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LETTER

First Evidence of a Functional Interaction between DNA Quadruplexes and Poly(ADP-ribose) Polymerase-1

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ABSTRACT We discovered that the abundant human nuclear protein poly(ADP-ribose) polymerase-1 (hPARP-1) binds to intramolecular DNA quadruplexes *in vitro* with high affinity and with a stoichiometry of two proteins for one quadruplex. Using an enzymatic assay, we have shown that hPARP-1 gets catalytically activated upon binding to G-quadruplexes localized at the c-kit promoter and human telomere regions. This is the first example of a truly functional quadruplex—protein interaction, which has possible implications in understanding hPARP-1 mediated mechanisms of transcription regulation and telomere end protection.

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It has been known for several decades that DNA sequences containing a high density of contiguous guanines grouped as clusters are able to adopt four-stranded secondary structures named guanine (G)-quadruplexes or tetraplexes (*1*). Converging *in silico* and *in vitro* data have recently revealed a high prevalence of such G-rich DNA sequences throughout the human genome (*2–4*). The identification of prokaryotic and eukaryotic proteins that interact with these motifs also reinforces the hypothesis that quadruplexes do form *in vivo* and that their formation is biologically relevant (*5–7*). Although several lines of evidence link G-quadruplexes with DNA recombination (*8*), telomere maintenance, (*9*) and more recently, regulation of gene transcription (*10–12*), there is a noticeable absence of examples for DNA quadruplex-induced modulation of nuclear protein(s) function. When exploring protein—quadruplex interactions, attention has been focused almost exclusively on the effects of the natural DNA binding protein on the quadruplex structure and proteins have been classified according to their ability to stabilize, destabilize, or promote the formation of DNA quadruplexes (*6, 7*). Unnatural Cys_2His_2 zinc finger proteins have also been engineered that bind to quadruplex DNA with high affinity (*13, 14*) and block the biochemical functions of DNA polymerase and telomerase *via* quadruplex stabilization (*15*). However, to our knowledge, there is no example of a quadruplex-induced protein catalytic activation, which would provide an additional proof of quadruplex existence *in vivo* and contribute to our understanding of quadruplex functions in nuclei. Human poly(ADP-ribose) polymerase (hPARP-1), a protein abundant in chromatin of eukaryotic cells, has been regarded as an intracellular sensor of DNA strand breaks (*16*). Upon binding to DNA breaks *via* its two zinc finger-containing DNA-binding domain (DBD), activated hPARP-1 cleaves NAD^+ into nicotinamide and ADP-ribose and polymerases the latter onto nuclear acceptor proteins and hPARP-1 itself. It has also been implicated in the transcription of eukaryotic genes possibly by perturbating chromatin structure after being recruited to non-B DNA structures in gene regulating sequences (*17–19*). Although important, only limited data is available regarding interactions between hPARP-1 and undamaged genomic DNA.

An increasing number of oncogenes have been reported that possess a quadruplex-forming sequence in their promoter (*20*). An attractive hypothesis suggests that formation of these promoter quadruplexes *in vivo* could interfere with the transcription machinery, thus leading to a change of the level

