Transfer Ribonucleic Acid Nucleotidyltransferase from Escherichia coli

II. PURIFICATION, PHYSICAL PROPERTIES, AND SUBSTRATE SPECIFICITY*

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SUMMARY
Transfer RNA nucleotidyltransferase (EC 2.7.7.25) from Escherichia coli has been purified to near homogeneity. The enzyme catalyzes the addition of CMP and AMP to the 3'-OH end of tRNA after complete or partial removal of the -PC-C-A sequence (2-6). Transfer RNA with an intact 3' terminus cannot serve as substrate while tRNA from which one to three nucleotides have been removed, for example by venom phosphodiesterase (7), can be completely repaired by the enzyme and the corresponding nucleoside triphosphates (1, 2, 4, 5). However, no reconstitution is possible after removal of the fourth nucleotide from the 3' terminus (2, 7, 8). Since only partially purified tRNA-nucleotidyltransferase preparations were obtained in earlier studies, the question remained whether the same enzyme or possibly two different ones catalyze the addition of AMP and CMP to tRNA. The turnover of these nucleotides in tRNA in vivo differs significantly (9-11) and a measurable CMP turnover was only observed in eukaryotic cells (12-15). Differences in the chemical and kinetic properties between the AMP- and CMP-incorporating activities of the rabbit muscle enzyme had been observed (16). These studies suggested the presence of two sites, but it could not be ascertained whether these sites were on the same or different proteins.

Here we report the purification of tRNA-nucleotidyltransferase from E. coli to near homogeneity. A protein, which migrates as a single band in polyacrylamide gel, catalyzes the incorporation of only CMP and AMP into venom phosphodiesterase-treated tRNA. All tRNA species of the same organism as well as heterologous tRNA can serve as substrate.

MATERIALS AND METHODS

General—Frozen cells of E. coli B, grown to half-log phase in enriched medium, were obtained from Grain Processing Corporation. Polyethylene glycol 6000 was obtained from Union Carbide; dextran T500, Sephadex, and Sepharose were from Pharmacia. Whatman DEAE-cellulose (DE-52) and glass fiber filters (934 AH) were obtained from Reeve Angel Company. Hydroxylapatite was from Bio-Rad or prepared according to Tiselius, Bjerten, and Levin (17). Tritium-labeled nucleoside triphosphates and uniformly labeled L-[14C]-amino acids were obtained from Schwarz BioResearch; venom phosphodiesterase was obtained from Worthington. For the estimation of tRNA concentration, an average molecular weight of 26,000 and an absorbance in 0.2 M NaCl of A260 = 220 was used (18). Protein was determined according to Lowry et al. (19) with bovine serum albumin (Armour) as standard.

Transfer RNA—Transfer RNA from E. coli B was purchased from Schwarz BioResearch and purified as described (20). Polyacrylamide gel electrophoresis of the rechromatographed tRNA gave the characteristic single broad band.† Yeast tRNA was obtained from Schwars BioResearch and was further purified by chromatography on Sephadex G-100 (20). Transfer RNA from rabbit reticulocytes was prepared from the postribosomal supernatant solution (21) by protamine sulfate precipitation (22). The precipitated tRNA was suspended in 20 ml of 50 mM Tris-HCl (pH 7.1)-2 M LiCl, stirred, and centrifuged at 15,000 rpm for 10 min. The supernatant solution was then applied to a column of DEAE-Sephadex A-50. The column was washed with 50 mM Tris-HCl (pH 7.1) and the tRNA was eluted with 50 mM Tris-HCl (7.1)-0.8 M NaCl. The tRNA was precipitated

