Telomere G-Strand Structure and Function Analyzed by Chemical Protection, Base Analogue Substitution, and Utilization by Telomerase in Vitro†

Eric R. Henderson,*,‡ Michael Moore,§ and Bruce A. Malcolm‖

Department of Zoology, Iowa State University, Ames, Iowa 50011, and Cancer Research Center and Department of Biochemistry, University of California, Berkeley, California 94720

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ABSTRACT: Eukaryotic telomeres have a 12–16 nucleotide long deoxyguanosine (dG) rich single-stranded overhang at their molecular termini. Some of the unique features of telomeres are probably attributable to a specialized structure formed by this overhang. In the ciliated protozoan *Tetrahymena thermophila*, the dG-rich overhang is comprised of approximately two repeats of the sequence d(TTGGGG). Previous work has shown that the synthetic oligonucleotide $d(TTGGGG)_4$ can form an unusual non-Watson–Crick base-paired structure (the "G-strand structure") containing G-G base pairs and *syn*-guanines. We have tested the susceptibility of various dGs in this structure to methylation by DMS. At 0–10 °C one dG residue is hypersensitive to methylation while others are particularly resistant. By systematically substituting deoxyinosine (dI) for dG in $d(TTGGGG)_4$ we identify N_2 groups of guanine essential for formation of the G-strand structure. We show that dI-substituted molecules that cannot form the G-strand structure nonetheless function as substrates for telomere repeat addition in vitro by the telomere lengthening enzyme, telomerase. The implications of these data are discussed.

All eukaryotic telomers examined so far share several structural features. These include a deoxyguanosine (dG)[1] rich strand (G-strand) and a deoxycytosine (dC) rich strand (C-strand) in the duplex region of the telomere, a defined orientation of the two strands of the duplex (the G-strand running 5′ to 3′ toward the end of the chromosome), and a G-strand 3′ overhang protruding beyond the telomeric duplex (Blackburn & Szostak, 1984; Klobutcher et al., 1981; Pluta et al., 1982; Henderson et al., 1988; Henderson & Blackburn, 1989). Single-stranded synthetic oligonucleotides corresponding to one or more repeats of the G-strand overhang serve as primers for telomere G-strand repeat addition in vitro by *Tetrahymena* telomerase, an enzyme involved in telomere length regulation (Greider & Blackburn, 1985, 1987, 1989). Similar enzymatic activities have been reported in other organisms (Zahler & Prescott, 1988; Shippen-Lentz & Blackburn, 1989), suggesting that telomere repeat addition to the G-strand overhang is a conserved mechanism for maintenance of eukaryotic chromosomal termini. Synthetic oligonucleotides corresponding to two or more repeats of the G-strand overhangs of telomeres from phylogenetically diverse organisms share the ability to form novel G-strand structures (identified as fast-migrating species in nondenaturing polyacrylamide gels) that are stabilized by non-Watson–Crick base pairs (Henderson et al., 1987). Hence, it is of interest to determine the details of these structures and whether they are involved in recognition by telomerase or other telomere binding proteins.

The studies presented here provide new information that should be useful in understanding the structure of the *Tetrahymena* G-strand sequence $d(TTGGGG)_4$ and the relationship between this structure and its potential role in telomere function. The structure of this oligonucleotide has been probed by chemical protection and methylation interference assays.

Chart I: Oligonucleotides Used in This Study

Parent molecule:

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                1 2 3 4    5 6 7 8    9101112    13141516
5'-TTGGGGTTGGGGTTGGGGTTGGGG-3'
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Base analog substituted molecules:

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#1. 5'-TTIIIITTIIIITTIIIITTIIII-3'
#2. 5'-TTIIIITTIIIITTGGGGTTGGGG-3'
#3. 5'-TTGGGGTTGGGGTTIIIITTIIII-3'
#4. 5'-TTIIIITTGGGGTTGGGGTTGGGG-3'
#5. 5'-TTGGGGTTIIIITTGGGGTTGGGG-3'
#6. 5'-TTGGGGTTGGGGTTIIIITTGGGG-3'
#7. 5'-TTGGGGTTGGGGTTGGGGTTIIII-3'
#8. 5'-TTGGIGTTGGIGTTGGIGTTGGIG-3'
#9. 5'-TTGGGITTGGGITTGGGITTGGGI-3'
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We have synthesized a series of deoxyinosine (dI) substituted derivatives of the *Tetrahymena* G-strand sequence (Chart I) and tested them using nondenaturing gel electrophoresis for the ability to form G-strand structures analogous to that formed by the parent molecule. These oligonucleotides have been further scrutinized by chemical protection analysis. Finally, we show that G-strand derivatives that are incapable of forming the G-strand structure still function efficiently as primers for telomerase-mediated telomere repeat addition in vitro, suggesting that the G-strand structure is not required by telomerase.

