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# Targeted cleavage of mRNA *in vitro* by RNase P from *Escherichia coli*

**(external guide sequence/model substrates)**

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ABSTRACT **External guide sequences (EGSs) complementary to mRNAs that encode β-galactosidase from *Escherichia coli* and nuclease A from *Staphylococcus aureus* can target these RNAs for cleavage *in vitro* by RNase P from *E. coli*. Specific cleavage occurs at locations predicted by the nucleotide sequences of the EGSs. EGSs with regions complementary to the mRNAs that are as short as 13 nucleotides function efficiently and turn over slowly during incubation with the target substrate and the enzyme. EGSs composed of deoxyribonucleotides as well as those composed of ribonucleotides are effective, but cleavage of the targeted substrate with DNA as an EGS is about 10-fold less efficient than that with RNA as an EGS. An RNA EGS inhibited the formation of β-galactosidase activity in a crude extract (S30) of *E. coli* that was capable of catalyzing coupled transcription–translation reactions.**

RNase P is a ubiquitous enzyme that cleaves tRNA precursors to generate the 5′ termini of mature tRNA molecules (1). This enzyme is a ribonucleoprotein (2), and its RNA subunit from *Escherichia coli* (M1 RNA) and from other eubacteria (3, 4) has catalytic activity *in vitro*. RNase P from *E. coli* is capable of cleaving small model substrates that consist of a stem–loop structure flanked by a single-stranded region on the 5′ side and a 3′ terminal sequence of ACCA (5). Recently, it was shown that RNase P can cleave modified model substrates in which no loop component is present and which consist simply of two base-paired oligonucleotides (6). These observations led to the hypothesis that any RNA can serve as a substrate for RNase P provided that it is hybridized to an "external guide sequence" (EGS) that is complementary to the proposed target over a region of several nucleotides, and provided that the EGS terminates in the sequence ACCA, which is found in most tRNA precursor molecules. We now show that this hypothesis is correct: RNase P from *E. coli* or its catalytic RNA subunit, M1 RNA, in the presence of an appropriately designed EGS, can cleave *in vitro* the mRNA for β-galactosidase from *E. coli* and the mRNA for nuclease A from *Staphylococcus aureus* with the predicted specificity. Furthermore, we demonstrate that β-galactosidase activity can be inhibited in a coupled transcription–translation system *in vitro* by this technique.

The methods that are described here may be of general use in the cleavage of any selected RNA *in vitro* or, in conjunction with appropriately designed expression systems, *in vivo*.

## MATERIALS AND METHODS

**Materials.** T7 RNA polymerase and restriction endonucleases were purchased from New England Biolabs; DNA polymerase I Klenow fragment, RNasin (ribonuclease inhibitor), and *E. coli* S30 coupled transcription–translation system from Promega; RNase-free DNase I from Cooper Biomedical; T4 RNA ligase and nucleoside triphosphates from Pharmacia; and radiolabeled chemicals from Amersham. RNase III was a generous gift from A. W. Nicholson (Wayne State University). Partially purified RNase P from *Saccharomyces cerevisiae* was a gift from D. Engelke (University of Michigan) and partially purified RNase P from HeLa cells (7) was prepared in our laboratory by S. K. Chou. C5 protein (8) was prepared in our laboratory by D. Wesolowski and J. Arnez. M1 RNA was prepared as described (8).

**Plasmids and Oligonucleotides.** pLZBH, a plasmid carrying the *lacZ* gene from *E. coli* under the control of a bacteriophage T7 promoter, was a gift from P. Einat and F. Ruddle (Yale University). pUT7SNTM, a plasmid carrying the gene for nuclease A from *S. aureus* under control of the T7 promoter, was a gift from J. Flanagan and D. Engelman (Yale University). pGEM β-gal, a plasmid carrying the *lacZ* gene under control of an *E. coli* promoter, was purchased from Promega with the S30 system.

RNA and DNA oligonucleotides were made by automated synthesis and purified by electrophoresis in 12% or 15% polyacrylamide sequencing gels. The concentration of the purified oligonucleotides was calculated from measurements of their absorbance at 260 nm. The theoretical melting temperature of each complex between an mRNA and its EGS was calculated as described (9).

