Formations of hairpin and tetrahedral structures by the trinucleotide repeat sequence d(CGG)n might contribute to its expansion in fragile X syndrome. Here we show that tetraplex structures of d(CGG)n are destabilized by two mammalian heterogeneous nuclear ribonucleoprotein-related tetraplex telomeric DNA-binding and -stabilizing proteins, quadruplex telomeric DNA-binding protein 42 (qTBP42) (Sarig, G., Weisman-Shomer, P., Eritzki, R., and Fry, M. (1997) J. Biol. Chem. 272, 4474–4482) and unimolecular quadruplex telomeric DNA-binding protein 25 (uqTBP25) (Eritzki, R., and Fry, M. (1997) J. Biol. Chem. 272, 15881–15890). Blunt-ended and 3′-tailed or 3′- and 5′-tailed bimolecular tetraplex structures of d(CGG)n and guanine-sparse 20-46-mer partial DNA duplex were progressively destabilized by increasing amounts of qTBP42 or uqTBP25 in time-dependent and ATP- or Mg2+-independent reactions. By contrast, tetraplex structures of telomeric and IgG sequences or guanine-rich double-stranded DNA resisted destabilization by qTBP42 or uqTBP25. Increased stability of tetraplex d(CGG)n in the presence of K+ or Na+ ions or at lowered reaction temperature diminished the destabilizing activity of uqTBP25. The contrasting stabilization of tetraplex telomeric DNA and destabilization of tetraplex d(CGG)n by qTBP42 and uqTBP25 suggested that sequence or structural differences between these tetraplexes might serve as cues for the differential stabilizing/destabilizing activities.

Fragile X syndrome is the most common inherited mental retardation disorder with an incidence of 0.4–0.6/1000 in males and 0.2–0.6/1000 in females (1). This syndrome is caused by an expansion of a d(CGG)/d(GCC) trinucleotide repeat tract and of an adjacent CpG island located 5′ to FMR1 gene (2–5). Whereas normal individuals have 6–54 copies of the repeat sequence, carrier females and transmitting males carry a premutation of >50 to 200 copies, and affected offspring of carrier females have a full mutation of >200 to over 2000 repeats (reviewed in Ref. 6). Full expansion of the d(CGG) sequence is accompanied by methylation of the trinucleotide repeat tract and of an adjacent CpG island located 5′ to FMR1 (7–10). As a result of d(CGG) expansion and hypermethylation, transcription of the FMR1 gene is silenced (8, 11, 12), and replication of a chromosomal section spanning 400 kilobase pairs 5′ and up to 350–600 kilobase pairs 3′ to the expanded d(CGG)n tract is delayed (13).

The genetic instability in fragile X syndrome is thought to be instigated by the formation of unusual structures of either or both d(CGG) and d(GCC) strands. Oligonucleotides, d(CGG)n or d(GCC)n, fold in vitro into unmolecular hairpin structures under physiologically like conditions (Refs. 14–18 and reviewed in Ref. 19). Hairpins of d(CGG)n also assemble in vitro into multimolecular tetraplex structures that are stabilized by non-canonical guanine-guanine hydrogen bonds (Refs. 20–22 and reviewed in Refs. 19 and 23). Recent evidence obtained in yeast demonstrated formation in vivo of secondary structures of d(CGG)n that evade cellular mechanisms for the detection and repair of single-stranded loops (24). Secondary structures of trinucleotide repeat sequences were proposed to be a causative factor in their expansion. Pausing of DNA polymerases at hairpin or tetraplex structures of d(CGG)n was observed both in vitro (25, 26) and in vivo (27). This pausing increases the probability of polymerase slippage and expansion of the trinucleotide sequence (24). Alternatively, looped-out secondary structures of single-stranded d(CGG)n or d(GCC)n might cause expansion by displacement synthesis of an Okazaki fragment (28, 29). Once expanded, hairpin or tetrahedral structures of d(CGG)n become thermodynamically stable (14) and are likely to block the transcription and replication machineries and thus prevent FMR1 expression and delay its replication.

