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JOURNAL OF THE AMERICAN CHEMICAL SOCIETY
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Cross-Chiral, RNA-Catalyzed Exponential Amplification of RNA

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Cite This: https://doi.org/10.1021/jacs.1c09233
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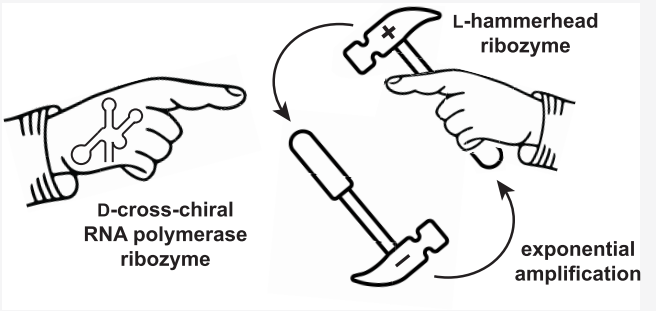
ABSTRACT: Informational macromolecules in biology are composed of subunits of a single handedness, D-nucleotides in nucleic acids and L-amino acids in proteins. Although this chiral uniformity may be expedient, it is not a chemical necessity, as demonstrated by the recent example of an RNA enzyme that catalyzes the RNA-templated polymerization of RNA molecules of the opposite handedness. This reaction, when carried out iteratively, can provide the basis for exponential amplification of RNA molecules and the information they contain. By carrying out thermal cycling, analogous to the polymerase chain reaction, and supplying oligonucleotide building blocks that comprise both the functional strand of RNA and its complement, cross-chiral exponential amplification was achieved. This process was used to amplify the L-RNA form of the hammerhead ribozyme, catalyzed by the D-RNA form of the polymerase. The resulting L-hammerhead exhibits the expected activity in cleaving a corresponding L-RNA substrate. Exponential amplification was also carried out within individual droplets of a water-in-oil emulsion. The ability to amplify enantio-RNAs, both in bulk solution and within compartments, provides a means to evolve cross-chiral RNA polymerases based on the function of the RNAs they produce.



INTRODUCTION

Exponential growth is a dynamic requirement of replicative molecular systems that undergo Darwinian evolution.[1,2] To achieve such growth, a parental molecule serves as a template to direct the synthesis of either an identical or complementary product, the latter requiring that the complementary product in turn direct the synthesis of a new copy of the parental molecule. In most synthetic chemical replication systems, however, exponential growth does not occur at constant temperature because the template−product complex must first dissociate to allow each molecule to serve as a template in the next round of replication. Instead there is subexponential amplification that depends on the spontaneous dissociation of the template−product complex to make available new template molecules.[3]

In biology, the complex machinery of genome replication ensures that complementary strands of RNA or DNA become separated without the need for thermal denaturation. In biochemical exponential amplification systems, such as the polymerase chain reaction (PCR), repeated cycles of heating and cooling are carried out to enable successive rounds of template copying.[4] In either case, the information content of the starting template is carried over to the succession of products. Thus, the information is amplified, as well as the molecules that embody it.

The PCR relies on a polymerase protein to catalyze repeated rounds of DNA-templated synthesis of DNA, each round involving extension of a DNA primer through the addition of deoxyribonucleoside 5′-triphosphates (dNTPs). There also is one reported example of an RNA polymerase ribozyme that catalyzes the exponential amplification of short RNAs, similarly extending an RNA primer on an RNA template by adding ribonucleoside 5′-triphosphates (NTPs), a process that has been termed "ribo-PCR".[5]

It usually goes without saying that the polymerase enzyme and the DNA or RNA being amplified are of the usual biological handedness; that is, the protein is composed of L-amino acids and the nucleic acid is composed of D-nucleotides. It is understood that if the mirror were reversed, the result would be equivalent, and there are examples in which a chemically synthesized polymerase protein composed of D-amino acids has been shown to synthesize, and even amplify, nucleic acids composed of L-nucleotides.[6−8] But would it be possible to use a polymerase of the biological chirality to amplify nucleic acids of the nonbiological chirality?