**Preparation of mRNA and Hybridization of EGS with Its Target mRNA.** T7 RNA polymerase was used to transcribe a fragment of plasmid pLZBH that was generated by digestion with *Bgl* I restriction endonuclease and carried the *lacZ* gene. The digested DNA was treated with the Klenow fragment of DNA polymerase prior to the transcription reaction that generated *Bgl* I RNA (mRNA for *lacZ*). This step was included as a consequence of the manufacturer's instructions (Promega), in order to eliminate extraneous high molecular weight transcripts that appear when DNA templates with 3′ protruding ends are used. RNA polymerase was also used to transcribe plasmid pUT7SNTM that had been linearized with *Hin*dIII to generate NHIII RNA (mRNA for *nucA*). Conditions for transcription have been described (8). Uniformly labeled RNA was prepared by transcription in the presence of [α-$^{32}$P]GTP and purified by electrophoresis in denaturing 5% polyacrylamide gels.

To observe hybridization between an mRNA and its EGS, the EGS was labeled either at its 5′ end, by using [γ-$^{32}$P]ATP and T4 polynucleotide kinase, or at its 3′ end, by using [5′-$^{32}$P]pCp and T4 RNA ligase (10). The labeled EGS was then annealed (see below) to the unlabeled fragment of mRNA and the annealed samples were loaded onto a nondenaturing 5% polyacrylamide gel. Hybrids were detected by an appropriate shift in the mobility of the labeled EGS.

**Assay of RNase P Activity on EGS–Substrate (mRNA) Complexes.** EGS and the mRNA substrate were annealed,

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Abbreviation: EGS, external guide sequence.

unless otherwise indicated, in 100 mM Tris·HCl, pH 8.3, at 80°C for 4 min and then cooled on ice before assay. All assays were carried out at 37°C under standard conditions, as described elsewhere (11). RNase P holoenzyme (5 or 10 nM M1 RNA and 100 or 200 nM C5 protein) and M1 RNA (100 nM) were added as appropriate. After incubation for 30 min or 60 min, the reactions were stopped by addition of a mixture of 10 M urea, 0.05% bromophenol blue, and 0.05% xylene cyanol, and the samples were loaded on a composite 4–8% polyacrylamide sequencing gel. The products of the reaction were visualized by autoradiography.

**Determination of the Cleavage Sites and Analysis of the End Groups of the Cleavage Products.** Both products of cleavage of uniformly labeled mRNA by RNase P were extracted from preparative polyacrylamide gels, digested to completion with RNase T1 (5,000 units for 30 min at 37°C), and analyzed in a 25% polyacrylamide sequencing gel. A complete RNase T1 digest of the precursor to $tRNA^{Tyr}$ from *E. coli* and the products generated by cleavage of this precursor by RNase P were used as markers. The large 5′-proximal cleavage product contained all the expected oligonucleotides, including pppGp, which is the nucleotide present at the transcription initiation site. All oligonucleotides, in the appropriate molar ratios, were present that were expected to be present in the 3′-proximal cleavage product of the particular mRNA under study. These included pGp, which was the expected 5′-terminal nucleotide of the small product produced by cleavage with RNase P.

**Determination of Rates of Turnover of EGSs.** The activity of RNase P (10 nM M1 RNA and 200 nM C5 protein) was measured by direct mixing assay in the presence of various concentrations of labeled substrate (mRNA, from 15 to 300 nM) and a fixed concentration of EGS (10 nM). No annealing step was performed and the incubation was carried out at 37°C. Aliquots were taken 3, 6, 9, and 12 min after the start of incubation and the products of the reaction were resolved in a 4% polyacrylamide gel that contained 7 M urea. The gel was dried and the amount of radioactivity in substrates and products was quantified with a Betascope blot analyzer (Betagen, Waltham, MA).

**Inhibition of β-Galactosidase Activity in the S30 Expression System.** The reactions (25 μl) were performed in accordance with instructions from the manufacturer, with the exception that 1 μl of RNasin was included in the reaction mixture and the S30 extract was always the last component added just prior to the start of incubation. EGSs or other unrelated oligoribonucleotides were added to the reaction mixture 10 min after the start of incubation at 37°C, and incubation was continued for a further 20 min. Reactions were stopped by placing tubes in an ice bath, and enzyme activity was measured in accordance with the protocol supplied with the kit. For kinetic measurements of the appearance of β-galactosidase activity, aliquots were removed at 5-min intervals from the reaction mixture.

