REFERENCES AND NOTES

1. A. E. Strong and E. P. McClain, *Bull. Am. Meteorol. Soc.* 65, 138 (1984).
2. A. E. Strong, in preparation.
3. ———, *Geofis. Int.* 23, 129 (1984).
4. E. M. Rasmusson and J. M. Wallace, *Science* 222, 1195 (1983).
5. S. G. H. Philander, *J. Geophys. Res.* 83, 3679 (1978).
6. R. Legeckis, W. Pichel, G. Nesterczuk, *Bull. Am. Meteorol. Soc.* 64, 133 (1983).
7. H. R. Gordon *et al.*, *Appl. Opt.* 22, 20 (1983).
8. R. M. Laurs, P. C. Fiedler, D. R. Montgomery, *Deep Sea Res.* 31, 1085 (1984).
9. S. R. Schneider, *NASA Tech. Pap.* 1827 (1981).
10. A. Rango and J. Martinec, *Nordic Hydrol.* 10, 225 (1979).
11. J. Martinec, A. Rango, E. Major, *NASA Ref. Publ.* 1100 (1983).
12. M. Matson and D. R. Wiesnet, *Nature (London)* 289, 451 (1981).
13. K. F. Dewey and R. Heim, Jr., *NOAA Tech. Rep. NESS-87* (1981).
14. D. R. Wiesnet, D. F. McGinnis, J. A. Pritchard, *Wat. Resour. Bull.* 10, 1040 (1974).
15. C. P. Berg and D. F. McGinnis, paper presented at the 46th Annual Meeting of the American Society of Photogrammetry, St. Louis, MO (1980).
16. C. P. Berg *et al.*, in *Satellite Hydrology*, M. Deutsch, D. R. Weisnet, A. Rango, Eds. (American Water Resources Association, Sioux Falls, SD, 1981), pp. 309–315.
17. *Red River of the North Post Flood Report 1978* (U.S. Army Corps of Engineers, St. Paul, MN, 1979).
18. D. F. McGinnis and S. R. Schneider, *Photogramm. Eng. Remote Sens.* 44 (No. 1), 57 (1978).
19. R. A. Scofield, paper presented at the Tenth Conference on Weather Forecasting and Analysis, Clearwater Beach, FL (25–29 June 1984).
20. ——— and L. E. Spayd, Jr., *NOAA Tech. Memo. NESDIS 8* (1984).
21. L. E. Spayd, Jr., and R. A. Scofield, *NOAA Tech. Memo. NESDIS 5* (1984).
22. J. F. Bartholic and R. A. Sutherland, *Proc. Fla. State Hort. Soc.* 91, 334 (1978).
23. J. D. Martsolf, paper presented at the Conference on Cooperative Climate, Tallahassee, FL (1983).
24. ———, *Citrus Ind.* 64, 24 (1983).
25. C. R. Griffith *et al.*, *Mon. Weather Rev.* 106, 1153 (1978).
26. L. J. Heitkemper, paper presented at the International Conference on Interactive Information and Processing Systems for Meteorology, Oceanography, and Hydrology, Los Angeles (7 to 11 January 1985).
27. J. F. Moses, paper presented at the International Conference on Interactive Information and Processing Systems for Meteorology, Oceanography, and Hydrology, Los Angeles (7 to 11 January 1984).
28. E. C. Barrett and D. W. Martin, *The Use of Satellite Data in Rainfall Monitoring* (Academic Press, New York, 1981).
29. P. A. Davis and J. D. Tarpley, *J. Clim. Appl. Meteorol.* 22, 369 (1983).
30. J. D. Tarpley, *J. Appl. Meteorol.* 18, 1172 (1979).
31. C. G. Justus and J. D. Tarpley, paper presented at the American Meteorological Society Atmospheric Radiation Conference, Baltimore (1983).
32. C. J. Tucker, J. A. Gatlin, S. R. Schneider, *Photogramm. Eng. Remote Sens.* 50, 53 (1984).
33. J. D. Tarpley, S. R. Schneider, R. L. Money, *J. Clim. Appl. Meteorol.* 23, 491 (1984).
34. S. R. Schneider and D. F. McGinnis, Jr., paper presented at the Eighth International Symposium on Machine Processing of Remotely Sensed Data, Purdue University (1982).
35. C. J. Tucker, J. R. G. Townshend, T. E. Goff, *Science* 227, 369 (1985).

**Research Articles**

# The Intervening Sequence RNA of *Tetrahymena* Is an Enzyme

ARTHUR J. ZAUG AND THOMAS R. CECH

**A shortened form of the self-splicing ribosomal RNA (rRNA) intervening sequence of *Tetrahymena thermophila* acts as an enzyme in vitro. The enzyme catalyzes the cleavage and rejoining of oligonucleotide substrates in a sequence-dependent manner with $K_m = 42$ $\mu M$ and $k_{cat} = 2$ min$^{-1}$. The reaction mechanism resembles that of rRNA precursor self-splicing. With pentacytidylic acid as the substrate, successive cleavage and rejoining reactions lead to the synthesis of polycytidylic acid. Thus, the RNA molecule can act as an RNA polymerase, differing from the protein enzyme in that it uses an internal rather than an external template. At *p*H 9, the same RNA enzyme has activity as a sequence-specific ribonuclease.**

IN RNA SELF-SPLICING, THE FOLDED STRUCTURE OF AN RNA molecule mediates specific cleavage-ligation reactions (*1–5*). Self-splicing exemplifies intramolecular catalysis (*6*) in that the reactions are accelerated many orders of magnitude beyond the basal chemical rate (*7, 8*). The reactions are highly specific, as seen in the choice of a free guanosine nucleotide as a substrate in the self-splicing of the *Tetrahymena* ribsomal RNA precursor (pre-rRNA) and other RNA's containing group I intervening sequences (*1–3, 7*). Furthermore, the cleavage-ligation activity mediates a series of splicing, cyclization, and reverse cyclization reactions, suggesting that the active site is preserved in each reaction (*9, 10*). However, the RNA is cleaved and rejoined during self-splicing; because the RNA is not regenerated in its original form at the end of the reaction, it is not an enzyme. The RNA moiety of ribonuclease P, the enzyme responsible for cleaving transfer RNA (tRNA) precursors to generate the mature 5′ end of the tRNA, has been the only example of an RNA molecule that meets all criteria of an enzyme (*11–13*).

