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Reconstitution of enzymatic activity from fragments of M1 RNA

(RNA catalysis/RNA–RNA complementation/RNA–RNA interaction/RNase P)

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ABSTRACT **Certain fragments of M1 RNA, the catalytic subunit of RNase P from *Escherichia coli*, either have no enzymatic activity at all or have altered substrate specificity compared with that of the intact catalytic RNA. After simple mixing *in vitro*, many of these fragments of M1 RNA can reassociate with other fragments to form complexes that have enzymatic activity typical of wild-type M1 RNA. Furthermore, inactive M1 RNA molecules with internal deletions can be complemented *in vitro* by other inactive derivatives of M1 RNA that have nonoverlapping deletions. Thus, two inactive molecules of M1 RNA can interact to form an active RNA enzyme. Functional attributes can be assigned to various regions of M1 RNA when the reconstitution process is combined with assays for activity with different substrates.**

RNase P, the enzyme responsible for the biosynthesis of the 5′ termini of mature tRNA molecules in *Escherichia coli* (1, 2), is composed of an RNA subunit (M1 RNA) and a protein subunit (C5 protein), both of which are essential for the activity of RNase P *in vivo* (3–6). M1 RNA can carry out the catalytic reaction *in vitro* (7) and is capable of processing a variety of RNA substrates, such as precursors to tRNAs and to 4.5S RNA, small model substrates, and derivatives of plant virus RNAs (reviewed in ref. 1). In this paper we demonstrate that various large regions of M1 RNA can be deleted without loss of ability to catalyze cleavage of some, but not all, substrates. Moreover, using an assay *in vitro* for the activity of reconstituted M1 RNA, we have confirmed that certain parts of M1 RNA, by contrast with the RNA subunit of RNase P from *Bacillus subtilis* (8), are essential for wild-type catalytic activity with specific substrates.

Catalytic RNA activities other than M1 RNA have been reconstituted from RNA fragments with little or no function under "standard" conditions *in vitro* (9–13). In the case of M1 RNA, a true enzyme, active complexes may contain certain fragments that have no activity whatsoever and/or other fragments that cleave only certain substrates.

MATERIALS AND METHODS

Materials. Nucleoside triphosphates and T4 RNA ligase were purchased from Pharmacia; ^{32}P-labeled nucleotides from Amersham; SP6 RNA polymerase, DNA polymerase I Klenow fragment, and RNasin (RNase inhibitor) from Promega; T7 RNA polymerase and restriction endonucleases from New England Biolabs; the DNA sequencing kit from United States Biochemical; and RNase-free DNase I from Cooper Biomedical. C5 protein was prepared in our laboratory by D. Wesolowski and J. Arnez.

Construction of Truncated Derivatives of M1 RNA. Plasmid pJA2′ (14) was used to generate an *Eco*RI–*Hin*dIII insert that contained the bacteriophage T7 promoter followed by the gene for M1 RNA. This DNA insert was digested with various restriction endonucleases and the appropriate fragments were ligated into pUC19 that had been digested with *Eco*RI and *Hin*dIII. The ligation mixture was used to transform *E. coli* DH5α. DNAs from all appropriate transformants were sequenced to verify the extent of the deleted sequences. The derivatives of M1 RNA obtained in this way are indicated by the symbol Δ followed by numbers between square brackets; these numbers indicate the extent of the deletion within the nucleotide sequence of M1 RNA (see Fig. 2*A* and Fig. 4).

Preparation of RNA. Plasmids encoding variant M1 RNAs were linearized with appropriate restriction endonucleases and transcribed by T7 RNA polymerase, as described (15). RNA substrates were labeled with [α-^{32}P]GTP during transcription and purified by electrophoresis in denaturing polyacrylamide gels.

Assays of Catalytic Activity. Cleavage of RNA substrates (concentration range, 1 nM to 1 μM) was performed in 50 mM Tris·HCl, pH 7.5/100 mM NH_4Cl/10 mM $MgCl_2$ (buffer A) when the RNase P holoenzyme was assayed (M1 RNA plus C5 protein) and either in buffer A supplemented with 90 mM $MgCl_2$ and 4% PEG (buffer B) or in buffer C [50 mM Tris·HOAc, pH 7.5/3 M NH_4OAc/50 mM $Mg(OAc)_2$] when M1 RNA or fragments thereof were used as the source of catalytic activity.

