Evidence for an RNA Binding Region in the Escherichia coli processing Endoribonuclease RNase E*

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The processing endoribonuclease RNase E (rne), which is encoded by the rne gene, is involved in the maturation process of messenger RNAs and a ribosomal RNA. A number of deletions were constructed in order to assess functional domains of the rne gene product. The expression of the deletion constructs using a T7 promoter/RNA polymerase overproduction system led to the synthesis of truncated Rne polypeptides. The smallest gene fragment in this collection that was able to complement a temperature sensitive rne-3071 mutation and to restore the processing of 9 S RNA was a 2.3-kilobase pair fragment with a 1.9-kilobase pair N-terminal coding sequence that mediated synthesis of a 70.8-kDa polypeptide. Antibodies raised against a truncated 110-kDa polypeptide cross-reacted with the intact Rne polypeptide. Antibodies raised against a truncated 80-kDa polypeptide did not bind to the protein. The two mutated Rne polypeptides expressed from the cloned gene, is involved in the

Out of over 20 ribonucleases identified so far in Escherichia coli (Deutscher, 1993) there are only three enzymes known to possess processing endonuclease activity. They catalyze very specific reactions and act only on a subpopulation of RNA molecules. The enzymes are RNase III, RNase P, and RNase E (Deutscher, 1985, 1993; Apirion et al., 1992; Apirion and Miczak, 1993). They cleave RNA in such a manner as to generate 3'-hydroxy termini and require a divalent cation for catalysis. Each of these enzymes seems to have limited substrate specificity and defined cleavage sites. Genetic evidence suggests that the contribution of these enzymes (RNase III, RNase P, and RNase E) is unique, since in each case it was found that when the enzyme activity was abolished by mutation, the cleavage ascribed to it could not be accomplished by any other enzyme in the E. coli cells. The rnc gene encoding RNase III is not essential for bacterial survival (Takiff et al., 1989), whereas the genes encoding RNase P (rmp) and RNase E (rne/ams/hmp) are. RNase P cleaves all of the tRNA precursors to generate the 5'-phosphoryl terminus of mature tRNA (Altman, 1989). Both RNase E and RNase III are involved in processing of the precursor molecules of ribosomal and messenger RNAs (Apirion et al., 1992; Apirion and Miczak, 1993; Court, 1993). RNase III is specific for double-stranded RNA. It introduces a double cleavage in each of two stems that produce 16 S and 23 S rRNA (Ginsburg and Steltz, 1975; Taskad et al., 1978; Gegeheimer and Apirion, 1980) and contributes to the decay and stability of some mRNAs (Gitelman and Apirion, 1980; Regnier and Grunberg-Manago, 1989; Bardwell et al., 1989; Faubladier et al., 1990; Regnier and Grunberg-Manago, 1990; Robert Le Meur and Portier, 1992; Hajnitsdf et al., 1994). RNase K has been defined as the enzyme implicated in the growth rate-dependent regulation of the expression of ompA mRNA by introducing a cleavage in the 5' leader of the ompA message (Lundberg et al., 1990). Recent data suggest that RNase K is a proteolytic product of RNase E.1

RNase E was initially defined as a processing ribonuclease that catalyzes the maturation of 5 S rRNA (Apirion, 1978; Ghora and Apirion, 1978). This enzymatic activity also cleaves RNA I, a small RNA that controls the replication of ColE1 plasmid DNA (Tomcsanyi and Apirion, 1985) and is involved in the maturation and turnover of many bacterial and bacteriophage T4 mRNAs (Mudd et al., 1988; Lundberg et al., 1990; Nilsson and Uhihn, 1991; Regnier and Hajnitsd, 1991; Mackie, 1991, 1992; Klug et al., 1992; Gaper and Haas, 1993; Deutscher, 1993). Inactivation of RNase E has a stabilizing effect on the bulk of mRNAs (Ono and Kuwano, 1979; Babitzke and Kushn, 1991; Mudd et al., 1990). The entire rne gene, suggested to be the RNase E structural gene, was cloned and sequenced (Casaregola et al., 1992), but the exact size of its open reading frame remained unclear. Recently RNase E has been purified and shown to be the rne gene product. It is now established that the E. coli rne/ams/hmp locus encodes the RNA processing endonuclease RNase E (Cormack et al., 1993; Carposis et al., 1994; Taraseviciene et al., 1994). There were some discrepancies in the molecular size of the gene product in different reports. According to the latest corrections2 intact Rne protein consists of 1061 amino acids. The molecular mass calculated from the DNA sequence amounts to 118 kDa, while the protein in SDS-polyacrylamide gel electrophoresis migrates