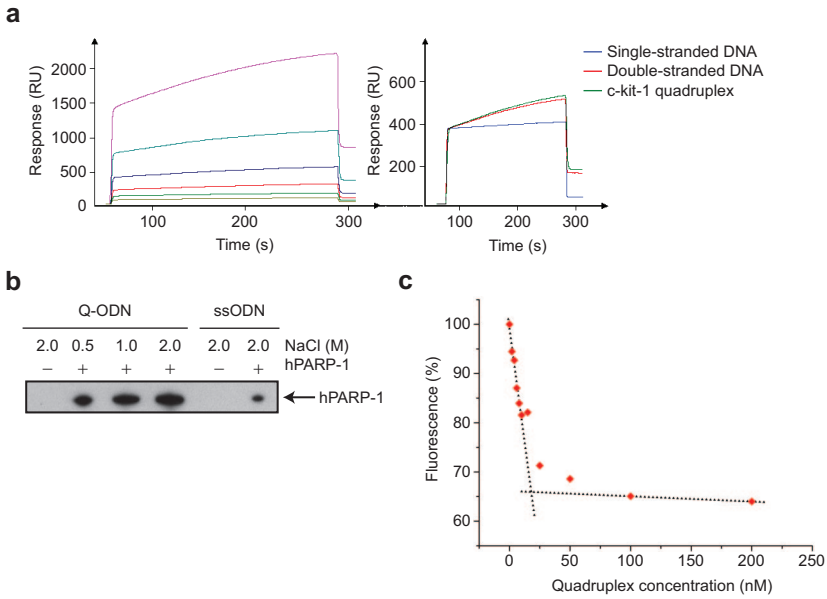

Figure 1. hPARP-1 binds to the c-kit-1 quadruplex *in vitro*. a) Left: sensorgram overlay for hPARP-1 binding to immobilized c-kit quadruplex at six different hPARP-1 concentrations (0.98−31.25 nM bottom to top). Right: sensorgram overlay for hPARP-1 (3.9 nM) binding to single-stranded DNA, double-stranded DNA, and c-kit-1 quadruplex. b) Purified recombinant hPARP-1 was bound to streptavidin-coated magnetic beads coupled with either 5′-biotinylated c-kit-1 quadruplex oligonucleotides (Q-ODN) or control single-stranded quadruplex-free oligonucleotide (ssODN). Bound proteins were eluted with increasing concentrations of NaCl (0.5–2.0 M) and analyzed by Western blotting using monoclonal anti-hPARP-1 antibodies. c) Titration curve for binding of hPARP-1 to the c-kit-1 quadruplex. The hPARP-1 concentration was 40 nM. The linear parts of the binding curve are fitted and extrapolated separately with a linear fitting (····). The intersection at a quadruplex concentration of 20 nM indicates a protein:DNA stoichiometry of 2:1.

of expression of the corresponding gene. Although a significant body of *in vitro* and indirect evidence have recently been gathered that all support this idea of a quadruplex mediated regulatory mechanism (*10*–*12*), the exact role quadruplexes may play remains unknown. Herein, we discovered that the abundant human nuclear protein poly(ADP-ribose) polymerase (hPARP-1) binds to intramolecular DNA quadruplexes *in vitro* with high affinity and gets catalytically activated upon binding to G-quadruplexes. This is the first example of a truly functional quadruplex−protein interaction, which has possible implications in understanding hPARP-1 mediated mechanisms of transcription regulation and telomere end protection.

Our first goal was to demonstrate the interaction between hPARP-1 and an intramolecular promoter DNA quadruplex. Two G-rich sequences have been identified within the nuclease hypersensitive region of the human proto-oncogene c-kit that encodes for a tyrosine kinase receptor. Both sequences are 31 nucleotides apart from each other and were shown to form G-quadruplex structures *in vitro* (*21, 22*). As a model system we have chosen to study in detail the interaction between hPARP-1 and the quadruplex from the c-kit promoter that proved the most stable (c-kit-1) (*21*). However, we also investigated the interaction with other intramolecular quadruplexes, including the second c-kit quadruplex (c-kit-2) (*22*) or the human telomeric quadruplex (Htelo). Surface plasmon resonance (SPR) was first used to simultaneously evaluate the affinity of purified hPARP-1 for biotinylated quadruplex, duplex, and single-stranded DNAs immobilized on a streptavidin-coated sensor chip (Figure 1, panel a) (*14*). The sequence of the single-stranded oligonucleotide control was analogous to that of the c-kit-1 quadruplex but carrying five G⟶T mutations to prevent quadruplex formation. While no detectable binding to single-stranded DNA was observed up to high protein concentrations, responses of comparable amplitude and proportional to protein concentration were observed for both c-kit-1 quadruplex and double-stranded DNA, indicative of a specific interaction with either structural transitions (quadruplex) or structural discontinuities (free ends) in DNA. Corrected sensorgrams were analyzed using a langmuirian global fit model, and hPARP-1 was found to bind to double-stranded DNA break and to the c-kit-1 quadruplex with apparent K_d values of 18 and 65 nM, respectively. We also found that hPARP-1 could bind to the human telomeric quadruplex, although with a significantly weaker affinity than to the c-kit-1 quadruplex (apparent K_d value of 5.8 μM). hPARP-1 specific binding to the DNA quadruplexes was confirmed by a pull-down assay using the same single-stranded and c-kit-1 quadruplex biotinylated oligonucleotides as for the SPR experiments and streptavidin-coated magnetic beads. Data indicate that only folded quadruplex sequences provide a binding site for hPARP-1, thus further supporting the notion that hPARP-1 binding to the c-kit-1 sequence requires DNA structure specific recognition (Figure 1, panel b).

