Sequencing and expression of the *rne* gene of *Escherichia coli*

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ABSTRACT

RNase E is a major endonucleolytic RNA processing enzyme in *Escherichia coli*. We have sequenced a 3.2 kb *EcoRI*-*Bam*HI fragment encoding the *rne* gene, and identified its reading frame. Upstream from the gene, there are appropriate consensus sequences for a putative promoter and a ribosome binding site. We have translated this gene using a T7 RNA polymerase/promoter system. We determined 25 amino acids from the N-terminal of the translated product and they are in full agreement with the DNA sequence. The translated product of the *rne* gene migrates in SDS containing polyacrylamide gels as a 110,000 Da polypeptide, but the open reading frame found in the sequenced DNA indicates a much smaller protein. The entity that migrates as a 110,000 Da contains RNA, which could account, at least partially, for the migration of the *rne* gene product in SDS containing polyacrylamide gels.

INTRODUCTION

RNase E, a processing endoribonuclease, was partially purified by Misra and Apirion (1). It matures 5S rRNA from its precursors from all the rRNA genes (2–4). The enzyme also cleaves RNA I (5), a molecule that controls the replication of ColE1 plasmid DNA. RNase E is also involved directly or indirectly in the metabolism of many *E. coli* mRNAs (6) as well as T4 mRNA (7). *E. coli* strains carrying the *rne-3071* mutation fail to mature 5S rRNA at 43°C (2, 8) and do not grow at this temperature; they contain a temperature sensitive RNase E activity (9). Using an *rne-3071* mutant the *rne*⁺ gene was cloned by complementation of the temperature sensitivity. Initially, a 1.6 kb fragment that 'complements' the *rne-3071* mutation in a *rec*⁺ genetic background was cloned (10). Subsequently, a larger fragment, approximately 3.2 kb, was cloned (11) that complements the *rne-3071* mutation in *rec*⁺ or *rec*⁻ genetic backgrounds.

In order to define the gene for the RNase E enzyme, we have sequenced the entire 3.2 kb fragment. On expression of the 3.2 kb DNA fragment, using the system developed by Tabor and Richardson (12), a 110 kDa product was detected.

MATERIALS AND METHODS

Plasmids and Strains

Plasmids: pBluescript (Stratagene), pGP1-2 (12); pT7-5, pGP1-4 (S. Tabor, personal communication). pT7-5 is very similar to pT7-1 (12); both are used for cloning DNA fragments next to a T7 promoter (12). pGP1-4 is similar to pGP1-2 (12); both are used for the controlled expression of T7 RNA polymerase (12). M13mp18/19 (13), pRE117 (11); pRE141, pT7-5 containing the *EcoRI*-*Bam*HI 3.2 kb fragment from pRE117, this fragment includes the whole *rne* gene; pRE145, pRE149, and pRE150 (present study). pNA12, is pT7-5, which contains an *EcoRI*-*Bam*HI fragment from pNA9 (14), it codes for RNase III. Strains: JM109, XL-1-Blue (Stratagene); N 3438, *rne-3071*, *recA*; N3433, *rne*⁺ *recA*⁺.

Reagents and Chemicals

Restriction enzymes used in the present study were purchased from United State Biochemicals, Boehringer Mannheim and New England Biolabs. T4 DNA ligase (USB), polynucleotide kinase (BMB), SequenaseTM kit (USB), pBluescript Exo/Mung kit (Stratagene). All other chemicals and reagents were either from Sigma Chemical or from Fisher.

Construction of Plasmids

An *EcoRI*-*Bam*HI fragment, approximately 3.2 kb, from pRE117, was subcloned in the *EcoRI*-*Bam*HI sites of plasmid pBluescript to prepare pRE145; this is the same 3.2 kb fragment found in pRE141. Plasmid pRE149 was constructed by subcloning a 2.5 kb *EcoRI*-*Nru*I fragment into *EcoRI*-*EcoRV* sites of pBluescript and pRE150 by subcloning a 2.2 kb *EcoRI*-*Pvu*II into *EcoRI*-*EcoRV* sites of pBluescript. In all these plasmids (pRE145, 149 and 150), the *E. coli* DNA was under the control of a T7 bacteriophage promoter.