In view of the key role of hairpin or tetraplex structures of d(CGG)n, in the expansion of this sequence (27, 30–32), there is a special interest in proteins that can unwind such DNA secondary structures. SV40 T-antigen (33), human Bloom’s syndrome DNA helicase (34), and yeast Sgs1 helicase (35) were shown to unwind tetrahedral structures of guanine-rich telomeric DNA and of IgG switch region sequences. More specifically, we demonstrated recently that while failing to unwind tetrahedral structures of telomeric and IgG switch region DNA, human Werner syndrome DNA helicase resolved bimolecular tetraplex structures of d(CGG)n, at an efficiency that exceeded the unwinding of a partial DNA duplex (36). Unwinding of tetraplex d(CGG)n by Werner helicase was ATP- and Mg2+-dependent and required the presence of an unpaired single-stranded tail at the 3′ or 5′ end of the tetrahedral structure (36).

In this paper we report that two hnRNP-related nuclear DNA-binding proteins from rat liver, quadruplex telomeric

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The abbreviations used are: hnRNP, heterogeneous nuclear ribonucleoprotein; TEMED, N,N,N,N′-tetramethylenediamine; G’2 DNA, bimolecular tetraplex DNA; G4 DNA, tetramolecular tetraplex DNA; qTBP42, quadruplex telomeric DNA-binding protein 42; uqTBP25, unimolecular quadruplex telomeric DNA-binding protein 25; RRM, RNA recognition motif.

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DNA-binding protein 42 (qTBP42) (37, 38), and unimolecular quadruplex telomeric DNA-binding protein 25 (uqTBP25) (39) destabilized tetraplex structures of d(CGG)₇. Previously identified as proteins that tightly bind and stabilize quadruplex telomeric DNA (37–39), it is shown here that qTBP42 and uqTBP25 destabilized bimolecular tetraplex structures of d(CGG)₇ without detectably binding these tetrahelices. Destabilization of tetraplex d(CGG)₇ by either protein did not require ATP or Mg²⁺ or the presence of an unpaired single-strand tail at the ends of the tetrahelix. The extent of destabilization of a d(CGG)₇ tetraplex structure by uqTBP25 was inversely proportional to its alkali ion-mediated thermal stability.

**EXPERIMENTAL PROCEDURES**

**Materials and Enzymes**—Isotopically 5'-labeled [γ-³²P]ATP (~3000 Ci/mmol) was provided by Amersham Pharmacia Biotech. Bacteriophage T4 polynucleotide kinase was a product of Promega. Operon Technologies supplied synthetic DNA oligomers, listed in Table I. Filter paper, DE81 and 3, was purchased from Whatman. Amresco supplied acrylamide/bisacrylamide (19:1 or 30:1.2). TEMED, N,N,N',N'-tetramethylethylenediamine. N,N,N',N'-tetramethylethylenediamine, and xylene cyanol FF were the products of IBI. Proteins qTBP42 and uqTBP25 were produced by Operon Technologies. Human Werner syndrome DNA helicase was purified to apparent homogeneity as proteins that tightly bind and stabilize quadruplex telomeric DNA (37–39), it is shown here that qTBP42 and uqTBP25 destabilized bimolecular tetraplex structures of d(CGG)₇.

**Preparation of Double-stranded and Tetraplex Forms of DNA Oligomers**—DNA oligomers were purified by electrophoresis through a denaturing 8–12% polyacrylamide gel in 0.5 M 32P-5'-labeled TeR2 or 3'-tail d(CGG)₇ in TE buffer containing 500 mM NaCl to precipitate at 4 °C under 80–120 V through a non-denaturing 8–12% polyacrylamide gel in 0.5 × TBE buffer (1.25 × Tris borate buffer, pH 8.3) that contained 10 mM of a specified salt. The resolved DNA bands were visualized by exposing to autoradiographic film gels that were dried on Whatman DE81 filter paper. Amounts of tetraplex or double-stranded DNA and of displaced single strands were quantified by exposing gels dried on Whatman DE81 filter paper to phosphomager plate (Fuji).