1 A. von Gabain, personal communication.
2 G. A. Mackie, personal communication.
RNA Binding by RNase E

### Table I

| Strain or plasmid | Characteristic or genotype | Reference or source |
|------------------|---------------------------|---------------------|
| **Strains**      |                           |                     |
| K38              | lacZ43, relA1, spoT1, thi-1 | Goldblum and Apirion (1981) |
| N3433            | lacZ43, relA1, spoT1, thi-1, rne-3071(Ts) | Goldblum and Apirion (1981) |
| N3431            | lacZ43, relA1, spoT1, thi-1, rne-3071(Ts), recA | Goldblum and Apirion (1981) |
| CH1928           | araB139, a(araABC-leu7697T, cI(AC)-74), galU, galK, hsdR, rpsL150, thi, amS1, zoe276.74 | Mudd et al. (1990) |
| **Plasmids**     |                           |                     |
| pGP1–2           | Contains bacteriophage T7 RNA polymerase gene under the inducible promoter | Tabor and Richardson (1985) |
| pT7–5            | Plasmid carrying T7 promoter | Tabor and Richardson (1985) |
| pRE171           | 6.0 kb PstI rne chromosomal fragment in pT7–5 | Tarasoviciene et al. (1994) |
| pRE141           | 3.2 kb EcoRI-BamHI insert in pT7–5 | Tarasoviciene et al. (1994) |
| pRE152           | 3.0 kb EcoRI-SphI insert in pT7–5 | This study |
| pRE153           | 2.6 kb EcoRI-MluI insert in pT7–5 | This study |
| pRE154           | 2.5 kb EcoRI-NruI insert in pT7–5 | This study |
| pRE155           | 1.9 kb EcoRI-BanII insert in pT7–5 | This study |
| pRE156           | 1.7 kb EcoRI-Aval insert in pT7–5 | This study |
| pRE157           | 1.6 kb EcoRI-Pvull insert in pT7–5 | This study |
| pRE158           | 2.8 kb XhoI-BamHI insert in pT7–5 | This study |
| pRE160           | 1.2 kb EcoRI-HindIII insert in pT7–5 | This study |
| pRE181           | 6.0 kb PstI rne-3071 chromosomal fragment in pT7–5 | This study |
| pRE182           | 6.0 kb PstI amS1 chromosomal fragment in pT7–5 | This study |
| pRE183           | 3.6 kb PstI-BamHI amS1 insert in pT7–5 | This study |
| pRE184           | 858 bp EcoRV deletion in pRE171 | This study |
| pRE185           | 858 bp EcoRV deletion in pRE141 | This study |
| pPAP5            | Contains the papBA intercistronic region | Nilsson and Uhlén, submitted for publication |
| pPAN5            | 113 bp deletion in pPAP5 |                     |

**Materials and Methods**

**Bacterial Strains and Plasmids** — The bacterial strains and plasmids used in this study are listed in Table I.

**DNA Manipulations** — All DNA manipulations (restriction enzyme digestion, fill-in reactions, transformation, ligation, and DNA preparations) were performed as described by Sambrook et al. (1989) unless otherwise stated.

**Preparation of Cell Extracts** — Cells containing the coupled T7 polymerase/promoter system (Tabor and Richardson, 1985) were grown at 30 °C in LB medium. At a cell density of about 2 × 10^9 cells/ml, the culture was shifted to 42 °C for 25 min to induce the transcription of T7 RNA polymerase and then shifted back again to 30 °C for 1 h. The whole cell extract for protein analysis was prepared by boiling the cells in a lysis buffer (60 mM Tris-HCl, pH 6.8, 1% SDS, 1% β-mercaptoethanol, 10% glycerol, and 0.01% bromphenol blue) for 3 min prior to loading onto the SDS-polyacrylamide gel. For testing the enzymatic activity, cells were lysed in a buffer containing 0.1 M Tris-HCl, pH 8.0, 0.01 M MgCl₂, 0.01 M KCl, and 7 mM β-mercaptoethanol by six ultrasonic bursts of 20 s with intervals of 30 s in an ISAD 2000 Sonicator ultrasonic processor using the smaller probe. Cell debris and intact cells were removed by centrifugation at 5000 × g for 10 min followed by centrifugation at 20,000 × g for 30 min. The supernatant was removed, and the P-20 pellet was resuspended in the same buffer and used to test the enzymatic activity.

