Single-stranded telomere-binding protein employs a dual rheostat for binding affinity and specificity that drives function

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T he proper management of ssDNA in the cell is required for numerous aspects of chromosome biology. In all kingdoms of life, ssDNA is formed transiently during the execution of many essential cellular processes including transcription, DNA replication, recombination, and repair. To coordinate these numerous activities, a diverse array of proteins has evolved to bind ssDNA, to facilitate normal events such as DNA replication, or to signal the appearance of inappropriate ssDNA and initiate repair (1). Several of these ssDNA-binding proteins function in genome-wide maintenance (1, 2). Widely studied examples include the bacterial single-strand–binding protein (SSB) and its functional equivalent in eukaryotes, replication protein A (RPA) (3–5). SSB and RPA are both essential for DNA replication, binding nascent ssDNA that is generated when duplex DNA is unwound and thereby preventing reannealing and/or the formation of secondary structures that would impede progression of the replisome. Both proteins are also central to the cellular response to DNA lesions. Although RPA and SSB exhibit no sequence homology, they each employ an array of OB-folds for contacting ssDNA. Detailed analysis of RPA has revealed that it utilizes these OB-folds to contact ssDNA in distinct modes, engaging differing lengths of ssDNA with different subunits, presumably to orchestrate higher-order manipulations (6–8). Thus, to interact consistently throughout the genome, RPA also needs to bind ssDNA indiscriminately. Commensurate with this expectation, RPA displays little obvious sequence preference in vitro, binding ssDNA tenaciously with single-digit nanomolar affinities (6, 7).

In contrast to the genome-wide and the apparently sequence-nonspecific role performed by the canonical RPA complex (2), proteins that interact with ssDNA overhangs at telomeres exhibit sequence specificity tuned to the G-rich telomeric repeats (9–11). These telomere-dedicated proteins also show exceptional affinities for their ssDNA ligands, ranging from the tight nanomolar binding by human Pot1 to single-digit picomolar binding by the Saccharomyces cerevisiae Cdc13 protein (9, 11, 12). Remarkably, the Cdc13 protein performs its telomere-dedicated role as a subunit of a heterotrimeric complex with a domain architecture that closely parallels that of RPA (13). In both the canonical and telomere-dedicated RPA, the large subunit is constitutively associated with two smaller proteins, Stn1/Ten1 with Cdc13 and Rpa32/Rpa14 with RPA70. In the CST complex, high affinity for ssDNA is conferred by the large subunit, whereas in RPA high

![Significance](https://www.pnas.org/lookup/suppl/doi:10.1073/pnas.1722147115/-/DCSupplemental)

Proteins that bind nucleic acids are frequently categorized as being either specific or nonspecific, with interfaces to match that activity. In this study, we have found that a telomere-binding protein exhibits a degree of specificity for ssDNA that is finely tuned for its function, which includes specificity for G-rich sequences with some tolerance for substitution. Mutations of the protein that dramatically impact its affinity for single-stranded telomeric DNA are lethal, as expected; however, mutations that alter specificity also impact biological function. Unexpectedly, we found mutations that make the protein more specific are also deleterious, suggesting that specificity and nonspecificity in nucleic acid recognition may be achieved through more nuanced mechanisms than currently recognized.

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affinity is achieved through multivalency (2, 4, 8, 12–15). However, ssDNA binding by the telomere-dedicated RPA complex (t-RPA) is notably distinct from RPA, suggesting that these structurally similar domains have taken on distinct biochemical roles. Unlike RPA70, which uses two OB-fold domains for its core recognition of ssDNA, Cdc13 employs a single OB-fold augmented by an unusually long β2–3 loop (Fig. 1) (16) to contact DNA with exceptionally tight picomolar affinity. Furthermore, Cdc13 binds ssDNA with exquisite specificity for G-rich sequences (17, 18), which it achieves through recognition of a GxGT motif embedded in a larger oligonucleotide (10, 12). Nevertheless, Cdc13 presumably needs to show sequence flexibility to accommodate the heterogeneity of yeast telomeres (19), although the mechanism by which Cdc13 achieves this flexibility has not previously been elucidated.