## RESULTS

**Specific Cleavage of mRNAs Directed by EGSs.** Two genes that have been cloned into convenient expression systems for transcription *in vitro* (see *Materials and Methods*) were chosen for study of their mRNAs as potential substrates for RNase P—namely, the *lacZ* gene from *E. coli* and the *nucA* gene from *S. aureus*. Segments of each gene, several hundred nucleotides in length, were transcribed to provide target molecules, and appropriate EGSs were designed and synthesized in each case, as shown in Fig. 1. Initially, EGSs were selected with a high G+C content, with sufficient length to make stable hybrids at 37°C, and with the ability to make a G·C base pair on the 3′ side of the expected site of cleavage by RNase P. Since model substrates with a hairpin structure

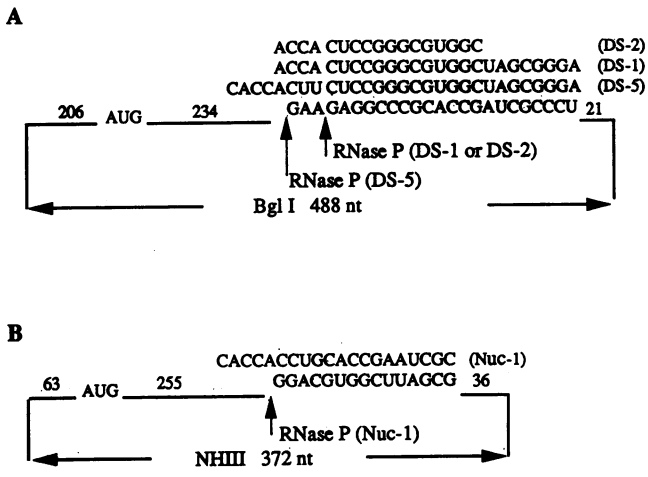


FIG. 1. Complementary sequences in EGSs and target mRNAs. (*A*) *Bgl* I RNA (target mRNA for *lacZ*) and DS-1, DS-2, and DS-5 (EGSs) form 21, 13, and 24 base pairs, respectively. (*B*) NHIII RNA (target mRNA for *nucA*) and Nuc-1 (EGS) form a stem of 15 base pairs. Numbers above the solid line indicate the number of nucleotides (nt) present in the particular sequence. Arrows show the predicted sites of cleavage.

ending in the sequence AGCA rather than ACCA are not cleaved *in vitro* by RNase P (5), EGSs that terminated in the sequence AGCA were also tested. Before assaying the activity of RNase P on these EGS–substrate complexes, we examined the hybridization between DS-1, DS-2, DS-5, Nuc-1, and their target mRNAs by heating and reannealing; all of them hybridized efficiently to their target mRNA (data not shown).

The results shown in Fig. 2 indicate that specific cleavage of the test substrates occurs only in the presence of the "correct" EGS (lanes 4 and 10); no cleavage is seen when the EGS (lanes 1, 2, 7, and 8) or the enzyme (lanes 3, 5, 9, and 11) is omitted from the reaction mixture. Furthermore, when EGSs ending with the 3′-terminal sequence AGCA (DS-1G or Nuc-1G) are used, the efficiency of cleavage by RNase P is reduced to about 10–20% of that seen with DS-1 or Nuc-1 (Fig. 2*B*, lanes 6 and 12). The decrease in efficiency of cleavage was anticipated but is not as severe as expected from our results with the model substrates that have hairpin structures, mentioned above. Note that the mRNA for *nucA* (Fig. 2*B*, lanes 4 and 10) is cleaved much more efficiently than the mRNA for *lacZ* (Fig. 2*A*, lanes 4 and 10). Cleavage of the mRNA for *lacZ* by M1 RNA alone is barely detectable in this experiment, even though in separate experiments cleavage was also detected with M1 RNA alone (data not shown). The RNase P holoenzyme, which generally cleaves substrates with at least 10-fold higher efficiency than does M1 RNA alone (12), is able to cleave both substrates. When excess M1 RNA or RNase P was added to these reaction mixtures, complete cleavage of the target substrate was achieved. The precise locations of the sites of cleavage by RNase P, as well as the nature of the end groups produced, were determined. In both instances, the results indicated that the site and nature of the cleavage event were those anticipated from previously determined characteristics of the cleavage of substrates by RNase P as shown in Fig. 1.

