Expanded View Figures

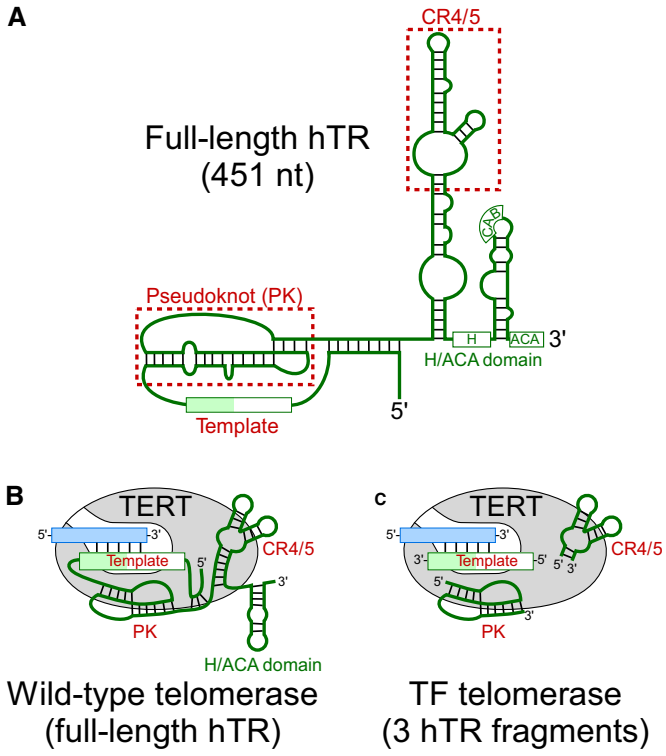

Figure EV1. Comparison of wild-type and template-free (TF) telomerase reconstitution systems.

A Schematic of the 451-nt full-length hTR. The hTR secondary structure comprises three major structural domains: the pseudoknot (PK), conserved regions 4/5 (CR4/5), and box H/ACA domain. The template region and the two structural domains, PK, and CR4/5 (red), are minimally required for reconstituting telomerase activity *in vitro*.

B The wild-type telomerase core enzyme comprises the full-length hTR (green) and the catalytic TERT protein (gray). The substrate for wild-type telomerase is a single-stranded DNA primer (blue).

C TF telomerase comprises the minimally required PK and CR4/5 hTR fragments (green). The substrate for TF telomerase is duplex of a single-stranded DNA primer (blue) pre-annealed with an RNA template.

Figure EV2. Km measurement for nucleotide incorporations at specific template positions.

A Sequences of the DNA/RNA hybrid substrates, position of the pause signal, and pause site (red). *In vitro*-reconstituted TF telomerase was analyzed with specific DNA/RNA hybrid substrates to determine the K_M for nucleotide incorporation. Numbers below the DNA/RNA duplexes indicate positions corresponding to the telomerase template and the order of nucleotides incorporated. Non-telomeric template residues (orange) are denoted. The nucleotide incorporation K_M values measured are indicated (black arrow). The DNA products were labeled with a radioactive ^{32}P-dNTP (asterisk) followed by the nucleotide incorporation whose K_M is specifically measured.

B Representative gels for K_M measurements. A ^{32}P end-labeled DNA recovery control (r.c.) was added before product purification and precipitation. The nucleotide incorporation K_M values measured are indicated (black arrow).

C Plots derived from the normalized intensity of the denoted (black arrow) nucleotide incorporation product over the total intensity of products for the specified nucleotide concentration. The Michaelis–Menten equation, $Y = V_{max}*X/(K_M+X)$, was used to fit the nonlinear curve to determine the K_M. Two or three replicates were performed for each measurement.