There is no known instance of an all-L-polynucleotide in biology, but such material can be synthesized chemically. There is one example of an RNA polymerase ribozyme, which was obtained by *in vitro* evolution, that catalyzes the templated

Received: August 31, 2021

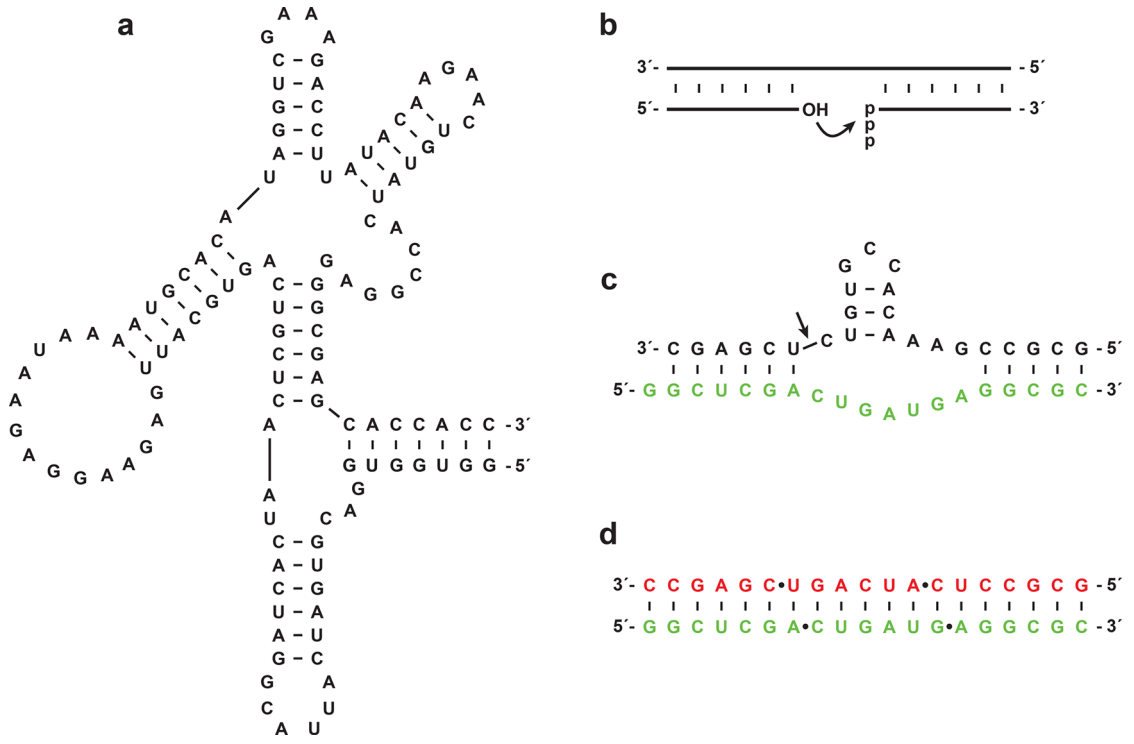


Figure 1. Composition of the cross-chiral polymerase and hammerhead ribozymes. (a) Sequence and secondary structure of the 27.3t polymerase.[10] (b) The polymerase catalyzes the RNA-templated joining of an oligonucleotide 3′-hydroxyl and an adjacent 5′-triphosphorylated mono- or oligonucleotide, forming a 3′,5′-phosphodiester linkage and releasing inorganic pyrophosphate. The curved arrow indicates the site of reaction. (c) Sequence and secondary structure of the RNA-cleaving hammerhead ribozyme, which can be split into two strands, with one strand (green) containing most of the nucleotides that are essential for catalysis and the other strand (black) containing the cleavage site (arrow). (d) Both the functional plus strand of the hammerhead and a complementary minus strand (red) were divided into three component oligonucleotides, requiring the cross-chiral polymerase to catalyze four joining reactions (black dots) to synthesize the two strands during the course of exponential amplification.

synthesis of RNA molecules of the opposite handedness.[9] Recently, this cross-chiral polymerase was optimized so that it can accept 5′-triphosphorylated mono- or oligonucleotide substrates of all possible sequence combinations at the site of addition.[10]

The present study used the improved cross-chiral polymerase, termed "27.3t" (Figure 1a,b), to carry out exponential amplification of RNAs of the opposite handedness. The object of amplification was the hammerhead ribozyme, which catalyzes the site-specific cleavage of RNA.[11] Through repeated thermal cycles, both the L-hammerhead and its complement were synthesized from a total of six component L-oligonucleotides. The resulting L-hammerhead exhibits the expected catalytic activity, cleaving an L-RNA substrate in a site-specific manner.