The sequence specificity and affinity displayed by Cdc13 provide a unique system for investigating how these two biochemical properties contribute to function in vivo. To do so, this study examined an extensive panel of mutations across the DNA-binding interface for their effects on both binding affinity and specificity and subsequently determined how perturbations in either property affected Cdc13 function in vivo. Not surprisingly, substantial reductions in Cdc13-binding affinity were lethal in vivo, whereas less severe declines in affinity were better tolerated. Unexpectedly, this approach also identified a second category of mutations that had little effect on affinity but large effects on specificity in vitro. These specificity mutations reduced the ability of Cdc13 to tolerate variations in telomere sequence, which substantially impaired Cdc13 function in vivo. Moreover, the magnitude of the in vivo defect closely correlated with the extent to which specificity was altered, thereby demonstrating that both affinity and sequence tolerance contribute to biological function. Thus, by conducting a systematic analysis of the Cdc13 DNA-binding domain (DBD) interface, we have uncovered a finely tuned binding rheostat of specificity and affinity that confers optimal biological function.

Results
Systematic Mutagenesis of the DNA-Binding Interface of Cdc13 Identifies a 35-Fold Span in Affinity. To address how the biochemical features of Cdc13 allow it to perform its biological roles, we introduced a set of eight alanine mutations into the DBD across the binding interface (16, 20), with an emphasis on the aromatic residues that play key roles in affinity and specificity, and measured the impact of these changes on binding characteristics (Fig. 1A). The change in binding affinity to the minimal Tel11 substrate (GTGTGGGTGTG) exhibited by these mutant proteins was measured at the physiological salt conditions identified previously using an EMSA binding assay (SI Appendix, Fig. S1) (12). The DBD constructs exhibited a range of binding affinities, from slightly tighter than the very tight WT apparent $K_d$ of 2.1 pm (equivalent to a reduced value of 71 pM) (Fig. 1B and SI Appendix, Table S1). These defects in binding cannot be attributed to a change in protein structure or stability. Circular dichroic and NMR analysis suggest no alterations in secondary or tertiary structure (SI Appendix, Figs. S2 and S3). Furthermore, most of the mutations did not significantly alter the melting temperature of the protein, and the observed minor changes show no correlation with biochemical activity, presumably because they are all well above the temperature at which the binding and in vivo studies were conducted (SI Appendix, Fig. S2). Thus, the impact on the binding affinity to the Tel11 substrate exhibited by these mutant proteins spanned almost 35-fold, creating a set of proteins exhibiting a wide range, or rheostat, of binding affinities.

Large Defects in Binding Affinity Correlate with Substantial Impacts on in Vivo Viability. This range of binding affinities allowed us to ask whether the unusually tight affinity exhibited by Cdc13 was required and, indeed, what level of DNA binding was necessary, for function in vivo. To do so, the mutations described above were examined for their effects in vivo by integrating each mutation into the genome of a diploid strain of yeast in place of one copy of the WT CDC13 gene. This panel of diploid strains was used to generate $\text{cdc13-DBD}^{-}$ haploid strains, which revealed a gradient of viability (Fig. 2). Changes in viability were not explained by changes in protein levels (SI Appendix, Fig. S4).

Not unexpectedly, the $\text{cdc13-Y522A}$ and $\text{cdc13-K622A}$ mutant strains, which exhibited greatly reduced binding, were capable of only two to five cell divisions (Fig. 2A), consistent with the 15- to 34-fold reduction in binding affinity for the minimal Tel11 substrate associated with these two mutations (Fig. 1B). For both strains, this severe growth defect was partially suppressed by $\text{exol-}\Delta$ and $\text{rad9-}\Delta$ mutations (Fig. 2A); this recapitulates the behavior of previously characterized $\text{cdc13}^{-}$ mutations (21–23), arguing that defects in Cdc13 DNA binding behaved in a manner comparable to other loss-of-function mutations in CDC13. Notably, the growth of the $\text{cdc13-K622A}$ mutant strain (with a 15-fold reduction in binding affinity) was reproducibly less impaired than the $\text{cdc13-Y522A}$ strain (with a 34-fold reduction in vitro binding), providing a strong correlation between the in vitro biochemical properties of these two mutations and their in vivo phenotypes.