**RESULTS**

**Formation of Bimolecular Tetraplex Structures of d(CGG)₇ and Their Ion-dependent Stability**—Trinucleotide repeat sequences d(CGG)₇ readily fold into hairpin structures (14–19) and also assemble into multimolecular tetrahelical complexes (19–23). As shown in Fig. 1A, incubation of 32P-5'-labeled DNA (37) or 3'-tail d(CGG)₇ in TE buffer at 37 °C in the presence of 300 mM NaCl resulted in the generation of electrophoretically retarded forms specific for each oligomer. Similar incubation of an equimolar mixture of the two oligomers yielded the two slowly migrating forms of each oligomer and a third hybrid species with intermediate slowed mobility. All the retarded DNA structures were accumulated with a second order kinetics, indicat-
Telomeric DNA-binding Proteins Destabilize Tetraplex d(CGG)$_n$

Fig. 1. Stoichiometry and heat lability of d(CGG)$_7$ tetraplex structures. A, stoichiometry of d(CGG)$_7$ tetraplex. Tetrahelical DNA was formed at 4 °C for 20 h in mixtures that contained 47 μM 32P-5'-labeled d(CGG)$_7$ or 3'-tail d(CGG)$_7$ or a 1:1 mixture thereof in 10 μl of TE buffer, 300 mM NaCl. Aliquots of the reaction mixtures were either heated at 100 °C for 10 min or kept on ice prior to electrophoresis. Single-stranded d(CGG)$_7$ (43). The tetrahelical nature of similarly generated forms of 9-folded, antiparallel dimeric hairpin G$_2$ tetraplex structures. Based on results shown in A and on previously described findings (20, 21, 36), bimolecular d(CGG)$_7$ tetrahelices are schematically depicted as dimers of two hairpins bonded by guanine quartets. Shown are a G'2 bimolecular tetraplex of d(CGG)$_7$, a tetraplex of d(CGG)$_7$ carrying a non-d(CGG) single-stranded tail at its 3’ end (dashed lines), and a bimolecular mixed complex of the two oligomers. Only two pairs of stacked guanine quartets are outlined in each tetraplex. The two hairpins are aligned against each other in one of several possible orientations (21). C, effect of alkali ions on rates of heat denaturation of G’2 3’-tail d(CGG)$_7$. Tetraplex G’2 forms of 32P-5'-labeled 3’-tail d(CGG)$_7$ (100 fmol/10 μl reaction mixture) were prepared in the presence of 300 mM of LiCl, KCl, or NaCl and incubated at the indicated temperatures for 10 min in the presence of 50 mM of the respective salt. Heat denaturation was terminated by rapid cooling of the mixtures to 4 °C, and single-stranded 3’-tail d(CGG)$_7$ was resolved from the remaining tetraplex structure by electrophoresis at 4 °C through a nondenaturing 10% polyacrylamide gel containing 10 mM of the respective salt. Amounts of tetraplex and single-stranded 3’-tail d(CGG)$_7$ were quantified by phosphorimaging. D, effect of the type and concentration of alkali ion on the melting temperature of G’2 3’-tail d(CGG)$_7$. Rates of heat denaturation of 32P-5'-labeled 3’-tail d(CGG)$_7$ were measured in the presence of 0, 10, 50, or 100 mM of LiCl, KCl, or NaCl as in C. Melting temperatures at which 50% of the input tetraplex structure was denatured were derived from the denaturation curves.

Bimolecular Tetraplex Forms of d(CGG)$_n$ but Not of a Telomeric Sequence Are Destabilized by qTBP42 and uqTBP25—

The hnRNP-related proteins qTBP42 and uqTBP25 tightly bind and stabilize single-stranded and tetraplex telomeric sequences (37, 39). However, a survey of the interaction of qTBP42 and uqTBP25 with tetraplex structures of different DNA sequences revealed that neither protein detectably bound d(CGG)$_n$ tetrahelices. Moreover, in clear contrast to their stabilizing effect on tetraplex telomeric DNA, both proteins destabilized bimolecular tetraplex structures of d(CGG)$_n$. As seen in Fig. 2, A and B, neither qTBP42 nor uqTBP25 formed detect-
Telomeric DNA-binding Proteins Destabilize Tetraplex d(CGG)$_n$