The ability of a polymerase ribozyme to amplify functional RNAs of the opposite handedness suggests that the Darwinian evolution of such a polymerase could be based on fitness criteria pertaining to the function of the enantio-RNA products that it synthesizes. Ultimately, if those products could also function as a cross-chiral polymerase, then it would enable a cross-chiral biology in which the two enantiomers of a biopolymer work in concert to propagate heritable information.

RESULTS

The hammerhead ribozyme is widely distributed in biology and is one of the best studied catalytic RNAs.[12,13] The minimal catalytic motif consists of three stem elements that are connected by three short single-stranded regions.[14,15] One of these single-stranded regions contains a single nucleotide that defines the cleavage site. Another contains a stretch of three purine nucleotides that position a general base to assist in catalysis.[16,17] The third contains a region of seven highly conserved nucleotides that are essential for catalysis,[18] including a key guanylate residue that functions as a general acid.[16,17]

A common format for RNA-catalyzed RNA cleavage by the hammerhead ribozyme places the region of seven highly conserved nucleotides, which has the sequence 5′-CUGAUGA-3′, within the "enzyme" strand, flanked by nucleotides that form two regions of Watson−Crick pairing with a separate RNA substrate (Figure 1c). This format was chosen for the present study, requiring the cross-chiral polymerase to synthesize both the enzyme portion of the hammerhead (plus strand) and its complement (minus strand), each from three component L-oligonucleotides (Figure 1d).

For both the plus and minus strands, the oligonucleotide at the 5′ end contained seven nucleotides and was fluorescently labeled; the other two component oligonucleotides contained six nucleotides and were chemically 5′-triphosphorylated. Thus, a total of four RNA-catalyzed joining events were required to complete the synthesis of the two strands. On the basis of prior studies, the polymerase ribozyme is expected to have a rate of 0.01−0.1 min^{-1} for these various joining reactions.[10]

Thermal cycling was carried out, employing an excess of D-polymerase ribozyme, 0.1 nM starting amount of the complete

https://doi.org/10.1021/jacs.1c09233
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minus-strand L-RNA, 1 μM each of the two fluorescently labeled L-oligonucleotides, and 0.5 μM each of the four unlabeled L-oligonucleotides. Due to the slow catalytic rate of the polymerase ribozyme compared to protein polymerases, the extension time in each cycle was 4 h, carried out at 10 °C. Denaturation was carried out at 64 °C for 2 s, with temperature ramping of 1.5 °C/s. Unlike the protein polymerases used in the PCR, the polymerase ribozyme is not thermostable. Instead, it too becomes denatured during the heating step but then refolds each time the temperature is reduced.

Following 24 thermal cycles, the yield of fluorescently labeled, full-length, plus-strand products was 2.0% relative to the starting amount of the fluorescently labeled 5′-oligonucleotide, which corresponds to ~200-fold more material than the starting amount of minus-strand template (Figure 2a,b). The yield of full-length, minus-strand products was 2.7% in the same reaction mixture. These yields are 4.1% and 5.4%, respectively, relative to the starting amount of the four unlabeled oligonucleotides. There was no detectable yield of either product in control reactions that were maintained at 10 °C for the same amount of time.