Moderate Defects in Binding Affinity only Partially Correlate with in Vivo Viability. Surprisingly, the correlation between in vitro and in vivo behavior did not extend to other mutations introduced into the DBD interface of Cdc13. For example, two mutations,
The above results strongly suggested that af-

cdc13-Y561A and cdc13-Y626A, with only modest declines in affinity for the Tel11 substrate, nevertheless exhibited pronounced growth defects. Both these haploid mutant strains gave rise to barely visible colonies (Fig. 2 A and SI Appendix, Fig. S5A) which were accompanied by a high percentage of inviable individual cells for both strains, resulting in a long delay in forming visible colonies. Thus, despite having only a 3.5-fold effect on in vitro binding affinity, the cdc13-F539A and cdc13-Y626A mutant strains exhibited a severe degree of in vivo telomere dysfunction.

Mutations That Increase Cdc13-Binding Affinity Show Growth Defects in Strains Sensitized to Telomere Dysfunction. Equally striking was the behavior of strains expressing mutant proteins that slightly increased the affinity for the Tel11 substrate relative to the affinity of the WT protein for Tel11 (Fig. 1 B and SI Appendix, Table S1). In an otherwise WT yeast background, strains bearing mutations in Y556, I578, or Y651 exhibited a growth phenotype that was indistinguishable from that of a WT strain (Fig. 2 A and SI Appendix, Fig. S5B). However, when cdc13-Y561A or cdc13-I578A mutations were introduced into a strain background that is impaired for an additional aspect of telomere homeostasis (a telomerase deficiency), these mutant proteins were incapable of conferring the same level of function as the WT Cdc13 protein. Immediately following the loss of telomerase, the growth of a telomerase-defective strain is initially indistinguishable from that of a telomerase-proficient strain, although a decline in viability eventually becomes evident with continued propagation (24). In contrast, a newly generated telomerase-defective strain that also bore either a cdc13-Y561A or a cdc13-I578A mutation exhibited an immediate decline in viability (Fig. 2 B). Similarly, these same mutations also exhibited a pronounced synthetic growth defect when combined with a mutation in the Ku heterodimer; the cdc13-Y556A yku80Δ, cdc13-I578A yku80Δ, and cdc13-Y561A yku80Δ double-mutant strains were close to inviable (SI Appendix, Fig. S5C). The synthetic lethality due to these mutations in the DBD interface were not readily explained by their small increase in affinity for the Tel11 substrate.

Binding Specificity Is Profoundly Altered by Mutations in the DNA-Binding Interface. The above results strongly suggested that affinity was not the only important biochemical feature required for Cdc13 function in vivo. We therefore asked whether an additional biochemical property, binding specificity for telomeric substrates, was altered by these mutations. We have previously assessed Cdc13 specificity by measuring binding affinities for oligonucleotides with substitutions for the “pool” of the three other bases at specific positions within the minimal Tel11 oligonucleotide (10). This approach revealed a “specificity profile” defined by the relative loss of affinity when the identity of a base in the ligand is altered. The larger the loss in affinity for the pool relative to the cognate ligand, the more specifically the cognate base is recognized. This strategy revealed that bases at positions G1, G3, and T4 within the Tel11 (GTGGGAGTGTG) substrate are the most specifically recognized by both the Cdc13 DBD and the full-length Cdc13 protein (10, 12). Substitutions at these three positions in the Tel11 sequence led to a significant loss of affinity (up to 87-fold) by the WT protein, whereas the change in affinity upon substitution at G9, a site which is less specifically recognized, was more modest (Table 1 and SI Appendix, Fig. S1C).

To determine how specificity is impacted by mutations across the DBD interface, binding to these pools of oligonucleotides was performed with all mutant proteins (Fig. 3 A, Table 1, and SI Appendix, Table S1). A wide range of effects was observed when the pool of bases was substituted at positions in the Tel11 oligo, with the reductions in affinity ranging from 4.5-fold to nearly 3,000-fold.

