Nucleotide Sequence of an Arginine Transfer Ribonucleic Acid from Bacteriophage T4*

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The nucleotide sequence was determined by analysis of RNA, uniformly labeled in vitro, according to the conventional techniques. In addition, RNA synthesized in vitro in the presence of α32P-labeled nucleoside triphosphates was analyzed through the use of nearest neighbor sequencing techniques. Although a unique sequence could not be determined by this latter analysis, restrictions on the sequence imposed by nearest neighbor data and secondary structure common to tRNA molecules allowed prediction of the correct nucleotide sequence.

Bacteriophage T4 induces the synthesis of eight tRNA species upon infection of Escherichia coli (1); these include the acceptor activities for arginine, glycine, isoleucine, leucine, and proline (2, 3). The T4 tRNA system has proved amenable to the analysis of tRNA biosynthesis, a process which involves post-transcriptional modification of precursor molecules to yield mature tRNA species. Integral to this analysis has been the determination of nucleotide sequences of T4 tRNAs and their precursors, since an understanding of mechanisms involved in precursor RNA maturation depends, in part, upon knowledge of molecular structures of the tRNAs and their biosynthetic intermediates. To date, the nucleotide sequences of T4 tRNAs specific for serine (4), proline (4), glycine (5, 6), glutamine (7), and leucine (8) have been determined, as have the nucleotide sequences to the precursors to proline and serine (4) and glutamine and leucine (9). The nucleotide sequence of a stable, low molecular weight RNA of 140 residues coded by T4 has also been established (10).

In this work we report the nucleotide sequence of a low molecular weight T4 RNA species of RNA mobility previously designated ε (1). The sequence was determined by analysis of the RNA, uniformly labeled in vitro, by the procedures developed by Sanger described in Ref. 11. In addition, the RNA was synthesized in vitro using α32P labeled nucleoside triphosphates (12). Nucleotide sequences of products of ribonuclease T1 and A digests of the RNA synthesized in vitro confirmed the results obtained in vivo; although analysis of the limited digestion products of the in vitro synthesized RNA was not performed, the nucleotide sequence was predicted from nearest neighbor data and from the secondary structure common to tRNA molecules which provide strong constraints on the final sequence. The deduced sequence was identical to that determined by analysis of RNA synthesized in vivo.

This RNA consists of 75 nucleotide residues and can be arranged in the cloverleaf structure typical of tRNAs, with an anticodon corresponding to codons for arginine. The RNA is therefore presumed to represent the arginine acceptor activity of T4 identified previously (3), although the ability of this RNA to accept amino acid has not yet been tested.

EXPERIMENTAL PROCEDURES

Bacterial and Bacteriophage Strains (Wisconsin) - Sequence determination was performed on arginine tRNA from several strains of bacteriophage T4, including the wild type T4D (13) and derivatives of T4 carrying various mutations in serine tRNA. Bacterial hosts were Escherichia coli strains B/9 (14) and BN (15). No sequence

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‡ To whom requests for reprints should be sent.

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Nucleotide Sequence of tRNA\textsuperscript{\textsc{arg}} from Phage T4

**Figure 1.** Component nucleotides of tRNA\textsuperscript{\textsc{arg}}, \textsuperscript{32}P-labeled tRNA\textsuperscript{\textsc{arg}} was digested with ribonuclease T\textsubscript{1}, and the products were fractionated by two-dimensional thin layer chromatography. Products N1, N2, N3, and N4, collectively referred to as Up, probably represent derivatives of Up that occur in the first position of the anticodon sequence (see legend to Fig. 6). There was no evidence for the presence of minor nucleotides \textsuperscript{s}Up in this tRNA, nor for modification of the Ap adjacent to the 3' side of the anticodon. The dotted circles show the positions of nucleotides present in too low a yield to reproduce photographically.