We explored further the nature of the protein−DNA interaction using atomic force microscopy (AFM) and a 200-base-long oli-

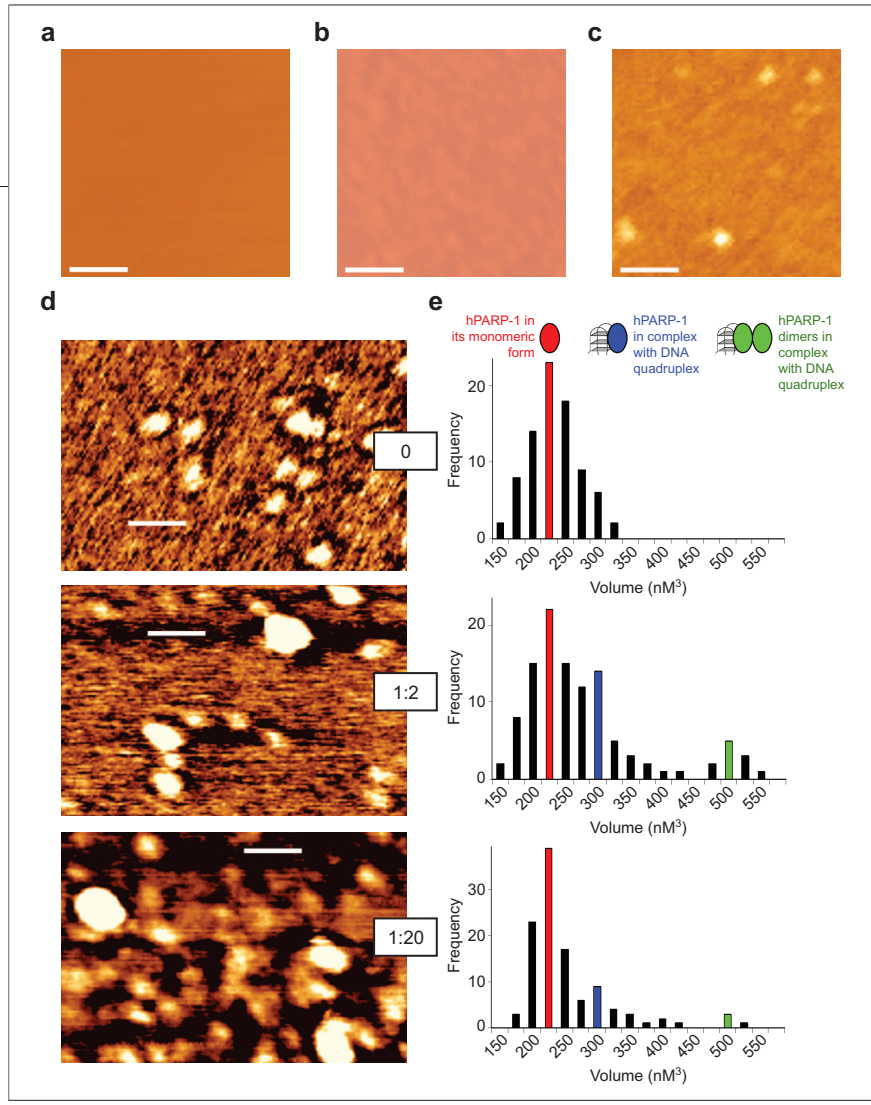

Figure 2. AFM analysis of hPARP-1 binding to DNA quadruplexes. Oligonucleotides (200 bp) carrying c-kit quadruplex-forming sequences and purified human hPARP-1 protein were used in the binding assays. a) Mica before deposition. b) Heat-denatured (unfolded) oligonucleotides in the K^+,Na^+-free buffer. c) Folded DNA quadruplexes. d) Representative AFM images of free hPARP-1 proteins (top panel) and hPARP-1-quadruplex binding reactions (middle and bottom panels). The length of the scale bars is 50 nm. e) Histograms representing the distribution of molecular volume frequencies in samples shown in panel d. The molar ratios of DNA to protein are indicated on the linkers between the AFM image and the corresponding histogram. Pictograms on the top indicate hPARP-1 protein in its monomeric form, hPARP-1 in complex with the DNA quadruplex, and hPARP-1 dimers formed upon binding to DNA.

gonucleotide from the c-kit promoter gene carrying both natural quadruplex-forming sequences (bases 76−94 and 125−146). Quadruplex-free single-stranded oligonucleotides are invisible or indistinguishable from the background under the commonly employed conditions of AFM observation in tapping mode and unmodified tip (*23*). Therefore neither single-stranded nor quadruplex arms appear on corresponding panels. In the absence of DNA or in the presence of unfolded single-stranded DNA, hPARP-1 molecular volume has the typical unimodal distribution (*24*) with the maximum at 200−225 nm^3. When hPARP-1 was incubated in the presence of the quadruplex-containing oligonucleotide, the distribution of frequencies has quasi-trimodal character due to the appearance of 1:1 hPARP-1−quadruplex complexes (maximum at 275−300 nm^3) and hPARP-1 dimerization on DNA leading to the formation of 2:1 complexes with the maximum volume at 475−500 