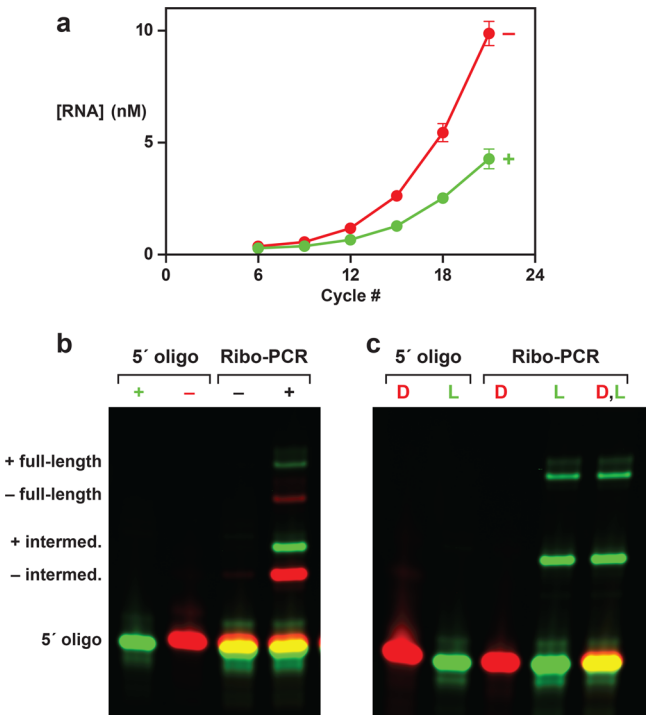

Figure 2. Cross-chiral amplification of RNA. (a) Thermal cycling was carried out to generate copies of both the plus- and minus-strand RNA (green and red, respectively). Values are based on three replicates, with error bars representing SE. (b) Products obtained after 24 cycles of ribo-PCR were analyzed by PAGE, in comparison to the unreacted 5′-oligonucleotide component of each strand and to a reaction mixture that was not thermocycled but instead maintained at 10 °C. (c) The D-polymerase amplifies L- but not D-RNA in the presence of either the chiral components or a racemic mixture of components. Only the plus strand of L- and D-RNA are labeled (false color green for fluorescein and red for cyanine-5, respectively). Reaction conditions: 10 μM D-ribozyme, 0.1 nM starting minus-strand RNA, 1 μM each 5′-oligonucleotide, either 0.1 μM (a) or 0.5 μM (b, c) each middle and 3′-oligonucleotide, 50 mM $MgCl_2$, and 0.9 M TPA at pH 8.3, with either 24 (a, b) or 30 (c) thermal cycles of 10 °C for 4 h and then 64 °C for 2 s.

An authentic standard of full-length, plus-strand material, which was prepared in a simple batch reaction using the D-polymerase and excess template, has the same gel mobility as the products generated by thermal cycling (Figure S1a). The plus-strand products were gel purified and analyzed by liquid chromatography/high-resolution mass spectrometry (LC/HRMS), which gave a mass of 7808.333 Da, matching the expected value of 7808.330 (Figure S1b).

Cross-chiral amplification was also carried out for 48 thermal cycles, comparing reactions with either none or 1 nM starting amount of the complete minus-strand RNA. By the 48th cycle, the yield of both plus- and minus-strand products had leveled off, with an excess of the minus strand throughout the amplification process (Figure S2). Following a lag period of ~10 cycles, reactions that contained no starting amount of template also generated both plus- and minus-strand products. There is a one base-pair overlap between the complementary oligonucleotide substrates (Figure 1d), which enables the cross-chiral polymerase, at a low level of efficiency,[10] to catalyze their joining to form full-length materials. These materials then serve as templates to initiate exponential amplification. The reaction with no starting template gives about half the yield of full-length products after 24 cycles compared to the template-seeded reaction. The time needed to complete 48 thermal cycles is 8 days, by which point only ~60% of the polymerase molecules remain intact (Figure S2). Also by that time, up to 30% of the oligonucleotide substrates have been consumed. Together, these two factors likely explain why the reaction levels off in the later cycles.

The plus-strand products were generated by thermal cycling in the same manner as above but instead using a biotinylated 3′-terminal oligonucleotide component of the plus strand to enable capture of the double-stranded products on streptavidin-coated beads. The minus strand was then removed by washing with alkali, and the plus strand was recovered by elution with hot formamide. Amplification was carried out in 80 μL volume in either the presence or absence of 1 nM starting amount of minus-strand L-RNA for 18 thermal cycles. The resulting recovered materials were tested for their ability to function as a hammerhead ribozyme in cleaving a corresponding L-RNA substrate. After 24 h of incubation, either 14% or 0.8% of the substrate was cleaved using materials obtained from the amplification reactions with or without starting minus-strand L-RNA, respectively (Figure 3). A positive control reaction using 200 nM L-hammerhead that had been prepared by solid-phase synthesis gave 82% cleavage under the same conditions, whereas no cleavage was detected in the absence of the hammerhead ribozyme.

The specific activity of the amplified products was determined by comparison to the synthetic standard. Eight different concentrations of synthetic L-hammerhead were tested in the RNA-cleavage reaction to obtain a saturation profile (Figure S3a), which in turn was used to predict the concentration of L-hammerhead obtained from cross-chiral amplification based on the observed RNA-cleavage activity. The true concentration of the full-length, plus-strand amplification products was determined based on their fluorescence intensity following separation by PAGE (Figure S3b). For three replicate amplification reactions, the ratio of actual to predicted concentration of the products was 98 ± 18%. Thus, the specific activity of the amplified materials is

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J. Am. Chem. Soc. XXXX, XXX, XXX−XXX