Complementation Assays. Fragments of M1 RNA were incubated together for 30 min at 37°C, unless otherwise noted, in the presence of substrate in buffer B or C. The cleavage products were analyzed by electrophoresis in denaturing gels. The relative specific activity of a complex is defined as the initial velocity of the cleavage reaction catalyzed by the complex divided by the amount of RNA in the complex in the particular reaction mixture.

RESULTS

Substrate Specificity of Fragments of M1 RNA. In the presence of C5 protein, fragments of M1 RNA with small deletions, such as Δ[45–54] RNA or Δ[273–281] RNA, still retain activity that is close to wild type when the precursor to tRNA^{Tyr} (pTyr) is the substrate (Fig. 1). For example, the K_m and k_{cat} values for the cleavage of pTyr by Δ[273–281] RNA are approximately the same as those obtained with wild-type M1 RNA (data not shown), and the relative rate of cleavage of the precursor to 4.5S RNA (p4.5S), a natural substrate that has a simple stem–loop structure (16), is about half that of pTyr (Fig. 1). However, we found that, on the small model substrate pAT1 (derived from tRNA^{Phe}; ref. 17), which has a stem–loop structure similar to that of p4.5S, k_{cat} was lower by a factor of 10 whereas K_m was essentially unchanged. Thus, the deleted region, nucleotides 273–281, is not essential for activity, though nucleotides 276–279 have been reported to be involved in a phylogenetically conserved pseudoknot interaction (18). Its presence or absence, how-

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Abbreviations: pTyr and p4.5S, precursors to tRNA^{Tyr} and 4.5S RNA, respectively, from *Escherichia coli*.

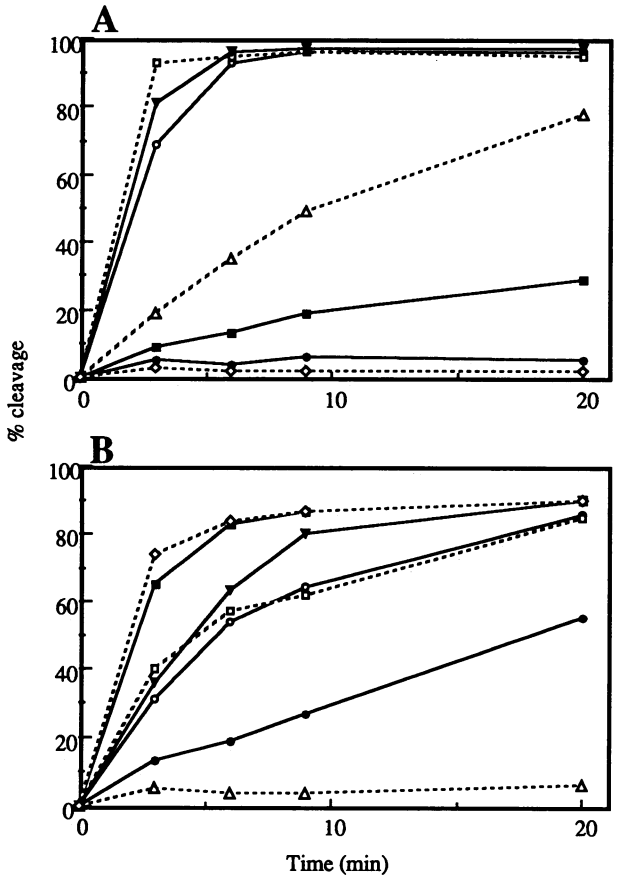

FIG. 1. RNase P activity on different substrates. M1 RNA or derivatives of M1 RNA were mixed with C5 protein and the activity of the ribonucleoprotein complexes was assayed with pTyr (*A*) and p4.5S (*B*) as substrates. The concentrations of the RNAs were as follows: M1 RNA (○), 0.5 nM; Δ[65] RNA (△), 25 nM; Δ[92] RNA (■), 5 nM; Δ[273–281] RNA (□), 25 nM; Δ[45–54] RNA (▼), 25 nM; Δ[156–205] RNA (●), 50 nM; Δ[94–204] RNA (◇), 25 nM. Aliquots were taken at 3, 6, 9, and 20 min, and the products of the reaction were separated by electrophoresis in an 8% polyacrylamide gel that contained 7 M urea. The gels were dried and the amounts of substrates and products were quantified with a Betascope blot Analyzer (Betagen, Waltham, MA). Calculations of kinetic parameters for the most active complexes were made from similar experiments in which more data points were taken in the linear range of kinetics.

ever, clearly affects the ability of M1 RNA to interact with particular non-tRNA precursor substrates with varying efficiency. Accordingly, we analyzed the activity of several other derivatives of M1 RNA with p4.5S as substrate, as well as with pTyr, to locate those regions of M1 RNA that are important in the reaction with each substrate. Two fragments that have single nucleotide deletions, Δ[65] RNA and Δ[92] RNA, were included in this study (14, 19).