The spectrum of products N1 to N4 has not been previously observed in any bacteriophage T4 tRNA. There is no evidence for the presence of minor nucleotide \textsuperscript{s}Up in this tRNA, nor for modification of the Ap adjacent to the 3' side of the anticodon. The dotted circles show the positions of nucleotides present in too low a yield to reproduce photographically.

**Table I.** Extent of nucleotide modification (Wisconsin)

<table>
<thead>
<tr>
<th>Site</th>
<th>Unmodified</th>
<th>Modified</th>
<th>Extent of Modification (%)</th>
<th>How Determined</th>
</tr>
</thead>
<tbody>
<tr>
<td>17</td>
<td>Up</td>
<td>\textsuperscript{32}P</td>
<td>10-15</td>
<td>1</td>
</tr>
<tr>
<td>20</td>
<td>Up</td>
<td>\textsuperscript{32}P</td>
<td>10-20</td>
<td>1, 2</td>
</tr>
<tr>
<td>34</td>
<td>Up</td>
<td>\textsuperscript{32}P</td>
<td>90-100</td>
<td>2</td>
</tr>
<tr>
<td>40</td>
<td>Up</td>
<td>\textsuperscript{32}P</td>
<td>90-100</td>
<td>2</td>
</tr>
<tr>
<td>53</td>
<td>Up</td>
<td>\textsuperscript{32}P</td>
<td>90-100</td>
<td>2</td>
</tr>
<tr>
<td>54</td>
<td>Up</td>
<td>\textsuperscript{32}P</td>
<td>90-100</td>
<td>2</td>
</tr>
</tbody>
</table>

The extent of modification in the appropriate \textsuperscript{32}P-labeled ribonuclease fragment was determined in two ways: (1) when an oligonucleotide containing a modified residue separated from the unmodified one during electrophoresis, the amount of radioactivity in the modified one compared to the total provided a qualitative measurement; (2) the extent of modification of a chosen nucleotide fragment could be estimated by digesting the fragment to mononucleotides with ribonuclease T\textsubscript{1}, separating the products by two-dimensional thin layer chromatography, and visually comparing the amount of radioactivity in modified and unmodified residues. Up refers to modified nucleotides N1, N2, N3, and N4 collectively (see Fig. 1), and its position at residue 34 was inferred by analogy with other tRNA sequences (see legend to Fig. 6). There was no evidence for the presence of the minor nucleotide \textsuperscript{s}Up at residue 8 in this tRNA, nor for modification of residue 37, the Ap adjacent to the 3' side of the anticodon.
Nucleotide Sequence of tRNA\textsuperscript{ArQ} from Phage T4

B

FIG. 2. Products of ribonuclease T\textsubscript{1}, digestion of tRNA\textsuperscript{ArQ}. The sequences of the numbered oligonucleotides are given in Table II. A and B show the products of ribonuclease T\textsubscript{1}, digestion of \textsuperscript{32}P-labeled tRNA\textsuperscript{ArQ} isolated from bacteriophage T\textsubscript{4}D grown on Escherichia coli strain B/3. The products were fractionated in the first dimension on a cellulose/acetate strip in pyridine acetate buffer (pH 3.5) and in the second dimension by homochromatography (A) or on DEAE-paper in 7% formic acid (B). In B, the unmarked products which move just ahead of T15a in the first dimension are presumed to be additional modified forms of the T15 oligonucleotides, based on the results of analysis of these products with ribonuclease A. C shows the products of ribonuclease T\textsubscript{1}, digestion of tRNA\textsuperscript{ArQ} synthesized in vitro with [\textsuperscript{3}H]GTP; fractionation was as for B. Oligonucleotide T15 is seen as a single spot because the pyrimidine in the anticodon which is modified in vivo is not modified in the in vitro tRNA product. Similarly, oligonucleotide T13b, which contains the modified nucleotide G\textsuperscript{m2}p, is missing in this preparation. Because oligonucleotide T2 does not contain a Gp residue, it was not labeled in this preparation.

digestion with ribonuclease T\textsubscript{1}, followed by two-dimensional thin layer chromatography of the products. The latter analysis permitted identification of minor nucleotides.