Table 1. Apparent \( K_d \) values for WT and mutant Cdc13 DBD proteins to Tel11 variants

| Protein Tel 11, pM | H1*, pM | H3*, pM | V4*, pM | H9*, pM |
|-------------------|---------|---------|---------|---------|
| WT                | 2.1 ± 0.2 | 31 ± 5  | 85 ± 20 | 180 ± 40 | 9 ± 1   |
| Y556A             | 0.6 ± 0.1 | 300 ± 30| 310 ± 50| 570 ± 200| 43 ± 4  |
| I578A             | 1.0 ± 0.2 | 420 ± 20| 130 ± 20| 280 ± 70  | 20 ± 2  |
| Y561A             | 1.6 ± 0.2 | 15 ± 30  | 430 ± 80 | 380 ± 30  | 16 ± 2  |
| Y565A             | 4.7 ± 0.8 | 330 ± 50 | 260 ± 20 | 370 ± 70  | 34 ± 5  |
| Y626A             | 7.2 ± 0.9 | 1,230 ± 60| 2,500 ± 60| 290 ± 70  | 180 ± 10|
| F539A             | 7.4 ± 0.2 | 2,100 ± 100| 2,600 ± 100| 400 ± 130 | 180 ± 10|
| K622A             | 31 ± 3    | 2,400 ± 400| 1,200 ± 200| 450 ± 1,000| 210 ± 60|
| Y522A             | 71 ± 16   | 430 ± 50 | 770 ± 200| 6,000 ± 1,000| 1,200 ± 300|

\( * \)H refers to an equimolar pool of A, C, and T; V refers an equimolar pool of G, C, and A.

Glustrom et al.

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these effects were simply additive, when assessing binding of the mutant protein (Y522A) to the modified ligand the reduction in affinity would be predicted to be 2,958-fold, which is quite similar to the observed value of 2,800-fold (SI Appendix, Table S1). The fact that these sites behave independently is consistent with their ∼15-Å separation in the structure (30). Y522, however, is physically proximal to G1. Here, the impact on binding in the doubly substituted Y522A/H1 complex was less than additive, with the observed net affinity down 200-fold relative to WT, whereas the additive effect would be ∼500-fold. This deviation from a simply additive result supports the prediction from the structure that Y522 specifically recognizes G1.

To visually identify protein/nucleic acid pairs whose combined alteration deviates from thermodynamic additivity, we divided the affinities for the binding of the doubly substituted pairs by the binding affinity of each mutant protein for Tel11 (Fig. 3B and SI Appendix, Table S1). Thermodynamic additivity would predict that, with this normalization, the mutant proteins would show the same specificity profiles as observed for WT Cdc13. This scaled specificity profile indeed revealed that, in mutants for which the effects were close to additive, such as Y522A, the specificity profile mirrored that of WT. Exceptions included sites of direct contact where the impact on binding was smaller than expected, as discussed above for the Y522A/H1 pair.

Several mutant Cdc13 proteins unexpectedly exhibited a deviation from additivity in which the pairs led to a greater loss of affinity than predicted by additivity. This was particularly evident for Y626A and F539A and to a lesser extent for Y561A (Fig. 3B and SI Appendix, Table S1). A case in point was the interaction of F539A with H1, where the combined reduction in affinity (600-fold) was about 11-fold greater than the product of the F539 vs. Tel 11 (3.5-fold) and WT vs. H1 (15-fold) differences between WT binding with Tel 11. This was also the case for the interaction between F539A and position H3 in the DNA: F539A exhibited a 1,300-fold loss in binding affinity at this site even though F539A exhibited only a 3.5-fold reduction in Tel11 binding. Again, the net impact of the combination of amino acid change and oligonucleotide substitution was highly nonadditive. This binding profile for the F539A mutation argues that the Cdc13-F539A protein was more specific for the Tel11 sequence, as substitution of the oligonucleotide base led to greater losses in binding than observed for the WT protein. A key observation is that this enhanced specificity was not due to tighter binding of the mutant proteins to Tel11; rather, it arose from a decreased tolerance for the substitutions in the oligonucleotide. Notably, these effects were not manifest only at the base closest to the site of alanine mutation in the structure (Fig. 14), suggesting that long-range effects across the DBD interface dictate binding specificity. F539A illustrates this nicely: Although this substitution had a large, nonadditive impact on H1 and H3 binding, it is poised between T4 and G5 in the structure of the complex (30).