**Factors Affecting the Efficiency of Cleavage.** RNAs in solution can adopt complex conformations. Tertiary structure can, therefore, sterically hinder the accessibility of certain sequences in a potential target to an EGS. EGSs chosen to be complementary to certain sequences in the potential substrates might hybridize more or less strongly than those at other locations in the nucleotide sequence of the target. We examined this possibility in an attempt to explain

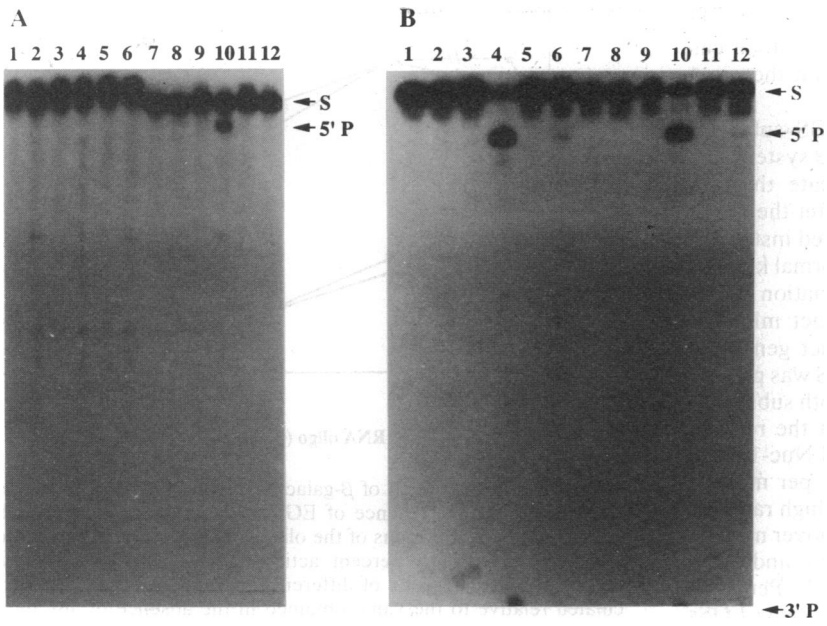


FIG. 2. Cleavage of *Bgl* I RNA and NHIII RNA (see legend to Fig. 1) that have been rendered susceptible to cleavage by their interactions with specific EGSs. Each labeled mRNA (10 nM) was annealed to its EGS (200 nM), and then the complex was incubated in the presence of RNase P or M1 RNA under standard assay conditions. After a 1-hr incubation at 37°C, the reactions were stopped and the reaction products were resolved in a composite denaturing 4–8% polyacrylamide gel. (*A*) *Bgl* I RNA as substrate. (*B*) NHIII RNA as substrate. Lanes 1–6, assays with M1 RNA; lanes 7–12, assays with the RNase P holoenzyme; lanes 1, 2, 7, and 8, no EGS present; lanes 3, 4, 9, and 10, DS-1 as EGS (*A*) and Nuc-1 as EGS (*B*); lanes 5, 6, 11, and 12, DS-1G as EGS (*A*) and Nuc-1G as EGS (*B*); lanes 1, 3, 5, 7, 9, and 11, no enzyme added; lanes 2, 4, 6, 8, 10, and 12, enzyme added. The substrate (S) and products of cleavage (P) are marked. The 3′-proximal product of cleavage of *Bgl* I RNA is visible only in the original film.

the differences in efficiencies of cleavage of the two mRNAs, as seen in Fig. 2. We denatured the mRNAs by heating and reannealing them in the presence of the EGSs, presuming that steric hindrance due to tertiary structure would no longer prevent hybridization of the EGS to the target. Annealing was carried out as described for assays of RNase P activity (see *Materials and Methods*). Compared with the results of direct mixing of the substrate and EGS, the annealing procedure clearly improved the efficiency of cleavage of the *lacZ* mRNA from 20% to 30%, but it had little effect on the cleavage of *nucA* mRNA (Fig. 3), as expected from the results cited above. We also tested EGSs that were chosen to be complementary to a different region of the *lacZ* mRNA (nucleotides 781–800): no cleavage by RNase P was detected. Thus, the particular choice of cleavage site in a target does play a role in determining the efficiency of the cleavage reaction in the presence of an EGS, as a result of differences in steric accessibility by the EGS or by RNase P itself.

