Volume 16 Number 14 1988 Nucleic Acids Research

# *Escherichia coli* RNase D: sequencing of the *rnd* structural gene and purification of the overexpressed protein

Jiren Zhang and Murray P.Deutscher

Department of Biochemistry, University of Connecticut Health Center, Farmington, CT 06032, USA

Received February 1, 1988 Accession no. X07055

## ABSTRACT

We have determined the nucleotide sequence of a 1.4-kb-pair fragment of the *E. coli* chromosome that carries the complete *rnd* gene encoding RNase D, a putative tRNA processing enzyme. The coding region of *rnd* extends for a total of 1128 nucleotides beginning at an initiator UUG codon and terminating at a UAA codon, and encodes a 375-amino acid polypeptide of 42,679 daltons, consistent with the known size of RNase D. A rapid purification procedure was developed for isolation of RNase D from strains overexpressing the enzyme. The N-terminal sequence and the amino acid composition of the homogenous protein were in excellent agreement with those derived from the sequence of the *rnd* gene.

## INTRODUCTION

Processing of the 3' terminus of tRNA precursors in *Escherichia coli* is thought to involve an initial endonucleolytic cleavage downstream from the mature 3' end followed by one or two exonucleolytic trimming reactions that expose the encoded -CCA sequence (1). Despite the identification of several potential processing enzymes, the details and proteins involved in 3' end maturation have not yet been conclusively established (1). A prime candidate for the 3' trimming enzyme is the exoribonuclease, RNase D (2). The enzyme has been purified to homogeneity (3), and *in vitro* is capable of rapidly removing extra residues from the 3' terminus of tRNA precursors (4), generating functional tRNAs. This, and the fact that RNase D acts much more slowly on mature tRNA (2), suggest a physiological role for the enzyme in tRNA maturation. However, mutants deficient in RNase D, *rnd* mutants, grow normally and show no defect in tRNA processing (5). Thus, the role of this enzyme *in vivo* is as yet unclear.

To learn more about the structure, function and regulation of

RNase D, we have recently cloned the *rnd* gene, provided evidence that it is the structural gene for RNase D and confirmed that a putative deletion mutant devoid of RNase D (5) is missing the *rnd* gene (6). We have also shown that elevated levels of RNase D are deleterious to *E. coli* (6). Here, we present the complete nucleotide sequence of the *rnd* gene and describe a purification procedure for obtaining large amounts of homogeneous RNase D from strains overexpressing the enzyme. We have also used the purified protein to confirm various aspects of its derived amino acid sequence, thereby establishing that *rnd* is the structural gene for RNase D.

## MATERIALS AND METHODS

### Bacterial strains, phages and plasmids

*Escherichia coli* strain UT481 (Δ*lac-pro*),*hsd*S($r^-m^-$),F'*lacI*$^q$, Δ*lacM15*) was used for growth of phages and plasmids. The multiple RNase-deficient strain, 20-12E/18-11 (RNase I$^-$,II$^-$,D$^-$,BN$^-$,T$^-$) (7) transformed with pDB14 was used for purification of RNase D. Plasmid pUC18 (8) was used for construction of *rnd* clones and subclones. Plasmid pDB14 is a derivative of pUC18 that carries a 1.4-kb-pair insert containing the *rnd*$^+$ gene (6). Phages M13mp8 and M13mp19 (9) were used for sequencing of the *rnd*$^+$ gene.

### Culture conditions

Cells were routinely grown at 37$^o$C in YT medium or on YT-agar plates (10). Antibiotics, when added, were at the following concentrations: ampicillin, 50 μg/ml; tetracycline, 12.5 μg/ml. Growth in liquid culture was followed by absorbance at 550 nm.

### Materials

[ $\alpha^{32}$P]dATP (800 Ci/mmol) and $^{32}$Pi were purchased from NEN and [$^3$H]poly(A) was from Amersham. Restriction endonucleases, T4 DNA ligase and Bal31 exonuclease were obtained from New England Biolabs. DNA polymerase I (Klenow fragment) was from Boehringer-Mannheim Biochemicals and modified T7 DNA polymerase (Sequenase) was from United States Biochemical. Corp. DEAE Sephadex A-50 was purchased from Pharmacia, hydroxyapatite (Bio-Gel HT) and Affi-Gel Blue from Bio-Rad Laboratories and Ultrogel AcA44 from LKB. [$^{32}$P] phosphodiesterase-treated tRNA, tRNA-C-C-[$^{14}$C]A and tRNA-CCA-[$^{14}$C]Cn were prepared as described previously (5,11). All other chemicals were reagent grade.

