A human nuclease specific for G4 DNA

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We have identified a human nuclease that specifically cleaves four-stranded DNA stabilized by G quartets (G4 DNA). This nuclease, GQN1 (G quartet nuclease 1), cuts within the single-stranded region 5′ of the barrel formed by stacked G quartets. GQN1 does not cleave duplex or single-stranded DNA, Holliday junctions, or G4 RNA. Cleavage depends on DNA structure and not on flanking sequence. Activity is elevated in but not restricted to B cells, making GQN1 a strong candidate for function in immunoglobulin heavy chain class switch recombination. Identification of a mammalian nuclease that specifically cleaves G4 DNA provides further support for the notion that DNA structures stabilized by G quartets form *in vivo* and function in regulated recombination and genomic evolution.

The mammalian genome contains domains that are characteristically G-rich, including the immunoglobulin heavy chain (IgH) class switch regions, the rDNA, and the telomeres. These domains all have specialized properties in recombination. The IgH switch (S) regions participate in a process of regulated DNA deletion, during which one or more constant regions are excised to join the expressed variable to a new constant region (1–3). The rDNA exists as several hundred repeats, which must undergo recombination to erase mutations which would otherwise accumulate with each cell division (4). The telomeric repeats [$(TTAGGG)_n$] are normally established and maintained by telomerase, but there also exist alternative pathways for telomere maintenance that depend on recombination (5).

DNA oligonucleotides that are G-rich can interact *in vitro* to form four-stranded structures, called G4 DNA (6, 7). The repeating unit of G4 DNA is a G quartet (Fig. 1*A*), a planar array of four guanines joined by hydrogen bonds, with a monovalent cation at the center (8). Hydrogen bonds between guanines in each quartet and stacking of hydrophobic G quartets on one another contribute to the stability of G4 DNA. Runs of three or more guanines are sufficient to allow G4 DNA formation. G quartets form readily and spontaneously *in vitro*, and G-rich synthetic oligonucleotides typically exist in solution as equilibrium mixtures of single strands and G4 DNA. Although G4 DNA has not been directly observed *in vivo*, eukaryotic cells contain enzymes that specifically recognize and attack G4 DNA, further consistent with the hypothesis that this structure occurs during normal replication, transcription, or recombination. Mammalian proteins that bind with high affinity and specificity to G4 DNA and appear to function in G-rich chromosomal domains include the major nucleolar protein, nucleolin (9, 10), and the candidate telomere binding protein, hnRNP D (9, 11). Strikingly, the eukaryotic RecQ family helicases BLM and Sgs1p preferentially unwind G4 DNA (12, 13). Both BLM (14) and Sgs1p (15) localize to the nucleolus, where the G-rich rDNA is transcribed into rRNA. Deficiency in Sgs1 causes nucleolar fragmentation and deletion of rDNA circles (15, 16), and Sgs1 participates in telomere maintenance in the absence of telomerase (17–19).

The specialized functions in recombination of G-rich genomic regions led us to ask whether mammalian cells contain a nuclease that specifically recognizes structures formed by G-rich DNA. We have discovered and characterized a human endonuclease specific for DNA containing G quartets, GQN1 (G quartet nuclease 1). The biochemical properties and substrate specificity of GQN1 identify it as an endonuclease that cleaves G4 DNA but does not cleave duplex or single-stranded DNA, Holliday junctions, or G4 RNA. GQN1 levels are elevated in B cells, implicating this activity in IgH class switch recombination. More generally, the existence of a mammalian nuclease specific for G4 DNA provides further support for the notion that DNA structures stabilized by G quartets form *in vivo* and contribute to recombination in somatic cells and genomic evolution.

Materials and Methods

Nuclear Extract Preparation. Cells (10^{10}) were collected by centrifugation, washed in PBS, and resuspended for 10 min in lysis buffer (10 mM Hepes·NaOH, pH 7.9/1.5 mM $MgCl_2$/10 mM KCl/0.5 mM DTT, and a protease inhibitor mixture of 0.7 μg/ml pepstatin/1 μg/ml aprotinin/2 μg/ml leupeptin/0.5 mM PMSF). These and subsequent steps and reagents were at 4°C. Cells were spun at 1,000 × *g* for 10 min, resuspended in 30 ml of lysis buffer, homogenized with 10 strokes of a Dounce homogenizer, then centrifuged at 500 × *g* for 10 min. The supernatant was discarded, and pellets centrifuged at 20,000 × *g* for 20 min and then resuspended in 20 ml of extraction buffer (25 mM Hepes·NaOH, pH 7.9/25% glycerol/1.5 mM $MgCl_2$/0.2 mM EDTA/1 mM DTT/0.42 M KCl/protease inhibitors). Pellets were loosened by using a Dounce homogenizer, nutated in extraction buffer for 40 min, then centrifuged at 90,000 × *g* for 60 min. The resulting supernatant was passed through a 10-ml DEAE-Sepharose FF column equilibrated with 50 mM Tris·HCl, pH 7.4/0.2 mM EDTA/1 mM DTT/500 mM KCl. The flow through was dialyzed against 1 liter of buffer containing 20 mM Tris·HCl, pH 7.5/1 mM EDTA/20% glycerol/0.5 mM DTT/0.5 mM PMSF/50 mM KCl/0.02% Nonidet P-40 for 5 h, and clarified by centrifugation at 20,000 × *g* for 30 min, and the supernatant was either flash frozen and stored at −70°C or further purified immediately.