Under the conditions that we used for targeted cleavage, the overall efficiencies of the reactions were independent both of the melting temperatures of the theoretical complexes, which varied from 83°C (Nuc-1) to 99°C (DS-5), and the length of the complementary sequences in the EGSs (13–24 nucleotides) that we tested (Fig. 1). However, the chemical nature of the EGS did affect the efficiency of the reaction. Studies of model substrates (J.-P. Perreault and S.A., unpublished experiments) revealed that EGSs composed of DNA support cleavage, albeit inefficiently, of target substrates. Accordingly, we tested the ability of DNA EGSs to target both mRNAs for cleavage by RNase P. As shown in Fig. 4, with a concentration of RNase P that was 10-fold higher than the one used in the experiments for which results are shown in the previous figures, cleavage of the mRNA for *nucA* is readily apparent. A previously unobserved product is also apparent (lanes 4 and 5) at this high enzyme concentration. No significant cleavage of the mRNA for *lacZ* was

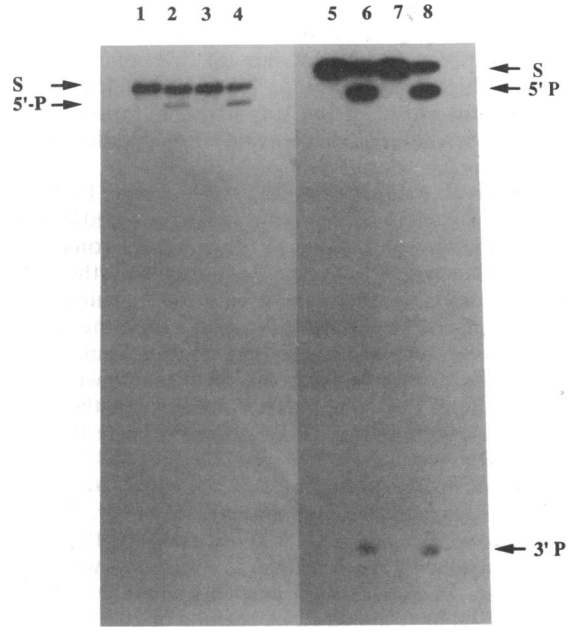


FIG. 3. Effects of annealing conditions on the activity of the RNase P holoenzyme. Labeled *Bgl* I RNA or NHIII RNA was used in all assays. Lanes 1–4, *Bgl* I RNA plus DS-1 as substrate; lanes 5–8, NHIII RNA plus Nuc-1 as substrate; lanes 1, 2, 5, and 6, direct mixing of EGS and target mRNA at 37°C; lanes 3, 4, 7, and 8, annealing of EGS and target mRNA after heating at 80°C (see *Materials and Methods*); lanes 1, 3, 5, and 7, no enzyme added; lanes 2, 4, 6, and 8, RNase P added.

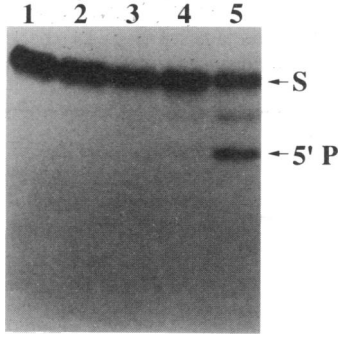


FIG. 4. Cleavage by RNase P of NHIII RNA targeted by an EGS composed of deoxyribonucleotides. Lane 1, labeled NHIII RNA alone; lane 2, with EGS but no enzyme; lane 3, with EGS and C5 protein, but no M1 RNA; lane 4, with no EGS, but with RNase P; lane 5, with EGS and RNase P added. In this experiment the amount of the enzyme used was 10-fold more (0.1 μM M1 RNA and 2 μM C5 protein) than usually used. The substrate(s) and the 5′-proximal product of cleavage (5′ P) are shown. The 3′-proximal product of cleavage has run off the bottom of the gel.

detected (data not shown), presumably because of the lower efficiency of the latter reaction. Even M1 RNA alone can cleave the complex made with a DNA EGS and the *nucA* mRNA (data not shown).

