1-24-2006

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Identification of the Determinants for the Specific Recognition of Single-Strand Telomeric DNA by Cdc13†

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Received July 1, 2005; Revised Manuscript Received October 17, 2005

ABSTRACT: The single-strand overhang present at telomeres plays a critical role in mediating both the capping and telomerase regulation functions of telomeres. The telomere end-binding proteins, Cdc13 in *Saccharomyces cerevisiae*, Pot1 in higher eukaryotes, and TEBP in the ciliated protozoan *Oxytricha nova*, exhibit sequence-specific binding to their respective single-strand overhangs. *S. cerevisiae* telomeres are composed of a heterogeneous mixture of GT-rich telomeric sequence, unlike in higher eukaryotes which have a simple repeat that is maintained with high fidelity. In yeast, the telomeric overhang is recognized by the essential protein Cdc13, which coordinates end-capping and telomerase activities at the telomere. The Cdc13 DNA-binding domain (Cdc13-DBD) binds these telomere sequences with high affinity (3 pM) and sequence specificity. To better understand the basis for this remarkable recognition, we have investigated the binding of the Cdc13-DBD to a series of altered DNA substrates. Although an 11-mer of GT-rich sequence is required for full binding affinity, only three of these 11 bases are recognized with high specificity. This specificity differs from that observed in the other known telomere end-binding proteins, but is well suited to the specific role of Cdc13 at yeast telomeres. These studies expand our understanding of telomere recognition by the Cdc13-DBD and of the unique molecular recognition properties of ssDNA binding.

Recognition of single-strand DNA (ssDNA)[1] is essential for many fundamental cellular activities, including recombination, repair, replication, transcription, translation, and telomere maintenance. Structural and biochemical studies of the proteins that mediate ssDNA-binding activity have provided key insights into the mechanism of recognition. Generally, small domains containing mixed α/β topologies, such as the OB fold and KH domain, are used for ssDNA recognition, and binding is achieved through base and backbone interactions that are completely distinct from those used in double-strand DNA recognition (*1*−*5*). In many biological contexts, DNA needs to be uniformly recognized; thus, it is appropriate for this recognition to be non-sequence-specific. However, in a few cases, including telomere maintenance, transcription, and viral packaging, it is imperative that recognition be exquisitely sequence-specific (*3*, *6*−*8*).

Telomeres, the DNA, RNA, and protein complexes at the ends of chromosomes, play important roles in many essential cellular functions. They regulate the proliferative lifetime of the cell and protect chromosomes from degradation or end-to-end fusion, as well as participate in meiotic segregation and chromatic silencing (reviewed in refs *9*−*13*). Telomeres are critical to the maintenance of the genome and normal development. Thus, telomere malfunction can have dramatic adverse impacts on human health, leading to cancer, aging disorders, and increased human mortality (*14*−*19*). Therefore, it is essential to understand the mechanisms through which telomeres are properly maintained.

In all organisms studied thus far, telomeres terminate in a highly regulated single-strand overhang comprised of the GT-rich strand. This end must be sequestered from cellular factors to prevent chromosomal degradation, as well as to discriminate the end structure from damaged DNA (*20*−*22*). A family of single-strand DNA-binding proteins with binding specificity for the GT sequences at telomeres has been identified, the functions of its members include protection of the overhang from degradation and regulation of telomerase activity (*8*, *23*−*26*). Members of this family include the Pot1 (protection of telomeres) proteins from organisms ranging from *Schizosaccharomyces pombe* to human (*24*), the TEBPs (telomere end-binding proteins) from ciliates (*27*, *28*), and the Cdc13 proteins from budding yeasts (*8*).

Cdc13 is strictly required for proper *Saccharomyces cerevisiae* telomere function (*8*, *29*, *30*) and mediates the activity of two primary telomere functions: end protection and telomerase regulation. Deletion of Cdc13 is lethal due to the loss of the essential end protection function. Loss of Cdc13 activity leads to dramatic resection of the C-rich strand of the chromosome (up to 10 000 bases) (*31*). This DNA damage activates the Rad9 DNA damage pathway, resulting in cell-cycle arrest at the G_2 phase (*32*). In addition to end protection, Cdc13 is both a positive and negative regulator of telomere length (*23*, *33*). Loss of the ability of Cdc13 to

† Financially supported by NIH Grant GM59414, the Arnold and Mabel Beckman Foundation, and the Keck Foundation.

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[1] Abbreviations: BSA, bovine serum albumin; DBD, DNA-binding domain; DTT, 1,4-dithiothreitol; EDTA, ethylenediaminetetraacetic acid; OB, oligonucleotide/oligosaccharide binding; Pot, protection of telomeres; ssDNA, single-strand DNA; TEBP, telomere end-binding protein; thio-G, 6-thioguanosine; WC, Watson−Crick.

10.1021/bi0512703 CCC: $33.50
Published on Web 12/22/2005

interact with telomerase leads to progressive erosion of telomeres, resulting in a delayed lethal phenotype (*34*, *35*). Negative regulation of telomere length is less well understood, but is believed to involve the interplay of additional end capping proteins (*33*). Extensive biochemical and genetic data indicate that Cdc13 serves as the primary anchor point for assembly of the functional complexes at the telomere through its specific interaction with the G-rich tail of telomeres. This ssDNA binding activity is performed by a centrally located domain within the full-length protein that spans residues 497−694 (Cdc13-DBD) (*36*).

High-fidelity recognition of *S. cerevisiae* telomeres presents a biochemical challenge due to the intrinsic heterogeneity of the sequence. Rather than consisting of a simple repeat that is maintained with high integrity, yeast telomeres are a mixture of sequential guanines interspersed within runs of GTs. Sequence patterns can be summarized with the consensus sequence $\{(TG)_{0-6}TGGGTGTG(G)_n\}$ (*39*). This heterogeneity can be understood by examining the features of the unusually large telomerase RNA templating sequence (here 3′−5′), ACACACACCCACACCAC (*41*). The variability observed in yeast telomeres can be completely explained by a combination of abortive reverse transcription of this template and the ability of the template to bind substrate in more than one register (*39*). The Cdc13-DBD must recognize these variable sequences and also remain specific for telomeric DNA to ensure proper telomere maintenance and to prevent aberrant telomerase activity at other sites, such as double-strand breaks.

Cdc13-DBD binds single-strand telomeric DNA with an unusually high affinity, with a dissociation constant of 3 pM at low ionic strength (*36*). The NMR structure of the Cdc13-DBD−DNA complex revealed that the Cdc13-DBD adopts an oligosaccharide/oligonucleotide binding motif (OB fold), the topology present in both the Pot1 (*42*, *43*) and TEBP (*44*) proteins. An extensive binding interface is formed between the Cdc13-DBD and the minimal telomeric DNA (GTGTGGGTGTG, Tel-11) (*45*−*47*). Specific sites in the protein that contribute to affinity for DNA were identified by a complete alanine scan of the protein−DNA interface (*48*). Analysis of the mutations that abolish DNA binding in vivo revealed that most are lethal, highlighting the requirement for appropriate Cdc13 DNA binding activity for cellular viability (R. B. Cervantes, L. C. Ricks, J. N. Roberts, D. S. Wuttke, and V. Lundblad, unpublished data). Here, we report the in vitro binding affinity of the Cdc13-DBD for a series of DNA ligands containing several natural and unnatural base substitutions to probe the molecular requirements for the unusual specificity necessary for biological function. Our results indicate that, while a surprisingly small set of bases confers sequence-specific binding, this minimal set is enough to ensure the biologically required telomere localization. The specificity exhibited by the Cdc13-DBD is distinct from that observed in other telomere end-binding proteins, illustrating the diversity exhibited by the OB fold in performing this specialized function.

