A rat liver nuclear protein, unimolecular quadruplex telomere-binding protein 25, (uqTBP25) is described that binds tightly and specifically single-stranded and unimolecular tetraplex forms of the vertebrate telomeric DNA sequence 5'-d(TTAGGG) 3', A near homogeneous uqTBP25 was purified by ammonium sulfate precipitation, chromatographic separation from other DNA binding proteins, and three steps of column chromatography. SDS-polyacrylamide gel electrophoresis and Superdex 200 gel filtration disclosed for uqTBP25 subunit and native $M_r$ values of 25.4 ± 0.5 and 25.0 kDa, respectively. Sequences of uqTBP25 tryptic peptides were closely homologous, but not identical, to heterogeneous nuclear ribonucleoprotein A1, heterogeneous nuclear ribonucleoprotein A2/B1, and single-stranded DNA-binding proteins UP1 and HDP-1. Complexes of uqTBP25 with single-stranded or unimolecular quadruplex 5'-d(TTAGGG) 3', respectively, had dissociation constants, $K_d$, of 2.2 or 13.4 nM. Relative to d(TTAGGG) 4, complexes with 5'-r(UUAGGG) 3, blunt-ended duplex telomeric DNA, or quadruplex telomeric DNA had >10 to >250-fold higher $K_d$ values. Single base alterations within the d(TTAGGG) repeat increased the $K_d$ of complexes with uqTBP25 by 9–215-fold. Association with uqTBP25 protected d(TTAGGG) 4 against nuclease digestion, suggesting a potential role for the protein in telomeric DNA transactions.

Linear eukaryotic chromosomes end with a specialized DNA-protein structure termed the telomere that guards the chromosome terminus against degradative attack or fusion with ends of other chromosomes (1–4). Telomeric DNA consists of evolutionarily conserved short, tandemly repeated nucleotide sequences. The telomeric DNA strand, oriented 5' to 3' toward the chromosome end (“G-strand”) in all vertebrates, alime molds, filamentous fungi, and Trypanosoma, is a repeated 5'-d(TTAGGG)-3' sequence paired to a complementary 5'-r(UUAGGG) 3, blunt-ended duplex telomeric DNA with 12–16-nucleotide-long segments of telomeric DNA with each round of replication of somatic cells (26–28) suggests that exonucleolytic degradation of the terminus of telomeric DNA may also contribute to its progressive trimming. It has been suggested, therefore, that telomeric DNA binding proteins that were identified in diverse species may participate in the complex dynamics of elongation and shortening of telomeric DNA (4, 29). Some such proteins from different species bind tightly single-stranded telomeric DNA (30–33). Other proteins bind to or mediate the formation of tetraplex forms of telomeric DNA (33–42). Proteins of a third category selectively associate with the duplex region of telomeric DNA (43–46).

In this work we describe the purification from rat hepatocytes and characterization of a 25-kDa monomeric protein, termed uqTBP25, that binds tightly and in a sequence-specific fashion single-stranded and unimolecular tetraplex forms of the G-strand of telomeric DNA. A partial amino acid sequence of uqTBP25 is closely homologous but not identical with sequences of hnRNP A1 and hnRNP A2/B1 and their respective derivative single-stranded DNA-binding proteins UP1 and HDP-1. Protein uqTBP25 is distinguished from hnRNP A1 and A2/B1 by its molecular size, preferential binding to DNA over RNA, and sequence-specific binding to the telomeric DNA G-strand. This protein also differs from UP1 and HDP-1 by its selective binding to the G-strand of telomeric DNA and

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| Oligomer designation | Length | Nucleotide sequence |
|----------------------|--------|---------------------|
| TeR-3*               | 5-mer  | -                    |
| TeR-4*               | 23-mer | -                    |
| TeR-5*               | 20-mer | -                    |
| rTeR-4*              | 23-mer | -                    |
| TeR-4 C*             | 17-mer | -                    |
| TeR-3 C*             | 24-mer | -                    |
| TeR-4 C*             | 24-mer | -                    |
| TeR-4 C*             | 23-mer | -                    |
| TeR-4 C*             | 24-mer | -                    |
| TeR-4 C*             | 24-mer | -                    |
| TeR-4 C*             | 24-mer | -                    |
| Mut1 TeR-4*          | 24-mer | -                    |
| Mut2 TeR-4*          | 24-mer | -                    |
| Hook TeR-4*          | 32-mer | -                    |
| Hook TeR-4 C*        | 27-mer | -                    |
| Q*                   | 20-mer | -                    |
| Single Q*            | 7-mer  | -                    |
| Anti-Q*              | 20-mer | -                    |

a) Clusters of guanine residues are underlined.
b) Clusters of cytosine residues are underlined.
c) A loci of a substituted nucleotide relative to TeR-4 DNA are doubly underlined.

d) Loci of a substituted nucleotide relative to TeR-4 DNA are doubly underlined.

by its failure to significantly stimulate the activity of DNA polymerase α.

**EXPERIMENTAL PROCEDURES**

**Materials and Enzymes—**Radioactively 5'-labeled (γ-32P)ATP (~3000 Ci/mmol), (α-32P)dGTP (~3000 Ci/mmol), Klenow fragment of *Escherichia coli* polymerase I, and molecular mass Rainbow™ marker proteins were products of Amersham Corp. Synthetic DNA oligomers, listed in Table I, were purchased from Operon Technologies. The HPLC-purified RNA oligomer r(UUAGGG)₄ (Table I) was a product of Midland Science. Scopes of calf thymus DNA polymerase α was the gift of Dr. L. A. Loeb (University of Washington). Kodak XAR5 and BioMax MR-1 autoradiographic film, urea, TEMED, bromphenol blue, and xylene cyanol FF were supplied by IBL. Hitrap Blue HPLC column and Supepdex™ 200 HPLC gel filtration column were provided by Pharmacia Biotech Inc. Econo-Pac 10 HPLC cartridge, reagents for sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), and molecular weight protein standards were the products of Bio-Rad. Novex provided Millipore™ molecular size protein standards. Biotrace polyvinylidene difluoride binding matrix membranes were supplied by Gelman Sciences.