The sequences of the small products (T1, T3, T4, T5, and T8) were determined by their fingerprints, their nucleotide compositions, and from the products of digestion with ribonuclease A. The salient features of the sequence analysis of the remaining larger fragments, summarized in Table II, are described fully below.

A-U-A-C-C-A\textsubscript{m1} (T2) - Ribonuclease T\textsubscript{1}, digestion of this product gave Ap(2), Up(1), and Cp(2), while complete digestion with snake venom phosphodiesterase gave pA(2), pC(2), and pU(1), yielding the partial sequence A(U, A, C, C)\textsubscript{m1}. Digestion with ribonuclease A gave the products A-Up(1), A-Cp(1), and Cp(1). The sequence was established by limited digestion with spleen phosphodiesterase, which gave the products A-U-A-C-C-A\textsubscript{m1}, A-U-A-C-C-A\textsubscript{m1}, A-U-A-C-C-A\textsubscript{m1}, and A-U-A-C-C-A\textsubscript{m1}; the sequences of these latter products were determined by complete digestion with snake venom phosphodiesterase and with ribonuclease T\textsubscript{1}.

C-U-Gp (T6) - Ribonuclease A digestion gave the composition Cp(1), Up(1), and Gp(1). Modification of the oligonucleotide with CMCT\textsuperscript{2} followed by ribonuclease A digestion gave the products Cp and U-Gp, thereby establishing the sequence.

U-C-C-Gp (T7) - Ribonuclease A digestion gave the composition Up(2), Cp(1), and Gp(1). CMCT modification followed by ribonuclease A digestion gave the products U-Gp, C-Gp, and C-Gp. The sequence was established by limited digestion with spleen phosphodiesterase, which gave the products U-C-C-Gp.

1 The abbreviation used is: CMCT, N-cyclohexyl-N'-((\beta)-morpholinyl-(4)-ethyl)-carbodiimide-methyl-p-toluene sulfonate.

Gp, C-C-Gp, C-C-Gp, and C-Gp; these latter products were identified by their electrophoretic mobilities and their nucleotide compositions.

C-A-U-A-C-Gp (T9) - Digestion with ribonuclease A gave the products A-Up(1), A-Cp(1), Gp(1), and Gp(1). The sequence was established by digestion with ribonuclease U, which gave the products C-Ap, U-Ap, and C-Gp. Additional confirmation of the sequence was obtained by ribonuclease A digestion following modification with CMCT; this operation yielded the products C-gp, C-gp, and C-gp.

A-U-C-C-C-A-Gp (T10) - Ribonuclease A digestion gave the composition A-Up(1), C-C-Ap(1), and A-C-Gp. The sequence was established by ribonuclease U, digestion which gave the products A-U-C-C-C-A-Gp, C-A-Gp, and C-A-Gp. The sequence of the latter oligonucleotide was determined by sequential digestion of it with spleen phosphodiesterase. Digestion of the intact oligonucleotide with spleen phosphodiesterase served to confirm the sequence; products of this digestion, identified by electrophoretic mobilities and compositions, were A-U-C-C-C-A-Gp, C-A-C-A-Gp, and C-A-C-A-Gp.

U-C-C-U-Gp (T11) - Ribonuclease A digestion gave the composition Up(2), Cp(1), and Gp(1). After modification with CMCT, digestion with ribonuclease A gave the products U-Gp, C-Gp, and C-Gp. The sequence was established by partial digestion of the oligonucleotide with spleen phosphodiesterase, which gave the products U-C-C-U-Gp, C-C-U-Gp, and C-C-U-Gp.