Protein Purification. Nuclear extract from 10^{10} cells was fractionated on a 10-ml heparin-Sepharose column (Amersham Pharmacia) equilibrated with TEDGK buffer (20 mM Tris·HCl, pH 7.5/1 mM EDTA/1 mM DTT/10% glycerol/50 mM KCl) plus 0.04% Nonidet P-40 with a linear gradient of 0.05–1.0 M KCl. The flow-through fractions were directly loaded onto a 10-ml Q-Sepharose column equilibrated with TEDGK, active fractions were precipitated by addition of solid $(NH_4)_2SO_4$ to a final concentration of 1.2 M, stirred at 4°C for 1 h, and centrifuged at 8,000 × *g* for 20 min. The supernatant was fractionated on a 1-ml phenyl-Superose column equilibrated with 25 mM Tris·HCl, pH 7.5/1 mM EDTA/1 mM DTT/10% glycerol/100 mM KCl/1.2 M $(NH_4)_2SO_4$ and eluted with a linear gradient of $(NH_4)_2SO_4$ (descending from 1.2 to 0 M) and pH (ascending from 7.5 to 8.0).

Abbreviations: IgH, immunoglobulin heavy chain; ssDNA, single-stranded DNA; GQN1, G quartet nuclease 1; S region, Ig switch region; C region, constant region.

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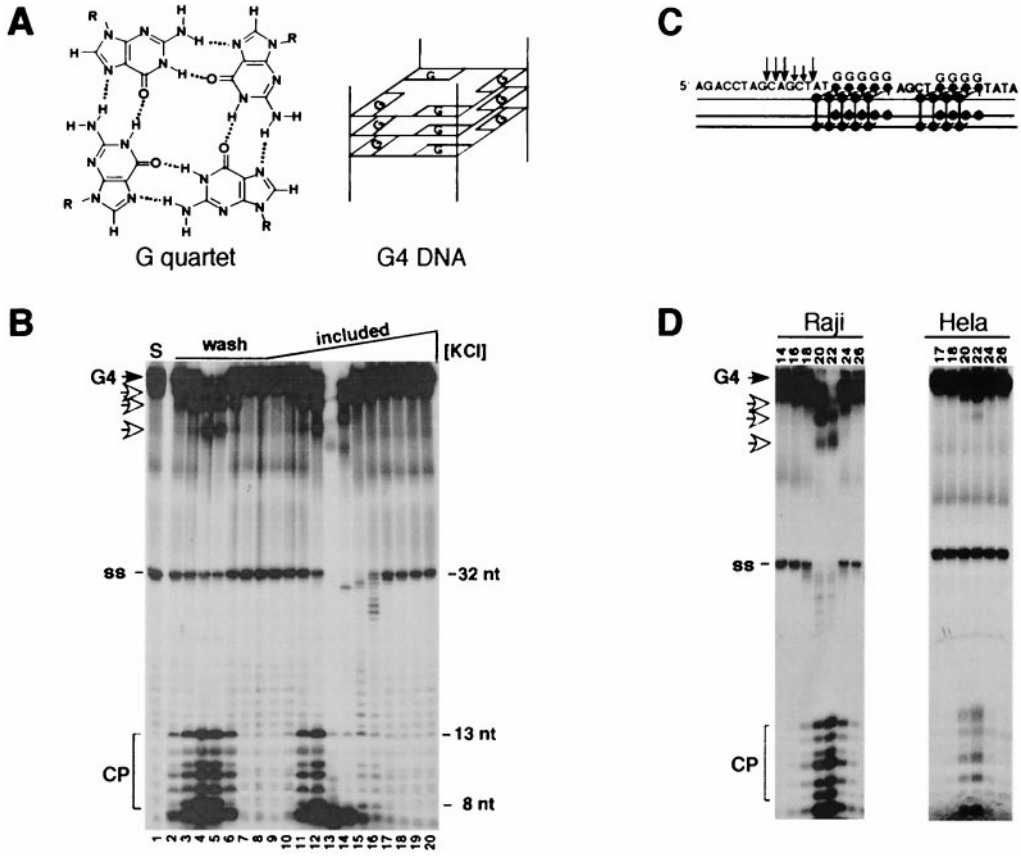