Following self-splicing of the *Tetrahymena* rRNA precursor, the excised IVS RNA (*14*) undergoes a series of RNA-mediated cyclization and site-specific hydrolysis reactions. The final product, the L − 19 IVS RNA, is a linear molecule that does not have the first 19 nucleotides of the original excised IVS RNA (*9*). We interpreted the lack of further reaction of the L − 19 species as an indication that all potential reaction sites on the molecule that could reach its active site (that is, intramolecular substrates) had been consumed; and we argued that the activity was probably unperturbed (*9*). We have now tested this by adding oligonucleotide substrates to the L − 19 IVS RNA. We find that each IVS RNA molecule can catalyze the cleavage and rejoining of many oligonucleotides. Thus, the L − 19 IVS RNA is a true enzyme. Although the enzyme can act on RNA molecules of large size and complex sequence, we have found that studies with simple oligoribonucleotides like $pC_5$ (pentacytidylic acid) have been most valuable in

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revealing the minimum substrate requirements and reaction mechanism of this enzyme. These studies are presented below.

**The L − 19 IVS RNA catalyzes the cleavage and rejoining of oligonucleotides.** Unlabeled L − 19 IVS RNA was incubated with 5′-$^{32}$P–labeled $pC_5$ in a solution containing 20 m*M* $MgCl_2$, 50 m*M* tris-HCl, *p*H 7.5. The $pC_5$ was progressively converted to oligocytidylic acid with both longer and shorter chain length than the starting material (Fig. 1A). The longer products extended to at least $pC_{30}$, as judged by a longer exposure of an autoradiogram such as that shown in Fig. 1A. The shorter products were exclusively $pC_4$ and $pC_3$. Incubation of $pC_5$ in the absence of the L − 19 IVS RNA gave no reaction (Fig. 1C).

Phosphatase treatment of a 60-minute reaction mixture resulted in the complete conversion of the $^{32}$P radioactivity to inorganic phosphate, as judged by polyethyleneimine thin-layer chromatography (TLC) in 1*M* sodium formate, *p*H 3.5 (*15*). Thus, the 5′-terminal phosphate of the substrate does not become internalized during the reaction, and the substrate is being extended on its 3′ end to form the larger oligonucleotides. When $C_5\overset{*}{p}C$ was used as the substrate and the products were treated with ribonuclease $T_2$ or ribonuclease A, the $^{32}$P radioactivity was totally converted to $C\overset{*}{p}$ (*15*). Thus, the linkages being formed in the reaction are exclusively 3′,5′-phosphodiester bonds. The products of the $C_5\overset{*}{p}C$ reaction were totally resistant to phosphatase treatment.

The reaction was specific for ribonucleotides, no reaction taking place with d-$pC_5$ (Fig. 1B) or d-$pA_5$ (*15*). Among the oligoribonucleotides, $pU_6$ was a much poorer substrate than $pC_5$ or $pC_6$ (Fig. 1D), and $pA_6$ gave no reaction (*15*).

No reaction occurred when magnesium chloride was omitted. The enzyme activity was approximately constant in the range 5 to 40 m*M* $MgCl_2$ (*15*). The 20 m*M* concentration was routinely used to circumvent the potential effect of chelation of $Mg^{2+}$ by high concentrations of oligonucleotide substrates.

The L − 19 IVS RNA is regenerated after each reaction, such that each enzyme molecule can react with many substrate molecules. For example, quantitation of the data shown in Fig. 1G revealed that 16 pmol of enzyme converted 1080 pmol of $pC_5$ to products in 60 minutes. Such numbers underestimate the turnover number of the enzyme; because the initial products are predominantly $C_6$ and $C_4$, it is likely that the production of chains of length greater than six or less than four involves two or more catalytic cycles. Quantitation of the amount of radioactivity in each product also provides some indication of the reaction mechanism. At early reaction times, the amount of radioactivity (a measure of numbers of chains) in products larger than $pC_5$ is approximately equal to that found in $pC_4$ plus $pC_3$, consistent with a mechanism in which the total number of phosphodiester bonds is conserved in each reaction. As the reaction proceeds, however, the radioactivity distribution shifts toward the smaller products. This is most likely due to a competing hydrolysis reaction also catalyzed by the L − 19 IVS RNA, as described below.

The rate of conversion of 30 μ*M* $pC_5$ to products increases linearly with L − 19 IVS RNA enzyme concentration in the range 0.06 to 1.00 μ*M* (*15*). At a fixed enzyme concentration (Fig. 1, E to G), there is a hyperbolic relation between the reaction rate and the concentration of $pC_5$. The data are fit by the Michaelis-Menten rate law in Fig. 2. The resulting kinetic parameters are $K_m$ = 42 μ*M* and $k_{cat}$ = 1.7 $min^{-1}$.

The stability of the enzyme was determined by preliminary incubation at 42°C for 1 hour in the presence of $Mg^{2+}$ (standard reaction conditions) or for 18 hours under the same conditions but without $Mg^{2+}$. In both cases, the incubated enzyme had activity indistinguishable from that of untreated enzyme tested in parallel, and no degradation of the enzyme was observed on polyacrylamide gel electrophoresis (*15*). Thus, the L − 19 IVS RNA is not a good substrate. The enzyme is also stable during storage at −20°C for periods of months. The specific activity of the enzyme is consistent between preparations.