nm^3 (Figure 2). In addition to confirming hPARP-1 affinity for DNA quadruplexes, this AFM experiment also suggests that the protein binds to DNA as a dimer. However, because of the presence of two quadruplex-forming sequences within the fragment of the c-kit promoter, one cannot completely rule out that the 2:1 protein−DNA complexes observed by AFM correspond to the coexistence of both quadruplexes, each bound to one hPARP-1 protein.

To unambiguously clarify the stoichiometry of the complexes observed on a surface, we subsequently employed a spectroscopic method that could allow us to further characterize the nature of individual complexes in solution. hPARP-1 contains multiple tryptophan residues and, in particular, four in its DBD that are highly sensitive to DNA binding (*25, 26*). We observed the formation of the protein−DNA complexes by monitoring the tryptophan fluorescence quenching upon addition of different DNA sequences. Titrations were carried out using both c-kit quadruplexes, the human telomeric quadruplex, the c-myc quadruplex, and a single-stranded mutated DNA fragment that cannot adopt a quadruplex conformation and which was used as a negative control. Although no significant fluorescence changes were observed with single-stranded DNA even at high DNA concentration, a DNA concentration-dependent fluorescence quenching was obtained for all four quadruplexes tested (c-kit-1, c-kit-2, c-myc, and Htelo), which all showed similar profiles. An initial fast fluorescence decrease was observed at low quadruplex concentration until a plateau was slowly reached at higher quadruplex concentration (Figure 1, panel c and Supplementary Figure 1). The titration experiment was repeated with two initial protein concentrations (40 and 60 nM), and the binding stoichiometry was determined by the "tangent" method. In both cases, quenching saturation was obtained with 0.5 equiv of quadruplex, indicating the implication of two proteins for one quadruplex per