Experimental Procedures

DNA Synthesis and Purification. Oligonucleotides were synthesized by an Applied Biosystems automated synthesizer. Following deprotection, oligonucleotides were purified by electrophoresis through a 12% polyacrylamide [0.3% or 0.8%

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* Author to whom correspondence should be addressed.
‡ Department of Zoology, Iowa State University.
§ Cancer Research Center, University of California.
‖ Department of Biochemistry, University of California.

[1] Abbreviations: dG, deoxyguanosine; dI, deoxyinosine; DMS, dimethyl sulfate; NMR nuclear magnetic resonance.

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bis(acrylamide)] gel containing 50% (w/v) urea and 89 mM Tris–89 mM boric acid (pH 8.3) (1× TBE) buffer. DNA was visualized by UV shadowing and eluted from the gel in 10 mM Tris–1 mM EDTA (pH 7.5) (TE). Samples were desalted by C_{18} (Waters) chromatography. DNA was repurified by the same procedure after radiolabeling.

DNA Radiolabeling. A total of 100 ng of purified oligonucleotide was incubated for 20 min at room temperature with 50 mM Tris (pH 7.5), 1 mM $MgCl_2$, 1 mM DTT, 100–300 μCi of [γ-^{32}P]ATP (7000 Ci/mmol), and 5 units of T4 polynucleotide kinase. Following incubation samples were immediately loaded onto a 12% polyacrylamide gel and purified as described above.

Chemical Protection, Methylation Interference, and Piperidine Cleavage. For chemical protection studies radiolabeled DNA was denatured at 90 °C for 1 min and equilibrated at the desired temperature for 15–30 min prior to incubation with dimethyl sulfate (DMS) (typical dilution in water 1:1 to 1:100) for 5–15 min in 50 mM sodium cacodylate, pH 7.0, or buffer indicated in the text and figures. Reaction volume was 10 μL; 10 μL of ice-cold NaOAc (500 mM) containing 10 μg of tRNA was added to the sample followed immediately by 50 μL of ice-cold ethanol (95%). Samples were allowed to precipitate for 16 h at −20 °C and collected by centrifugation. Methylation interference differed from this protocol in that samples were incubated with DMS at high dilution (1:100 in water) and temperature (55 °C) for 3 or 12 min and then treated as above. Following collection of the methylated DNA, samples were electrophoresed at 4 °C under nondenaturing conditions as described previously (Henderson et al., 1987). Bands were eluted in TE and collected by ethanol precipitation at −20 °C using carrier tRNA (100 μg/mL) and NaOAc (300 mM). All samples were cleaved with piperidine (1:10 fresh dilution in water) at 90 °C for 30 min, dried in a Speed-Vac (Savant), redissolved in water, and dried two more times. Samples were analyzed by electrophoresis through a 40-cm, 12% polyacrylamide [0.3% bis(acrylamide)] gel containing 50% (w/v) urea and 1× TBE.

Nondenaturing Gel Electrophoresis. Conditions were similar to those previously described (Henderson et al., 1987) with the exception of the experiment shown in Figure 3b in which 50 mM NaCl was included in the electrophoresis buffer, gel buffer, and loading mixture. All samples were denatured at 90 °C for 1 min and allowed to equilibrate at the desired temperature for 15–30 min prior to electrophoretic separation.

Telomerase Assay. This was performed as described by Greider and Blackburn (1985). A total of 100 ng of gel-purified DNA was used for each assay. Reactions contained 50 mM Tris, pH 8.0, 100 mM NaOAc, 2 mM $MgCl_2$, 0.5 unit of RNasin, 0.1 mM spermine, 1 mM spermidine, 5 mM 2-mercaptoethanol, 200 mM dTTP, and 10 μCi of [α-^{32}P]dGTP (400 Ci/mmol) in 10 μL to which 10 μL of partially purified telomerase [100000*g* extract supernatants (S-100) fractionated by Sephacryl S-500 (Pharmacia) gel filtration followed by heparin–agarose (Bio-Rad) chromatography] was added. Samples were incubated at 30 °C for 60 min; 20 μL of 200 mM EDTA was added to stop the reaction. This was followed by two phenol extractions and precipitation from ethanol with NH_4OAc (2.5 M). The labeled material was collected by centrifugation and resuspended in 95% formamide–5 mM EDTA. The samples were heated to 90–100 °C for 1 min and immediately analyzed by electrophoresis through an 8% polyacrylamide sequencing gel (40 cm).