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† G. R. Philips, T. W. Muna, and J. Timko, data to be published.
with ethanol, centrifuged, and redissolved in 5 ml of 1 M Tris-HCl (pH 8.0). After incubation for 1 hour at 37°, the tRNA was again precipitated by ethanol, centrifuged, and dissolved in 10 mM Tris-HCl (pH 7.0)-10 mM Mg*+ acetate-2 mM EDTA. It was further purified by chromatography on Sephadex G-100 in the same buffer. Transfer RNA from rat liver was prepared from the postribosomal supernatant solution by phenol extraction (23). It was further purified by chromatography on Sephadex G-100 as described above. The tRNA was then stripped from amino acids by incubation in 1 M Tris, pH 8.0, and reprecipitated with ethanol. All tRNA samples were dissolved in H2O and stored frozen. Digestion of tRNA by venom phosphodiesterase to yield the different substrates for the enzymatic assay was similar as described (7). To produce tRNA-X-C-C2 a two-step procedure was required. Venom phosphodiesterase digestion was performed for 1 hour at 20°. This removed all of the terminal AMP and approximately 65% of the penultimate CMP. The tRNA was deproteinized and mononucleotides were removed (7). CMP was then reincorporated in a 5-ml reaction mixture containing 5 mM glycine-NaOH (pH 9.2), 10 mM Mg*+ acetate, 1 mM glutathione, 2.4 μmoles of tRNA, 20 μmoles of CTP, 1 mg of bovine serum albumin, and 50 μg of purified tRNA-nucleotidyltransferase (Fraction VI). After 90 min at 37° the solution was deproteinized, chromatographed on Sephadex G-100, and precipitated with ethanol.

\[ \text{tRNA-Nucleotidyltransferase Assay} \]

The standard assay contained in 0.1 ml: 50 μmoles of glycine-NaOH (pH 9.2), 1.0 μmole of Mg*+ acetate, 1.0 μmole of glutathione, 1.6 μmoles of venom phosphodiesterase-treated tRNA, and 0.01 to 0.5 μg of protein. For AMP incorporation 20 nmoles (25 nCi) of ATP and tRNA X C C were used. The assay for CMP incorporation contained 20 nmoles (33 nCi) of 5-[3H]-CTP and tRNA X C C were used. All assays were conducted under conditions which gave kinetically valid data as determined by pilot experiments. After 5 to 30 min at 37°, the reaction was stopped by addition of 2 ml of 20 mM EDTA containing 0.2 mM ATP, followed by 2 ml of 10% trichloroacetic acid. For a control, an identical reaction mixture was precipitated at zero time. The precipitate was collected on glass fiber filters and washed with 3.5% trichloroacetic acid and 95% ethanol. The filters were dried and counted in 3 ml of a toluene-based scintillator (20). One unit of enzyme was defined as that amount which incorporated 1.0 n mole of nucleotide into tRNA in 1.0 min at 37°.

\[ \text{Aminoacylation of tRNA} \]

A crude aminoacyl-tRNA synthetase preparation was obtained from E. coli B as described by Muench and Berg (24) except that ribosomes and tRNA were first removed by liquid polymer phase fractionation. The crude enzyme fraction was stored at -10° in 10% (v/v) glycerol and gently shaken for 3 hours at 4°. The gel suspensions were centrifuged and 50 μl of the supernatant solutions were assayed for enzymatic activity. For those slices that contained enzymatic activity the corresponding larger gel pieces were combined and homogenized in 2.0 ml of 50 mM sodium phosphate (pH 7.1). The gel suspension was incubated for 3 hours at 37° and centrifuged, and the supernatant was withdrawn. The extraction was repeated twice and the combined supernatants were lyophilized. The protein was dissolved in 50 μl of 0.1 mM sodium phosphate (pH 7.1)-1.0% SDS-1.0% 2-mercaptoethanol and subjected to SDS-gel electrophoresis as described by Shapiro, Vinuela, and Maizel (28).

\[ \text{Analytical Gel Filtration} \]

Analytical gel filtration was performed with Sephadex G-100 according to Whitaker (27) or Sepharose 4B in 6 M guanidine hydrochloride similar to Fish, Mann, and Tanford (28).

\[ \text{RESULTS} \]

\[ \text{Purification of tRNA-Nucleotidyltransferase} \]