T-\psi-C-Gp (T12) - Ribonuclease T\textsubscript{1}, digestion gave 1 mol each of Tp, \psi-p, Cp, and Gp. The products of digestion with spleen phosphodiesterase, T-\psi-C-Gp, \psi-C-Gp, and C-Gp, established the sequence, which was additionally confirmed by digestion of the oligonucleotide with ribonuclease A following...
TABLE II

Sequence determination of ribonuclease T1 digestion products of tRNA<sup>Arg</sup> (Wisconsin)

Oligonucleotide fragments are digestion products shown in Fig. 2. The theoretical molar yield is based on the sequence shown in Fig. 8. The experimental molar yield was calculated by normalizing the radioactivity in each nucleotide to that in fragment T14 which was assumed to occur only once in the molecule. Calculations were made from several tRNA preparations with similar results; calculations shown here are derived from a single preparation of above average purity (>90% as estimated from the yield of oligonucleotides unrelated to arginine tRNA). When two or more forms of the same oligonucleotide occur, the relative yield of each form varies with the preparation (see Table I). The composition of each oligonucleotide was determined in two ways: (a) alkaline hydrolysis followed by electrophoretic fractionation of the products; molar yield of each nucleotide is given relative to Gp; (b) digestion with ribonuclease T1, followed by two-dimensional thin layer chromatography of the products, which permitted identification of minor nucleotides. The modified residues collectively referred to as Up were contained in oligonucleotide T15; however, the location of Up within the oligonucleotide was not determined by nucleotide sequence analysis. Our provisional placement of Up within oligonucleotide T15, which situates it in the first position of the anticodon in the complete tRNA sequence, is by analogy with other known l.tRNA sequences (20).

Ribonuclease A - Figures in parentheses are molar yields relative to guanosine-containing products. Products were identified by composition and by electrophoretic mobility.

Ribonuclease U<sub>2</sub> - Products were identified by composition and by electrophoretic mobility. The nucleotide sequences U-C-C-C-Ap (from Fragment T8) and U-C-C-U, U-C-U-Ap (from Fragment T15c) were determined by further digestion of these oligonucleotides with spleen phosphodiesterase.

CMCT + Ribonuclease A - Products were identified by composition. A "*' above Up or Gp indicates modification by CMCT.

Spleen Phosphodiesterase (SP'ase) - Products were identified by composition and by electrophoretic mobility. Numbers in brackets give electrophoretic mobilities of products on DEAE-paper in pyridine acetate buffer at pH 3.5 (oligonucleotide T2) or in 7% formic acid (oligonucleotides T7, T10, T11, T12, T14). Mobilities were measured relative to xylene cyanol dye (= 1.00). Products of digestion of T15 are shown in Fig. 3.

Venom Phosphodiesterase (VP'ase) - Products were identified by their mobilities in a two-dimensional thin layer chromatographic system. Figures in parentheses indicate relative molar yield of each product.