Assay of RNase D activity

Standard reaction mixtures contained in 0.1 ml: 20 mM glycine-NaOH, pH 8.9, 5 mM $MgCl_2$, 20 µg [$^{32}$P] diesterase-treated tRNA and enzyme fraction. Incubation was at 37°C, and acid-soluble radioactivity was determined as described (11).

Recombinant DNA procedures

Plasmid DNA isolation, restriction endonuclease and Bal31 exonuclease digestions, DNA ligation, transformation, hybridization and gel electrophoresis were carried out by standard techniques described in Maniatis *et al.* (12). Phage DNA for DNA sequencing was purified as described by Dale *et al.* (13).

DNA sequencing

The sequence of the *rnd* gene was determined by the dideoxy chain termination method (14) using either DNA polymerase I or T7 DNA polymerase. Compression problems in GC-rich regions were overcome by increasing the reaction temperature to 50°C, by using acrylamide gels with 40% formamide, by substituting dITP for dGTP, or by a combination of these methods. Both strands were sequenced completely and all fragments were overlapped.

Purification of RNase D

RNase D was purified from the overexpressing strain, 20-12E/18-11 transformed with plasmid pDB14. This strain is mutant for five RNases, and overexpresses RNase D from the plasmid to a maximal level about 20 times wild type. Overexpressing cells were prepared by growth in a fermentor to an $A_{550}$ of 0.8, and stored frozen at -20°C until use. At this early stage of growth cells have a higher specific activity of RNase D because at later times the culture tends to be overgrown by faster-growing cells with a lower plasmid copy number and less RNase D (6).

Thawed cells (40 g wet weight) were suspended in 2 volumes of buffer A (20 mM Tris, pH 7.5, 5 mM $MgCl_2$, 0.1 mM dithiothreitol, 0.1 mM phenylmethylsulfonylfluoride) containing 150 mM KCl, and ruptured by two passages through an Aminco French press at about 10,000 p.s.i. The crude extract was treated with DNase I (10 µg/ml) for 45 min at 0°C and centrifuged for 40 min at 17,000 xg. The supernatant fraction was then centrifuged for 2 hours at 45,000 rpm in a Beckman 50.2 rotor.

The resulting supernatant fraction II was dialyzed against buffer A containing 40 mM KCl to lower the KCl concentration, and applied to a

DEAE Sephadex column (5 x 18 cm) equilibrated with buffer A containing 60 mM KCl. RNase D was eluted with a 7-liter linear gradient from 60 to 300 mM KCl in buffer A. Fractions containing RNase D were combined, dialyzed against buffer A and applied to a column of Affi-Gel Blue (2.5 x 10 cm) equilibrated with buffer A. RNase D was eluted with a 4-liter linear gradient from 0 to 0.5 M KCl in buffer A. Active fractions were combined, concentrated with an Amicon YM-10 membrane in a Diaflo filtration apparatus, and dialyzed against buffer A. The dialyzed sample (10 ml) was applied to a hydroxyapatite column (1.5 x 5 cm) equilibrated with buffer A, and RNase D was eluted with a 150-ml linear gradient from 10 to 200 mM potassium phosphate, pH 7.5. The active fractions were concentrated, as above, to 3 ml and applied in two 1.5 ml portions to a column of Ultrogel AcA44 (1.5 x 80 cm) equilibrated with buffer A containing 0.2 M KCl. Active fractions were frozen at -20$^{o}$C until further use. A summary of the purification scheme is presented in RESULTS.

Amino acid analysis and N-terminal sequence analysis

Samples were precipitated with 20% trichloroacetic acid, washed once with HCl-acetone (1:500) and lyophilized. For amino acid analysis samples of 10 µg were hydrolyzed for 1 hour with 6 N HCl-2% phenol at 150$^{o}$C and applied to a Beckman System 7300 Amino Acid Analyzer. For cysteine determination as cysteic acid, samples were first oxidized with performic acid prior to hydrolysis. For N-terminal sequencing a sample of 40 µg (~ 1 nmol) was applied to an updated Applied Biosystem 470A protein sequencer.

