

Supplemental Material

**RNA 3'-phosphate cyclase (RtcA) catalyzes ligase-like adenylation
of DNA and RNA 5'-monophosphate ends**

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Supplemental Figures S1 and S2

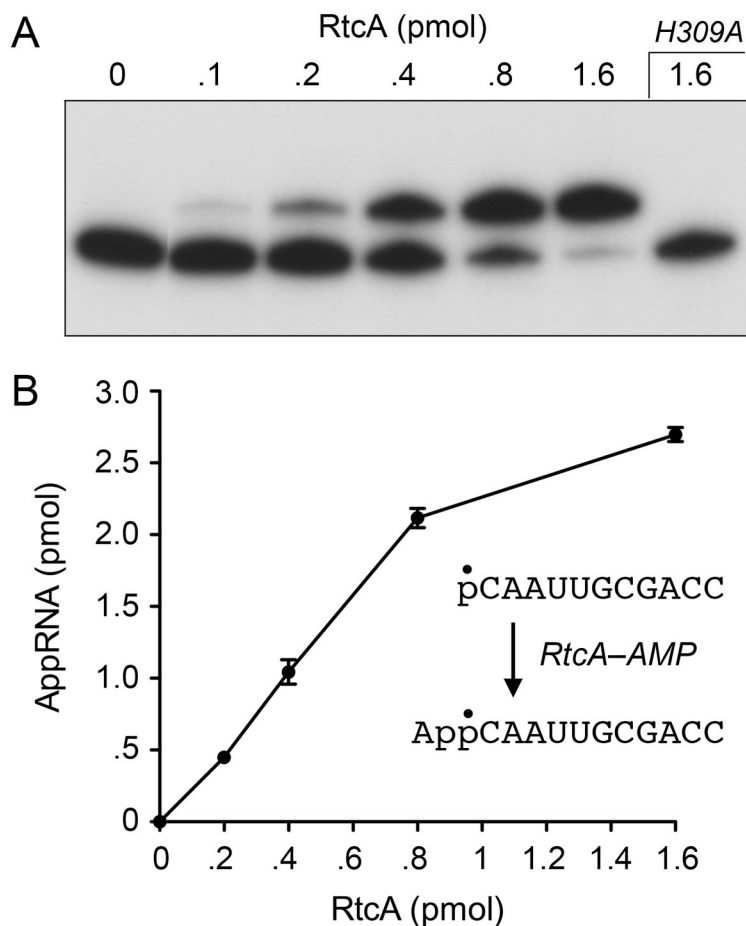


Figure S1. **RtcA adenylylates a 5'-phosphate RNA end.** (A) Reaction mixtures (10 μ l) containing 50 mM Tris-acetate (pH 6.0), 1 mM DTT, 2 mM MgCl_2 , 1 mM ATP, 3 pmol (300 nM) 5' ^{32}P -labeled 12-mer RNA (5'-pCAAUUGCGACC_{OH}), and RtcA as specified were incubated for 30 min at 37°C. The products were analyzed by urea-PAGE and visualized by autoradiography. (B) The extents of conversion of the pDNA strand to AppDNA product were quantified and plotted as a function of input RtcA. Each datum is the average of three independent enzyme titration experiments \pm SEM.

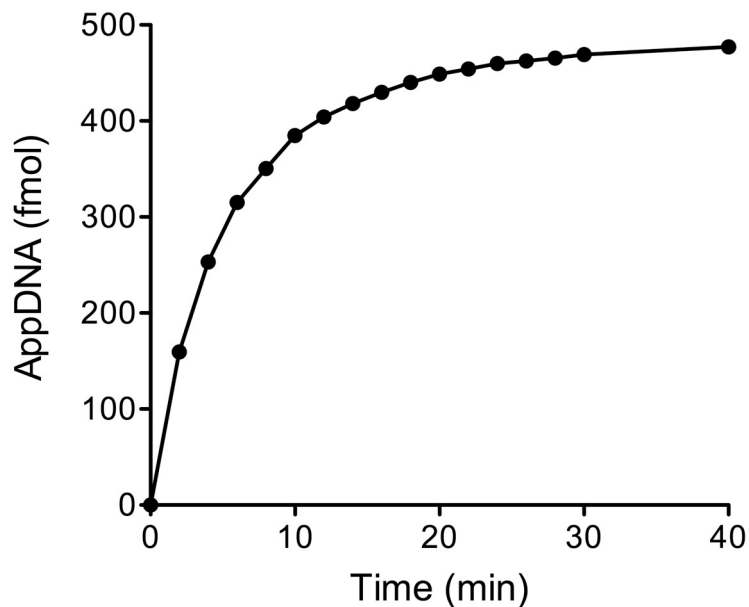


Figure S2. **5'-Adenylation in the absence of magnesium and ATP.** A reaction mixture (190 μ l) containing 50 mM Tris-HCl (pH 7.4), 5 mM DTT, 9.5 pmol (50 nM) 5' 32 P-labeled 12-mer RNA (5'-pCAAUUGCGACC_{OH}), and 266 pmol (1.4 μ M) RtcA [corresponding to \sim 630 nM RtcA-AMP] was incubated at 37°C. Aliquots (10 μ l, containing 0.5 pmol of 12-mer DNA) were withdrawn at the times specified and quenched immediately with formamide-EDTA. The time 0 sample was withdrawn prior to adding the RtcA. The extents of conversion of the pDNA strand to AppDNA product are plotted as a function of reaction time.