Proc. Nat. Acad. Sci. USA
Vol. 72, No. 12, pp. 4790–4794, December 1975
Biochemistry

A method for the isolation of specific tRNA precursors

(tRNA affinity chromatography)

GABRIEL VÖGELI, HENRI GROSJEAN*, AND DIETER SÖLL

Yale University, Department of Molecular Biophysics and Biochemistry, New Haven, Connecticut 06520

Communicated by Joseph G. Gall, September 11, 1975

ABSTRACT **tRNA affinity chromatography, based on complex formation between tRNAs with complementary anticodons, has been applied to the isolation of specific tRNA precursors. When [^{32}P]RNA, isolated from an *Escherichia coli* strain containing a thermolabile ribonuclease P, was chromatographed on resin-bound yeast phenylalanine tRNA, precursor tRNA^{Glu} (possessing the complementary anticodon) was specifically retained. Likewise, precursor tRNA^{Phe} was isolated from a column of resin-bound *E. coli* glutamate tRNA. Both precursor tRNAs isolated were monomeric and may be processed products of an originally larger RNA precursor. Both tRNA precursors contain additional nucleotides beyond the 5′-end of the mature tRNA and have all modified bases found in mature tRNA. The method can be extended to isolate other tRNA precursors by affinity chromatography with different tRNAs. Since the principle of complementary anticodon interaction is not restricted to any particular organism, specific precursor tRNAs from other sources may also be isolated in this way.**

Like other stable RNA species, tRNA in bacterial and mammalian cells is formed via a larger RNA precursor molecule which is subsequently cleaved to mature size tRNA by the action of special nucleases. The terminal nucleotidyltransferase is responsible for formation of the C-C-A_{OH} terminus of some tRNAs. An additional part of this "maturation process" is the formation of modified nucleosides in tRNA, which proceeds as post-transcriptional nucleotide modification. At present the detailed scheme of the steps involved in these processes is not fully known (1–4). The lack of a variety of characterized RNA substrates has been a major obstacle in unraveling these processes. The transient nature of precursor tRNAs complicates the isolation of these molecules. Two factors have aided the isolation so far: (*i*) some bacteriophages have been constructed (5–7) or occur in nature (e.g., refs. 3 and 8) which harbor tRNA genes in their genome. Upon infection or induction these genes are transcribed with high frequency and give rise to a sufficient concentration of tRNA precursors to allow their isolation (9). (*ii*) More recently, temperature-sensitive mutants affecting at least one of the precursor tRNA processing nucleases (RNase P) have been obtained (10, 11). When these mutant strains are grown at the nonpermissive temperature, tRNA precursors accumulate. However, it is not easy to isolate specific individual tRNA precursors even from such mutant strains.

Very stable complexes of tRNA species with complementary anticodons have been found (12). Recently this principle was used for the rapid isolation of individual tRNA species of high purity by affinity chromatography. If one of the tRNAs of such a pair is permanently fixed to a solid column support, it will selectively bind the complementary tRNA contained in a mixture (13). The best studied pair of tRNAs with complementary anticodons is the complex of tRNA^{Phe} and tRNA^{Glu} (12–14). We reasoned that if in the precursor tRNA the anticodon conformation resembles that of the mature tRNA, then it should be possible to isolate specific tRNA precursors from a crude RNA mixture by tRNA affinity chromatography. In this paper we describe the isolation of specific tRNA precursors using affinity chromatography with resin-bound tRNA^{Phe} or tRNA^{Glu} (15).

Abbreviation: ptRNA, precursor tRNA.

* Present address: Laboratoire de Chimie Biologique, Faculté des Sciences, Universite Libré de Bruxelles, Rue des Chevaux, 67, 1640 Rhode-St-Genèse, Belgium.

MATERIALS AND METHODS

Bacterial Strains. *E. coli* A49 carrying a thermolabile RNase P (10) was used throughout this work.

Preparation of RNA (for Precursor tRNA Isolation). A 200-ml culture of *E. coli* A49 was grown at 30° in low phosphate medium (16) to a cell density of 2.5×10^8 cells per ml. The culture was then shifted to 42°. After 30 min 25 mCi of carrier-free [^{32}P]orthophosphate was added. After 10 min the cells were collected by centrifugation, resuspended in 5 ml of 20 mM Tris·HCl (pH 7.5)–5 mM EDTA and extracted three times with phenol. Special care was taken to perform all operations up to the first phenolization step at about 42°. The RNA was precipitated with ethanol and then subjected to affinity chromatography.

