Supporting Information

Das et al. 10.1073/pnas.1314289110

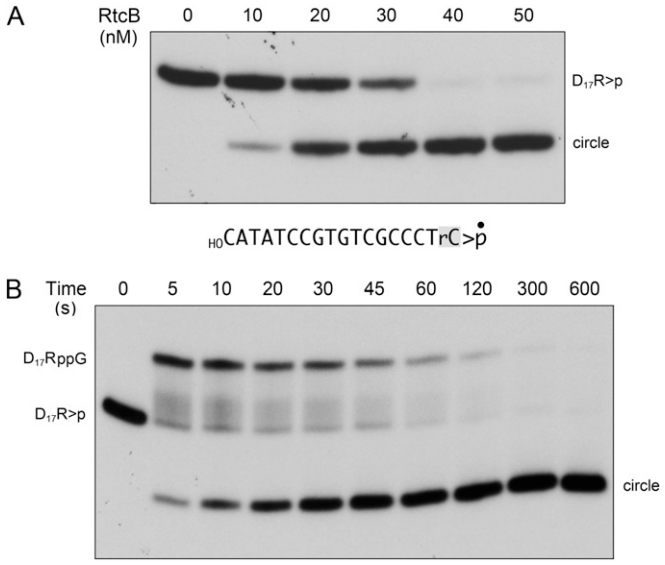

Fig. S1. RtcB splices 5′-OH DNA with a ribonucleoside-2′,3′-cyclic-PO_4 terminus. (*A*) Reaction mixtures (10 μL) containing 50 mM Tris·HCl (pH 8.0), 2 mM $MnCl_2$, 100 μM GTP, 0.02 μM $_{HO}$D17R>p (shown with the 3′ ribonucleoside shaded and the ^{32}P-label denoted by •), and RtcB as specified were incubated at 37 °C for 30 min. The products were analyzed by urea-PAGE and visualized by autoradiography. The positions of the linear substrate and ligated circle are indicated (*Right*). (*B*) Reaction mixture containing 50 mM Tris·HCl (pH 8.0), 2 mM $MnCl_2$, 100 μM GTP, 1 μM RtcB, and 0.02 μM $_{HO}$D17R>p was incubated at 37 °C. Aliquots were withdrawn at the times specified and quenched immediately. The products were analyzed by urea-PAGE and visualized by autoradiography. The positions of the linear substrate and guanylylated intermediate are indicated (*Left*); the position of the ligated circle is indicated (*Right*).