<table>
<thead>
<tr>
<th>Oligonucleotide Sequence</th>
<th>Theoretical Yield</th>
<th>Experimental Yield</th>
<th>C</th>
<th>A</th>
<th>G</th>
<th>U</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1 Gp</td>
<td>0.9 + 2.4</td>
<td>0.9 + 2.4</td>
<td>1.0</td>
<td>A-Gp(1.0)</td>
<td>A-Gp</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T2 A=U-A-C-C-Ap</td>
<td>0.7 2.4</td>
<td>0.7 2.4</td>
<td>1.0</td>
<td>A-Gp(1.0)</td>
<td>A-Gp</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T3 Gp</td>
<td>1.1 + 0.9</td>
<td>1.1 + 0.9</td>
<td>1.0</td>
<td>A-Gp(1.0)</td>
<td>A-Gp</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T4 Gp</td>
<td>1.0 + 1.2</td>
<td>1.0 + 1.2</td>
<td>1.0</td>
<td>A-Gp(1.0)</td>
<td>A-Gp</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T5 Gp</td>
<td>1.0 + 1.0</td>
<td>1.0 + 1.0</td>
<td>1.0</td>
<td>A-Gp(1.0)</td>
<td>A-Gp</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T6 Gp</td>
<td>1.0 + 1.2</td>
<td>1.0 + 1.2</td>
<td>1.0</td>
<td>A-Gp(1.0)</td>
<td>A-Gp</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T7 Gp</td>
<td>1.0 + 1.0</td>
<td>1.0 + 1.0</td>
<td>1.0</td>
<td>A-Gp(1.0)</td>
<td>A-Gp</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T8 A-U-A-Gp</td>
<td>1.0 + 1.0</td>
<td>1.0 + 1.0</td>
<td>1.0</td>
<td>A-Gp(1.0)</td>
<td>A-Gp</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T9 C-C-A-U-A-C-Gp</td>
<td>0.8 2.3</td>
<td>0.8 2.3</td>
<td>1.5</td>
<td>A-Gp(1.0)</td>
<td>A-Gp</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T10 U-C-C-C-A-C-Gp</td>
<td>0.8 2.0</td>
<td>0.8 2.0</td>
<td>1.0</td>
<td>A-Gp(1.0)</td>
<td>A-Gp</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T11 U-C-C-Gp</td>
<td>0.9 1.9</td>
<td>0.9 1.9</td>
<td>1.9</td>
<td>A-Gp(1.0)</td>
<td>A-Gp</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T12 T-K-C-Gp</td>
<td>1.0 1.0</td>
<td>1.0 1.0</td>
<td>1.0</td>
<td>A-Gp(1.0)</td>
<td>A-Gp</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

CMCT modification. The latter operation yielded the products (T, Up,Cp and Gp.

U-A-A-U-Gp (T13a) and U-A-A-U-Gm<sup>12</sup>-Gp (T13b) - The oligonucleotide U-A-A-U-Gp presumably arises from undermethylation of the Gm<sup>12</sup>-Gp, thereby allowing cleavage by ribonuclease T1. Ribonuclease T1 digestion gave the composition Up(2), Ap(2), and Gm<sup>12</sup>-Gp(1); the identity of Gm<sup>12</sup>-Gp was established by its mobility on a two-dimensional thin
FIG. 3. Sequential digestion of the oligonucleotide A-U-C-C-U-U-C-U-A-A-Gp by spleen phosphodiesterase. The oligonucleotide was digested and the products were fractionated in two dimensions by homochromatography. All visible products were characterized by further digestion with ribonuclease A in order to identify the limited digestion products of exonucleolytic cleavage. This was essential for following the path of successively shorter fragments; for example, the prominent spots at the top of the chromatogram do not contain A-A-Gp and therefore are not limited digestion products of exonucleolytic cleavage. The position of Up was not established.

FIG. 4. Relative electrophoretic mobilities of 32P-labeled CMCT-blocked oligonucleotides, fractionated by electrophoresis on Whatman No. 3MM paper at pH 3.5. The "" above U and G indicates the presence of the carbodiimide reagent on the residue. Blue marks the position of the xylene cyanole dyed anodic end at the top.

FIG. 5. Products of ribonuclease A digestion of 32P-labeled arginine tRNA. Two-dimensional fractionation of the products was as described for Fig. 2B. The positions of the modified nucleotides collectively referred to as Up were not determined. The sequences of the numbered oligonucleotides are given in Table III. Arginine tRNA used in this analysis was isolated from bacteriophage strain T4D grown in Escherichia coli strain B/5.

layer chromatography system. Ribonuclease A digestion gave the products Up(1), A-A-Up(1), and Gm2'-Gp(1). The sequence was established by digestion with ribonuclease U, which gave the products U-Ap, Ap, and U-Gm2'-Gp.

ψ-U-U-Gp (T14) —Digestion with ribonuclease T, gave the composition ψp(1), Up(2), and Gp(1). The sequence was established by partial digestion with spleen phosphodiesterase which gave the products ψ-U-U-Gp, U-U-Gp, and Up-Gp; these products were identified by their nucleotide compositions as determined by digestion with ribonuclease T,.