Preparation of RNA (for tRNA Isolation). Total RNA was extracted from *E. coli* A49 grown at 30° and labeled for 8 hr with ^{32}P [orthophosphate].

Affinity Chromatography. tRNA affinity chromatography was carried out according to Grosjean *et al.* (13). Two columns (0.3 ml bed volume each) were used in which either pure *E. coli* tRNA^{Glu} (0.4 mg) or pure yeast tRNA^{Phe} (0.4 mg) was bound to hydrazinyl-polyacrylamide (13). The columns were used at least 10 times without loss of capacity.

The radioactive RNA was dissolved in 0.15 ml of adsorption buffer [10 mM sodium acetate (pH 5.5)–10 mM $MgSO_4$–0.135 M NaCl], renatured at 60° for 2 min, and then applied to the affinity column at 0° at a flow rate of 2.5 ml/hr. The column was then washed with 10 ml of adsorption buffer at a flow rate of 8 ml/hr. The bound RNA was released from the columns by raising the temperature to 30° and eluting with 10 mM sodium acetate (pH 4.8)–10 mM EDTA–0.135 M NaCl. The pooled RNA-containing fractions were dialyzed against glass-distilled water.

RESULTS

Isolation of precursor tRNAs

For the isolation of precursor tRNAs we made use of an *E. coli* mutant defective in tRNA maturation (10). This strain contains a thermolabile form of RNase P (10), the enzyme which cleaves precursor tRNAs at the 5′-end of the mature tRNA molecule (4, 20). Upon shifting the strain to the nonpermissive temperature, precursor tRNAs accumulate. Since

tRNA affinity chromatography is very specific (13) we decided to use crude RNA in our attempts to purify tRNA precursors. Pulse-labeled RNA formed at the nonpermissive temperature was extracted and fractionated on a $tRNA^{Phe}$ column. As expected, a small amount (approximately 0.8%) of the nucleic acid material was retained on the column. The flow-through material was passed through a $tRNA^{Glu}$-column and a similar amount of material was retained. The purified samples were analyzed by polyacrylamide gel electrophoresis and compared with the starting material (Fig. 1). The tRNA affinity chromatography resulted in a remarkable purification of the precursors to $tRNA^{Glu}$ (panel 1) and $tRNA^{Phe}$ (panel 3) compared to the crude extract (panel 2). The precursor $tRNA^{Glu}$ preparation contains two major bands which migrate somewhat slower than tRNA. They may represent different conformations of the same molecule since sequence analysis revealed that both RNAs have the same sequence†. The precursor $tRNA^{Phe}$ preparation contains one major band in the 4S region. Thus the major species in our precursor tRNA preparations are monomeric tRNA precursors. These major species isolated from polyacrylamide gel were designated $ptRNA^{Glu}$ and $ptRNA^{Phe}$. Some minor, more slowly migrating bands are visible on the gel between the origin and the 4S RNA region. These may be dimeric or multimeric tRNA precursors‡. Some material, presumably high-molecular-weight RNA, did not penetrate the gel and remained at the origin. This material was not further analyzed.

In order to prove that the isolated RNA species were indeed precursor tRNAs, two experiments were performed—cleavage of the RNAs with *E. coli* RNase P and nucleotide sequence analysis.

Cleavage of precursor tRNAs with ribonuclease P

Since the RNAs were isolated from an RNase P defective strain, we wanted to test whether this enzyme could convert our RNA preparations to mature tRNA size. When pure *E. coli* RNase P was incubated with either $ptRNA^{Phe}$ or $ptRNA^{Glu}$, an endonucleolytic cleavage occurred. Polyacrylamide gel electrophoresis in 7 M urea (Fig. 2) revealed two species—an oligonucleotide fragment and a tRNA-size fragment. Sequence analysis showed the latter species to be indistinguishable from mature tRNA. As can be seen in Fig. 2, the RNase P reaction was incomplete. This was observed in several experiments and may be a consequence of different conformations of the RNA. It is known that RNase P action is dependent on the proper tertiary structure of the molecule (2). Since RNase P treatment yielded mature tRNA, we concluded that the isolated RNA species were indeed tRNA precursors.

