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Apparent involvement of ribonuclease D in the 3′ processing of tRNA precursors*

(tRNA biosynthesis/ribonuclease II/-C-C-A sequence)

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ABSTRACT **_Escherichia coli_ RNase D and RNase II have been purified to homogeneity and compared for their ability to remove extra nucleotides following the -C-C-A sequence in tRNA precursors. RNase D and RNase II are single-chain proteins with molecular weights of 38,000 and 78,000, respectively. Both enzymes require a divalent cation for activity on tRNA precursors, but, in addition, RNase II is stimulated by monovalent cations. RNase D specifically removes mononucleotide residues from a mixture of tRNA precursors to generate amino acid acceptor activity for essentially all amino acids. Although RNase II can also remove precursor-specific residues, no amino acid acceptor activity is recovered. Similarly, RNase D action on the _E. coli_ $tRNA^{Tyr}$ precursor is limited, whereas RNase II causes extensive degradation. In contrast to the processive mode of hydrolysis by RNase II, RNase D removes nucleotides randomly and slows down greatly at the -C-C-A sequence, thereby allowing the tRNA to be aminoacylated and protected from further degradation. These results suggest that RNase D is the 3′-processing nuclease _in vivo_ and that RNase II is a nonspecific degradative enzyme. The importance of RNA conformation for correct processing is also discussed.**

The biosynthesis of functional tRNA molecules requires the participation of specific nucleases that remove extra nucleotides at the 5′ and 3′ termini of tRNA precursors (1). Although the nuclease involved in processing at the 5′ terminus, RNase P, has been known for a number of years (2), the enzyme(s) responsible for final trimming at the 3′ end has not been conclusively identified. This has come about because the cumbersome electrophoretic procedures previously used to assay 3′-processing enzymes have precluded extensive purification and study of activities identified in cell extracts. Nevertheless, several activities capable of generating tRNA-size molecules from tRNA precursors that contain extra residues following the -C-C-A sequence (termed type I precursors) have been partially purified from *Escherichia coli*. It was suggested initially that the known exonuclease, RNase II, was the enzyme involved in processing the 3′ terminus of type I precursors (3–5). Subsequently, Bikoff *et al.* (6) identified another nuclease, RNase PIII, distinct from RNase II, that was required for synthesis of *E. coli* su^{+}_{III} $tRNA^{Tyr}$; and Shimura *et al.* (7) described two activities, termed RNase Q and RNase Y, that also acted on type I precursors. However, these putative processing activities have not been well characterized, and their relationship to one another is not known.

In addition to the type I precursor, which is prevalent in *E. coli* (4, 8, 9), phage-infected *E. coli* (10) and eukaryotic cells (11, 12) contain a second type of tRNA precursor in which the -C-C-A sequence is replaced by other nucleotides (type II precursor). A mutant of *E. coli*, termed BN, has been isolated (13); it fails to process type II precursors when the cells are infected by phage (14), and this defect is associated with the loss of a nucleolytic activity in extracts (ref. 15; unpublished results). These results suggest that at least two 3′-processing nucleases are present in *E. coli*, one for each type of precursor.

In order to overcome the difficulties associated with purifying and studying the 3′ nucleases, and to determine which ones may actually have the specificity to function in processing, we have developed procedures for synthesizing large amounts of synthetic type I and type II tRNA precursors that are radioactively labeled only in the nucleotide residues to be removed by the processing nucleases (16). Using these substrates and simple assay procedures involving solubility in acid, we previously described the partial purification of two enzymes capable of removing the extra nucleotides from type I precursors (17). One of these enzymes was RNase II (18, 19), and the other was RNase D, a nuclease that we had shown earlier is active on tRNAs with altered structures (20).

We have now purified each of these enzymes to homogeneity and have compared their mode of action on tRNA precursors. Our results indicate that RNase D specifically removes the extra 3′ residues from type I precursors to generate amino acid acceptor activity and has the properties expected for the type I processing nuclease. RNase II, on the other hand, appears to function as a nonspecific degradative enzyme.

