Supporting Information

Experimental Procedures

rNTP and dNTP hydrolysis assays

rNTPase assays

Gel purified ^{32}P end-labeled 12GT four-stranded G-quadruplex or its denatured form at 10-20 μM were incubated in the presence of ~2.5 nM immunopurified recombinant telomerase eluate and 1 mM non-radioactive ATP or GTP and 33 nM [γ^{32}P]-ATP or [γ^{32}P]-GTP (Perkin Elmer; 6000 Ci/mmol; 10 μCi/μl) in Telomerase buffer, 100 mM NaCl and 100 μg/ml BSA for 30-40 min at 25°C in a total volume of 10 μl. The reaction was terminated with the addition of 10 μl of 50 mM EDTA. An aliquot (1 μl) from each reaction mixture was spotted onto a polyethyleneimine (PEI) cellulose plate (Merck) which was developed in 0.8 M lithium chloride (LiCl). The amount of [^{32}P]-orthophosphate released was visualized using a PhosphorImager and measured using ImageQuant TL software. Free $^{32}P_i$ (Perkin Elmer; 1 mCi/ml) was used as a migration marker.

dNTPase assays

Gel purified ^{32}P end-labeled 12GT four-stranded G-quadruplex or its denatured form at 10-20 μM were incubated in the presence of ~2.5 nM immunopurified recombinant telomerase eluate and either dGTP (264 nM [α^{32}P]-dGTP and 9.7 μM non-radioactive dGTP) or dTTP (264 nM [α^{32}P]-dTTP and 100 μM non-radioactive dTTP) individually or in combination ([α^{32}P]-dGTP/non-radioactive dGTP and nonradioactive dTTP or [α^{32}P]-dTTP/non-radioactive dTTP and nonradioactive dGTP) in Telomerase buffer and 100 mM NaCl for 30-40 min at 25°C in a total reaction volume of 10 μl. [α^{32}P]-dGTP and [α^{32}P]-dTTP are from Perkin Elmer, at 3000

Ci/mmol and 10 μCi/μl. The reaction was terminated with the addition of 10 μl of 50 mM EDTA. An aliquot (1 μl) from each reaction mixture was spotted onto a PEI cellulose plate which was developed in 0.8 M LiCl. The amounts of [α^{32}P]-dGDP or [α^{32}P]-dTDP released were visualized using a PhosphorImager and measured using ImageQuant TL software. Free $^{32}P_i$ was used as a migration marker. Note that the position of dNDPs on the plate was deduced from the migration of the corresponding dNTP. The position of dNTPs, in turn, was judged from their mobility on a 12 % polyacrylamide sequencing type gel (data not shown).