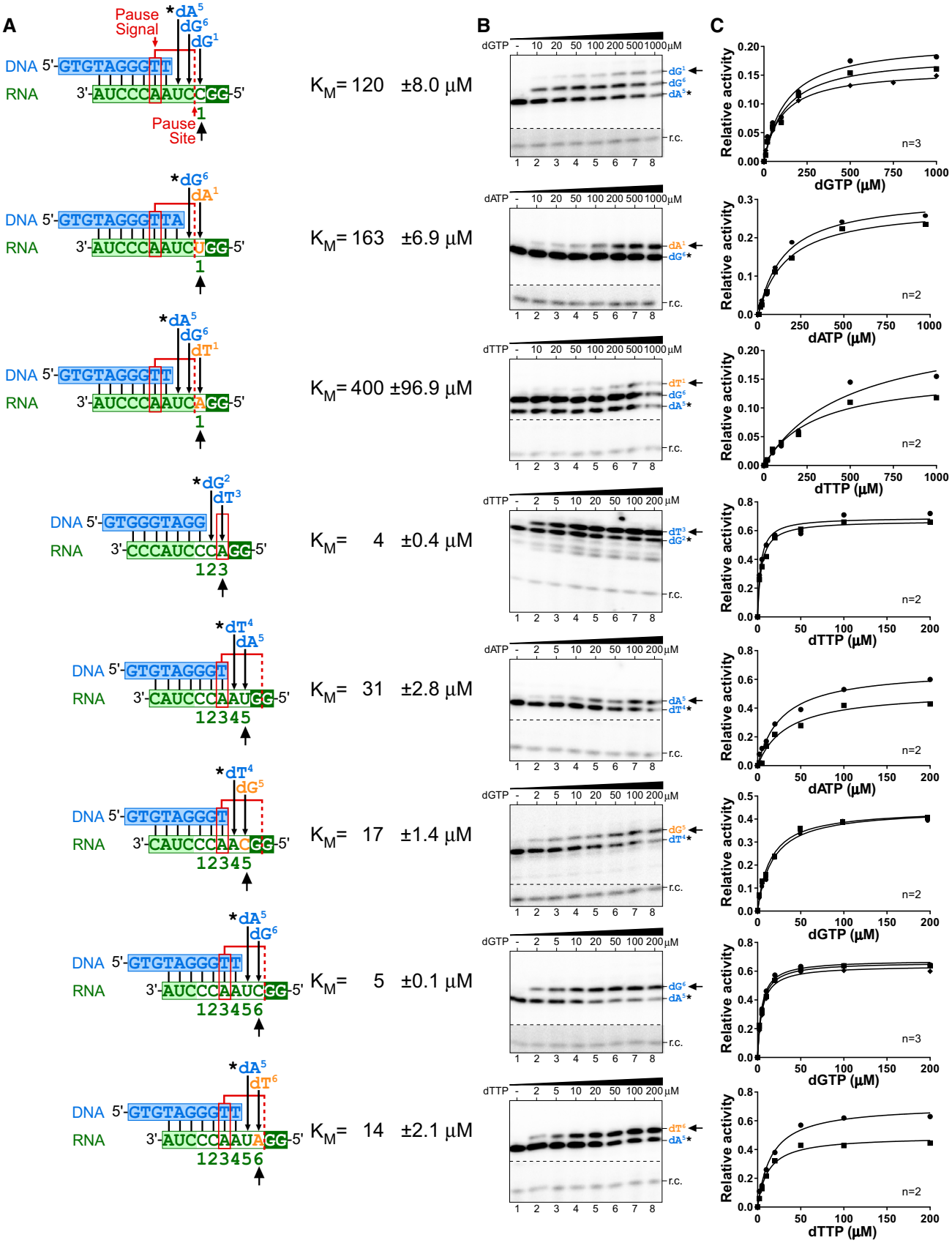
A
B
C
Pause Signal
Pause Site
DNA 5'-GTGTAGGGTT
RNA 3'-AUCCCAAUCCGG-5'
K_M= 120 ±8.0 μM
DNA 5'-GTGTAGGGTTA
RNA 3'-AUCCCAAUCUGG-5'
K_M= 163 ±6.9 μM
DNA 5'-GTGTAGGGTT
RNA 3'-AUCCCAAUCAGG-5'
K_M= 400 ±96.9 μM
DNA 5'-GTGGGTAGG
RNA 3'-CCCAUCCCAGG-5'
K_M= 4 ±0.4 μM
DNA 5'-GTGTAGGGT
RNA 3'-CAUCCCAAUGG-5'
K_M= 31 ±2.8 μM
DNA 5'-GTGTAGGGT
RNA 3'-CAUCCCAACGG-5'
K_M= 17 ±1.4 μM
DNA 5'-GTGTAGGGTT
RNA 3'-AUCCCAAUCGG-5'
K_M= 5 ±0.1 μM
DNA 5'-GTGTAGGGTT
RNA 3'-AUCCCAAUAGG-5'
K_M= 14 ±2.1 μM
dGTP - 10 20 50 100 200 500 1000 μM
dATP - 10 20 50 100 200 500 1000 μM
dTTP - 10 20 50 100 200 500 1000 μM
dTTP - 2 5 10 20 50 100 200 μM
dATP - 2 5 10 20 50 100 200 μM
dGTP - 2 5 10 20 50 100 200 μM
r.c.
1 2 3 4 5 6 7 8
Relative activity
dGTP (μM)
dATP (μM)
dTTP (μM)
n=3
n=2