Results

DMS Modification of d(TTGGGG)$_4$ Identifies N_7 Groups of dG That Are Resistant, Sensitive, and Hypersensitive to Methylation in the G-Strand Structure. DMS reacts primarily with the N_7 group of guanine (Maxam & Gilbert, 1980). We reasoned that if an N_7 group is involved in base pairing, it will show resistance to methylation by DMS. d(TTGGGG)$_4$ was radiolabeled at the 5′ end and subjected to limited methylation as a function of temperature. This treatment resulted in a very specific pattern of susceptible and protected guanines at temperatures up to 40 °C (Figure 1, lanes 1–15). This pattern is lost as 55 °C, indicative of unfolding of the structure (Figure 1, lanes 16–18). These observations corroborate the previously reported melting temperature for this structure determined by other methods (Henderson et al., 1987). One guanine (position 9 in Chart I and Figure 1) is hypersensitive to DMS modification. It is possible that the enhanced susceptibility of this guanine is a consequence of it being unpaired, and possibly strained in a loop in the structure. Placing G9 in a loop puts a constraint on possible secondary structures. The number of nucleotides in the loop is probably three or less since G8 and G10 are well protected relative to G9. The two blocks of four guanines at the 3′ end of the structure and more resistant to DMS modification than those at the 5′ end. The block of four guanines at the 5′ end appears to be particularly susceptible to methylation above 10 °C (although G2 and G3 are resistant to methylation below 10 °C). We cannot rule out the possibility that some of this enhanced susceptibility to methylation is due to multiple DMS modifications in some molecules, which would give a cleavage pattern biased toward the radiolabeled 5′ end.

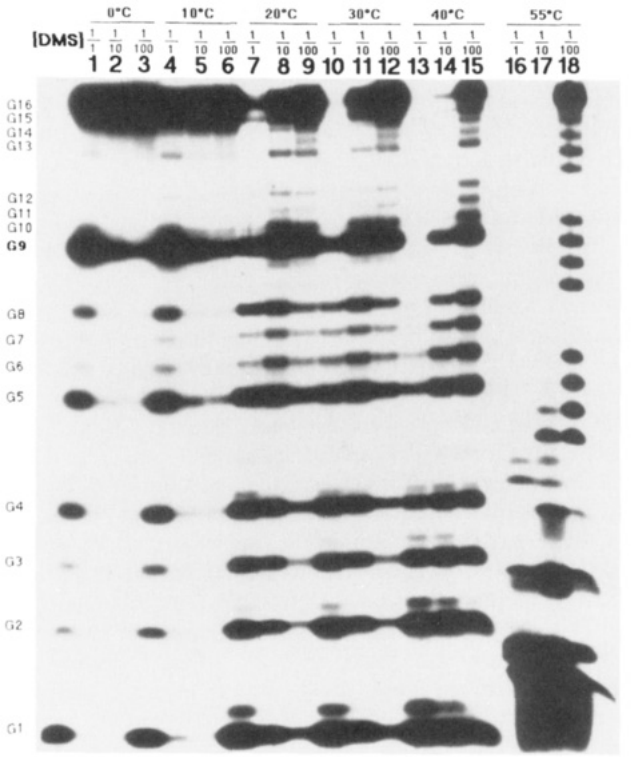

FIGURE 1: Differential susceptibility of guanines in d(TTGGGG)$_4$ to methylation as a function of temperature. Three DMS dilutions were used at each temperature (1:1, 1:10, 1:100). At 0 °C (lanes 1–3), 10 °C (lanes 4–6), 20 °C (lanes 7–9), 30 °C (lanes 10–12), and 40 °C (lanes 13–15) a specific cleavage pattern is seen indicating which N_7 groups are resistant or sensitive to methylation. At 55 °C (lanes 16–18), above the T_m of the structure (40–45 °C), no specific protection pattern is observed. At elevated temperatures and high concentrations of DMS, extensive cleavage occurs, resulting in most of the radiolabel migrating to the bottom of the gel (e.g., lane 16). Lanes 1–15 and 16–18 do not line up because the figure is a composite of two gels.