**Protein Refolding Assay** — Whole cell extracts containing overexpressed polypeptide were separated on 5%–8% SDS-polyacrylamide gel. Proteins were visualized with ice-cold 0.25 M KCl containing 1 mM DTT. The gel was rinsed several times with deionized water containing 1 mM DTT. The bands containing overexpressed polypeptide were excised from the gel. Proteins were eluted in 4 volumes of acetone and recovered by centrifugation. Proteins were denatured with 6 M guanidine hydrochloride in buffer D (0.05 M Tris-HCl, pH 8.0, 0.1 mg/ml bovine serum albumin, 0.15 M NaCl, 1 mM DTT, and 0.1 mM EDTA) (Hager and Burgess, 1980). Renaturing of the proteins was performed by 50-fold dilution of the guanidine hydrochloride in buffer D for 1–2 h at room temperature. After the refolding, guanidine hydrochloride was removed by dialysis against buffer containing 0.05 M Tris-HCl, pH 8.0, 0.15 M NaCl, 1 mM DTT and 0.005 mM EDTA. The protein solution was concentrated against 50% glycerol in the same buffer and used for the enzymatic activity test as described below.

**Assay for RNase E Activity** — 32P-Labeled RNA transcripts (1–2 × 10^5 cpm) were incubated in 20 μl of a mixture containing 10 mM Tris-HCl, pH 8.0, 100 mM NH₄Cl, 0.1 mM Na₂EDTA, 5 mM magnesium acetate, 50 μg/ml yeast tRNA, and cell extract (10–15 μg). To inactivate the enzyme encoded by the chromosomal gene copy, protein extracts from the rne(Ts) were preincubated for 20 min at 43 °C. The reaction mixtures were incubated at 30 °C for 30 min, and reactions were terminated by the addition of 5 μl of loading buffer (50 mM Na₂EDTA, 1% SDS, 50% glycerol, 0.01% bromphenol blue). Samples were heated at 95 °C for 3 min and subjected to electrophoresis in a 5%–10% tandem polyacrylamide gel containing 7 M urea. The bands were visualized and quantitated on the PhosphorImager (Molecular Dynamics).

**Western Blot Analyses** — Rabbit anti-Rne antibodies were raised against the 110 kDa protein overexpressed from the plasmid pRE141 using a T7 polymerase/promoter system (Tabor and Richardson, 1985). Affinity purification of the antibodies and the Western blot procedure were performed as described earlier (Tarasoviciene et al., 1994).

**Preparation of Substrates for RNase E Activity Test** — As templates for RNA synthesis in vitro, we have polymerase chain reaction-derived DNA fragments corresponding to 9 S RNA and intercistronic region of...
papBA with a sequence corresponding to the T7 promoter at the 5'-end (see Fig. 1B). The following primers were used: Pr1, 5'-ATAGCTACCGCACCGGCA, for the intercistronic region of papBA (sense); Pr7, 5'-ATTAGCTACGGACCTGAA-3', and Pr6, 5'-ATAGCTACGCGAAGC for the intercistronic region of papBA (antisense); Pr7, 5'-ATAGCTACGGACCTGAA-3', and Pr6, 5'-ATTAGCTACGCGAAGC for the intercistronic region of papBA (antisense) DNA (Cormack et al., 1993). 32P-Labeled RNA probes (1 x 10^6 cpm) generated from in vitro transcription were added, and the hybridization was performed for 2 h at 44°C. The membranes were washed 5 times for 10 min in TEN50 buffer. To visualize the proteins the membranes were stained with 0.1% Amido Black in 25% isopropanol, 10% acetic acid for about 3 min and destained in 25% isopropanol, 10% acetic acid. The blots were dried, and radioactive bands were detected by PhosphorImager (Molecular Dynamics).