**Turnover of the EGS.** If cleavage of the EGS–substrate complex is occurring as expected *in vitro* in the system that utilizes pure enzyme, then we might anticipate that the hybridized oligonucleotides would dissociate after the cleavage event takes place (either because of increased instability at the cleaved end of the hybrid or because of normal kinetics of dissociation and reassociation). Such dissociation would allow the EGS to be reused for targeting intact mRNAs. Therefore, we measured the amount of product generated during cleavage under conditions where the EGS was present in limiting quantities. We found that EGSs for both substrates turn over, but to extents that appear to reflect the relative accessibility of the targeted sites. That is, EGS Nuc-1 has a turnover number of about 0.35 mol of product per mole of EGS per minute as determined from the data at high ratios of mRNA to EGS (Fig. 5), and EGS DS-1 has a turnover number about 3-fold lower (data not shown). These data, and those from parallel studies with model substrates (J.-P. Perreault and S.A., unpublished experiments), show that some EGSs can indeed be used repeatedly, but at a low rate, *in vitro*.

**Inhibition of β-Galactosidase Activity *in Vitro*.** Prior to determining whether the method of RNA inactivation described herein can be utilized *in vivo*, we tested it in an extract of *E. coli* that most nearly emulates conditions found *in vivo*—namely, in a coupled transcription–translation system capable of expressing the *lacZ* gene. In this system, a plasmid DNA that encodes the *lacZ* gene product is added to the other components of the system together with the EGS. About 75% of radioactively labeled EGS remained intact in the crude extract after a 30-min incubation at 37°C (data not shown). In this crude extract, both DS-1 and DS-1G, EGSs complementary to the mRNA for *lacZ*, efficiently inhibited the development of β-galactosidase activity (Fig. 6). DS-2 also showed the same kinetics of inhibition as did DS-1 (data not shown). Other, noncomplementary RNAs (43-mer and Nuc-1; Fig. 6) partially inhibited the activity, but only when present at high concentration. In the latter cases, the inactivation may be due to nonspecific effects on transcription and translation of high concentrations of added RNA.

Even though DS-1G is less efficient than DS-1 in directing cleavage of the *lacZ* mRNA by RNase P in the purified system, it is apparent that in the crude extract either DS-1G performs more efficiently as an EGS or RNase P is less sensitive to the presence of the altered 3′-terminal sequence, thus enabling this EGS to inhibit the formation of β-galactosidase as efficiently as does DS-1. In the crude extracts, therefore, the target complexes with 3′-terminal AGCA sequences behave more like full-length tRNA precursors that contain 3′-terminal sequences other than ACCA and that are cleaved efficiently *in vitro* by RNase P (13).

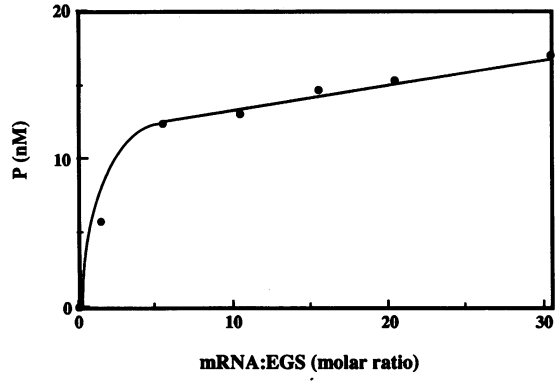


FIG. 5. Determination of the turnover of Nuc-1 in the presence of RNase P. For each substrate concentration the amount of product obtained 5 min after the start of incubation was determined.