EXPERIMENTAL PROCEDURES

Cdc13-DBD Protein. The minimal DNA-binding domain of the telomere end-binding protein from *S. cerevisiae*, Cdc13, spans residues 497−694 of the full-length protein (*36*). The C-terminally His-tagged Cdc13-DBD was expressed in *Escherichia coli* and purified using a nickel-charged HiTrap Chelating HP column (GE Healthcare/Amersham Biosciences) as previously described (*36*). This His-tagged protein binds to the single-strand DNA ligand Tel-11 (dGTGTGGGTGTG) with affinity indistinguishable from that of the non-His-tagged version. Protein concentrations were determined by absorbance at 280 nm ($\epsilon = 14\,320$ M^{-1} cm^{-1}).

DNA Ligands. DNA ligands were purchased on the microgram scale (Operon) and purified by reversed-phase HPLC (*36*). DNA concentrations were determined by absorbance at 260 nm using standard extinction coefficients calculated from the sequence. Binding reaction mixtures contained ^{32}P-labeled single-strand DNA ligands (*36*) at concentrations (30−50 pM) at least 100-fold below the measured dissociation constants.

Nitrocellulose Filter Binding Studies. Equilibrium binding reactions were performed with a fixed concentration of DNA ligand by varying the concentration of protein over a broad range in binding buffer [5 mM HEPES (pH 7.8), 600 mM LiCl, 2.5 mM $MgCl_2$, 0.1 mM Na_2EDTA, 2 mM DTT, and 0.1 mg/mL BSA] (*36*, *48*). This buffer is identical to that used in our previous studies with the exception of the monovalent salt (*36*, *48*). In this study, we substituted 600 mM LiCl for 750 mM KCl because LiCl has been shown to reduce the propensity of G-quadruplex formation (*49*, *50*). Although these structures have not been observed in our gel electrophoresis assays with the canonical ligand, Tel-11 (GTGTGGGTGTG), we wanted to minimize the possibility of G-quadruplex formation for some of the G-rich mutant ligands in this study. The binding constant for the Cdc13-DBD with Tel-11 in 600 mM LiCl (2.7 nM) was comparable to the binding constant in 750 mM KCl (1 nM). While the possibility that substitutions in the Tel-11 DNA cause structural changes in the Cdc13-DBD exists, preliminary NMR data show that the global structure of the Cdc13-DBD is not significantly altered when several different DNA variants are bound (M. P. Latham and D. S. Wuttke, unpublished data).

The 96-well MultiScreen MAHA N4550 nitrocellulose filter plates (Millipore) were prepared for binding via prewetting for 1 min with 80 μL of the binding buffer lacking DTT and BSA. Samples containing protein and DNA were incubated for 1 h on ice and were then loaded onto the filter paper in the wells and incubated for 1 min before being filtered with a MultiScreen Resist Vacuum manifold. The wells were washed twice with 200 μL of buffer without DTT and BSA, air-dried, and then exposed to a Phosphorimager screen. ImageQuant (Molecular Dynamics) was used to quantify radioactivity on the filters. Data were fit to the standard two-state binding model $y = C_1[x/(x + K_d)] + C_2$, where y is the magnitude of radioactivity bound to the protein, x is the protein concentration, C_2 is the baseline or background counts, C_1 is the total change in counts (value at the plateau minus the background counts), and K_d is the apparent equilibrium constant. The reported $\Delta\Delta G$ values of the ensemble in the degenerate experiment and in the single-substitution experiment are an average of experiments repeated under the same conditions. $\Delta\Delta G$ values for the single-substitution experiment were determined using an average value for the wild-type K_d. In the case of the

degenerate library experiment, the $\Delta\Delta G$ values were calculated using a value for the wild-type K_d determined for each plate to correct for a systematic difference in sample binding from plate to plate.

RESULTS

Identification of "Hot Spots" for Cdc13 Specificity within the Telomere Sequence. To efficiently identify the bases in Tel-11 critical for recognition, we substituted an approximately equimolar mixture of the three other noncanonical natural bases for the wild-type base at a given position in the 11-mer (Table 1). Specificity was assessed by comparing the apparent binding affinities for the cognate and randomized sequences. Surprisingly, the effects of substitution varied dramatically throughout Tel-11. The relative binding affinities spanned a wide range, from slight changes to a 450-fold reduction in affinity. The effects partitioned into three classes: large (>100-fold), small (4−100-fold), and negligible (<2-fold) (Table 1). Figure 1 shows representative binding data from these binding experiments using one example from each class compared to wild-type Tel-11. Large reductions in affinity were observed for substitutions to bases in positions 1, 3, and 4 at ~100-, ~450-, and ~400-fold, respectively. Replacing bases at positions 2, 7, and 9 has a small effect (~4−6-fold) on affinity, while substitution of any of the remaining positions (5, 6, 8, 10, and 11) results in minor effects on measured binding affinity. Figure 2 highlights the distinction between the bases at the 5′ end of Tel-11 required for specificity and the bases at the 3′ end that are not individually required for specificity (although they are required for high-affinity binding, vide infra).

Table 1: Affinity Changes for Binding of the Cdc13-DBD to Tel-11 Containing a Degenerate Library Substitution at Each Site in the DNA

sample	sequence	ΔΔG (kcal/mol) ensemble	average relative K_d
WT	GTGTGGGTGTG		1.0
1	**A** **C**TGTGGGTGTG **T**	2.5 ± 0.3	95
2	**A** G**C**GTGGGTGTG **G**	1.0 ± 0.1	5.9
3	**A** GT**C**TGGGTGTG **T**	3.4 ± 0.1	450
4	**A** GTG**C**GGGTGTG **G**	3.2 ± 0.3	360
5	**A** GTGT**C**GGTGTG **T**	-0.6 ± 0.1	0.4
6	**A** GTGTG**C**GTGTG **T**	0.1 ± 0.1	1.1
7	**A** GTGTGG**C**TGTG **T**	0.9 ± 0.1	5.0
8	**A** GTGTGGG**C**GTG **G**	-0.5 ± 0.2	0.4
9	**A** GTGTGGGT**C**TG **T**	0.8 ± 0.2	4.1
10	**A** GTGTGGGTG**C**G **G**	0.2 ± 0.2	1.4
11	**A** GTGTGGGTGT**C** **T**	-0.1 ± 0.02	0.9