**Covalent intermediate.** When $C_5\overset{*}{p}$ was used as a substrate, radioactivity became covalently attached to the L − 19 IVS RNA (Fig. 3A) (*16*). This observation, combined with our previous knowledge of the mechanism of IVS RNA cyclization (*9, 10, 17,*

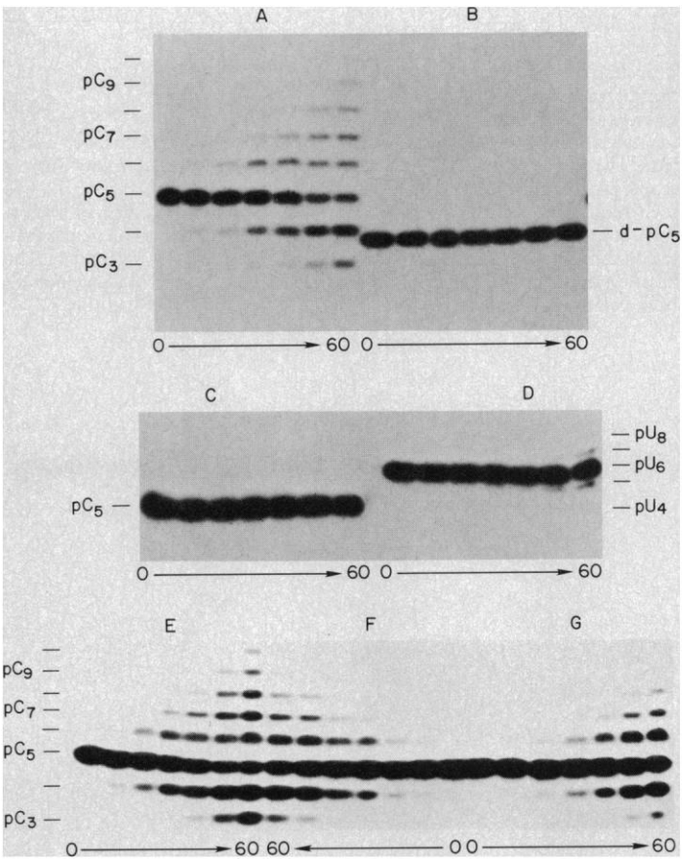


Fig. 1. The L − 19 IVS RNA catalyzes the cleavage and rejoining of oligoribonucleotide substrates; (A) 10 μ*M* $pC_5$ and (B) 10 μ*M* d-$pC_5$, both with 1.6 μ*M* L − 19 IVS RNA; (C) 45 μ*M* $pC_5$ in the absence of L − 19 IVS RNA; (D) 45 μ*M* $pU_6$ with 1.6 μ*M* L − 19 IVS RNA; (E) 10 μ*M* $pC_5$, (F) 50 μ*M* $pC_5$ and (G) 100 μ*M* $pC_5$, all with 1.6 μ*M* L − 19 IVS RNA. Oligonucleotides were 5′-end labeled by treatment with [γ-$^{32}$P]ATP and polynucleotide kinase; they were diluted with unlabeled oligonucleotide of the same sequence to keep the amount of radioactivity per reaction constant. The L − 19 IVS RNA was synthesized by transcription and splicing in vitro. Supercoiled pSPTT1A3 DNA (*30*) was cut with Eco RI and then transcribed with SP6 RNA polymerase (*31*) for 2 hours at 37°C in a solution of nucleoside triphosphates (0.5 m*M* each), 6 m*M* $MgCl_2$, 4 m*M* spermidine, 10 m*M* dithiothreitol, 40 m*M* tris-HCl, *p*H 7.5, with 100 units of SP6 RNA polymerase per microgram of plasmid DNA. Then NaCl was added to a final concentration of 240 m*M* and incubation was continued at 37°C for 30 minutes to promote excision and cyclization of the IVS RNA. Nucleic acids were precipitated with three volumes of ethanol and redissolved in 50 m*M* CHES, *p*H 9.0; $MgCl_2$ was added to a final concentration of 20 m*M*, and the solution was incubated at 42°C for 1 hour to promote site-specific hydrolysis of the circular IVS RNA to give L − 19 IVS RNA (*9*). The reaction was stopped by the addition of EDTA to 25 m*M*. The L − 19 IVS RNA was purified by preparative gel electrophoresis and Sephadex G-50 chromatography. Labeled oligonucleotides were incubated with unlabeled L − 19 IVS RNA at 42°C in 20 m*M* $MgCl_2$, 50 m*M* tris, *p*H 7.5, for 0, 1, 2, 5, 10, 30, and 60 minutes. Reactions were stopped by the addition of EDTA to a final concentration of 25 m*M*. Products were analyzed by electrophoresis in a 20 percent polyacrylamide, 7*M* urea gel, autoradiograms of which are shown.

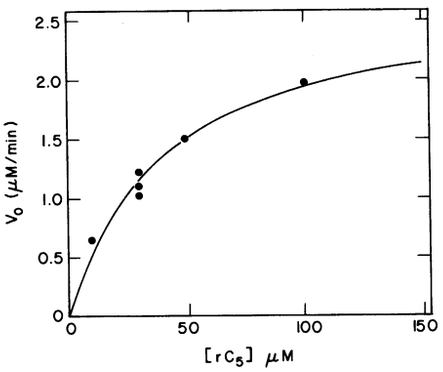


Fig. 2. Kinetics of conversion of $pC_5$ to larger and smaller oligonucleotides with 1.6 $\mu M$ L − 19 IVS RNA. Products were separated by polyacrylamide gel electrophoresis. With the autoradiogram as a guide, the gel was cut into strips and the radioactivity in each RNA species was determined by liquid scintillation counting. The amount of reaction at each time was taken as the radioactivity in $pC_3 + pC_4 + pC_6 + pC_7 + \ldots$ divided by the total radioactivity in the lane. The initial velocity of product formation, $V_o$, was determined from a semilogarithmic plot of the fraction of reaction as a function of time. $V_o$ was then plotted as a function of substrate concentration; the line is a least-squares fit to the Michaelis-Menten equation. The resulting kinetic parameters are $K_m = 42\ \mu M$, $V_{max} = 2.8\ \mu M\ min^{-1}$, and $k_{cat} = 1.7\ min^{-1}$. The kinetic parameters for the first and second steps in the reaction have not yet been determined separately.