complex, which is in agreement with our AFM experiments. hPARP-1 was previously demonstrated to dimerize in solution only at high (submicromolar) protein concentration, suggesting that the observed protein dimerization happens upon binding to quadruplex only. The observed level of fluorescence quenching at saturating quadruplex concentration (40–50%) is consistent with that observed by others when investigating hPARP-1 DBD binding to double-stranded DNA breaks. This result, in addition to our previous studies on engineered zinc fingers binding to quadruplexes, (*13–15*) suggests that hPARP-1 could possibly bind to DNA quadruplexes via its zinc finger DBD. Protein dimerization has been previously demonstrated for hPARP-1 binding to 5′-recessed DNA ends, although considering the DBD of hPARP-1 only (*25, 26*). Our observation that native human hPARP-1 protein dimerizes upon interaction with DNA quadruplexes is in line with a previously reported suggestion on hPARP-1 dimerization derived from an enzyme kinetics study (*27*). This supports the notion that protein dimer formation is required for hPARP-1 catalytic activation and further substantiates the recent reports that hPARP-1 function is not limited to DNA damage repair and also has a prominent role in normal cell physiology.

Cellular proteins have been identified that bind to quadruplexes and exhibit either a stabilizing or destabilizing effect on the DNA structure. To assess the effect of hPARP-1 on quadruplex stability and conformation, we have carried out fluorescence resonance energy transfer (FRET) experiments using a c-kit-1 quadruplex dually labeled with two fluorophores, fluorescein (FAM) and tetramethyl-rhodamine (TAMRA), at the 5′ and 3′ termini, respectively. When quadruplex is formed, fluorescence emission of FAM is efficiently quenched by the neighboring TAMRA. When the quadruplex unfolds, the distance between the fluorophore and the quencher increases and the emissions of both FAM and TAMRA become independent of each other (*28*). Fluorescence experiments were carried out by titrating in a solution of dual-labeled c-kit-1 quadruplex with increasing amounts of hPARP-1 and monitoring the fluorescence emission of both fluorophores while exciting fluorescein at 470 nm. No significant change in the fluorescence spectra was observed even at high protein concentration (up to 4 equiv), suggesting that hPARP-1 does not unwind quadruplexes upon binding (data not shown).

We finally investigated whether hPARP-1–quadruplex interaction was functional by measuring the effect of quadruplex DNA on hPARP-1 catalytic activity in the reaction of autopoly(ADP-ribosyl)ation. The formation of poly(ADP-ribose) induced by the interaction of hPARP-1 with the c-kit-1 and Htelo quadruplexes was compared to that obtained in the presence of a nuclease-treated salmon testes DNA acting as a hPARP-1 activator and used as a reference (Figure 3). We showed that DNA quadruplex induced the *in vitro* hPARP-1 catalyzed poly(ADP-ribose) synthesis, whereas single-stranded oligonucleotides containing mutated sequences that cannot form a quadruplex exhibited no potential to stimulate hPARP-1 enzymatic activity. These results therefore establish DNA quadruplex as an effective coenzymatic activator of hPARP-1 and indicate that poly(ADP-ribose) synthesis is affected by quadruplex structure. Furthermore, a hPARP-1 activation experiment was also carried out using a fixed protein concentration and varying concentrations of two distinct DNA activators: a B-DNA fragment carrying one accessible dsDNA end and the c-kit-1 quadruplex. The results (Supplementary Figure 2) show that quadruplex DNA is an equally potent hPARP-1 activator as a duplex DNA end. These data are in line with previously reported observations on high coenzymatic efficiency of other non-B DNA structures (*27, 30*). Although the requirement of DNA cofactor for hPARP-1 activity is well established in many studies, (*29*) the