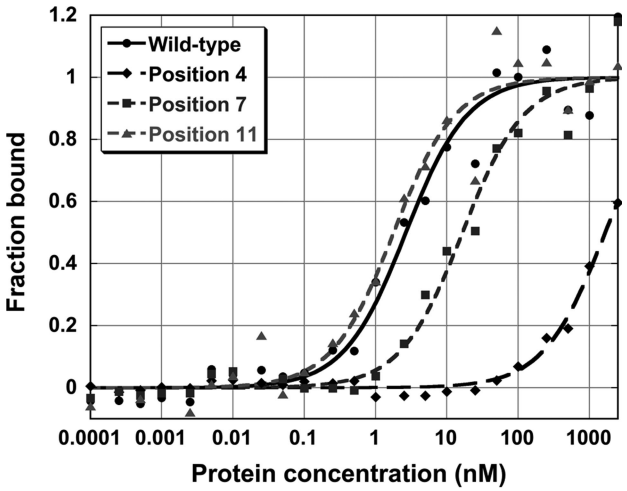

FIGURE 1: Binding curves from the degenerate library experiment illustrating the range of measured binding affinities. Data are fit to a two-state model as described in Experimental Procedures. Wild-type binding data are shown with filled circles. Data obtained from replacement of the canonical base with a library of alternate natural bases for positions 4, 7, and 11 are shown with diamonds, squares, and triangles, respectively.

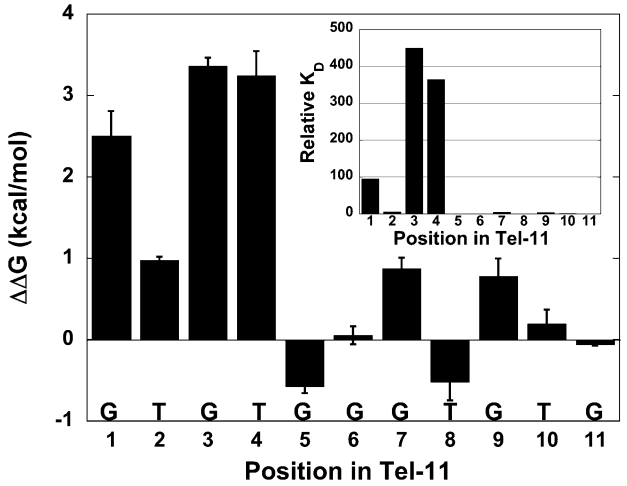

FIGURE 2: Graphical representation of the ensemble average $\Delta\Delta G$ values for substitution of individual bases with a library of other natural bases. The inset shows the relative binding constants at each position. Sequence of the parent Tel-11 oligonucleotide is shown at the bottom of the graph.

Role of the 3′ End of Tel-11. None of the bases at the 3′ end of Tel-11 are individually required for binding specificity since substitution at each position with the pool of natural bases has only weak effects on affinity. However, the 3′ bases are known to be important for maintaining affinity since binding to shorter ligands results in weakened binding (data not shown and ref *45*). Loss of the terminal base results in a 20-fold reduction in the level of binding, and no binding is observed for an 8-mer ligand as assessed by analogous filter binding experiments (data not shown). In addition, changing every site that is not strongly specifically recognized to its complementary base (creating the GAGTCCCACAC substrate) results in a complete loss of binding. This

indicates that, while a wholesale substitution of the 3′ region is not tolerated by the Cdc13-DBD, this region maintains a preference for GT-rich sequences. The striking difference in the magnitude of affinity effects depending on the position in Tel-11 led to further study of atomic determinants for specificity at the 5′ end of the DNA ligand.

Single-Substitution Experiments Delineate the Chemical Moieties Required for Specific Recognition at the 5′ End. The chemical moieties required for specific binding at the 5′ end of the DNA were identified using a series of singly substituted ligands (see Materials and Methods). Bases with the largest affinity reductions in the library experiment (positions 1, 3, and 4) were individually substituted with the three alternative natural bases and several non-natural variants. The natural base substitutions test for the importance of base size and hydrogen bond donor−acceptor patterns, while the non-natural variants test specific chemical moieties. For example, inosine is identical to guanine but lacks the extracyclic amine at the 2 carbon. Inosine was substituted at positions 1 and 3 to test the thermodynamic importance of the extracyclic amine on binding by guanine. The variant thio-G, in which the 6-carbonyl oxygen is replaced with sulfur, was tested to determine the importance of the oxygen as a hydrogen-bonding acceptor. Thio-G reports on hydrogen bonding because the hydrogen bond accepting potential of the sp^2-hybridized sulfur is much weaker than that of oxygen (*51*). Deoxyuracil was substituted at position 4 to test the importance of the ring methyl of thymine on binding. Qualitatively, the aggregate binding data from this single-substitution experiment mirrored the results from the library experiment. Substitution of any other natural base at positions 1, 3, and 4 resulted in a marked decrease in binding affinity. The differences in the relative affinities between the substituted ligands and the base variants provide insight into the base features required for specific recognition by the Cdc13-DBD. Data are summarized in Table 2 and Figure 3.

Table 2: Affinity Changes for Binding of the Cdc13-DBD to Tel-11 Containing Single Substitutions

sample	sequence	average $\Delta\Delta G$ (kcal/mol)	average relative K_d
wild type	GTGTGGGTGTG	0	1
A1	**A**TGTGGGTGTG	2.9 ± 0.1	200
C1	**C**TGTGGGTGTG	3.3 ± 0.1	370
T1	**T**TGTGGGTGTG	2.8 ± 0.1	170
S1	**S**TGTGGGTGTG	2.8 ± 0.2	160
I1	**I**TGTGGGTGTG	0.7 ± 0.1	3.8
A3	GT**A**TGGGTGTG	3.3 ± 0.1	410
C3	GT**C**TGGGTGTG	4.3 ± 0.1	>2300
T3	GT**T**TGGGTGTG	3.5 ± 0.4	>540
S3	GT**S**TGGGTGTG	3.2 ± 0.4	340
I3	GT**I**TGGGTGTG	0.2 ± 0.1	1.4
A4	GTG**A**GGGTGTG	3.9 ± 0.1	>1100
C4	GTG**C**GGGTGTG	3.7 ± 0.1	>830
G4	GTG**G**GGGTGTG	2.8 ± 0.1	170
U4	GTG**U**GGGTGTG	0.4 ± 0.1	2.2

G1 Substitutions. Guanine has several possible specificity determinants, including the size of the purine base and specific interactions with hydrogen bond acceptors and/or donors. Recognition of the base could occur through the Watson−Crick (WC) interaction face, which includes the extracyclic amine, the 1-imino, and the 6-carbonyl oxygen, or the Hoogsteen face, which includes N7 and the 6-carbonyl