Fig. 2. Bimolecular tetraplex forms of d(CGG)$_n$ but not of telomeric DNA are destabilized by qTPB42 and uqTPB25. A, destabilization of G'2 d(CGG)$_n$ and G'2 3'-tail d(CGG)$_n$ by qTPB42. End-labeled $^{32}$P-5'-G'2 d(CGG)$_7$, or G'2 3'-tail d(CGG)$_7$, at 100 or 70 fmol, respectively, were incubated at 37°C for 30 min with 6.3 units of qTPB42 in DNA destabilization reaction mixtures (see “Experimental Procedures”). Control samples that did not contain qTPB42 were either incubated at 37°C for 30 min to visualize the input tetraplex forms or were boiled for 10 min to obtain their constituent component single strands. Destabilization of the G'2 d(CGG)$_7$ tetraplexes was observed both before and after the proteins were removed by SDS (Fig. 2, A and B). In contrast, protein-3',5' TeR2 DNA complexes were seen before qTPB42 or uqTPB25 deproteinization, and SDS deproteinization of the complex revealed that amounts of the telomeric tetraplex DNA were not decreased as a result of interaction with either protein (Fig. 2C). This result is in line with the previously reported stabilization, and not destabilization, of tetraplex telomeric DNA by qTPB42 (37) and uqTPB25 (39).

Requirements and Specificity of G'2 d(CGG)$_n$ Destabilization by qTPB42 and uqTPB25—Destabilization of bimolecular tetraplex forms of d(CGG)$_n$ by qTPB42 or uqTPB25 was time-dependent, and its extent was directly proportional to the amount of added protein (Fig. 3). Tetraplex structures of d(CGG)$_n$ were fully or substantially destabilized by qTPB42 at molar ratios of protein to tetraplex substrate of up to 1:100, indicating turnover of the destabilizing protein (see “Discussion”). Destabilization of tetraplex structures of d(CGG)$_n$ sequences by the qTPB42 and uqTPB25 reached a maximum at 37°C and became undetectable below 30°C (results not shown). However, results summarized in Table II demonstrated that neither protein required ATP nor Mg$^{2+}$ for the destabilization of G'2 3'-tail d(CGG)$_7$. In fact, tetraplex destabilization by both proteins was suppressed in the presence of Mg$^{2+}$, presumably due to stabilization of the tetraplex by the ion. In control experiments, G'2 3'-tail d(CGG)$_7$ was unwound by human Werner helicase in ATP- and Mg$^{2+}$-dependent reaction such that the enzyme failed to destabilize this tetraplex DNA substrate when either ATP or Mg$^{2+}$ were omitted (data not shown, see also Ref. 36). The ATP- and Mg$^{2+}$-independent destabilization of tetraplex DNA by qTPB42 and uqTPB25 also contrasted the ATP and Mg$^{2+}$ requiring unwinding of tetraplex DNA by SV40 virus, yeast, and human DNA helicases (see “Discussion”). ATP-independent unwinding of duplex DNA is the hallmark of helix-destabilizing single-stranded DNA-binding proteins. However, control experiments indicated that tetraplex unwinding was not an activity shared by all single-stranded DNA-binding proteins. Molar excess of Escherichia coli SSB and human RPA did not detectably destabilize G'2 3'-tail d(CGG)$_7$ under the tested conditions with or without ATP and Mg$^{2+}$ (results not shown).

Results presented in Table III showed that both qTPB42 and uqTPB25 were capable of destabilizing to a comparable extent G'2 forms of d(CGG)$_n$ with or without non-d(CGG) single-stranded tails at their 3' ends or 3' and 5' ends. In addition, the two proteins destabilized a bimolecular tetraplex form of the methylated oligomer d($^5$mCGG)$_n$. By contrast, neither protein detectably resolved a tailed bimolecular tetraplex structure of the telomeric sequence 3',5' TeR2 or a tailed tetramolecular tetraplex form of the IgG class switch sequence oligomer Q (Table III). In measuring the destabilization of double-stranded DNA, we found that a guanine-sparsed 20-46-mer partial DNA duplex was resolved by qTPB42 to a significant extent but that it was inefficiently destabilized by uqTPB25. Neither protein was capable, however, of destabilizing the guanine-rich 3',5'-tail d(CGG)$_7$-3',5'-tail d(CGG)$_7$, blunt-ended duplex or the TeR3-anti-TeR 5'-tailed duplex (Table III).

