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28 February 1983; accepted 31 October 1983

Catalytic Activity of an RNA Molecule Prepared by Transcription in vitro

Abstract. *Ribonuclease P is a ribonucleoprotein that cleaves precursors to transfer RNA (tRNA) molecules to yield the correct 5′ terminal sequences of the mature tRNA's. The RNA moiety M1 RNA of ribonuclease P from* Escherichia coli *and the unprocessed transcript prepared in vitro of the gene for M1 RNA can both perform the cleavage reactions of the canonical enzyme in the absence of the protein moiety. When the transcript of the M1 RNA gene is combined with the protein moiety not only is a tRNA precursor cleaved but also the precursor to 4.5S RNA from* Escherichia coli.

The transcripts of all transfer RNA (tRNA) genes in *Escherichia coli* are processed by ribonuclease P to yield the correct 5′ terminal sequences of the tRNA's (*1*). Ribonuclease P consists of a protein moiety, C5, and an RNA moiety, M1 (*2*), but it has been shown that M1 RNA alone can cleave precursors of tRNA molecules with the correct site specificity (*3*). In vivo M1 RNA is generated from a longer gene transcript (abbreviated pM1 RNA) by the removal of 36 or 37 nucleotides from the 3′ terminus (*4*, *5*). We now report that pM1 RNA, prepared by transcription in vitro of the gene for M1 RNA, can perform the same cleavage of the precursor to tyrosine tRNA ($tRNA^{Tyr}$) from *E. coli* as can M1 RNA purified from *E. coli*. These experiments were undertaken to eliminate the possibility that a protein contaminant in the M1 RNA preparations could account for the observed catalytic activity. Our results show that an unprocessed gene transcript, prepared in vitro, has the properties of a biochemical catalyst and extend our previous observation that an RNA molecule can execute enzymatic functions previously thought to be reserved for proteins.

pM1 RNA made in vitro by transcription of the gene for M1 RNA (*4*) can cleave the precursor to *E. coli* $tRNA^{Tyr}$ (Fig. 1, lane 2). The products of this reaction (which is carried out in buffer containing 60 m*M* Mg^{2+}) have the same mobility as the products generated by cleavage of the same substrate by M1 RNA or ribonuclease P (Fig. 1, lanes 4, 5, and 9). In the experiments shown in Fig. 1, lanes 2 and 9, pM1 RNA and M1 RNA were present in the respective reaction mixtures in equal amounts. Portions of these mixtures were assayed during the period of linear kinetics of product accumulation. It is apparent that the initial rates of both reactions are similar (Fig. 1, lanes 2 and 9). However, when reconstituted ribonuclease P complexes (*3*, *6*) consisting of pM1 RNA and the protein subunit, C5, of *E. coli* ribonuclease P were assayed for activity, the rate of reaction was about seven times faster than with pM1 RNA alone (compare Fig. 1, lane 2 to lane 4 and lane 5 to lane 7) (*7*). When C5 protein was mixed with mature M1 RNA in equimolar amounts to reconstitute ribonuclease P, there is only a twofold stimulation in the rate of reaction as compared to the rate when M1 RNA is used alone (*3*). In the experiments we describe here, C5 protein is added in hundredfold molar excess. Under these conditions the rates of the reactions carried out by complexes made with pM1 RNA or with M1 RNA are stimulated to an equal extent (data not shown). This stimulatory effect, which we are studying further, may be due to the alteration by C5 protein of the effective concentration of the RNA's (by disaggregation of RNA complexes) or it may be an indication that C5 protein is needed in more than equimolar amounts for optimum activity of the protein-RNA complexes.

The products generated by cleavage of the precursor to $tRNA^{Tyr}$ by pM1 RNA, by the pM1 RNA–C5 protein complex, or by M1 RNA alone have the same electrophoretic mobilities. Analyses of two dimensional oligonucleotide separation patterns (fingerprints) of the reaction products generated by either pM1 RNA alone or by the pM1 RNA–C5 protein complex show that the products are the same as those produced by M1 RNA or by cleavage by ribonuclease P of the precursor to $tRNA^{Tyr}$. In particular, the 5′ terminus of the large fragment containing the tRNA sequence is pGGU as expected (G, guanine; U, uracil) (*8*).