M1 RNAs with large deletions, such as Δ[156–205] RNA and Δ[94–204] RNA, when mixed with C5 protein, also cleaved p4.5S efficiently, even though they were unable to cleave pTyr in the presence (Fig. 1) or absence of C5 protein. Thus, these fragments of M1 RNA retain a region essential for interaction with the protein moiety (15) and must include both the catalytic site and the binding site for p4.5S.

In our collection of fragments of M1 RNA, only those that include the entire region from C92 to C204 are able to cleave pTyr. Even M1 RNA with a single nucleotide deletion, Δ[92] RNA, by itself or in combination with C5 protein is also more active with p4.5S than with pTyr as substrate. Thus C92, which was found to be important in the cleavage by M1 RNA of pTyr without the 3′-terminal CCA sequence (14), is absolutely unnecessary for cleavage of p4.5S RNA. Furthermore, previous work has shown that pTyr unfolds when interacting with M1 RNA (20); such interactions may not be necessary with p4.5S or other small substrates. Therefore, Δ[156–205] RNA and Δ[94–204] RNA lack sequences that are involved in a critical step in the binding of pTyr, but they still clearly retain the catalytic center of the enzyme and the binding site for p4.5S, as demonstrated by their ability to cleave p4.5S.

Many M1 RNAs with very large deletions were inactive with all substrates tested (see Fig. 4), regardless of the presence or absence of C5 protein. However, only Δ[65] RNA, when reconstituted with C5 protein, can cleave pTyr (19) but *not* p4.5S (Fig. 1), perhaps because the binding site for the latter substrate is affected by the deletion of A65.

These results provide a rough definition of the functional subdomains of M1 RNA. We asked next whether different subdomains could reassociate to form complexes with full catalytic activity.

Complementation *in Vitro* of Fragments of M1 RNA. To demonstrate the feasibility of reconstitution, we first mixed the 5′ half (Δ[156–377] RNA) of M1 RNA with the 3′ half (Δ[1–163] RNA). These two half-molecules of M1 RNA provide ample opportunity for the wild-type structure to reform through conventional Watson–Crick hydrogen bonding (Fig. 2*A*), just as do separated halves of tRNA molecules (22). The two halves are inactive alone but, when they are incubated in the presence of the substrate (without an annealing step), a complex with specific activity similar to that of intact M1 RNA is formed regardless of the substrate used and independently of the presence or absence of C5 protein (Figs. 3 and 4). Similar results have been obtained with halves of the RNA subunit of RNase P from *B. subtilis* (12).

Two inactive derivatives of M1 RNA that have internal deletions might interact in a complex with a substrate in a way that would "mimic" the active conformation of the wild-type M1 RNA, in particular if a dimer of M1 RNA is the active form of the enzyme (23) in the absence of C5 protein. Indeed, the combination of several pairs of inactive derivatives of M1 RNA that had internal or terminal deletions resulted in the formation of an active enzyme (Fig. 4). To our surprise, even an M1 RNA with a large internal deletion, such as Δ[94–281] RNA (referred to as the "bottom domain" of M1 RNA; Fig. 2*C*), could complement M1 RNA that lacked both the 5′ and 3′ terminal sequences (Δ[1–73, 273–377] RNA) or the "top domain" (see Figs. 2*B* and 3). We note that these two latter derivatives, which cannot interact by Watson–Crick pairing in any obvious way according to the structures portrayed in Fig. 2, could represent two separate functional domains, as identified in our study of substrate specificity of individual fragments. Furthermore, at least part of the actual structures formed by the interacting fragments of RNA resembles that of intact M1 RNA more closely than do the structures of the fragments alone, as determined by sensitivity to attack by RNase T1 of two of the complexes we studied, Δ[62–108] RNA plus Δ[155–377] RNA and Δ[62–108] RNA plus Δ[1–54, 107–377] RNA (data not shown).