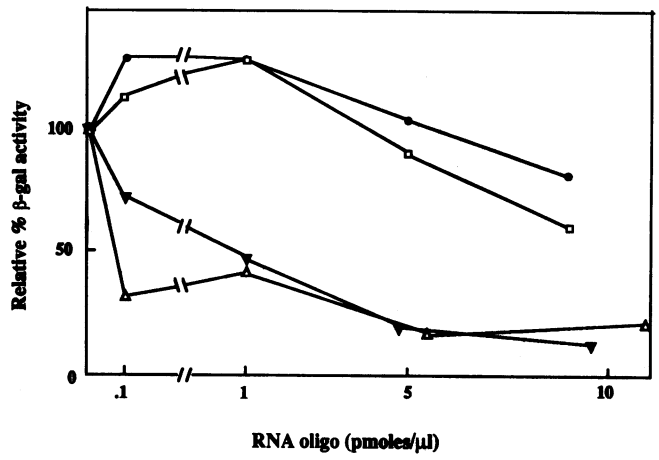


FIG. 6. Measurement of β-galactosidase activity in S30 expression system in the presence of EGSs or unrelated oligoribonucleotides. The concentrations of the oligoribonucleotides are indicated on the abscissa. The percent activity of β-galactosidase (β-gal) obtained in the presence of different oligoribonucleotides was calculated relative to the value obtained in the absence of any oligoribonucleotides, in parallel control experiments. □, An oligoribonucleotide (43-mer) not complementary to the mRNA for *lacZ*; ●, Nuc-1 (EGS complementary to the mRNA for *nucA*); ▼, DS-1 (EGS complementary to the mRNA for *lacZ*; △, DS-1G (EGS identical to DS-1 except for the 3′-terminal sequence, which is AGCA rather than ACCA).

An examination of high molecular weight RNA synthesized in the crude extract during incubation with EGSs revealed that no RNA sufficiently large to code for intact β-galactosidase was present in significant quantities when DS-1 was present (data not shown). However, RNAs with a distribution of sizes that ranged from that of the virtually intact mRNA for *lacZ* to that of very small oligonucleotides were present in equal amounts in extracts with no added EGS or with added Nuc-1 (data not shown). We conclude, therefore, that at high concentrations Nuc-1 exerts its inhibitory effect on some aspect of the transcription–translation processes that does not include cleavage and degradation of the mRNA for *lacZ*.

It is conceivable that the inhibitory effect of an EGS on the formation of β-galactosidase activity in the coupled transcription–translation system is due to the action of some RNase other than RNase P on the complex formed with the mRNA. RNasin was included in reaction mixtures to minimize the effects of nonspecific RNases. We also tested the ability of purified RNase III to degrade the double-stranded RNA complexes that formed between the mRNAs that we used and their EGSs under the same buffer conditions as those in the coupled transcription–translation system. RNase III cleaves the *lacZ* mRNA in a complex with DS-1 or DS-5, but this cleavage does not occur at precisely the same sites as those induced by RNase P (data not shown). However, RNase III does not cleave the *lacZ* mRNA in a complex with DS-2 and it does not cleave the *nucA* mRNA in a complex with Nuc-1. Since RNase III cleaves only certain complexes—namely, those with double-stranded RNA regions of 20 base pairs or more (14)—this enzyme cannot be responsible for all the effects that we ascribe to the action of RNase P on targeted substrates.

## DISCUSSION

We have demonstrated that mRNA can be targeted for cleavage by RNase P from *E. coli* through the use of an EGS.

The general properties of an appropriate EGS were predicted earlier (6) on the basis of studies of the interaction between small model substrates and RNase P in a purified system *in vitro*. These features are as follows: (*i*) a region of complementarity of several base pairs between the EGS and the target molecule; (*ii*) a 3′-terminal ACCA sequence in the EGS; and (*iii*) a single-stranded region, in the targeted substrate, upstream from the beginning of the regions of complementarity with the EGS (or upstream from the cleavage site). Potential substrates, such as the mRNAs for *lacZ* from *E. coli* and *nucA* from *S. aureus*, with these properties are cleaved *in vitro* with the correct specificity by RNase P from *E. coli* in the presence of the appropriate EGSs. However, as expected, not all sequences selected within these mRNAs form complexes that are cleaved with equal efficiency. While the precise sequences of the EGSs appear not to be very important, provided that their length is greater than 12 nucleotides (as required for adequate specificity in the total population of RNAs in eubacteria), some general method must be used for the rapid identification of accessible regions in mRNA. For example, mild digestion of a potential target mRNA with RNase T1 can reveal regions of single-strandedness. Modeling of the secondary structure of such RNAs might also provide a useful adjunct to such experiments.