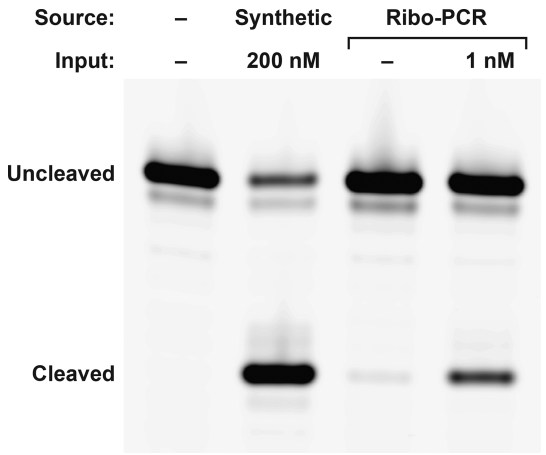

Figure 3. Functional products of cross-chiral amplification. L-Hammerhead ribozymes were produced either synthetically or by 18 cycles of ribo-PCR under the conditions described in Figure 2 but with 0.1 μM each middle and 3′-oligonucleotide, then used to cleave a 5′-labeled L-RNA substrate. Reaction conditions: 2 μM L-substrate and 20 mM $MgCl_2$ at pH 8.0 and 37 °C for 24 h.

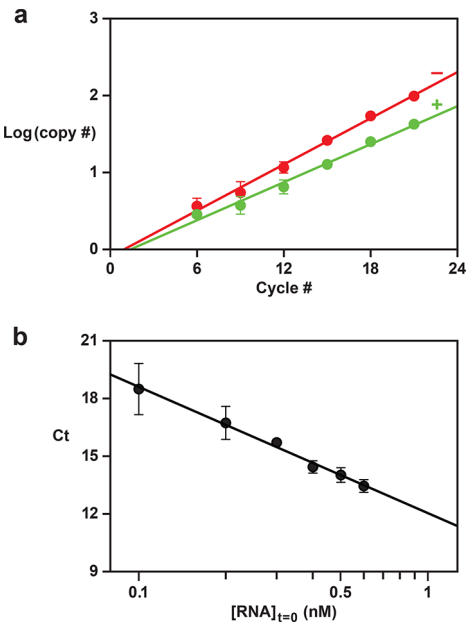

Figure 4. Cross-chiral exponential amplification of RNA. (a) The yield of plus- and minus-strand RNA (green and red, respectively) was determined as a function of thermal cycle number. (b) Number of thermal cycles required to reach a threshold of 2 nM products as a function of the starting concentration of minus-strand L-RNA. Reaction conditions as in Figure 2 but with 0.1 μM each middle and 3′-oligonucleotide. Values are based on three replicates, with error bars representing SE.

comparable to that of the chemically synthesized L-hammerhead.

It is possible that some of the full-length, plus-strand L-RNA was assembled incorrectly due to misincorporation of an oligonucleotide component. Looking more closely at the LC/HRMS data, there are small amounts of two other products, one having a mass of 7770.287 Da and present in 0.3% relative abundance and the other having a mass of 7792.339 Da and present in 0.1% relative abundance. These compounds correspond to "mutant" products, the first with the 3′-terminal oligonucleotide component replaced by another copy of the middle component (expected mass 7770.292) and the second with the middle component replaced by the middle component of the minus-strand (expected mass 7792.335).

Cross-chiral RNA amplification is chiroselective. When the D-polymerase ribozyme was given a mixture of the complete set of L- and D-oligonucleotide substrates, labeling the 5′ component of the D- and L-RNA plus strands with fluorescein and cyanine-5, respectively, the yield of L-RNA products was comparable to that seen with the L-substrates alone and there was no detectable yield of D-RNA products (Figure 2c).

To determine whether amplification is truly exponential, the starting amount of minus-strand L-RNA was varied and the yield of newly formed plus- and minus-strand L-RNA products was measured as a function of the number of thermal cycles. As above, the 5′ component of each strand was differentially labeled, enabling quantitation of both products from a common reaction mixture. A semilog plot of RNA copy number versus cycle number was linear for both measured products, consistent with exponential growth (Figure 4a). The slope of this plot corresponds to $\log(1 + f)$, where f is the fractional yield of product per cycle. The calculated values of f are 0.21 (r^2 = 0.97) and 0.26 (r^2 = 0.98) for the plus- and minus-strand, respectively. Thus, it requires 4−5 thermal cycles to achieve a doubling of the amplified materials.