To demonstrate further the surprising ability of several fragments of M1 RNA to form a complex with enzymatic activity, we mixed the bottom domain of M1 RNA with two fragments, Δ[1–73, 169–377] RNA and Δ[1–163, 273–377] RNA, which correspond together to the top domain of M1 RNA ("triple" complex). We also mixed the top domain with two fragments that together correspond to the bottom domain, Δ[95–377] RNA and Δ[1–281] RNA (triple complex), and successfully formed a complex with enzymatic activity (see Fig. 3, lane 2). Even four RNA fragments, Δ[95–377] RNA, Δ[1–73, 169–377] RNA, Δ[1–163, 273–377] RNA, and Δ[1–281] RNA, were able to form an active complex. Such a

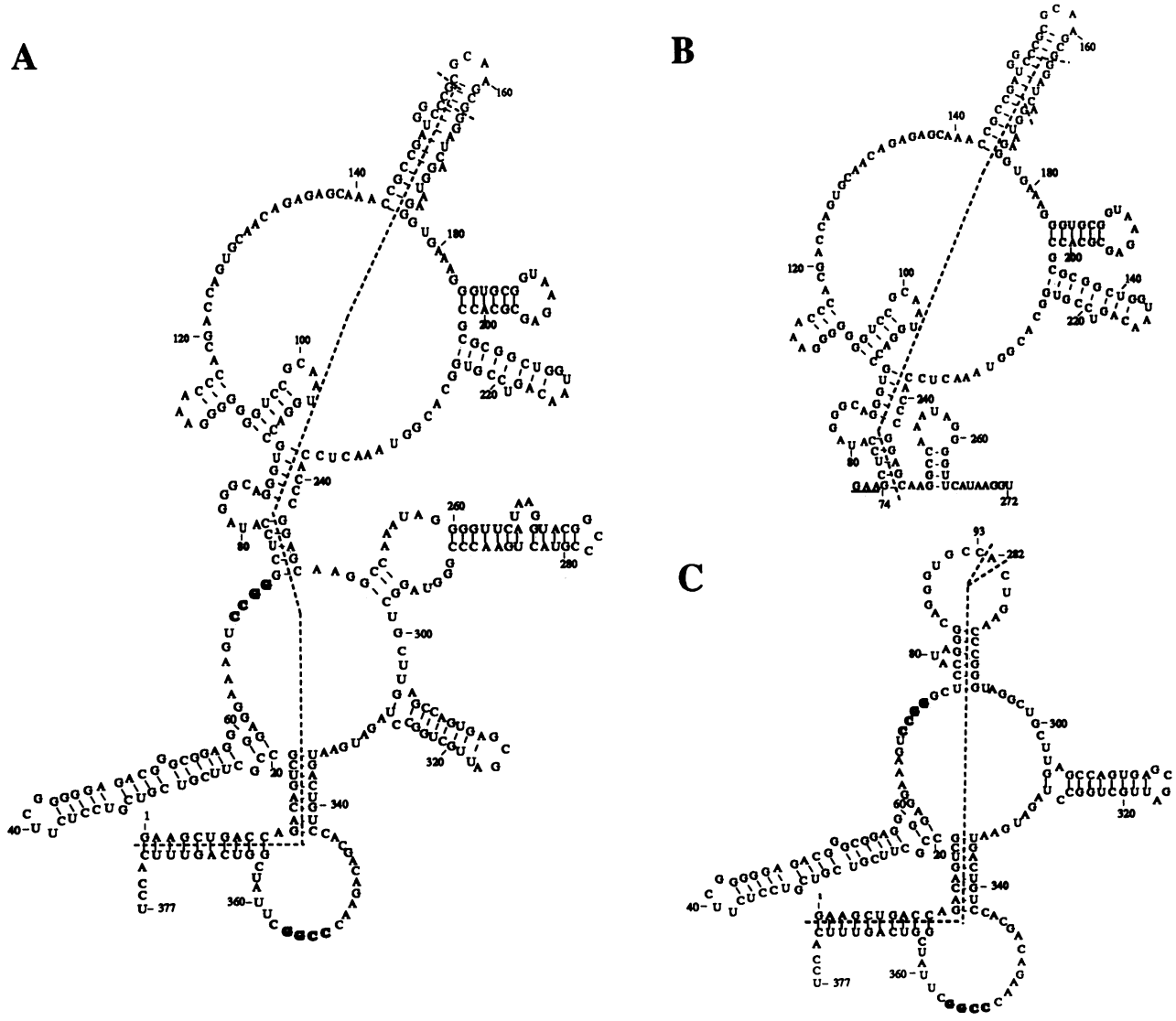

FIG. 2. Schematic representation of the secondary structure of M1 RNA (*A*) and two fragments of M1 RNA referred to as "top" (*B*) and "bottom" (*C*) in the text. The bold letters in *A* and *C* represent nucleotides involved in pseudoknot formation (21). The numbering in *B* and *C* corresponds to the numbering in *A*. Deleted sequences are indicated as discontinuities in the numbering. The dashed lines through the structures indicate the boundaries of "half" and "quarter" fragments of M1 RNA used in the experiments reported here. The underlined sequence, GAA, in *B* is derived from the beginning of the intact M1 RNA sequence but is found in this position as a consequence of the cloning procedure.

four-fragment, "quadruple" reconstituted complex was not active when the first fragment, Δ[95–377] RNA, was replaced by Δ[65, 95–377] RNA, another indication that the presence of A65 is important in catalysis.

The quadruple combination, according to Watson–Crick base-pairing schemes, appears more likely to generate an active complex than does either three-fragment combination. The specific activity (about 10% that of wild-type M1 RNA) and the lag period of the triples and the quadruple were all about the same (data not shown). However, since the generation of enzymatic activity with one test pair of fragments exhibited a first-order dependence on the concentration of each derivative (data not shown), the rate of reassociation may be limited by simple diffusion.

