Purification and Properties of a Specific *Escherichia coli* Ribonuclease which Cleaves a Tyrosine Transfer Ribonucleic Acid Precursor

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**SUMMARY**

Precursor molecules of *Escherichia coli* wild type and mutant tyrosine tRNA's contain at both their 5' and 3' termini extra nucleotides in addition to those of the mature tRNA molecule. The early steps of processing these precursor molecules must involve specific ribonuclease cleavage. We report the isolation from *E. coli* extracts of the specific endonucleolytic RNase which cleaves only a single phosphodiester bond of the 129 nucleotide tyrosine tRNA precursor molecule. This cleavage removes all extra nucleotides present at the 5' terminus of the precursor as a 41 nucleotide fragment, exposing the 5' end of the mature tRNA. After sufficient purification, this activity has no effect upon the extra nucleotides at the 3' end of the tRNA precursor. Therefore processing of the two ends of this molecule must be carried out by different enzymatic activities.

This novel RNase activity, which we have called RNase P, has been purified by washing ribosomes with 0.2 M NH₄Cl, followed by ammonium sulfate fractionation and chromatography on DEAE-Sephadex and phosphocellulose. At this stage it shows no evidence of other *E. coli* RNase activities. RNase P requires both monovalent and divalent cations for optimal activity, and has a pH optimum of 8.0. In the course of purifying RNase P, we have discovered in other subcellular fractions of *E. coli* RNase activities potentially responsible for additional steps of precursor tRNA processing.

**EXPERIMENTAL PROCEDURE**

**Materials**

**Bacterial Strains**—*E. coli* MRE600 was kindly provided by Dr. B. F. C. Clark of this laboratory. The RNase I- strain 180, a derivative of A19, was the gift of Dr. D. Morse, Harvard Medical School, Boston, Mass. *E. coli* MB831 was that used by Altman (1) to prepare tyrosine tRNA precursor. *E. coli* BF200 (9), the gift of Dr. P. Primakoff, Stanford University, Palo Alto, California, was used interchangeably with strain MB831.

**Bacteriophage Strains**—Mutant A25 of bacteriophage φ80 was that reported by Smith et al. (10). This strain carries a mutant in the structural gene for tyrosine tRNA and has been used in the preparation of tyrosine tRNA precursor.
Enzymes—Electrophoretically purified pancreatic DNase, DPF, was obtained from Worthington Biochemical Corp., Freehold, N. J. RNase T1 was from Sankyo Corp., Tokyo. RNase III was purified from E. coli MRE600 according to the procedure of Robertson et al. (11). Rho factor (12) was the kind gift of Dr. J. Roberts of this laboratory. Rabbit hemoglobin was the gift of Dr. M. B. Mathews of this laboratory, and catalase was purchased from Sigma Chemical Co., St. Louis, Mo.

Chemicals—All routine chemicals were of reagent grade. Ammonium sulfate was the ultra pure “Aristar” grade, British Drug Houses, Poole, England.

Chromatographic Media—DEAE-Sephadex A-50 and Sephadex G-25 were obtained from Pharmacia, Uppsala, Sweden. Phos- phocellulose was from Whatman. The DEAE-Sephadex was washed and prepared for use according to Robertson et al. (11), whereas the phosphocellulose was washed extensively in 1 M Tris-HCl, pH 7.9.

Tyrosine tRNA Precursor—32P-labeled tyrosine tRNA pre- cursor was prepared and fractionated on polyacrylamide gels as described by Altman (1). The precursor, and other RNA bands on polyacrylamide gels, were eluted as follows. The region depicting the desired area was cut from an exposed x-ray film, which was used as a template for excising the desired regions of the 20 × 40 cm slab gel. The gel band was mechanically homogenized and eluted in 0.1 M Tris-HCl, pH 9.1, 0.5 M NaCl, 0.01 M EDTA. Equal volumes (1 to 5 ml) of disrupted polyacrylamide suspended in the above buffer and water-saturated phenol were homogenized mechanically, centrifuged for 10 min at 10,000 × g, and the aqueous layer taken; the other phase was re-extracted with an equal volume of the above buffer by stirring, and the pooled aqueous phases were filtered through a 0.45-μm Millipore filter. One A260 unit of E. coli tRNA was added per ml of aqueous phase, and the RNA was precipitated with 2.5 volumes of absolute ethanol at −20°, centrifuged, and the pellet collected and resuspended in 0.1 ml of distilled water. Specific activity of the precipitate was 0.5 to 2 × 106 cpm per μg.