## RESULTS

Sequence analysis of the *rnd* gene

Previously, we described the cloning and isolation of the *rnd* gene as a 1.4-kb-pair fragment in plasmid pUC18 (6). In maxicells, the resulting plasmid, termed pDB14, directed the synthesis of a 41-kDa protein, the known size of RNase D (4), and also led to elevated expression of RNase D activity (6). Since cloning of this fragment in either orientation led to the same level of overexpression, it apparently carries the promoter as well as the complete coding region of *rnd*.

The 1.4-kb fragment and subclones derived from it by Bal31 or restriction nuclease digestion were transferred to M13mp8 and M13mp19 for sequencing by the dideoxy method (14). The sequencing

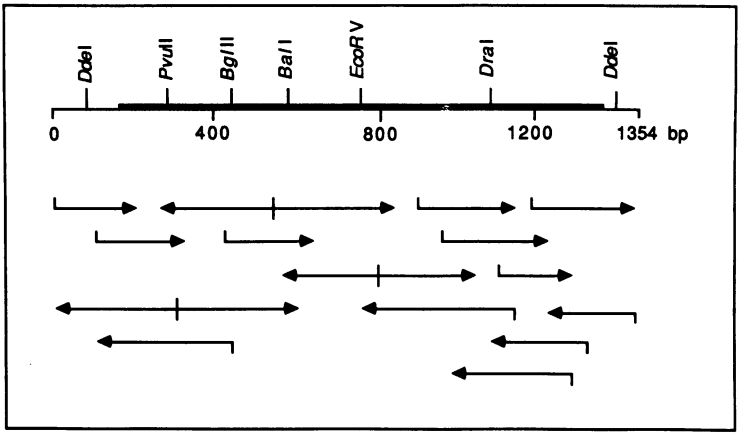


Fig. 1. Sequencing strategy and restriction map of the 1.4-kb-pair fragment carrying *rnd*. The position of the *rnd* coding sequence on the fragment is presented as a bold line. Each arrow shows the beginning, end, and direction of the fragment used for sequencing.

strategy and restriction map of the 1.4-kb fragment are presented in Fig. 1. Both strands were sequenced completely and agreed throughout.

Examination of the nucleotide sequence (Fig. 2) revealed only a single open reading frame of sufficient length to encode RNase D extending from nucleotide 157 to a UAA termination codon beginning at nucleotide 1294. Four possible in frame, translation start sites, 3 UUG and 1 AUG, are present within the first 126 nucleotides of the open reading frame, any one of which would lead to a protein close to the size of RNase D. The most likely candidate, however, was the most upstream UUG at position 169-171 since only that start site is preceded by strong Shine-Delgarno sequence, UAAAGAG (6 out of 7 nucleotides compared to the consensus sequence, underlined in Fig. 2). Confirmation of this translation start site was made by N-terminal sequence analysis of purified RNase D (see below). The resulting open reading frame of 1125 nucleotides encodes a 375-amino acid polypeptide of 42,679 daltons.

The accuracy of the nucleotide sequence was supported by identification of all the expected restriction sites within the sequence (data not shown) and by determination of the amino acid composition of homogeneous RNase D. As shown in Table 1, the experimentally determined amino acid composition of RNase D agrees very well with