Sequencing

A 3.2 kb fragment of DNA, containing the *rne* gene was sequenced using the Sanger's dideoxy chain termination method (15). Both strands were sequenced by subcloning small fragments in M13mp18/19, generated by digesting with different enzymes, *EcoRV*, *Cl*aI, *Hae*III, and *Pvu*II. In addition, some of the inaccessible regions were reached by deletions generated by using *Exo*III and S1 nuclease. The deletions were introduced into

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plasmids pRE145, pRE149 and pRE150 from the 5' ends. When necessary, the 3' ends were protected either by digestion with *Pst*I or by thio NTP filling, using the Klenow fragment of DNA polymerase, and pBluescript enhanced kit from Stratagene, as described by the manufacturer.

Expression of Plasmids

Strain JM109 was cotransformed with pGP1-4 along with one of the following plasmids: pRE145, 3.2 kb *Eco*RI-*Bam*HI fragment and pRE149, 2.5 kb *Eco*RI-*Nru*I fragment. As a negative control, the plasmid pBluescript without insert was used. For protein synthesis, DNA fragments were expressed from a T7 promoter (12). Briefly, the co-transformed cells were grown in 3 ml of LB in presence of 100 µg/ml ampicillin and 25 µg/ml kanamycin. At 0.4 O.D. at A₆₀₀ cells were washed with M9 salt solution and resuspended in M9 medium supplemented with 0.01% amino acids except cysteine and methionine. Cells were grown at 30°C for one hour, thereafter shifted to 43°C for 20 min and rifampin 300 µg/ml was added to one of the tubes. After another 10 minutes at 43°C the cultures were shifted to 30°C. The cultures were further incubated for 20 minutes and labeled with [³⁵S] methionine for 10 minutes. Thereafter, cells were pelleted, boiled in sample loading buffer, and subjected to electrophoresis in 5%/10% tandem SDS-PAGE (16), along with molecular weight markers.

Sequencing the RNaseE Protein

Proteins to be sequenced were prepared exactly as described under *Expression of plasmids*, except that no radioactive label was used, and the medium was LB (17). The proteins to be sequenced were transferred from the gel to a polyvinylidene difluoride (PVDF) membrane (18), using a Bio Rad trans blot apparatus in presence of Tris glycine buffer containing 20% methanol. The proteins were stained with coomassie brilliant blue

