domains is not due to incorrect predictions of gene boundaries, as examination of upstream regions did not reveal the existence of reading frames that had been missed during automated gene annotation. Furthermore, analysis of syntenic relationships provided evidence of substantial genomic rearrangements upstream of the *CDC13* genes in the *Candida* clade.[17]

In addition to loss of the N-terminal half of the protein, changes in the Cdc13 DBD were also observed. Examination of the Cdc13 DBDs from the *Saccharomyces* and *Kluveromyces*

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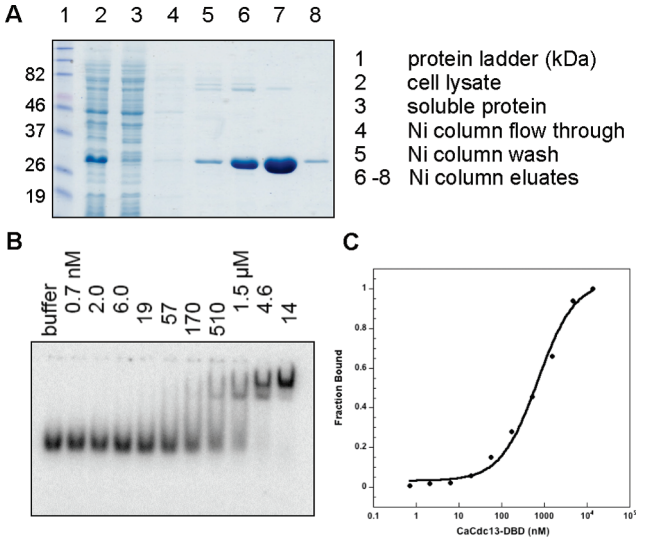

Figure 2. Expression, purification, and representative binding data of the *C. albicans* DBD with a 26-mer telomeric oligomer (Ca T1; see Table S1 of the Supporting Information). Affinity purification (A) and electrophoretic mobility shift assays (B) were performed as described in the Supporting Information, and the data were fit to a standard two-state binding model (C).

clades revealed a significant degree of similarity both at the sequence level and in the predicted secondary and tertiary structures. In contrast, the same domain from the *Candida* clade had undergone substantial sequence divergence, both within the clade and when compared to the rest of the Saccharomycotina subphylum. Nevertheless, structure predictions indicated with a high degree of confidence that the *Candida* clade DBDs adopted an OB fold that was structurally similar to that of the *S. cerevisiae* Cdc13 DBD[18] (Figure 1B and Supporting Information). Comparison of the predicted *C. albicans* structure with the *S. cerevisiae* DBD structure revealed a key difference, however. In the *S. cerevisiae* Cdc13 DBD, a "hot spot" for binding affinity is formed by a cluster of four DNA contact residues,[19] which are conserved among members of the *Saccharomyces* clade. In contrast, this hot spot cluster was not observed in the predicted *C. albicans* DBD (Figure 1B). The absence of this hot spot was not restricted to the *C. albicans* DBD, as a high frequency of substitutions occurred at these four amino acid positions among all members of the *Candida* clade.

These changes at the predicted ssDNA−protein interface suggested that the properties of interaction with ssDNA would be substantially different. To examine this, a panel of Cdc13 DBDs were expressed in *E. coli* and initially examined for solubility; three DBDs (*Candida glabrata* from the *Saccharomyces* clade and *Candida parapsilosis* and *C. albicans* both from the *Candida* clade) were chosen for further analysis (see Figure S1 of the Supporting Information for a phylogenetic tree of Cdc13 sequences). Figure 2 and Table 1 illustrate the binding properties of the *C. albicans* Cdc13 DBD with a telomeric 26-mer, compared to nontelomeric ssDNA oligomers of comparable length. Surprisingly, the *C. albicans* DBD bound telomeric ssDNA oligomers with an affinity that was reduced by ~1000-fold, when compared to the affinity of the binding of the *S. cerevisiae* DBD to comparable oligomers.[4] Furthermore, the affinity of the *C. albicans* DBD for a nontelomeric substrate relative to a telomeric oligomer was reduced only ~5-fold, in contrast to *S. cerevisiae* Cdc13, which

Table 1. K_D Values for the Wild-Type *C. albicans* Cdc13 DBD, or the DBD Bearing the Indicated Mutations, Bound to Single-Stranded Oligonucleotides

C. albicans DBD	oligonucleotide[a]	K_D (μM)	*x*-fold change
wild type	Ca T1 (26-mer)	0.65 ± 0.10	–
	Ca T2 (13-mer)	1.8 ± 0.27	2.8
	R1 (26-mer)	2.0 ± 0.58	3.1
	R2 (46-mer)	1.90 ± 0.24	3.2
	Ca T3 (46-mer)	0.59 ± 0.09	0.9
R23A	Ca T3 (46-mer)	2.70 ± 0.33	4.6
K78A	Ca T3 (46-mer)	1.90 ± 0.15	3.2
Y135A	Ca T3 (46-mer)	3.20 ± 0.33	5.4
Y137A	Ca T3 (46-mer)	1.20 ± 0.17	2.0