Fig. 1. Identification of G4 DNA endonuclease activity in human cells. (*A*) G quartets and G4 DNA. Four guanines interact in a planar array to form a G quartet (*Left*). G quartets, in turn, stabilize interactions between DNA strands that produce G4 DNA (*Right*). (*B*) G4 DNA cleavage activity after heparin-agarose chromatography of Raji nuclear extract. Assays of representative fractions are shown. The ^{32}P-labeled TP-S G4 DNA substrate (G4; closed arrow) and single-stranded DNA (ssDNA) 32-mer are indicated; essentially all G4 DNA preparations contain a small amount of ssDNA. Structured G4 DNA digestion products (open arrows) migrated slightly faster than the G4 DNA substrate; shorn single-stranded cleavage products (CP) were 8 to 13 nt in length. (*C*) The sequence of TP-S is shown, with cleavage sites marked by arrows. Gs in quartets are represented as ●. (*D*) Phenyl-Superose chromatography of G4 DNA cleavage activities in Raji and HeLa extracts. G4 DNA cleavage assays tested representative fractions purified from each cell type by heparin agarose, Mono-Q, and then phenyl-Superose chromatography. Single-stranded competitor DNA concentrations were 20 μg/ml (Raji) or 2 μg/ml (HeLa).

Active fractions were pooled, dialyzed against 50 vol of MEDGN buffer (25 mM Mes·NaOH, pH 6.5/1 mM EDTA/1 mM DTT/10% glycerol/50 mM NaCl) at 4°C for 5 h, then fractionated on a 1-ml Mono-S HR5/5 FPLC column eluted with a linear gradient of 50–800 mM NaCl in MEDGN. Active fractions were pooled, diluted 6-fold with buffer TEDGK (pH 8.8), and loaded onto a 1-ml DEAE-Sepharose column, and the flow-through was loaded directly onto a Mono-Q HR 5/5 FPLC column, which was washed and then eluted with a linear gradient of 50–600 mM KCl in TEDGK (pH 8.8). Fractions containing nuclease activity were collected and stored at −70°C. At this step, GQN1 was purified at least 6,000-fold.

Preparation and Labeling of DNA and RNA Substrates. Substrates were derived from the following synthetic oligonucleotides: TP (49-mer), TGGACCAGACCTAGCAGCTATGGGGGAG-CTGGGGAAGGTGGGAATGTGA; M (47-mer), TAGTCCAGGCTGAGCAGGTACGGGGGAGCTGGGGTAGA-TGGGAATGT; TP-S (32 mer): AGACCTAGCAGCTATGGGGGAGCTGGGGTAGA;rTP-S (32-mer):AGACCU-AGCAGCUAUGGGGGAGCUGGGGUAGA; OX-1 (32-mer), ACTGTCGTACTTGATATTTTGGGGTTTTGGGG; cTP (49-mer), TCACATTCCCACCTTCCCCAGCTCCCCCATAGCTGCTAGGTCTGGTCCA; cTP-1 (21-mer), ATAGCTGCTAGGTCTGGTCCA; cTP-2 (19-mer), AGCTGCTAGGTCTGGTCCA; cTP-3 (16-mer), TGCTAGGTCTGGTCCA; M-13 (33 mer): AAACGACGGCCAGTGCCAAGCTTGCATGCCTGC; J1 (49-mer), GACGCTGCCGAATTCTGGCGTTAGGAGATACCGATAAGCTTCGGCTTAA; J2 (49-mer), CTTAAGCCGAAGCTTATCGGTATCTTGCTTACGACGCTAGCAAGTGATC; J3 (49-mer), TGATCACTTGCTAGCGTCGTAAGCAGCTCGTGCTGTCTAGAGACATCGA; J4 (49-mer), ATCGATGTCTCTAGACAGCACGAGCCCTAACGCCAGAATTCGGCAGCGT.

Oligonucleotides TP and M bear sequences from the murine IgH Sγ2b and Sμ switch region consensus repeats. TP-S is a truncated version of TP; and rTP-S is a synthetic RNA oligonucleotide corresponding in sequence to TP-S. OX-1 carries the *Oxytricha* telomeric repeat sequence (TTTTGGGG) and 16 nt of random sequence to prevent structuring at the 5′ end. Oligonucleotides cTP1-cTP4 are complementary to TP.

Folded or duplex substrates were generated, gel purified, and 5′-end-labeled with [γ-^{32}P]ATP and T4-polynucleotide kinase, as previously described (12); or 3′ end-labeled by incubating 6 pmol of G4 DNA in a reaction mixture containing 200 mM potassium cacodylate, 25 mM Tris·HCl (pH 6.6), 0.25 mg/ml BSA, 2.5 mM $CoCl_2$, 50 μCi of [α-^{32}P]ddATP (1 Ci = 37 GBq), and 25 units of terminal transferase, at 37°C for 60 min. G4 RNA was formed from oligonucleotide rTP-S under conditions identical to those used for formation of G4 DNA. G4 DNA/duplex chimeras were prepared by annealing TP-G4 DNA to oligonucleotides cTP-1, cTP-2, or cTP-3, which are complementary to

the 5′ end. Holliday junctions were prepared by annealing oligonucleotides J1, J2, J3, and J4 (20); J2 was 5′-end-labeled before Holliday junction formation to facilitate gel purification. Structures of G4 DNA and G2′ DNA were confirmed by footprinting with dimethyl sulfate (12, 13, 21), and guanine residues involved in G-quartet formation are underlined in the oligonucleotide sequences shown above.