**Preparation of Single-stranded, Double-stranded, and Tetraplex DNA Oligomers—**Full-length DNA oligomers were purified by electrophoresis through a 5% x 20 cm 15% PAGE gel (acryl/bis acrylamide) (19:1) as we described previously (47). The purified DNA or RNA oligomers were labeled at their 5’-end with 32P in a bacteriophage T4 polynucleotide kinase and RNasin were provided by Promega. Acryl/ bisacrylamide (19:1 or 30:1:2) was purchased from Amresco. Bacteriophage T4 gene 32 protein was purchased from Boehringer Mannheim. Immunoaffinity-purified calf thymus DNA polymerase α was the gift of Dr. L. A. Loeb (University of Washington). Kodak XAR5 and BioMax MR-1 autoradiographic film, urea, TEMED, bromphenol blue, and xylene cyanol FF were supplied by IBL. Hitrap Blue HPLC column and Supepdex™ 200 HPLC gel filtration column were provided by Pharmacia Biotech Inc. Econo-Pac 10 HPLC cartridge, reagents for sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), and molecular weight protein standards were the products of Bio-Rad. Novex provided Millipore™ molecular size protein standards. Biotrace polyvinylidene difluoride binding matrix membranes were supplied by Gelman Sciences.

**Preparation of Single-stranded, Double-stranded, and Tetraplex DNA Oligomers—**Full-length DNA oligomers were purified by electrophoresis through a 5% x 20 cm 15% PAGE gel (acryl/bisacrylamide) (19:1) as we described previously (47). The purified DNA or RNA oligomers were labeled at their 5’-end with 32P in a bacteriophage T4 polynucleotide kinase-catalyzed reaction (48). Oligomers were maintained in their single-stranded conformation as a 0.25–0.70 μM solution in 1.0 mM EDTA, 10 mM Tris-HCl buffer, pH 8.0 (TE buffer), and were boiled immediately prior to use. Double-stranded telomeric DNA was prepared by annealing a 1.25-fold molar excess of a cytosine-rich sequence with a complementary guanine-rich oligomer, and duplex DNA molecules were electrophoretically resolved from residual DNA single strands as we described (33). Labeling of a protruding end of the annealed duplex DNA was catalyzed by the Klenow fragment of *E. coli* polymerase I using 5’[α-32P]dGTP as we described previously (47). Unimolecular (G 4) and bimolecular (G 2) tetraplex forms of TeR DNA were prepared, their stoichiometry was verified, and their stabilization by Hoogsteen bonds was demonstrated, as we described in detail elsewhere (33). Parallel G4 quadruplex forms of oligomers Q and single Q were prepared according to Sen and Gilbert (48), and their stoichiometry was shown to be tetramolecular as recently described (33). The parallel quadruplex form of d(CGG)₉ was prepared, and its structure was verified as previously detailed (49).

**Electrophoretic Mobility Shift Assays, SDS-PAGE, and Southwestern Blotting—**The DNA binding activity of uqTB25 was monitored by electrophoretic mobility shift assay as we described previously (32, 50). In a typical assay for the binding of single-stranded TeR-4 or TeR-2 DNA, 5.0–15.0 ng of 32P-5'-labeled TeR-2 or TeR-4 DNA was incubated at 4 °C for 20 min with 30–3000 ng of purified or crude protein fraction in a 15-μl final volume of buffer D (0.5 mM EDTA, 20% glycerol in 25 mM Tris-HCl buffer, pH 7.5). The binding mixture was electrophoresed in a Mini PROTEAN II electrophoresis system (Bio-Rad) at 4 °C under 10 V/cm through a nondenaturing 6% polyacrylamide gel (acryl/bisacrylamide, 30:1:2) in 0.6 × TBE buffer (1.2 mM EDTA in 0.54 mM Tris borate buffer, pH 8.3) until a bromphenol blue tracking dye migrated 2.5–4.0 cm into the gel. The gels were dried on DE-81 filter paper and exposed to x-ray film or to a phosphor imaging plate (Fuji). The proportion of free and uqTB25-bound TeR DNA was determined by phosphor imaging, and their amounts were deduced from the known specific activity of the labeled DNA probe. One unit of uqTB25 DNA binding activity was defined as the amount of uqTB25 that bound 66 pmol of single-stranded TeR-2 DNA under the described standard conditions. Standard assay conditions were also employed for the binding of tetramolecular G4 quadruplex DNA and of double-stranded DNA. Binding of tetraplex G2 TeR DNA or G4 TeR DNA was assayed as described above except that 50 mM KCl or 50 mM NaCl, respectively, was added to the binding mixture. The electrophoretic structure of the DNA, the 0.6 × TBE gel running buffer contained 50 mM KCl or 50 mM NaCl as necessary, and electrophoresis was performed at 4 °C.

**SDS-PAGE and silver or Coomassie Blue staining of resolved protein bands was carried out as we described previously (50). Molecular size protein markers were the Amersham Rainbow™, Novex Multimark™, or Bio-Rad prestained or unstained molecular weight standards.

**Southwestern analysis was conducted according to Petracek et al. (31) with the minor modifications that we recently introduced (33). TeR-4 DNA binding activity was detected in nuclear extracts by exposing the electrophoretically resolved proteins to 0.85 μg of 32P-5'-labeled TeR-4 DNA in the presence of 50 mM NaCl.**
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Purification of the Telomeric DNA-binding Protein