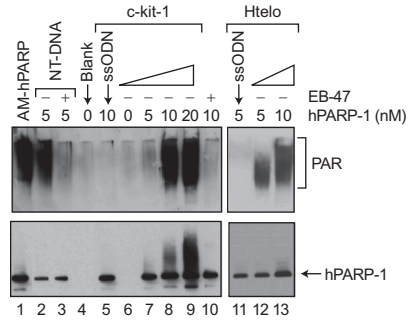

Figure 3. hPARP-1 enzymatic activity is stimulated by DNA G-quadruplexes. Immunoblot analyses of the *in vitro* poly(ADP-ribose) (PAR) synthesis by human PARP-1 stimulated by different types of DNA substrates in the presence of 133 μM βNAD$^+$. *In vitro* poly(ADP-ribosyl)ated hPARP-1 (AM-hPARP) and PAR synthesis stimulated by 16.6 ng μL^{-1} nuclease-treated salmon testes DNA (NT-DNA) are shown as a reference (lanes 1–3). In lane 4, the reaction mixture contained no hPARP-1 and DNA (Blank). The formation of PAR induced by the interaction of hPARP-1 with c-kit-1 promoter (c-kit-1, lines 5–10) and human telomeric (Htelo, lines 11–13) quadruplexes (8.3 nM) was evaluated in the presence of increasing hPARP-1 content. c-kit-1 and Htelo DNA quadruplexes both induced the *in vitro* hPARP-1 catalyzed PAR synthesis, while corresponding single-stranded oligonucleotides (lines 5 and 11) containing mutated sequences that cannot form a quadruplex exhibited no potential to stimulate hPARP-1 enzymatic activity. The inhibitor of hPARP-1 enzyme, EB-47 (400 nM), prevents hPARP-1 activation by both NT-DNA and c-kit-1 quadruplex (lines 3 and 10). Immunodetection of hPARP-1 in the corresponding samples is shown in the bottom panel.

precise mechanisms for enzymatic transition from inactive to active state are yet to be investigated. Our data, together with previous observations, indicate that hPARP-1 can utilize different types of DNA cofactors (DNA strand interruptions, non-B-DNA and DNA quadruplexes) to catalyze poly(ADP-ribosyl)ation (*27, 29, 30*). The structural diversity of enzyme cofactors that can be formed in the genome in several physiological contexts and following various patho-

physiological stimuli provides a rationale for pleiotropic functions of hPARP-1 in eukaryotic cells.

Our data provide the first reported example of a functional interaction linking a promoter DNA quadruplex and an abundant natural protein. This has implications in the yet to be demonstrated existence of promoter quadruplexes *in vivo* and also in understanding the role hPARP-1 may play in undamaged cells and in regulation of gene transcription in particular. It has been proposed that hPARP-1 may exert its function in transcription regulation through direct binding to the gene-regulating sequences (*18, 19*). However, the mechanism by which hPARP-1 is recruited to gene promoters in the absence of DNA damage remains unknown. We propose that promoter quadruplexes may act as hPARP-1 recruiting elements as part of its mechanism of transcription regulation. Our finding that at least two genes (pS2 and iNOS linked to breast cancer and oxidative stress, respectively) for which transcription is known to be regulated by hPARP-1 (*31, 32*) possess in their promoter a sequence that forms extremely stable quadruplexes *in vitro* (Supplementary Figure 3) supports this novel hypothesis that we are now in the process of exploring. This hPARP-1−quadruplex interaction may also have implications in the mechanisms of telomere maintenance. Using SPR, we have demonstrated that hPARP-1 could also bind the intramolecular quadruplex formed by the single-stranded 3′-end of the human telomeric DNA although with a significantly weaker (90-fold) affinity (Supplementary Figure 4). This would support the recent suggestion that hPARP-1 may be activated by and directly bind eroded telomeres as part of a DNA damage repair process (*33, 34*).

METHODS

DNA Oligonucleotide Preparation. The dual-labeled oligonucleotide used for the FRET experiment and the 200-base oligonucleotide used for AFM were purchased from IBA (Germany). All other oligonucleotides were purchased from Sigma Genosis. All concentrations were expressed in strand molarity with a nearest-neighbor approximation for the absorption concentrations of the unfolded species.