Figure EV2.

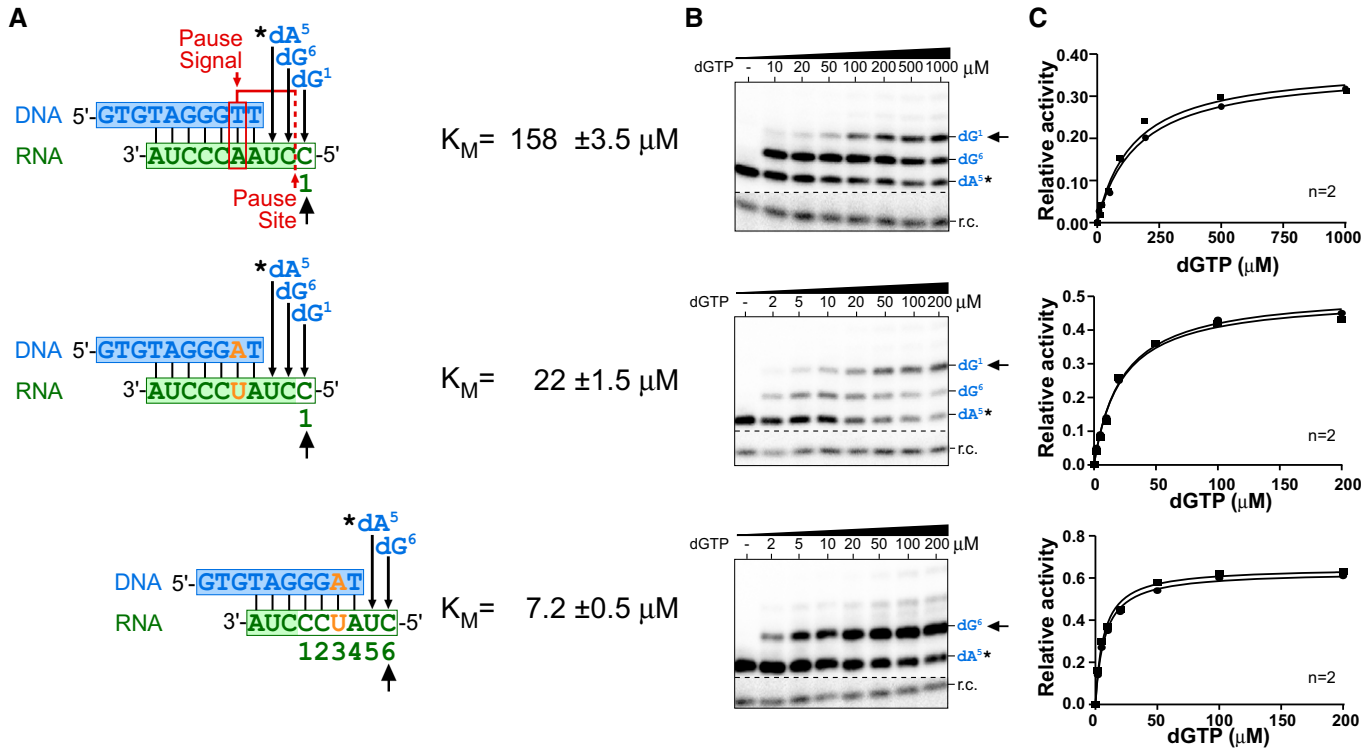

Figure EV3. ***K*M measurement for nucleotide incorporations in the presence or absence of the pause signal.**

A Sequences of the DNA/RNA hybrid substrates, position of the pause signal, and pause site (red). *In vitro*-reconstituted TF telomerase was analyzed with specific DNA/RNA hybrid substrates to determine the K_M for each nucleotide incorporation. Numbers below the DNA/RNA duplexes indicate positions corresponding to the telomerase template and the order of nucleotides incorporated. Mutations to disrupt the pause signal denoted (orange). The DNA products were labeled by incorporating a ^{32}P-dATP (0.165 μM, asterisk) prior to the K_M measurement.

B Representative gels for K_M measurements. An ^{32}P end-labeled DNA recovery control (r.c.) was added before product purification and precipitation. The nucleotide incorporation K_M values measured are indicated (black arrow).

C Plots derived from the normalized intensity of the denoted (black arrow) nucleotide incorporation product over the total intensity of products for the specified nucleotide concentration. The Michaelis–Menten equation, $Y = V_{max}*X/(K_M+X)$, was used to fit the nonlinear curve to determine the K_M. Two or three replicates were performed for each measurement.