Methylation Inhibits G-Strand Structure Formation. To determine if methylation of particular dGs could inhibit G-strand structure formation, we performed methylation interference assays. d(TTGGGG)$_4$ was radiolabeled at the 5′ end and partially methylated with DMS above the T_m of the G-strand structure so that all dGs were equally accessible to methylation. The methylated DNA was denatured and incubated at 4 °C, and the products were analyzed by nondenaturing polyacrylamide gel electrophoresis to determine whether methylation of certain guanines could interfere with G-strand structure formation. The subpopulation of methylated molecules still capable of forming the G-strand structure is expected to migrate the same distance as the unmodified structure. Figure 2a shows a nondenturing gel separation of methylated molecules. Lanes 1 and 2 contain d(TTGGGG)$_4$ methylated for 3 and 12 min, respectively. Longer periods of methylation resulted in fewer fast-migrating (i.e., G-strand structure) molecules, as expected. Lane 3 contains the unmodified parental molecules, the majority of which were in the fast-migrating conformation (arrow). The fast-migrating species from the methylated DNA lanes was isolated and the DNA cleaved at the position of methylation with piperidine. Cleavage patterns of slower migrating forms (bracketed in Figure 2a) are not shown because these molecules existed as heterogeneous populations, making correlation of the cleavage pattern and the structure difficult. Figure 2b shows the results of piperidine cleavage of the methylated G-strand structure displayed on a denaturing polyacrylamide gel. Guanines in positions 4, 5, 9, and 10 were heavily methylated and therefore do not contain N_7s critical for structure formation. Conversely, guanines in positions 1–3, 6–8, 11, 12, and 14–16 were very weakly methylted, indicating that addition of a methyl group to N_7 at these positions impairs structure formation. Guanine at position 13 was variably metylated in different experiments. dGs that inhibit G-strand structure formation when methylated, yet are accessible for methylation after the structure has formed (G1, G2, G8, G12, and G16), are marked with filled circles in the figure.

dI Substitutions Reveal N_2 Groups Important for G-Strand Structure Formation. To further analyze the atomic requirements for formation of the G-strand structure, we replaced guanine with hypoxanthene (i.e., replacement of dG with dI) at various positions in the telomeric G-strand parent sequence (Chart I). Hypoxanthene lacks the N_2 group of guanine, thus reducing the strength of base pairing across the Watson–Crick face [N_1, (±)N_2, and O_6] of the base. However, the base-pairing potential of the Hoogsteen face (N_7 and O_6) of hypoxanthene is identical with that of guanine.

Nondenaturing gel electrophoretic analysis of the dI-substituted oligonucleotides in a Tris-borate buffer system identical with that originally used to demonstrate the existence of the G-strand structure (Henderson et al., 1987) showed that none of them formed structures that comigrated with that formed by the parent molecule under these conditions (Figure 3a). However, small changes in the mobility of some of the dI-substituted oligonucleotides were observed, suggesting the occurrence of subtle structural changes as a function of temperature (Figure 3a). To test the ability of dI-substituted oligonucleotides to form the G-strand structure under conditions comparable to those used in the DMS protection assays (see below), we performed the same analysis in a Tris–borate buffer system including 50 mM NaCl. Under these conditions several of the dI-substituted molecules were capable of forming fast-migrating species potentially analogous to the G-strand structure (Figure 3b). These results show that the presence