**Binding Specificity in Conjunction with Affinity Predicts in Vivo Phenotypes More Accurately.** We noted above that in vivo phenotypes correlated roughly with severe losses of binding affinity but that more moderate changes in binding affinity did not fully explain the phenotypes. The reduced tolerance by F539A and Y626A for deviations from the Tel11 sequence (i.e., increased specificity) provides a biological explanation for their in vivo phenotypes, which were significantly functionally impaired, particularly when contrasted with the similarly affinity-impaired Y565A. The severe growth defects associated with the cdc13-F539A and cdc13-Y626A strains, as well as the more subtle growth defects in the cdc13-I578A, cdc13-Y561A, and cdc13-Y556A yeast strains (with mutations that conferred increased affinity but reduced specificity) demonstrate that binding specificity contributes substantially to Cdc13 function.
Discussion

In this study, we have performed a systematic analysis of the ssDNA-binding surface of Cdc13 by generating a panel of alanine mutations that span the interface and then probing the impact of these mutations on binding affinity and specificity. This detailed biochemical analysis was combined with an in vivo phenotypic screen—to show that even minor differences in function, revealing a gradient, or rheostat, of functionality. As expected, strains expressing mutant proteins with a reduction in binding affinity of more than 15-fold were inviable, demonstrating that high-affinity DNA binding is an essential function of the yeast t-RPA complex which contains the Cdc13 protein. Surprisingly, this systematic analysis identified a second category of mutations that did not confer substantial changes in ssDNA-binding affinity but altered the ssDNA-binding specificity of the Cdc13-binding interface, such that the surface was less tolerant of changes in the ssDNA. This second category of cdc13 mutations also had a substantial impact on viability, thereby revealing that sequence tolerance is as important as binding affinity for biological function in vivo.

Typically, mutating a contacting amino acid increases sequence tolerance by removing the H-bond donors and acceptors and steric interactions that enforce specific recognition. In contrast, mutations in a subset of residues of the Cdc13-binding interface confer a decrease in sequence tolerance. Notably, mutating these amino acids impacts recognition of bases 10 Å away (Fig. 1A), indicating that long-range effects across this interface contribute to specificity. Furthermore, removal of these aromatics from the interface does not make binding more promiscuous, suggesting that these side chains are not driving local specificity and instead are accommodating sequence diversity. We therefore propose a model in which DNA binding by Cdc13 employs a highly cooperative interface with sequence diversity accommodated through plastic binding modes. This argues that ssDNA binding employs localized contacts between a subset of amino acids and adjacent bases that are important for binding affinity as well as long-range effects across the interface that are critical for sequence tolerance.

Analysis of the biochemical data in the context of the Cdc13 DBD/Tel11 structure points to three distinct functional parts of the interface (30). The first region is the segment of the OB-fold barrel that interacts with the 5′ end identified in previous mutagenesis studies as driving both affinity and specificity of interaction. This region includes Y522 and K622, the residues whose substitution has the largest impact on affinity without significant changes in specificity. The second is the long β3 loop (highlighted in blue in Fig. 1A), encompassing mutations spanning residues Y556A to Y565A, that interacts with the 3′ end of the ligand. Mutations in this loop have more moderate impacts on affinity and specificity, consistent with a “Velcro-like function,” that is, a sticky surface suited to binding any sequence. The final structural region bridges these two, spanning the middle part of the barrel. Here substitution of two key aromatic residues, F539A and Y626A, results in a modest loss in affinity but a significant increase in specificity characterized by the dramatic loss of tolerance of substitutions at the rather distant sites of G1 and G3. Thus, this middle region appears to control the plasticity of the recognition so that Cdc13′s ability to accommodate sequence alterations is impaired upon loss of these aromatic residues. The behavior is reminiscent of another sequence-tolerant telomere end-binding protein, Pot1p of Schizosaccharomyces pombe, in which sequence tolerance is implemented through new binding modes that thermodynamically compensate for base substitutions through alternate stacking interactions and new H-bonding networks (28). Our data suggest that the loss of key aromatic residues in this middle region impacts the ability of the protein to tolerate alternative sequences, perhaps due to the ability of the aromatic amino acids to stack on the exposed bases of ssDNA and affect plasticity.