Δ[94–281] RNA plus Δ[1–73, 272–377] RNA, as well as the triples and quadruple, did not form active complexes in the presence of C5 protein (Fig. 3, lanes 5–8). Thus, although two or more RNA molecules together can form an active enzyme, these results suggest that in the holoenzyme, which requires only one RNA molecule for activity (23), the protein does not generally interact efficiently with a complex of more than one RNA molecule. However, C5 protein does protect two sites on intact M1 RNA (15), and some complexes of two RNA molecules did function in the presence of C5 protein if one of the fragments included both sites that C5 protein protects.

Substrate Specificity of Reconstituted Complexes. If a particular fragment of M1 RNA were to exhibit a strong preference for one of our test substrates, we might anticipate that this preference would be modified or eliminated in a reconstituted, active complex in which the previously missing segment of M1 RNA was now present. Accordingly, we surveyed the complexes listed in Fig. 4 with pTyr and pAT1 as substrates. The results, summarized symbolically, show that all active combinations were able to hydrolyze both substrates. In general, as with intact M1 RNA, all complexes tested had slightly higher activity with pAT1 than with pTyr.

In a few cases, a marked preference for particular substrates was exhibited by the various complexes, showing that not all functional defects in the individual fragments were repaired in the complexes. For example, the complex of Δ[1–54] RNA and Δ[273–377] RNA, as well as the triple and quadruple complexes, cleaved pAT1 much less efficiently than they cleaved pTyr.

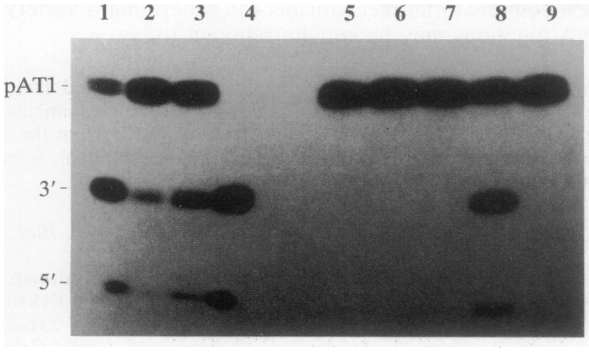

FIG. 3. Complementation assays. Two, three, or four fragments of M1 RNA were mixed together and the resulting complex was assayed with pAT1 as substrate (0.2 μM) in buffer C or in the presence of C5 protein in buffer A. The reaction mixture was incubated at 37°C for 30 min. The products of the reaction were analyzed in a 12% polyacrylamide gel that contained 7 M urea. Lanes 1–4, reactions in buffer C; lanes 5–9, reactions in buffer A; lanes 1 and 5, Δ[94–281] RNA (0.1 μM) and Δ[1–73, 273–377] RNA (0.1 μM), or "top" plus "bottom"; lanes 2 and 6, Δ[1–73, 273–377] RNA (0.1 μM), Δ[95–377] RNA (0.1 μM), and Δ[1–281] RNA (0.1 μM), or "triple" complex; lanes 3 and 7, Δ[1–73, 169–377] RNA (0.1 μM), Δ[1–163, 273–377] RNA (0.1 μM), Δ[95–377] RNA (0.1 μM), and Δ[1–281] RNA (0.1 μM), or "quadruple" complex; lanes 4 and 8, Δ[156–377] RNA (0.01 μM) and Δ[1–163] RNA (0.01 μM), or 5' half plus 3' half; lane 9, pAT1 alone.