A

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1  AATTCATGTTTGTACAGCTTATCATCGATACGTTGCCCGCTTCGTCAGCGGTGATAGC 60
61  AACAACTTTTACCGATGGAGTCTCTGTTTATCATGGTTGGCGATTCTAATTTACGCCAACAGG 120
121  ATTCGCGGCACCTATTTTCTATGCTTATATTTTACACCTTATTTACTTCACTCGGG 180
181  TGATCACTTTTATGATGGTTTATTAACCAATCAGCAAGAAAGTGAAGAAATCGTAGT 240
241  AAGCGGGTGATAAATGGTAAAGTCATCTTGCTATAAAGGCTTGCACTGGAATAATGA 300
301  GGCGGTTTCCGTGTCATCTTGTGTTAAACAAAGAAATTTACGGAATAACCCATTTCGC 360
361  CGACCGATCATCCACGACGAATGGCGTAAGAGCTATTGATCTTTCAGGCAGTTAGCGGG 420
421  CTGCGGGTTGCAGTCTTACCGGTAGATGGAATATTTCTGGAGAGTAATACCCAGTCTG 480
481  TTTCTTTGATAATTGCGCTGTGTTTCCGCATGAAACCGGGCAACGACACTCTCGCGCT 540
541  CTTTGAGCTGACGATAACCGTGAGGTTGGCGACGCGACTAGACACGAGGCCATCGGTTCA 600
601  CACCCGGAAGGCGTACTTTGCCCGCAGCTTAGTCGTCAATGTAAGAATAATGAGTAAG 660
661  TTACGATGAAAAGAAATGTTAATCAACGCACTCAGCAGGAAGAGTTGCGGCTTGCCTTG 720
    MetLysArgMetLeuLeuAsnAlaThrGlnGlnGluGluLeuArgValAlaLeu
721  TAGATGGGCGCTCTGATGACCTGGATTCGAAAGTCCAGGGCAGCAGCAAGAAAGG 780
    AlaAspGlnArgLeuTyrAspLeuAspLeuGluSerProGlyHisGluGlnLysLys
781  CAAACATCTACAAAGGTAATACCCGCATTAACCGAGTCTGGAAGTCTGTTTGTG 840
    laAsnIleTyrLysGlyLysIleThrArgIleGluProSerLeuGluAlaAlaPheVal
841  ATACGGCGCTGAACGTCACGGTTTCTCCATCAAAAGAAATGCGCGCAATATTTCC 900
    sPTyrGlyAlaGluArgHisGlyPheLeuProLeuGlyGluIleAlaArgGluTyrPheP
901  CTGCTAACTACAGTGCTCATGGTCTGCTCAACATTAAGATGTGTTGCGTGAAGGTCAG 960
    roAlaAsnTyrSerAlaHisGlyArgProAsnIleLysAspValLeuArgGluGlnG
961  AAGTCATTGTTTCAGATCGATAAAGAGAGCGCGCAACAAAGCGCGGCAATTAACCACT 1020
    luValIleValGlnIleAspLysGluGluArgGlyAsnLysGlyAlaAlaLeuThrThrP
1021  TTATCAGTCTGGCGGTAGCTATCTGCTGATGCGCAACACCGCGCGCGGTGGCA 1080
    heIleSerLeuAlaGlySerTyrLeuValLeuMetProAsnAsnProArgAlaGlyGlyI
1081  TTTCTCGCGTATCGAAGGCGCAGCAGCTACCGAATTAAGAAAGCACTGGCAAGCCTTG 1140
    leSerArgArgIleGluGlyAspAspArgThrGluLeuLysGluAlaLeuAlaSerLeuG
1141  AACTGCCGGAAGGCTGGGGCTTATCGTGGCACCCTGGCGTGGGCAATCTGCTGAGG 1200
    lLeuProGluGlyMetGlyLeuIleValArgThrAlaGlyValGlyLysSerAlaGluA
1201  CGCTCAATGGATTTAAGCTTCCGCTCGAAGCACTGGGAAGCCATCAAAAAGCGCTG 1260
    laLeuGlnTrpAspLeuSerPheArgLeuLysHisTrpGluAlaIleLysLysAlaAlaG