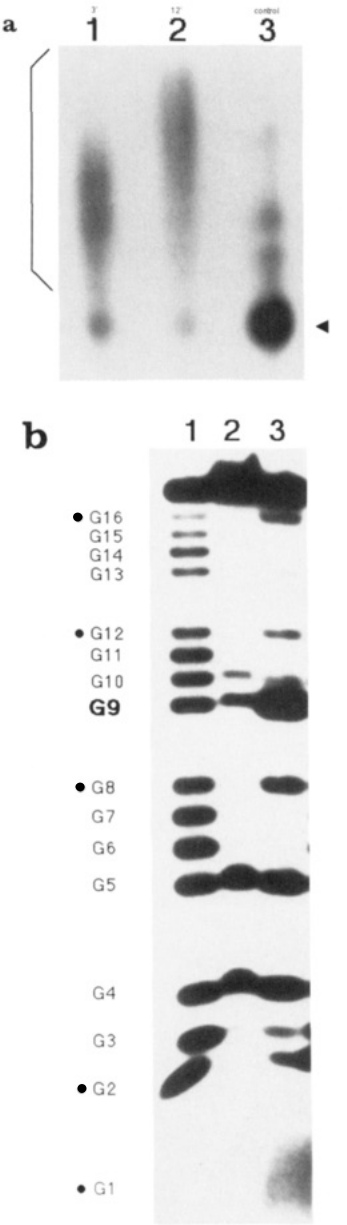

FIGURE 2: (a) After methylation of d(TTGGGG)$_4$ at 55 °C, above the T_m of the G-strand structure, molecules still able to form the structure were isolated by nondenaturing gel electrophoresis at 4 °C. Lanes 1 and 2 contain molecules methylated for 3 and 12 min, respectively. Lane 3 is the unmethylated control. Lanes 1 and 2 contain the same amount of radiolabeled d(TTGGGG)$_4$, but lane 3 does not. The position of the G-strand structure (the fast-migrating species) is indicated with an arrowhead. Bracketed area is material unable to form the structure after methylation. (b) Molecules able to form the G-strand structure were eluted from the nondenaturing gel, cleaved with piperidine, and analyzed electrophoretically. Lane 1 contains the "complete" methylated sample (not fractionated by nondenaturing gel electrophoresis). Lane 2 is the methylated material isolated from the G-strand structure region of the gel in part a. Lane 3 is a methylation protection pattern diagnostic for the G-strand structure (as in Figure 1) for comparison to the methylation interference pattern in lane 2. In the absence of piperidine no cleavage above background levels for the methylated DNA was observed (not shown).

of dIs at the 3′ end (oligonucleotides 1, 3, and 7) causes a greater decrease in stability of the structure, resulting in a reduction in migration rate, than the presence of dIs at the 5′ end. Moreover, comparison of dI-substituted oligonucleotides 8 and 9 showed that dI in positions 4, 8, 12, and 16 (oligonucleotide 9) allows condensed structure formation while a shift of these four dIs to positions 3, 7, 11, and 15

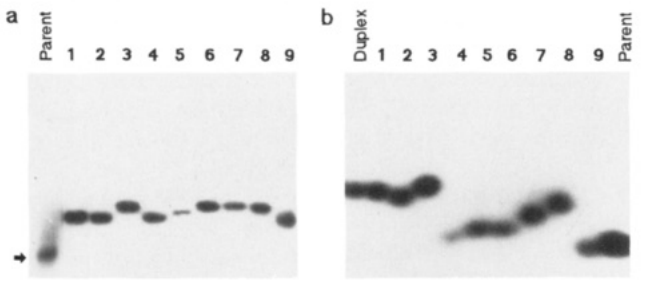

FIGURE 3: (a) Nondenaturing gel electrophoresis of dI-substituted molecules under low-salt conditions. None of the dI-containing molecules form fast-migrating species analogous to that formed by the parent molecule under these conditions. The lane numbers correspond to the oligonucleotide numbers in Chart I. The lane containing the parent molecule was part of the same gel as the rest of the samples but was overexposed for better visualization of the G-strand structure band. (b) Nondenaturing gel electrophoresis of dI-substituted molecules in the presence of 50 mM NaCl. Under these conditions several of the dI-containing molecules (4–7 and 9) can form faster migrating condensed structures probably analogous to that formed by the parent molecule. The lane numbers correspond to the numbered oligonucleotides in Chart I. Duplex = G-strand/C-strand 24-mer duplex marker.

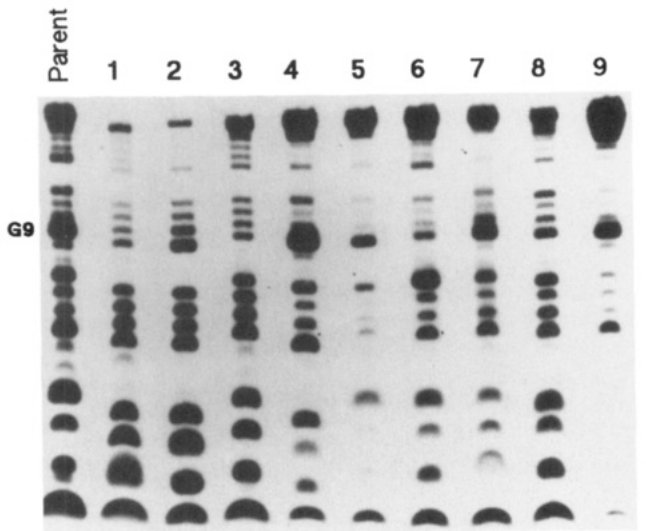

FIGURE 4: Chemical protection of dI-substituted molecules. Lane numbers correspond to the numbered oligonucleotides in Chart I. The dI-substituted molecules that form condensed structures under nondenaturing electrophoretic conditions (4–7 and 9) have a methylation protection pattern at low temperature (4 °C) similar to that of the parent molecule, especially at the 3′ end. Several molecules have protected 5′ ends, unlike the parent molecule. Oligonucleotide 6 shows a shift in the position of the hypersensitive, putative loop, guanine from G9 to G8.

(oligonucleotide 8) completely precludes it. The ability to detect these ion effects illustrates the sensitivity of the nondenaturing gel assay.