RESULTS

Construction and Analysis of Deletions in the rne Gene—Plasmid pRE171 contains a 6-kilobase pair PstI-PstI chromosomal DNA fragment encoding the entire rne gene (Tarasewiczi et al., 1994). The rne gene deletions were constructed by cloning DNA fragments from a smaller derivative pRE141 (Tarasewiczi et al., 1991) using different restriction enzymes. Deletion constructs were expressed in the coupled T7 polymerase/promoter system (plasmid vector pT7-5) described by Tabor and Richardson (1985). The deletion constructs are shown in Fig. 1. E. coli conditionally lethal (temperature sensitive) mutant strains N3438 (rne3071, recA+), N3438 (rne3071, recA+), and CH1828 (ams-1, recA+) were transformed with the different deletion constructs and the ability of the plasmids to complement the Ts mutations was tested. As shown in Table I, only the plasmids pRE171, pRE141, pRE152, pRE153, pRE154, and pRE156 were able to complement the Ts mutations in a recA- background. In recA+ strains N3431 and CH1828, even plasmid pRE160 carrying a 1.2-kilobase pair insert with the coding sequence of about 600 nucleotides reversed the temperature sensitivity, since recombination events could occur. The plasmids pRE184 and pRE185 containing a 858-nucleotide deletion at the 5'-end of the gene (starting at codon 29) did not overcome the temperature sensitivity and did not restore enzymatic activity. The results are fully consistent with the recent findings that the rne3071 and ams-1 mutations in the rne gene are located near the 5' terminus within the first 600 nucleotides (the ams-1 mutation is a G→A transition in codon 66 and the rne3071 mutation is a C→T transition in codon 68 of the rne gene (McDowall et al., 1993)). Plasmid pRE158 contains a deletion in the promoter region, and it suppressed the Ts phenotype only in recA+ strains, since it fails to express the protein (data presented below).

The expression of the cloned fragments in vivo led to the synthesis of truncated proteins (Table II). The yields of the synthesized proteins amounted to as much as 10–20% of the total cell proteins (Fig. 2a). The smaller polypeptides seemed to be truncated at the 3'-end, while the larger were synthesized on the ribosome.
be present in relatively higher amounts than the larger ones (data not shown). The antibodies raised against the truncated protein expressed from the plasmid pRE141, containing the N-terminal two-thirds of the intact Rnase sequence, cross-reacted with all the polypeptides expressed from the deletion constructs (Fig. 2b). Even the smallest (184 amino acids encoded from the rne cistron) polypeptide expressed from pRE160 was recognized by the antibodies (Fig. 2, lane 6), suggesting that the N-terminal part of the protein contains strong immunological epitopes. The expressed polypeptides migrated with some discrepancy on the SDS-polyacrylamide gel in comparison with the molecular weight calculated from the cloned sequence. The largest inconsistency was exhibited by the protein expressed from the plasmid pRE171 containing the intact rne gene sequence (Table I).

Enzymatic Activity of the Truncated Products—The protein extracts prepared from the deletion constructs expressed in E. coli strain N3438 (rne-3071) were tested for enzymatic activity using as substrates 9 and 7 S RNAs transcribed in vitro. As is shown in Fig. 3 and Table II, extracts from the strains containing plasmids pRE171, pRE141, pRE153, and pRE154 exhibited endonucleolytic activity. Extracts prepared from the strains containing the plasmids with smaller inserts (less than 2.5 kilobase pairs), pRE155–160, and with the deletion at the N terminus (pRE184 and pRE185), were not able to process 9 or 7 S RNA (Fig. 3, A and B, and data not shown). Extracts from the cells containing the plasmid pRE158 also were lacking ribonucleolytic activity, since no protein was expressed from this plasmid (see Table II).

The overexpressed truncated polypeptides were purified by eluting the proteins from the SDS-polyacrylamide gel. The eluted polypeptides were at least 95% pure, as only traces of other polypeptides were visible when the samples were overloaded on the gel (data not shown). The proteins were subjected to a denaturation-renaturation procedure as described under “Materials and Methods.” Enzymatic activity was exhibited by the renatured polypeptides expressed from the plasmids pRE171 (the entire Rnase protein), pRE141, and pRE154 (Fig. 3C). No enzymatic activity was detected with the renatured proteins expressed from the plasmids pRE155 and pRE160 (Fig. 3C). This is in good agreement with the activity test of the protein extracts. Taken together, our data from the enzymatic activity tests of deletion mutants suggest that the endonucleolytic activity is encoded by the N-terminal part of the RNase E protein molecule.