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1261  AAAGCCGCGGCCCCCTTCTGATTTCATCAGGAGACCAACGTAATCGTTCGCGCATTCC 1320
    luSerArgProAlaProPheLeuIleHisGlnGluSerAsnValIleValArgAlaPheA
1321  CGGATTCTTACGTCAGGACATCGCGGAAATCCTTATCGATAACCGAAGTCTCGAAC 1380
    rgAspTyrLeuArgGlnAspIleGlyGluIleLeuIleAspAsnProLysValLeuGluL
1381  TGGACGTCAGCATATCGCTGCATTAGTTCGCCGGATTTCAGCAGCAAAATCAAATGA 1440
    euAlaArgGlnHisIleAlaAlaLeuGlyArgProAspPheSerSerLysIleLysLeuA
1441  ACACCGCGAGATCCCGCTGTTTCAGCCATACAGATCGAGTCACAGATCGATCCGCT 1500
    snThrGlyGluIleProLeuPheSerHisTyrGlnIleGluSerGlnIleGluSerAlaP
1501  TCCAGCGTGAAGTTCGCTGCGCTTGGTTCATTGTTATCGACAGCACCGAAGCGT 1560
    heGlnArgGluValArgLeuProSerGlyGlySerIleValIleAspSerThrGluAlaL
1561  TAACGCCATCGACATCAACTCCGACGCGGACCGCGCGCGGATATCGAAGAAACCG 1620
    euThrAlaIleAspIleAsnSerAlaArgAlaThrArgGlyGlyAspIleGluGluThrA
1621  CGTTTAACACTAACCTCGAAGCTGCCGATGAGATTGCTCGTCAGCTGCGCTCGCTGACC 1680
    laPheAsnThrAsnLeuGluAlaAlaAspGluIleAlaArgIleLeuArgLeuArgAspL
1681  TCGCGCGCTGATTGTTATCGATTTCATCGATGACGCGCAGTACGCCACACGCGTGGG 1740
    euGlyGlyLeuIleValIleAspPheIleAspMetThrProValArgHisGlnArgAlaV
1741  TAGAAAACCGTCTCGGTGAAGCGGTGCGTCAGACCGTGGCGCTATTCAATCAACGATA 1800
    alGluAsnArgLeuArgGluAlaValArgGlnAspArgAlaArgIleGlnIleSerHisI
1801  TTTCTCGCTTGGCGCTGCTGGAATGTCGCGTCAGCGCTGAGCCCATCTCACTGGTGAAT 1860
    leSerArgPheGlyLeuLeuGluMetSerArgGlnArgLeuSerProSerLeuGlyGluS
1861  CCAGTCATCAGTGTGTCGCGCTGTTCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTG 1920
    erSerHisHisValCysProArgCysSerGlyThrGlyThrValArgAspAsnGluSerL
1921  TGTCGCTCTCTATTCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTG 1980
    euSerHisSerIleLeuArgLeuIleGluGluGluAlaLeuLysGluAsnThrGlnGluV
1981  TTCACGCAATGTTCTGTCGCAATCGCTTCTTACCTGCTGAATGAAGAAAGCTCTCGGG 2040
    alHisAlaIleValProValProIleAlaSerTyrLeuLeuAsnGluLysArgSerAlaV
2041  TAAATGCCATTGAACTCGTCAGGACGGTGGTGGTGAATTTGTCGCAACGATCAGA 2100
    alAsnAlaIleGluThrArgGlnAspGlyValArgCysValIleValProAsnAspGlnH
2101  TGGAAACCCGCACTACCACTGCTGCGGTCGCTGCTGCTGCTGCTGCTGCTGCTGCTGCT 2160
    etGluThrProHisTyrHisValLeuArgValArgLysGlyGluGluThrProThrLeuS
2161  GCTACATGCTCCGAAGCTGCATGAAGAGCGATGGCGCTGCGCTGCTGAAGAAGAGTTCG 2220
    ertyrMetLeuProLysLeuHisGluGluAlaMetAlaLeuProSerGluGluGluPheA
2221  CTGAACGTAAGCGTCCGGAACCACTGCGCTGCAACCTTTGCTATGCGGATGTCGCCGC 2280
    laGluArgLysArgProGluGlnProAlaLeuGlnProLeuProCysArgMetCysArgL
2281  TGGCCCAACGCCAGCTGAACCTGCGCGCTGTTGATGCTCAGCAGCAAGAGCTGCACC 2340
    euArgGlnArgGlnLeuAsnLeuProArgLeuLeuEnd
2341  GGCAACACCCAGCAGCTCTGCACAACCTGGGCTGTTGAGCGCTTCTTCCGCGCACTGAA 2400
2401  AGCGCTGTTTACAGGCTGTTGAAGAAACCAACCGCAGCAACCGACCGAAGCAGA 2460
2461  AGCGAAACCGAACGTCACAGGATCGTCGCAAGCTCTCAGAACCAACCGCGTACCG 2520
2521  TAATGAGCGCCGCGACCCGTAGTGAACGTAAGTGAAGCGAGCGATAATCGCGAAGAAA 2580
2581  CCGTCGTAATCGTCGCGAGGACAGCAGCAGAGCTGCGGAGACCGGTGAGAGCGCTGAG 2640
2641  GGCTGAGGTAACGGAAGCGTACCGCGCAGCAGCAGCAAGCGCGCGCTGTAACGTA 2700
2701  GCCCGCCCGTAAATGATGATAAACCTGAGCGCAACAAAGAGCGAAGCGCTGAATGTTG 2760
2761  AAGAGCAATCTGTTTACGAAACCGAACGGAAGAAAGCGTACGCTCGGCTTACGCGCGCT 2820
2821  GTAACAGCGTACGCTCAATCAGAAAGTGGTTACGAGCAAGCGTACGCGAAGAGCGGT 2880
2881  AGTCGACCGGTGGTTGAAGAACTGCGTCCGCGCAACCAATTTGTCAGGAAGCGCAGC 2940
2941  TCCACGCAAGAACTGGTGAAGTCCGCTGCGCAGCTGTAAGCGCAACCTGCAAGCAACA 3000
3001  GCAAGAAGAGAACTGCTGATAACCGTGACACCGTGGCATCGCTGCTGCTGCTGCTGCT 3060
3061  CGCTCGTACCTGCGCGTAAAGTGGTCAGCGTCTGCTGCTGCTGCTGCTGCTGCTGCTG 3120
3121  CAACCCAGTCCGCAATGCCGTTGACCGTAGCTGCGCGCTCCGGAACCTCGCGCTGCG 3178