DMS Modification of dI-Substituted Oligonucleotides. To analyze the effects of the dI substitutions on the G-strand structure at nucleotide resolution, a DMS protection assay was performed on the dI-substituted molecules. In this experiment we assumed that the reaction rates and cleavage efficiencies were similar for dG and dI. The dI-substituted molecules showed various protection patterns at 4 °C (Figure 4). Cleavage patterns for oligonucleotides 4, 5, 7, and 9 were very similar to that seen with the parent molecule. Oligonucleotide 8 showed a weak pattern of protection at the 3′ end. Oligonucleotide 6, containing dIs in positions 9–12, showed a shift in the position of the putative loop purine from I(G)9 to G8. The most heavily dI-substituted oligonucleotides, 1, 2, and 3, showed cleavage biased toward the 5′ end of the molecule but

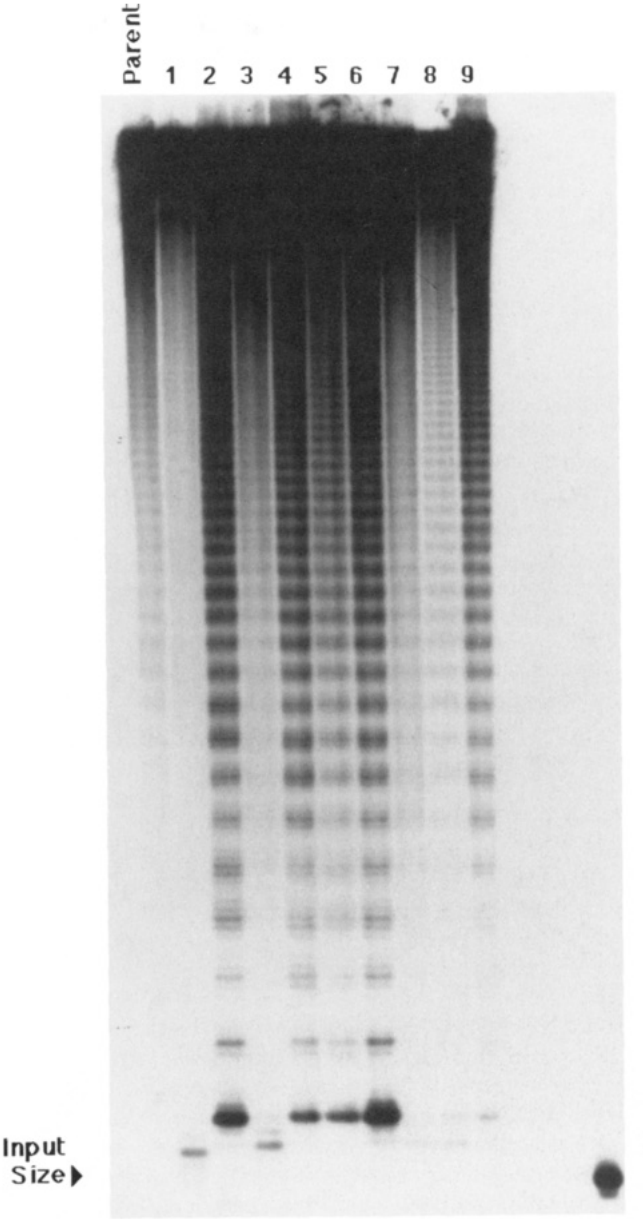

FIGURE 5: Addition of telomere repeats to dI-substituted oligonucleotides shown in Chart I. *Tetrahymena* telomerase added telomeric repeats to all primers regardless of their ability to form the G-strand structure. Those molecules lacking dIs at the 3′ end (2 and 4–6) incorporated more radiolabel into the addition products, suggesting that 3′ dGs may enhance telomerase activity. Lane numbers correspond to oligonucleotide numbers in Chart I.

lacking the pattern diagnostic for the G-strand structure.

G-Strand Structure Is Not Required for Telomerase-Mediated Telomere Repeat Addition in Vitro. We tested the ability of telomerase to add telomeric repeats to the dI-substituted oligonucleotides in vitro to explore the possibility that the G-strand structure was required for telomerase activity. As shown in Figure 5, all of the dI-substituted oligonucleotides were elongated by telomerase. Although the results shown are not quantitative, the efficiency of elongation of the dI-substituted molecules, with the exception of fully dI-substituted oligonucleotide 1, appears to be at least comparable to that of the parent molecule.