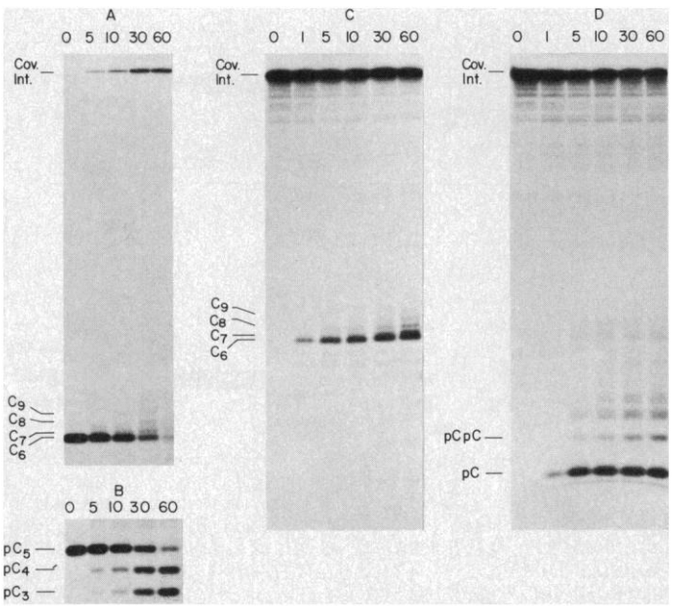


Fig. 3. Formation and resolution of the covalent enzyme-substrate intermediate. (A) To drive the formation of the covalent L − 19 IVS RNA-substrate intermediate, 8.5 n$M$ $C_5\overset{*}{p}C$ was treated with 0.16 $\mu M$ L − 19 IVS RNA under standard reaction conditions for 0 to 60 minutes. (B) $\overset{*}{p}C_5$ (0.01 $\mu M$) was reacted with 0.16 $\mu M$ L − 19 IVS RNA. Cleavage occurred normally, but there was very little rejoining. (C) Labeled covalent intermediate was prepared as in (A) (60 minutes) and purified by electrophoresis in a 4 percent polyacrylamide, 8$M$ urea gel. It was then incubated with 10 $\mu M$ unlabeled $C_5$ under standard reaction conditions for 0 to 60 minutes. The product designated $C_6$ comigrated with labeled $C_6$ marker (not shown). (D) Isolated covalent intermediate as in (C) was incubated under site-specific hydrolysis conditions (20 m$M$ $MgCl_2$, 50 m$M$ CHES, $p$H 9.0) at 42°C for 0 to 60 minutes. Positions of labeled mono- and dinucleotide markers are indicated. In the 10- and 30-minute lanes of (A) and the 10-, 30-, and 60-minute lanes of (C), band compression (reduced difference in electrophoretic mobility) is seen between $C_6$ and $C_7$ and to a lesser extent between $C_7$ and $C_8$. This is due to the absence of a 5′ phosphate. Thus, the charge-to-mass ratio is increasing with chain length, whereas with 5′-phosphorylated oligonucleotides the charge-to-mass ratio is independent of chain length. When such products were phosphorylated by treatment with polynucleotide kinase and ATP, the distribution was converted to the normal spacing as in Fig. 1 (*15*).

*18*), led to a model for the reaction mechanism involving a covalent enzyme-substrate intermediate (Fig. 4).

This reaction pathway is supported by analysis of reactions in which a trace amount of $\overset{*}{p}C_5$ was incubated with a large molar excess of L − 19 IVS RNA. The cleavage reaction occurred with high efficiency, as judged by the production of $\overset{*}{p}C_4$ and $\overset{*}{p}C_3$, but there was very little synthesis of products larger than the starting material (Fig. 3B; compare to Fig. 1A). These data are easily interpreted in terms of the proposed reaction pathway. The first step, formation of the covalent intermediate with release of the 5′-terminal fragment of the oligonucleotide, is occurring normally. The first step consumes all the substrate, leaving insufficient $C_5$ to drive the second transesterification reaction.

The model shown in Fig. 4 was tested by isolating the covalent enzyme-substrate complex prepared by reaction with $C_5\overset{*}{p}C$ and incubating it with unlabeled $C_5$. A portion of the radioactivity was converted to oligonucleotides with the electrophoretic mobility of $C_6$, $C_7$, $C_8$, and higher oligomers (Fig. 3C). In a confirmatory experiment, the covalent complex was prepared with unlabeled $C_5$ and reacted with $\overset{*}{p}C_5$. Radioactivity was again converted to a series of higher molecular weight oligonucleotides (*15*). In both types of experiments the data are readily explained if the covalent complex is a mixture of L − 19 IVS RNA's terminating in . . .GpC, . . .GpCpC, . . .GpCpCpC, and so on. Because they can react with $C_5$ to complete the catalytic cycle, these covalent enzyme-substrate complexes are presumptive intermediates in the reaction (Fig. 4). A more detailed analysis of the rate of their formation and resolution is needed to evaluate whether or not they are kinetically competent to be intermediates. We can make no firm conclusion about that portion of the enzyme-substrate complex that did not react with $C_5$. This unreactive RNA could be a covalent intermediate that was denatured during isolation such that it lost reactivity, or it could represent a small amount of a different enzyme-substrate complex that was nonproductive and therefore accumulated during the reaction.

The $G^{414}$-$A^{16}$ linkage in the C IVS RNA, the $G^{414}$-$U^{20}$ linkage in the C′ IVS RNA, and the $G^{414}$-$U^{415}$ linkage in the pre-rRNA are unusual phosphodiester bonds in that they are extremely labile to alkaline hydrolysis, leaving 5′ phosphate and 3′-hydroxyl termini (*9*, *19*). We therefore tested the lability of the $G^{414}$-C linkage in the covalent enzyme-substrate intermediate by incubation at $p$H 9.0 in a $Mg^{2+}$-containing buffer. This treatment resulted in the release of products that comigrated with pC and pCpC markers and larger products that were presumably higher oligomers of pC (Fig. 3D). Thin-layer chromatography was used to confirm the identity of the major products (*15*). In those molecules that released pC, the release was essentially complete in 5 minutes. Approximately half of the covalent complex was resistant to the $p$H 9.0 treatment. Once again, we can make no firm conclusion about the molecules that did not react. The lability of the $G^{414}$–C bond forms the basis for the L − 19 IVS RNA acting as a ribonuclease (Fig. 4).

