Supporting Information

Ligand-dependent Exponential Amplification of a Self-replicating L-RNA Enzyme

Charles Olea, Jr., David P. Horning, and Gerald F. Joyce

Departments of Chemistry and Molecular Biology and The Skaggs Institute for Chemical Biology

The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, California 92037

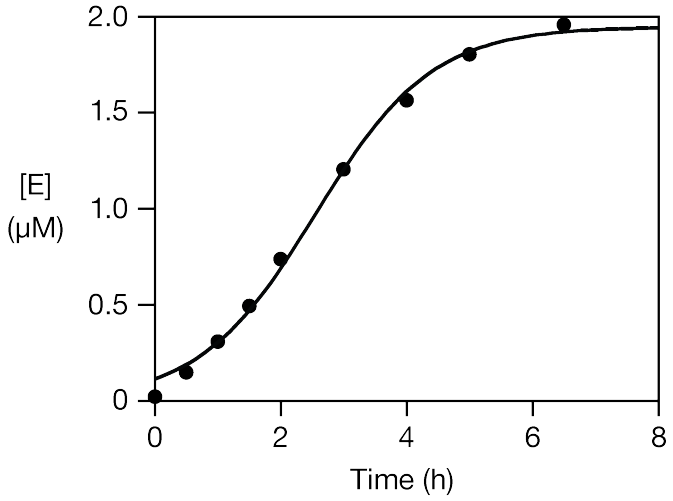

Figure S1. Exponential amplification of self-replicating D-RNA enzymes, employing materials prepared by *in vitro* transcription. The data were fit to the logistic growth equation (see Materials and Methods). The exponential growth rate was 1.1 h^{-1} and the maximum extent was 1.9 μM. Reaction conditions: 2.5 μM A, 20 μM B, 0.25 μM E, 25 mM $MgCl_2$, pH 8.5, 42 °C.

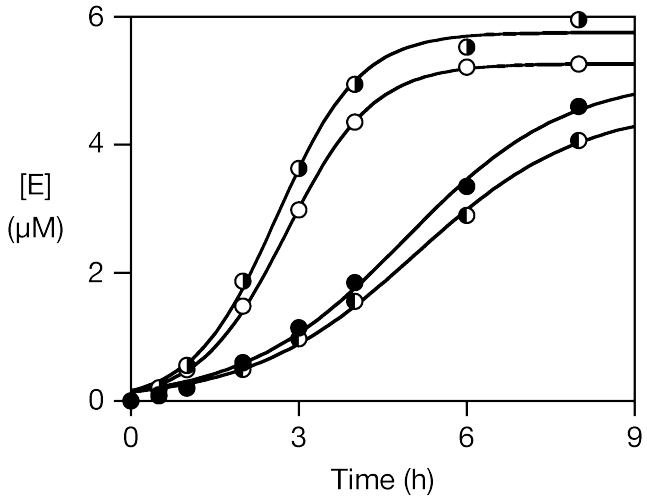

Figure S2. Exponential amplification of self-replicating D-RNA enzymes, employing substrates prepared by either solid-phase synthesis (filled circles) or *in vitro* transcription (open circles). The left and right half of the circles (filled or open) correspond to the A and B substrates, respectively. The data were fit to the logistic growth equation (see Materials and Methods). Reaction conditions: 10 μM A, 10 μM B, no starting E, 25 mM $MgCl_2$, pH 8.5, 42 °C.