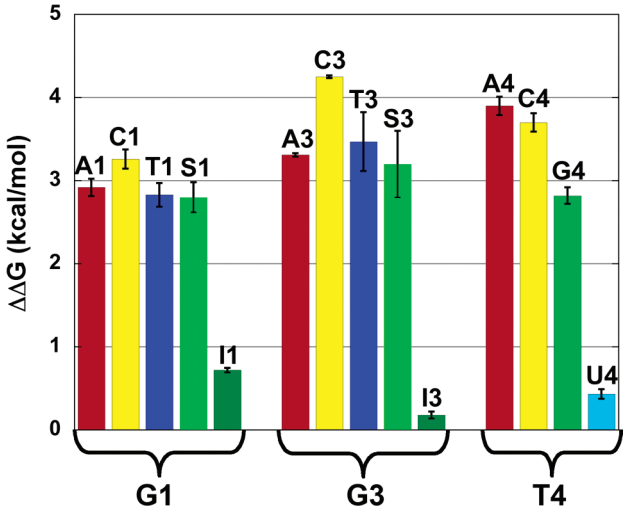

FIGURE 3: Graphical representation of the energetic impact of single substitutions on binding to the Cdc13-DBD. The alternative bases substituted for each of the first four bases in Tel-11 are color coded by base type, with data for adenine in red, data for cytosine in yellow, data for thymine in blue, and data for guanine in green. Data for analogues of guanine are in alternate shades of green: light green for thio-G (S) and dark green for inosine (I). Data for the analogue of thymine, uracil (U), are shown in light blue.

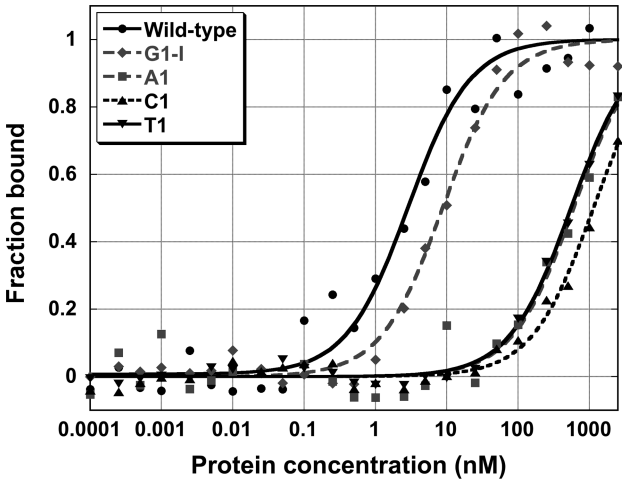

FIGURE 4: Binding curves for site-specific substitutions at G1. Replacement of G1 with other natural bases has a strong effect on binding affinity, while inosine has a modest effect. Data are fit to a two-state model as described in Experimental Procedures. Wild-type data are shown with circles, inosine data with diamonds, and A, C, and T data with squares, triangles, and upside-down triangles, respectively.

oxygen. Figure 4 illustrates the large range of binding affinities observed when G1 is substituted with natural and non-natural ligands. Replacing G1 with inosine results in a small reduction in affinity (~3-fold), indicating that the extracyclic amine is not required for recognition or affinity. In contrast, the 6-carbonyl oxygen is very important for recognition, since substitution of G1 with thio-G results in an ~150-fold reduction in affinity. Substitution with adenine represents a complete change in the WC acceptors and donors and leads to an ~200-fold decrease in affinity. This loss of binding is approximately equal to swapping the 6-carbonyl oxygen for sulfur (thio-G), indicating that the 1-imino donor contributes minimally to binding. Also, this result suggests that the Hoogsteen hydrogen bond acceptor (N7) cannot compensate for the loss of the 6-carbonyl oxygen. We

substituted thymine for G1 to test the importance of the size and/or shape of the purine base on binding, while preserving a WC-face carbonyl oxygen hydrogen bond acceptor. A decrease in affinity of 160-fold indicates that the size and shape of the purine base are important for binding. This conclusion is supported by the cytosine substitution, which causes a reduction in affinity (~370-fold) that is more than twice the effect of the thymine or thio-G substitutions and nearly double that of the adenine substitution. These data indicate that both the purine base geometry and the 6-carbonyl oxygen are critical for recognition and binding of the guanine at position 1.

G3 Substitutions. Guanine at position 3 is absolutely essential for recognition of Tel-11 by the Cdc13-DBD as no other natural base is tolerated at this position. The trends in affinity changes upon substitution closely parallel those observed for substitution at position 1. Substitution with adenine, thymine, and thio-G have large effects on affinity (410-, 540-, and 340-fold decreases, respectively), while substitution with inosine results in essentially no change in affinity (~1.3-fold decrease). Also, replacing G3 with a cytosine leads to the greatest reduction in the apparent K_d of any substitution in the study (>2000-fold decrease). Therefore, both the purine base geometry and the 6-carbonyl oxygen are critical for recognition and affinity. Interestingly, thymine is less well tolerated here compared to position 1, suggesting that the geometry of the purine base is more important for specificity and affinity at position 3. Nucleotide G3 is the most important base for recognition and specificity, since position 3 has a much lower tolerance for altering any of these determinants than any other site in Tel-11.

T4 Substitutions. The thymine at position 4 is highly specifically recognized by the Cdc13-DBD. The thermodynamic effects of substitution at position 4 are second in magnitude only to those at G3 (Table 2), revealing that the GT pair at position 3 is a stringent specificity determinant for Cdc13. Substitution of T4 with cytosine results in a >830-fold reduction in binding affinity, suggesting that this pyrimidine ligand cannot be accommodated in the binding pocket to satisfy either steric or hydrogen bonding requirements. Substitution of T4 with deoxyuracil produces a very small change (~2.2-fold decrease), indicating that the ring methyl is not important for specificity. Purine bases are not well tolerated at this position, with the level of binding by adenine decreased by >1100-fold and guanine somewhat better tolerated, exhibiting a 170-fold decrease in affinity. Thus, base size is clearly an important discriminant at this position. The pronounced lack of tolerance for substitution at this site suggests that the alternate bases cannot reorient to find favorable steric or hydrogen bonding interactions, consistent with limited conformational malleability at this site. In contrast to the pronounced effects of substitution at T4, modification from T at position 2 to any other base is well-tolerated and results in modest (<30-fold) decreases in affinity (data not shown), consistent with the data from the pooled oligonucleotide experiment.

DISCUSSION

A Cdc13-DBD Specificity Motif within Tel-11. A subset of bases at the 5′ end of Tel-11, G1, G3, and T4, are specifically recognized by the Cdc13-DBD. Substitution of these bases with either a mixture of alternate bases or each of the other natural bases results in sizable decreases in binding free energy (see Figures 1−4). In contrast, individual substitution of any of the bases at the 3′ end (5−11) with a library of the other natural bases results in only modest changes in binding (Table 1 and Figure 2). Thus, the $G_1X_2G_3T_4$ motif is the specificity element for Cdc13. Recognition of the two guanines requires a hydrogen bond acceptor, the 6-carbonyl oxygen, and favors the purine base geometry. The hydrogen bonding pattern on T4 is also required for recognition with base size or shape being less important. In contrast, all of the bases in Tel-11 are required for high-affinity binding as the Cdc13-DBD binds weakly to shorter ligands, suggesting that nonspecific backbone and/or stacking interactions are dominant in the 3′ end of the Tel-11 ligand.