MATERIALS AND METHODS

tRNA Precursors. tRNA-C-C-A-[^{14}C]C_n was prepared from *E. coli* tRNA and [^{14}C]CTP by using rabbit liver tRNA nucleotidyltransferase as described (16). The preparation used in these studies was characterized (16) and was found to contain an average of three CMP residues beyond the C-C-A. The ^{32}P-labeled precursor to *E. coli* $tRNA_I^{Tyr}$ (8) was kindly supplied by Ryszard Kole and Sidney Altman, Yale University.

Enzymes. RNase II was purified by a modification of our previously described procedure (17). Chromatography on DEAE-Sephadex was substituted for the second DEAE-cellulose step, and chromatography on Affi-Gel blue (Bio-Rad Laboratories) replaced the Sephadex G-100 step. This procedure led to an overall purification from the S30 fraction of about 1500-fold, and a specific activity of about 10,000 μmol of AMP released from poly(A) per hr per mg of protein (10,000 units), which is nearly 5-fold higher than previously reported.

RNase D was also purified as described earlier (17), with minor modifications. As above, DEAE-Sephadex was used to separate RNase II and RNase D, and an additional step employing chromatography on Ultrogel AcA 44 (obtained from LKB) was introduced at the end of the purification. The final specific activity with diesterase-treated [^{32}P]tRNA as substrate was about 300 μmol/hr per mg of protein, which is about 7-fold higher than our earlier preparation. Details of the purification procedures will be published elsewhere.

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E. coli aminoacyl-tRNA synthetases were prepared from strain 35-10R (deficient in tRNA nucleotidyltransferase) as described (21). Alkaline phosphatase was purchased from Worthington. RNase P was a gift from Ryszard Kole and Sidney Altman.

Assays. Nuclease activity was determined by measurement of trichloroacetic acid-soluble radioactivity as described (17). Generally, reaction mixtures contained in 0.1 ml: 10 mM Tris·HCl at pH 7.5, 5 mM $MgCl_2$, 0.1 M KCl, a radioactive tRNA precursor, and RNase II or RNase D. Details of individual experiments are presented in the legends. Amino acid acceptor activity was measured in the same reaction mixture, which also contained 1 mM ATP, about 10 μCi (1 Ci = 3.7×10^{10} becquerels) of a ^{3}H-labeled reconstituted protein hydrolysate containing 15 amino acids (Schwarz/Mann) and sufficient aminoacyl-tRNA synthetases to reach maximal incorporation in 5 min.

RESULTS

Purity and Structural Properties of RNase D and RNase II. The modified purification procedures for RNase II and RNase D resulted in preparations of greatly increased specific activity and apparent homogeneity, as judged by electrophoresis in 7.5% and 10% acrylamide gels containing sodium dodecl sulfate (10% gel shown in Fig. 1). The specific activity of the purified RNase II, on the basis of hydrolysis of poly(U), is 2500 units/mg under the conditions of Singer and Tolbert (18) and 800 units/mg when assayed as described by Gupta *et al.* (19). In each case these values are about 4-fold higher than those of the preparations reported by these workers. The molecular weights of RNase D and RNase II, determined from electrophoresis under denaturing conditions (Fig. 1), are 38,000 and 78,000 respectively. Because the native molecular weights determined by chromatography on Ultrogel AcA 44 are about 40,000 for RNase D, and 80,000–85,000 for RNase II (data not shown), these results suggest that both enzymes are single-chain proteins.

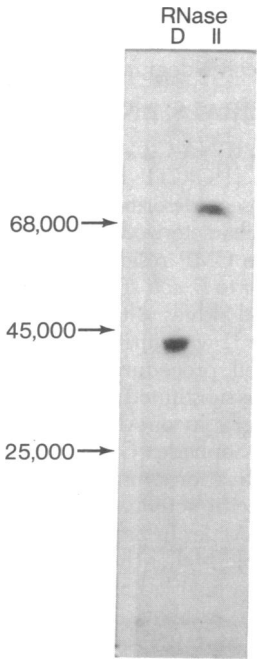

FIG. 1. Acrylamide gel electrophoresis of purified RNase D and RNase II under denaturing conditions. Electrophoresis was performed on a 10% acrylamide gel containing sodium dodecyl sulfate as described by Laemmli (21). Samples were run at a constant current of 20 mA per tube until the tracking dye, bromphenol blue, was 1 cm from the end of the gel. The gel was stained overnight in a solution of 0.22% Coomassie brilliant blue in 40% methanol/20% acetic acid (vol/vol) and destained by diffusion in 45% methanol/10% acetic acid. Bovine serum albumin, ovalbumin, and chymotrypsinogen were used as molecular weight markers.