The DNA quadruplexes were prepared in buffer containing 50 mM Tris·HCl at pH 7.4 and 100 mM KCl by heating to 95 °C for 5 min. After slow cooling for 6 h to RT, the oligonucleotide was stored at 4 °C. Biotinylated oligonucleotides of identical sequences, quadruplex or single-stranded oligonucleotides, were used for the SPR, "pull-down", and hPARP-1 activity assays.

Surface Plasmon Resonance. All experiments were carried out on a Biacore 2000 Biosensor using freshly filtered and degassed buffers. The experiments were performed using four different immobilized DNA targets: two quadruplexes, (c-kit-1) of sequence d(biotin-[$C_3G_3CG_3CGCGAG_3AG_4AG_2$]) and (Htelo) of sequence d(biotin-[$GT_2A(G_3T_2A)_4G_2$]); one double-stranded DNA (ds DNA) comprising the oligonucleotide d(biotin-[$G_2CATAGTGCGT-G_3CGT_2AGC$]) hybridized with its complementary sequence; and one unstructured single-stranded DNA of sequence comparable to that of c-kit-1 but carrying G→T mutations to prevent quadruplex formation, d(biotin-[$C_3GTGCGTGCGCGAGTGAGTTG$]) (ss DNA). Biotinylated oligonucleotides were loaded onto four separate lanes of a SA chip (Biacore) using the MANUAL INJECT command (Biacore 2000 control software) at a flow rate of 10 μL min^{-1} in loading buffer (50 mM Tris pH 7.4, 100 mM KCl). Running buffer (50 mM Tris pH 7.4, 100 mM KCl, 1 mM DTT, 50 μM zinc acetate) was then run through the four channels at a fow rate of 10 μL min^{-1} for 1 h before any protein was injected. hPARP-1 proteins were diluted in running buffer to concentrations of 0.98, 1.95, 3.9, 7.8, 15.6, 31.2, 62.5, and 125 nM. The KINJECT command was used to inject 60-μL protein samples for a period of 3 min followed by a dissociation phase of 180 s. In between successive protein injections, the chip was regenerated by injecting running buffer containing 1 M KCl for 1 min.

Oligonucleotide "Pull-down" Assay. Streptavidin-coated polystyrene beads (Dynabeads M-280, Dynal Biothech) were incubated with a 2.5 μM solution of either c-kit quadruplex (c-kit-1) or mutated singled-stranded oligonucleotide (ss ODN) at RT for 30 min in accordance with manufacturer's instructions. Protein binding assays were carried out in the presence of 12 nM recombinant full-length human PARP-1 protein (specific activity 10 U μg^{-1}, Trevigen) for 30 min with gentle agitation at RT in the binding buffer (50 mM Tris-HCl (pH 7.4), 50 mM KCl, 2 mM $MgCl_2$, 1 mM DTT, 50 μM $Zn(OAc)_2$). The protein-bound beads were separated using a magnetic separator (Dynal) and washed five times with 50 μL of low-salt buffer (50 mM Tris-HCl, pH 7.4, 100 mM KCl). Bound hPARP-1 proteins were eluted with 20 μL of NaCl (0.5–2.0 M) and subsequently analyzed by Western blotting using mouse anti-PARP-1 antibody (1:500, clone 42, BD Transduction Laboratories). Signals were detected using an enhanced chemoluminescence system (Amersham Biosciences).