Sequence analysis

Final proof of the nature of the precursors came from sequence analysis (18) of the ptRNAs, their RNase P cleavage products, and of mature tRNAs isolated from the same strain.

† It is known that *E. coli* $tRNA^{Glu}$ can exist in a native and denatured conformation [Bina-Stein, M. (1974) Ph.D. Dissertation, Yale University].

‡ Some larger RNA species in the $ptRNA^{Phe}$ preparation contain pppG- and all RNase T1 oligonucleotides of $ptRNA^{Phe}$ in addition to other oligonucleotides. This suggests that they may be dimeric ptRNAs. Rapid methods of RNA extraction from the cells, e.g., fast phenol extraction (9), did not improve the yield of these presumed dimeric ptRNAs.

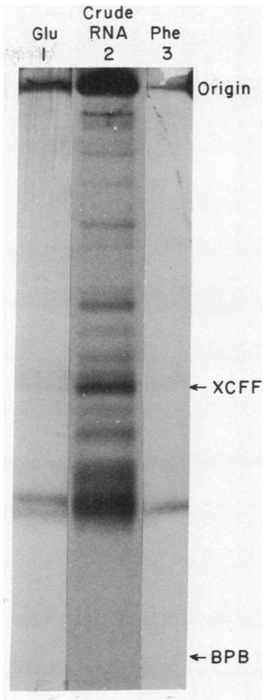

FIG. 1. Polyacrylamide gel electrophoresis of precursor tRNA (ptRNA) preparations. $ptRNA^{Glu}$ (panel 1), $ptRNA^{Phe}$ (panel 3), and crude RNA (panel 2). The RNA from the affinity chromatography was dissolved in 0.03 ml of a 20% sucrose solution containing xylene cyanol FF (XCFF) and bromophenol blue (BPB) and applied to a 10% polyacrylamide gel, pH 8.3 (17). After electrophoresis (the bromophenol blue marker migrated 23 cm from the origin), the RNA bands were detected by autoradiography.

$ptRNA^{Glu}$ and $tRNA^{Glu}$ were digested with RNase T1 and analyzed by the standard procedures (17). The fingerprints (Fig. 3) differ in only a few oligonucleotides. Their sequences and molar yields are shown in Table 1. $tRNA^{Glu}$

Table 1. T1 RNase end products of $ptRNA^{Glu}$

Fragment no.	Sequence	Molar yields	
		$tRNA^{Glu}$	$ptRNA^{Glu}$
1	G-	12.4 (10)	12.2 (10)
2	C-G-	1.5 (1)	1.7 (1)
3	A-G-	1.8 (1)	1.4 (1)
4	A-C-G-	1.0 (1)	0.9 (1)
5	C-C-C-A-G-	1.0 (1)	0.9 (1)
6	A-C-A-C-C-G-	0.9 (1)	0.9 (1)
7	*U-G-*	*0.1 (0)*	*1.0 (1)*
8	*pG-*	*0.8 (1)*	*0 (0)*
9	U-A-A-C-A-G-	0.9 (1)	1.0 (1)
10	T-ψ-C-G-	1.3 (1)	1.4 (1)
11	U-C-ψ-A-G-	1.3 (1)	1.1 (1)
12	C-C-C-U-S-U-C-m^2A-C-G-	0.7 (1)	0.5 (1)
13	A-A-U-C-C-C-C-U-A-G-	0.9 (1)	1.1 (1)
14	U-C-C-C-C-U-U-C-G-	1.1 (1)	1.1 (1)
15	*C-U-C-U-U-C-C-C-G-*	*0.0 (0)*	*0.7 (1)*
16	C-C-A_{OH}	1.1 (1)	1.3 (1)

The oligonucleotides are numbered as shown in Fig. 3. The fingerprint contains some faint spots which are not part of the mature tRNA sequence and were discarded as contaminants. The numbers indicate the measured molar yields; those deduced from the final sequence are in parentheses. Italicized entries denote fragments which differ in ptRNA and tRNA.