A-U-C-C-U-U-C-U-A-A-Gp (T15 a, b, and c) —This product was located in at least three positions on the fingerprint, depending upon the state of the Up residue. Although the exact position of Up could not be determined by sequence analysis, we have distinguished the Up residue from Up in the nucleotide sequence for convenience of discussion of multiple forms of oligonucleotide T15 and of the further digestion products derived from T15a, b, and c. By analogy with other known tRNA sequences (20), Up is placed arbitrarily such...
that its position in the T15 oligonucleotides corresponds to the first position of the anticodon of tRNA<sup>Arg</sup>.

Sequence determination was complicated by the presence of the Up residues, which are resistant to ribonuclease A cleavage and often show a tendency to undergo apparent nonspecific degradation which obscures electrophoretic resolution of digestion products. These factors consequently affected quantitation of molar yields of secondary digestion products; molar yields of these products were somewhat variable, reflecting heterogeneity with respect to modification of the Up residue. In a single experiment, digestion of the intact oligonucleotide with ribonuclease A gave the products A-Up(1), A-A-Gp(1), Cp(2), Yp(1), and UP(2); although the molar yield of Up was not determined, its presence was inferred from the spectrum of unidentified minor products observed.

Ribonuclease U<sub>2</sub> digestion gave the products Ap, Gp, and C-C-G-U-C-U-A-A-Gp; the sequence of the 3 residues of the latter product was determined by digestion with spleen phosphodiesterase followed by two-dimensional fractionation of the resulting products as described by Seidman et al. (7). Digestion of the intact oligonucleotide with spleen phosphodiesterase followed by two dimensional fractionation of the products (Fig. 3) gave A-U-C-C-U-C-U-A-A-Gp, U-C-C-U-U-C-U-A-A-Gp, C-C-U-C-U-A-A-Gp, U-U-C-U-A-A-Gp, and U-A-U-A-A-Gp; these were identified by further digestion with ribonuclease A. Because these ribonuclease A digestions were performed with excess enzyme, the position of Up was not established. Arginine tRNA used for this analysis was isolated from bacteriophage strain T4D grown on E. coli strain BN. The sequence was further established by digestion of the oligonucleotide with ribonuclease A following modification with CMCT, which yielded the products A-U-Cp, Cp, U-U-Cp, and A-U-A-Gp; Fig. 4 shows the electrophoretic mobilities of these products.

Sequence Analysis of Products of Ribonuclease A Digestion

The oligonucleotides generated by ribonuclease A digestion are shown in Fig. 5. Table III gives the sequence of each of these oligonucleotides together with their theoretical and experimental molar yields. Alkaline hydrolysis and digestion with ribonucleases T<sub>1</sub> and T<sub>2</sub> established the sequences of all the products.

Limited Digestion to Obtain Overlapping Fragments

Two techniques were used to establish the relative order of ribonuclease T<sub>1</sub> and ribonuclease A fragments in the molecule. Limited digestion of the tRNA with ribonuclease T<sub>1</sub> generated large fragments which were separated in the first dimension on a cellulose/acetate strip and in the second dimension by homochromatography. The products were then analyzed by complete digestion with ribonucleases T<sub>1</sub> and A; these digestion products were subsequently analyzed by ribonucleases T<sub>2</sub> and T<sub>1</sub>, respectively. The tRNA was also subjected to digestion with nuclease S1 (as described by Harada and Dahlberg (17)), which acts specifically at the single-stranded 3' terminus and