\( uqTBP25 \)—Activities that bind single-stranded and tetraplex forms of the vertebrate telomeric sequence TeR-4, 5'-d(TTAGGG)\(_n\), were detected by electrophoretic mobility shift analysis in extracts of nonhistone nuclear proteins from rat hepatocyte. Southwestern analysis of the unimolecular tetraplex G\(_4\) TeR-4 DNA binding activity detected in replicate extracts a major protein—G\(_4\) TeR-4 DNA complex band of \(-24\) kDa and two minor bands of 31 and 33 kDa, respectively (for a typical analysis, see Fig. 1A). To isolate the \(-24\)-kDa binding activity, the nuclear extract was initially fractionated by ammonium sulfate precipitation. The major portion of an activity that bound single-stranded TeR-4 or unimolecular tetraplex G\(_4\) TeR-4 DNA was detected by electrophoretic mobility shift analysis in the 50–70% (NH\(_4\))\(_2\)SO\(_4\) precipitate. To resolve TeR DNA-specific binding activity from other proteins that bind nonspecifically to single-stranded DNA, the resuspended and dialyzed 70% ammonium sulfate precipitate was incubated with denatured salmon sperm DNA and then chromatographed on a DE-52 column. DNA and DNA-protein complexes strongly adsorb to the anion exchanger, whereas some proteins that do not bind to denatured DNA adsorb weakly to DE-52 (53). Electrophoretic mobility shift analysis of G\(_4\) TeR-4 DNA binding activity and SDS-PAGE resolution of UV-cross-linked G\(_4\) TeR-4 DNA complexes revealed an electrophoretically retarded 34-kDa complex band in fractions that were eluted from DE-52 by 50 mM NaCl (see “Experimental Procedures”). Several additional proteins, including \( qTBP42 \) (33), that formed complexes with denatured DNA and tightly adsorbed to DE-52 were eluted from the column by 225 mM NaCl (see “Experimental Procedures”). The TeR-4 DNA binding activity that was eluted from DE-52 by 50 mM NaCl was further purified by successive steps of chromatography on columns of Econo-Pac S, HiTrap Blue and phenyl-Sepharose. Elution profiles of the TeR DNA binding activity from the different columns, as revealed by electrophoretic mobility shift analysis were identical when \([32P]\)dGMP into DNA was measured as described previously (53). Measurement of the Effect of \( uqTBP25 \) on Polymerase \( \alpha \)-Catalyzed DNA Synthesis—DNA synthesis was conducted for 30 min at 37 °C in a reaction mixture that contained in a final volume of 25 \( \mu \)l Tris-HCl buffer, pH 7.5, 3.0 mM MgCl\(_2\), 1.0 mM DTT, 2.1 units of immunoaffinity-purified \( \alpha \)-pol III, and 1.0 mM of buffer D containing 50 mM NaCl. The protein-loaded column was washed by five column volumes of the equilibration buffer, and bound proteins were eluted by two packed column volumes each of 100 and 225 mM NaCl in buffer D. Electro-photography, mobility shift analysis and SDS-PAGE separation of UV cross-linked TeR-4 DNA-protein complexes, respectively, revealed TeR DNA-specific binding activity and UV-cross-linked TeR-4 DNA-protein complex of 34 kDa in the 50 mM NaCl wash fractions. Similar analysis did not reveal DNA-protein complexes in the 100 mM NaCl fraction; instead, multiple complex bands, the dominant of which was \( qTBP42 \) (33), were present in the 225 mM NaCl eluate. The 50 mM NaCl eluate fractions were pooled together and dialyzed overnight against \(-50\) volumes of buffer P (0.5 mM EDTA, 20% glycerol in 25 mM NaPO\(_4\) buffer, pH 7.0). The dialyzed fractions were loaded at a ratio of 24.0 mg of protein/ml of packed resin onto a 0.5-ml Econo-Pac S column equilibrated in buffer P and mounted on a GradiFrac low pressure chromatography device (Pharmacia). The loaded column was washed with 7.5 packed resin volumes of buffer P, and bound proteins were eluted from the column by a linear gradient of 19.5 column volumes of 0.0–1.0 mM NaCl in buffer P. Fifty fractions were collected, and as done in every subsequent chromatography, aliquots were dialyzed overnight against 150 volumes of buffer D and then assayed for G\(_4\) TeR-4 DNA binding. G\(_4\) TeR-4 DNA binding activity of \( uqTBP25 \) was detected in the 150–320 mM NaCl eluate both by electrophoretic mobility shift analysis and by the identification in SDS-PAGE of a \(-34\)-kDa UV-cross-linked protein—TeR-4 DNA complex. The active fractions were pooled together, dialyzed overnight against 150 volumes of buffer P and loaded onto a 0.5-ml HiTrap Blue HPLC column equilibrated in buffer P and mounted on a GradiFrac device. The loaded column was washed by six column volumes of the equilibration buffer, and adsorbed proteins were eluted by a 21-packed column volume linear gradient of 0.0–4.0 mM NaCl in buffer P. Fifty fractions were collected and aliquots were dialyzed, and the HiTrap Blue binding activity was detected by electrophoretic mobility shift analysis and SDS-PAGE resolution of UV-cross-linked protein—TeR-4 DNA complexes in fractions that were eluted from HiTrap Blue by 2.5–3.5 mM NaCl. Fractions containing the binding activity were pooled together and dialyzed overnight against \(-50\) volumes of 4.0 mM NaCl in buffer S (1.0 mM EDTA in 25 mM Tris-HCl buffer, pH 7.5) and loaded at a ratio of 1.0 mg of protein/1.0 ml of packed resin onto a 1.0-m1 Phenyl-Sepharose column equilibrated in buffer S. The loaded column was washed with two packed column volumes of the equilibration buffer, and bound proteins were eluted by a stepwise gradient of 0.0–0.5 mM NaCl in buffer S followed by a 40% ethylene glycol wash to elute proteins that remained adsorbed to Phenyl-Sepharose at 0.0 mM NaCl. Fractions were collected into Nuncid P-10 (0.05% final concentration), and the activity of \( uqTBP25 \) was detected in fractions that were eluted from the phenyl-Sepharose column by 1.0–0.5 mM NaCl. Silver and Coomassie Blue staining of the eluted proteins indicated that a 25-kDa species was the major protein eluted by 1.0–0.5 mM NaCl (Fig. 2C), whereas the majority of the proteins that were loaded onto the column were eluted by 40% ethylene glycol. Determination of the protein content of the collected fractions by silver or Coomassie Blue staining of SDS-PAGE-resolved protein bands was directly performed on fractions that were dialyzed against water. Fractions that were used for the assay of DNA binding activity were stabilized by the immediate addition soybean trypsin inhibitor protein (200 \( \mu \)g/ml final concentration), and following their dialysis overnight against \(-20\) volumes of buffer D, they were stored in aliquots at \(-80\) °C. Under these storage conditions, the DNA binding activity was fully preserved for at least 4 months.