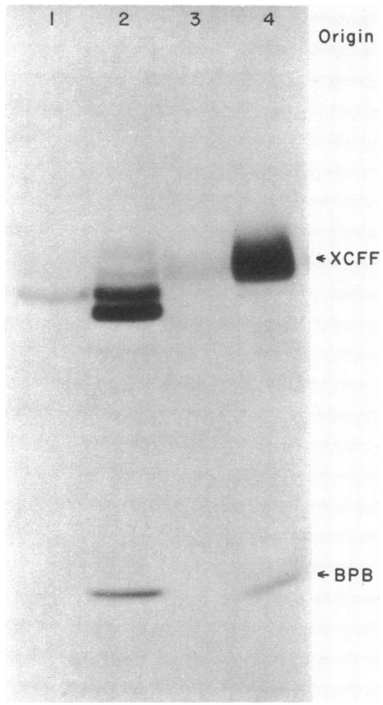

FIG. 2. Separation by polyacrylamide gel electrophoresis of ptRNAs after RNase P treatment. (Panel 1) $ptRNA^{Glu}$; (panel 2) $ptRNA^{Glu}$ after RNase P treatment; (panel 3) $ptRNA^{Phe}$; (panel 4) $ptRNA^{Phe}$ after RNase P treatment. [^{32}P]ptRNA (7×10^5 cpm) and carrier tRNA (20 μg) in 0.01 ml of 0.01 M Tris·HCl (pH 8.0)–0.1 M NH_4Cl–0.005 M $MgCl_2$–10^{-4} M EDTA was incubated with 1 μg of RNase P (31) for 2 hr at 37°. The samples were run on a 9% gel containing 7 M urea until the bromophenol blue (BPB) marker migrated 22 cm from the origin. In order to visualize the small oligonucleotide fragment formed upon RNase P treatment, gels in panels 2 and 4 contain ten times as much radioactive RNA as those in panels 1 and 3. The slower moving contaminating bands (panel 2) are also contained in $ptRNA^{Glu}$.

contains a 5′-terminal pG- (fragment 8) residue, which is lacking in $ptRNA^{Glu}$. However, the precursor contains two additional oligonucleotides, U-G- (fragment 7) and C-U-C-U-U-C-C-C-C-G- (fragment 15). A similar analysis of the pancreatic digest (Table 2) shows $ptRNA^{Glu}$ to contain 3 moles of G-U- (fragment 6), whereas $tRNA^{Glu}$ has only one in addition to a pG-U- (fragment 10) terminus. The 3′-end of $ptRNA^{Glu}$, fragment 16 (not shown in Fig. 1), is C-C-A_{OH} as in the mature tRNA. Clearly, the two additional oligonucleotides are at the 5′-end. Since the nucleotide sequence of *E. coli* $tRNA^{Glu}$ had been previously determined (21), only the sequence at the 5′-end of $ptRNA^{Glu}$ was in question. The

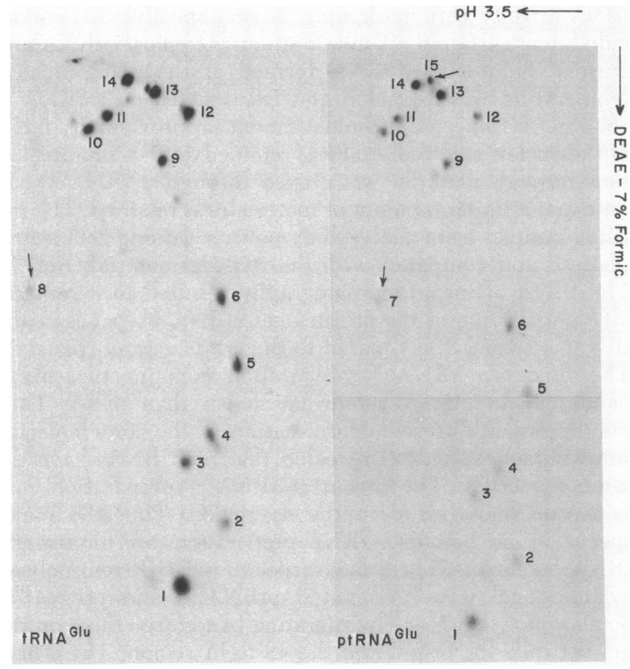

FIG. 3. Fingerprint of a complete RNase T1 digest of $ptRNA^{Glu}$ and $tRNA^{Glu}$. Arrows indicate the fragments that differ between the two RNAs. The 3′-terminal oligonucleotide C-C-A_{OH} is not on the fingerprint.