Other Polynucleotides—32P-labeled mixed tRNAs was prepared from the same cultures as the tRNA precursor and purified in a similar fashion. 18S rRNA was grown and purified according to Dahlberg (13). [3H]poly(A) copolymer, specific activity 24.3 μCi per μmole, was that used by Robertson et al. (11). Poly(G), Poly(C), Poly(U), Poly(A), and Poly(I) were prepared using RNase polymerase with unlabeled poly(C) as template, and had a specific activity of 255 μCi per μmole. A small RNA species present in ϕX174-infected cells was labeled with 32P and purified in a manner identical with the precursor. Denatured calf thymus DNA was the kind gift of Dr. F. Galibert of this laboratory.

Methods

Standard Assays for Enzymatic Cleavage of tRNA Precursor—Unless otherwise noted, all reactions were carried out in a final volume of 0.1 ml in silicone-treated tubes at 37° for 90 min. Each reaction contained 0.01 M Tris-HCl, pH 8.0; 0.1 M NH₄Cl; 0.005 M MgCl₂; 10−4 M EDTA; 10−3 M 2-mercaptoethanol; substrate (ordinarily an amount of tyrosine tRNA pre- curser containing 5,000 to 15,000 cpm of 32P), and enzyme (an amount of the fraction to be tested appropriate to give at least 25% cleavage of the precursor during the incubation period). Reactions were terminated by adding 0.1 ml of 0.4 M EDTA, pH 9.4, containing 0.05% bromphenol blue. Since more highly purified enzyme fractions contain 5% sucrose, all reactions were normally made 5% in sucrose at this point. Samples were then evaporated to dryness in vacuo, resuspended in 25 μl of distilled water, and layered on a 10% polyacrylamide slab gel (20 × 40 × 0.3 cm). Electrophoresis was carried out in a continuous buffer system containing 10.8 g of Tris base, 0.93 g of disodium-EDTA, 5.5 g of boric acid per liter, pH 8.3, for 16 hours at 400 volts and 4°. Positions of the radioactive RNA bands in the gels were determined by radioautography.

In certain experiments it was desirable to follow the extensive degradation of RNA substrates. In these, the amount of RNA remaining precipitable in 5% trichloroacetic acid was determined as described by Robertson et al. (11).

Fingerprinting analysis of RNA’s was carried out using the methods of Sanger and his collaborators (15, 16) as applied by Goodman et al. (17).