As in standard PCR, one can determine the number of thermal cycles required to achieve a threshold level of amplification (the Ct value), which has a log−linear relationship to the starting concentration of material. These data in turn can be used to calculate the starting concentration of material in an unknown sample, based on the observed Ct value. Choosing a threshold value of 2 nM products and testing a range of starting concentrations for the minus-strand L-RNA of 0.1−0.6 nM, there was good log−linearity (Figure 4b; r^2 = 0.90). These results provide further evidence that RNA-catalyzed, cross-chiral amplification is truly exponential.

The spatial separation of individual PCR amplicons by "colony PCR" or "emulsion PCR" has provided the basis for impactful technologies, including for applications in next-generation DNA sequencing and high-throughput screening of DNA-encoded libraries. An emulsion PCR approach was applied to the cross-chiral polymerase ribozyme. A water-in-oil emulsion was generated, with the individual droplets having a mean diameter of 7.1 ± 1.5 μm (Figure 5a). Each droplet contained an average of 11 seed molecules of minus-strand L-RNA, together with the D-ribozyme and the L-oligonucleotide substrates required for exponential amplification. The aqueous droplets suspended in the oil phase remained stable for several days under the thermal cycling conditions. The products of emulsion-based amplification did not differ significantly from those obtained in the solution-phase reaction (Figure 5b,c).

DISCUSSION

Chemical processes that entail exponential amplification have the special property of generating abundant copies of a small amount of starting material, limited only by the supply of component building blocks. When the material being amplified carries information, then that information is also exponentially amplified. The propagation of heritable information provides the basis for Darwinian evolution in biology and has many biotechnological applications. All previous examples of the amplification of informational macromolecules have utilized materials of the standard biological chirality or in some cases

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J. Am. Chem. Soc. XXXX, XXX, XXX−XXX

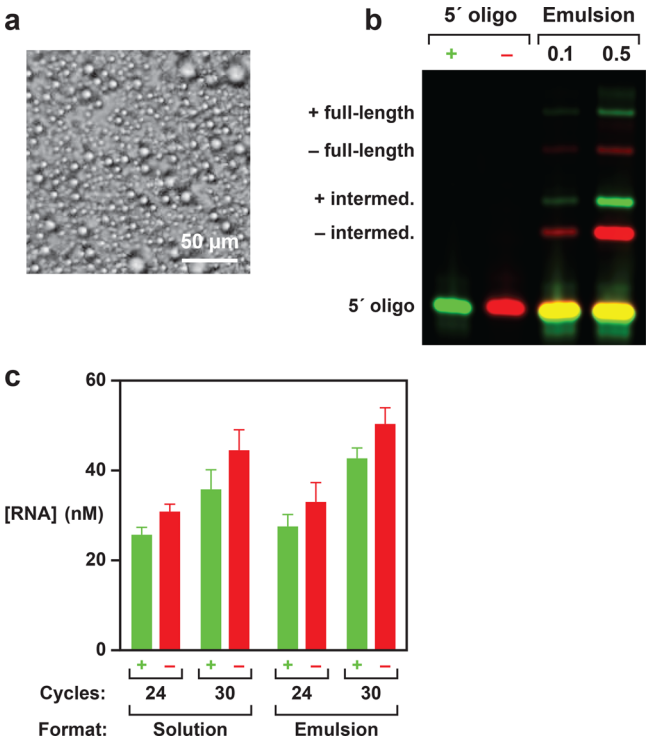

Figure 5. Emulsion-based, cross-chiral amplification. (a) Light microscopy image of droplets after 24 cycles of ribo-PCR. (b) Emulsion ribo-PCR with either 0.1 or 0.5 μM each middle and 3′-oligonucleotide component. (c) Comparison of solution- and emulsion-based ribo-PCR, initiated with 0.1 nM minus-strand RNA and 0.5 μM each middle and 3′-oligonucleotide component, carried out for either 24 or 30 cycles. Reaction conditions were otherwise as in Figure 2.

with a complete reversal of that chirality. The present study demonstrates that this need not be the case; the material being amplified and the material bringing about amplification can be of the opposite chirality.