Nuclease Assays. Nuclease activity was assayed in 20-μl reaction mixtures containing 25 mM Hepes·NaOH, pH 7.5/5 mM $MgCl_2$/7.5% glycerol/0.25 mM DTT/50 mM KCl/0.1 mg/ml BSA/20 μg/ml denatured *Escherichia coli* DNA/5 fmol of end-labeled G4 DNA. The amount of *E. coli* DNA carrier was reduced to 1 μg/ml at the final steps of purification. Reactions were incubated at 37°C for 30 min, terminated by addition of proteinase K and SDS to final concentrations of 1 mg/ml and 1%, respectively, and incubated at 55°C for 15 min to ensure complete proteolysis. Samples were then extracted once with 1:1 phenol/chloroform, once with 1-butanol, dried under vacuum, resuspended in 95% formamide containing 0.01% bromophenol blue and xylene cyanol, and analyzed on 15% polyacrylamide gels (19:1 acrylamide/bisacrylamide) containing 7.0 M urea and 1× TBE (9 mM Tris-borate/2.5 mM EDTA, pH 8.3). G4 DNA structures were stable under these conditions of loading and electrophoresis.

Results

Identification of G4 Nuclease Activity in Human Cell Lines. Initial attempts to identify G4 DNA nuclease activity in unfractionated nuclear extracts were not successful. We therefore fractionated nuclear extracts on heparin-Sepharose and assayed the fractions for cleavage of G4 DNA. In extracts of Raji, a human Epstein–Barr virus-transformed B cell line, activity that cleaved G4 DNA was identified in both the flow-through and included fractions (Fig. 1*B*). This activity cleaved TP-G4 to produce single-stranded cleavage products 8–13 nt in length, corresponding to cleavage in a zone 2–7 nt 5′ of the G quartets (Fig. 1*C*). The size of the G4 DNA substrate diminished on cleavage, as evidenced by its increased gel mobility (Fig. 1*B*, wash, lanes 3–6; KCl eluate, lanes 11–12) in fractions that contained nuclease activity. In these lanes, G4 DNA appears as four distinct labeled bands, corresponding to starting substrate (filled arrow) and digestion products that retain the 5′ end-label in 1, 2, or 3 DNA strands (open arrows). Activity was more abundant in the flow-through, and was well resolved from contaminating nucleases that overlapped the activity peak in the included fractions, destroying the small amount of single-stranded DNA typically present in G4 DNA preparations (e.g., Fig. 1*B*, lanes 13 and 14). Flow-through fractions were therefore pooled and further purified by successive steps of chromatography on phenyl-Superose, Mono-S, DEAE-Sepharose, and Mono-Q. This effected a 6,000-fold purification of nuclease activity. We refer to this nuclease as GQN1.

G4 DNA Cleavage Activity Is Not Cell-Type-Specific. To ask whether G4 DNA cleavage activity is restricted to B cells, nuclear extracts from the human cervical carcinoma, HeLa, were fractionated and assayed. Comparison of phenyl-Superose column profiles of cleavage activities from Raji and HeLa (Fig. 1*D*) showed that HeLa cells contain an activity that chromatographed like GQN1 from B cells, and that cleaved G4 DNA to produce essentially identical products. Activity was on the order of 20-fold more abundant in Raji than in HeLa extracts, and further experiments focused on the enzyme from B cells.

Properties of GQN1. Purified GQN1-cleaved G4 DNA increased with time to plateau after 30 min at 37°C (Fig. 2*A*). The optimal temperature for cleavage was 37°C, and activity was significantly reduced at lower temperatures (data not shown). G quartets are stabilized by the presence of a monovalent cation at the center of the quartet, and K^+ confers particular stability (reviewed by ref. 22). GQN1 activity was unaltered at K^+ concentrations from 35 to 190 mM (Fig. 2*B*), well below and above physiological concentrations (140 mM). GQN1 was active in 2–5 mM Mg^{2+}, but replacement of Mg^{2+} with 2 mM Mn^{2+} resulted in a significant reduction of activity, and 5 mM Mn^{2+} abolished cleavage; 2–5 mM Ca^{2+} supported only a very low level of cleavage (Fig. 2*C*). Ionic requirements distinguish GQN1 from FEN1, another structure-specific nuclease in mammalian cells. FEN1 is a multifunctional nuclease that cleaves three-way junctions and is involved in repair and processing of Okazaki fragments. Unlike GQN1, FEN1 is inhibited by relatively low concentrations (50 mM) of K^+ and is stimulated by Mn^{2+} concentrations as high as 10 mM (23). Cleavage activity was neither enhanced nor diminished in the presence of ribonucleoside triphosphates or deoxynucleoside triphosphates at 2–5 mM (data not shown), which distinguishes GQN1 cleavage activity from the G4 helicases, Sgs1p and BLM, both of which require ATP for activity (12, 13).