Preparation of Subcellular Fractions—All operations were carried out at 4°. Five grams of E. coli MRE600 or 180 were ground with 10 g of leviogated alumina with a previously chilled (−20°) mortar and pestle until a paste was formed. Five milliliters of Buffer A (0.05 M Tris-HCl, pH 7.5, 0.06 M NH₄Cl, 0.01 M MgCl₂, 0.006 M 2-mercaptoethanol) were added, and 10 μg per ml of pancreatic DNase were added to the resultant slurry. After 30 min at 4°, the mixture was centrifuged for 10 min at 8,000 rpm in 12 ml glass centrifuge tubes in the SS34 rotor of the Sorvall RC-2-B centrifuge. The supernatant was then centrifuged for 40 min at 15,500 rpm as above; the resulting 30,000 × g supernatant is called S 30 and was that in which precursor cleaving activity was previously detected (2). Ribosomes were prepared from this S 30 supernatant by centrifugation for 4 hours at 45,000 rpm in the type 65 rotor of the Beckman model L ultracentrifuge. The upper two-thirds of the resulting S 100 supernatant was removed with a Pasteur pipette, and the rest was discarded. The ribosomal pellet was rinsed with 2 ml of Buffer A, which was discarded, and the pellet was then redis- solved in 2 ml of fresh Buffer A. After removal of an aliquot of resuspended ribosomes for assays, the ribosomes were washed with the desired concentration of NH₄Cl as follows. An appropriate amount of 4 M NH₄Cl in Buffer A was added, and the mixture was transferred to a Beckman cellulose nitrate centrifuge tube (1 × 2 inch). This tube was attached to the cup of a variable-speed Vortex mixer (Lab-Line Instruments) with vinyl tape and allowed to agitate gently overnight at 4°. The volume was increased to 5 ml with Buffer A containing the appropriate NH₄Cl concentration, and the mixture was centrifuged for 4 hours in the SW39 or SW50 rotor of the Beckman model L ultracentrifuge at 37,000 rpm. The upper two-thirds of the resulting supernatant was removed and retained, while the rest was discarded. The ribosomes were again resuspended in Buffer A, an aliquot removed, and a further washing step initiated. Protein concentra- tions were determined by the procedure of Lowry et al. (18).

Determination of Radioactivity—Samples dried on glass fiber filters or on paper were assayed for radioactivity using the toluene-based scintillation fluid of Robertson et al. (11). Quantitative analysis of the kinetics of tRNA precursor reactions was
performed after cutting out the appropriate bands from the gel as described above. The radioactivity in each sample was assayed by placing it in an empty vial and measuring the Cerenkov radiation fluid and 20% for Cerenkov radiation.

Sucrose Density Gradient Centrifugation—Sucrose density gradients (7 to 25%) were prepared in a buffer containing 0.01 M Tris-HCl, pH 8.0, 0.005 M MgCl₂, 10⁻⁴ M EDTA, and 10⁻⁴ M 2-mercaptoethanol. Centrifugation was carried out for 10 hours in the SW50 rotor of the Beckman L265B ultracentrifuge at a temperature of 5°.

RESULTS

Partial Purification of a Specific Precursor-cleaving Activity—We attempted to devise a purification procedure which avoided harsh treatment of subcellular components for as long as possible. In particular we attempted not to disrupt ribosomes. This approach was used successfully by Robertson et al. in their purification of E. coli RNase III (11) and was also an alternative purification of E. coli RNase II suggested by Spahr (19). An S 30 extract from 5 g of E. coli MRE600 was prepared as described under “Experimental Procedure,” and the ribosomes were isolated and washed as described under “Experimental Procedure.” The resuspended ribosomes and the supernatants, after washing at various NH₄Cl concentrations, were assayed for their ability to cleave the tRNA precursor.

Fig. 1 shows the effect of various subcellular fractions upon the tyrosine tRNA precursor. The large change in mobility of the precursor upon specific cleavage is almost entirely due to the removal of the 41 nucleotides located to the 5’ side of the tRNA sequence in the precursor, since the 5’ terminal region accounts for 41 of the 44 extra nucleotides in the precursor. A comparison of Lanes 8 and 11 shows that the S 30 extract contains an activity which cleaves the precursor to yield a major product and was later shown to be RNase III (11). The specific endonucleolytic activity does not elute until 0.5 M NH₄Cl is added (Lanes 16 to 18), and the relative yield of the putative 5’-terminal fragment is markedly improved in comparison to that observed with the 30 to 50% ammonium sulfate fraction (cf. Lanes 16 and 19). Subsequent experiments (not shown) have demonstrated that the specific RNase activity does not elute from such a column in the presence of Buffer B containing 0.4 M NH₄Cl, but it is eluted at about 0.42 M NH₄Cl.