Table 2. Pancreatic RNase end products of $ptRNA^{Glu}$

Fragment no.	Sequence	Molar yields: $tRNA^{Glu}$	Molar yields: $ptRNA^{Glu}$
1	C-	13.6 (19)	12.7 (23)
2	U- + ψ-	6.3 (8)	5.6 (12)
3	m^2A-C-	1.2 (2)	1.4 (2)
4	A-A-C-	0.9 (1)	1.1 (1)
5	G-C-	2.0 (2)	1.9 (2)
6	*G-U-*	*1.3 (1)*	*2.8 (3)*
7	G-G-C-	1.0 (1)	1.0 (1)
8	G-A-A-U-	1.1 (1)	1.1 (1)
9	A-G-G-A-C-	1.2 (1)	1.0 (1)
10	*pG-U-*	*0.5 (1)*	*0 (0)*
11	G-G-U-	1.4 (1)	1.4 (1)
12	A-G-A-G-G-C-	0.9 (1)	0.8 (1)
13	A-G-G-G-G-A-C-	0.2 (1)	0.2 (1)
14	A-G-G-G-G-T-	0.3 (1)	0.1 (1)

The numbers indicate the measured molar yields; those deduced from the final sequence are in parentheses. Italicized entries denote fragments which differ in ptRNA and tRNA.

Table 3. T1 RNase end products of $ptRNA^{Phe}$

Fragment no.	Sequence	Molar yields: $tRNA^{Phe}$	Molar yields: $ptRNA^{Phe}$
1	G-	9.5 (9)	7.6 (9)
2	A-G-	2.7 (2)	1.9 (2)
3	C-A-G-	1.0 (1)	1.0 (1)
4	C-C-C-G-	0.9 (1)	0.7 (1)
5	*C-A-C-C-*A_{OH}	*0.9 (1)*	*0.2–0.9 (1)*
6	*pG-*	*0.7 (1)*	*0 (0)*
7	D-C-G-	0.9 (1)	0.7 (1)
8	D-A-G-	1.0 (1)	1.1 (1)
9	U-C-C-G-	0.9 (1)	0.8 (1)
10	C-U-C-A-G-	1.0 (1)	1.0 (1)
11	A-U-A-G-	0.4 (1)	0.9 (1)
12	*U-U-G-*	*0.2 (0)*	*1.0 (1)*
13	T-ψ-C-G-	1.3 (1)	1.0 (1)
14	A-ψ-U-G-	1.0 (1)	1.2 (1)
15	A-U-U-C-C-G-	1.1 (1)	1.1 (1)
16	A-A-msiA-A-ψ-C-C-C-C-G-	1.0 (1)	1.0 (1)
17	U-m^7G-X-C-C-U-U-G-	0.8 (1)	1.0 (1)
18	*pppG-*	*0 (0)*	*1.0 (1)*

The numbers indicate the measured molar yields; those deduced from the final sequence are in parentheses. Italicized entries denote fragments which differ in ptRNA and tRNA.