**RESULTS**

**Purification of the Telomeric DNA-binding Protein**

\( uqTBP25 \)—Activities that bind single-stranded and tetraplex forms of the vertebrate telomeric sequence TeR-4, 5'-d(TTAGGG)\(_n\), were detected by electrophoretic mobility shift analysis in extracts of nonhistone nuclear proteins from rat hepatocyte. Southwestern analysis of the unimolecular tetraplex G\(_4\) TeR-4 DNA binding activity detected in replicate extracts a major protein—G\(_4\) TeR-4 DNA complex band of \(-24\) kDa and two minor bands of 31 and 33 kDa, respectively (for a typical analysis, see Fig. 1A). To isolate the \(-24\)-kDa binding activity, the nuclear extract was initially fractionated by ammonium sulfate precipitation. The major portion of an activity that bound single-stranded TeR-4 or unimolecular tetraplex G\(_4\) TeR-4 DNA was detected by electrophoretic mobility shift analysis in the 50–70% (NH\(_4\))\(_2\)SO\(_4\) precipitate. To resolve TeR DNA-specific binding activity from other proteins that bind nonspecifically to single-stranded DNA, the resuspended and dialyzed 70% ammonium sulfate precipitate was incubated with denatured salmon sperm DNA and then chromatographed on a DE-52 column. DNA and DNA-protein complexes strongly adsorb to the anion exchanger, whereas some proteins that do not bind to denatured DNA adsorb weakly to DE-52 (53). Electrophoretic mobility shift analysis of G\(_4\) TeR-4 DNA binding activity and SDS-PAGE resolution of UV-cross-linked protein—G\(_4\) TeR-4 DNA complexes revealed an electrophoretically retarded 34-kDa complex band in fractions that were eluted from DE-52 by 50 mM NaCl (see “Experimental Procedures”). Several additional proteins, including \( qTBP42 \) (33), that formed complexes with denatured DNA and tightly adsorbed to DE-52 were eluted from the column by 225 mM NaCl (see “Experimental Procedures”). The TeR-4 DNA binding activity that was eluted from DE-52 by 50 mM NaCl was further purified by successive steps of chromatography on columns of Econo-Pac S, HiTrap Blue and phenyl-Sepharose. Elution profiles of the TeR DNA binding activity from the different columns, as revealed by electrophoretic mobility shift analysis were identical when \([32P]\)dGTP (specific activity, 5,450 cpm/pmol) or 0.5 \( \mu \)g of bacteriophage M13mp2 single-stranded DNA primed by a 17-mer universal primer and 25 \( \mu \)M concentration of each of the four dNTPs and \([32P]\)dGTP; specific activity 1,260 cpm/pmol). Increasing amounts of phenyl-Sepharose-purified \( uqTBP25 \) were added to the reaction mixtures as described in “RESULTS.” DNA synthesis was terminated, acid-insoluble DNA was precipitated, and incorporation of \([32P]\)dGMP into DNA was measured as described previously (53).

**Determination of the Amounts of Protein**—The Bio-Rad protein assay kit was used to determine amounts of protein.
Sequence-specific Telomeric DNA-binding Protein

**Fig. 1. Southwestern analysis of G′4 TeR-4 DNA binding activity in nuclear extract and protein purification.** A, Southwestern blotting of unimolecular tetraplex G′4 TeR-4 DNA binding activity in nuclear extract from rat hepatocyte. Aliquots of nonhistone nuclear protein extract from rat hepatocytes were either boiled in the presence of 55 mM β-mercaptoethanol (β-ME) or were left untreated and without β-mercaptoethanol. The protein samples were electrophoresed through an SDS-10% polyacrylamide gel, the resolved proteins were reanimated and exposed to 32P-5′-labeled TeR-4 DNA in the presence of 50 mM NaCl, and unbound probe was washed (see “Experimental Procedures”). Shown is an autoradiogram of the dried blotted gel. An arrow marks the position of a major G′4 TeR-4 DNA-binding protein band of ~24 kDa. B, SDS-PAGE analysis of proteins in successively purified fractions of uqTBP25. Approximately 6 μg of protein of crude nuclear extract and of each partially purified fraction of uqTBP25 were electrophoresed through an SDS-13% polyacrylamide gel, and the resolved protein bands were stained with silver (see “Experimental Procedures”). A 25-kDa protein band that became detectable in the HiTrap Blue fraction and was enriched in the phenyl-Sepharose fraction is marked with an arrow.

binding activity. As seen in Fig. 2A, TeR-4 DNA binding activity was detected by mobility shift electrophoresis in phenyl-Sepharose fractions 10–14 (1.0–0.5 m NaCl eluate). Covalent UV cross-linking of labeled DNA to the phenyl-Sepharose-resolved proteins revealed in fractions 10–14 a 94-kDa protein–TeR-4 DNA complex (Fig. 2B), whose amount corresponded to the TeR DNA binding activity (Fig. 2A). Finally, SDS-PAGE resolution of the phenyl-Sepharose protein fractions showed that the intensity of a Coomassie Blue-stained 25-kDa band, which constituted >80% of the protein content of fractions 11–13 (Fig. 2C) was well correlated with the level of TeR-4 DNA binding activity in fractions 10–14 (Fig. 2A) and with the amount of the UV-cross-linked TeR-4-protein complex in these fractions (Fig. 2B). The ~25-kDa size of the unimolecular tetraplex G′4 TeR-4 DNA binding activity as detected in nuclear extracts by Southwestern analysis (Fig. 1A) as well as the 25-kDa molecular mass of the highly purified active protein (Fig. 2C) and the 34-kDa size of its complex with TeR-4 DNA (Fig. 2B) strongly suggested that the 25-kDa protein represented uqTBP25. Details of a typical purification scheme summarized in Table II indicated that, relative to crude nuclear extract, the phenyl-Sepharose fraction of uqTBP25 was purified more than 1000-fold with a final yield of 2.2%.

Chemical-Physical Properties of uqTBP25—Some properties of uqTBP25 are presented in Table III. That uqTBP25 was proteinaceous was demonstrated by its heat lability, inactivation by SDS, and sensitivity to proteinase K. The resistance of uqTBP25 to digestion by micrococcal nuclease (Table III) suggested that it did not require an essential nucleic acid component. Binding of TeR-4 DNA by uqTBP25 was not affected by exposure to 8.5 mM MalNEt (Table III), indicating that reduced protein sulfhydryl groups were not directly involved in the protein interaction with DNA. This was also corroborated by the equally efficient renaturation of the 25-kDa protein in Southwestern blotting with or without the presence of β-mercaptoethanol (Fig. 1A).

The highly purified uqTBP25 migrated in SDS-PAGE as a 25.4 ± 0.4-kDa polypeptide (n = 6). An apparent molecular size of 25.0 kDa (n = 2), which was found for native uqTBP25 by Superdex 200° gel filtration, suggested that uqTBP25 was a 25-kDa monomeric protein.