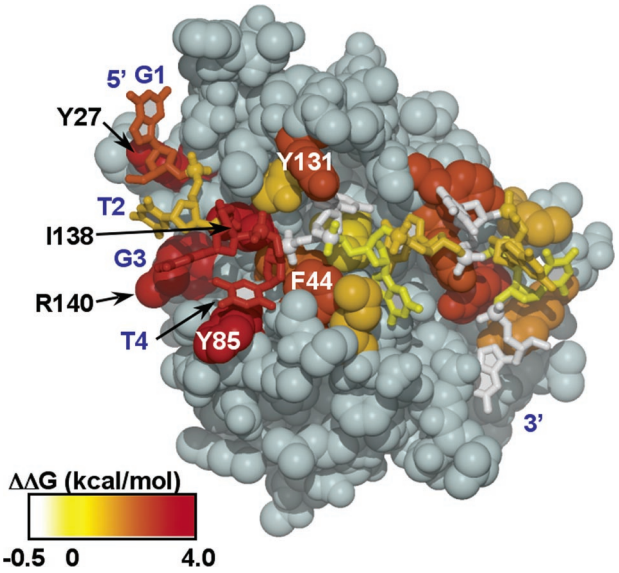

FIGURE 5: Energetic contributions of each DNA base and individual protein residues to binding mapped onto the Cdc13-DBD−Tel-11 structure. The magnitude of the change in binding free energy is illustrated using a color ramp from white to red, for −0.5 to 4.0 kcal/mol. Values for $\Delta\Delta G$ are from the ensemble average values obtained in the degenerate library experiment and from the alanine mutagenesis study by Anderson et al. (*48*). This figure was prepared using MOLSCRIPT (*63*).

Coincidence of the Protein and DNA Determinants of Binding. The specificity-determining region of Tel-11, the 5′ $G_1X_2G_3T_4$ interaction site, overlaps precisely with the hot spot of affinity on the protein surface identified by the complete alanine-scanning mutagenesis of the residues at the interface (*48*). The spatial coincidence of the thermodynamically important bases in Tel-11 and residues in the Cdc13-DBD is striking when the average $\Delta\Delta G$ values resulting from mutation of both the DNA and protein are simultaneously mapped on the structure of the complex (Figure 5). Therefore, the direct contacts between the important protein residues and the critical DNA bases confer specificity as well as affinity.

Analysis of Protein Determinants of Specificity. The structure of the Cdc13-DBD−Tel-11 complex identified the contact partners but not the precise geometry of the protein−DNA contacts. With the knowledge of the specificity-determining features of the DNA, the structure can be re-examined to predict the protein elements that confer DNA

binding specificity. The two features of the G1 base that confer specificity are the shape of the purine base and the oxygen at the 6 carbon. The orientation of this base with respect to the protein surface is not well defined in the structure due to fraying near the end; therefore, we cannot unambiguously identify the protein residues involved in recognition. There are, however, several candidates that may participate in a hydrogen bond interaction with G1. A nearby residue, Y27, has one of the largest decreases in binding affinity (650-fold) when mutated to alanine, and this mutation is lethal to the cell (R. B. Cervantes, L. C. Ricks, J. N. Roberts, D. S. Wuttke, and V. Lundblad, unpublished data) (*48*). The side chain hydroxyl of Y27 is close to the phosphate backbone of G1 and does not appear to participate in a direct H-bonding interaction with the base. One possibility is a conformational rearrangement of Y27 to place the aromatic ring in line to form a hydrogen bond (H-bond) contact with G1. Alternate H-bonding partners for G1 include lysine 5, which would require the side chain to rotate toward G1, or lysine 26, where G1 would have to flip the solvent-exposed 6-carbonyl oxygen inward toward the protein. There are no obvious structural constraints on the size or shape of the base, but perhaps the requirement for a purine at this position is due to proper alignment of the 6-carbonyl oxygen for hydrogen bonding interactions or for stacking interactions with Y27.

The important recognition elements of G3 are the 6-carbonyl oxygen and the geometry of the purine base. The requirement for an oxygen atom at position 6 suggests that a hydrogen bonding interaction between the protein and the DNA is important for the specific recognition of this critical base. The side chain of R140 likely provides the hydrogen bond donor. In the structure, R140 is poised to interact directly with the base of G3. Mutation of this conserved residue to alanine decreases the affinity by 540-fold and is lethal in vivo (R. B. Cervantes, L. C. Ricks, J. N. Roberts, D. S. Wuttke, and V. Lundblad, unpublished data) (*48*). Similarly, a substitution of adenine for G3, which results in a loss of a hydrogen bond acceptor at position 6, decreases the affinity by 380-fold. Furthermore, mutating R140 to lysine restores binding and cellular viability, implying a requirement for a hydrogen bond donor at this position. Another candidate hydrogen bonding residue is K127 since mutation to alanine is barely viable to cells and reduces binding affinity (R. B. Cervantes, L. C. Ricks, J. N. Roberts, D. S. Wuttke, and V. Lundblad, unpublished data). However, K127 is closer to T2 in the structure. Since T2 is not strongly recognized by Cdc13, the role of K127 may be to form a hydrogen bonding interaction with the oxygen at position 4 to correctly orient T2 for a stacking interaction with G3.

The specific recognition of T4 appears to be achieved by a combination of space complementarity and hydrogen bonding interactions. Examination of the structure reveals a hydrophobic pocket at this site formed by Y85, F44, and I83. Tyrosine 85, one of the most important residues for binding DNA (a 700-fold reduction in the level of binding is observed in Y85A), is involved in a stacking interaction with T4 and forms one side of a buried pocket that accommodates T4. Additionally, the side chain hydroxyl of Y85 is in position to hydrogen bond to the oxygen on the carbon at position 4, which may direct specificity for a T at this position. I138 and F44 also play a role in maintaining affinity (replacement with alanine results in 710- and 170-fold reductions in affinity, respectively) perhaps by sterically constraining the pocket to accommodate the smaller pyrimidine base.

Recognition Trends in the Telomere End-Binding Protein Family. Specific recognition of telomeric ssDNA by the telomere end-binding proteins from different organisms, Cdc13 from budding yeast, TEBP from ciliated protozoa, and Pot1 from fission yeast and humans, follows similar but not identical trends (*42*−*44*, *46*, *47*). All members of this family specifically recognize a subset of the bases in their canonical ligand. The cognate sequence for the *Sp*Pot1 N-terminal DBD is GGTTAC. Substitution of any of the first five bases with the complementary base results in large decreases in affinity, ranging from 200-fold (G1) to greater than 1000-fold (G2−A5) (*7*). This specificity has been attributed to the unusual nonsequential G-T hydrogen bonding interactions that occur within the compact DNA ligand (*42*). The full-length *Sp*Pot1 protein binds a larger substrate containing at least two GGTTAC repeats, with relaxed specificity for the second repeat relative to the first (*52*). Similarly, hPot1, which binds the sequence T**TAGG**GTTA**G**, is most sensitive to substitutions to the five underlined bases (*43*). The 5′ end of the ligand is specifically recognized, and the final 3′ end guanine is sequestered within the protein. Thus, the Cdc13-DBD specificity requirements mirror those observed for the Pot1 proteins, whereby only a subset of the canonical ligand, $G_1X_2G_3T_4$, is specifically recognized by the Cdc13-DBD. In contrast, TEBP$\alpha\beta$ is distinct from the other end-binding proteins in that only two bases in the minimal 12-mer (GGGGTTTTG**GG**G) produce large affinity changes upon substitution (*44*, *53*, *54*). These affinity changes range from 10- to 80-fold, several orders of magnitude smaller than the changes observed in the binding of Pot1 or Cdc13-DBD to noncognate sequences.