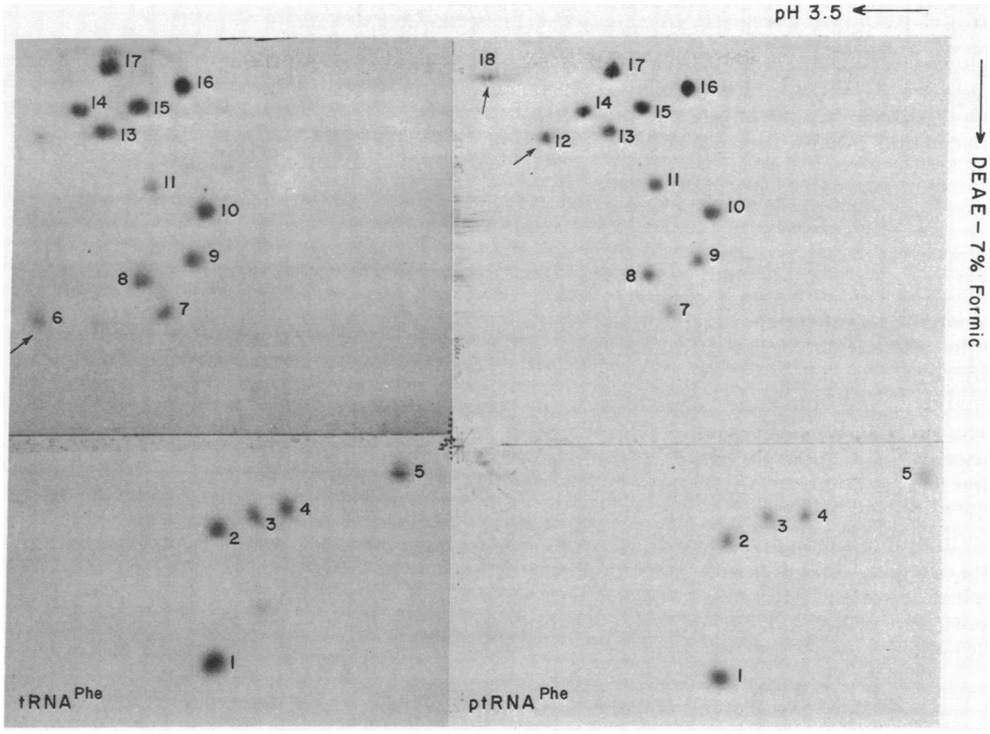

FIG. 4. Fingerprint of a complete RNase T1 digest of ptRNA^{Phe} and tRNA^{Phe}. Arrows indicate the fragments that differ between the two RNAs.

task of ordering the additional two oligonucleotides was aided by the analysis of the products obtained from RNase P cleavage, the cleaved ptRNA^{Glu} and the small oligonucleotide (Fig. 2). Cleaved ptRNA^{Glu} yielded the same RNase T1 fingerprint as mature tRNA; in particular it contains the 5′-end pG-. Therefore the small cleavage product must possess a 2′,3′-hydroxyl end. Upon digestion with RNase T1, C-U-C-U-U-C-C-C-G- was formed, while RNase A action produced U-, C-, and G-U. This information is sufficient to deduce the sequence of the ptRNA^{Glu} shown in Fig. 5. We could not detect a terminal 5′-phosphate in ptRNA^{Glu} although most tRNA precursors contain a terminal phosphate (see, e.g., ref. 2). The lack of a 5′-triphosphate end group suggests that the isolated ptRNA^{Glu} is already a cleavage product of the primary transcript.

Table 4. Pancreatic RNase end products of ptRNA^{Phe}

Fragment no.	Sequence	Molar yields	
		tRNA^{Phe}	ptRNA^{Phe}
1	C-	15.7 (14)	11.0 (14)
2	U- + ψ	5.6 (6)	5.2 (8)
3	A-C-	1.0 (1)	0.7 (1)
4	m^{7}G-X-C-	0.8 (1)	1.0 (1)
5	*G-C-*	*0.4 (0)*	*0.9 (1)*
6	A-G-C-	1.2 (1)	1.0 (1)
7	G-U-	1.4 (1)	1.3 (1)
8	*pG-C-*	*0.7 (1)*	*0 (0)*
9	G-A-U-	1.0 (1)	1.2 (1)
10	A-G-D-	1.3 (1)	1.0 (1)
11	G-G-T- + G-G-D-	1.0 (1)	1.1 (1)
12	G-G-G-C-	0.6 (1)	0.5 (1)
13	A-G-A-G-C-	1.0 (1)	1.0 (1)
14	G-G-A-U- + G-A-G-U-	1.0 (1)	1.1 (1)
15	G-A-A-msiA-A-ψ-	1.1 (1)	1.1 (1)
16	A-G-G-G-G-A-ψ-	0.3 (1)	0.2 (1)
17	*pppG-U-*	*0 (0)*	*0.9 (1)*

The numbers indicate the measured molar yields; those deduced from the final sequence are in parentheses. Italicized entries denote fragments which differ in ptRNA and tRNA.