Exponential amplification that depends on complementarity between a plus and minus strand (e.g., for nucleic acids) requires the synthesis of both strands to complete a cycle of amplification. The rate of synthesis of the two strands is usually not identical, leading to an excess of one of the two strands after repeated cycles. This asymmetry is apparent in the PCR when the two primers are not present in equal concentration[19] and in the *in vitro* amplification of RNA when one of the two strands is either more stable or more difficult to copy.[20,21] In the present study, there was an excess of minus strand over plus strand, with a fractional yield per cycle of 0.26 and 0.21, respectively. The oligonucleotide components of the two strands were present in equal concentration and have similar affinity for the complementary template. The predicted rate constants for the joining reactions are comparable for the two strands.[10] The only apparent advantage for the minus strand is that addition of the middle oligonucleotide appears to be more favorable, as evidenced by the higher yield of this intermediate compared to that of the plus strand (Figure 2b).

The complexity of the amplification reaction is modest, with only six component oligonucleotides required to complete the synthesis of the two strands. The cross-chiral polymerase is able to catalyze the RNA-templated joining of 5′-triphosphorylated RNA substrates of any length, although it operates more efficiently with short oligonucleotides compared to mononucleotides.[10] Thus, one might be able to use a larger number of shorter components than was the case here, even though that format would surely result in a lower fractional yield per thermal cycle and likely result in a lower fidelity of assembly. In the presence of the six component oligonucleotides, the L-hammerhead ribozyme was assembled with a fidelity of >99.5%. With a larger number of components there would be more opportunity for mismatched incorporation, as well as for the oligonucleotide substrates to pair with each other in competition with their binding to the template. It appears that the best opportunity to increase the complexity of cross-chiral amplification will be to increase the efficiency of the polymerase ribozyme through further *in vitro* evolution.

It is notable that oligonucleotide components having the same chirality as the polymerase are neither substrates for the polymerase nor inhibitors of cross-chiral amplification (Figure 2c). The interaction between plus- and minus-strand components depends on Watson−Crick pairing, which cannot occur for oligonucleotides of the opposite handedness.[22,23] The cross-chiral polymerase recognizes its substrates entirely through tertiary interactions, which are highly enantioselective and do not involve Watson−Crick pairing.[9,10] Thus, the D- and L-polymerases can operate simultaneously and without cross-interference within a common reaction mixture.

Under evolutionary pressure, the D-polymerase could be selected based on the properties of the L-RNA it produces, while the L-polymerase could be selected based on the properties of the D-RNA it produces. The L- and D-RNA products need not have the same sequence or even the same function. A special case would involve the synthesis of the L-polymerase by the D-polymerase and *vice versa*. Even then, the two polymerases need not have the same sequence. Together, however, they could provide the basis for a cross-chiral genetic system, with both hands working together to propagate heritable information. It should be noted, however, that such a system would be metastable and that chiral symmetry breaking would occur once either hand of evolving RNAs gained the ability to operate as a homochiral system.

■ CONCLUSION

A cross-chiral RNA enzyme with RNA-dependent RNA polymerase activity is able to catalyze a special form of the polymerase chain reaction in which the enzyme amplifies RNA molecules of the opposite chirality. The D-RNA enzyme catalyzes the amplification of L-RNAs using L-oligonucleotide building blocks. Amplification is truly exponential, and the amplified products exhibit the mirror-image function of their D-RNA counterparts.

The ability to carry out cross-chiral exponential amplification of functional RNAs opens the door to directed evolution experiments where the cross-chiral polymerase is selected based on the function of the RNA it produces. This approach has proven to be powerful in optimizing the activity of a homochiral RNA polymerase ribozyme.[5,24,25] By comparison, the cross-chiral polymerase is a relatively young enzyme that has not had yet the benefit of extensive evolutionary optimization.

■ EXPERIMENTAL SECTION

See Supporting Information methods section for sources of materials used in this study and for methods used to prepare synthetic oligonucleotides and the 27.3t form of the D-polymerase ribozyme.

https://doi.org/10.1021/jacs.1c09233
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Cross-Chiral Amplification of RNA. A mixture of RNA components at twice their final concentration, which contained 20 μM D-polymerase, 0.2−2 nM minus-strand L-RNA, 2 μM each 5′-oligonucleotide, 1.0 μM each middle and 3′-oligonucleotide, and 50 mM Tris-HCl (pH 8.3), was heated at 95 °C for 3 min, then slowly cooled to 23 °C. Amplification was initiated by adding to this mixture an equal volume of 100 mM $MgCl_2$, 1.8 M TPA, and 50 mM Tris-HCl (pH 8.3), followed by thermal cycles of 10 °C for 4 h and then 64 °C for 2 s. The thermal cycler has a temperature ramp rate of 1.5 °C per second, thus requiring 30−40 s to ramp up to 64 °C or ramp down to 10 °C.