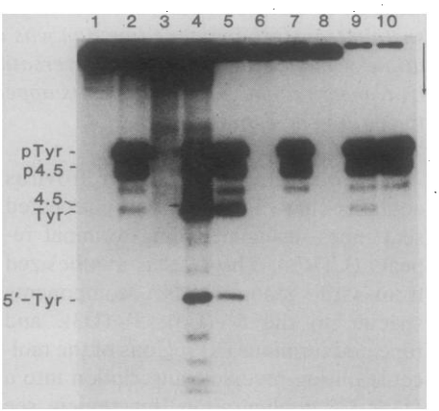

Fig. 1. Cleavage of precursor to $tRNA^{Tyr}$ by pM1 RNA. pM1 RNA was made in vitro generally as described (*4*) with sufficient unlabeled ribonucleoside triphosphate added so that the product had a specific radioactivity of 7×10^6 to 8×10^6 count/min per microgram of RNA (*12*). Assays for ribonuclease P activity were carried out and analyzed in a 10 percent polyacrylamide gel (*3*); the buffer compositions are given in (*13*). The substrate was a mixture of the precursor to $tRNA^{Tyr}$ (abbreviated pTyr; 560 fmole was added where indicated below) and the precursor to 4.5*S* RNA (abbreviated p4.5). The substrate preparations and procedures for the reconstitution of ribonuclease P have been reported in some detail (*3*). The cleavage products generated in the reactions shown in the figure are abbreviated as Tyr and 4.5 for the segments containing the mature RNA sequences and 5′ Tyr for the fragment of the precursor to $tRNA^{Tyr}$ that contains precursor-specific nucleotides. The 5′-terminal fragment of the 4.5*S* precursor molecule is not distinguishable from fragments of pM1 RNA generated by radioautolysis in lane 3 or from background in lane 4. (Lane 1) pM1 RNA (7.5 fmole) assayed in buffer C, no pTyr was added; (lane 2) as lane 1 except that pTyr was added; (lane 3) pM1 RNA (7.5 fmole) reconstituted with C5 protein (750 fmole) by direct mixing (*3*) in buffer B and no pTyr was added; (lane 4) as lane 3, but pTyr was added; (lane 5) pM1 RNA (15 fmole) was reconstituted with C5 protein (1500 fmole) by dialysis (*3*) in 20 μl and 2 μl was assayed in buffer A, and pTyr was added; (lane 6) as lane 5 except that pTyr was not added; (lane 7) pM1 RNA (15 fmole) was treated as in the reconstitution experiments for lanes 5 and 6 and assayed in buffer C, with pTyr added; (lane 8) as in lane 7 except that pTyr was not added; (lane 9) nonradioactive M1 RNA (7.5 fmole) was assayed in buffer C, with pTyr added; (lane 10) pTyr only.

Ribonuclease P, the M1 RNA–C5 protein complex, cleaves not only precursors to tRNA's but also another substrate, the precursor to 4.5*S* RNA, a small stable RNA found in *E. coli* (*9*). The pM1 RNA–C5 protein complex can also effect this reaction. The arrow in Fig. 1, lane 3, points to the upper part of a doublet band in Fig. 1, lane 4. We have shown, using material isolated in preparative cleavage experiments, that the upper band in Fig. 1, lane 4, corresponds to mature 4.5*S* RNA and that it has the expected oligonucleotide, pGGGGC, at its 5′ terminus. Fingerprints of the cleavage products generated by pM1 RNA from both of our substrates are identical to those obtained when M1 RNA was used as the source of ribonuclease P activity [figure 7 in (*3*)].

The pM1 RNA used in our experiments was prepared by transcription in vitro and extracted with phenol prior to assay for nuclease activity. We earlier showed (*3*) that mature M1 RNA preparations, treated in the same way, lacked detectable protein. In the case of pM1 RNA, the only possible source of contaminating protein in the preparation is the transcription mixture used to synthesize the RNA. This mixture has no nuclease activity when incubated with the substrates we used. Thus, the ability of pM1 RNA preparations to cleave the precursor to $tRNA^{Tyr}$ and generate the 5′ terminus of the mature tRNA lies in the pM1 RNA itself. Furthermore, the molar concentration of product generated in reactions such as those illustrated in Fig. 1, lane 2, is greater than the concentration of pM1 RNA in the reaction mixture (*10*). In preparative reactions, pM1 RNA can generate a more than tenfold molar excess of product compared to its own concentration. pM1 RNA is unchanged in size during the course of the reaction (data not shown). These data indicate that pM1 acts catalytically, as is the case for M1 RNA (*3*).