Requirements of RNase D and RNase II for Hydrolysis of tRNA Precursors. Both RNase D and RNase II can release labeled residues from the synthetic type I tRNA precursor. However, the ionic requirements of the two enzymes are quite different (Table 1). RNase D requires a divalent cation for activity, and this requirement can be satisfied by Mg^{2+}, and less well by Mn^{2+}. The optimum Mg^{2+} concentration is 1–3 mM (data not shown). RNase II also shows a requirement for a divalent cation, but it can be demonstrated only in the presence of EDTA. This result suggests that the enzyme contains a tightly bound metal ion, although it remains to be proven. RNase II activity is also stimulated by the monovalent cations K^+ and Na^+. In contrast, RNase D is slightly inhibited by addition of these cations, most likely due to the increased ionic strength. Similar requirements are also observed with the *E. coli* $tRNA_1^{Tyr}$ precursor as substrate (unpublished data). The ionic requirements for hydrolysis of the tRNA precursors differs somewhat from those with other substrates (19, 20). This variation in requirements depending on the nucleic acid substrate has been seen previously with RNase II (20).

Products of Hydrolysis of tRNA Precursors by RNase D and RNase II. Because both nucleases could remove extra residues from tRNA precursors, it was important to determine the specificity of this hydrolysis. Whichever enzyme functions as the 3′-processing nuclease for type I precursors *in vivo* must stop at the -C-C-A sequence, because our laboratory has previously shown that mutants defective in tRNA nucleotidyltransferase and unable to repair the 3′ terminus have little or no defect in tRNA biosynthesis (23, 24). Thus, in order to determine whether RNase D or RNase II has the specificity expected of the 3′-processing nuclease, we have tested each enzyme for its ability to regenerate amino acid acceptor activity concomitant with removal of extra residues from the tRNA precursor.

The data in Fig. 2 show that the tRNA precursor preparation is essentially devoid of amino acid acceptor activity and that incubation without the addition of nuclease releases no labeled residues from the precursor and does not increase amino acid acceptor activity. Addition of RNase D leads to release of greater than 80% of the extra residues over a period of 30 min, and, coincident with this hydrolysis, close to 60% of the amino acid acceptor activity of the original tRNA preparation used to construct the precursor is restored. Because the aminoacy-

Table 1. Ion requirements of RNase D and RNase II with tRNA-C-C-A-[^{14}C]C_n as substrate

Assay conditions	Acid-soluble product, nmol/5 min	
	RNase D	RNase II
No addition	0.03	0.14
$+Mg^{2+}$	1.41	0.17
$-Mg^{2+}$, +EDTA	<0.01	<0.01
$-Mg^{2+}$, $+Mn^{2+}$	0.67	0.04
$+Mg^{2+}$, $+K^+$	1.14	0.39
$+Mg^{2+}$, $+Na^+$	1.03	0.57

Assays were carried out in the presence of 50 μg of tRNA precursor and 36 milliunits of RNase D and 43 milliunits of RNase II (based on activity with diesterase-treated tRNA). Divalent cations and EDTA were added at 5 mM and monovalent cations at 0.1 M. Incubation was for 5 min at 37°C.