The reaction was quenched by adding EDTA in excess of Mg^{2+}, then the reaction materials were precipitated with ethanol and analyzed by PAGE. To ensure complete recovery of the fluorescently labeled oligonucleotides during ethanol precipitation, 0.6 mg/mL of glycogen was added to the mixture prior to precipitation with 0.3 M NaOAc and 2.5 volumes of ethanol. For reactions that used a 5′-oligonucleotide with upstream photocleavable linker, the products were photolyzed (350 nm) at 4 °C for 20 min prior to analysis. Before loading of the gel, the reaction products were incubated in the presence of 95% formamide and 25 mM EDTA at 95 °C for 5 min to separate the complementary strands of RNA, which remained separated in a gel containing 8 M urea.

Catalytic Activity of the Amplified Products. After quenching the amplification reaction with EDTA, the mixture was added to 10 μg of streptavidin-coated magnetic beads per 1 pmol of biotinylated 3′-oligonucleotide and incubated with gentle agitation at 23 °C for 1 h. The beads had been preblocked by incubating with 1 mg/mL tRNA. The bead-bound materials were washed twice with 300 mM NaCl, 1 mM EDTA, and 10 mM Tris-HCl (pH 8.0), then the minus-strand RNA was removed by washing with 20 mM NaOH, 1 mM EDTA, and 0.05% Tween-20. The remaining plus-strand RNA was washed with 8 M urea, 1 mM EDTA, and 10 mM Tris-HCl (pH 8.0), then eluted with 95% formamide and 0.25 mM EDTA at 95 °C, followed by ethanol precipitation. The precipitated materials were dissolved in an aqueous solution containing the L-RNA substrate for the hammerhead ribozyme, lyophilized, and redissolved in 50 mM Tris-HCl (pH 8.0), which was heated at 65 °C for 1 min, then slowly cooled to 23 °C. The hammerhead cleavage reaction was initiated by adding 20 mM $MgCl_2$ and 50 mM Tris-HCl (pH 8.0), then incubated at 37 °C for 24 h. The reaction was quenched with EDTA, precipitated with ethanol, and the products were analyzed by PAGE.

LC/HRMS Analysis. LC/HRMS was performed by Novatia (Newtown, PA) using 50 pmol of full-length, plus-strand amplification products that had been purified by PAGE. Analyses were performed by electrospray ionization LC/MS on the Oligo HTCS platform, which achieves mass accuracy of 0.01−0.02%.

Emulsion-Based Amplification. The oil phase contained KF-6038, mineral oil, and DMF-A-6cs (4:20:76 w/w/w), which was prepared gravimetrically and gently rotated for 2 h prior to use. Water-in-oil emulsions were prepared using a homogenizer to agitate 50 μL of the cross-chiral amplification mixture, 200 μL of the oil mixture, and a single polytetrafluoroethylene ball (0.125 in. diameter) in a 1.7 mL screw-cap microtube at 2500 rpm for 65 s. The emulsion was transferred through a wide bore tip to a reaction tube and subjected to either 24 or 30 thermal cycles of 10 °C for 4 h and then 64 °C for 2 s. The top oil layer was removed, then the reaction mixture was quenched with EDTA, diluted with water, and extracted three times with chloroform. The products were analyzed by PAGE.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/jacs.1c09233.

Additional methods information, a table listing the sequences of all nucleic acids used in this study, a figure showing the electrophoretic mobility and LC/HRMS analysis of the L-hammerhead product obtained from cross-chiral amplification, a figure showing the yield of amplification products over 48 thermal cycles, and a figure showing the data used to determine the specific activity of the L-hammerhead product relative to a synthetic standard (PDF)

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Author Contributions

The manuscript was written through contributions of both authors. Both authors have given approval to the final version of the manuscript.

Funding

This work was supported by Grant 80NSSC19K0481 from NASA, Grant 078731668 from NSF, and Grant 287624 from the Simons Foundation.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The authors are grateful to Wesley Cochrane for assistance with emulsion-based amplification.

■ ABBREVIATIONS

PAGE, denaturing polyacrylamide gel electrophoresis; TPA, tetrapropylammonium chloride

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https://doi.org/10.1021/jacs.1c09233
J. Am. Chem. Soc. XXXX, XXX, XXX−XXX