In vivo, the phenotypes displayed by strains bearing mutations in the DBD interface showed numerous similarities to previously described mutations in CDC13 that confer viability defects, arguing that this set of cdc13-DBD mutations is impacting the primary Cdc13 function. Severe impaired cdc13-DBD strains displayed a DNA damage response and impaired cell cycle progression (SI Appendix, Fig. S5 D and E) comparable to that of previously characterized cdc13-defective strains (32). This panel of cdc13-DBD mutant strains also exhibited a profile of genetic interactions in response to rad9-Δ and exo1-Δ mutations (Fig. 2) that recapitulated the behavior of cdc13 temperature-sensitive (cdc13-ts) strains (23, 32). However, there was one notable difference. In cdc13-ts strains grown at nonpermissive temperatures, there is a marked increase in the extent of exposed telomeric G-strand ssDNA (33, 34). This observation, combined with the enhanced DNA damage response observed in cdc13- impaired strains, led to the prediction that telomeric ssDNA creates a specific signal that elicits a cell-cycle checkpoint (32, 33). In contrast, none of the cdc13-DBD mutant strains—even those that were severely impaired—exhibited any detectable increase in the extent of G-strand ssDNA at chromosome ends (SI Appendix, Fig. S5F). This suggests that the primary DNA lesion eliciting a checkpoint response in cdc13-impaired cells may not be ssDNA but in fact may be some other intermediate that arises during DNA replication stress.

The behavior of yeast strains expressing three mutations highlights the in vivo double role of rigidly binding specificity: F539A, Y565A, and Y626A all exhibit similar reductions in affinity (2.2- to 3.5-fold) (Fig. 1B) but vary markedly with regard to specificity (Fig. 3B). Y656A is modestly more specific than WT, while F539A and Y626A are significantly more specific. This in vitro gradient of specificity generates a comparable in vivo gradient, as a strain expressing the mutant Cdc13-Y656A protein is slightly less functional than WT (SI Appendix, Fig. S5 B and C), whereas cdc13-F539A and cdc13-Y626A are severely impaired (Fig. 2A and SI Appendix, Fig. S5A). This surprising result shows that a gain in specificity in fact can be deleterious to function. We also underscore that ssDNA-binding specificity is a binary (specific vs. nonspecific) trait; rather there is a continuum of specificity that is critical to the biological functioning of many DNA-binding proteins. Specifically, our results indicate that the recognition of ssDNA by Cdc13 relies on a finely tuned balance of both affinity and specificity to ensure that the t-RPA complex can readily localize to a limited region of the genome and still accommodate the sequence heterogeneity present at yeast telomeres.