Atomic Force Microscopy. Oligonucleotide (200 bases long) carrying c-kit quadruplex-forming sequence (0.5 pmol) was incubated with hPARP-1 (0.1–10 pmol) in 10 μL of binding buffer (50 mM KOAc, 20 mM Tris-AcOH pH 7.9, 10 mM $Mg(OAc)_2$, 1 mM DTT) for 10 min at 25 °C, and the complex was cross-linked with 0.8% glutaraldehyde for 15 min. The reaction was terminated by the addition of 5 μL of 2 M Tris-HCl (pH 7.7). High molecular weight components were transferred in the binding buffer using G-25 spin columns, and samples were diluted 5-fold with this buffer, placed onto freshly cleaved mica (Ted Pella), rinsed with deionized water, and dried with argon. In some experiments oligonucleotides were heat-denatured at 70 °C for 10 min and subsequently annealed in the K^+,Na^+-free deposition buffer (10 mM bis-Tris-propane·HCl, 10 mM $MgCl_2$, 1 mM DTT, pH 7.0) to achieve DNA quadruplexes unfolding. AFM images were acquired in dry air with a Nanoscope III AFM Instrument (Veeco/Digital Instruments) in close contact (tapping) mode using tapping mode etched OMCL-AC160TS silicon AFM probes (Olympus Optical). The images were processed, and the measurements were performed with version 2.2.85(5.1) Femtoscan software (Advanced Technologies Center). The histograms were created from the measurements of at least 80 unobstructed objects.

Fluorescence Spectroscopy. Fluorescence emission spectra were recorded in quartz cells at 20 °C on a Jobin Yvon Fluorolog 3.22 instrument. The excitation and emission bandwidths were fixed to 8 and 5 nm, respectively. FRET experiments were carried out using a 500-μL quartz cuvette containing a solution of 10 nM dual-labeled annealed c-kit quadruplex in 50 mM Tris, pH 7.4, 100 mM KCl, 50 μM $Zn(OAc)_2$, and 1 mM DTT. Concentrated protein aliquots (from a 1 μM stock solution in buffer) were directly added to the quadruplex solution. The spectra were recorded between 485 and 680 nm while exciting at 470 nm.

Fluorescence titration experiments were carried out in a 500-μL quartz cuvette containing a solution of 500 μL of hPARP-1 (40 nM) in 50 mM Tris, pH 7.4, 100 mM KCl, 50 μM zinc acetate, and 1 mM DTT. Concentrated DNA quadruplex aliquots (from 500 nM, 5 μM, and 50 μM stock solutions in buffer) were directly added to the protein solution. The spectra were recorded between 300 and 550 nm while exciting at 295 nm. Prior to the experiment, the protein at the desired concentration was kept in a low-binding Eppendorf tube at 0 °C for 1 h. It was then transferred into a quartz cell for the time of the experiment. Fluorescence of the free protein was shown to be stable for the average duration of an experiment, proving that photobleaching and protein adsorption on cell walls previously observed by others (*24, 25*) were negligible within our experimental conditions.

hPARP-1 Activity Assays. Reactions of autopoly(ADP-ribosyl)ation were carried out in a buffer containing 50 mM Tris-HCl (pH 7.4), 50 mM KCl, 2 mM $MgCl_2$, 1 mM DTT, 50 μM $Zn(OAc)_2$ in the presence of 133 μM NAD^+ (Trevigen) and structurally different types of DNA (see figure legend) as a cofactor. ADP-ribosylation was induced

by the addition of recombinant full-length hPARP-1 (specific activity 10 U μg^{-1}, Trevigen), and the reaction mixture (15 μL volume) was incubated at 25 °C for 30 min. In some reactions, the hPARP-1 inhibitor EB-47 (Axxora) was added in final concentration of 400 nM. The reactions were terminated by adding 2X SDS-PAGE loading buffer, samples were heated at 100 °C for 5 min and resolved by SDS-PAGE in a 4–12% gradient bis-Tris gel (NuPAGE, Invitrogen). *In vitro* automodified hPARP-1 (Biomol) was loaded onto the gel (50 ng $lane^{-1}$) alongside the reaction samples as an immunoblotting standard. The extent of hPARP-1 autopoly(ADP-ribosyl)ation was determined by Western blot analysis with a mouse monoclonal antipoly(ADP-ribose) antibody (1:500, Biomol). For immunodetection of hPARP-1 the blots were reprobed with mouse anti-PARP antibody (1:500, clone 42, BD Transduction Laboratories). Signals were detected using an enhanced chemiluminescence system (Amersham Biosciences).

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Supporting Information Available: This material is free of charge *via* the Internet.

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