Structures of the TEBP$\alpha\beta$ complex with noncognate sequences reveal the basis for the observed tolerance for base substitution. When additional bases are either deleted or inserted into the sequence, the ligand reorients such that the sequence register retains some cognate character. The result of this "nucleotide shuffling" is a relatively small change in binding energy, despite large structural rearrangements (*54*). This type of structural malleability may explain the relatively weak effects of individual substitutions in the 3′ end of the Tel-11 ligand but the apparent requirement for GT-rich sequence in Cdc13 binding. When all of the 3′ bases are substituted with their complement, however, no binding by the Cdc13-DBD is observed. However, at any given position in the 3′ end, substitution with a pool of alternate bases produces little change in binding, in contrast to the strict sequence requirements at the 5′ end of the ligand. The biochemical implication that the binding site at the 5′ end is fixed while the binding site at the 3′ end is more malleable can be understood in the context of the structure. The 5′ end of the cognate DNA interacts with the β-barrel and $\beta1-\beta2$ and $\beta4-\beta5$ loops, the canonical ligand binding site used by almost all OB fold proteins (*1*). However, the 3′ end of the DNA interacts with the unusually large ordered loop (between $\beta2$ and $\beta3$), a feature present in only the Cdc13-DBD and, interestingly, the second OB fold of hPot1 (*43*, *46*). This loop serves to provide a higher-affinity binding

site and may be important for the recognition of heterogeneous sequences.

While Cdc13, hPot1, *Sp*Pot1, and TEBPαβ all employ OB folds in recognizing ssDNA, the chemical nature of the protein−DNA interface is surprisingly diverse among the members of this family. Cdc13 and *Sp*Pot1 each possess a single OB fold that alone exhibits high-affinity and specific binding to DNA, while hPot1 and TEBPαβ require two or more of these protein modules for interaction with telomeric DNA in vitro. Interestingly, only hPot1 and TEBPαβ are currently known to recognize the extreme 3′ terminus by sequestering the final base within the protein structure. Cdc13 and *Sp*Pot1 may also have a role in end recognition as both proteins have been proposed to contain at least one more OB fold (*52*, *55*, *56*). Like that of the Cdc13-DBD, the DNA recognition interface of TEBPαβ contains a large number of aromatic and hydrophobic residues; 19 aromatic and hydrophobic residues cluster at the protein−DNA interface in TEBPαβ and 12 for the Cdc13-DBD (*44*, *46*, *53*, *57*). In TEBPαβ, the aromatic residues participate in a contiguous extended stacking structure with guanine bases, which is further stabilized by extensive hydrophobic packing. For Cdc13, nearly all, nine of ten that were tested, of the interfacial aromatic or hydrophobic residues are crucial for high-affinity binding; however, the stacking interactions are localized (*47*, *48*). The ten aromatic and hydrophobic interfacial residues in the hPot1 complex flank the base stacking pairs and also do not form an extended stacked system (*43*). In contrast to the other end-binding proteins, there are only five aromatic and hydrophobic residues at the *Sp*Pot1 interface. These residues form stabilizing stacking interactions similar to those observed in hPot that flank the highly compact DNA structure (*42*).

One unusual feature in Cdc13 is a dearth of charged or polar residues at the protein−DNA interface that can participate in specific hydrogen bonding interactions. In contrast, *Sp*Pot1 and hPot1 are rich in these types of residues which form specific hydrogen bonding interactions between the DNA and protein. In *Sp*Pot1, there are 13 sequence-specific hydrogen bonding interactions that form a network arising from positively charged and polar side chains in the protein to the Watson−Crick donors or acceptors of the first four bases, GGTT (*42*). Similarly, hPot1 has 22 sequence-specific hydrogen bonding interactions over two OB folds to nine of the ten bases that are recognized (*43*). TEBPαβ is less hydrophilic with nine hydrogen bonding interactions from charged and polar residues to specific moieties on the WC face of the bases within the 12-mer DNA ligand (*44*). There are only seven charged residues at the interface between the Cdc13-DBD and the Tel-11 DNA and only one polar amino acid. Two of these residues, R140, which is predicted to hydrogen bond to G3, and K127, are the only charged or polar residues that greatly weaken binding when mutated to alanine. Several other lysines along the interface have only modest reductions (<8-fold) in affinity when altered to alanine, suggesting that they are not important for hydrogen bonding interactions. Tyrosine is well-represented across the interface between the Cdc13-DBD and Tel-11 and can contribute a hydrogen bonding donor through the side chain hydroxyl. G1 may be specifically recognized by Y27 through a hydrogen bonding interaction with the 6-carbonyl oxygen. The absence of traditional hydrogen-bonding partners may assist in the recognition of heterogeneous yeast telomeres.

FIGURE 6: Potential Cdc13 binding sites mapped onto representative yeast telomere sequences (*40*). Multiple Cdc13 binding sites are present in the heterogeneous telomere sequence.

Recognition of Heterogeneous Yeast Telomere Sequences. Cdc13 binds specifically to the heterogeneous yeast telomeric sequence with high affinity. While an 11-mer of DNA sequence is necessary for high-affinity binding, we have shown that the determinants of specificity reside in the first four bases with the ideal binding site, $G_1X_2G_3T_4$. Examination of the consensus sequence representing yeast telomeres [$(TG)_{0-6}$TGGGTGTG(G)] reveals that despite the heterogeneity, yeast telomeres always contain a consensus Cdc13 recognition site (that is, a $G_1X_2G_3T_4$ sequence followed by GT-rich sequence). Inspection of several typical yeast telomere sequences (*40*), shown in Figure 6, reveals that there are many potential binding sites for the Cdc13-DBD contained within the telomere sequence. Thus, the specificity exhibited by the Cdc13-DBD is therefore ideally suited for Cdc13 to fulfill its essential role at the yeast telomere, as a consensus binding sequence is invariably present. The unusual specificity exhibited by Cdc13 also provides an alternate explanation for the intolerance of budding yeast to changes in the templating region of telomerase RNA (*58*, *59*). Complete mutation of a region of the TLC1 template indicates that yeast tolerate only conservative A/C-rich sequence in the telomerase RNA even though the reverse transcriptase is still enzymatically active. While some of this tolerance could be attributed to the loss of the double-strand binding protein Rap1 at telomeres, the patterns of viability are also consistent with the binding preferences exhibited by Cdc13 (*58*, *59*). In addition, the Cdc13-DBD binding specificity reported here explains why a short stretch of GT repeats is sufficient for the Cdc13-dependent recruitment of telomerase to double-strand breaks (*60*−*62*), leading to telomere-mediated healing of the DNA break.