Discussion

The structure of the *Tetrahymena* telomeric G-strand sequence $d(TTGGGG)_4$ has been probed by methylation protection, methylation interference, and base analogue (hypo-

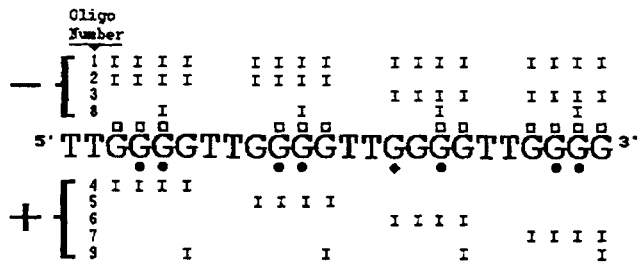

FIGURE 6: Summary of the locations of DMS-resistant and -sensitive dGs in d(TTGGGG)$_4$ and the locations of dIs that do and do not inhibit condensed structure formation. (◆) Methylation-hypersensitive dG; (□) dGs that inhibit G-strand structure formation when methylated at N_7; (●) dGs that are resistant to methylation at N_7 in the G-strand structure; (−) oligonucleotides containing dI substitutions that inhibit condensed structure formation; (+) oligonucleotides containing dI substitutions that allow condensed structure formation.

xanthene) substitution experiments. Figure 6 and the following text summarize our results. dGs 2, 3, 6, 7, 11, 14, and 15 are resistant to methylation at low temperature while dG 9 is hypersensitive. Methylation of dGs 1–3, 6–8, and 11–16 impairs formation of the structure. Replacement of the following dGs with dI impairs structure formation as assayed by nondenaturing gel electrophoresis: dGs 1–16 (oligonucleotide 1); dGs 1–8 (oligonucleotide 2); dGs 9–16 (oligonucleotide 3); dGs 3, 7, 11, and 15 (oligonucleotide 8). Replacement of dGs 4, 8, 12, and 16 (oligonucleotide 9) with dIs does not impair formation of a condensed structure that comigrates with the parental structure in 50 mM NaCl. Condensed structures with migration rates different than that of the parent molecule form when dG is replaced with dI at positions 1–4 (oligonucleotide 4), 5–8 (oligonucleotide 5), 9–12 (oligonucleotide 6), and 13–16 (oligonucleotide 7). Methylation of the dI-substituted molecules reveals that several of them have methylation patterns similar to that of the parent molecule (oligonucleotides 4, 5, 7, and 9) while others show altered patterns (oligonucleotides 6 and 8) or apparent loss of structure altogether (oligonucleotides 1, 2, and 3). All of the dI-substituted molecules function as primers for telomers-mediated telomere repeat addition in vitro. The following suggestions regarding the nature of the G-strand structure are based on these findings: (1) G9 is probably unpaired in a loop; (2) the N_7 group of dGs 1, 2, 3, 6, 7, 8, 11, 12, 13, 14, 15, and 16 is involved in structure formation or stabilization; (3) the N_2 group of dGs 3, 7, 11, and 15 is required for structure formation while the N_2 groups of dGs 4, 8, 12, and 16 are not; (4) formation of a condensed structure is not required for telomerase substrates in vitro.

Methylation Protection and Interference Reveal Two Types of dG N_7 Requirements. The DMS protection and methylation interference experiments provide overlapping and complementary data regarding the disposition of various dGs in the G-strand structure. Methylation protection reveals N_7 groups that are resistant to methylation after the structure has formed [dGs 2, 3 (at 10 °C and below), 6, 7, 11, 14 and 15] while methylation interference reveals N_7 groups that inhibit structure formation if methylated (dGs 1–3, 6–8, and 11–16). dGs that are both resistant to methylation in the G-strand structure and inhibit structure formation when methylated are probably those using the N_7 group for base pairing. Conversely, dGs that inhibit structure formation when methylated but are accessible to methylation once the structure has formed are likely to be arranged such that the N_7 group is important for structure stabilization although not directly involved in a base pair. It is possible that methylation of these N_7 groups causes steric interference or disrupts stabilizing hydrogen bonds to water (Dickerson et al., 1982; Kopka et al., 1983; Hunter et al., 1986, 1987).

dI Substitutions Reveal Essential and Nonessential N_2 Groups for G-Strand Structure Formation. The N_2 group of dG is only necessary for formation of a hydrogen bond on the Watson–Crick face of the nucleotide (N_1, N_2, and O_6). dGs that are paired on the Hoogsteen face should not suffer base-pair instability when the N_2 group is removed (unless this group has a stabilizing function other than hydrogen bonding between bases). While replacements of blocks of dG with dI in d(TTGGGG)$_4$ caused loss of the ability of the oligonucleotide to form condensed structures in some cases, it is not clear precisely which dIs were responsible. The general conclusion from these experiments was that blocks of dIs at the 5′ end were better tolerated than those at the 3′ end in terms of condensed structure formation. Comparison of dI-substituted molecules 8 and 9 is more informative since a single dI residue in each block of four purines either allows condensed structure formation of precludes it completely in a position-dependent fashion. Hence, the N_2 group of dGs in positions 3, 7, 11, and 15 is probably involved in base pairing while the N_2 group of dGs in positons 4, 8, 12, and 16 is not.