Specificity in nucleic acid recognition by proteins has been studied from both a biochemical and structural perspectives (reviewed in ref. 35), which have suggested that the nucleic acid-binding interface is malleable. For example, single-stranded recognition interfaces can be remodeled to match different substrates to achieve specific recognition for both DNA and RNA, as exemplified by the Oxytricha nova telomere end-binding protein (36), the S. pombe Pot1 protein (28, 37), and, in RNA recognition, the PUF protein (38) and the MS2 coat protein (39). This malleability could be dynamic in origin (40, 41). Lacking in all these prior studies, however, has been a demonstrated link between the requirement for recognition malleability and function. The observation that mutations in the DNA-binding interface of Cdc13 render the protein more specific and less functional was unexpected. While it is common to observe loss of function upon loss of a biochemical activity, the enhancement of specificity leading to a substantial reduction in biological function has not been reported previously in nucleic acid recognition, to the best of our knowledge. As a systematic evaluation of the binding specificity of mutant proteins is not commonly undertaken, this disruption of multiple biochemical behaviors may be a broader phenomenon than previously appreciated. A case in point is the human CST complex, a heterotrimer with a domain organization very similar to that of the yeast t-RPA complex (42–44). Unlike t-RPA, the CST complex is not a telomere-dedicated protein; although it displays a preference for G-rich sequences, the arrangement of guanosine nucleotides needed for high-affinity binding does not correspond to the repeat characteristic of
1. Dickey TH, Altschuler SE, Wutkie DS (2013) Single-stranded DNA-binding proteins: Multiple functions for a single protein. Structure 21:1074–1084.
2. Fanning E, Klimovich V, Nager AR (2006) A dynamic model for replication protein A (RPA) function in DNA processing pathways. Nucleic Acids Res 34:4126–4137.
3. Lohman TM, Ferrari ME (1994) Escherichia coli single-stranded DNA-binding protein: Multiple DNA-binding modes and cooperativities. Annu Rev Biochem 63:527–570.
4. Wold N (1997) Replication protein A: A heterotrimeric, single-stranded DNA-binding protein required for eukaryotic DNA metabolism. Annu Rev Biochem 66:61–92.
5. Bochkarev A, Bochkareva E (2004) From RPA to BRCA2: Lessons from single-stranded DNA binding by the OB-fold. Curr Opin Struct Biol 14:46–53.
6. Fan J, Pavlecht NP (2012) Structure and conformational change of replication protein A heterotrimer bound to ssDNA. Genes 26:2337–2347.
7. Broyse CA, et al. (2013) A new structural framework for integrating replication protein A into DNA processing machinery. Nucleic Acids Res 41:2313–2327.
8. Chen R, Subramanyam S, Ebock AH, Spies M, Widl MS (2016) Dynamic binding of replication protein A is required for DNA repair. Nucleic Acids Res 44:5758–5772.
9. Lei M, Poddar ER, Cech TR (2004) Structure of human POF1 bound to telomeric single-stranded DNA provides a model for chromosome end protection. Nat Struct Mol Biol 11:1223–1229.
10. Elderidge AM, Halsey WA, Wutkie DS (2006) Identification of the determinants for the specific recognition of single-stranded telomeric DNA by Cdc13. Biochemistry 45:871–879.
11. Crow JE, Wutkie DS (2006) Themes in ssDNA recognition by telomere-end protection proteins. Trends Biochem Sci 31:516–525.
12. Lewis KA, Pfaff DA, Earley JN, Altschuler SE, Wutkie DS (2014) The tenacious recognition of yeast telomere sequence by Cdc13 is fully exerected by a single OB-fold domain. Nucleic Acids Res 42:475–484.
13. Gao H, Cervantes RB, Mandell EK, Otero JH, Lundblad V (2007) RPA-like proteins mediate yeast telomere function. Nat Struct Mol Biol 14:208–214.
14. Gélinas AD, et al. (2009) Telomere capping proteins are structurally related to replication protein A with an additional telomere-specific domain. Proc Natl Acad Sci USA 106:1929–1930.
15. Sun J, et al. (2009) Stn1-Ten1 is an Rpa2-Rpa3-like complex at telomeres. Genes Dev 23:2900–2914.
16. Mitton-Fry RM, Anderson EM, Hughes TR, Lundblad V, Wutkie DS (2002) Conserved structure for single-stranded telomeric DNA recognition. Science 296:145–147.
17. Nugent CI, Hughes TR, Lue NF, Lundblad V (1996) Cdc13p: A single-strand telomeric DNA-binding protein with a dual role in yeast telomere maintenance. Science 274:249–252.
18. Lin J-J, Zakian VA (1996) The Saccharomyces CDC13 protein is a single-strand TG1-3 telomeric DNA-binding protein in vitro that affects telomere behavior in vivo. Proc Natl Acad Sci USA 93:13760–13765.
19. Förstmann K, Liniger, J Cell Substructures. Structure 21:1074–1084.
20. Anderson EM, Halsey WA, Wutkie DS (2003) Site-directed mutagenesis reveals the thermodynamic requirements for single-stranded DNA recognition by the telomere-binding protein Cdc13. Biochemistry 42:3751–3758.