Supporting Figure Legends

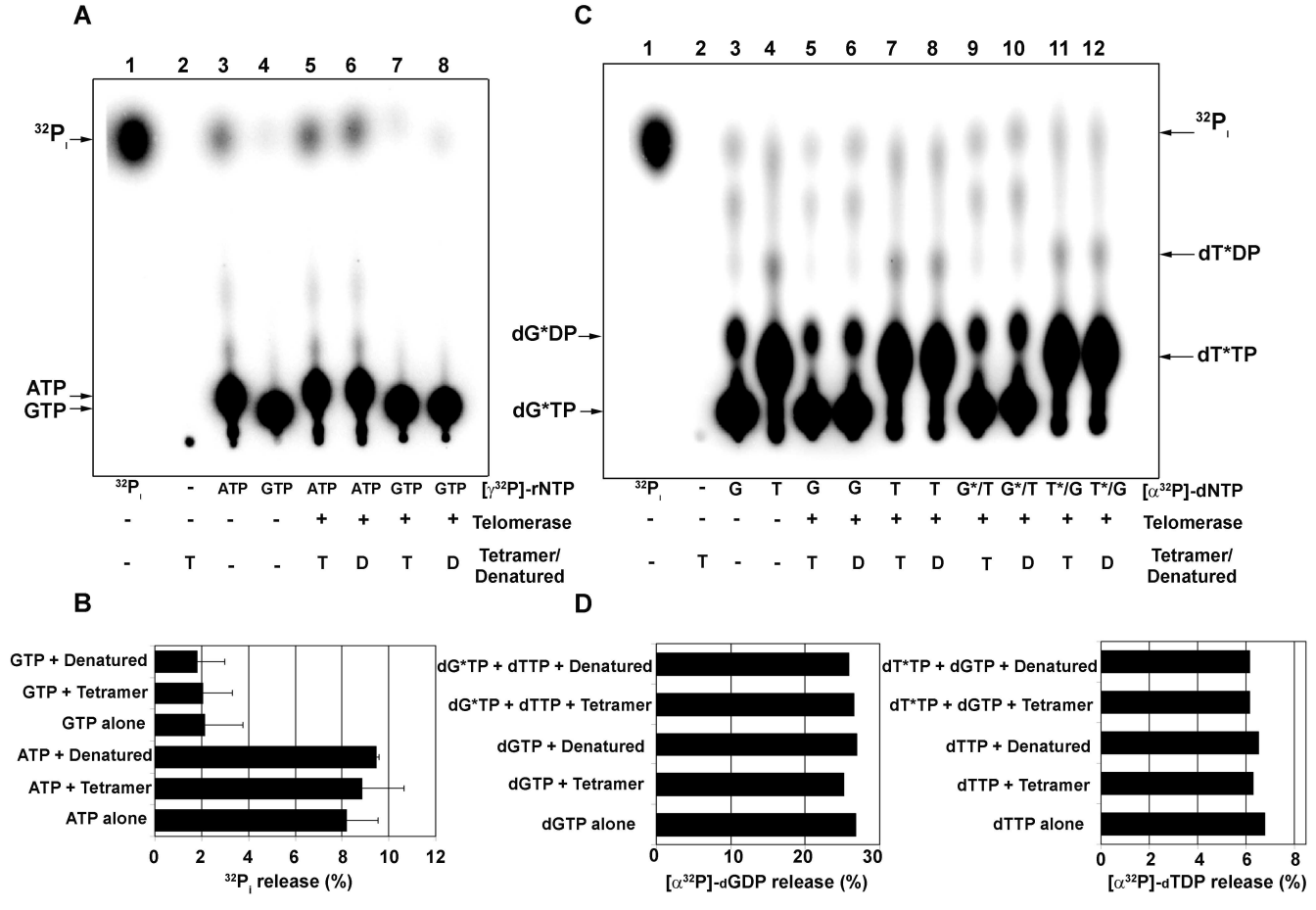
Supporting Figure 1

Telomerase does not utilize rNTPs or dNTPs as an energy source. **(A)** Test of ATP (lanes 5 and 6) and GTP (lanes 7 and 8) hydrolyzing capacity of recombinant *Tetrahymena* telomerase. [γ^{32}P]-ATP or [γ^{32}P]-GTP (33 nM) in combination with 1 mM cold ATP or GTP, respectively, were incubated with ~ 2 nM recombinant *Tetrahymena* telomerase in the presence of 10-20 μM ^{32}P-end labeled gel purified 12GT tetramer (T; lanes 5 and 7) or its denatured counterpart (D; lanes 6 and 8). Lane 2: 12GT Tetramer alone. Lanes 3 and 4: ATP and GTP respectively in the absence of telomerase and DNA. **(B)** The amount of free $^{32}P_i$ released was quantitated and normalized to total signal. The error bars represent range from two independent experiments. **(C)** Test of dGTP (G) and dTTP (T) hydrolyzing capacity of recombinant *Tetrahymena* telomerase. dGTP (264 nM [α^{32}P]-dGTP and 9.7 μM cold dGTP) or dTTP (264 nM [α^{32}P]-dTTP and 100 μM cold dTTP) individually (lanes 5, 6 and 7, 8 respectively) or together (lanes 9-12) were incubated with ~ 2 nM recombinant *Tetrahymena* telomerase in the presence of 10-20 μM ^{32}P end-labeled gel purified 12GT tetramer (T; lanes 5, 7, 9, 11) or denatured counterpart (D; lanes 6, 8, 10, 12). Lane 2: 12GT Tetramer alone. Lanes 3 and 4: dGTP and dTTP respectively in the absence of telomerase and DNA. *indicates the dNTP that carries the radiolabel when dGTP and dTTP are used in combination. **(D)** The proportions of [α^{32}P]-dGDP and [α^{32}P]-dTDP released were quantitated and normalized to total signal. The experiment shown is representative of two independent experiments. In (A) and (C) the products were separated on a polyethyleneimine (PEI) cellulose plate. Free ^{32}Phosphate ($^{32}P_i$; lane 1) was loaded as a size marker.

Supporting Figure 2

Telomerase RNA is not required for telomerase interaction with G-quadruplex DNA. Biotinylated gel-purified 12GT tetramer G-quadruplex and its denatured counterpart at 0, 0.2, 0.5, 1, 2.5, 10, 15, 20 μM concentrations or non-telomeric control (Bio-PBR) at 2.5 μM were incubated with ^{35}S-methionine labeled full-length recombinant TERT in the absence of telomerase RNA and recovered on NeutrAvidin beads. 'Input' represents 50 % of starting material in the bound lanes. 'LC' is a ^{33}P-labeled PBR48-Bio oligonucleotide used as a loading and recovery control.

Supporting Figure 1



Supporting Figure 2