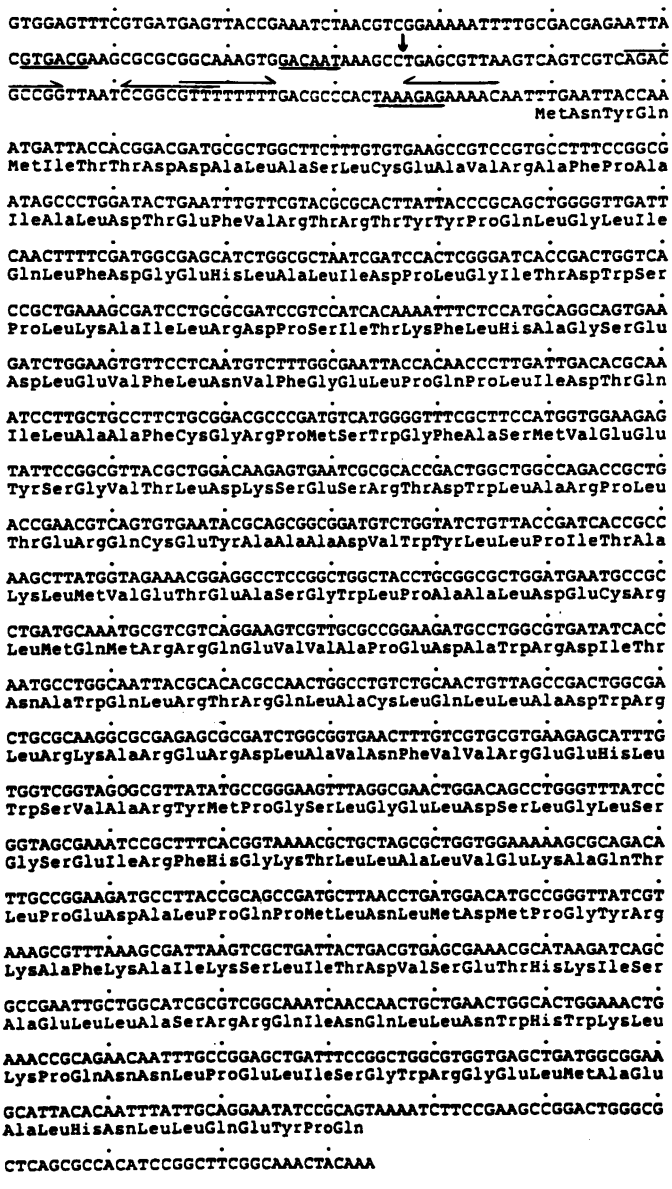


Fig. 2. Nucleotide sequence of the 1.4-kb fragment containing the *rnd* gene. Only the sequence of the noncoding DNA strand is shown together with the derived amino acid sequence of RNase D. The putative ribosome binding site and -10 and -35 regions of the promoter are underlined. The arrows above the nucleotide sequence upstream of the coding region show the location of stems of possible stem-loop structures. The vertical arrow shows the position of cleavage by *Dde*1.

Table 1
Predicted and experimentally determined amino acid composition of *E. coli* RNase D

| Amino acid residue | Predicted from nucleotide sequence | Determined from protein hydrolysate[a] |
|---|---|---|
| | residues/protein | |
| Alanine | 37 | 38 |
| Arginine | 25 | 25 |
| Aspartic acid + Asparagine | 31 | 32 |
| Glycine | 17 | 18 |
| Glutamic acid + Glutamine | 47 | 49 |
| Histidine | 7 | 8 |
| Isoleucine | 17 | 16 |
| Leucine | 58 | 58 |
| Lysine | 13 | 13 |
| Methionine | 11 | 9 |
| Phenylalanine | 11 | 11 |
| Proline | 20 | 21 |
| Serine | 20 | 20 |
| Threonine | 19 | 19 |
| Tryptophan | 12 | ND |
| Tyrosine | 9 | 9 |
| Valine | 16 | 15 |
| Cysteine | 5 | 5[b] |

[a]Calculated relative to phenylalanine
[b]Determined after performic acid oxidation

the predicted composition derived from the DNA sequence. Codon usage for the *rnd* gene is given in Table 2. Optimal codon usage, as defined by Ikemura and Ozeki (15) is 62.2%, suggestive of a relatively poorly expressed gene. Rare codons in the *rnd* gene are found at a slightly elevated level compared to total *E. coli* genes sequenced. Whereas the codons CGA, CGG, AGA, AGG, CUA, ACA, CCC, GGA, AUA are found as 1.9% of total codons in *E. coli* genes (16), they are present as 3.7% of the codons in *rnd*, again indicative of a more poorly expressed gene.

Location of the Translation Start and Stop Sites and the Promoter Region

To determine which of the 4 possible translation start sites is used for RNase D synthesis, approximately 1 nmole of homogeneous protein was subjected to 6 cycles of automated N-terminal sequence analysis in a gas-phase sequenator. The first six amino acids with their absolute yield in pmoles are: methionine (439), asparagine (311),