Determination of the kinetics of destabilization of blunt-ended or tailed G'2 d(CGG)$_7$ indicated that after 60 min at
Whereas blunt-ended and tailed G\textsubscript{qTBP42} were incubated at 37 °C for 30 min with 250 fmol of either \textsuperscript{32}P-5\textsuperscript{-labeled d(CGG)\textsubscript{2}}, \textsuperscript{3}-tail d(CGG)\textsubscript{2}, or 3',5'-tail d(CGG)\textsubscript{2}, respectively. DNA destabilization was terminated by cooling the mixtures to 4 °C and by the addition of SDS to 0.25%. Tetraplex and displaced single strands were resolved by electrophoresis at 4 °C through a nondenaturing 8% polyacrylamide gel. Amounts of tetraplex and single-stranded oligomers were quantified by phosphor imaging. Extent of tetraplex destabilization was calculated as the ratio of tetraplex remaining in mixtures incubated with qTBP42 to tetraplex in mixtures identically incubated without protein.

Proteins, qTBP42 (1.25 units) or uqTBP25 (18.5 units), were incubated at 37 °C for 30 min with 250 fmol of either \textsuperscript{32}P-5\textsuperscript{-labeled G'2 3'-tail d(CGG)\textsubscript{2}}, \textsuperscript{3}-tail d(CGG)\textsubscript{2}, or 3',5'-tail d(CGG)\textsubscript{2}, by 12.5 units of uqTBP25 and electrophoretic resolution of the displaced single strands were conducted as in A. C, titration of the tetraplex and double-stranded DNA destabilizing activity of qTBP42. Increasing amounts of qTBP42 were incubated at 37 °C for 30 min with 250 fmol of either \textsuperscript{32}P-5\textsuperscript{-labeled G'2 3'-tail d(CGG)\textsubscript{2}}, or 20-/46-mer partial DNA duplex. The DNA destabilization reactions were terminated as in A, and tetraplex or partial DNA duplex were resolved from their corresponding displaced single strands by electrophoresis at 4 °C through a nondenaturing 12% polyacrylamide gel that contained 10 mM NaCl. Extent of DNA destabilization was calculated as the ratio of tetraplex or partial DNA duplex destabilized in the presence of the indicated amounts of qTBP42 to tetraplex in mixtures incubated without protein at 37 °C for 30 min. Insets, autoradiograms of gels separating G'2 3'-tail d(CGG)\textsubscript{2}, and 20-46-mer partial DNA duplex destabilized by increasing amounts of qTBP42. D, titration of the tetraplex and partial DNA duplex destabilizing activity of uqTBP25. Increasing amounts of uqTBP25 were incubated at 37 °C for 30 min with 250 fmol of either \textsuperscript{32}P-5\textsuperscript{-labeled G'2 3'-tail d(CGG)\textsubscript{2}}, or 20-/46-mer partial DNA duplex. Reactions were terminated, and DNA was electrophoretically resolved, and results were analyzed as in C. Insets, autoradiograms of gels separating G'2 3'-tail d(CGG)\textsubscript{2}, and 20-46-mer partial DNA duplex destabilized by increasing amounts of uqTBP25.

### Table II

**ATP and Mg\textsuperscript{2+} are dispensable for destabilization of G'2 3'-tail d(CGG)\textsubscript{2} by qTBP42 and uqTBP25**

Proteins, qTBP42 (1.25 units) or uqTBP25 (18.5 units), were incubated at 37 °C for 15 min with 250 fmol of \textsuperscript{32}P-5\textsuperscript{-labeled G'2 3'-tail d(CGG)\textsubscript{2}}, in standard tetraplex DNA destabilization assay mixtures (see “Experimental Procedures”) with or without the indicated additions. Single-stranded and G'2 3'-tail d(CGG)\textsubscript{2}, were resolved by electrophoresis at 4 °C through a nondenaturing 10% polyacrylamide gel, and their respective bands were quantified by phosphorimaging.

| Addition | G'2 3'-tail d(CGG)\textsubscript{2} destabilizing activity | qTBP42 | uqTBP25 |
|----------|-------------------------------------------------------|--------|---------|
| None     | 1.00\textsuperscript{a}                              | 1.00\textsuperscript{a} |         |
| 1 mm ATP | 1.17                                                 | 0.48   |         |
| 4 mm MgCl\textsubscript{2} | 0.36                                              | 0.0    |         |
| 1 mm ATP + 4 mm MgCl\textsubscript{2} | 0.20                                             | 0.0    |         |

\textsuperscript{a} Normalized values of 1.00 correspond to destabilization of 66 and 42% of the input G'2 3'-tail d(CGG)\textsubscript{2} substrate by qTBP42 and uqTBP25, respectively.