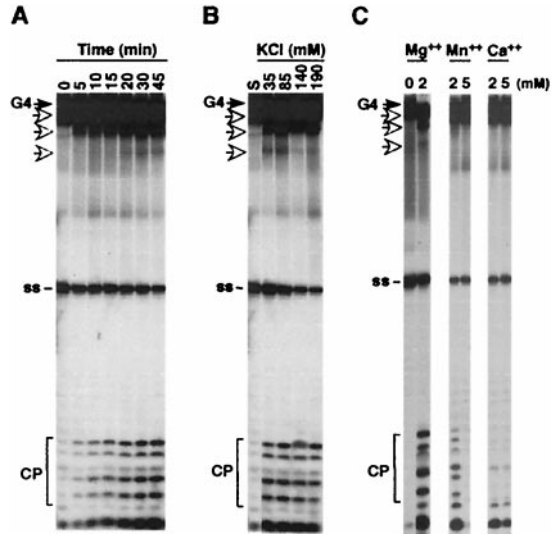

Fig. 2. Kinetics and salt sensitivity of GQN1. (*A*) Time course of cleavage by highly purified GQN1. (*B*) Assays of cleavage in reaction mixtures containing 35–190 mM K^+. S, DNA substrate with no added enzyme. (*C*) Assays of cleavage in reaction mixtures containing Mg^{2+}, Mn^{2+}, or Ca^{2+}. Symbols as in Fig. 1.

GQN1 Specifically Cleaves DNA Containing G Quartets. GQN1 did not cleave the single-stranded M13 oligonucleotide (Fig. 3*A Left*), which contains no G runs and does not become structured in solution; nor did it cleave single-stranded TP oligonucleotide (Fig. 3*A Center*), although it was very active on G4 DNA generated from TP (Fig. 3*A Right*). Cleavage of single-stranded DNA (ssDNA) was assayed in the absence of ssDNA carrier, which probably explains the small amount of phosphatase activity observed in reactions with single-stranded substrates.

GQN1 cleavage of duplex DNAs was assayed on standard duplexes and chimeric substrates, generated by annealing TP G4 DNA to complementary oligonucleotides 16, 19, or 21 nt in length. None of the chimeras was cleaved, either on the G-rich strand (Fig. 3*B*, lanes a, b, and c, respectively) or the complementary strand (not shown). Cleavage of duplex DNA was assayed in reactions containing substrate that had been 5′-labeled on both strands, then analyzing denatured cleavage products to detect either nicks or double-strand breaks. The denatured duplexes ran as intact single strands (Fig. 3*B*, lane ds).