Table 2
Codon Usage in the *E. coli rnd* gene

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Gly | GGC | 2 | Leu | CTG | 30 | Arg | CGG | 1 | Met | ATG | 11 |
| Gly | GGA | 2 | Leu | CTA | 3 | Arg | CGA | 2 | Ile | ATA | 1 |
| Gly | GGT | 6 | Leu | CTT | 4 | Arg | CGT | 11 | Ile | ATT | 6 |
| Gly | GGC | 7 | Leu | CTC | 3 | Arg | CGC | 10 | Ile | ATC | 10 |
| Glu | GAG | 7 | Leu | TTG | 9 | Arg | AGG | 0 | Thr | ACG | 7 |
| Glu | GAA | 22 | Leu | TTA | 9 | Arg | AGA | 1 | Thr | ACA | 3 |
| Asp | GAT | 12 | Phe | TTT | 7 | Ser | AGT | 3 | Thr | ACT | 3 |
| Asp | GAC | 9 | Phe | TTC | 4 | Ser | AGC | 4 | Thr | ACC | 6 |
| Val | GTG | 6 | Ser | TCG | 4 | Lys | AAG | 5 | Trp | TGG | 12 |
| Val | GTA | 2 | Ser | TCA | 2 | Lys | AAA | 8 | End | TGA | 0 |
| Val | GTT | 3 | Ser | TCT | 1 | Asn | AAT | 5 | Cys | TGT | 3 |
| Val | GTC | 5 | Ser | TCC | 6 | Asn | AAC | 5 | Cys | TGC | 2 |
| Ala | GCG | 17 | Pro | CCG | 16 | Gln | CAG | 8 | End | TAG | 0 |
| Ala | GCA | 4 | Pro | CCA | 2 | Gln | CAA | 10 | End | TAA | 1 |
| Ala | GCT | 3 | Pro | CCT | 1 | His | CAT | 4 | Tyr | TAT | 6 |
| Ala | GCC | 13 | Pro | CCC | 1 | His | CAC | 3 | Tyr | TAC | 3 |

tyrosine (361), glutamine (354), methionine (363) and isoleucine (294); no other amino acid was present above 50 pmoles in any of the cycles. This sequence agrees exactly with the derived amino acid sequence beginning at nucleotide 169, and confirms that RNase D synthesis begins at the first UUG codon in the open reading frame. These data also indicate that RNase D is not subject to processing at its N-terminus.

The approximate location of the translation termination codon in the *rnd* gene was verified by Bal31 deletion and maxicell analysis of the product of the truncated gene. We have previously shown (6) that removal of ~ 100 nucleotides from one end of the 1.4-kb fragment leads to the synthesis of a RNase D several thousand daltons larger than the wild type protein. Sequencing of this truncated gene revealed that 85 nucleotides were removed from the downstream end, resulting in removal of 8 amino acids from the C-terminus of RNase D, and addition of 54 amino acids due to nucleotide sequences in pUC18 before another stop codon was reached. These findings confirm the reading frame at the C-terminus of the derived sequence, and verify that the translation stop site is in the last 85 nucleotides of the 1.4-kb fragment, as is suggested by the sequence.

The fact that the 1.4-kb-pair fragment is active for RNase D synthesis in either orientation in pUC18 (6), and is not dependent on IPTG, suggests that the *rnd* promoter is located on this insert. This is supported by the finding that upstream of the coding region are the sequences GACAAT and GTGACG, spaced 17 nucleotides apart, that are consistent with the -10 and -35 regions of a promoter. Analysis of this region by the method of Mulligan *et al.* (17) gave a homology score of 53, well within the range of known *E. coli* promoters. Furthermore, treatment of the 1.4-kb fragment with the restriction enzyme, *Dde* I, which cuts between the putative promoter region and the Shine-Delgarno sequence (see Fig. 1), renders RNase D expression from the shortened fragment dependent on the *lacZ* promoter in pUC18. Synthesis of RNase D occurs only when the fragment is inserted in the proper orientation, and is greatly stimulated by the presence of IPTG. Removal of additional nucleotides by cleavage with *Pvu*II (see Fig. 1), renders the *rnd* gene inactive. Based on these data, it is likely that the proposed upstream region is the *rnd* promoter, although nuclease S1 mapping of the transcription start site will be necessary to conclusively establish this point. No rho-independent transcription termination site or palindromic unit (rep sequence) (18) was found in the short region following the coding sequence on the 1.4-kb fragment.