### Table III

**Specificity of DNA destabilization by qTBP42 and uqTBP25**

Standard DNA destabilization reaction mixtures contained 100 fmol of \textsuperscript{32}P-5\textsuperscript{-labeled tetraplex or double-stranded (ds) DNA and 2.5 or 12.5 units of qTBP42 or uqTBP25, respectively. The mixtures were incubated at 37 °C for 30 min, and displaced single strands were resolved from remaining tetraplex or double-stranded DNA by electrophoresis through a nondenaturing 12% polyacrylamide gel. Phosphorimaging was used to quantify the amounts of destabilized tetraplex or double-stranded DNA.

| DNA | DNA destabilized |
|-----|-----------------|
| qTBP42 | uqTBP25 |
| G'2 d(CGG)\textsubscript{2} | 1.00\textsuperscript{a} | 1.00\textsuperscript{a} |
| G'2 3'-tail d(CGG)\textsubscript{2} | 0.74 | 1.47 |
| G'2 3',5'-tail d(CGG)\textsubscript{2} | 0.78 | 1.18 |
| G'2 3',5'-tail TeR2\textsuperscript{b} | 0.53 | 0.63 |
| G'2 3',5'-tail Q\textsuperscript{c} | 0.0 | ND |
| 20-/46-mer partial DNA duplex | 0.0 | 0.0 |
| ds [TeR3 + anti-TeR3] | 0.0 | 0.0 |
| ds [3',5'-tail d(CGG)\textsubscript{2}, 3',5'-tail d(CCG)\textsubscript{2}] | 0.0 | ND |

\textsuperscript{a} Normalized values of 1.00 correspond to destabilization of 54 and 34 fmol of G'2 d(CGG)\textsubscript{2}, substrate by qTBP42 and uqTBP25, respectively.

\textsuperscript{b} Bimolecular G'2 tetraplex structure of 3',5'-tail TeR2 oligomer and a tetramolecular G4 structure of 5',5'-tail Q oligomer were prepared and characterized as described (37, 39).

\textsuperscript{c} ND, not done.

**Only Weakly Bonded G'2 d(CGG)\textsubscript{2} Tetraplex Structures Are Destabilized by uqTBP25**—To assess the potency of destabilization of tetraplex DNA by uqTBP25, the protein was incu-
bated with $^{32}$P-5'-labeled G'2 3'-tail d(CGG)$_7$ that was prepared in the presence of Li$^+$, K$^+$, or Na$^+$ ions and subsequently suspended in reaction mixtures that lacked or contained the respective ion. As demonstrated in Fig. 1, C and D, the different alkali ions conferred different heat stability on this tetraplex DNA. Results shown in Fig. 4 indicated that in the absence of salt in the reaction mixtures, G'2 3'-tail d(CGG)$_7$ was electrophoretically resolved from displaced single strands as in Fig. 2A. Amounts of tetraplex and single-stranded 3'-tail d(CGG)$_7$ were quantified by phosphorimaging, and extent of tetraplex destabilization was calculated as in Fig. 3C.