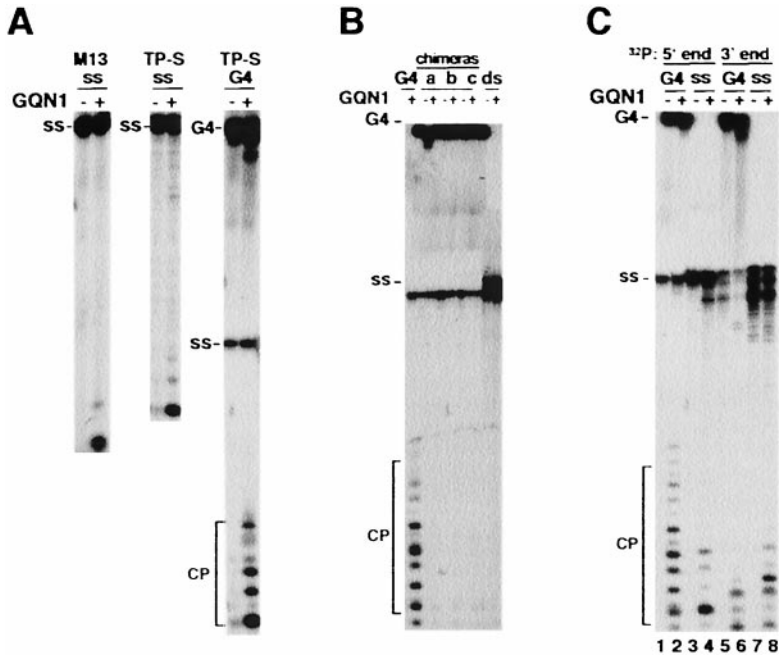

Fig. 3. Substrate specificity of GQN1. (*A*) GQN1 does not cleave ssDNA. Assays of GQN1 cleavage of ^{32}P-lableled ssDNA oligonucleotides M13 and TP-S, and TP-S G4 DNA. (*B*) GQN1 does not cleave duplex/G4 DNA chimeras or duplex DNA. Assays of GQN1 cleavage of ^{32}P-labeled G4 DNA generated from the TP 49-mer oligonucleotide (G4); TP-G4 DNA/partial duplex chimeras carrying 21 (a), 19 (b), or 16 (c) nt annealed at the 5′ end; or a TP duplex substrate (ds). (*C*) GQN1 cleaves 5′ but not 3′ of the G quartets. Assays of GQN1 cleavage of G4 DNA and ssDNA generated from the TP oligonucleotide and ^{32}P-labeled at the 5′ or 3′ end. Symbols as in Fig. 1.

We conclude that GQN1 is inactive on the duplex DNA substrate.

GQN1 Cleaves 5′ and Not 3′ of the G Quartets. Incubation of 3′-labeled G4 DNA with GQN1 truncated the G4 DNA, as evidenced by increased gel mobility (Fig. 3*C*, lane 6), but generated no single-stranded products that were not also produced on incubation of GQN1 with ssDNA (Fig. 3*C*, compare lanes 6 and 8). Similar results were obtained with other 3′-labeled oligonucleotide substrates (not shown). A small amount of exonuclease activity is present in the purified enzyme preparation, as ssDNA is somewhat degraded during the course of the reactions (lanes 4 and 8). This may be a contaminant or an associated activity, as we observed exonuclease activity in highly purified preparations of GQN1 produced by several different purification schemes.

GQN1 Cleaves G4 DNA but Not RNA. G4 RNA substrates were generated from the synthetic RNA oligonucleotide rTP-S, which bears the RNA sequence corresponding to that of the DNA oligonucleotide TP-S, incubated with GQN1, then denatured and visualized by gel electrophoresis. The G4 RNA was not cleaved by GQN1 (Fig. 4*A*, compare lanes 2 and 4). The absence of G4 RNA cleavage was not due to inhibition or inactivation of GQN1 by the G4 RNA substrate, because G4 DNA was cleaved in assays containing both G4 RNA and G4 DNA substrates (Fig. 4*A*, lanes 5 and 6). GQN1 is therefore an endonuclease specific for G4 DNA and inactive on G4 RNA.

GQN1 Does Not Cleave Holliday Junctions. The Holliday junction represents an intermediate in homologous recombination and is composed of four base-paired stems surrounding a central unpaired region. GQN1 did not cleave a Holliday junction substrate in standard reaction conditions (Fig. 4*B*, lane 2), and its inactivity was unaffected by addition of NTPs or dNTPs (Fig. 4*B*, lanes 3–9); nor did GQN1 resolve Holliday junctions, as single-stranded products were not evident on denaturing gel electrophoresis (data not shown). GQN1 is therefore distinct from the structure-specific mammalian activity that resolves Holliday junctions (20, 31).

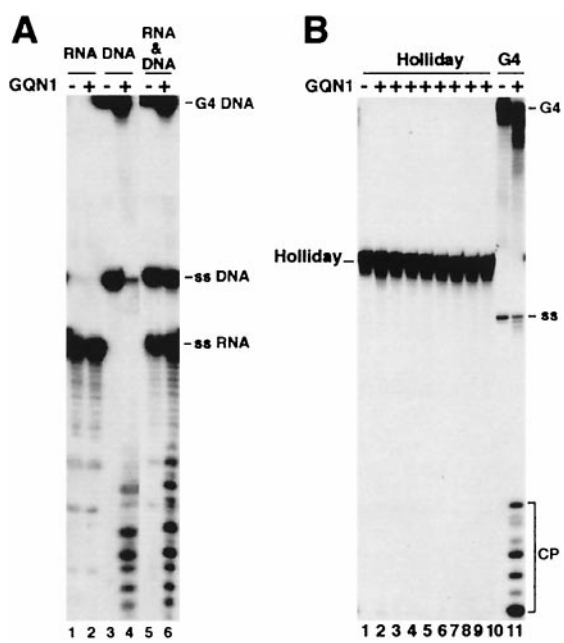

Fig. 4. GQN1 does not cleave G4 RNA or Holliday junctions. (*A*) Comparison of cleavage of ^{32}P-labeled G4 RNA and G4 DNA substrates. G4 RNA was generated from rTP-S, and G4 DNA was generated from TP. Reaction mixtures contained G4 RNA (lanes 1 and 2), G4 DNA (lanes 3 and 4), and both G4 RNA and G4 DNA (lanes 5 and 6). (*B*) GQN1 does not cleave Holliday junctions. Cleavage of ^{32}P-labeled Holliday junctions was assayed in the absence of nucleotides (lanes 1, 2, 10, and 11) and in the presence of 2 mM ATP, CTP, GTP, UTP, dATP, dCTP, or dGTP, respectively (lanes 3 to 9). Cleavage of G4 DNA was assayed as a control (lanes 10 and 11).