Purification of Overexpressed RNase D

Transformation of *E. coli* with plasmid pDB14 leads to about a 20 to 30-fold maximal overexpression of RNase D (6). The level of overexpression varies with growth of the cells apparently due to selection for faster-growing cells containing less of the nuclease (6). Thus, the highest specific activities of RNase D are found when cells are at relatively low densities, necessitating a compromise between large amounts of cells and high activity in large-scale preparations.

For the purification scheme described here, strain 20-12E/18-11, which is devoid of most of the known *E. coli* exoribonucleases, was used to lower the amount of potential contaminating activities. The purification of RNase D from 40 g of these cells containing pDB14 is summarized in Table 3 (details are presented in Materials and Methods). The overall purification gave about 6 mg of homogeneous RNase D and required four chromatographic steps. However, since contaminating RNases were largely eliminated by use of the mutant strain, RNase D of sufficient purity for most studies, and free of nucleic acids, could be

Table 3
Summary of purification of RNase D from 18-11/pDB14

| Step | Total activity | Total protein | Specific activity | Recovery | Purification |
|---|---|---|---|---|---|
| | units | mg | units/mg | % | -fold |
| 1. Supernatant I | 2,200 | 1220 | 1.80 | 100 | 1.0 |
| 2. Supernatant II | 2,100 | 828 | 2.54 | 95 | 1.4 |
| 3. DEAE-Sephadex | 3,700 | 260 | 14.1 | 168 | 7.8 |
| 4. Affi-Gel Blue | 5,200 | 54 | 96.6 | 236 | 54 |
| 5. Hydroxyapatite | 4,300 | 15.9 | 271 | 195 | 150 |
| 6. Ultrogel AcA44 | 2,100 | 6.4 | 326 | 95 | 180 |

obtained in large amounts with just two columns (Fig. 3).

Homogeneous RNase D could be obtained with a purification of about 180-fold (Fig. 3). It was not possible to determine recovery of RNase D during the purification procedure since, as shown in Table 3, activity in the first 3 steps is underestimated due to inhibition by the

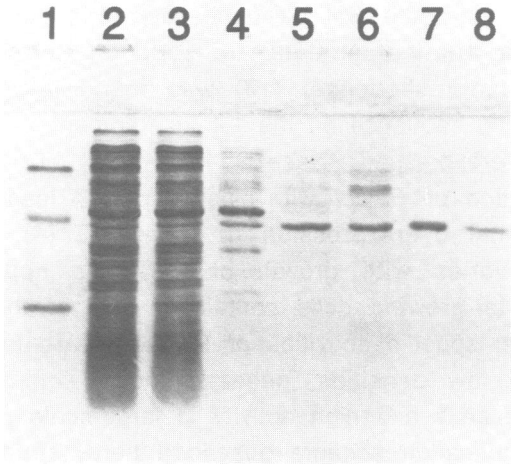


Fig. 3. SDS-polyacrylamide gel electrophoresis of RNase D during purification from strain 18-11/pDB14. Samples and gels were prepared as described by Dreyfuss *et al.* (23) using 10% gels, and stained with Coomassie brilliant blue R-250. Lane 1, standard proteins: bovine serum albumin (68 kDa), ovalbumin (43 kDa) and trypsin inhibitor (20 kDa); lane 2, supernatant I, 3 µg; lane 3, supernatant II, 3 µg; lane 4, DEAE Sephadex fraction, 3 µg; lane 5, Affi-Gel Blue fraction, 2.7 µg; lane 6, hydroxyapatite fraction, 3.4 µg; lane 7, Ultrogel AcA44 fraction, 2.5 µg; lane 8, purified RNase D from strain A19 (3), 1.6 µg.

large amounts of nucleic acid present. The specific activity of the purified enzyme, 326 U/mg, is essentially identical to the 300 U/mg previously obtained by conventional methods (3), and indicates the similarity of the overexpressed protein to its chromosomal counterpart. This is also indicated by the same size of the two proteins on SDS gels (Fig. 3), and by their same relative activities against a variety of RNA substrates (data not shown). The large amounts of purified RNase D that can be prepared by the procedures described here should facilitate structural and functional studies of this protein.

## DISCUSSION

The complete nucleotide sequence of the *rnd* gene, the proposed structural gene for RNase D, has been determined. The gene encodes an acidic, 375-amino acid protein of about 42,500 daltons which is consistent with the isoelectric point (pI = 6.2) and molecular mass (Mr = 40,000) of RNase D determined previously (3). Likewise, the N-terminal sequence and amino acid composition determined for homogeneous RNase D are in complete agreement with the amino acid sequence derived from the *rnd* gene. These findings confirm that *rnd* is the structural gene for RNase D, and suggest that RNase D is not processed after its synthesis.