21. Weinert TA, Kiser GL, Hartwell LH (1994) Mitotic checkpoint genes in budding yeast and the dependence of mitosis on DNA replication and repair. Genes Dev 8:652–665.
22. Zuber MK, Guillard S, Lydall D (2004) Exo1 and Rad24 differentially regulate generation of ssDNA at telomers of Saccharomyces cerevisiae cdc13-1 mutants. Genetics 168:103–115.
23. Paschini M, et al. (2012) A naturally thermostable activity compromises genetic analysis of telomere function in Saccharomyces cerevisiae. Genetics 191:79–93.
24. Lendvay TS, Morris DK, Sah J, Balasubramanian B, Lundblad V (1996) Senescence mutants of Saccharomyces cerevisiae with a defect in telomere replication identify three additional EST genes. Genetics 144:1399–1412.
25. Carter PJ, Winters G, Wilkinson AJ, Ferst AR (1984) The use of double mutants to detect structural changes in the active site of the tyrosyl-tRNA synthetase (Saccharomyces cerevisiae). Cell 38:835–840.
26. Wells JA (1990) Additivity of mutational effects in proteins. Biochemistry 29:8509–8517.
27. Schreiber G, Fersht AR (1995) Energetics of protein-protein interactions: Analysis of the barnase-barstar interface by single mutations and double mutant cycles. J Mol Biol 248:478–486.
28. Dickey TH, McKeever MA, Wutkie DS (2013) Non-specific recognition is achieved in Port1p through the use of multiple binding modes. Structure 21:121–132.
29. Lloyd NR, Dickey TH, Hom RA, Wutkie DS (2016) Tying up the ends: Plasticity in the recognition of single-stranded DNA at telomeres. Biochemistry 55:5226–5340.
30. Mitton-Fry RM, Anderson EM, Theobald DL, Glisant LW, Wutkie DS (2004) Structural basis for telomeric single-stranded DNA recognition by yeast Cdc13. J Mol Biol 338:241–255.
31. Wu Z, Baglioni F, Kemble JL, Wetsore SD (2014) DNA-protein e-interactions in nature: Abundance, structure, composition and strength of contacts between aromatic amino acids and DNA nucleobases or deoxyribose sugar. Nucleic Acids Res 42:6726–6741.
32. Lydall D, Weinert T (1995) Yeast checkpoint genes in DNA damage processing: Implications for repair and arrest. Science 270:1488–1491.
33. Garvik B, Canoni M, Hartwell L (1995) Single-stranded DNA arising at telomeres in cdcl3 mutants may constitute a specific signal for the RAD9 checkpoint. Mol Cell Biol 15:6128–6138.
34. Booth C, Griffith E, Brady G, Lydall D (2001) Quantitative amplification of single-stranded DNA (QADS) demonstrates that cdcl3-1 mutants generate ssDNA in a telomere to centromere direction. Nucleic Acids Res 29:4414-4422.
35. Rois R, et al. (2010) Origins of specificity in protein-DNA recognition. Annu Rev Biochem 79:233–269.
36. Theobald DL, Schulte SC (2003) Nucleotide shuffling and ssDNA recognition in Oxytricha nova telomere end-binding protein complexes. EMBO J 22:4214–4224.
37. Lloyd NR, Wutkie DS (2018) Discrimination against RNA backbones by a ssDNA binding protein. Structure 26:722–733.e2.
38. Hall TMT (2014) Expanding the RNA recognition code of PUF proteins. Nat Struct Mol Biol 21:653–655.
39. Johansson HE, et al. (1998) A thermodynamic analysis of the sequence-specific binding of RNA by bacteriophage MS2 coat protein. Proc Natl Acad Sci USA 95:9244–9249.
40. Deka P, Rajan PK, Perez-Canadillas JM, Varani G (2005) Protein and RNA dynamics play key roles in determining the specific recognition of G-rich polyadenylation regulatory elements by human Cstf-64 protein. J Mol Biol 347:719–733.
41. Casino P, et al. (2018) Conformational dynamics for DNA interaction in the Salmonella Rsbi response regulator. Nucleic Acids Res 46:456–472.
42. Canteel DE, et al. (2009) A DNA polymerase-α primase cofactor with homology to replication protein A-32 regulates DNA replication in mammalian cells. J Biol Chem 284:5807–5818.
43. Miyake Y, et al. (2009) RNA-like mammalian Ctcl1-Stn1-Ten1 complex binds to single-stranded DNA and protects telomeres independently of the Pot1 pathway. Mol Cell 36:193–206.
44. Surovtseva YV, et al. (2009) Conserved telomere maintenance component 1 interacts with STN1 and maintains chromosome ends in higher eukaryotes. Mol Cell 36:207–218.
45. Wan B, et al. (2015) The Tetrahymena telomerase p75-p45-p19 subcomplex is a unique CST complex. Nat Struct Mol Biol 22:1023–1026.
46. Hom RA, Wutkie DS (2017) Human CST prefers G-Rich but not necessarily telomeric sequences. Biochemistry 56:4210–4218.
47. Chastain M, et al. (2016) Human CST facilitates genome-wide RAD51 recruitment to G-rich repetitive sequences in response to replication stress. Cell Rep 16:2048.
48. Bhattacharjee A, Wang Y, Diao J, Price CM (2017) Dynamic DNA binding, junction recognition and G4 melting activity underlie the telomeric and genome-wide roles of human CST. Nucleic Acids Res 45:12311–12324.
49. Bhattacharyya S, et al. (2002) Characterization of binding-induced changes in dynamics suggests a role for sequence-specific binding of ssDNA by replication protein A. Protein Sci 11:2136–2135.