G-Strand Structure. A previous report suggested five possible secondary structures for the G-strand structure, four of which were duplex hairpins and one of which was a triplex pseudoknot (Henderson et al., 1987). These studies showed by 2D NMR analyses that at 9 °C there are four to six *syn*-guanines in d(TTGGGG)$_4$ (Henderson et al., 1987). If the G-strand structure is a simple hairpin, then of the four possible G-G base pairs (Hobza & Sandorfy, 1987), the type in this structure may contain one *anti*- and one *syn*-guanine since this is consistent with an antiparallel strand rearrangement (Aboul-ela & Tinoco, 1985). In this instance one would expect that the hydrogen-bonded N_7 group of the *syn*-dGs would be resistant to methylation by DMS. Thus, for a simple hairpin model, the data presented here support the proposal that dGs 2, 3, 6, 7, 11, 14 and 15 are in the syn conformation. The interpretation of the methylation experiments presented above is consistent with this observation if one considers that dGs 2 and 3 only show methylation resistance at the lowest tempertures, and hence, the stability of these putative syn residues may have been insufficient to allow their detection by NMR. Other structural models are also consistent with these data. One alternative explanation for methylation resistance is offered by a model proposed by J. Williamson and T. Cech (personal communication, submitted for publication) based on the well-documented observation that guanine- and deoxyguanine-containing polymers form quartet structures in which each base is paired simultaneously on the Hoogsteen and Watson–Crick faces to its two neighbors [reviewed in Saenger (1984)]. Further studies are necessary to differentiate between a simple hairpin and a quartet model for telomeric G-strand oligonucleotides.

The discovery of an enzyme that specifically adds telomeric repeat sequences to telomeric G-strands and the finding that G-strand oligonucleotides form novel non-Watson–Crick structures have led to speculation regarding the existence of these structures in vivo and their biological role. We show here that dI-substituted oligonucleotides incapable of forming the G-strand structure serve at least as well as the parent molecule as primers for telomerase-mediated telomere repeat addition in vitro. Greider and Blackburn (1989) have shown that *Tetrahymena* telomerase contains an RNA component that includes the sequence 5′...CAACCCCAA...3′. These authors have demonstrated that this sequence must be intact

for telomerase function and have suggested that it serves as an internal template for G-strand sequence addition. Furthermore, they have shown that some telomerase RNA antisense deoxyoligonucleotides, unrelated to telomere sequences, serve as substrates for telomerase in vitro if they hybridize to a region of the telomerase RNA adjacent to the internal template. If there is a structural element of the G-strand overhang that is recognized by telomerase in vivo, it is not reflected in the in vitro assay since all of the primers used here functioned as substrates for telomerase-mediated telomere repeat addition. The fundamental requirement appears to be the ability to hybridize to the appropriate region of the telomerase RNA. However, while there is no absolute requirement for a non-Watson–Crick structure, differences in kinetic parameters (K_m and V_{max}) for some of the dI-containing oligonucleotides used in this study (M. Lee and E. Blackburn, personal communication) suggest that utilization of the substrate by telomerase may be somewhat compromised by the inability of the substrate to form a particular structure.

In contrast to the in vitro situation, addition of telomeric repeats is highly attenuated in the cell, implying that the telomerase activity observed in vitro is not representative of the entire telomere length regulation machinery. In fact, proteins that lack telomerase activity but bind to telomeres have been identified in several organisms. Some of these proteins have nontelomeric cellular functions including transcriptional activation (Buchman et al., 1988) and ultrastructural roles (Shoeman et al., 1988). Others appear to bind specifically to the duplex (Gottschling & Cech, 1984; Gottschling & Zakian, 1986; Berman et al., 1986) while another class bind specifically to the G-strand overhang (Gottschling & Zakian, 1986; Price & Cech, 1987; B. Mixan, P. Qiu, and E. Henderson, unpublished results). It may be in the latter context that an unusual G-strand DNA structure is critical, providing the recognition signal for formation of a telomeric protein "cap" that must be modified or displaced to allow telomere length regulation.

The data presented here should provide useful information for the solution of the telomere G-strand structure. From a more global perspective, since dG-rich sequences are found throughout the genome, understanding this structure and its dynamic properties may have ramifications beyond the elucidation of the mechanism of telomere function.

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