It is also evident in Fig. 3 that Buffer B containing 0.1 M NH₄Cl has removed significant amounts of RNase activity with properties different from that eluted in 0.5 M NH₄Cl. In light of the results shown in Fig. 1, it is likely that the activity eluted in 0.1 M NH₄Cl corresponds to the latent nonspecific RNase components, the majority of which were not removed from ribosomes until they were washed in 0.5 M NH₄Cl. Activity present in fractions eluted from DEAE-Sephadex in 0.5 M NH₄Cl could be concentrated by addition of 0.6 g of ammonium sulfate per ml of enzyme solution, followed by centrifugation as described in the legend to Fig. 2, and dialysis of the resuspended pellet against Buffer B containing 0.02 M NH₄Cl. This activity could be stored unfrozen on ice and is stable for several weeks.
FIG. 1 (top left). Subcellular fractionation of tyrosine tRNA precursor cleavage activity. Ribosomes were prepared from 5 ml of an S 30 supernatant of Escherichia coli MRE600 and washed with the indicated NH₄Cl concentrations as described under "Experimental Procedure." At each stage small aliquots were retained for assay purposes, and equivalent volumes, normalized to the S 30 starting volume, were added to each reaction. This means, for example, that about 100 µg of protein were added to the reaction in Lane 1 and correspondingly lesser amounts to the other reactions. Each reaction contained 2 × 10⁴ cpm of ³²P-labeled tyrosine tRNA precursor, and the assays were incubated and analyzed as described under "Experimental Procedure."

FIG. 2 (top right). Ammonium sulfate fractionation of the 0.2 M NH₄Cl ribosomal wash. Four milliliters of a 0.2 M NH₄Cl ribosomal wash (Fig. 1, Lane 4) were subjected to ammonium sulfate fractionation as described in the text, and the precipitates were resuspended in 0.5 ml of Buffer A. After dialysis of each resuspended fraction and the final supernatant against Buffer B containing 0.02 M NH₄Cl, 10 µl fractions were assayed for activity against 1 × 10⁴ cpm of tyrosine tRNA precursor as described under "Experimental Procedure." The ammonium sulfate percentages in the figure refer to the number of grams of ammonium sulfate added per 100 ml of original enzyme solution. Each ammonium sulfate fraction was assayed twice. Those reactions depicted in the left of each pair received 1 µl of enzyme solution, whereas those on the right received 5 µl.

Fig. 3 (bottom). DEAE-Sephadex fractionation of the 30 to 50% ammonium sulfate fraction. A DEAE-Sephadex column (1 × 8 cm) in Buffer B containing 0.02 M NH₄Cl was set up as described in the text, and 0.2 ml of the dialyzed 30 to 50% ammonium sulfate fraction (Fig. 2, Lanes 7 and 8) was added. Four column volumes of Buffer B with 0.02 M NH₄Cl were collected in 2-ml fractions, followed by equal volumes of Buffer B containing increasing amounts of NH₄Cl as indicated. Each fraction was made 5% in sucrose upon collection, and 25 µl of the first three 2-ml fractions to be collected at each NH₄Cl concentration were assayed for activity against tyrosine tRNA precursor (1 × 10⁴ cpm) as described under "Experimental Procedure."
Fig. 4. Products of digestion of 32P-labeled A25 precursor by the precursor-cleaving enzyme. a, separation of the products on polyacrylamide gel electrophoresis together with a marker of mature tRNA*. b, ribonuclease T1 products from the 'tRNA' band. The 3' end includes the additional 3'-terminal nucleotides of the precursor; all other products are from the tRNA sequence. c, ribonuclease T1 products from the 5' fragment. The numbered nucleotides from the precursor segment are: 1, Gp; 2, ApGp; 3, CpApGp; 4, CpCpApGp; 5, ApUpApApGp; 6, UpApApApaP5p; 7, CpUpUpCpCpGpGp; 8, CpApUpApApCpCpGpGp. Separation is by electrophoresis on cellulose acetate in pyridine acetate, 7 M urea, pH 3.5, from right to left; and on DEAE paper in 7% formic acid (v/v) from top to bottom.