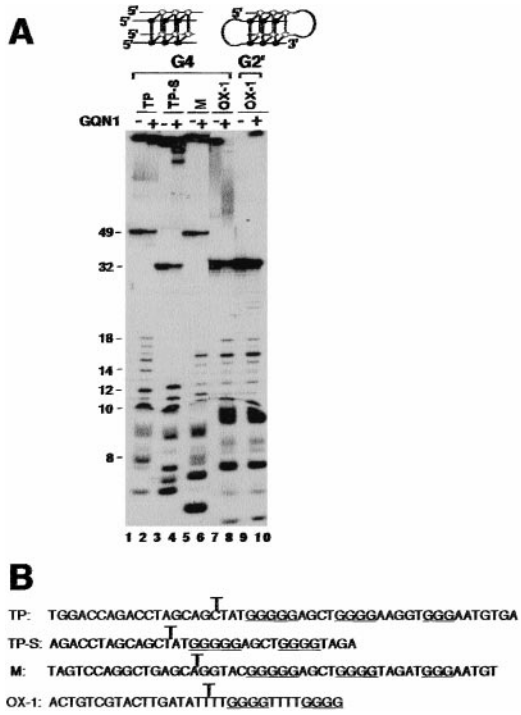

Fig. 5. GQN1 cleavage is independent of DNA sequence. (*A*) Cleavage assays using as substrates ^{32}P-labeled four-stranded parallel G4 DNA formed from oligonucleotides TP, TP-S, M, and OX-1; and a bimolecular and antiparallel ''G2′ DNA'' substrate formed from OX-1 (lanes 9 and 10). The top of the figure shows schematic representations of structures of four-stranded and two-stranded substrates. Symbols as in Fig. 1. (*B*) Cleavage sites in oligonucleotides assayed in *A*.

GQN1 Cleavage Depends on DNA Structure, Not Sequence. GQN1 cleaved G4 DNAs derived from the IgH Sγ2b (TP and TP-S) and Sμ (M) switch region consensus sequences and the *Oxytricha* telomeric repeat (Fig. 5*A*, lanes 1–8). Mapping of cleavage sites showed that, with each substrate tested, cleavage occurred in the single-stranded region 2–5 nt 5′ of the barrel formed by the stacked G quartets, regardless of sequence (Fig. 5*B*). Cleavage appeared to be independent of sequence, and cleavage products were heterogeneous in size. This heterogeneity may be due to the presence of an exonuclease activity (either a contaminant or intrinsic to GQN1) that attacks the initial cleavage product, or it may reflect relatively unconstrained choice of cleavage site.

G quartets can stabilize several distinct DNA structures. One, sometimes called G2′ DNA, consists of two antiparallel strands, forms readily and is stable in physiological conditions (13, 32, 33). GQN1 cleaved G2′ DNA formed from the OX-1 oligonucleotide to produce products identical to those generated on cleavage of OX-1 G4 DNA (Fig. 5*A*, compare lanes 10 and 8, respectively).

Discussion

We have identified a human endonuclease that specifically cleaves four-stranded DNA structures stabilized by G quartets (G4 DNA). We refer to this activity as GQN1 (G quartet nuclease 1). The properties of GQN1 identify it as a novel mammalian structure-specific deoxynuclease. GQN1 cleaves 5′ of a block of G quartets, generating single-stranded 5′ fragments and a shorn product that retains its structure despite the truncation. GQN1 cleaves DNA containing G quartets regardless of whether the substrate is four-stranded or two-stranded, and does not cleave ssDNA, duplex DNA, G4 RNA, or Holliday junctions. Cleavage requires a single-stranded region, and does not occur if the DNA within the cleavage zone is duplex rather than single-stranded. GQN1 is active at physiological K^+ concentrations, which is important because the monovalent cation K^+ fits snugly within a quadruplex structure and is readily coordinated by G quartets to stabilize G4 DNA structure (reviewed by ref. 22).

Liu, Gilbert, and coworkers (24, 25) characterized a nuclease in *Saccharomyces cerevisiae* that cleaves G4 DNA. This nuclease, Kem1p, is encoded by a gene identified in five unrelated screens. Under the guise of Xrn1p, it functions a cytoplasmic exonuclease active on RNA substrates (refs. 27 and 28; reviewed by ref. 29). The murine homolog of Kem1p, mXrn1, is a cytoplasmic nuclease which cleaves RNA but not DNA (30), and is therefore biochemically distinct from mammalian GQN1, that cleaves G4 DNA but not RNA (Fig. 5*A*). Most enzymes of DNA metabolism found in yeast have functional homologs in mammalian cells. The inability of the mammalian Kem1p homolog to cleave G4 DNA raised the question of whether mammalian cells contain a G4 DNA nuclease. The identification of G4 DNA cleavage activity in mammalian cells now fills this gap in the mammalian enzymatic repertoire.