All procedures were performed at 4°. Frozen E. coli B cells (200 g) were thawed and suspended in 200 ml of Buffer I. The suspension was passed through a French pressure cell at 15,000 psi. Bentonite (3 mg) was added and the mixture was stirred for 10 min. The lysate was centrifuged for 30 min at 18,000 rpm in a Sorvall SS 34 rotor and the supernatant solution (315 ml) (Fraction I) was subjected to the following fractionation. To Fraction I were added 80 ml of 42% polyethylene glycol (w/w) in Buffer I, 80 ml of 12% dextran (w/w) in Buffer I, and 50 g NaCl. After stirring for 2 hours the mixture was centrifuged to separate the two phases. The polyethylene glycol-containing upper phase (380 ml) (Fraction IIa) was collected and saved. The dextran-containing lower phase (95 ml) was re-extracted by addition of 80 ml of 42% polyethylene glycol in Buffer I, 300 ml of Buffer I, and 44 g of NaCl. After stirring for 2 hours, the mixture was centrifuged and the upper phase (390 ml) (Fraction IIb) was removed and combined with Fraction IIa. This pooled material (Fraction II) was dialyzed for 1 hour against three successive 4-liter volumes of Buffer I. The dialysis was performed with sufficient agitation to keep the material within the dialysis...
bag homogeneous. The dialed Fraction II (620 ml) was brought to 30% of saturation in (NH₄)₂SO₄ (105 g). The mixture was centrifuged to separate the phases. The lower phase was removed from beneath the upper phase with a syringe. The upper phase (125 ml), a highly viscous solution of polyethylene-glycol containing virtually all of the enzyme, was immediately applied to a Sephadex G-25 column (5 x 30 cm) previously equilibrated with Buffer I. It was developed with Buffer I. After a void volume of 540 ml, the next 490 ml which contained all enzymatic activity were pooled (Fraction III). Fraction III was immediately applied to a DEAE-cellulose column (3 x 3 cm) previously equilibrated with Buffer I. The column was washed with Buffer II (10 mM Tris-HCl (pH 7.8)-10 mM Mg²⁺-acetate-5 mM 2-mercaptoethanol-10% glycerol) until the absorbance at 280 nm of the effluent was less than 0.01. The enzyme was then eluted in 70 ml with Buffer II containing 0.15 M KCl to yield Fraction IV. Fraction IV was immediately applied to a hydroxylapatite column (3 x 2 cm) equilibrated with Buffer I. The column was developed at a flow rate of 1 ml per min with a linear gradient of 0 to 0.4 M NaCl in Buffer I (100 ml total volume). All enzymatic activity was eluted before the bulk of the other proteins (Fig. 1). Usually all fractions containing enzymatic activity were pooled (Fraction VI). In a typical experiment, the volume was 7 ml. An equal volume of a 5 mg per ml solution of bovine serum albumin was added and the enzyme was stored at -20°.

The purification is summarized in Table I. The combined polyethylene-glycol extracts (Fractions II) and Fraction III each apparently contained more activity than Fraction I. This might have been due to substances in Fraction I which inhibited the enzyme and which were removed during these purification procedures. The incorporating activities for CMP and AMP exhibited approximately the same extent of purification throughout all stages of purification and eluted in the same fractions from the second DEAE-cellulose column. A 200-fold purification was routinely obtained for Fraction VI, while the peak fraction was about 4000-fold enriched.

Physical Characterization of Purified Enzyme

Polyacrylamide Gel Electrophoresis—The peak fraction from the second DEAE-cellulose column in Fig. 1 gave a single protein band upon gel electrophoresis at pH 9.3 (Fig. 2A). The peak fraction from another DEAE-cellulose column obtained in the absence of glycerol and magnesium was subjected to SDS-polyacrylamide gel electrophoresis in the presence of 2-mercaptoethanol at pH 7.1. It also showed a single protein band; however, when a large amount of this protein (30 μg) was applied, a minor component could be seen (Fig. 2C).

To determine whether Fraction VI contained more than one enzyme it was subjected to preparative gel electrophoresis. The gel was sliced and assayed as described under "Materials and Methods." The protein pattern of this gel is shown in the upper portion of Fig. 3. The results of the enzymatic assay of

![Fig. 1. Elution profile of the second DEAE-cellulose column](http://www.jbc.org/)

### Table I

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Total protein (mg)</th>
<th>Specific activitya</th>
<th>Recoverya</th>
</tr>
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<tr>
<td></td>
<td></td>
<td>AMP</td>
<td>CMP</td>
</tr>
<tr>
<td>I. Crude extract</td>
<td>11,400</td>
<td>0.11</td>
<td>0.19</td>
</tr>
<tr>
<td>IIa. First liquid polymer extract</td>
<td>3,600</td>
<td>0.38</td>
<td>0.48</td>
</tr>
<tr>
<td>IIb. Second liquid polymer extract</td>
<td>1,180</td>
<td>0.37</td>
<td>0.39</td>
</tr>
<tr>
<td>III. Sephadex G-25 column</td>
<td>250</td>
<td>6.0</td>
<td>15</td>
</tr>
<tr>
<td>IV. First DEAE-cellulose column</td>
<td>21.4</td>
<td>15</td>
<td>31</td>
</tr>
<tr>
<td>V. Hydroxylapatite column</td>
<td>4.6</td>
<td>64</td>
<td>120</td>
</tr>
<tr>
<td>VI. Second DEAE-cellulose column</td>
<td>0.3</td>
<td>207</td>
<td>426</td>
</tr>
<tr>
<td>VI. Peak fraction</td>
<td>0.04</td>
<td>480</td>
<td>611</td>
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</tbody>
</table>