**A competitive inhibitor.** Deoxy $C_5$, which is not a substrate for L − 19 IVS RNA–catalyzed cleavage, inhibits the cleavage of $pC_5$ (Fig. 5A). Analysis of the rate of the conversion of $pC_5$ to $pC_4$ and $pC_3$ as a function of d-$C_5$ concentration is summarized in Fig. 5, B and C. The data indicate that d-$C_5$ is a true competitive inhibitor with the inhibition constant $K_i = 260\ \mu M$. At 500 $\mu M$, d-$A_5$ inhibits the reaction only 16 percent as much as d-$C_5$. Thus, inhibition by d-$C_5$ is not some general effect of introducing a deoxyoligonucleotide into the system but depends on sequence.

The formation of the covalent enzyme-substrate intermediate (EpC) can be represented as

$$E + C_5 \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} E \cdot C_5 \xrightarrow{k_2} EpC + C_4$$

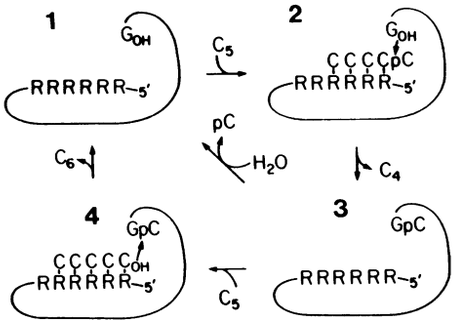


Fig. 4. Model for the enzymatic mechanism of the L − 19 IVS RNA. The RNA catalyzes cleavage and rejoining of oligo(C) by the pathway **1** → **2** → **3** → **4** → **1**. The L − 19 IVS RNA enzyme (**1**) is shown with the oligopyrimidine binding site (RRRRRR, six purines) near its 5′ end and $G^{414}$ with a free 3′-hydroxyl group at its 3′ end. The complex folded core structure of the molecule (*23, 24, 32*) is simply represented by a curved line. The enzyme binds its substrate ($C_5$) by Watson-Crick base-pairing to form the noncovalent enzyme-substrate complex (**2**). Nucleophilic attack by $G^{414}$ leads to formation of the covalent intermediate (**3**). With the pentanucleotide $C_5$ as substrate, the covalent intermediate is usually loaded with a single nucleotide, as shown; with substrates of longer chain length, an oligonucleotide can be attached to the 3′ end of $G^{414}$. If $C_5$ binds to the intermediate (**3**) in the manner shown in (**4**), transesterification can occur to give the new product $C_6$ and regenerate the enzyme (**1**). Note that all four reactions in this pathway are reversible. When acting as a ribonuclease, the L − 19 IVS RNA follows the pathway **1** → **2** → **3** → **1**. The covalent intermediate (**3**) undergoes hydrolysis, releasing the nucleotide or oligonucleotide attached to its 3′ end (in this case pC) and regenerating the enzyme (**1**).

If $k_{-1} >> k_2$, then $K_m = k_{-1}/k_1$, the dissociation constant for the noncovalent E · $C_5$ complex. The observation that the $K_i$ for d-$C_5$ is within an order of magnitude of the $K_m$ for $C_5$ can then be interpreted in terms of d-$C_5$ and $C_5$ having similar binding constants for interaction with the active site on the enzyme. This fits well with the idea that the substrate binds to an oligopurine ($R_5$) sequence in the active site primarily by Watson-Crick base-pairing, in which case the $C_5 \cdot R_5$ duplex and the d-$C_5 \cdot R_5$ duplex should have similar stability.

**Enzyme mechanism and its relation to self-splicing.** The stoichiometry of the reaction products (equimolar production of oligonucleotides smaller than and larger than the starting material), the lack of an ATP or GTP (adenosine triphosphate; guanosine triphosphate) energy requirement, the involvement of a covalent intermediate, the specificity for oligoC substrates, and the competitive inhibition by d-$C_5$ lead to a model for the enzyme mechanism (Fig. 4). The L − 19 IVS RNA is proposed to bind the substrate noncovalently by hydrogen-bonded base-pairing interactions. A transesterification reaction between the 3′-terminal guanosine residue of the enzyme and a phosphate ester of the substrate then produces a covalent enzyme-substrate intermediate.

Transesterification is expected to be highly reversible. If the product $C_4$ rebinds to the enzyme, it can attack the covalent intermediate and reform the starting material, $C_5$. Early in the reaction, however, the concentration of $C_5$ is much greater than the concentration of $C_4$; if $C_5$ binds and attacks the covalent intermediate, $C_6$ is produced (Fig. 4). The net reaction is 2 $C_5 \rightarrow C_6 + C_4$. The products are substrates for further reaction, for example, $C_6 + C_5 \rightarrow C_7 + C_4$ and $C_4 + C_5 \rightarrow C_3 + C_6$. The absence of products smaller then $C_3$ is explicable in terms of the loss of binding