Possible Functions of GQN1 *in Vivo*. One likely function for GQN1 is in IgH class-switch recombination. Switch recombination is a regulated process of DNA deletion that results in joining of a new constant (C) region to the expressed heavy chain variable region. In mammalian cells, G-rich S regions 2–10 kb in length are located upstream of each C region that participates in switch recombination. Cδ is the only C region that lacks an associated S region, and expression of Cδ is regulated by RNA processing, not DNA recombination. Switch recombination is region-specific, not sequence-specific, and the junctions produced are heterogeneous in position and sequence at the endpoints (34). Transcription of both S regions targeted for recombination is prerequisite for switch recombination (reviewed by ref. 1), and the transient DNA denaturation that occurs during transcription could allow G quartets to form. Structure-specific cleavage of DNA by GQN1 could produce the heterogeneous endpoints characteristic of switch recombination junctions.

We previously proposed that structures formed by the G-rich S regions are the targets for enzymes that carry out class switch recombination (2, 9). Several different observations led to this hypothesis: (*i*) The S regions contain tandem repeats of G-rich sequences, which readily form G4 DNA *in vitro*. (*ii*) The transcription of the switch regions that must precede recombination could promote G-quartet formation by denaturing S regions in switching B cells *in vivo*. (*iii*) BLM helicase preferentially unwinds G4 DNA substrates (12), and this helicase is missing in Bloom's syndrome, a genetic disease associated with immunodeficiency because of impaired switch recombination (35–37). (*iv*) The factor LR1, which is induced in switching B cells and binds tightly to duplex S region sequences, is composed of two polypeptides with very high affinity for DNA containing G quartets, nucleolin and hnRNP D (9, 10, 38–40). Identification of a nuclease specific for G4 DNA provides further biochemical support for a pathway of switch recombination that depends on formation of unusual DNA structures.

GQN1 cleaves DNA containing G quartets to produce a single-strand lesion 5′ of the barrel formed by the G quartets. Additional steps in the recombination pathway might involve cleavage of the C-rich strand. A stable RNA/DNA hybrid forms during S-region transcription *in vitro* (42, 43), and if a hybrid similarly forms *in vivo*, it may be a target for other structure-specific nucleases. Both XPF-ERCC1 and XPG can cleave a DNA strand at its junction with an RNA/DNA hybrid (41), and either of these nucleases could be a candidate for cutting the C-rich strand. Alternatively, transfer of the single-strand end produced by GQN1 cleavage to the region that will serve as the

recombination acceptor could create a structure that is targeted by other activities.

The genomic structure of the mammalian IgH loci argues for the importance of G-rich S regions in mammalian switch recombination, but this genomic structure does not appear to be evolutionarily conserved. In *Xenopus*, the DNA regions that support a recombination event analogous to mammalian switch recombination are not G-rich, but A + T-rich (44). This observation suggests an interesting parallel between switch recombination in mammals and frogs: A + T-rich DNA, like G-rich DNA, is able to form unusual and characteristic structures that may function as targets of recombination enzymes.

Ubiquitous Functions of GQN1. GQN1 levels are elevated in Raji, an Epstein–Barr virus-transformed B cell line that supports recombination of extrachromosomal switch substrates (45). An apparently identical activity is present, albeit at much lower levels, in the cervical adenocarcinoma line HeLa. Analysis of additional cell lines is necessary to determine whether GQN1 activity is ubiquitous, and whether activity is consistently highest in B cells that can support switch recombination. If GQN1 proves to be present in most mammalian cell types, that would support a generalized role in recombination of G-rich genomic regions, including the rDNA and the telomeres.

G4 DNA *in Vivo*. The readiness with which G quartets and structures containing G quartets form *in vitro* (6, 8) suggests that these structures also form *in vivo*. This notion is further supported by identification of factors in eukaryotic cells that recognize, generate, or dynamically alter G4 DNA with remarkable specificity. These include proteins that bind G4 DNA; proteins that enhance G4 DNA formation *in vitro*; and, perhaps most compellingly, proteins that dynamically alter G4 DNA structure, including Kem1p (24, 25) and GQN1 nucleases, and Sgs1p (13) and BLM (12) helicases. The most straightforward conclusion from these results is that G4 DNA is produced with sufficient frequency *in vivo* that enzymes have specialized in recognizing, altering, and eliminating it.

One of the surprises emerging from sequence analysis of the human genome has been that average base composition is nonuniform (46, 47). The lumpiness of the genomic landscape is likely to have functional correlates. The ability of G quartets to stabilize intermolecular interactions independent of DNA homology led to suggestions that G runs may promote DNA/DNA contacts critical to recombination in meiosis (6, 48). As we learn more about sequences of individual human genomes, the contribution of G-rich regions to evolution will become evident.

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