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B

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      -35      -10      S/D
      TTGACA      TATAAT
621  GGCCTTACTTTGCGGTCAGCTAGTCGTAATGAACTAATAATGAGTAACTACGTAAGAAAGATGTTA

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Figure 1. (A) Nucleotide sequence of a 3.2kb fragment of *Escherichia coli* encoding the *rne* gene. The possible reading frame encoding a protein with an apparent mass of 62 kDa starts at nucleotide 666 and terminates at nucleotide 2315. The sequenced 25 amino acids from the N-terminal are underlined. (From the first 27 amino acids, two could not be determined.) **(B)** A putative promoter and a ribosome binding site. A possible promoter, a Shine-Dalgarno sequence, and an ATG initiation codon, are indicated.

R-250 and the band was cut out from the membrane and transferred to a protein sequencing facility. The protein was sequenced by the Edmond degradation procedure.

Ribonuclease Treatment

Cells were grown at 30°C to an A₆₀₀ of 0.3–0.4. the temperature was shifted to 43°C for 25 min to induce the

synthesis of T7 RNA polymerase, and rifampin (300 $\mu\text{g/ml}$) was added to inhibit *E. coli* RNA polymerase. Cells were labeled with Tran [^{35}S] methionine (1100 Ci/mmol) cysteine (1100 Ci/mmol), or [^{32}P] H_3PO_4 (carrier-free), and opened by lysis in an SDS containing buffer; the contents separated in a 5%/10% tandem SDS-PAGE.

RESULTS

A 3.2 kb fragment of *E. coli* DNA has been sequenced (Fig. 1A) using Sanger's dideoxy chain termination method (15). For sequencing purposes, the 3.2 kb fragment was subcloned after digestion with the DNA restriction endonucleases *Cla*I, *Eco*RV, *Pvu*II, and *Hae*III. The fragments obtained after the digestions were cloned into the *Sma*I site of M13mp18 and M13mp19 and sequenced, using M13 universal and reverse primers. In some cases the fragments were shortened using *Exo*III and S1 nuclease deletions prior to sequencing.