The 0.5 M NH₄Cl fraction from the DEAE-Sephadex column was dialyzed against Buffer B containing 0.02 M NH₄Cl and 5% sucrose. A 1-ml aliquot was further analyzed on a phosphocellulose column 1 cm in height prepared in a Pasteur pipette. Stepwise elution of this column was carried out exactly as described for the DEAE-Sephadex step, using Buffer B containing 5% sucrose and the NH₄Cl concentrations indicated in Fig. 3. We find that all of the activity is recovered in the flow-through of the column (Buffer B containing 0.02 M NH₄Cl). At this point the amount of protein in the active fractions is not detectable. However, an estimate of the minimum extent of purification through the DEAE-Sephadex step is presented below.

Specificity of Precursor-cleaving Activity—We have previously shown that crude E. coli extracts split the precursor to give the tRNA sequence (with partial loss of the extra nucleotides at the 3' end) a fragment comprising the first 22 to 23 nucleotides from the precursor 5' end, and mono- and dinucleotides (2). These products could have resulted from a specific single cleavage splitting off the 41 nucleotide fragment which was subsequently partly degraded by other enzymes in the crude extracts. This interpretation has now been shown to be correct.

Fig. 4e shows that the more highly purified enzyme gives only two major RNA products on polyacrylamide gel electrophoresis. One ('tRNA') migrates slightly behind mature tRNA, while the second (5' fragment) moves more slowly than did the 22 nucleotide fragment described before (2). The products of digestion of these two bands with T1 and pancreatic ribonucleases were examined; Fig. 4, b and c, shows the separation of the T1 ribonuclease products. The 'tRNA' band contains the entire tRNA sequence from the terminal pGpG . . . , and includes the extra nucleotides at the 3' end of the precursor. Partial loss of these nucleotides results in an additional 3'-terminal T1 ribonuclease product which is present when the crude extracts are used for processing but which is absent in Fig. 4b.

The digestion products of the 5' fragment (Fig. 4e) are those expected from the complete 41 nucleotide 5' segment. GpUp is absent from the pancreatic ribonuclease products obtained with this fragment; instead an additional nucleotide migrating in the position of GpU is found. An alkaline hydrolysis of this gave Gp, and it is tentatively identified as GpU. This pancreatic ribonuclease product would be expected if the specific RNase splits the 3' phosphodiester bond of the last nucleotide in the precursor 5' segment.

These results indicate that the enzymatic activity which we are studying here has a single simple mode of action on the precursor; we designate this activity ribonuclease P. We can further conclude that RNase P purified to this stage is already free of the activity or activities which degrade the 5'-terminal fragment and remove the extra nucleotides from the 3' end of the precursor. The sequence of tyrosine tRNA precursor and its cleavage point by RNase P are shown in Fig. 5.
Fig. 5. Primary structure of 380 A25 tyrosine tRNA precursor. The arrow indicates the cleavage point of RNase P and the underlined segments indicate those nucleotides not normally found in the mature tRNA. The nucleotides not underlined are frequently depicted in the cloverleaf configuration.

FIG. 6. Kinetics of RNase P digestion. A, kinetics of RNase P activity purified through DEAE-Sephadex. Tyrosine tRNA precursor (3.6 × 10⁶ cpm) was incubated with 15 μl of RNase P, purified through DEAE-Sephadex and concentrated by ammonium sulfate precipitation, under standard conditions in a 0.1 ml reaction. At the times indicated, 15 μl aliquots were taken and added to 10 μl of 0.4 M EDTA containing bromphenol blue and left on ice until the desiccation step. The samples were run on an acrylamide gel such as that shown in Figs. 1 to 3, and the bands corresponding to each species were cut out and their radioactivity determined as described under "Experimental Procedure." ○—○, tRNA precursor; O—O, tRNA moiety; ▲—▲, 5' fragment. Less than one-tenth of the indicated radioactivity found at 90 min in the tRNA or 5' fragment positions was observed in a sample incubated for 90 min at 37° without added enzyme.