Sequence Homology among uqTBP25, Two hnRNP Species, and Their Derivative Single-stranded DNA-binding Proteins—To find out whether or not uqTBP25 represented a known protein, sequences of five tryptic peptides of uqTBP25 were determined. A computerized search through GenBankTM revealed all five uqTBP25 peptide sequences to be closely homologous, although not identical, to conserved amino acid sequences in hnRNP A1 and A2/B1 and in their derivative aminoterminal proteolytic fragments, calf thymus single-stranded DNA-binding proteins UP1 (58, 61, 64) and mouse HDP-1 (58, 64), respectively. The sequence of hnRNP A1 that remained identical in four mammalian species (Table IV) and of its cognate single-stranded binding protein UP1 (58, 61, 64) differed from the corresponding partial sequence of uqTBP25 by 4 amino acids out of 43 sequenced residues. One dissimilar amino acid represented a nonconservative G to Q substitution in uqTBP25 peptide IV (Table IV). Most notable, amino acid sequences of uqTBP25 were clearly distinct from those of cloned rat hnRNPA1 and of rat UP1 (Table IV; Refs. 61 and 62). The dissimilarity between uqTBP25 and rat hnRNPA1 extended to their different molecular sizes of 25 and 34.2 kDa, respectively (see above, under “Results”; Ref. 61), and uqTBP25 differed from both hnRNPA1 and UP1 by its nucleic acid binding preferences (see below, under “Results” and “Discussion”). Hence, despite their close sequence similarity, uqTBP25, hnRNPA1, and UP1 was each a distinct protein. An extensive sequence similarity was also found for uqTBP25 and human hnRNPA2/B1 (Table IV) and its derivative fragment, the DNA-binding protein HDP-1 (58, 64). However, six amino acid alterations were noted among the 43 sequenced uqTBP25 residues, two of which, E to A and N to A in uqTBP25 peptide V, constituted nonconservative substitutions (Table IV). Differences between rat uqTBP25 and human hnRNPA2/B1 also extended to their different molecular sizes of 25 and 36–37.4 kDa, respectively (Table IV; Ref. 54), and both hnRNPA2/B1 and HDP-1 differed from uqTBP25 by their nucleic acid binding specificity (see below, under “Results” and “Discussion”).
**Sequence-specific Telomeric DNA-binding Protein**

**Table II**

| Purification of uqTBP25 |
|-------------------------|
| **Purification step** | **Total protein** | **Binding activity** | **Specific activity** | **Yield** | **Fold** |
| Crude extract | 12,400.0 | 3,100.0 | 0.25 | 100.0 | 1.0 |
| Ammonium sulfate | 1,296.0 | 896.0 | 0.69 | 28.9 | 2.8 |
| DNA-DEAE cellulose | 480.0 | 393.6 | 0.82 | 12.7 | 3.3 |
| Econo-Pac S | 42.83 | 215.4 | 5.03 | 6.9 | 20.1 |
| HiTrap Blue | 5.81 | 77.3 | 13.30 | 2.5 | 53.2 |
| Phenyl-Sepharose | 0.26 | 69.6 | 267.70 | 2.2 | 1070.8 |

**Table III**

**Properties of uqTBP25**

Binding of 32P-5'-labeled TeR-4 by phenyl-Sepharose-purified uqTBP25 was conducted under standard conditions without or with the indicated treatments. The uqTBP25-G'4 TeR-4 DNA complex was resolved by mobility shift electrophoresis, and its amount was quantified by phosphor imaging.

| Treatment | Percentage of initial activity |
|-----------|------------------------------|
| None | 100.0 |
| 100 °C, 2 min | 52.1 |
| 100 °C, 8 min | 20.5 |
| Proteinase K digestion | 1.5 |
| 0.25% SDS | 0.0 |
| Micrococcal nuclease | 120.6 |
| 8.5 mM MalNEt | 98.0 |

### Notes

* a uqTBP25 protein (5.9 binding units) was incubated at 37 °C for 60 min with 26.7 μg/μl proteinase K and than incubated with 32P-5'-labeled G'4 TeR-4 DNA. Shown is an average result of four independent determinations.

* b uqTBP25 protein (9.2 binding units) was incubated at 37 °C for 50 min with 15.0 μg/ml micrococcal nuclease in the presence of 1.0 mM CaCl2. Digestion was terminated by the addition of EGTA and thymidine 3',5'-diphosphate to final concentrations of 5.0 and 4.0 mM, respectively, and the treated protein was incubated with 32P-5'-labeled G'4 TeR-4 DNA. Shown is an average result of six determinations.

* c uqTBP25 protein (2.5 binding units) was incubated at 4 °C for 15 min with 8.5 mM MalNEt, and the reaction was terminated by the addition of 15.0 mM DTT. The average result of three experiments is shown.

**Fig. 2.** TeR-4 DNA binding activity and SDS-PAGE of proteins in phenyl-Sepharose-purified fractions of uqTBP25. A HiTrap Blue-purified fraction of uqTBP25 (1.3 mg of protein) was loaded onto a column of phenyl-Sepharose (1.3-ml packed volume), proteins were eluted by a stepwise gradient of 4.0–0.0 M NaCl, and fractions were collected into 0.05% Nonidet P-40 (final concentration) as described under “Experimental Procedures.” A, mobility shift electrophoresis of fractions resolved by phenyl-Sepharose. Fractions were assayed for 32P-5'-labeled G'4 TeR-4 DNA binding activity as detailed under “Experimental Procedures.” B, SDS-PAGE of UV-cross-linked phenyl-Sepharose-resolved proteins. To bind G'4 TeR-4 DNA to uqTBP25, 6.0-μl aliquots of each phenyl-Sepharose-resolved fraction were incubated at 4 °C for 15 min with 1.05 pmol of 32P-5'-labeled G'4 TeR-4 DNA in the presence of 50 mM NaCl in a final volume of 15.0 μl. Protein-DNA complexes were covalently cross-linked by irradiating the samples at 4 °C for 5 min in a microtiter plate at a distance of 6 cm from a UVP (San Gabriel, CA) UV light source (254 nm, 580 microwatts/cm2 at 6 inches). The irradiated samples were electrophoresed through an SDS, 13% polyacrylamide gel, which was dried and exposed to autoradiographic film. The position of a 34-kDa protein-DNA complex present in fractions 10–14 is marked with an arrow. C, Comassie Blue staining of SDS-PAGE-resolved phenyl-Sepharose proteins. Electrophoresis and protein staining were conducted as described under “Experimental Procedures.” An arrow marks the position of the 25-kDa band that was eluted into fractions 10–14.
Table IV