Translation of *rnd* is initiated at an infrequently used UUG codon. Only a few other *E. coli* genes have been shown to utilize UUG for initiation (19), and interestingly, one of these is the gene for another exoribonuclease, polynucleotide phosphorylase (20). The significance of this observation is not known, however. In one situation, the *cya* gene encoding adenylate cyclase, the presence of a UUG codon has been shown to limit gene expression (21). Based on its probable promoter sequence and on its codon usage, the *rnd* gene would already fall into the category of a relatively poorly to moderately expressed gene. Thus, it is not clear what additional limitation might be imposed by the presence of the UUG codon.

Analysis of sequences upstream of the *rnd* coding region has revealed a number of potential regulatory regions. First of all, 8 nucleotides upstream of the initiator UUG is the sequence UAAAGAG which matches the consensus Shine-Delgarno sequence in 6 of 7 nucleotides (19), and is the appropriate distance away to be the ribosome binding site. Further upstream are the hexanucleotide

sequences GACAAT and GTGACG, spaced 17 nucleotides apart, that by computer analysis look like a promoter (17), and that most likely serve as a *rnd* promoter. Experimentally, it is known that the 1.4-kb pair fragment contains a promoter that functions *in vivo* for *rnd* expression, and that removal of the hexanucleotide regions by cleavage at the *Dde*1 site abolishes promoter activity of the fragment.

A puzzling feature of the regulatory region is that a potentially strong transcription termination sequence (22) (noted by inverted arrows above the sequence in Fig. 2) is located between the putative promoter and the ribosome binding site. This sequence in the RNA would contain a 6 to 9-base pair GC-rich stem, a 5-base loop, and is followed by a run of as many as 8 U residues. At present, it is not clear whether this site serves to regulate transcription from the putative *rnd* promoter, especially since the distance between them is very short, or whether it might act as a terminator for a transcript originating upstream of *rnd*. It is known that genes for at least two other proteins are immediately upstream of the *rnd* gene (6), and the ends of two possible open reading frames are found in the first 100 or so nucleotides of the sequence shown in Fig. 2, either of which could represent the C-terminus of one of these unknown proteins (6). A second possible stem-loop structure (noted by inverted arrows in Fig. 2), overlapping the terminator and including much of the ribosome binding site, was also noted. These structures, as well as other longer-range possible complementary sequences (not shown) in the region upstream of *rnd*, raise the possibility of regulation of RNase D expression. Further studies to analyze regulation in this system are underway.

Analysis of the derived amino acid sequence of RNase D indicates that it is a relatively hydrophobic protein, particularly for one that interacts with a highly-charged RNA substrate. Over 43% of the residues in RNase D are hydrophobic amino acids (Ala, Ile, Leu, Met, Phe, Trp, Val). A similar calculation for proteins, in general, yields a value of about 36% (24), and for the other sequenced RNases, RNase H (25), RNase III (26) and PNPase (20), this value varies from 34 to 40%. As might be expected from the number of hydrophobic amino acids in RNase D, a number of distinct hydrophobic regions are present in the protein based on hydropathy plots (27). Despite these findings, however, there is no evidence that RNase D interacts with membranes, and most likely,

the hydrophobic regions are buried and simply serve as part of the internal structure of the protein.

Although RNase D acts on RNA, it has an overall negative charge. However, while the acidic amino acids are distributed evenly throughout the protein, the basic amino acids are more clustered. Twenty percent of the amino acids between residues 180 and 240 are basic, whereas only 8% of the remaining residues of the protein are arginine or lysine. This distribution focuses attention on this central region as a possible part of the RNA binding site of the protein.

Finally, the derived amino acid sequence of RNase D was examined for similarity to the sequences of RNase H (25), RNase III (26) and PNPase (20). No extended regions of similarity were found in comparisons of RNase D with RNase H or RNase III; the longest stretch of identical or conservatively replaced amino acids was four. Comparison of RNase D with the exoribonuclease, PNPase, however, revealed two regions of 16 amino acids each that were 44% identical, one of which had, in addition, 19% related amino acids. Though RNase D and PNPase do not appear to be closely related, it is possible that these small regions of similarity between the two proteins have some structural or functional significance.