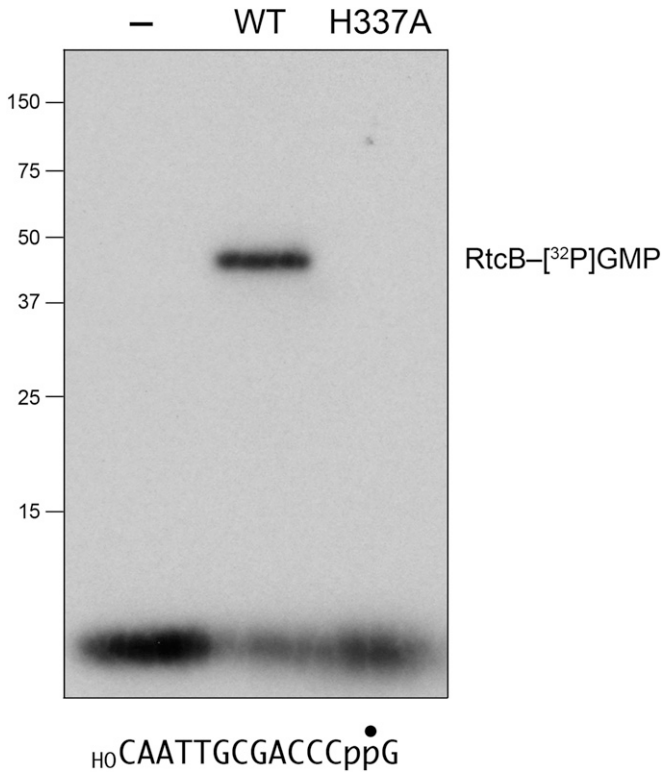

Fig. S2. Transfer of guanylate from DNAppG to RtcB to form RtcB–GMP. Reaction mixtures (10 μL) containing 50 mM Tris·HCl (pH 8.0), 2 mM $MnCl_2$, 0.1 μM of gel-purified ^{32}P-GMP–labeled DNAppG (shown with the ^{32}P-label denoted by •), and either no enzyme (lane –) or 2 μM wild-type RtcB (WT) or H337A mutant were incubated at 37 °C for 20 min. The products were analyzed by SDS/PAGE; an autoradiograph of the dried gel is shown. The RtcB–GMP adduct is indicated (*Right*). The positions and sizes (in kilodaltons) of marker polypeptides are indicated (*Left*).

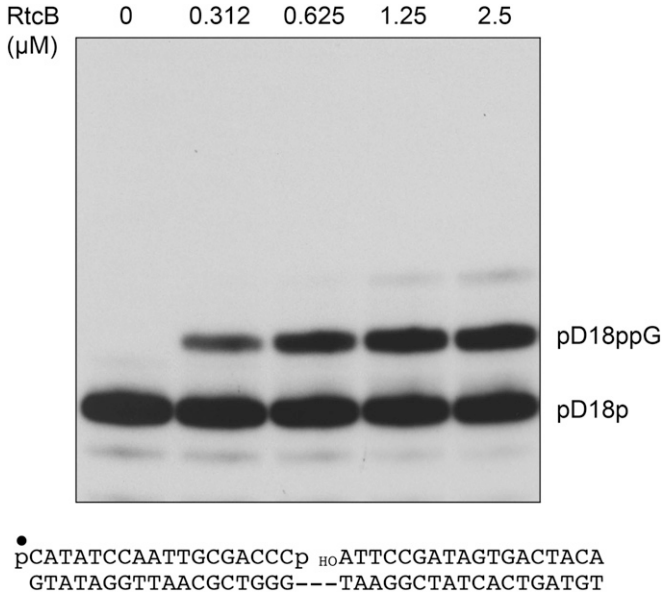

Fig. S3. *E. coli* RtcB guanylylates but does not seal a 3′-PO_4/5′-OH nick in duplex DNA. The 5′ ^{32}P-labeled 3′-PO_4/5′-OH nicked duplex DNA substrate is shown (*Lower*) with the ^{32}P-label denoted by •. The substrate was prepared by annealing the labeled 18-mer pDNAp strand (pD18p) and the unlabeled 18-mer $_{HO}DNA_{OH}$ strand to the unlabeled 36-mer template strand and then separating the nicked duplex from single strands and two-strand tailed duplexes by native polyacrylamide gel electrophoresis. The three-strand nicked duplex was located by autoradiography and then eluted from an excised gel slice by soaking overnight at 4 °C in 10 mM Tris·HCl (pH 8.0), 1 mM EDTA. RtcB reaction mixtures (10 μL) containing 50 mM Tris·HCl (pH 8.0), 2 mM $MnCl_2$, 100 μM GTP, 0.1 μM nicked duplex DNA, and RtcB as specified were incubated at 37 °C for 30 min. The products were analyzed by urea-PAGE and visualized by autoradiography. No labeled species corresponding to a 36-mer nick sealing product was detected.