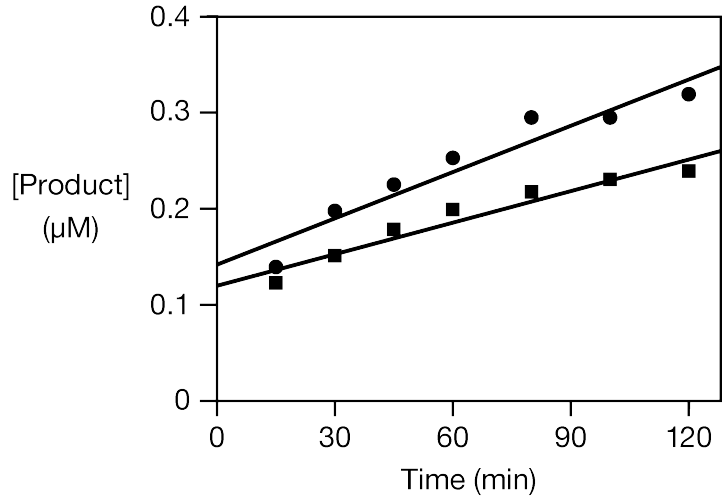

Figure S3. Catalytic activity of the D- and L-RNA enzymes, measured in a simple ligation reaction employing an oligonucleotide substrate having the sequence 5′-GGUCUCAUAU-3′ in place of substrate A. This oligonucleotide is complementary to a portion of E that binds A during self-replication. The reaction is biphasic, with a rapid initial burst followed by a slower linear phase.[1] The data were obtained during the latter phase and fit to a linear equation, giving observed rates for the D- (squares) and L- (circles) RNA-catalyzed reactions of 0.26 and 0.39 h^{-1}, respectively. Reaction conditions: 2.5 μM oligonucleotide, 20 μM B, 0.25 μM E, 25 mM $MgCl_2$, pH 8.5, 42 °C.

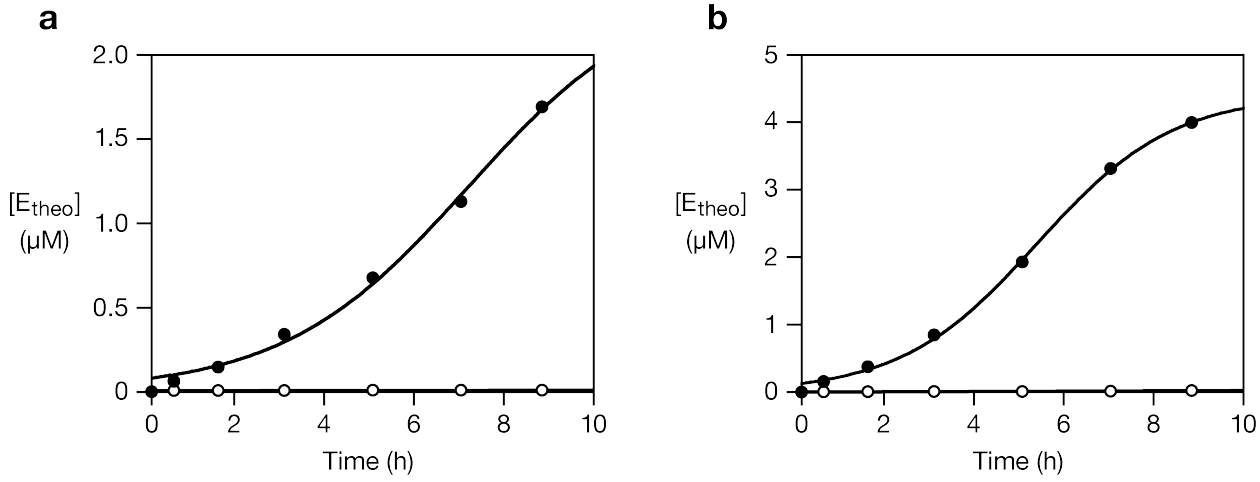

Figure S4. Ligand-dependent exponential amplification of self-replicating RNA enzymes, employing either (a) synthetic D-A_{theo} and D-B, or (b) synthetic L-A_{theo} and L-B. The reactions were carried out in either the presence (filled circles) or absence (open circles) of 5 mM theophylline. The data were fit to the logistic growth equation (see Materials and Methods). Reaction conditions: 10 μM A_{theo}, 10 μM B, no starting E, ± 5 mM theophylline, 25 mM $MgCl_2$, pH 8.5, 42 °C.

Materials and Methods

Materials. Oligonucleotides were prepared by solid-phase synthesis using an Expedite 8909 DNA/RNA synthesizer, with all reagents and nucleoside phosphoramidites purchased from Glen Research (Sterling, VA) except L-2′-*t*-butyldimethylsilyl RNA phosphoramidites, which were from ChemGenes (Wilmington, MA). All oligonucleotides were purified by denaturing polyacrylamide gel electrophoresis (PAGE) and subsequent ethanol precipitation. Histidine-tagged T7 RNA polymerase and M1 RNA were prepared as described previously.[2,3] T4 polynucleotide kinase and Antarctic phosphatase were purchased from New England Biolabs (Ipswich, MA), bovine pancreatic DNase I was from Roche Applied Science (Indianapolis, IN), and Superscript II reverse transcriptase was from Life Technologies (Carlsbad, CA). Yeast inorganic pyrophosphate, nucleoside and deoxynucleoside 5′-triphosphates, and theophylline were from Sigma-Aldrich (St. Louis, MO), [γ-^{32}P]ATP (6 μCi/pmol) was from Perkin-Elmer (Waltham, MA), and human serum (off-clot) was from MP Biomedicals (Solon, OH).