**ACKNOWLEDGMENT**

This work was supported by Grant GM16317 from the National Institutes of Health. This is paper 52 in the series, "Reactions at the 3' Terminus of tRNA". The previous paper in this series is ref. 28.

**REFERENCES**

1. Deutscher, M.P. (1984) Crit. Rev. Biochem. *17*, 45-71.
2. Ghosh, R.K. and Deutscher, M.P. (1978) J. Biol. Chem. *253*, 997-1000.
3. Cudny, H., Zaniewski, R. and Deutscher, M.P. (1981) J. Biol. Chem. *256*, 5627-5632.
4. Cudny, H. and Deutscher, M.P. (1980) Proc. Natl. Acad. Sci. U.S.A. *77*, 837-841.
5. Blouin, R.T., Zaniewski, R. and Deutscher, M.P. (1983) J. Biol. Chem. *258*, 1423-1426.
6. Zhang, J. and Deutscher, M.P. (1988) J. Bacteriol., in press.
7. Deutscher, M.P., Marlor, C.W. and Zaniewski, R. (1985) Proc. Natl. Acad. Sci. U.S.A. *82*, 6427-6430.
8. Yanisch-Perron, C., Vieira, J. and Messing, J. (1985) Gene *33*, 103-109.

9. Messing, J. (1983) Methods Enzymol. *101*, 20-78.
10. Miller, J.H. (1972) Experiments in Molecular Genetics. pp. 431-435. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.
11. Deutscher, M.P. and Ghosh, R.K. (1978) Nucleic Acids Res. *5*, 3821-3829.
12. Maniatis, T., Fritsch, E.F. and Sambrook, J. (1982) Molecular Cloning. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.
13. Dale, R.M.K., McClure, B.A. and Houchins, J.P. (1985) Plasmid *13*, 31-40.
14. Sanger, F., Nicklen, S. and Coulson, A.R. (1977) Proc. Natl. Acad. Sci. U.S.A. *74*, 5463-5464.
15. Ikemura, T. and Ozeki, H. (1982) Cold Spring Harbor Symp. Quant, Biol. *47*, 1087-1097.
16. Maruyama, T., Gojobori, T., Aota, S. and Ikemura, T. (1986) Nucleic Acids Res. *14*, r151-r197.
17. Mulligan, M.E., Hawley, D.K., Entriken, R. and McClure, W.R. (1984) Nucleic Acids Res. *12*, 789-800.
18. Gilson, E., Clement, J.M., Perrin, D. and Hofnung, M. (1987) Trends Gen. *3*, 226-230.
19. Gold, L. and Stormo, G. (1987) in Neidhardt, F.C. (ed.) Escherichia coli and Salmonella typhimurium-Cellular and Molecular Biology, Amer. Soc. Microbiol., Washington, D.C., pp. 1302-1307.
20. Regnier, P., Grunberg-Manago, M. and Portier, C. (1987) J. Biol. Chem. *262*, 63-68.
21. Reddy, P., Peterkofsky, A. and McKenney, K. (1985) Proc. Natl. Acad. Sci. U.S.A. *82*, 5656-5660.
22. Yager, T.D. and von Hippel, P.H. (1987) in Neidhardt, F.C. (ed.) Escherichia coli and Salmonella Typhimurium - Cellular and Molecular Biology, Amer. Soc. Microbiol., Washington, D.C., pp. 1241-1275.
23. Dreyfuss, G., Adam, S.A. and Choi, Y.D. (1984) Mol. Cell. Biol. *4*, 415-423.
24. Doolittle, R.F. (1986) Of URFS and ORFS. pp. 49-60. University Science Books, Mill Valley, CA.
25. Kanayo, S. and Crouch, R.J. (1983) J. Biol. Chem. *258*, 1276-1281.
26. March, P.E., Ahnn, J. and Inouye, M. (1985) Nucleic Acids Res. *13*, 4677-4685.
27. Kyte, J. and Doolittle, R.F. (1982) J. Mol. Biol. *157*, 105-132.
28. Deutscher, M.P., Marshall, G.T. and Cudny, H. (1988) Proc. Natl. Acad. Sci. U.S.A., in press.