In fact, a similar analysis of tetraplex destabilization by qTBP42 was deflected by uniform direct suppression of the protein DNA binding activity by all three ions (results not shown). To examine whether the alkali ions acted through their effect on the intrinsic stability of the tetraplex substrate, $^{32}$P-5'-labeled G'2 3'-tail d(CGG)$_7$ was reacted with uqTBP25 in the presence of Li$^+$, K$^+$, or Na$^+$ at 37 or 42 °C. As seen in Fig. 5, although tetraplex destabilization at 37 °C was increasingly diminished by ions in the order Na$^+$ > K$^+$ > Li$^+$, elevation of the incubation temperature to 42 °C resulted in significantly enhanced
destabilization rates in the presence of K⁺ or Na⁺ ions. Hence, uqTBP25 was able to destabilize only weakly bonded G‘2 3’-tail d(CGG)₇. Quadruplex 3’-tail d(CGG)₂ of low stability in the presence of Li⁺ ions or at an elevated temperature was efficiently destabilized by uqTBP25, whereas the increased stability of this tetraplex in K⁺ or Na⁺ at or at 37 °C restricted its protein-mediated destabilization. Therefore, under the tested conditions, destabilization by uqTBP25 was limited to only G‘2 3’-tail d(CGG)₂ of low inherent stability. Notably, however, the choice between stabilization and destabilization of tetraplex DNA by qTBP42 or uqTBP25 was not solely determined by the stability of the tetraplex substrate. Whereas the high stability of G₄ 5’-tail Q DNA (Tm >70 °C) correlated with its failure to be unwound by the proteins (Table III), G‘2 3’,5’-tail TeR₂ DNA that was stabilized, rather than destabilized, by the proteins (Fig. 2 and Table III), was less stable (Tm = 38.5 °C) than G‘2 3’-tail d(CGG)₂ (Fig. 1, C and D).

**DISCUSSION**

In this paper we demonstrated that proteins qTBP42 and uqTBP25 destabilized tetraplex structures of the fragile X syndrome expanded repeat sequence d(CGG)ₙ. Both proteins were originally isolated on the basis of their tight binding to single-stranded and tetraplex forms of the vertebrate telomeric sequence d(TTAGGG)ₙ. Furthermore, by forming complexes with single-stranded and tetraplex telomeric DNA, qTBP42 and uqTBP25 imparted increased stability on the bound DNA. Hence, the observed failure of these two proteins to bind detectably tetraplex structures of d(CGG)ₙ and their facile destabilization of these tetrahelices demonstrated that they have opposite sequence- or structure-specific effects on different types of quadruplex DNA.

Protein qTBP42, a 42-kDa monomer (37), was identified as a close homologue of the mouse CArG box-binding protein CBF-A that also shares extensive homology with several hnRNP-related RNA and single-stranded DNA-binding proteins (38). Protein qTBP42 formed tight complexes (Kᵈₙ values of 5–14 × 10⁻⁹ mol/liter) with single-stranded and unimolecular or bimolecular tetraplex forms of the vertebrate guanine-rich telomeric sequence (37). Association of qTBP42 with tetraplex telomeric DNA increased the heat and nuclelease stability of the bound tetrahelix (37). The 25-kDa uqTBP25 protein (39) was identified as a close homologue of hnRNP A1 and hnRNP A2/B1 and of their derivatives, the DNA helix-destabilizing proteins UP1 (44) and HDP-1 (45) that span the N-terminal two-thirds of the respective hnRNP molecules. Protein uqTBP25 formed tight complexes with single-stranded and unimolecular tetraplex forms of d(TTAGGG)ₙ (Kᵈₙ values of 2 to 13 × 10⁻⁹ mol/liter), in a DNA sequence and structure-specific fashion (39). Binding to uqTBP25 increased the nuclelease resistance of single-stranded and unimolecular tetraplex forms of telomeric DNA (39).