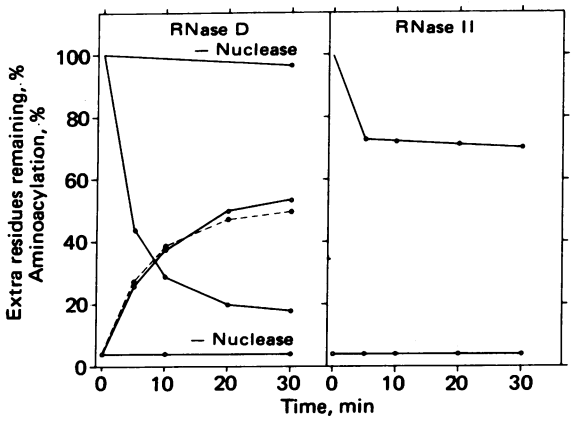

FIG. 2. Comparison of nucleotide release and generation of amino acid acceptor activity by RNase D and RNase II. Assays were carried out with 50 μg of tRNA precursor and 0.1 unit of RNase D or RNase II (based on activity with diesterase-treated tRNA). After the indicated period of nuclease action at 37°C, an aminoacyl-tRNA synthetase mixture was added and the extent of aminoacylation in 5 min was determined. Acid-precipitable material was collected on Whatman GF/C filters, and its radioactivity was measured under double-label conditions. Nuclease hydrolysis was determined from the decrease in ^{14}C cpm (●—●), and aminoacylation, from the level of ^{3}H cpm (○—○). The percent hydrolysis is relative to a zero time sample, and the percent aminoacylation is relative to a sample of the original tRNA used to synthesize the precursor. The theoretical level of aminoacylation calculated from the Poisson distribution at each level of hydrolysis (see text) is given by ●- - ● in the RNase D panel.

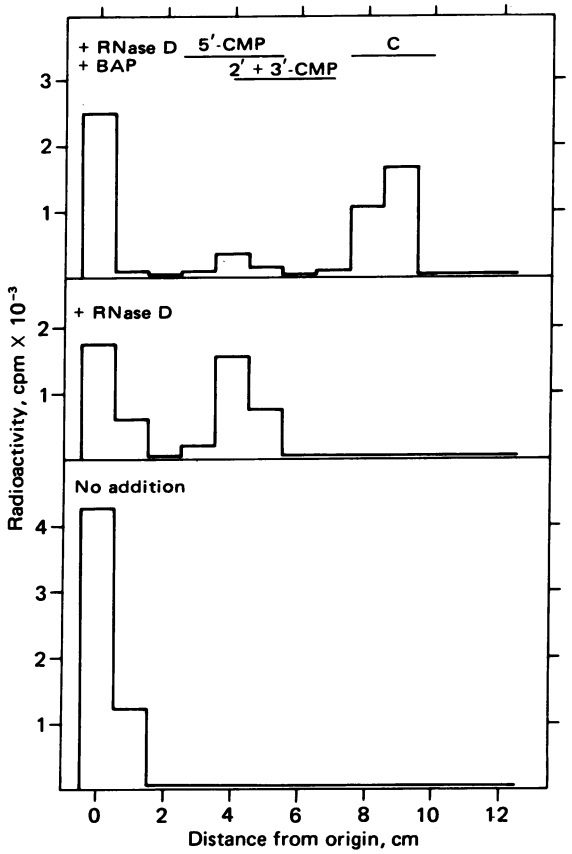

FIG. 3. Chromatographic analysis of the product generated by RNase D hydrolysis. tRNA precursor was incubated in the presence (60% hydrolysis) or absence (zero hydrolysis) of RNase D. An aliquot of each reaction mixture was spotted on Whatman 3 MM paper and the chromatogram was developed with 95% ethanol/1 M ammonium acetate (60:40, vol/vol) for 3 hr. A second aliquot of the enzyme-treated sample was heated to inactivate RNase D and then incubated for 5 min at 37°C with about 15 μg of RNase-free bacterial alkaline phosphatase (BAP) prior to chromatography. After drying, 1-cm strips were cut out, beginning 0.5 cm behind the origin, and assayed for radioactivity. Standards of 5'-CMP, cytidine, and 2'- and 3'-CMP were included in each track and located by ultraviolet light.

lation experiments are carried out with a mixture of 15 amino acids, the results indicate that RNase D is capable of specifically removing the extra residues from most, if not all, tRNA precursors. In addition, because the aminoacyl-tRNA synthetase preparation lacks tRNA nucleotidyltransferase, RNase D must stop without entering the -C-C-A sequence. This conclusion is supported by the observation that addition of tRNA nucleotidyltransferase leads to only a small increase (≈5%) in the extent of aminoacylation. In contrast to the results with RNase D, RNase II action on the tRNA precursors reaches a limit at 30–40% removal of extra residues, and there is no restoration of amino acid acceptor activity associated with this hydrolysis (Fig. 2).