Note the change of scale on the right-hand ordinate. B, comparison of the kinetics of RNase P activity in fractions of different purity. An experiment identical with that described in A was performed using 3 μl of the S 30 extract from which the RNase P fraction used in A was obtained. Since the 5' fragment is degraded by the S 30 fraction, the total amount of radioactivity recovered in each aliquot was estimated by adding the radioactivity in the precursor band to 1.5 times the radioactivity in the tRNA moiety. •—•, precursor band in reaction with S 30; O—O, tRNA moiety in reaction with S 30. Superimposed on these data are the data from A with the scale on the ordinate magnified 4-fold. ■—■, precursor band from A; □—□, tRNA moiety from A.

Table I

Ionic requirements of purified RNase P

RNase P purified through the DEAE Sephadex step was concentrated by ammonium sulfate precipitation, and aliquots were assayed under standard conditions except that the assay mixture contained not more than 0.001 M MgCl₂ or 0.0013 M NH₄Cl after addition of enzyme. The various substances listed under Additions in the table were present in the following final concentrations: 0.005 M MgCl₂; 0.1 M NaCl; 0.1 M NH₄Cl; 0.02 M EDTA; 0.1 M KCl; 0.01 M MnCl₂; 0.001 M ATP. Relative activity was estimated by visual inspection of autoradiographs of slab gels which resemble those shown in Fig. 3. A dash indicates complete absence of the tRNA band and the 5' fragment band.

| Tube number | Additions        | Relative activity |
|-------------|------------------|-------------------|
| 1           | None             | —                 |
| 2           | MgCl₂            | +                 |
| 3           | MgCl₂, NaCl      | ++                |
| 4           | MgCl₂, NH₄Cl     | +                 |
| 5           | NH₄Cl, EDTA      | —                 |
| 6           | MgCl₂, KCl       | +                 |
| 7           | NaCl             | —                 |
| 8           | KCl              | —                 |
| 9           | NH₄Cl            | —                 |
| 10          | MnCl₂            | —                 |
| 11          | ATP, MgCl₂, NH₄Cl| ++                |
| 12          | MgCl₂, 2 × NH₄Cl | +                 |
| 13          | MgCl₂, NaCl, NH₄Cl| —               |
| 14          | No enzyme        | —                 |

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Properties of RNase P—We have studied the kinetics of the cleavage reaction by RNase P, both in crude extracts and after purification through the DEAE-Sephadex step, by quantifying the release of specific RNA products with time. In this way, we hope not only to find whether the properties of the reaction are altered by the removal of components during the purification, but also to estimate the minimum extent of purification. Results obtained with the more highly purified enzyme fraction, shown in Fig. 6A, indicate that the recovery of products accounts quantitatively for the loss of tRNA precursor. In addition, the ratio of radioactivity recovered in tRNA to that in the 5' fragment at any given time is close to that expected for two RNA's of identical specific activity which have their size ratio (88 to 41 nucleotides). Fig. 6B illustrates a comparison of the kinetics of RNase P activity in fractions of different purity. After appropriate normalization of the data obtained with the S 30 extract, as described in the legend to Fig. 6B, to facilitate comparison, we find that both the kinetics of disappearance of the tRNA precursor and the appearance of the tRNA are identical. This result is one indication that the properties of the RNase P cleavage reaction have not been grossly altered by the purification steps. Furthermore, 154 µg of S 30 protein were added to one reaction shown in Fig. 6, while less than 1 µg of protein from the RNase P DEAE-fraction was added to the other. Since the initial rate of tRNA precursor cleavage by S 30 is 2.8 times that of the DEAE-fraction as determined from the data on which this comparison is based, we conclude that the enzyme in this fraction has been purified more than 50-fold, but the real figure is undoubtedly higher.