Computer analysis of the sequence revealed a number of possible reading frames, the longest could potentially code for a polypeptide with a molecular mass of about 62 kDa starting with an AUG at nucleotide 666 (Fig. 1A). This reading frame ends up at nucleotide 2315 where the termination codon TAG is encountered. Previous studies indicated that the 3.2 kb DNA fragment contains a promoter (11). Analysis of the upstream nucleotides, from the reading frame, revealed a sequence which can act as a promoter (19). It contains the -10 and -35 consensus sequences AATAAT and TTGCCC respectively. The sequence AATAAT depicts a highly conserved -10 consensus sequence, with only one nucleotide variation. Similarly four nucleotides, TTGC, are conserved from the -35 consensus promoter sequence. However, since the beginning of the *me* gene transcript was not determined, and since the two putative parts of the promoter, are relatively far apart (19), it is possible that another sequence serves as the promoter. Located at -6, to the initiation codon (AUG), is the sequence TAAG which can serve as a ribosomal binding site (see Fig. 1B; 20). Close examination of the 3' end of the gene did not reveal a sequence that can give rise to a termination stem and loop structure (21, 22).

In order to characterize the product of the *rne* gene, various size fragments of the sequenced *coli* DNA were introduced to the pBluescript, and put under the control of a T7 promoter (Fig. 2). In all cases where the *rne-3071* mutation was complemented, we observed a 110,000 Da product (Fig. 3). The smallest fragment that tested positive contained a 2.6 kb DNA. It is obvious that a 2.6 kb DNA fragment can not code for a 110,000 Da protein. When the coding frame was disrupted at the *HindIII* site by a *tet* gene (at nucleotide 1217 Fig. 1a), the RNase E band disappeared, and the gene was rendered non-functional. In order to find out if the *rne* gene product is derived from the identified ORF we sequenced the N terminal of the translated product, and found that all the amino acids sequenced (twenty five, see Fig. 1) were in full agreement with the DNA sequence. Thus, it is clear that we have identified correctly, at least one end of the reading frame of the *rne* gene.

When the *rne* gene was expressed in the Maxi cell system (11), it showed a very unusual behavior. Rather than the customary single polypeptide, a number of polypeptides, all related structurally, ranging in size from about 40,000 to 130,000 Da, were found. Moreover, even when the current system (the T7 promoter/polymerase system) is employed, the behavior of the *rne* gene product(s) could be extremely unorthodox.

While after the cells were opened by hot sodium dodecyl

Figure 2. Plasmid constructs related to DNA fragments encoding RNase E. These fragments complement the *rne-3071* mutation.

Figure 3. Identification of the RNase E protein. Labeling was carried out with [³⁵S] methionine, using in each case a two plasmid system as described under Materials and Methods. In each case the first lane (1,3 and 5) contained 400µg/ml rifampicin and the second (2,4 and 6) did not. The first two lanes contain material from a strain carrying the two plasmids but no *coli* insert. The size of the *rne* gene product is around 110,000 Da as determined by protein markers.

sulphate the product of the RNase E gene could be shown to migrate as a single band of about 110 kDa (Fig. 3), material subjected to sonication shows a number of products all related to RNase E. These products, including the main band (110,000 Da), are unlikely to be aggregates. As can be seen in Fig. 4, when the *rne* gene products were exclusively labeled by [³⁵S]methionine (in presence of rifampicin) for a prolonged time,

Figure 4. An SDS polyacrylamide (5%/9% tandem) gel electrophoresis of the proteins synthesized in strain N3433 using the T7 polymerase/promoter system (12). In all cases the strains contained two plasmids, one being pGP1-2. Lane 1: control (pT7-5), lane 2: pNA12 (a plasmid coding for RNase III), lanes 3-8: pRE141. Lane 4, sample buffer contained 3% sodium N-lauroyl sarcosinate; lane 5, 5% SDS; lane 6, 7 M urea; lane 7, 6 M guanidine HCl; lane 8, sample was boiled for 5 minutes with the sample buffer instead of the regular 2 minutes. In all cases the samples, after the different treatments, were loaded directly onto the gel. [^{35}S] methionine was used to label the proteins for 60 minutes, and the synthetic medium contained 15% LB medium. The size of the RNase III protein is 25,000 Da and the size of the largest band in lanes 3-8 is around 110,000 Da, as determined by protein markers.

numerous bands related to the *rne* gene were observed. However, under similar conditions the expression of the *mc* (RNase III) gene appears as a single band (Fig. 4) of the appropriate size (25,000 Da). Interestingly, subjecting the RNase E material to various treatments, such as higher concentration of SDS, or guanidine HCl, did not change the pattern, indicating that these bands did not represent aggregated material.