[a] Oligonucleotide sequences are listed in Table S1 of the Supporting Information.

discriminates telomeric ssDNA by at least 1000-fold.[3] The level of binding to a 13-mer telomeric ssDNA was reduced relative to that of the 26-mer substrate (Table 1), which further differentiates the *C. albicans* Cdc13 DBD from the *S. cerevisiae* DBD.[4]

The lack of strong discrimination for telomeric substrates was not specific for the *C. albicans* Cdc13 DBD, as the *C. parapsilosis* DBD also exhibited a similar binding affinity for a telomeric 26-mer (K_D = 0.21 ± 0.026 μM), which was enhanced only 7-fold relative to those of nontelomeric substrates.

As an additional point of comparison with the *S. cerevisiae* Cdc13 DBD, alanine missense mutations were introduced into the four residues indicated in Figure 1 of the *C. albicans* Cdc13 DBD. Alanine replacements in the four comparable residues of the *S. cerevisiae* Cdc13 DBD comprising the hot spot result in a 500−700-fold reduction in binding affinity, as shown previously.[19] In striking contrast, the four mutant *C. albicans* Cdc13 DBDs exhibited at most a 5-fold impact on binding affinity, relative to that of the wild-type DBD (Table 1).

These observations indicate that the *C. albicans* and *C. parapsilosis* Cdc13 DBDs exhibit biochemical properties that are quite distinct from those of the *S. cerevisiae* Cdc13 DBD, which correlates with the changes predicted for the ssDNA−protein interface for the *C. albicans* and *C. parapsilosis* DBDs (Figure 1 and data not shown). In addition to the loss of the affinity hot spot, the unusual $\beta2-\beta3$ loop that plays a central role in ssDNA recognition by the *S. cerevisiae* Cdc13 DBD[18] was shorter in the *C. albicans* and *C. parapsilosis* Cdc13 DBD models and also lacked the high content of aromatic residues that contributed to binding by the *S. cerevisiae* DBD. These two changes may be sufficient to account for the dramatic reduction in the level of binding to telomeric ssDNA substrates by the *C. albicans* and *C. parapsilosis* DBDs.

Assessment of the predicted ssDNA−protein interface for other Cdc13 members of the Saccharomycotina subphylum suggests that an attenuated preference for telomeric substrates may be a general feature, as Cdc13 proteins from several members of the *Kluveromyces* clade exhibited amino acid replacements at both the hot spot for binding affinity and the $\beta2-\beta3$ loop. We therefore examined the DNA binding properties of the Cdc13 DBD from *C. glabrata*, which diverged from the common ancestor of *Saccharomyces sensu stricto* species after whole-genome duplication occurred and is an outlier of the *Saccharomyces* clade.[20] The *C. glabrata* DBD bound a ssDNA 26-mer oligomer composed of G-rich telomeric DNA with a K_D of 0.079 ± 0.02 μM, which was

several orders of magnitude weaker than the affinity of the *S. cerevisiae* DBD for its telomeric substrate. Furthermore, the *C. glabrata* DBD favored a telomeric substrate by only 40−80-fold in binding affinity, relative to nontelomeric substrates. This suggests that a transition from nonspecific ssDNA binding to an increased affinity for telomeric ssDNA took place in the common ancestor of *C. glabrata* and the rest of the *Saccharomyces* clade, with increased specificity evolving along the branch leading to *S. cerevisiae*.

These observations raise the provocative question of whether telomere-specific localization of Cdc13, and its associated proteins Stn1 and Ten1, is restricted to members of the *Saccharomyces* clade. Specifically, we propose that only Cdc13 from *S. cerevisiae* (and presumably closely related species) possesses the sequence-specific DNA binding properties that are necessary to target this complex to a specific region of the genome. This proposal is also consistent with the absence of the N-terminal domain of Cdc13 within the *Candida* clade, which would prevent inappropriate recruitment of telomerase to nontelomeric regions of the genome. The corollary of this proposal is that the evolutionarily conserved task of Stn1 and Ten1 (and their associated large subunit) is a genome-wide role in DNA replication, rather than a direct role in telomere maintenance or chromosome end protection. Consistent with this hypothesis, the human Stn1 protein was first discovered as a subunit of a stimulatory factor for DNA polymerase α/primase.[11,12] The exclusive localization of this complex to telomeres in *S. cerevisiae* may reflect niche specialization, with the acquisition of specificity for telomeric ssDNA driven by evolutionary pressures within the *Saccharomyces* clade.

■ ASSOCIATED CONTENT

Supporting Information. Supplementary Table S1, Figure S1, and detailed experimental methods. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: lundblad@salk.edu. Phone: (858) 453-4100, ext. 1913. Fax: (858) 457-4765.

Author Contributions

E.K.M. and A.D.G. contributed equally to this work.

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