The effect of ionic conditions on the RNase P reaction has been surveyed using the same assay system already described for the purification. We have tested various salt and pH conditions with the intention of comparing the RNase P cleavage reaction with those carried out by other E. coli RNases. For this reason, and because of the cumbersome nature of the standard assay, we have only screened certain carefully chosen sets of conditions. The results which we have obtained for a purified RNase P preparation, summarized in Table I, show that RNase P has the following properties. (a) This enzyme requires magnesium or some divalent cation (Mn++, or may partially replace Mg++); (b) this activity is stimulated by K+ and NH₄⁺ (0.1 M) when Mg++ is present. No activity is observed in the presence of K⁺ and NH₄⁺ when Mg++ is absent. An equal concentration of Na⁺ (0.1 M) inhibits the extent of cleavage. ATP has no effect on the reaction, nor does the addition of excess bulk tRNA (data not shown).

A similar pattern of ionic requirements for RNase P was also observed with cruder preparations (e.g. the activity associated with 0.06 M NH₄Cl-washed ribosomes) as well as with RNase P preparations purified through phosphocellulose and desalted by passage through Sephadex G-25. Separate experiments have shown that the pH optimum for RNase P at various stages of the purification is in the neighborhood of 8.0, with lower activity observed both at pH 7.0 and pH 8.5. The size of RNase P was estimated by a velocity sedimentation through a 7 to 25% sucrose density gradient in the absence of NH₄Cl as described under “Experimental Procedure.” Purified catalase and hemoglobin were centrifuged in a separate tube as markers. In some experiments low levels of activity were recovered moving slightly ahead of catalase, which has a reported
graphic fractionation, both with respect to recovery and conditions of elution. Since the chromatographic patterns, unlike those of RNase P, are not simple, this activity may be a mixture of several different enzymes or a complex capable of assuming different active states in different ionic environments. Nevertheless, we have succeeded in demonstrating the following. The activity reduces tyrosine tRNA precursor to small fragments, as assayed by polyacrylamide gel electrophoresis (Fig. 7, Lane 1), and also renders it soluble in 5% trichloroacetic acid as determined according to "Experimental Procedure" (Fig. 8). Much activity is still observed in the presence of 0.04 M EDTA, suggesting that certain aspects of this activity must be independent of divalent cations. Furthermore it is not affected by monovalent cations (data not shown), in contrast to RNase P.

Some selectivity in substrates is shown by this enzyme fraction as demonstrated by the following experiments. The active preparation attacks tyrosine tRNA precursor but mature tyrosine tRNA or bulk tRNA isolated from infected cells are much more resistant to this digestion (Figs. 7 and 8). Fig. 8 shows that the activity degrades a small φ80-induced RNA molecule and phage f2 RNA to a more limited extent than tRNA precursor. The synthetic alternating ribonucleotide copolymer poly(AU) is solubilized by this activity, but the double-stranded homopolymers pair poly(C) poly(C) is resistant to this degradation. Some of the implications of these results are discussed below.

**Discussion**

A nuclease which specifically cleaves the tyrosine tRNA precursor has been isolated from *E. coli*. This nuclease, which we have called RNase P, is loosely associated with ribosomal particles, from which it can be readily removed and further purified. It appears to be highly specific for the endonucleolytic cleavage of the tRNA precursor at a single phosphodiester bond. Optimal activity requires the presence of both monovalent and divalent cations.

RNase P can be distinguished from previously reported *E. coli* ribonucleases as follows. Its requirement for monovalent and divalent cations enables us to distinguish it from RNase I (3) or RNase IV (6). Purified p factor (12) also had no RNase P-like activity. The specific endonucleolytic mode of action of RNase P rules out its identity with the major exonucleolytic activity associated with *E. coli* RNase II (4, 19, 21) or with poly-Nucleotide phosphorylase (8). In order to test whether RNase III of *E. coli* is also responsible for the RNase P activity, we incubated an excess of highly purified RNase III (11) with the tyrosine tRNA precursor under optimal conditions for both enzymes (which are almost exactly the same). We found that RNase III has no effect on the electrophoretic mobility of the tRNA precursor.