One explanation for at least some of these anomalies, is the possibility that the *rne* protein binds to RNA. This is rather likely since in the RNase E mutant a relatively large number of RNAs accumulate which are not substrates for RNase E (23-25).

To test for the possibility that RNA is included in the *rne* gene product, we carried out a number of experiments all of which suggest that indeed this is the case. For instance, after treatment of the *rne* entity with ribonuclease, it migrates faster in a polyacrylamide gel (Fig. 5). The entity can be labeled with ^{32}P i or [^{35}S] methionine. The ^{32}P i labeled material can be degraded by ribonuclease, suggesting that it is RNA and not just phosphorylation of the protein. In Fig. 5 such experiments are presented. We can see that after treatment of the cell extract with micrococcal nuclease or T1 ribonuclease, the 110,000 entity disappears and a new smaller band appears. We also can see that material labeled with ^{32}P i and electroeluted from the 110,000 Da region of the gel disappears after digestion with micrococcal

Figure 5. RNase E contains RNA. The *rne* gene was expressed from plasmid pRE141 in a coupled T7 RNA polymerase/promoter system (12). (A) The material presented here was from cells labeled with [^{35}S]. The material presented in lanes 1-4 was incubated for 3 hrs. Lane 1, 37°C (no nuclease); lane 2, MN, micrococcal nuclease (30 u in 20 μl); lane 3, T1 ribonuclease (400 u in 20 μl); lane 4, no nuclease 0°C incubation; lane 5 contains material from the same strain without induction and no rifampin. Notice that in lane 5 the RNase E band (single arrow on the left) does not appear. The arrows on the right indicate molecular weight markers in daltons: 1, 116,000; 2, 97,400; 3, 66,000; 4, 45,000. (B) [^{32}P] labeled cells (Lanes 6, 7). An extract from cells induced for RNase E expression was separated in a 5% tandem SDS-PAGE and stained with coomassie brilliant blue R-250. The band containing the *rne* gene product was cut from the gel and the material was isolated by electroelution. The RNase E entity was precipitated with ethanol, dissolved in 0.01 M Tris, 0.01 M MgCl₂ and 0.01 M KCl pH 8.0, treated with 30 units of MN (micrococcal nuclease) for 1 hr at 37°C and run in a 5%/10% tandem SDS-PAGE. Lane 6, without MN; lane 7, with MN; lanes 8 and 9 contain [^{35}S] labeled material from a similar experiment, lane 8 induced, lane 9 uninduced (no rifampin). Notice that the RNase E gene product appears as a band of 110,000 Da (single arrow, lanes 1, 4 and 8). After digestion with nucleases a smaller product appears (double arrow, lanes 2 & 3). The RNase E entity labels with ^{32}P i (Lane 6) and it is RNA, since the label is sensitive to RNases (lanes 2, 3 and 7). A and B were from two different gels that were run differently, B is derived from a shorter run. (In lane 5 there is much more counts than in lane 9; but the pattern is equivalent. For instance the bottom two bands in lane 9 correspond to the two bands that migrate in lane 5 much further.)

nuclease. Thus, it is clear that the entity that migrates as a 110,000 Da is not a pure protein.

DISCUSSION

The sequence of the 3.2 kb DNA (Fig. 1) that complements the RNase E mutation, suggests that the *rne* gene codes for a protein of 550 amino acids with a mass of 62,000 Da. However, the product which is synthesized from the *rne* gene migrates as an 110,000 Da polypeptide (Fig. 3). While we are certain about the amino terminal end, since it had been sequenced (Fig. 1), we did not determine the carboxyl end, and it is possible that the open reading frame in Figure 1 is incorrect. Regardless of whether or not the ORF is correct it is obvious that 2 kb DNA (2.6-0.6; since the protein starts at nucleotide 666, see Fig. 1) cannot code for a 110,000 Da protein. Moreover, the finding that the *rne* gene product(s) migrates in a number of positions in PAGE (11), the different molecular mass determinations for

RNase E (1,26), and some of the observations presented here, point out to some rather unusual features of the *rne* gene product. While the finding presented here, that the *rne* gene entity contains RNA, might explain some of these peculiarities, it might not account for all the features of the RNase E gene product.