| Protein uqTBP25 and homologue protein-derived peptides | Amino acid sequence | Reference |
|------------------------------------------------------|--------------------|----------|
| uqTBP25 peptide I                                    | LFVGGGIK           |          |
| Human hnRNP A2/B1"                                   | LFVGGGIK           |          |
| Human hnRNP A1                                      | LFVGGGIK           |          |
| Bovine hnRNP A1                                     | LFVGGGIK           |          |
| Mouse hnRNP A1                                      | LFVGGGIK           |          |
| Rat hnRNP A1                                        | LFVGGGIK           |          |
| Xenopus laevis hnRNP A1                             | LFVGGGIK           |          |
| uqTBP25 peptide II                                  | DYEFOYQCK          |          |
| Human hnRNP A2/B1                                    | DYEFOYQCK          |          |
| Human hnRNP A1                                      | DYEFOYQCK          |          |
| Bovine hnRNP A1                                     | DYEFOYQCK          |          |
| Mouse hnRNP A1                                      | DYEFOYQCK          |          |
| Rat hnRNP A1                                        | DYEFOYQCK          |          |
| X. laevis hnRNP A1                                  | DYEFOYQCK          |          |
| uqTBP25 peptide III                                 | IVLQK              |          |
| Human hnRNP A2/B1                                    | IVLQK              |          |
| Human hnRNP A1                                      | IVLQK              |          |
| Bovine hnRNP A1                                     | IVLQK              |          |
| Mouse hnRNP A1                                      | IVLQK              |          |
| Rat hnRNP A1                                        | IVLQK              |          |
| X. laevis hnRNP A1                                  | IVLQK              |          |
| uqTBP25 peptide IV                                  | SGKPGAHTVTYK       |          |
| Human hnRNP A2/B1                                    | SGKPGAHTVTYK       |          |
| Human hnRNP A1                                      | SGKPGAHTVTYK       |          |
| Bovine hnRNP A1                                     | SGKPGAHTVTYK       |          |
| Mouse hnRNP A1                                      | SGKPGAHTVTYK       |          |
| Rat hnRNP A1                                        | SGKPGAHTVTYK       |          |
| X. laevis hnRNP A1                                  | SGKPGAHTVTYK       |          |
| uqTBP25 peptide V                                  | (T)(V)(E)EVDAAMNAR |          |
| Human hnRNP A2/B1                                    | SM4A EVDAAMNAR     |          |
| Human hnRNP A1                                      | TV4E EVDAAMNAR     |          |
| Bovine hnRNP A1                                     | TV4E EVDAAMNAR     |          |
| Mouse hnRNP A1                                      | TV4E EVDAAMNAR     |          |
| Rat hnRNP A1                                        | TV4E EVDAAMNAR     |          |
| X. laevis hnRNP A1                                  | ST4D EVDAAMNAR     |          |

a The single-stranded binding protein HDP-1 is 100% homologous to the amino-terminal end of hnRNP A2/B1, which includes all five sequences shown in this table (58, 64).

b The single-stranded binding protein UP1 is 100% homologous to the amino-terminal end of hnRNP A1, which includes all five sequences shown in this table (58, 61, 64).

c Minor or alternate residues were detected in the NH2-terminal three positions of the peptide: D, H, or T, in the first position, T or V in the second, and E or I in the third.

Table V

| Unlabeled competitor DNA (50-fold molar excess) | Percentage of initial activity (n) |
|-----------------------------------------------|----------------------------------|
| d(GCC)8                                       | 77.3                             |
| d(CGG)8                                       | 71.5                             |
| d(C-T)31                                      | 85.0                             |
| d(GG-C)                                       | 76.5                             |
| d(CCGG)                                       | 88.5                             |
| d(CCCG)                                       | 68.5                             |
| d(GCCG)                                       | 122.4                            |
| d(AGCG)                                       | 105.9                            |
| d(GCGG)                                       | 95.7                             |
| d(AACG)                                       | 4.6 (4)                          |
| Total yeast RNA                               | 105.9                            |

The designation — marks an EcoRI recognition sequence AATTG and G, respectively, at the 5’- and 3’-ends of the competing oligomers.

The full sequence of these oligonucleotides is presented in Table I.

Based on an average molecular size of 6.5 kDa as determined by PAGE, the RNA was added at a 25-fold molar excess over Ter-R DNA.

The dissociation constant 

Kd values for complexes of uqTBP25 with single-stranded telomeric sequences that contained two or more d(TTAGGG) clusters had nanomolar range dissociation constants (Table V). However, a complex of uqTBP25 with an oligomer that contained a single d(TTAGGG) cluster had a dissociation constant 8.5- or 12.5-fold higher than the Kd values of complexes with oligomers that had two or four telomeric repeat units, respectively (Table V). As shown in Table V, binding of Ter-R DNA by uqTBP25 was highly sequence-specific, such that complexes of uqTBP25 with oligomers that contained single base substitutions within the Ter-R DNA repeat unit had considerably elevated Kd values. Substituting the single adenosine residue, d(TTAGGG), within the Ter-R DNA sequence with a guanine, d(TTAGGG), in TeT DNA increased the Kd value of the protein-Ter-R DNA complex 215-fold relative to the Kd of a uqTBP25-TeR-4 DNA complex (Table V). Similarly, altering the Ter-R DNA d(TTAGGG) repeat unit into d(TTAGGG) in Mut1 Ter-R DNA increased 85-fold the Kd value of the uqTBP25-Mut1 Ter-R DNA complex (Table V). Interestingly, an increase of only 9-fold in Kd value, was obtained when the Ter-R DNA sequence d(TTAGGG) was changed into d(TTAGGG) in Mut2 Ter-R DNA (Table V).

That uqTBP25 bound preferentially Ter-R DNA over the homologous rTer-R RNA sequence is evident by the 11.6-fold higher Kd of the protein-rTer-R complex (Table V). This preferential binding of DNA over RNA contrasts with the promiscuity of several other RNP species to bind RNA more efficiently than DNA (Ref. 64; see “Discussion”). A preference of uqTBP25 for single-stranded over double-stranded Ter-R DNA was demonstrated by the 30-fold lower Kd of its complex with single-stranded Ter-R DNA relative to the Kd of its complex with blunt-ended double-stranded telomeric DNA (Table V). However, when the double-stranded Ter-R DNA ended with a d(TTAGGG) single-stranded overhang, its association with uqTBP25 was as tight as that of single-stranded Ter-R DNA (Table V). Of the various forms of
tetraplex DNA, only a unimolecular antiparallel G'4 TeR-4 DNA structure bound tightly to uqTBP25. Although the $K_d$ value of the complex of uqTBP25 with G'4 TeR-4 DNA was 6-fold higher than that of a uqTBP25-TeR-4 DNA complex (Table VI), this difference was due to the required presence of 50 mM NaCl in the binding mixture. We found that the dissociation constant of a complex of uqTBP25 with TeR-2 DNA, which could not form a tetraplex structure, was also increased in the presence of 50 mM KCl from 3.2 $\pm$ 0.7 $\times$ 10$^{-9}$ mol/liter (Table VI) to 28.0 $\pm$ 0.3 $\times$ 10$^{-9}$ mol/liter ($n$ = 2) and to 36.0 $\pm$ 0.9 $\times$ 10$^{-9}$ mol/liter ($n$ = 2) in the presence of 50 mM NaCl. It was concluded, therefore, that the single-stranded and unimolecular quadruplex forms of TeR-4 DNA were bound by uqTBP25 with a very similar affinity. By contrast, a bimolecular G'2 TeR-2 tetraplex DNA bound poorly to uqTBP25, forming a complex having a $K_d$ 38-fold higher than the dissociation constant of a complex with TeR-2 DNA (Table VI). Parallel tetramolecular G4 quadruplex forms of oligomer Q (33, 50), single Q (48), or d(CGG)$_8$ at up to 123 nm (49) did not form detectable complexes with the protein (results not shown). Hence, our results indicated that uqTBP25 selectively bound single-stranded and unimolecular tetruplex forms of TeR DNA in a highly sequence-specific fashion.