An RNase activity associated with ribosomes which has been called RNase V (7) has been identified with an activity which degrades messenger RNA's in an exonucleolytic fashion from their 3' ends. This process apparently depends upon the state of activity of the ribosomes, and our RNase P preparations do not appear to have such an activity.

Several investigators have reported that subcellular fractions of *E. coli* can cleave the 17 S precursor to 16 S ribosomal RNA (22, 23). In particular, Corte et al. (23) have described an activity present in RNase II preparations which carries out such a reaction. However, the endonucleolytic step of this reaction displays different ionic requirements from those we have described for RNase P. It would be of interest to learn whether RNase P can also carry out cleavage of 17 S ribosomal RNA precursors.

We conclude that RNase P is a novel RNase activity of *E. coli*. This enzyme may cleave all *E. coli* tRNA precursors or only the one for tyrosine tRNA. The choice of one of these alternatives will await the isolation of additional *E. coli* tRNA precursors.

The subcellular fractionation of RNase P shown in Fig. 1 dem-

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2 D. Schlessinger, personal communication.
It demonstrates that processing of at least one of the tRNA precursors is carried out by different enzymatic activities. This finding is confirmed by the fingerprinting data shown in Fig. 4. The results shown in Fig. 1 also show that RNase P shares some of the properties of RNase P with regard to their removal from ribosomes by NH₄Cl. While association with ribosomes in extracts does not necessarily mean that these enzymes and factors are located upon these particles in vivo, analysis of the behavior of the other E. coli RNases during such gentle subcellular fractionation as that described here might reveal other interesting functional associations within the cell.

The behavior of RNase P upon ammonium sulfate fractionation (Fig. 2) and chromatography upon DEAE Sephadex (Fig. 3) and phosphocellulose is different from that of the bulk of E. coli RNases and has greatly facilitated our purification of this enzyme. In light of these properties, it is possible that the active form of RNase P, which must have a strong negative charge, could be associated with some nucleic acid.

In particular, the specific cleavage of a single phosphodiester bond within the 129 nucleotide tRNA precursor, in contrast to the behavior of other RNases, we conclude that RNase P is the first ribonuclease to be described which has such a high degree of specificity. Several examples of specific DNases that have been reported (25-27). The fact that RNase P creates a 5' phosphate end group also makes it unique among E. coli endonucleolytic RNases so far characterized. Altman and Smith (2) suggested on the basis of studying various mutated tyrosine tRNA precursors that base changes both near and far away from the point of cleavage can affect the rate of cleavage. Those studies as well as the ones reported here suggest that RNA secondary and tertiary structure may be as important as sequence in the action of RNase P. An assessment of the relative importance of these substrate properties in determining RNase P specificity could also be important in a more general study of RNA to protein interactions.

Although we have only performed a few experiments on the additional RNase activities present in 0.5 M NH₄Cl ribosomal washes (Figs. 7 and 8), their results are worthy of some comment. From the studies of mutant tRNA's, Altman and Smith (2) proposed the existence of a degradative pathway for tRNA precursor other than the one leading to mature tRNA. RNase activity present in 0.5 M NH₄Cl ribosomal wash fractions which can degrade tyrosine tRNA precursor but not mature tRNA (Figs. 7 and 8) may correspond to this proposed scavenger enzyme. In addition, the activity or activities responsible for degrading the 5'-terminal fragment of the tyrosine tRNA precursor in less pure RNase P fractions, as well as that which produces the 3' end of the precursor molecule (see Figs. 1 and 2), may also be present in this 0.5 m NH₄Cl wash. An interesting feature of this RNase activity is its latent nature. The existence of such latent RNases associated with ribosomes might help to account for reports of RNase activities which are dependent upon ribosomal configuration or protein synthetic activity (7).

Finally, we conclude that the use of natural substrates such as tRNA precursors as one aspect of the rigorous characterization of E. coli ribonucleases should be instrumental in revealing new aspects of the regulation of RNA metabolism.

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