Evidence presented in this paper confirmed that qTBP42 and uqTBP25 bound and maintained in stable forms, respectively, bimolecular and single-stranded d(TTAGGG)ₙ, tetrameric sequence (Fig. 2C). By contrast, while not forming detectable complexes with bimolecular tetraplex structures of the trinucleotide repeat sequence d(CGG)ₙ, qTBP42 and uqTBP25 transformed these tetrahelices into their component single strands (Fig. 2, A and B). That the observed conversion reflected bona fide destabilization of tetrahelices d(CGG)ₙ by qTBP42 or uqTBP25 was indicated by the time and protein amount dependence of the transformation (Fig. 3). Neither ATP nor Mg²⁺ was necessary for the destabilization of tetraplex d(CGG)ₙ by qTBP42 or uqTBP25 (Table II). This lack of ATP and Mg²⁺ dependence distinguished qTBP42 and uqTBP25 from the tetrahelical DNA unwinding activity of DNA helicases. SV40 DNA helicase (33), yeast Sgs1 helicase (35), and human Bloom’s syndrome helicase (34) required ATP and Mg²⁺ for the unwinding of tetrahelical structures of guanine-rich telomeric and IgG switch region sequences. Similarly, ATP and Mg²⁺ were also necessary for the unwinding of tetraplex d(CGG)ₙ by human Werner syndrome helicase (36). Proteins qTBP42 and uqTBP25 also differed from DNA helicases by their tetraplex DNA substrate requirements. Whereas SV40 DNA helicase (33), yeast Sgs1 DNA helicase (35), and human Bloom’s syndrome (34) resolved quadruplex structures of telomeric DNA and of IgG switch region sequence, qTBP42 and uqTBP25 failed to destabilize these tetrahelical structures (Table III). Additionally, short unpaired single-stranded tails at the 3’ ends (34, 35) or 3’ and 5’ ends of the tetraplex ends were necessary for unwinding by Sgs1 (35) Bloom’s syndrome (34) and Werner syndrome (36) DNA helicases. By contrast, blunt-ended G‘2 d(CGG)₂ and 3’ or 3’,5’-tailed G‘2 d(CGG)₂ were similarly destabilized by qTBP42 and uqTBP25 (Fig. 3 and Table III).

The ability of qTBP42 and uqTBP25 to destabilize tetraplex d(CGG)ₙ without requiring ATP and Mg²⁺ suggested that they acted as DNA helix-destabilizing proteins rather than as energy-requiring DNA helicases. Indeed, as expected from helix-destabilizing proteins, both qTBP42 and uqTBP25 also resolved a 20/46-mer partial DNA duplex in ATP- and Mg²⁺-independent fashion (Fig. 3 and Table III). Calculation of the stoichiometry of destabilization of tetraplex d(CGG)ₙ and 20/46-mer partial DNA duplex by qTBP42 revealed that full or substantial destabilization was attained at protein to DNA molar ratios of 1:100 (see “Experimental Procedures” and “Results”). These results, as well as the absence of detectable complexes between qTBP42 and tetraplex or single-stranded d(CGG)ₙ indicated that after destabilization qTBP42 was released from the product single strands and became available for destabilization of additional tetraplex molecules.

Protein uqTBP25 (39) was found to share close homology with the hnRNP-derived mammalian single-stranded DNA-binding proteins UP1 (44) and HDP-1 (45) that destabilize double-stranded DNA without requiring ATP or Mg²⁺. Protein qTBP42, (38) being most closely related to the mouse hnRNP homologue CArG box-binding protein CBF-A (46), also contained extensive regions of homology with hnRNP A/B (47) and with the single-stranded DNA-binding proteins ssDBF (48) and CRP1 (49). Both qTBP42 (38) and uqTBP25 (39), as well as their homologues, contained the highly conserved RNA recognition motifs (RRMs) that characterize the hnRNP family. What might be the structural basis for the contrasting abilities of qTBP42 and uqTBP25 to bind and stabilize tetraplex telomeric DNA and to destabilize tetraplex d(CGG)ₙ? An explanation for the tight binding of telomeric DNA by hnRNP A1 and UP1 was recently provided through an analysis at 2.1 Å resolution of co-crystals of UP1 with the telomeric sequence d(TTAGGG)ₙ (50). It was shown that dimeric UP1 bound two single strands of the telomeric DNA and that each strand contacted the antiparallel RRM1 of one monomer and RRM2 of the other (50). Sharing conserved RRM elements and tetraplex d(CGG)ₙ destabilizing activity, it is conceivable that the RRM motifs of qTBP42 and uqTBP25 are also involved in the interaction with these tetrahelices and their destabilization. Hence, whereas the geometry of interacting tetraplex sequences of some RRMs might serve as a cue for their binding and stabilization, spatial or sequence properties of other tetraplexes will dictate their destabilization. One is tempted to speculate that proteins such as qTBP42 and uqTBP25 might act in vivo bi-functionally. Whereas they may stabilize desirable tetrahelices, such as perhaps at telomere ends, they also can resolve unwanted quadruplex structures that might form during rep-
cation or transcription of exposed stretches of guanine-rich stretches of single-stranded DNA.

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