Preparation of enzyme and substrates by *in vitro* transcription. Transcribed forms of the D-RNA enzyme (E) and substrates (A and B) were prepared as described previously.[2,3] E was obtained by run-off transcription of a corresponding DNA template. A was generated from transcribed E using *E. coli* M1 RNA and an external guide RNA to give cleaved products of uniform length, ending in a 3′-hydroxyl.[2,4] B was transcribed with a self-cleaving hammerhead ribozyme at the 3′-terminus, which resulted in cleaved products of uniform length, ending in a 2′,3′-cyclic phosphate.[2] All transcribed materials were purified by PAGE and subsequent ethanol

precipitation. Following purification, A was [5′-^{32}P]-labeled by first dephosphorylating using Antarctic phosphatase then phosphorylating using T4 polynucleotide kinase and [γ-^{32}P]ATP.

The double-stranded DNA templates used to prepare E and B were obtained by cross-extension of two overlapping synthetic oligodeoxynucleotides, employing reverse transcriptase as a DNA-dependent DNA polymerase. For E the two oligodeoxynucleotides had the sequences 5′-GGCTAATACGACTCACTATAGGAAGTTGTGCTCGATTGTTACGTAAGTAAC-3′ and 5′-TAAGTTGCGGTCTCATACTTCAACCCATTCAAACTGTTACTTACGTAACAATC-3′; for B the sequences were 5′-GGCTAATACGACTCACTATTGAGACCGCAACTTATACGG-AAAC-3′ and 5′-CAACTTTTCGGCCTTTCGGCCTCATCAGTACGTTTCCGTATAAGT-3′ (T7 RNA polymerase promoter sequence underlined).

Preparation of enzyme and substrates by solid-phase synthesis. Substrates A, A_{theo}, and B were synthesized in both the D- and L-form. The enzymes E and E_{theo} were generated from the corresponding substrates by RNA-catalyzed self-ligation. D-A and D-A_{theo} were 5′-labeled with hexachlorofluorescein, and L-A and L-A_{theo} were 5′-labeled with fluorescein, incorporating the corresponding phosphoramidite (Glen Research) as the final coupling step.

The D- and L-B substrates were chemically triphosphorylated prior to their deprotection and cleavage from the solid support[5,6] (Horning & Joyce, unpublished data). Following oligonucleo-tide synthesis, the 5′-terminal dimethoxytrityl group was removed and the free 5′-hydroxyl was phosphitylated with salicyl phosphorochloridite. This was reacted with tributylammonium pyrophosphate to form the cyclic metaphosphite, then oxidized with iodine to generate the linear 5′-triphosphate. The 5′-triphosphorylated products were verified by MALDI-TOF mass

spectrometry, with a calculated MH^+ of 4,694 Da and measured values of 4,694 and 4,695 Da for D- and L-B, respectively.

Exponential amplification. All self-replication reactions were carried out in the presence of 25 mM $MgCl_2$ and 50 mM EPPS (pH 8.5) at 42 °C. The reactions were initiated by adding a buffered solution of $MgCl_2$ to a mixture of all of the RNA components, and the reactions were quenched by adding 25 mM Na_2EDTA. Aliquots were removed from the reaction mixture at various times and analyzed by PAGE, measuring the fraction of either [^{32}P]-labeled or fluorescently-labeled A that had been converted to labeled E. These data were fit to the logistic growth equation: $[E] = a / (1 + be^{-ct})$, where a is the final extent, b is the degree of sigmoidicity, and c is the exponential growth rate.

References

(1) Paul, N.; Joyce, G. F. *Proc. Natl. Acad. Sci. USA* 2002, *99*, 12733–12740.

(2) Lincoln, T. A.; Joyce, G. F. *Science* 2009, *323*, 1229–1232.

(3) Lam, B. J.; Joyce, G. F. *J. Am. Chem. Soc.* 2011, *133*, 3191–3197.

(4) Forster, A. C.; Altman, S. *Science* 1990, *249*, 783–786.

(5) Lebedev, A. V.; Koukhareva, I. I.; Beck, T.; Vaghefi, M. M. *Nucleosides Nucleotides Nucleic Acids* 2001, *20*, 1403–1409.

(6) Paul, N.; Springsteen, G.; Joyce, G. F. *Chem. Biol.* 2006, *13,* 329–338.