RNA–RNA Interactions During Reconstitution. The nature of the physicochemical interactions that govern the reassociation of fragments in reconstituted complexes was investigated by altering the composition of buffers in which fragments were mixed or by changing the physical conditions. For example, the specific activity of a test complex such as 5' half plus 3' half was virtually the same regardless of whether or not the fragments were denatured and reannealed prior to assays for activity. Furthermore, several of the reassociated complexes, but not all, formed complexes that could be visualized, as can be dimers of M1 RNA, by agarose gel electrophoresis (ref. 23; unpublished data).

	Δ[1-54]	Δ[1-163]	Δ[62-108]	Δ[94-204]	Δ[94-281]	Δ[94-290]	Δ[94-377]	Δ[156-281]	Δ[156-290]	Δ[155-377]	Δ[169-377]	Δ[1-73, 273-377]	Δ[1-54, 107-377]	Δ[65]	Δ[45-281]	Δ[1-54, 273-377]
Δ[1-54]	−		+	+						+	+					
Δ[1-163]		−	−	−				+	+	+	+			−		
Δ[62-108]	+	−	−	−			+	+	+	+	+	−	+			
Δ[94-204]	+	−	−	−				+			−	−				
Δ[94-281]					−							+		+		
Δ[94-290]						−						−				
Δ[94-377]			+				−							+		
Δ[156-281]		+	+	+				−				+				
Δ[156-290]		+	+						−		−	+		−		
Δ[155-377]	+	+	+							−				+		
Δ[169-377]	+	+	+	−					−		−			+		
Δ[1-73, 273-377]			−	−	+	−		+	+			−		−		
Δ[1-54, 107-377]			+										−	−		
Δ[65]		−			+		+		−	+	+	−	−	−		
Δ[45-281]															−	+
Δ[1-54, 273-377]															+	−

FIG. 4. Summary of functional assays with M1 RNA fragments by themselves or in reassociated complexes. All reactions were carried out in buffer C as described in *Materials and Methods*. Fragments and complexes were assayed with both pTyr and pAT1 as substrates. A plus sign (+) indicates that ≈10% of product or more compared with that generated by intact M1 RNA was produced from either substrate by the complex tested. A minus sign (−) indicates that no activity was recovered; i.e., the RNAs used did not complement each other. The combinations not tested are indicated by empty squares. The concentration of each fragment or complex used varied between 0.05 and 0.5 μM.

The activity of M1 RNA itself on various substrates was shown previously to be affected by the concentration and nature of the divalent cations present; by ionic strength, pH, and temperature; and by the presence of various denaturants (24, 25). We used a reconstituted complex containing three fragments as a test complex for these studies. We examined the activity of M1 RNA and of the bottom domain plus Δ[1–73, 169–377] RNA and Δ[1–163, 273–377] RNA with pTyr and pAT1 as substrates. Our results (data not shown) indicate that the triple reconstitution worked only when buffers B and C, which contain high concentrations of Mg^{2+} ions (see *Materials and Methods*), were used. Even 1 M urea in buffer B, or 10% ethanol in either buffer, was sufficient to abolish the activity of the complex. The addition of spermidine or other divalent metal ions, or a higher pH, failed to reveal any catalytic activity. Therefore, in comparison with native M1 RNA (24), the reconstituted triple complex is more susceptible to loss of its active configuration. In fact, the optimum temperature for the reaction with the two triple, the quadruple, and the top and bottom complexes is between 45°C and 50°C, whereas that for intact M1 RNA and the complex of the half molecules is between 55°C and 60°C (data not shown).

Finally, complexes that contained one half of M1 RNA and the DNA equivalent of the other half of the M1 sequence were not active (data not shown).