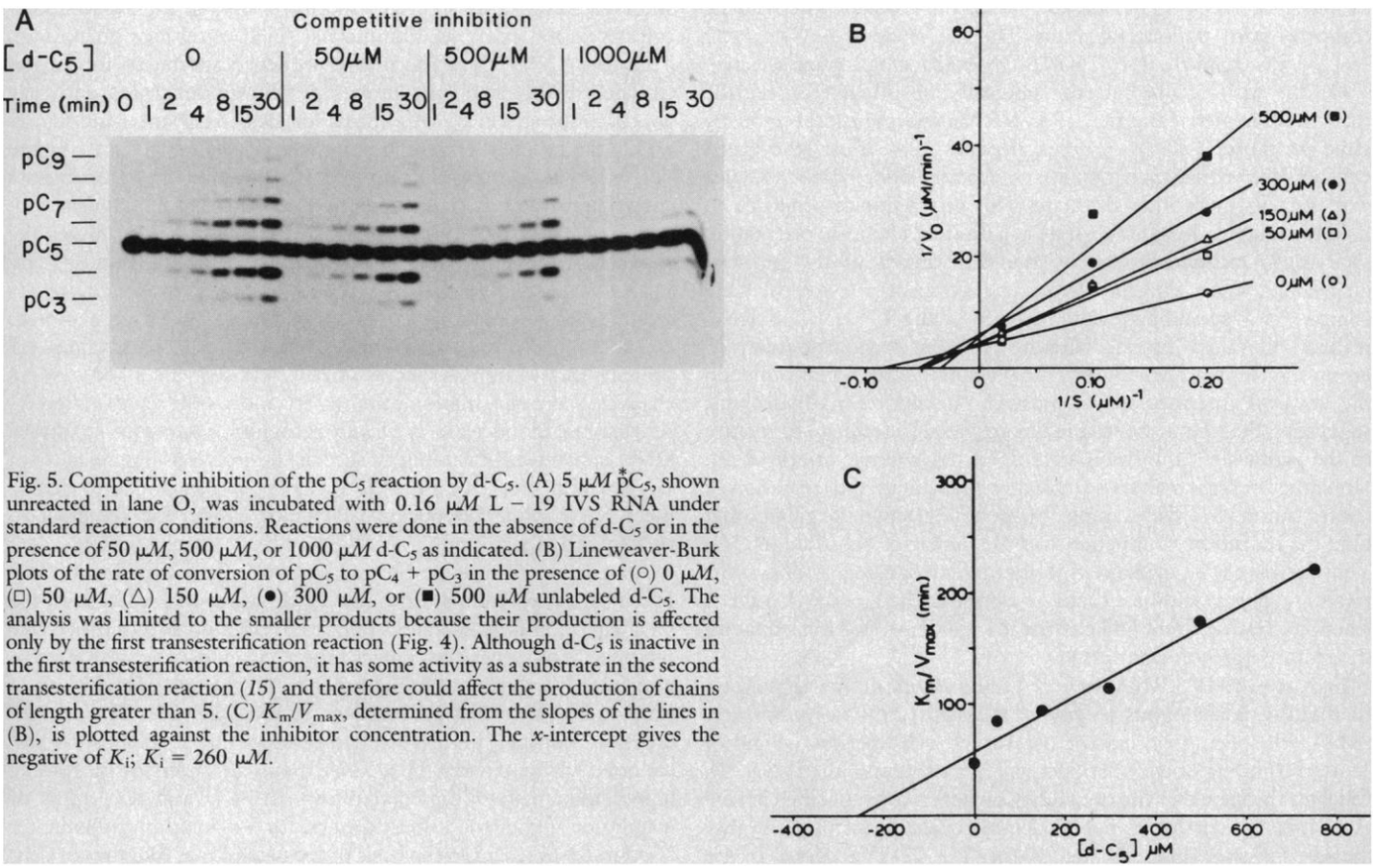


Fig. 5. Competitive inhibition of the $pC_5$ reaction by d-$C_5$. (A) 5 μ*M* $^*pC_5$, shown unreacted in lane O, was incubated with 0.16 μ*M* L − 19 IVS RNA under standard reaction conditions. Reactions were done in the absence of d-$C_5$ or in the presence of 50 μ*M*, 500 μ*M*, or 1000 μ*M* d-$C_5$ as indicated. (B) Lineweaver-Burk plots of the rate of conversion of $pC_5$ to $pC_4$ + $pC_3$ in the presence of (○) 0 μ*M*, (□) 50 μ*M*, (△) 150 μ*M*, (●) 300 μ*M*, or (■) 500 μ*M* unlabeled d-$C_5$. The analysis was limited to the smaller products because their production is affected only by the first transesterification reaction (Fig. 4). Although d-$C_5$ is inactive in the first transesterification reaction, it has some activity as a substrate in the second transesterification reaction (*15*) and therefore could affect the production of chains of length greater than 5. (C) $K_m/V_{max}$, determined from the slopes of the lines in (B), is plotted against the inhibitor concentration. The *x*-intercept gives the negative of $K_i$; $K_i$ = 260 μ*M*.

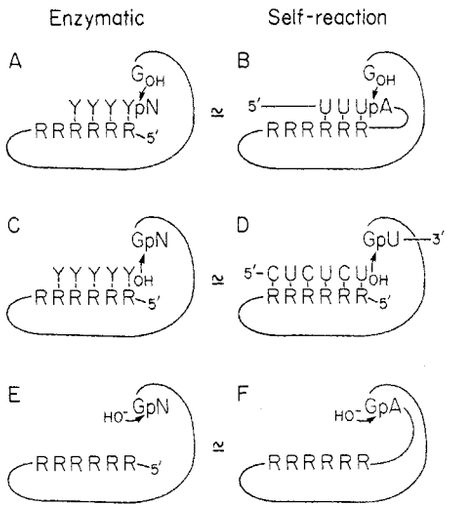


Fig. 6. Relation of reactions catalyzed by the L − 19 IVS RNA to self-splicing and the related IVS RNA-mediated reactions. Formation of the covalent enzyme-substrate intermediate (A) is analogous to IVS RNA autocyclization (B). Resolution of the enzyme-substrate intermediate (C) is analogous to exon ligation (D) or the reversal of cyclization (*10*). Hydrolysis of the enzyme-substrate intermediate (E) is analogous to site-specific hydrolysis of the circular IVS RNA (F) or of the pre-rRNA (*19*).

interactions relative to $C_4$ ($C_3$ could form only two base pairs in the binding mode that would be productive for cleavage).

The transesterification reactions are conservative with respect to the number of phosphodiester bonds in the system. Thus, RNA ligation can occur without an external energy source as is required by RNA or DNA ligase. Hydrolysis of the covalent intermediate competes with transesterification. The net reaction is $C_5 + H_2O \rightarrow C_4 + pC$, with the L − 19 IVS RNA acting as a ribonuclease.