**Stabilization of uqTBP25-associated Telomeric DNA against Nucleolytic Attack**—To inquire whether the stability of TeR DNA is affected by its association with uqTBP25, we compared the rate of digestion by micrococcal nuclease of unbound and protein-bound single-stranded TeR-4 DNA. Unbound single-stranded TeR-4 DNA or its complex with uqTBP25 were exposed for different lengths of time at 20 °C to 0.30 ng/µl micrococcal nuclease. The nucleolytic digestion was terminated by adding SDS to a final concentration of 0.25%, and the DNA samples were electrophoresed through a 6% nondenaturing polyacrylamide gel to separate the intact DNA oligomer from its digestion products, which migrated at the front of the gel. The kinetics of breakdown of the unbound or uqTBP25-bound single-stranded TeR-4 DNA indicated that whereas 53 or 77% of the unbound TeR-4 DNA was digested within 1 or 3 min, respectively, only 4 or 9% of the uqTBP25-bound TeR DNA was degraded after exposure to the nuclease for these periods of time (Fig. 4A). To assess the specificity of protection of TeR-4 DNA by uqTBP25 against nucleolytic attack, we examined the effect of the protein on the rate of digestion of the poorly bound TeR-4 DNA (Table VI). As seen in Fig. 4B, 25 or 39% of the unbound TeR-4 DNA were digested after a 1- or 3-min exposure to micrococcal nuclease, and similarly, 41 or 50% of the protein-associated TeR-4 DNA was degraded after digestion for the same periods of time. Hence, uqTBP25-associated TeR-4 DNA was not protected against nucleolytic attack, and its rate of breakdown was even modestly accelerated in the presence of the protein. It appeared, therefore, that the formation of a sequence-specific tight complex between TeR-4 DNA and uqTBP25 was responsible for the observed resistance of the protein-bound telomeric DNA to nuclease attack.

**The Effect of uqTBP25 on DNA Polymerase Activity**—The single-stranded DNA-binding protein UP1 and uqTBP25 are distinguished from one another by their closely homologous but nonidentical amino acid sequence (Table IV) and by their molecular sizes of 22 (58) and 25 kDa, respectively. One characteristic property of UP1 is its capacity to enhance the activity of calf thymus polymerase $\alpha$ (51). To further compare uqTBP25 with UP1, we examined the effect of uqTBP25 on polymerase $\alpha$-catalyzed DNA synthesis. Calf thymus polymerase $\alpha$ was incubated under DNA synthesis reaction conditions with increasing amounts of uqTBP25 and with either a poly(dG)–poly(dC) primer-template or with singly primed bacteriophage M13mp2 single-stranded DNA. DNA synthesis was determined by measuring the incorporation of $^{32}$P dGMP into acid-insoluble product DNA (see “Experimental Procedures”). As seen in Fig. 5, the copying of a poly(dC) template strand was inhibited by up to ~20%, whereas the copying of M13mp2 DNA was increased by less than 2-fold. Under the same reaction conditions, bacteriophage T4 gene 32 protein inhibited by up to 90% the copying of poly(dC) by polymerase $\alpha$ and stimulated the copying of M13mp2 DNA by more than 3-fold (results not shown). In modestly inhibiting polymerase $\alpha$-catalyzed copying of poly(dC) and stimulating M13mp2 DNA copying by less than 2-fold, uqTBP25 contrasted with UP1, which reportedly increased >5- or >10-fold the copying by polymerase $\alpha$ of poly(dC) or of E. coli exonuclease III-treated bacteriophage $\lambda$ DNA template, respectively (51). Hence, unlike UP1, uqTBP25 did not display a significant polymerase $\alpha$-stimulatory activity.

**Discussion**

The new mammalian telomeric DNA binding protein uqTBP25, which we describe in this manuscript, associates tightly and in a sequence-specific manner with single-stranded and unimolecular tetraplex forms of the G-strand of vertebrate telomeric DNA. Two or more (TTAGGG) telomeric DNA repeat units suffice for the formation of uqTBP25-DNA complexes that display nanomolar range dissociation constants (Table VI). Various single-stranded sequences, including DNA oligomers that do or do not contain guanine clusters as well as RNA sequences, fail to efficiently compete at a 50- or 75-fold molar excess with TeR-2 DNA for complex formation with uqTBP25 (Table V). The specific binding of vertebrate telo-
uqTBP25 Is Distinct from hnRNP A1 and hnRNP A2/B1—Four lines of evidence distinguish uqTBP25 from hnRNP A1 and hnRNP A2/B1. (i) The 25-kDa molecular mass of uqTBP25 (Fig. 2 and “Results”) differs from the 34- and 36–38-kDa molecular sizes of hnRNP A1 and hnRNP A2/B1, respectively (68). (ii) Out of 43 sequenced amino acid residues in uqTBP25, 4 or 6, respectively, are different from corresponding residues in hnRNP A1 or A2/B1 (Table IV). The amino acid sequence of hnRNP A1 is 100% conserved in human, bovine, mouse, and rat cells (Ref. 68, Table IV). The finding of different amino acids at matching positions in the rat cell-derived uqTBP25 and in the highly conserved hnRNP A1 (Table IV) indicates, therefore, that uqTBP25 does not represent rat hnRNP A1 or a derivative thereof. The six-residue difference between the amino acid sequences of rat uqTBP25 and human hnRNP A2/B1 (Table IV) strongly suggest that these two proteins are the products of distinct genes. Notably, a 100% identity exists between a partial sequence of mouse hnRNP A2/B1 and its human homologue (69). It is thus unlikely that the different amino acid sequences of uqTBP25 and of hnRNP A2/B1 are due to species diversity among homologous proteins. (iii) No detectable complex is formed between uqTBP25 and yeast total RNA (Table V; see “Results”), and a complex that does form between uqTBP25 and r(UUAGGG)₄ has a Kᵣ value 11.6-fold higher than that of a complex with d(TTAGGG)₄ (Table VI). The propensity of uqTBP25 for binding single-stranded DNA over RNA contrasts the preference of hnRNP A1 and hnRNP A2/B1 for association with RNA over single-stranded DNA (64). (iv) Data presented in Tables V and VI show that uqTBP25 binds d(TTAGGG)₄ sequences with a high degree of sequence specificity. By contrast, evidence shows that hnRNP A1 and A2/B1 bind RNA with a low sequence specificity (70), with a notable exception of a reported selective binding of d(TTAGGG)₄ by mouse liver hnRNP A2/B1 (69).