DISCUSSION

The catalytic activity of M1 RNA can be reconstituted from two or more fragments of the enzyme that, by themselves, are inactive with any substrate or are active with only some substrates and that do not necessarily have a previously designated function in the catalytic reaction. This phenomenon offers us the opportunity to learn more about the specific functions of defined pieces of M1 RNA, primarily through studies of substrate specificity, as discussed further below.

The reconstituted complexes, especially those in which there is no obvious interaction of fragments through extensive Watson–Crick base pairing, are fragile and have somewhat lower specific activity than intact M1 RNA.

Reconstitution of other catalytic RNAs from two or more fragments has also been demonstrated *in vitro* (9–13), and analysis of the ribosomes of certain eukaryotes has shown that the ribosomal RNAs of these organelles are assembled from multiple subfragments (26). In several of these cases also, Watson–Crick base pairing cannot always account for the stability of the new complexes. Indeed, the nature of the interactions that hold these complexes together is unknown. They may resemble the interactions that define the tertiary structure of tRNAs (27), in the sense that hydrogen bonding to sugar moieties of bases or to phosphate residues, stacking interactions, and non-Watson–Crick base-pairing schemes all come into play.

That reconstituted complexes, in most cases, have the ability to work reasonably efficiently with both precursor tRNA and other RNA substrates, whereas the starting fragments frequently cannot do so, indicates that the assignment of specific functions to certain fragments of M1 RNA is justifiable. [In fact, the substrates themselves may interact with specific regions of M1 RNA, or fragments thereof, in determining the structure of the active site of the enzyme, as has been suggested previously (14).] For example, M1 RNA

fragments that were unable to cleave pTyr were still able to process p4.5S (e.g., Δ[94–204] RNA). These derivatives, we propose, lack a region of M1 RNA that participates in an obligatory unfolding of the tertiary structure of pTyr that occurs prior to the subsequent, chemical steps of the catalytic reaction. This inference is strengthened by the fact that, in reconstituted complexes that now contain the missing sequences in question, the ability to act on pTyr is regained. Similarly, the deletion of a single nucleotide, A65, appears to alter the surrounding region in M1 RNA sufficiently to eliminate all ability to cleave p4.5S, whether or not C5 protein is present: this function is regained if the affected region is supplied by another fragment in the reconstituted complex. Of course, some very large deletions in M1 RNA completely abolish catalytic activity also.

We note that some active complexes listed in Fig. 4 lack certain nucleotide sequences found in intact M1 RNA—namely, nucleotides 156–163, 94–108, 156–204, 273–281, and 273–290 (in separate experiments we have found that nucleotides 45–54 and 256–263 are also not essential for activity). These sequences may participate in minor ways in optimizing the normal function of M1 RNA. In other complexes, which were judged to be entirely inactive, some of these same sequences, as well as others, are also missing: some combinations will never reassociate in the correct manner. However, many complexes did exhibit activity, and the variety of their composition shows how accommodating the spatial organization of sequences responsible for particular functions of M1 RNA can be. Our results do indicate that the regions in M1 RNA important for the preservation of *any* catalytic activity reside in the nucleotide sequences 1–45, 54–92, 204–273, and 290–377. These sequences encompass phylogenetically conserved structures (21, 28), regions important in substrate recognition (14) and in the binding of Mg^{2+} ions (25).

We previously reported (29) that certain derivatives, with up to 122 nucleotides absent from the 3′ terminus of the M1 RNA sequence, still exhibited low levels of activity. In our most recent experiments, derivatives with 3′ terminal deletions, prepared by transcription *in vitro* from defined fragments of DNA and without extra terminal nucleotides, were inactive. We ascribe the activity seen previously to the presence of plasmid-derived terminal sequences or, in certain cases, to low levels of contaminating, uniformly labeled fragments, of the same size, that were capable of reassociating with other fragments to form active complexes.

In the primordial world, RNA molecules with important catalytic functions might have first appeared as individual long, linear molecules that were occasionally cleaved by hydrolytic mechanisms (such as those associated with M1 RNA or other catalytic RNAs) to generate shorter molecules with a greater combinatorial potential for association into complexes with diverse functions. Conversely, a variety of short RNA molecules, with different functions, may have been ligated into longer molecules that, as a consequence, possessed multiple functions when acting alone (see ref. 1, p. 32) or in complexes with other molecules. In either case, the ease with which RNA fragments associate to form functional complexes lends support to hypotheses (9, 10, 30) that cleavage, reassortment, and ligation of RNA molecules was an important evolutionary mechanism in early living systems. Such combinatorial mechanisms for generating a variety of RNA functions may be equally relevant today.