a tRNA-X-C-C was used as substrate for AMP incorporation. C MP represents incorporation into tRNA-X, C MP represents incorporation into tRNA-X-C, while C MP represents incorporation into tRNA-X-C-C.
FIG. 2. Analytical polyacrylamide gel electrophoresis of purified tRNA-nucleotidyltransferase. A, peak fraction (5 μg) of Fig. 1 in a 7% gel. B, SDS-polyacrylamide gel of protein extracted from the active band in Fig. 3 (approximately 10 to 15 μg). C, SDS gel of peak fraction (30 μg) from second DEAE-cellulose column.

FIG. 3. Preparative gel electrophoresis of Fraction VI. Two hundred micrograms were applied to the gel and electrophoresis was performed as described under "Materials and Methods." Migration was toward the anode. A, diagram of protein staining pattern (cut). B, nucleotide-incorporating activity of proteins separated in the same electrophoresis. Transfer RNA-nucleotidyltransferase activity was assayed as described under "Materials and Methods." Dashed bar represents AMP incorporation into tRNA-X-C-C; the dotted bar represents CMP incorporation into tRNA-X; and the open bar CMP incorporation into tRNA-X-C.

TABLE II
Comparison of nucleotide incorporation by Fractions V and VI

The assay was described under "Materials and Methods" except that the extent rather than the rate of incorporation was measured. Two enzyme concentrations were used: for Fraction V 0.1 and 0.2 μg and for Fraction VI 0.02 and 0.04 μg of protein. The concentration of UTP and GTP was each 1.0 mM while ATP and CTP were 0.2 mM. Incubation was for 90 min at 37°C.

<table>
<thead>
<tr>
<th>tRNA substrate</th>
<th>Fraction V</th>
<th>Fraction VI</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMP</td>
<td>CMP</td>
<td>UMP</td>
</tr>
<tr>
<td>moles/mole tRNA</td>
<td>moles/mole tRNA</td>
<td>moles/mole tRNA</td>
</tr>
<tr>
<td>tRNA-X</td>
<td>0.00</td>
<td>2.85</td>
</tr>
<tr>
<td>tRNA-X-C</td>
<td>0.76</td>
<td>1.89</td>
</tr>
<tr>
<td>tRNA-X-C-C</td>
<td>0.97</td>
<td>0.28</td>
</tr>
</tbody>
</table>

Molecular Weight Studies—The molecular weight of tRNA-nucleotidyltransferase was estimated by SDS-gel electrophoresis. Each of the two SDS gels (Figs. 2, B and C) were run in parallel with gels containing molecular weight markers. The two SDS gels gave molecular weight values of 53,000 and 54,000, respectively. The plot of the log molecular weight against the distance of migration of the sample in Fig. 2B is shown in Fig. 4A. Two other determinations gave values of 57,000 and 51,000. The molecular weight was therefore estimated to be 54,000 ± 3000. SDS-gel electrophoresis performed with 8 M urea in the gel and electrode buffer (pH 7.1) also gave a single band and a molecular weight of 56,000.

Gel filtration on Sephadex G-100 (superfine) was also used to estimate the molecular weight of the native enzyme. From the plot of the log molecular weight against the elution volume the molecular weight appeared to be 45,000 (Fig. 4B). Three additional determinations gave values of 44,000, 48,000, and 43,000.

Analytical gel filtration with Sepharose 4B in 6 M guanidine hydrochloride gave values of 48,000, 46,000, and 45,000.
Methods” with 0.04 pg of Fraction V and 0.2 mM [3H]-NTP. The measured at 5, 10, and 15 min and the rate was determined from these values. Table depicts the concentration that gave 50% inhibition.