ACKNOWLEDGMENT

We thank Vicki Lundblad, Rachel Cervantes, and Leslie Ricks for providing data prior to publication and Douglas Theobald and Fiona Jucker for insightful discussions and thoughtful reading of the manuscript.

REFERENCES

1. Theobald, D. L., Mitton-Fry, R. M., and Wuttke, D. S. (2003) Nucleic acid recognition by OB-fold proteins, *Annu. Rev. Biophys. Biomol. Struct. 32*, 115−133.
2. Braddock, D. T., Baber, J. L., Levens, D., and Clore, G. M. (2002) Molecular basis of sequence-specific single-stranded DNA recognition by KH domains: Solution structure of a complex between hnRNP K KH3 and single-stranded DNA, *EMBO J. 21*, 3476−3485.
3. Braddock, D. T., Louis, J. M., Baber, J. L., Levens, D., and Clore, G. M. (2002) Structure and dynamics of KH domains from FBP bound to single-stranded DNA, *Nature 415*, 1051−1056.
4. Datta, S., Larkin, C., and Shildbach, J. F. (2003) Structural insights into single-stranded DNA binding and cleavage by F factor TraI, *Structure 11*, 1369−1379.

5. Desveaux, D., Allard, J., Brisson, N., and Sygusch, J. (2002) A new family of plant transcription factors displays a novel ssDNA-binding surface, *Nat. Struct. Biol. 9*, 512−517.
6. Stern, J. C., Anderson, B. J., Owens, T. J., and Shildbach, J. F. (2004) Energetics of the sequence-specific binding of single-stranded DNA by the F factor relaxase domain, *J. Biol. Chem. 279*, 29155−29159.
7. Lei, M., Baumann, P., and Cech, T. R. (2002) Cooperative binding of single-stranded telomeric DNA by the Pot1 protein of *Schizosaccharomyces pombe*, *Biochemistry 41*, 14560−14568.
8. Nugent, C. I., Hughes, T. R., Lue, N. F., and Lundblad, V. (1996) Cdc13p: A single-strand telomeric DNA-binding protein with a dual role in yeast telomere maintenance, *Science 274*, 249−252.
9. Cong, Y. S., Wright, W. E., and Shay, J. W. (2002) Human telomerase and its regulation, *Microbiol. Mol. Biol. Rev. 66*, 407−425.
10. Cech, T. R. (2004) Beginning to understand the end of the chromosome, *Cell 116*, 273−279.
11. Ferreira, M. G., Miller, K. M., and Cooper, J. A. (2004) Indecent exposure: When telomeres become uncapped, *Mol. Cell 13*, 7−18.
12. Smogorzewska, A., and de Lange, T. (2004) Regulation of telomerase by telomeric proteins, *Annu. Rev. Biochem. 73*, 177−208.
13. Blackburn, E. H. (2001) Switching and signaling at the telomere, *Cell 106*, 661−673.
14. Shiloh, Y., and Kastan, M. B. (2001) ATM: Genome stability, neuronal development, and cancer cross paths, *Adv. Cancer Res. 83*, 209−254.
15. Wong, K.-K., Maser, R. S., Bachoo, R. M., Menon, J., Carrasco, R. D., Gu, Y., Alt, F. W., and DePinho, R. A. (2003) Telomere dysfunction and ATM deficiency comprises organ homeostasis and accelerates aging, *Nature 421*, 643−648.
16. Maser, R. S., and DePinho, R. A. (2002) Connecting chromosomes, crisis, and cancer, *Science 297*, 565−569.
17. Cawthon, R. M., Smith, K. R., O'Brien, E., Sivatchenko, A., and Kerber, R. A. (2003) Association between telomere length in blood and mortality in people aged 60 years or older, *Lancet 361*, 393−395.
18. Harley, C. B. (2002) Telomerase is not an oncogene, *Oncogene 21*, 494−502.
19. Collins, K., and Mitchell, J. R. (2002) Telomerase in the human organism, *Oncogene 21*, 564−579.
20. Cervantes, R. B., and Lundblad, V. (2002) Mechanisms of chromosome-end protection, *Curr. Opin. Cell Biol. 14*, 351−356.
21. Harrington, L. (2004) Those dam-aged telomeres, *Curr. Opin. Genet. Dev. 14*, 22−28.
22. Lydall, D. (2003) Hiding at the ends of yeast chromosomes: Telomeres, nucleases and checkpoint pathways, *J. Cell Sci. 116*, (Part 20) 4057−4065.
23. Evans, S. K., and Lundblad, V. (2000) Positive and negative regulation of telomerase access to the telomere, *J. Cell Sci. 113* (Part 19), 3357−3364.
24. Baumann, P., and Cech, T. R. (2001) Pot1, the putative telomere end-binding protein in fission yeast and humans, *Science 292*, 1171−1175.
25. Loayza, D., and de Lange, T. (2003) POT1 as a terminal transducer of TRF1 telomere length control, *Nature 423*, 1013−1018.
26. Colgin, L. M., Baran, K., Baumann, P., Cech, T. R., and Reddel, R. R. (2003) Human POT1 facilitates telomere elongation by telomerase, *Curr. Biol. 13*, 942−946.
27. Gottschling, D. E., and Zakian, V. A. (1986) Telomere proteins: Specific recognition and protection of the natural termini of *Oxytricha* macronuclear DNA, *Cell 47*, 195−205.
28. Price, C. M., and Cech, T. R. (1987) Telomeric DNA-protein interactions of *Oxytricha* macronuclear DNA, *Genes Dev. 1*, 783−793.
29. Lustig, A. J. (2001) Cdc13 subcomplexes regulate multiple telomere functions, *Nat. Struct. Biol. 8*, 297−299.
30. Lin, J.-J., and Zakian, V. A. (1996) The *Saccharomyces CDC13* protein is a single-strand TG_{1-3} telomeric DNA-binding protein *in vitro* that affects telomere behavior *in vivo*, *Proc. Natl. Acad. Sci. U.S.A. 93*, 13760−13765.
31. Booth, C., Griffith, E., Brady, G., and Lydall, D. (2001) Quantitative amplification of single-stranded DNA (QAOS) demonstrates that *cdc13-1* mutants generate ssDNA in a telomere to centromere direction, *Nucleic Acids Res. 29*, 4414−4422.
32. Garvik, B., Carson, M., and Hartwell, L. (1995) Single-stranded DNA arising at telomeres in *cdc13* mutants may constitute a specific signal for the *RAD9* checkpoint, *Mol. Cell. Biol. 15*, 6128−6138.
33. Chandra, A., Hughes, T. R., Nugent, C. I., and Lundblad, V. (2001) Cdc13 both positively and negatively regulates telomere replication, *Genes Dev. 15*, 404−414.
34. Lendvay, T. S., Morris, D. K., Sah, J., Balasubramanian, B., and Lundblad, V. (1996) Senescence mutants of *Saccharomyces cerevisiae* with a defect in telomere replication identify three additional EST genes, *Genetics 144*, 1399−1412.
35. Evans, S. K., and Lundblad, V. (1999) Est1 and Cdc13 as comediators of telomerase access, *Science 286*, 117−120.
36. Anderson, E. M., Halsey, W. A., and Wuttke, D. S. (2002) Delineation of the high-affinity single-stranded telomeric DNA-binding domain of *Saccharomyces cerevisiae* Cdc13, *Nucleic Acids Res. 30*, 4305−4313.
37. Szostak, J. W., and Blackburn, E. H. (1982) Cloning yeast telomeres on linear plasmid, *Cell 29*, 245−255.
38. Förstemann, K., Hoss, M., and Lingner, J. (2000) Telomerase-dependent repeat divergence at the 3′ end of yeast telomeres, *Nucleic Acids Res. 28*, 2690−2694.
39. Förstemann, K., and Lingner, J. (2001) Molecular basis for telomere repeat divergence in budding yeast, *Mol. Cell. Biol. 21*, 7277−7286.
40. Teixeira, M. T., Arneric, M., Sperisen, P., and Lingner, J. (2004) Telomere length homeostasis is achieved via a switch between telomerase-extendible and -nonextendible states, *Cell 117*, 323−335.
41. Singer, M. S., and Gottschling, D. E. (1994) *TLC1*: Template RNA component of *Saccharomyces cerevisiae* telomerase, *Science 266*, 404−409.
42. Lei, M., Podell, E. R., Baumann, P., and Cech, T. R. (2003) DNA self-recognition in the crystal structure of the Pot1 (Protection of Telomeres)-ssDNA complex, *Nature 426*, 198−204.
43. Lei, M., Podell, E., and Cech, T. R. (2004) Structure of human Pot1 bound to telomeric single-stranded DNA provides a model for chromosome end-protection, *Nat. Struct. Mol. Biol. 11*, 1223−1229.
44. Horvath, M. P., Schweiker, V. L., Bevilacqua, J. M., Ruggles, J. A., and Schultz, S. C. (1998) Crystal structure of the *Oxytricha nova* telomere end binding protein complexed with single strand DNA, *Cell 95*, 963−974.
45. Hughes, T. R., Weilbaecher, R. G., Walterscheid, M., and Lundblad, V. (2000) Identification of the single-strand telomeric DNA binding domain of the *Saccharomyces cerevisiae* Cdc13 protein, *Proc. Natl. Acad. Sci. U.S.A. 97*, 6457−6462.
46. Mitton-Fry, R. M., Anderson, E. M., Hughes, T. R., Lundblad, V., and Wuttke, D. S. (2002) Conserved structure for single-stranded telomeric DNA recognition, *Science 296*, 145−147.
47. Mitton-Fry, R. M., Anderson, E. M., Glustrom, L. W., Theobald, D. L., and Wuttke, D. S. (2004) Structural basis for telomeric single-stranded DNA recognition by yeast Cdc13, *J. Mol. Biol. 338*, 241−255.
48. Anderson, E. M., Halsey, W. A., and Wuttke, D. S. (2003) Site-directed mutagenesis reveals the thermodynamic requirements for single-stranded DNA recognition by the telomere-binding protein Cdc13, *Biochemistry 42*, 3751−3758.
49. Williamson, J. R., Raghuraman, M. K., and Cech, T. R. (1989) Monovalent cation-induced structure of telomeric DNA: The G-quartet model, *Cell 59*, 871−880.
50. Meyer, M., and Suhnel, J. (2003) Interaction of cyclic cytosine-, guanine-, thymine-, uracil-, and mixed guanine-cytosine base tetrads with K^+, Na^+, and Li^+ ions: A density functional study, *J. Biomol. Struct. Dyn. 20*, 507−517.
51. Allen, F. H., Bird, C. M., Rowland, R. S., and Raithby, P. R. (1997) Resonance-induced hydrogen bonding at sulfur acceptors in $R_1R_2C{=}S$ and $R_1CS_2^-$ systems, *Acta Crystallogr. B53*, 680−695.
52. Trujillo, K. M., Bunch, J. T., and Baumann, P. (2005) Extended DNA binding site in Pot1 broadens sequence specificity to allow recognition of hetergeneous fission yeast telomeres, *J. Biol. Chem. 280*, 9119−9128.
53. Classen, S., Ruggles, J. A., and Schultz, S. C. (2001) Crystal structure of the N-terminal domain of *Oxytricha nova* telomere