Sequence analysis of the ptRNA^{Phe} was accomplished in a similar way. Fig. 4 shows a comparison of the RNase T1 fingerprints of ptRNA^{Phe} and tRNA^{Phe}. The two RNA species differ in three fragments. pppG- (fragment 18) and U-U-G- (fragment 12) are present only in ptRNA^{Phe}, whereas only the mature tRNA contains fragment 6, pG- (Fig. 4 and Table 3). Analysis of the products of an RNase A digest (Table 4) indicates that pppG-U- (fragment 17) and G-C- (fragment 5) occur only in the precursor, whereas the 5′-terminus of the mature tRNA is pG-C- (fragment 8). Table 3 and Fig. 4 show that the 3′-end of both tRNA^{Phe} and ptRNA^{Phe} is C-A-C-C-A$_{OH}$ (fragment 5). In several ptRNA^{Phe} preparations the yield of this oligonucleotide varied. However, the corresponding oligonucleotide C-A-C-C- without the terminal A was found. Cleaved ptRNA^{Phe}, obtained by RNaseP action (Fig. 2), has the same RNase T1 fingerprint as mature tRNA^{Phe}. Based on the known primary structure of tRNA^{Phe} (22) and on our results, the nucleotide sequence of ptRNA^{Phe} is deduced and shown in Fig. 5. Analysis of the 5′-terminal oligonucleotide released from ptRNA^{Phe} after RNase P treatment confirms this result.

CUCUUCCCGUGU····tRNA^{Glu}

pppGUUGC····tRNA^{Phe}

FIG. 5. Nucleotide sequence of *E. coli* ptRNA^{Glu} and ptRNA^{Phe}. The underlined nucleotides belong to the mature tRNA sequence.

Modified nucleotides in the precursor tRNA

Analysis by two-dimensional chromatography (19) of RNase T2 hydrolysates of the RNase T1 oligonucleotides from $ptRNA^{Glu}$, $ptRNA^{Phe}$, $tRNA^{Glu}$, and $tRNA^{Phe}$ showed the same extent of nucleotide modification in both precursor and mature tRNAs.

DISCUSSION

The successful isolation of specific tRNA precursors by tRNA affinity chromatography utilizing the complex formation of their complementary anticodons suggests that the anticodon structures in $ptRNA^{Phe}$ and $ptRNA^{Glu}$ must be similar to those occurring in the mature tRNAs. While no detailed physical studies have been undertaken to examine possible differences in the binding constants of mature tRNA and precursor tRNA in these complexes, it is obvious that the association is sufficiently strong to permit isolation of very pure ptRNAs by this method. Whether the anticodon structure in a multimeric precursor would also be able to engage in strong complex formation with the complementary tRNA is not clear at present. Affinity retardation studies of known multimeric precursors (1–4, 28) will be needed to learn whether this method extends to those classes of precursor tRNAs.

Although the methods for purification of any tRNA species are very advanced (e.g., ref. 23), practical limits to the selection at random of any desired ptRNA species by tRNA affinity chromatography are set by the stability of the tRNA:tRNA complex. It is known that not all pairs of tRNAs with complementary anticodons have the same high binding constant as the $tRNA^{Phe}$:$tRNA^{Glu}$ pair (24). Other tRNA pairs can be used for affinity chromatography; we have isolated precursor tRNAs with the $tRNA^{Pro}$:$tRNA^{Trp}$ pair. The stability of the tRNA:tRNA complex depends on the proper configuration of the anticodon, on the nature of the base pairs in the anticodon–anticodon complex, and on the hypermodified base adjacent to the anticodon (H. Grosjean, D. Söll, and D. M. Crothers, *J. Mol. Biol.*, in press). Since the presence of such a hypermodified base stabilizes the anticodon–anticodon complex, it is possible that tRNA affinity chromatography selects precursor tRNAs containing this modification.

In both $ptRNA^{Phe}$ and $ptRNA^{Glu}$ all modified nucleosides are inserted at the precursor stage. This is reminiscent of the T4 tRNA precursors, which are highly modified (3, 4), and it again indicates a tRNA-like structure of the ptRNA. It is pertinent to note that all pseudouridines are modified in $ptRNA^{Glu}$ and $ptRNA^{Phe}$, in contrast to the partial modification in $ptRNA^{Tyr}$ (25).