<table>
<thead>
<tr>
<th>Nucleotides incorporated</th>
<th>Inhibitor nucleotide</th>
<th>Concentration of tRNA substrate to produce 50% inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>H-ATP</td>
<td>CTP</td>
<td>0.21</td>
</tr>
<tr>
<td></td>
<td>UTP</td>
<td>2.1</td>
</tr>
<tr>
<td></td>
<td>ATP</td>
<td>0.6</td>
</tr>
<tr>
<td></td>
<td>UTP</td>
<td>8.0</td>
</tr>
<tr>
<td></td>
<td>ATP</td>
<td>0.032</td>
</tr>
<tr>
<td></td>
<td>CTP</td>
<td>0.029</td>
</tr>
<tr>
<td>H-CTP</td>
<td>CTP</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>UTP</td>
<td>0.7</td>
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<tr>
<td></td>
<td>ATP</td>
<td>0.014</td>
</tr>
<tr>
<td></td>
<td>CTP</td>
<td>0.025</td>
</tr>
</tbody>
</table>

**Effect of various nucleoside triphosphates on rate of incorporation of other nucleotides**

The assay was performed as described under “Materials and Methods” with 0.04 pg of Fraction V and 0.2 mM [3H]-NTP. The concentration of the inhibitors was varied over a range of 100 to a point which gave about 100% inhibition. Incorporation was measured at 5, 10, and 15 min and the rate was determined from these values. Table depicts the concentration that gave 50% inhibition.

<table>
<thead>
<tr>
<th>Nucleotides incorporated</th>
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<tr>
<td>H-CTP</td>
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<td>UTP</td>
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<tr>
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<td>ATP</td>
<td>0.014</td>
</tr>
<tr>
<td></td>
<td>CTP</td>
<td>0.025</td>
</tr>
</tbody>
</table>

**Substrate Specificity**

**Specificity of Nucleotide Incorporation**—Neither undigested tRNA nor tRNA which had been digested beyond the fourth nucleotide could serve as substrate for the highly purified enzyme; however, a small amount of UMP and CMP appeared to be incorporated by less pure enzymes. As compared to Fraction VI, Fraction V catalyzed the incorporation of an additional CMP and 1 UMP residue into tRNA-X and tRNA-X-C (Table II). Fraction V also incorporated 1 AMP residue into tRNA X C. Fraction VI, on the other hand, could only incorporate those nucleotides which had been previously removed by venom phosphodiesterase.

To determine whether the additional nucleotide-incorporating activities in Fraction V were similar to or different from the nucleotidyltransferase in Fraction VI, inhibition of “normal” and extraneous incorporating activities of Fraction V by nucleoside triphosphates was investigated. Table III shows the results; the data represent the concentration of inhibitor required to produce a 50% decrease in the rate of nucleotide incorporation. It can be seen that the concentration of CTP or UTP needed to produce a 50% inhibition of AMP incorporation was 5-fold lower for tRNA-X-C (extraneous AMP addition) than for tRNA-X-C-C. In comparison, CMP incorporation into tRNA-X-C-C (extraneous CMP addition) was much more sensitive to inhibition by ATP or UTP than was CMP incorporation into tRNA-X-C or tRNA-X. UMP incorporation into any of the three tRNA substrates was about 100-fold more sensitive to inhibition by ATP and CTP than was the incorporation of CMP.

**Lack of Specificity for Amino Acid-specific tRNA Species**—The following experiment was performed to see whether tRNA-nucleotidyltransferase could catalyze the addition of the -pC-C-A sequence to all amino acid-specific tRNA species. Unfractionated tRNA was digested with venom phosphodiesterase under conditions which removed all three terminal nucleotides which were then added back with purified tRNA-nucleotidyltransferase. The tRNA was isolated and the amino acid acceptor activity was compared to that of tRNA which had been treated with venom phosphodiesterase but not reconstituted, and to a tRNA sample which had not been treated at all. The results showed that after reconstitution virtually no acceptor activity remained. After reconstitution of the terminal -pC-C-A sequence, however, the acceptor activity for 17 amino acids tested could be fully restored. This showed that tRNA-nucleotidyltransferase could not discriminate between different amino acid-specific tRNA species.

**Lack of Specificity for Heterologous tRNAs**—When the Km values for the incorporation of CMP into tRNA-X-C from three different organisms were investigated, we found that yeast tRNA appeared to act as substrate for the E. coli enzyme very much like E. coli tRNA. Transfer RNA from rat liver and rabbit reticulocytes, on the other hand, gave Km values which were one order of magnitude higher than the Km for E. coli tRNA. It is not yet known whether these differences are significant.

**Absence of Other Enzymes**—Fraction V and Fraction VI did not contain any detectable RNase I, RNase II, polynucleotide phosphorylase, inorganic pyrophosphatase, ATPase, or CTPase activity. None of the aminosyl-tRNA synthetases was detected.