RNase E Contains an RNA Binding Region—The hydrophilicity plot of RNase E (Fig. 4A) from the computer structural analysis using the MacVector program revealed a highly
charged area between amino acids 580 and 720, which has been suggested to bind RNA (Casaregola et al., 1992). In order to test RNA binding by RNase E and deletion constructs, proteins expressed from the plasmids carrying the full-length wild-type, rne-3072, or amr-1 gene (pRE171, pRE181, pRE182) and truncated amr-1 gene (pRE183) were tested by Western-Northern blots. The proteins were separated by SDS-polyacrylamide electrophoresis and blotted onto a nitrocellulose membrane. The membrane was then hybridized with 32P-labeled 9 S RNA and RNA-protein binding was visualized by autoradiography. The data presented in Fig. 5 (lanes 1–4) clearly demonstrate that the full-length wild-type and mutated polypeptides were able to bind the classical Rnase E substrate 9 S RNA with high efficiency and specificity. Moreover, the 286-amino acid deletion (amino acids 29–314) at the N-terminal part of the protein (plasmids pRE184 and pRE185) did not affect the binding ability, suggesting that the binding occurs downstream of codon 315 and that the lack of catalytic activity of the mutated proteins does not abolish the RNA binding activity.

The overexpressed proteins from the deletion mutants (Table I) were tested for their activity to bind different RNAs: 9 S RNA, 7 S RNA, RNA E1 (untranslated region of Rne mRNA, see Fig. 1), tRNA\textsubscript{Val}, PAP5 RNA (papBA intercistronic region), PAN5 RNA (deletion in the papBA intercistronic region), aPAP5 RNA (antisense), and aPAN5 RNA (antisense). Data in Fig. 6 clearly demonstrate that only the proteins expressed from the plasmids pRE171, pRE141, and pRE154 (plasmids pRE152 and pRE153 were not tested) have the ability to bind RNA. The smaller truncated proteins (expressed from the plasmids pRE155 or pRE160) were not able to bind RNA. Therefore, the Rne molecule contains RNA binding activity, and the region for that activity is in the central part of the protein.

Furthermore, the RNA-protein hybridization experiments revealed another very interesting finding: only those RNAs that
Fig. 8. Secondary structure models of different RNAs used in this study. The RNA was folded using the FOLD program of Zuker and Stiegler (1981) run in the GCG package on a VAX computer. Established (지는) and putative (상호) RNase E cleavage sites are indicated.
were substrates for the RNase E endonucleolytic activity were bound by the proteins. As is shown in Fig. 6, the RNase E polypeptides exhibited an apparent high substrate specificity toward 9 S RNA, 7 S RNA, PAP5 RNA and RNA E1, while PANS RNA, tRNAval, antisense PAP5 and antisense PANS RNAs did not bind either to the intact Rne protein or to its truncated polypeptides. The data suggest that the substrate RNAs for RNase E also encode the determinant for RNA-protein recognition.

The RNA Binding Region Is Required for Expression of Endonucleolytic Activity—The ability of the active truncated polypeptides to cleave the RNA substrates was also tested. The experiments showed that all of the RNAs that were able to bind intact Rne protein were cleaved by RNase E (Fig. 7). On the other hand, mutations or deletions at the N terminus of the Rne polypeptide totally eliminated the catalytic activity, while the RNA binding activity was not affected (Fig. 5). Furthermore, only the polypeptides containing the intact N terminus, which were found to bind RNA, were able to perform the cleavage reactions. It was clear that the substrate specificity of the endonucleolytic activity and of the RNA binding were similar. Thus, it seems that both protein and RNA contain important determinants for recognition and for performing the enzymatic reaction.

**DISCUSSION**

The experiments presented here provide evidence that the catalytic domain of Escherichia coli processing endoribonuclease RNase E is located within the N-terminal half of the protein and that it includes an RNA binding region, which is likely to play a crucial role in the recognition and cleavage of specific substrate RNAs. The deletion analysis and RNA-protein blotting technique used in this study demonstrated that RNase E encodes an RNA binding region. A putative RNA binding motif, rich in arginines, was earlier predicted by computer analysis from the sequence similarity with the human U1 RNA-associated 70-kDa protein (Casaregola et al., 1992). Out of the 104 arginines present in the Rne protein, 31 (29%) are clustered between amino acid residues 601 and 731 of the polypeptide (Fig. 4A). The present results (Figs. 5 and 6) suggest that the RNA binding motif is located in the same region, i.e. in the central part of the protein molecule.