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1. Altman, S. (1989) *Adv. Enzymol. Relat. Areas Mol. Biol.* **62**, 1–36.
2. Baer, M., Lumelsky, N., Guerrier-Takada, C. & Altman, S. (1989) in *Nucleic Acids and Molecular Biology*, eds. Eckstein, F. & Lilley, D. M. J. (Springer, Berlin), Vol. 3, pp. 231–250.
3. Kole, R., Baer, M., Stark, B. C. & Altman, S. (1980) *Cell* **19**, 881–887.
4. Schedl, P. & Primakoff, P. (1973) *Proc. Natl. Acad. Sci. USA* **70**, 2091–2095.
5. Sakano, H., Yamada, S., Ikemura, T., Shimura, Y. & Ozeki, H. (1974) *Nucleic Acids Res.* **1**, 355–371.
6. Hansen, F. G., Hansen, E. B. & Atlung, T. (1985) *Gene* **38**, 83–93.
7. Guerrier-Takada, C., Gardiner, K., Marsh, T., Pace, N. & Altman, S. (1983) *Cell* **35**, 849–857.
8. Pace, N. R., Reich, C., James, B. D., Olsen, G. J., Pace, B. & Waugh, D. S. (1987) *Cold Spring Harbor Symp. Quant. Biol.* **52**, 239–248.
9. van der Horst, G., Christian, A. & Inoue, T. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 184–188.
10. Doudna, J. A., Couture, S. & Szostak, J. W. (1991) *Science* **251**, 1605–1608.
11. Jarrell, K. A., Dietrich, R. C. & Perlman, P. S. (1988) *Mol. Cell. Biol.* **8**, 2361–2366.
12. Pace, N. R., James, B. D., Reich, C., Waugh, D. S., Olsen, G. J. & Marsh, T. L. (1987) in *Molecular Biology of RNA: New Perspectives*, eds. Inouye, M. & Dudock, B. S. (Academic, San Diego), pp. 17–36.
13. Odai, O., Hiroaki, H., Sakata, T., Tanaka, T. & Uesugi, S. (1990) *FEBS Lett.* **267**, 150–152.
14. Guerrier-Takada, C., Lumelsky, N. & Altman, S. (1989) *Science* **246**, 1578–1584.
15. Vioque, A., Arnez, J. & Altman, S. (1988) *J. Mol. Biol.* **202**, 835–848.
16. Peck-Miller, K. A. & Altman, S. (1991) *J. Mol. Biol.* **221**, 1–5.
17. McClain, W., Guerrier-Takada, C. & Altman, S. (1987) *Science* **238**, 527–530.
18. Haas, E. S., Morse, D. P., Brown, J. W., Schmidt, F. J. & Pace, N. R. (1991) *Science* **254**, 853–856.
19. Lumelsky, N. & Altman, S. (1988) *J. Mol. Biol.* **202**, 443–454.
20. Knap, A. K., Wesolowski, D. & Altman, S. (1990) *Biochimie* **72**, 779–790.
21. James, B., Olsen, G., Lin, J. & Pace, N. (1988) *Cell* **52**, 19–26.
22. Mirzabekov, A. D., Lastity, D., Levina, E. S. & Bayev, A. A. (1971) *Nature New Biol.* **229**, 21–22.
23. Altman, S., Baer, M., Gold, H., Guerrier-Takada, C., Kirsebom, L., Lumelsky, N. & Vioque, A. (1987) in *Molecular Biology of RNA: New Perspectives*, eds. Inouye, M. & Dudock, B. S. (Academic, San Diego), pp. 3–15.
24. Guerrier-Takada, C., Haydock, K., Allen, L. & Altman, S. (1986) *Biochemistry* **25**, 1509–1515.
25. Kazakov, S. & Altman, S. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 9193–9197.
26. Schnare, M. N. & Gray, M. W. (1990) *J. Mol. Biol.* **215**, 73–83.
27. Rich, A. & RajBhandary, U. L. (1976) *Annu. Rev. Biochem.* **45**, 805–860.
28. Forster, A. C. & Altman, S. (1990) *Cell* **62**, 407–409.
29. Guerrier-Takada, C. & Altman, S. (1986) *Cell* **45**, 177–183.
30. Altman, S. (1990) *J. Biol. Chem.* **265**, 20053–20056.