end-binding protein α subunit both uncomplexed and complexed with telomeric ssDNA, *J. Mol. Biol. 314*, 1113−1125.
54. Theobald, D. L., and Schultz, S. C. (2003) Nucleotide shuffling and ssDNA recognition in *Oxytricha nova* telomere end-binding protein complexes, *EMBO J. 22*, 4314−4324.
55. Theobald, D. L., Cervantes, R. B., Lundblad, V., and Wuttke, D. S. (2003) Homology among telomeric end-protection proteins, *Structure 11*, 1049−1050.
56. Theobald, D. L., and Wuttke, D. S. (2004) Prediction of multiple tandem OB-folds in telomere end-binding proteins Pot1 and Cdc13, *Structure 12*, 1877−1879.
57. Classen, S., Lyons, D., Cech, T. R., and Schultz, S. C. (2003) Sequence-specific and 3′-end selective single-strand DNA binding by the *Oxytricha nova* telomere end binding protein α subunit, *Biochemistry 42*, 9269−9277.
58. Förstemann, K., Zaug, A. J., Cech, T. R., and Lingner, J. (2003) Yeast telomerase is specialized for C/A-rich RNA templates, *Nucleic Acids Res. 31*, 1646−1655.
59. Lin, J., Smith, D. L., and Blackburn, E. H. (2004) Mutant telomere sequences lead to impaired chromosome separation and a unique checkpoint response, *Mol. Biol. Cell 15*, 1623−1634.
60. Diede, S. J., and Gottschling, D. E. (2001) Exonuclease activity is required for sequence addition and Cdc13p loading at a *de novo* telomere, *Curr. Biol. 11*, 1336−1340.
61. Stellwagon, A. E., Haimberger, Z. W., Veatch, J. R., and Gottschling, D. E. (2003) Ku interacts with telomerase RNA to promote telomere addition at native and broken chromosome ends, *Genes Dev. 17*, 2384−2395.
62. Bianchi, A., and Negrini, S. (2004) Delivery of the yeast telomerase to a DNA break depends on the recruitment functions of Cdc13 and Est1, *Mol. Cell 16*, 139−146.
63. Kraulis, P. J. (1991) MOLSCRIPT: A program for display and analysis of macromolecular structure, *J. Appl. Crystallogr. 24*, 946−950.

BI0512703