RNe E is the largest ribonuclease identified so far in E. coli. The molecular mass of the protein calculated from the updated sequence is 118 kDa. The protein migrates in the SDS-polyacrylamide gel as a 180-kDa polypeptide. The deletion analyses used in this study allowed us to demonstrate that the Rne polypeptide lacking much of the molecule from the C terminus still maintained the enzymatic activity (Fig. 3). The calculated molecular mass of the smallest of our polypeptides still exhibiting endonucleolytic activity was 70.8 kDa (Table II; plasmid pRE154). Recently it was demonstrated that a degraded RNase E preparation may exhibit enzymatic activity (Carposis et al., 1994). Sedimentation analysis of a proteolysed Rne polypeptide on a glycerol gradient revealed that it contained 73- and 69-kDa polypeptides that correlated with the enzymatic activity. Presumably such partially degraded polypeptides retained the N terminus; this would be consistent with our present finding that the RNase E catalytic site is located in the N-terminal region of the Rne protein. Nucleotide sequencing of the two conditionally lethal temperature-sensitive mutations of the rne gene, rne-3071 and ams-1 (McDowell et al., 1993), revealed that these two mutations are located in a region near the 5' end of the gene and separated by only six nucleotides. The ams-1 mutation causes the change of glycine to serine at position of 66 in the predicted Rne amino acid sequence, while rne-3071 causes a conservative change of phenylalanine for leucine at position 68. Thus, one might suspect that glycine 66 and phenylalanine 68 are part of the active center located at the N-terminal part of the Rne molecule. The predicted two-dimensional structures of the mutant proteins suggested that mutation rne-3071 caused an enhancement of a helix in the region of the mutation, whereas mutation ams-1 did not affect helix, but introduced a new predicted β-turn in this region (Fig. 4B).

It has been established that 9 S RNA contains two cleavage sites at which RNase E acts. Our deletion analysis is consistent with the suggestion that both cleavages are caused by the same enzymatic activity since extracts from all of the deletion mutants that allowed the synthesis of a peptide with endonucleolytic activity could carry out both types of cleavage, while extracts from all the deletion mutants that failed to produce functional enzyme did not perform either cleavage (Fig. 3). The interesting question still remains if it is a sequential two-step reaction such that the substrate must be released (and subsequently be bound by the same or a different enzyme molecule) for the second cleavage to occur, or if both cleavages can occur while the RNA remains in the same active site but is repositioned for cleavage at the alternative sites.

The Rne protein seemed to bind RNA in a highly specific manner. Only those RNAs that were substrates for RNase E were bound by Rne protein (Figs. 6 and 7). Therefore, quite particular features of the RNA secondary structures presumably play a critical role in the specificity of the protein-RNA interaction. The importance of the stem-loop structure in the processing of 9 S RNA has been assessed earlier by in vitro experiments (Cormack and Mackie, 1992). Mutations affecting the 5' domain of 9 S RNA, which are likely to affect the secondary structure upstream of the first cleavage site, were tested for their effect on processing. Removal of the stem-loop region significantly slowed the processing efficiency, suggesting that secondary structural features adjacent to the cleavage site play a direct and critical role in RNase E recognition of its substrate (Cormack and Mackie, 1992). On the other hand, it has been shown that the addition of a 5'-terminal stem-loop structure (probably not in a correct/native context) can slow down RNase E cleavage of RNA I (Bouvet and Belasco, 1992). Recently it has been shown that the unusual longevity of the E. coli ompA transcript is determined by its untranslated region and that in some context the stem-loop structure might stabilize mRNA by impeding access of RNase E to downstream cleavage sites (Hansen et al., 1994). Plots of the predicted folded structures of different RNAs used as in vitro test substrates in the present study are shown in Fig. 8. In all cases there is potential for stem-loop formation, and the location and extent of the stem-loop near the cleavage site may be hypothesized to carry the features recognized by the RNase E protein. The RNase E polypeptides described here and our findings regarding protein-RNA binding specificity provide a basis for further localization and characterization of the specific functional domains in the structure of this important enzyme.

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