**Stability of Enzyme** When glycerol was omitted from all buffers during the preparation, the recovery of the enzyme decreased by 50% and the specific activity by 75%. When purification was performed in the absence of magnesium, Fraction V lost all activity in 1 week at 4°C. If magnesium was removed from the purified enzyme either by dialysis or by addition of EDTA all activity was lost, even though the assay was performed in the presence of Mg**++**. All attempts were unsuccessful to restore activity of the magnesium-depleted enzyme by dialysis against 10 mM Mg**++** or by addition of Mg**++** to a concentration of 10 mM in the absence or presence of 50 mM imidazole (pH 7.2). Dialysis of the purified enzyme against the assay buffer which contained 10 mM Mg**++** and 10 mM 2-mercaptoethanol for 6 hours at 4°C resulted in a 40% loss of activity. The purified enzyme was completely stable for at least 3 weeks when stored at −20°C in Buffer I containing about 0.1 M NaCl and 40% glycerol. The enzyme concentration of this sample was 20 μg per ml. Storage with 2.5 mg per ml of bovine serum albumin stabilized the enzyme such that no activity was lost up to 8 weeks of storage.

**DISCUSSION**

Transfer RNA nucleotidyltransferase from E. coli was purified to near homogeneity. The molecular weight of the purified enzyme was determined to be 34,000 ± 3,000 by SDS-gel electrophoresis and 45,000 ± 3,000 by gel filtration. The difference in results between the two methods was about 20%. We could not determine whether this difference was due to inaccuracies in either method (28, 29) or due to some inherent peculiarity of tRNA-nucleotidyltransferase. The enzyme appears to consist of a single protein and incorporates only 1 AMP and 2 CMP residues into tRNA-X. All amino acid acceptor species of E. coli tRNA as well as tRNA from eukaryocytes could serve as substrate for the enzyme. These results and those of Daniel and Littauer (8) thus suggest that tRNA-nucleotidyltransferase is not specific for any particular organism.

As was shown in Table II, Fraction V was able to incorporate 1 additional CMP residue into tRNA-X and tRNA-X-C. As shown earlier (7), this extraneous CMP incorporation appears to

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The dialysis tubings were heated to 100°C in the presence of distilled water.
be rather specific: in the absence of ATP exactly 2 CMP residues were incorporated into tRNA-X-C. Incorporation of the extraneous CMP was completely blocked in the presence of a 4- to 6-fold excess of ATP. Fraction V also catalyzed the addition of UMP to tRNA-X and tRNA-X-C in the absence of CTP and the incorporation was completely inhibited by a low concentration of CTP. Our results with the less pure Fraction V are thus in complete agreement with those of others (5, 8, 32-35) showing that partially purified tRNA-nucleotidyltransferase can make "mistakes" during reconstitution of the -pC-C-A sequence in vitro. On the other hand, the nearly homogeneous tRNA-nucleotidyltransferase is unable to catalyze any aberrant addition of nucleotides into tRNA-X, even in the absence of ATP. Preliminary results showed that Fraction V did not contain any other tRNA-nucleotidyltransferase. However, they do not exclude the possibility that a factor exists in this fraction which may alter the specificity of tRNA-nucleotidyltransferase. However, more extensive studies are needed to substantiate the presence of a factor which might copurify with tRNA-nucleotidyltransferase up to Fraction V and alter the specificity of the enzyme.

Numerous enzymes have been isolated which catalyze the addition of nucleoside monophosphates to the 3' end of oligo or polynucleotides (for review see Reference 36). Of these only the ATP-polynucleotide adenyltransferase (EC 2.7.7.19) has been purified and characterized to a special form of RNA polymerase (EC 2.7.7.6). A second enzyme, the tRNA-cytidyltransferase (EC 2.7.7.21) isolated of E. coli (41) indicate that the poly(A) polymerase might be only a special form of the latter enzyme. Its nucleotide and substrate specificity, however, make it unlikely that it is identical with tRNA-nucleotidyltransferase. Whether it represents a special form of the latter enzyme must await further studies.

REFERENCES
Transfer Ribonucleic Acid Nucleotidyltransferase from *Escherichia coli*: II. PURIFICATION, PHYSICAL PROPERTIES, AND SUBSTRATE SPECIFICITY
Jon P. Miller and Georg R. Philipps


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