In addition to tRNA-C-C-A, the other product released by the action of RNase D on the tRNA precursors is CMP. The CMP product was identified by its chromatographic mobility (Fig. 3) and by its conversion to cytidine after treatment with alkaline phosphatase (Fig. 3). These results confirm, also with the tRNA precursors, our previous suggestion that RNase D is an exonuclease releasing 5′ mononucleotides from the 3′ end of RNA chains (23).

Random Nature of RNase D Hydrolysis. It has been known for many years that RNase II hydrolyzes RNA chains in a processive manner (25); i.e., the enzyme acts on one chain completely before initiating hydrolysis of another chain. In contrast, RNase D appears to act randomly—i.e., dissociating from an RNA molecule after every hydrolytic event. This conclusion was initially suggested by the data in Fig. 2 and other similar experiments. Analysis of these results shows that the increase in aminoacylation lags behind the release of nucleotides, especially at early times. This is not the result expected if all three extra nucleotides were released processively, because under these conditions a given percentage hydrolysis should lead to the same percentage of chains capable of accepting amino acids, and the ratio of these two measurements should not change with time. However, assuming a random mode of nucleotide release and calculating the number of chains lacking all three extra residues (those chains that could be aminoacylated) from the Poisson distribution at various percentages of hydrolysis gives an excellent fit to the actual aminoacylation data (Fig. 2). The random nature of RNase D hydrolysis was confirmed by determination of the average chain length of extra residues remaining after various degrees of hydrolysis (Table 2). For a processive enzyme, the average chain length would always remain at the starting level of 2.9 residues, because the chains remaining would be untouched. However, as shown in Table 2, the average chain length of the extra residues associated with the remaining chains decreased continuously with increasing levels of hydrolysis, as expected for an enzyme acting randomly. In fact, these results predict that, even after the 82% hydrolysis of the extra residues, over 40% of the chains would still contain at least one CMP following the -C-C-A sequence, which would explain why the aminoacylation level in Fig. 2 approached only 60%.

Hydrolysis of the *E. coli* $tRNA_1^{Tyr}$ Precursor by RNase D and RNase II. In order to compare the action of RNase D and RNase II on a "natural" tRNA precursor, we have made use of the $tRNA_1^{Tyr}$ precursor that accumulates in the RNase P-negative strain A49 (3). This precursor differs from the synthetic precursors used here in that it contains a precursor-specific, 41-nucleotide sequence at the 5′ end in addition to the extra

Table 2. Random hydrolysis of tRNA-C-C-A-[^{14}C]C_n by RNase D

% hydrolysis	Average chain length of extra residues
0	2.9
37	2.2
58	1.9
71	1.7
82	1.4

Filters containing the acid-precipitable product from an experiment like that shown in Fig. 2 were subjected to alkaline hydrolysis, and the terminal cytidine was separated from the 2′- and 3′-CMP residues as described (16). The ratio of nucleotide (from internal residues) to nucleoside (from terminal residue) was used to calculate the average chain length of the extra residues remaining on the precursor after various degrees of hydrolysis.

residues at the 3′ end (8). However, because this precursor accumulates in cells that cannot remove the 5′ fragment, it appears that the normal course of processing *in vivo* requires removal of this fragment prior to final trimming at the 3′ end. Thus, we might expect that this precursor would be relatively resistant to hydrolysis by RNase D, if this enzyme were the 3′-processing nuclease. In fact, compared to RNase II, RNase D action on the precursor is quite limited, although it is significant (Fig. 4). Addition of RNase P stimulates hydrolysis by both RNase D and RNase II, but again it is relatively limited for RNase D compared to RNase II (Fig. 4). In the latter case, the large increase in acid-soluble radioactivity is due to complete digestion by RNase II of the 5′ fragment generated by RNase P cleavage (H. Cudny, M. P. Deutscher, and S. Altman, unpublished data). Hydrolysis of the intact precursor by RNase D is strongly dependent on the level of nuclease added and on the ionic strength of the reaction mixture. In contrast, the precursor is extremely sensitive to digestion by RNase II under a wide range of conditions (unpublished data). Treatment of the precursor with RNase P and RNase D generates a product of the size of mature $tRNA^{Tyr}$, as determined by high-resolution gel electrophoresis, whereas no such product is obtained with RNase II (H. Cudny, M. P. Deutscher, and S. Altman, unpublished data).