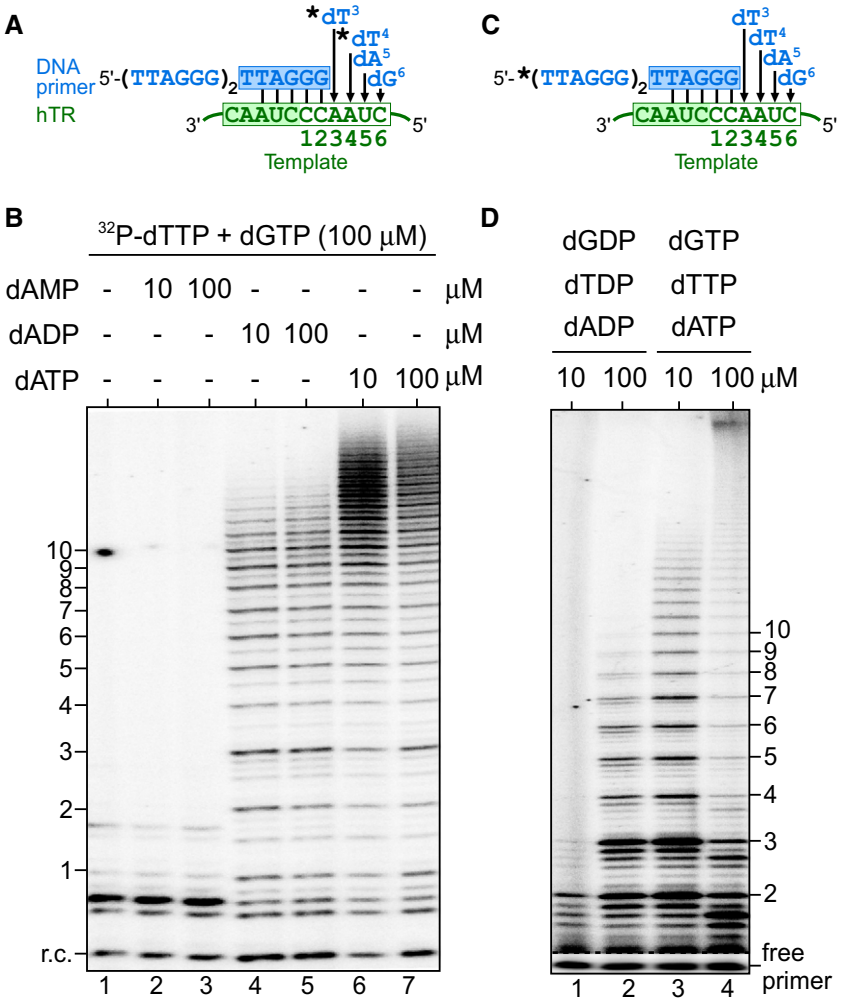

Figure EV4. The utilization of deoxynucleoside diphosphates as substrate for telomerase nucleotide addition.

A Schematic of nucleotide addition with wild-type telomerase. The order of the nucleotides, dT^3, dT^4, dA^5, and dC^6, added prior to first template translocation is depicted. The DNA products were labeled with 0.165 μM ^{32}P-dTTP (asterisk) and 10 μM dTTP.

B Telomerase was assayed in the presence of 0.165 μM ^{32}P-dTTP and 100 μM dGTP with either 10 or 100 μM of dAMP, dADP, and dATP. An ^{32}P end-labeled DNA recovery control (r.c.) was added before product purification and precipitation.

C Schematic of nucleotide addition with a ^{32}P end-labeled DNA primer and wild-type telomerase. The order of the nucleotides, dT^3, dT^4, dA^5, and dC^6, added prior to first