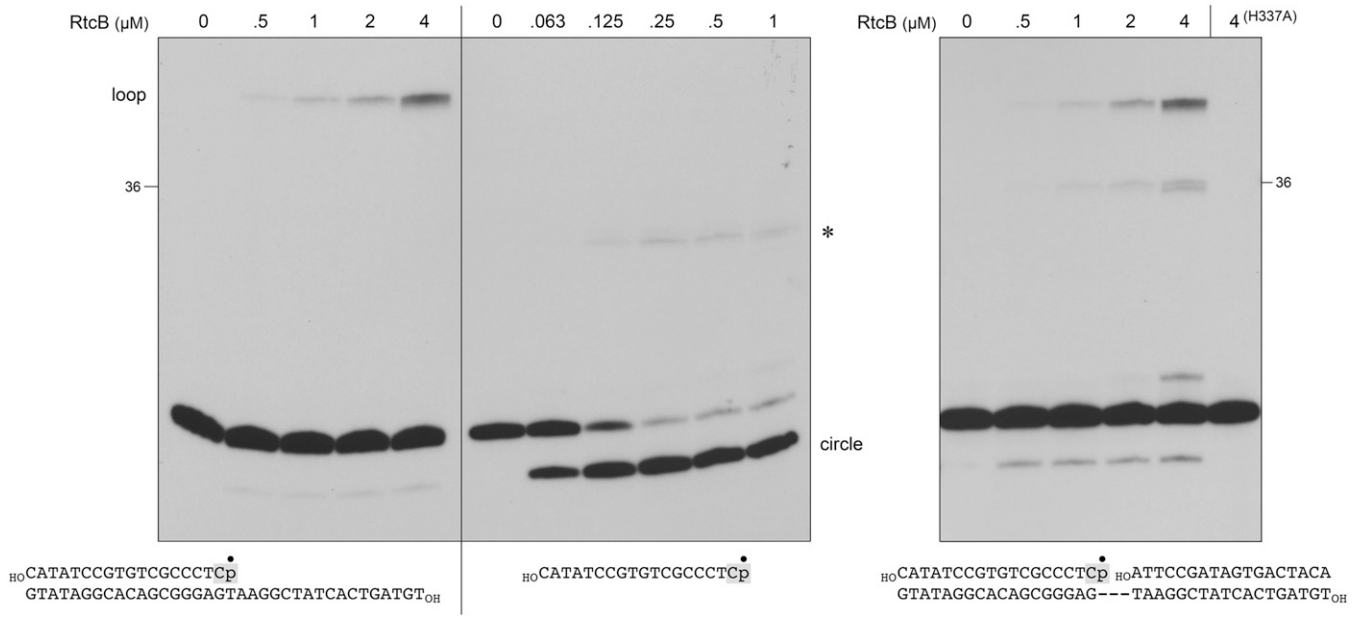

Fig. S4. Effect of duplex secondary structure on ligation of a $_{HO}$D17Rp strand. The 3′ ^{32}P-labeled D17Rp single strand is shown (*Lower Center*) with the ^{32}P-label denoted by • and the terminal ribonucleotide shaded in gray. The 5′-OH tailed duplex substrate (*Lower Left*) was prepared by annealing the labeled D17Rp strand to the unlabeled 36-mer template strand. The nicked duplex substrate (*Lower Right*) was prepared by annealing the labeled D17R1p strand and the unlabeled 18-mer $_{HO}$DNA$_{OH}$ strand to the unlabeled 36-mer template strand. The tailed duplex and three-strand nicked duplex were purified by native gel electrophoresis and eluted from excised gel slices by soaking overnight at 4 °C in 10 mM Tris·HCl (pH 8.0), 100 mM NaCl, 1 mM EDTA. RtcB reactions (10 μL) containing 50 mM Tris·HCl (pH 8.0), 2 mM $MnCl_2$, 0.1 mM GTP, 0.02 μM ^{32}P-labeled D17Rp single-strand, tailed-duplex, or nicked-duplex substrates and RtcB as specified were incubated at 37 °C for 30 min. The products were analyzed by urea-PAGE and visualized by autoradiography. The position of a 5′ ^{32}P-labeled 36-mer marker DNA strand (of identical sequence to the expected nick sealing product) that was analyzed in parallel is indicated (*Left* and *Right*). RtcB efficiently catalyzed intramolecular ligation of the D17Rp single strand to form a circular product that migrated faster than the linear substrate. A minor product corresponding to a dimer circle is denoted by the asterisk to the *Right* of the *Center* panel. Annealing of D17Rp to the 36-mer suppressed circularization, allowing for only inefficient joining of the 3′-PO_4 to the 5′-OH of the tailed duplex to form a stem-loop product (loop).