As far as the RNA is concerned, it is obvious that it is bound rather tightly to the protein. Unlike in the case of RNase P where the RNA and the protein dissociate in urea (27), in the case of RNase E they remain associated after electrophoresis in polyacrylamide gels containing urea and SDS. While it would be exciting to assume that the RNA found in RNase E is coded from the same region of the DNA as the protein (see below) this is not necessarily the case. Apparently, RNase E binds various RNAs (23–25), and therefore it is possible that newly synthesized RNA could appear in the RNase E band.

Since we established experimentally the amino terminal end of the RNase E protein, but not the carboxyl end, the maximum size polypeptide synthesized from the 2.6 kb fragment could be approximately 80,000 Da, and not the observed product that migrates in the gel as a 110,000 Da polypeptide (See Figs. 1, and 3). It is conceivable that the proteins synthesized from the shorter DNA fragments are not fully coded by the inserted *coli* DNA. However, it is most unlikely that in the different deletions tested they all will terminate in such a way as to produce an identical size polypeptide of 110,000 Da. Subjecting this protein to various agents such as urea, guanidine HCl and relatively high levels of sodium-dodecyl-sulfate did not reduce the molecular mass of this protein, suggesting that the large (110,000 Da) entity did not result from aggregation (Fig. 4).

The entity migrating in the 110,000 Da position contains RNA (Fig. 5), since it is sensitive to ribonucleases (T1, and micrococcal nuclease) and it labels with ^{32}P i and the label is degraded by ribonucleases (Fig. 5). Moreover, after sonication smaller products are generated from this band while other protein bands remain intact. Furthermore, when RNase E was purified, it significantly lost specific activity in some of the purification steps (26). (In one of the steps it lost more than ten fold in specific activity.) This could be explained if RNA is necessary for the function of RNase E and it was partially lost during the purification.

It is premature to speculate on the nature of the RNA found in RNase E, it could be for instance nonspecific RNA attached to the protein, since it is an RNA binding protein. However, there are a number of observations that indicate that this simple possibility is rather unlikely. The size of the *rne* gene product produced from the T7 promoter in presence or absence of rifampicin (blockage of host RNA synthesis) is the same, (see Fig. 3), suggesting that the RNA in RNase E originates from the same promoter as the polypeptide. Since we observed large products of RNase E (in the 100,000 Da size range) when we had used the maxicell system and RNase E was synthesized from its own promoter (11), it is unlikely that the association of the *rne* protein with RNA is related to the *rne* gene being expressed from a T7 promoter. Therefore, it is possible that the RNA moiety coincides or overlaps the message for the protein or is immediately contiguous to it. Since the relatively large size of RNase E can be observed also when it is overproduced and host RNA synthesis is blocked, it is unlikely that the size of RNase E is caused by association with non-specific RNA.

It is worthwhile to mention here that the *rne* gene is most likely the structural gene for RNase E, since the mutation *rne-3071* renders the activity temperature sensitive, (9, 26), and cloning the *rne*⁺ gene leads to increased RNase E activity in extracts

containing the cloned gene (11; Chauhan and Apirion, unpublished observations). Also, the *rne3071* mutation, and the 3.2 kb DNA fragment that complement it, do map to the same position (L. Taraseviciene, A. Miczak and D. Apirion, Manuscript in preparation).

Thus, the experiments presented here lead to the suggestion that RNase E is a unique kind of enzyme, where the protein is associated tightly with RNA. However, at present, we do not know if the attached RNA is specific or functional.

(A preliminary report of these findings was presented in the Cold Spring Harbor meeting on RNA Processing, 1990, p. 277.)

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