On the basis of our current understanding of the reaction, the catalytic strategies of the L − 19 IVS RNA enzyme appear to be the same as those used by protein enzymes (*20*). First, the RNA enzyme, like protein enzymes, forms a specific noncovalent complex with its oligonucleotide substrate. This interaction is proposed to hold the oligonucleotide substrate at a distance and in an orientation such as to facilitate attack by the 3′-hydroxyl of the terminal guanosine of the enzyme. Second, a covalent enzyme-substrate complex is a presumptive intermediate in the L − 19 IVS RNA reaction. Covalent intermediates are prevalent in enzyme-catalyzed group transfer reactions. Third, the phosphodiester bond formed in the covalent intermediate is unusually susceptible to hydrolysis, suggesting that it may be strained or activated to facilitate formation of the pentavalent transition state upon nucleophilic attack (*8, 9*). Similarly, protein catalysts are thought to facilitate the formation of the transition state, for example, by providing active site groups that bind the transition state better than the unreacted substrate (*6, 21*). Thus far there is no evidence that another major category of enzyme catalysis, general acid-base catalysis, occurs in the L − 19 IVS RNA reactions, but we think it likely that it will be involved in facilitating the required proton transfers.

Each L − 19 IVS RNA-catalyzed transesterification and hydrolysis reaction is analogous to one of the steps in *Tetrahymena* pre-rRNA self-splicing or one of the related self-reactions (Fig. 6). Thus, the finding of enzymatic activity in a portion of the IVS RNA validates the view that the pre-rRNA carries its own splicing enzyme as an intrinsic part of its polynucleotide chain. It seems likely that the $C_5$ substrate binding site of the L − 19 IVS RNA is the oligopyrimidine binding site that directs the choice of the 5′ splice site and the various IVS RNA cyclization sites (*10, 18, 19, 22*). Although the location of this site within the IVS RNA has not been proved, the best candidate is a portion of the "internal guide sequence" proposed by Davies and co-workers (*23*). Michel and Dujon (*24*) show a similar pairing interaction in their RNA structure model. The putative binding site, GGAGGG, is located at nucleotides 22 to 27 of the intact *Tetrahymena* IVS RNA and at positions 3 to 8 very near the 5′ end of the L − 19 IVS RNA. If this is the substrate binding site, site-specific mutation of the sequence should change the substrate specificity of the enzyme in a predictable manner.

**RNA polymerase or RNA restriction endonuclease?** With $C_5$ as a substrate, the L − 19 IVS RNA makes poly(C) with chain lengths of 30 nucleotides and longer. The number of P-O bonds is unchanged in the process. In the synthesis of poly(C) on a poly(dG) template by RNA polymerase, one CTP is cleaved for each residue polymerized. Thus, the RNA polymerase reaction is also conservative with respect to the number of P-O bonds in the system. The L − 19 IVS RNA can therefore be considered to be a poly(C) polymerase that uses $C_4$pC instead of pppC as a substrate. It incorporates pC units at the 3′ end of the growing chain and releases $C_4$; the $C_4$ is analogous to the pyrophosphate released by RNA polymerase. Synthesis is directed by a template, but the template is internal to the RNA enzyme. It may be possible to physically separate the template portion from the catalytic portion of the RNA enzyme with retention of activity. If so, the RNA enzyme could conceivably act as a primordial RNA replicase, catalyzing both its own replication and that of other RNA molecules (*25*).

In its ribonuclease mode, the L − 19 IVS RNA is expected to have specificity similar to that of the IVS RNA cyclization reaction (*10, 18*). That is, it recognizes three or more nucleotides in choosing a reaction site. Protein ribonucleases that are active on single-stranded RNA substrates have specificity only at the mononucleotide level (for example, ribonuclease $T_1$ cleaves after guanosine). Thus the L − 19 has more base-sequence specificity for single-stranded RNA than any known protein ribonuclease, and may approach the specificity of some of the DNA restriction endonucleases. An attractive feature of this new RNA ribonuclease is the possibility of completely and predictably changing its substrate specificity by altering the sequence of the internal binding site.

**How good an enzyme?** The L − 19 IVS RNA catalyzes the cleavage-ligation of $pC_5$ with $K_m = 42\ \mu M$, $k_{cat} = 2\ min^{-1}$, and $k_{cat}/K_m = 1 \times 10^3\ sec^{-1}\ M^{-1}$. The $K_m$ is typical of that of protein enzymes. The $k_{cat}$ and $k_{cat}/K_m$ are lower than those of many protein enzymes. However, $k_{cat}$ is well within the range of values for proteins that recognize specific nucleic acid sequences and catalyze chain cleavage or initiation of polymerization. For example, Eco RI restriction endonuclease cleaves its recognition sequence in various DNA substrates, including a specific 8-bp DNA fragment, with $k_{cat} = 1\ min^{-1}$ to $18\ min^{-1}$ (*26*). The $k_{cat}$ is also similar to that of the RNA enzyme ribonuclease P, which cleaves the precursor to tRNA with $k_{cat} = 2\ min^{-1}$ (*11, 13*).

Another way to gauge the catalytic effectiveness of the L − 19 IVS RNA is to compare the rate of the catalyzed reaction to the basal chemical rate. A transesterification reaction between two free oligonucleotides has never been observed, and hence the uncatalyzed rate is unknown. On the other hand, the rate of hydrolysis of simple phosphate diesters has been studied (*27, 28*). The second-order rate constant for alkaline hydrolysis of the labile phosphodiester bond in the circular IVS RNA (*8*) is 12 orders of magnitude higher than that of dimethyl phosphate (*27*) and ten orders of magnitude higher than that expected for a normal phosphodiester bond in RNA (*29*). On the basis of the data of Fig. 3D, the covalent

enzyme-substrate complex undergoes hydrolysis at approximately the same rate as the equivalent bond in the circular IVS RNA. Thus, we estimate that the L − 19 IVS RNA in its ribonuclease mode enhances the rate of hydrolysis of its substrate about $10^{10}$ times.