template translocation, was depicted. The DNA primer was ^{32}P end-labeled (asterisk) to eliminate the need of including ^{32}P-dTTP in the dNDP-only reaction.

D Telomerase was assayed in the presence of either 10 or 100 μM deoxynucleoside diphosphates (dGDP, dTDP, dADP) or deoxynucleoside triphosphates (dGTP, dTTP, dATP). Direct primer extension assays were performed with telomerase enzyme reconstituted *in vivo* and immuno-purified.

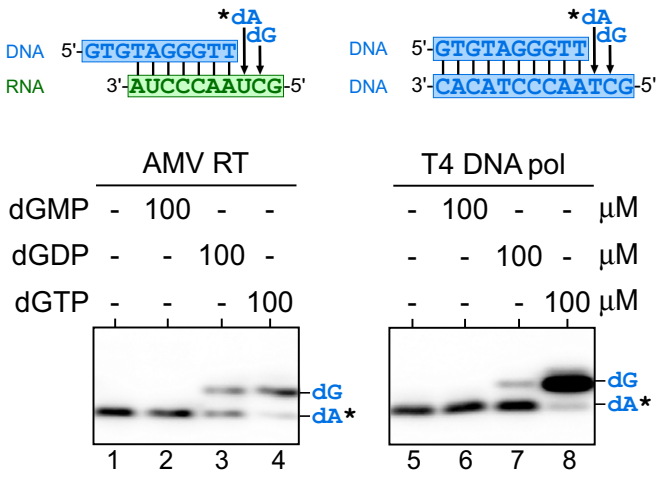

Figure EV5. Utilization of dGDP as substrate by the AMV RT and T4 DNA polymerase.

Sequences of the DNA/RNA or DNA/DNA hybrid substrates are shown above the gels. The templates of the substrates specify incorporation for a dA and a dG residue. The enzymes were assayed with 0.165 μM ^{32}P-dATP (asterisk) and either dGMP, dGDP, or dGTP at 100 μM.