uqTBP25 Is Distinct from the Single-stranded Binding Proteins UP1 and HDP-1—Four lines of evidence indicate that despite their close size and sequence similarity, uqTBP25 and UP1 or HDP-1 are distinct proteins. (i) The amino acid sequence of the single-stranded DNA binding proteins UP1 from calf thymus and HDP-1 from mouse myeloma (51) indicate that they are fully homologous to the amino-terminal portion of HnRNP A1 and HnRNP A2/B1, respectively (58, 64). Multiple
Fig. 4. uqTBP25 stabilizes TeR-4 DNA against nuclease digestion. Samples, 1.0 ng each, of 32P-5'-labeled single-stranded TeR-4 DNA or TeT-4 DNA were incubated at 4 °C for 20 min with or without phenyl-Sepharose-purified uqTBP25 (10.0 activity units). The naked or protein-bound DNA was exposed to 1.5 ng of micrococcal nuclease at 20 °C for the indicated periods of time in the presence of 1.0 mM CaCl2. Nuclease digestion was terminated, and TeR DNA was separated from its complex with uqTBP25 by the addition of SDS to a final concentration of 0.25%. The DNA samples were electrophoresed through a non-denaturing 6% polyacrylamide gel to separate intact DNA from its digestion products, which migrated at the front of the gel. Residual intact DNA was quantified by phosphor imaging analysis. A, kinetics of nuclelease digestion of TeR-4 DNA with or without uqTBP25. The average value and S.D. of three independent determinations at each time point are indicated. ▲ ——— ▲, nuclelease-treated uqTBP25-bound TeR-4 DNA; ■ ——— ■, nuclelease-treated unbound TeR-4 DNA. B, kinetics of nuclelease digestion of TeT-4 DNA with or without uqTBP25. The average value and S.D. of three independent determinations at each time point are indicated. A ——— A, nuclelease-treated uqTBP25-bound TeT-4 DNA; ■ ——— ■, nuclelease-treated unbound TeT-4 DNA.

Amino acid substitutions distinguish uqTBP25 from either UP1 or HDP-1 and from their respective progenitor proteins HnRNP A1 or A2/B1 (Table IV). Hence, it appears that uqTBP25 is not a product of proteolytic cleavage of either HnRNP A1 or A2/B1 as are UP1 or HDP-1, respectively. (ii) The 25-kDa size of uqTBP25 differs from the 22- and 27-kDa molecular masses of calf thymus UP1 (58) and mouse myeloma HDP-1 (65), respectively. (iii) Whereas UP1 or HDP-1 bind single-stranded DNA with little or no sequence preference (51, 65), uqTBP25 associates selectively with the telomeric sequence d(TTAGGG), (Tables V and VI). (iv) Unlike UP1, which stimulates >5- or >10-fold the copying by DNA polymerase α of poly(dC) or of single-stranded DNA templates, respectively (51), uqTBP25 slightly inhibits copying of poly(dC) and increases by less than 2-fold copying of single-stranded DNA (Fig. 5).

uqTBP25 Is Possibly Related to a Human Cell Telomeric DNA-binding Protein—A group of related proteins that bind the pre-mRNA 3' splice site r(UUAGG) as well as the telomeric sequence d(TTAGGG), was identified in HeLa cells (71). The size, antigenicity, nucleic acid binding preference, and partial amino acid sequence of most of these proteins suggested that they are identical or closely related to hnRNP type A2/B1, D, or E (71). However, a 26-kDa protein designated A26, which has stretches 18 amino acids long homologous to hnRNP A1, binds d(TTAGGG)α with a high sequence specificity. Resemblance between human A26 and rat uqTBP25 extends to their similar molecular mass, their preferential binding of single-stranded over blunt-ended double-stranded telomeric sequence, and their high sequence specificity of d(TTAGGG)α binding (71). Yet, some properties distinguish uqTBP25 from A26. Whereas binding competition results indicate that A26 binds r(UUAGGG)α more tightly than d(TTAGGG)α (71), the uqTBP25-d(TTAGGG)α complex has an 11.6-fold lower Kα than a uqTBP25-r(UUAGGG)α complex (Table VI). Additionally, unlike uqTBP25, which binds TeR-4 and TeR-2 DNA with a similar affinity (Table VI), A26 binds TeR-2 DNA less tightly than TeR-4 DNA (71). Last, A26 fails to bind the substituted homologues of r(UUAGGG)α (r(UUAGGG)α, r(UAGGAGG)α, r(UUGGGG)α), or r(UUAGGG)α, but it does bind r(UUAGAGG)α or r(UAGGAGG)α (71). By contrast, relative to d(TTAGG)α, uqTBP25 binds most weakly d(TTAGG)α and d(TTAGG)α, but it does associate relatively tightly with d(TTAGG)α (Table VI).

Potential Cellular Function of uqTBP25—The amino acid sequence of uqTBP25 indicates that it is probably a derivative of an hnRNP species that is closely related but not identical to hnRNP A1 or A2/B1 (Table VI). Likewise, uqTBP25 is related to but distinct from the single-stranded DNA-binding proteins UP1 and HDP-1. Based on its molecular size and telomeric DNA binding specificity, uqTBP25 is most closely similar to human protein A26 (71). It was argued that the prime target of protein A26 is the pre-mRNA splice site but that it could also be involved in the binding of telomeric DNA (71). In view of the preferential binding by uqTBP25 of telomeric DNA sequence over its RNA homologue and its lack of clear preference for an intact splice site (Table VI), it might be that this protein interacts primarily with the G-strand of telomeric DNA rather than with pre-mRNA. By binding the telomeric G-strand overhang, uqTBP25 may protect it against nuclease attack (Fig. 4). Additionally, uqTBP25 might be instrumental in the stabilization of specific structures of telomeric DNA. Hence, by binding tightly single-stranded or unimolecular tetraplex forms of d(TTAGG), while binding weakly its bimolecular or tetramolecular tetraplex forms, uqTBP25 may stabilize the monomolecular forms of the G-strand overhang and prevent the generation of multimolecular tetraplex structures.
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