REFERENCES AND NOTES

1. T. R. Cech, A. J. Zaug, P. J. Grabowski, *Cell* **27**, 487 (1981); K. Kruger *et al.*, *ibid.* **31**, 147 (1982).
2. G. Garriga and A. M. Lambowitz, *ibid.* **39**, 631 (1984).
3. G. Van der Horst and H. F. Tabak, *ibid.* **40**, 759 (1985).
4. F. K. Chu, G. F. Maley, M. Belfort, F. Maley, *J. Biol. Chem.* **260**, 10680 (1985).
5. C. L. Peebles *et al.*, *Cell*, in press; R. Van der Veen *et al.*, *ibid.*, in press.
6. A. Fersht, *Enzyme Structure and Mechanism* (Freeman, New York, ed. 2, 1985).
7. B. L. Bass and T. R. Cech, *Nature (London)* **308**, 820 (1984).
8. A. J. Zaug, J. R. Kent, T. R. Cech, *Biochemistry* **24**, 6211 (1985).
9. ———, *Science* **224**, 574 (1984).
10. F. X. Sullivan and T. R. Cech, *Cell* **42**, 639 (1985).
11. C. Guerrier-Takada *et al.*, *ibid.* **35**, 849 (1983).
12. C. Guerrier-Takada and S. Altman, *Science* **223**, 285 (1984).
13. T. L. Marsh, B. Pace, C. Reich, K. Gardiner, N. R. Pace, in *Sequence Specificity in Transcription and Translation*, R. Calendar and L. Gold Eds., *UCLA Symposium on Molecular and Cellular Biology* (Plenum, New York, in press); T. L. Marsh and N. R. Pace, *Science* **229**, 79 (1985).
14. Abbreviations: IVS, intervening sequence or intron; L − 19 IVS RNA (read "L minus 19"), a 395-nt RNA missing the first 19 nt of the L IVS RNA (the direct product of pre-ribosomal RNA splicing); $\overset{*}{p}$, $^{32}P$ within an oligonucleotide, that is, $C_5\overset{*}{p}C$ is $CpCpCpCpC^{32}pC$ and $\overset{*}{p}C_5$ is $^{32}pCpCpCpCpC$; d-$C_5$, deoxy$C_5$.
15. A. Zaug and T. Cech, unpublished data.
16. The radioactive phosphate was bonded covalently to the L − 19 IVS RNA as judged by the following criteria: it remained associated when the complex was isolated and subjected to a second round of denaturing gel electrophoresis; it was released in the form of a mononucleotide upon RNase $T_2$ treatment; and it was released in the form of a series of unidentified oligonucleotides upon RNase $T_1$ treatment (*15*). These results are consistent with a series of covalent enzyme-substrate complexes in which various portions of $C_5\overset{*}{p}C$ are linked to the L − 19 IVS RNA via a normal 3′,5′-phosphodiester bond. A more complete structural analysis of the covalent complexes is in progress.
17. A. J. Zaug, P. J. Grabowski, T. R. Cech, *Nature (London)* **301**, 578 (1983).
18. M. Been and T. R. Cech, *Nucleic Acids Res.* **13**, 8389 (1985).
19. T. Inoue, F. X. Sullivan, T. R. Cech, *J. Mol. Biol.*, in press.
20. W. P. Jencks, *Catalysis in Chemistry and Enzymology* (McGraw-Hill, New York, 1969).
21. T. N. C. Wells and A. R. Fersht, *Nature (London)* **316**, 656 (1985).
22. T. Inoue, F. X. Sullivan, T. R. Cech, *Cell* **43**, 431 (1985).
23. R. W. Davies *et al.*, *Nature (London)* **300**, 719 (1982); R. B. Waring, C. Scazzocchio, T. A. Brown, R. W. Davies, *J. Mol. Biol.* **167**, 595 (1983).
24. F. Michel and B. Dujon, *EMBO J.* **2**, 33 (1983).
25. T. R. Cech, in preparation.
26. P. J. Greene *et al.*, *J. Mol. Biol.* **99**, 237 (1975); P. Modrich and D. Zabel, *J. Biol. Chem.* **251**, 5866 (1976); R. D. Wells, R. D. Klein, C. K. Singleton, *Enzymes* **14**, 157 (1981); C. A. Brennan, M. B. Van Cleve, R. I. Gumport, in preparation; B. Terry, W. Jack, P. Modrich, in preparation.
27. J. Kumamoto, J. R. Cox, Jr., F. H. Westheimer, *J. Am. Chem. Soc.* **78**, 4858 (1956); P. C. Haake and F. H. Westheimer, *ibid.* **83**, 1102 (1961).
28. A. J. Kirby and M. Younas, *J. Chem. Soc. Ser. B.* (1970), p. 1165; C. A. Bunton and S. J. Farber, *J. Org. Chem.* **34**, 767 (1969).
29. The rate of nucleophilic attack by hydroxide ion on phosphate esters is sensitive to the $pK_a$ of the conjugate acid of the leaving group. A phosphate in RNA should be more reactive than dimethyl phosphate, because $pK_a$ = 12.5 for a nucleoside ribose and $pK_a$ = 15.5 for methanol [values at 25°C from P. O. P. Ts'o, *Basic Principles in Nucleic Acid Chemistry* (Academic Press, New York, 1974), vol. 1, pp. 462–463 and P. Ballinger and F. A. Long, *J. Am. Chem. Soc.* **82**, 795 (1960), respectively]. On the basis of the kinetic data available for the alkaline hydrolysis of phosphate diesters (*27, 28*), the slope of a graph of the logarithm of the rate constant for hydrolysis as a function of $pK_a$ can be roughly estimated as 0.6. Thus, RNA is expected to be more reactive than dimethyl phosphate by a factor of $10^{0.6\ (15.5-12.5)} = 10^{1.8}$. The estimate for RNA pertains to direct attack by $OH^-$ on the phosphate, resulting in 3′-hydroxyl and 5′-phosphate termini. Cleavage of RNA by $OH^-$-catalyzed transphosphorylation, producing a 2′,3′-cyclic phosphate, is a much more rapid (intramolecular) reaction but is not relevant to the reactions of the L − 19 IVS RNA.
30. J. V. Price and T. R. Cech, *Science* **228**, 719 (1985).
31. E. T. Butler and M. J. Chamberlin, *J. Biol. Chem.* **257**, 5772 (1982); D. A. Melton *et al.*, *Nucleic Acids Res.* **12**, 7035 (1984).
32. T. R. Cech *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 3903 (1983); T. Inoue and T. R. Cech, *ibid.* **82**, 648 (1985).


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