Our results confirm and extend our previous findings (*3*) that an RNA is capable of acting as a catalyst. It has also been shown that a precursor to ribosomal RNA in *Tetrahymena* has the ability to carry out enzymatic reactions (*11*). In this report, we have shown that the precursor to an RNA species has catalytic capability and that this precursor, as well as the processed, mature molecule, interacts with a protein cofactor to make an enzymatic complex with a higher rate of reaction and wider substrate range than that shown by the precursor RNA alone. Until studies of the kinetic characteristics of these reactions provide more information, the only function we can ascribe to the extra sequences in pM1 RNA is that of a signal for the termination of transcription (*4*).

CECILIA GUERRIER-TAKADA
SIDNEY ALTMAN
Department of Biology,
Yale University,
New Haven, Connecticut 06520

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10. Preparation of the precursor to *E. coli* $tRNA^{Tyr}$ and estimates of its concentration from specific radioactivity measurements were carried out as described (*8*). The reaction products in the experiments shown in Fig. 1 were analyzed on a 10 percent polyacrylamide gel in 89 m*M* tris-borate, 2.5 m*M* EDTA buffer. After autoradiography, the bands corresponding to intact precursor, processed tRNA and 5′ fragment were excised from the gel, and the radioactivity in each was determined by counting Cerenkov radiation. In preparative cleavage reactions with 18 fmole of pM1 RNA as enzyme and 6900 fmole of pTyr as substrate, 780 fmole of $tRNA^{Tyr}$ cleavage product are produced. In the experiment shown in Fig. 1, lane 2, at least 22 fmole of $tRNA^{Tyr}$ cleavage product results from the reaction catalyzed by 7.5 fmole of pM1 RNA.
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13. Buffer A: 30 m*M* tris-HCl, *p*H 7.0, 50 m*M* NH_4Cl, 5 m*M* $MgCl_2$, 0.1 m*M* EDTA, 0.1 m*M* 2-mercaptoethanol. Buffer B: 50 m*M* tris-HCl, *p*H 7.6, 60 m*M* NH_4Cl, 10 m*M* $MgCl_2$. Buffer C: 50 m*M* tris-HCl, *p*H 7.6, 100 m*M* NH_4Cl, 60 m*M* $MgCl_2$, 5 percent glycerol.
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18 November 1983; accepted 20 December 1983

Molecular Dissection of Transcriptional Control Elements Within the Long Terminal Repeat of the Retrovirus

Abstract. *The retroviral long terminal repeat (LTR) contains transcriptional control elements that affect viral gene expression. By deletion mutagenesis of the genome of the cloned Abelson murine leukemia virus, regulatory signals could be mapped to at least three domains within the LTR. A defective 5′ LTR that did not sustain transforming gene function was complemented by an intact LTR positioned at the 3′ end of the genome. This versatility of the retroviral genome with respect to its transcriptional control elements appears to provide a strong selective advantage for viral gene expression.*

The proviral genome of a retrovirus contains characteristic directly repeated sequences designated long terminal repeats (LTR's). The LTR is synthesized from viral genomic RNA components specific to the 5′ (U5), 3′ (U3), and repeated terminus (R) regions of the molecule during reverse transcription into a U3-R-U5 configuration [for review see (*1*)]. Because of their location within the viral genome, as well as their resemblance to transposable elements, retroviral LTR's have been said to function in processes including virus transcription, integration, replication, and genome rearrangement (*2, 3*).

With respect to viral RNA transcription, certain LTR functions have been tentatively assigned. Nucleotide sequence analysis of LTR's derived from a number of avian and mammalian retroviruses has revealed the existence of consensus promoter sequences in the U3 region located at similar distances from the RNA initiation site in the R region (*2, 3*). Moreover, evidence from eukaryotic cell-free transcription systems has indicated that viral RNA transcripts initiate within the LTR (*4*). Evidence is also accumulating for the existence of sequences with an "enhancer"-like function within the U3 region of the LTR [for review see (*5*)]. In other systems, enhancer elements, which can act at a distance from the RNA start site and independent of orientation, appear to play an essential role in efficient gene function (*6*).

Abelson murine leukemia virus, a prototypical acute transforming retrovirus,