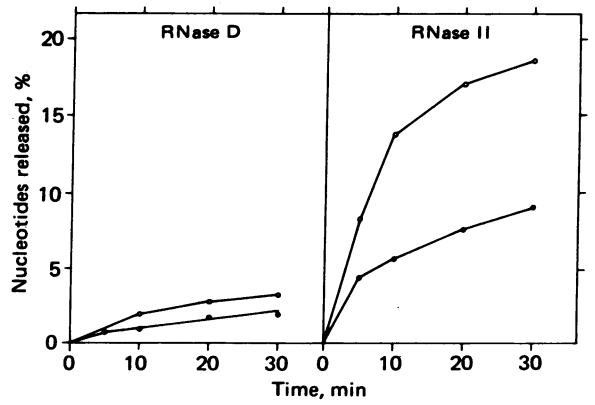

FIG. 4. Comparison of action of RNase D and RNase II on the *E. coli* $tRNA_1^{Tyr}$ precursor in the presence (○) and absence (●) of RNase P. Reactions were carried out with the addition of about 8000 cpm of ^{32}P-labeled precursor and either 31 milliunits of RNase D or 7 milliunits of RNase II (based on hydrolysis of diesterase-treated tRNA). When present, RNase P was at 0.15 μg. After the indicated period of incubation at 37°C, an aliquot was withdrawn and acid-soluble radioactivity was determined. Note that about four times as much RNase D activity, compared to RNase II, was present per assay.

DISCUSSION

The results presented here provide strong support for our previous suggestion (17) that RNase D, and not RNase II, is the enzyme that performs the final trimming to remove extra nucleotides after the -C-C-A sequence in *E. coli* type I tRNA precursors. Although RNase D and RNase II are both exonucleases acting at the 3′ end of RNA chains, several of the catalytic properties of these enzymes are markedly different. Thus, RNase D is highly specific for tRNA molecules, whereas RNase II is also active with rRNA and synthetic polynucleotides (20); RNase D degrades RNA chains randomly, whereas RNase II acts processively (25); and RNase D requires a higher concentration of Mg^{2+}, and is not dependent on a monovalent cation for optimal activity.

The properties of RNase D make it well suited to function as a 3′-processing nuclease for type I tRNA precursors. We have previously shown that RNase D acts extremely slowly on tRNA molecules terminating in -C-C-A (20). This property combined with its random removal of nucleotides would allow aminoacylation of a newly synthesized tRNA chain as soon as its -C-C-A sequence was exposed, thereby protecting the RNA from further hydrolysis. We have already shown that removal of the terminal nucleotides from tRNA depends on the level of aminoacylation (24). The properties of RNase II, on the other hand, are inconsistent with its being the 3′-processing nuclease. This enzyme's lack of specificity coupled with its processive removal of nucleotides suggests that it may function *in vivo* as a degradative enzyme to remove extraneous RNA molecules. Obviously, further studies of mutant strains deficient in RNase II (19) are required to determine whether this enzyme plays any role in RNA processing. Similarly, isolation of mutants devoid of RNase D would be extremely useful to conclusively establish whether this enzyme is involved in tRNA processing *in vivo*.

A feature of RNase D recognition that deserves some comment is its ability to remove nucleotides after the -C-C-A sequence and to degrade tRNA molecules lacking the -C-C-A sequence (20), whereas it is essentially inactive with intact tRNA. We have now shown (unpublished data) that the resistance of intact tRNA is not simply due to the presence of a resistant -C-C-A sequence, because a second -C-C-A sequence synthetically added to tRNA is rapidly removed by RNase D. These results strongly suggest that the resistance of intact tRNA to nuclease action is due to its three-dimensional structure, and that the -C-C-A sequence is relatively inaccessible to RNase D for initiation of hydrolysis. Furthermore, this raises the possibility that the function of precursor-specific sequences is to put precursor RNA molecules into conformations in which they will be recognized by correct processing nucleases but will be protected against nonspecific degradation during their conversion to mature resistant molecules. In fact, the increased sensitivity of the $tRNA_1^{Tyr}$ precursor to degradation by RNase II, and also RNase D, under certain ionic conditions (unpublished data) supports the idea that the strict conformational restraints are very important to ensure correct processing, and that even the normal processing nucleases may become nonspecific when these restraints are removed (26).