Since the anticodon-anticodon interaction is independent of the source of the tRNA species (24), this method should prove to be a general tool for the isolation of specific tRNA precursors from any organism. Although the isolation of tRNA precursors of short half-life in some organisms may require mutants having defective processing nucleases, tRNA precursors of yeast (26) and of mammalian cells (27) have been demonstrated. Thus, our method should allow the isolation of specific tRNA precursors from those eukaryotic cells. These precursor tRNAs should aid the isolation and characterization of processing nucleases from mammalian sources and also show whether the "leader" sequences are an immutable part of the tRNA genes and are conserved during evolution.

We are indebted to Dr. E. Bikoff for her generous gift of pure *E. coli* RNase P. This work was supported by NIH Grant GM 15401 and NSF Grant BMS 72-02400-A02.

1. Schaefer, K. & Söll, D. (1974) *Biochimie* **56**, 795–804.
2. Altman, S. (1975) *Cell* **4**, 21–29.
3. Seidman, J. G. & McClain, W. H. (1975) *Proc. Nat. Acad. Sci. USA* **72**, 1491–1495.
4. Guthrie, C. (1975) *J. Mol. Biol.* **95**, 529–547.
5. Smith, J. D., Abelson, J. N., Clark, B. F. C., Goodman, H. M. & Brenner, S. (1966) *Cold Spring Harbor Symp. Quant. Biol.* **31**, 479–485.
6. Andoh, T. & Ozeki, H. (1968) *Proc. Nat. Acad. Sci. USA* **59**, 792–799.
7. Squires, C., Konrad, B., Kirschbaum, J. & Carbon, J. (1973) *Proc. Nat. Acad. Sci. USA* **70**, 438–441.
8. Daniel, V., Sarid, S. & Littauer, U. Z. (1970) *Science* **167**, 1682–1688.
9. Altman, S. (1971) *Nature New Biol.* **229**, 19–21.
10. Schedl, P. & Primakoff, P. (1973) *Proc. Nat. Acad. Sci. USA* **70**, 2091–2095.
11. Sakano, H., Yamada, S., Ikemura, T., Shimura, Y. & Ozeki, H. (1974) *Nucleic Acids Res.* **1**, 355–371.
12. Eisinger, J. (1971) *Biochem. Biophys. Res. Commun.* **44**, 1135–1142.
13. Grosjean, H., Takada, C. & Petre, J. (1973) *Biochem. Biophys. Res. Commun.* **53**, 882–893.
14. Eisinger, J. & Gross, N. (1975) *Biochemistry* **14**, 4031–4041.
15. Vögeli, G., Grosjean, H. & Söll, D. (1975) *Fed. Proc.* **34**, 517 abstr.
16. Hayes, O. H., Hayes, F. & Guerin, M. F. (1966) *J. Mol. Biol.* **18**, 499–515.
17. Peacock, A. C. & Dingman, C. W. (1967) *Biochemistry* **6**, 1818–1827.
18. Barrell, B. G. (1971) *Progr. Nucleic Acid Res. Mol. Biol.* **2**, 751–779.
19. Kimball, M. E., Szeto, K. S. & Söll, D. (1974) *Nucleic Acids Res.* **1**, 1721–1732.
20. Robertson, H. D., Altman, S. & Smith, J. D. (1972) *J. Biol. Chem.* **247**, 5243–5251.
21. Ohashi, Z., Harada, F. & Nishimura, S. (1972) *FEBS Lett.* **20**, 239–247.
22. Barrell, B. G. & Sanger, F. (1969) *FEBS Lett.* **3**, 275–278.
23. McCutchan, T. F., Gilham, P. T. & Söll, D. (1975) *Nucleic Acids Res.* **2**, 853–864.
24. Eisinger, J. & Gross, N. (1974) *J. Mol. Biol.* **88**, 165–174.
25. Schaefer, K. P., Altman, S. & Söll, D. (1973) *Proc. Nat. Acad. Sci. USA* **70**, 3626–3630.
26. Blatt, B. & Feldman, H. (1973) *FEBS Lett.* **37**, 129–132.
27. Burdon, R. H. (1971) *Progr. Nucleic Acid Res. Mol. Biol.* **11**, 33–73.
28. Chang, S. & Carbon, J. (1975) *J. Biol. Chem.* **250**, 5542–5555.
29. Bikoff, E. K., LaRue, B. F. & Gefter, M. L. (1975) *J. Biol. Chem.* **250**, 6248–6255.