Coupled transcription–translation systems, while allowing expression of genes *in vitro*, are replete with nonspecific nucleolytic activities. It is, therefore, all the more remarkable that a variety of EGSs are relatively stable and can render mRNA susceptible to cleavage by RNase P in such a system. Furthermore, a decrease in the amount of RNA of molecular size sufficiently large to code for β-galactosidase can be easily detected in reaction mixtures that contain the appropriate EGS. The specific products of cleavage generated by the action of RNase P are, however, degraded too rapidly in this system to be observable.

Although it appears that it is RNase P, rather than other enzymes specific for cleavage of double-stranded RNA, such as RNase III, that is responsible for the cleavage of mRNA in targeted complexes, further experiments must be performed *in vivo* with suitably marked strains of *E. coli*. Our observations, coupled with those that show that the EGS turns over *in vitro* and can be composed of DNA, lead us to hope that this method can be used to some advantage *in vivo*. Indeed, if one compares the efficiency of this method with that of the method that involves use of antisense RNA (15), it appears that the stoichiometric ratio of the EGS to the target RNA is much lower than that of the analogous antisense RNA to the target RNA.

In preliminary experiments we have shown that DS-1, expressed from a synthetic gene encoded by a plasmid, effectively inhibits the expression of β-galactosidase activity in cultures of a strain of *E. coli* that harbors the plasmid. No extensive studies have yet been undertaken of the requirements for inhibition of gene function by an EGS under a variety of conditions *in vivo*. Such experiments would determine conclusively whether the inhibition of enzymatic activity *in vivo* is due to conventional antisense RNA action or the mechanism of RNase P cleavage we describe here. We may ask, however, why RNase P does not cleave many mRNAs in *E. coli*. The sequence NCCA should be found at the 3′ end of internal stem–loop structures in mRNA with a frequency of 1 in 64. While no survey can be easily made of the actual occurrence of structures with the requisite features for recognition by RNase P as a potential substrate in all mRNAs in *E. coli*, they must be sterically inaccessible to RNase P *in vivo* or they must have been selected against during evolution and do not exist.

Partially purified preparations of nuclear RNase P from *S. cerevisiae* and HeLa cells failed to cleave the mRNA–EGS complexes described above. However, EGSs with a more complex structure than those described here can render mRNA susceptible to cleavage *in vitro* by a crude preparation of RNase P from HeLa cells (Yan Yuan and S.A., unpublished experiments). Therefore, the method described here can be used to target any RNA for cleavage *in vitro* by using either eubacterial or mammalian RNase P, and it also offers a promising approach to the control of gene expression in both types of organism.

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1. Altman, S. (1989) *Adv. Enzymol.* **62,** 1–36.
2. Kole, R. & Altman, S. (1981) *Biochemistry* **20,** 1902–1906.
3. Guerrier-Takada, C., Gardiner, K., Marsh, T., Pace, N. & Altman, S. (1983) *Cell* **35,** 849–857.
4. Baer, M. & Altman, S. (1985) *Science* **228,** 999–1002.
5. McClain, W., Guerrier-Takada, C. & Altman, S. (1987) *Science* **238,** 527–530.
6. Forster, A. C. & Altman, S. (1990) *Science* **249,** 783–786.
7. Bartkiewicz, M., Gold, H. & Altman, S. (1989) *Genes Dev.* **3,** 488–499.
8. Vioque, A., Arnez, J. & Altman, S. (1988) *J. Mol. Biol.* **202,** 835–848.
9. Freier, S. M., Kierzek, R., Jaeger, J. A., Sugimoto, N., Caruthers, M. H., Neilson, T. & Turner, D. H. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 9373–9377.
10. Guerrier-Takada, C. & Altman, S. (1984) *Biochemistry* **23,** 6327–6334.
11. Guerrier-Takada, C., Lumelsky, N. & Altman, S. (1989) *Science* **246,** 1578–1584.
12. Baer, M., Arnez, J., Guerrier-Takada, C., Vioque, A. & Altman, S. (1990) *Methods Enzymol.* **181,** 569–582.
13. Guerrier-Takada, C., McClain, W. H. & Altman, S. (1984) *Cell* **38,** 219–224.
14. Robertson, H. D. (1982) *Cell* **30,** 669–672.
15. Cotten, M., Schaffner, G. & Birnstiel, M. L. (1989) *Mol. Cell. Biol.* **9,** 4479–4487.