These results still leave open the question of which enzyme acts *in vivo* on type II precursors. Although this type of precursor has not yet been found in *E. coli*, several examples are known in phage T4-infected *E. coli* (10), and a host mutant (BN) has been isolated in which processing of these tRNAs *in vivo* (13, 14) and *in vitro* (15) has been affected. Studies in our laboratory indicate that the BN strain contains normal levels of RNase D and RNase II (unpublished data), suggesting that these enzymes are not involved. However, because we have not yet been able to separate an additional nuclease specific for

tRNA precursors lacking the -C-C-A sequence, the possibility still exists that the "BN nuclease" is a mutated form of RNase D or RNase II that has lost only its ability to act on type II precursors. Because type II may be the exclusive form of tRNA precursors in eukaryotes, identification and study of a processing nuclease for this type of precursor would be extremely interesting.

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1. Altman, S. (1978) in *International Review of Biochemistry*, ed. Clark, B. F. C. (University Park Press, Baltimore, MD), Vol. 17, pp. 19–44.
2. Robertson, H. D., Altman, S. & Smith, J. D. (1972) *J. Biol. Chem.* **247**, 5243–5251.
3. Schedl, P. & Primakoff, P. (1973) *Proc. Natl. Acad. Sci. USA* **70**, 2091–2095.
4. Schedl, P., Roberts, J. & Primakoff, P. (1976) *Cell* **8**, 581–594.
5. Kitamura, N. H., Ikeda, Y., Yamada, Y. & Ishikura, H. (1977) *Eur. J. Biochem.* **73**, 297–306.
6. Bikoff, E. K., LaRue, B. F. & Gefter, M. L. (1975) *J. Biol. Chem.* **250**, 6248–6255.
7. Shimura, Y., Sakano, H. & Nagawa, F. (1978) *Eur. J. Biochem.* **86**, 267–281.
8. Altman, S. & Smith, J. D. (1971) *Nature (London) New Biol.* **233**, 32–39.
9. Chang, S. & Carbon, J. (1975) *J. Biol. Chem.* **250**, 5542–5555.
10. Barrell, B. G., Seidman, J. G., Guthrie, C. & McClain, W. H. (1974) *Proc. Natl. Acad. Sci. USA* **71**, 413–416.
11. Goodman, H. M., Olson, M. V. & Hall, B. D. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 5453–5457.
12. Valenzuela, P., Venegas, A., Weinberg, F., Bishop, R. & Rutter, W. F. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 190–194.
13. Maisurian, A. N. & Buyanovskaya, E. A. (1973) *Mol. Gen. Genet.* **120**, 227–229.
14. Seidman, J. G., Schmidt, F. J., Foss, K. & McClain, W. H. (1975) *Cell* **5**, 389–400.
15. Schmidt, F. J. & McClain, W. H. (1978) *Nucleic Acids Res.* **5**, 4129–4139.
16. Deutscher, M. P. & Ghosh, R. K. (1978) *Nucleic Acids Res.* **5**, 3821–3830.
17. Ghosh, R. K. & Deutscher, M. P. (1978) *Nucleic Acids Res.* **5**, 3831–3842.
18. Singer, M. F. & Tolbert, G. (1965) *Biochemistry* **4**, 1319–1330.
19. Gupta, R. S., Kasoi, T. & Schlessinger, D. (1977) *J. Biol. Chem.* **252**, 8945–8949.
20. Ghosh, R. K. & Deutscher, M. P. (1978) *J. Biol. Chem.* **253**, 997–1000.
21. Deutscher, M. P. & Evans, J. A. (1977) *J. Mol. Biol.* **109**, 593–597.
22. Laemmli, U. K. (1970) *Nature (London)* **227**, 680–685.
23. Morse, J. W. & Deutscher, M. P. (1975) *J. Mol. Biol.* **95**, 141–144.
24. Deutscher, M. P., Lin, J. J. C. & Evans, J. A. (1977) *J. Mol. Biol.* **117**, 1081–1094.
25. Nossal, N. G. & Singer, M. F. (1968) *J. Biol. Chem.* **243**, 913–922.
26. Sekiya, T., Contreras, R., Takeya, T. & Khorana, H. G. (1979) *J. Biol. Chem.* **254**, 5802–5816.