---

### Table III

<table>
<thead>
<tr>
<th>Oligonucleotide</th>
<th>Deduced Sequence</th>
<th>Molar Yield</th>
<th>Composition</th>
<th>Other Products of Further Digestion with Ribonuclease T&lt;sub&gt;1&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>Up + Yp</td>
<td>8</td>
<td>10.8</td>
<td>+</td>
</tr>
<tr>
<td>P2</td>
<td>Cp</td>
<td>13</td>
<td>13.7</td>
<td>+</td>
</tr>
<tr>
<td>P3</td>
<td>A-Cp</td>
<td>2</td>
<td>1.8</td>
<td>1.0</td>
</tr>
<tr>
<td>P4</td>
<td>G-Cp</td>
<td>2</td>
<td>1.9</td>
<td>1.0</td>
</tr>
<tr>
<td>P5</td>
<td>A-Up</td>
<td>1</td>
<td>1.8</td>
<td>1.1</td>
</tr>
<tr>
<td>P6</td>
<td>A-G-Cp</td>
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<td>0.8</td>
<td>1.0</td>
</tr>
<tr>
<td>P7</td>
<td>A-A-Up</td>
<td>1</td>
<td>1.0</td>
<td>2.0</td>
</tr>
<tr>
<td>P8</td>
<td>G-Up</td>
<td>1</td>
<td>1.4</td>
<td>1.0</td>
</tr>
<tr>
<td>P9</td>
<td>C-A-Gp</td>
<td>2</td>
<td>2.3</td>
<td>1.1</td>
</tr>
<tr>
<td>P10</td>
<td>A-A-G-up</td>
<td>1</td>
<td>(1)</td>
<td>1.6</td>
</tr>
<tr>
<td>P11</td>
<td>G-G-G-G-Cp</td>
<td>1</td>
<td>0.6</td>
<td>1.0</td>
</tr>
<tr>
<td>P12</td>
<td>G-G-A-Gp</td>
<td>1</td>
<td>*</td>
<td>1.3</td>
</tr>
<tr>
<td>P13a</td>
<td>G-G-G-Up</td>
<td>2</td>
<td>*</td>
<td>3.4</td>
</tr>
<tr>
<td>P13b</td>
<td>G-G-G-Up</td>
<td>1</td>
<td>*</td>
<td>1.7</td>
</tr>
<tr>
<td>P14</td>
<td>pG-Up</td>
<td>1</td>
<td>0.7</td>
<td>1.0</td>
</tr>
<tr>
<td>P15</td>
<td>G-G-G-A-Up</td>
<td>1</td>
<td>0.6</td>
<td>1.0</td>
</tr>
</tbody>
</table>

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sequence determination was complicated by the presence of the Up residues, which are resistant to ribonuclease A cleavage and often show a tendency to undergo apparent nonspecific degradation which obscures electrophoretic resolution of digestion products. These factors consequently affected quantitation of molar yields of secondary digestion products; molar yields of these products were somewhat variable, reflecting heterogeneity with respect to modification of the Up residue. In a single experiment, digestion of the intact oligonucleotide with ribonuclease A gave the products A-Up(1), A-A-Gp(1), Cp(2), Yp(1), and UP(2); although the molar yield of Up was not determined, its presence was inferred from the spectrum of unidentified minor products observed.

Ribonuclease U<sub>2</sub> digestion gave the products Ap, Gp, and C-C-G-U-C-U-A-A-Gp; the sequence of the 3 residues of the latter product was determined by digestion with spleen phosphodiesterase followed by two-dimensional fractionation of the resulting products as described by Seidman et al. (7). Digestion of the intact oligonucleotide with spleen phosphodiesterase followed by two dimensional fractionation of the products (Fig. 3) gave A-U-C-C-U-C-U-A-A-Gp, U-C-C-U-U-C-U-A-A-Gp, C-C-U-C-U-A-A-Gp, C-C-U-C-U-A-A-Gp, U-U-C-U-A-A-Gp, and U-A-U-A-A-Gp; these were identified by further digestion with ribonuclease A. Because these ribonuclease A digestions were performed with excess enzyme, the position of Up was not established. Arginine tRNA used for this analysis was isolated from bacteriophage strain T4D grown on E. coli strain BN. The sequence was further established by digestion of the oligonucleotide with ribonuclease A following modification with CMCT, which yielded the products A-U-Cp, Cp, U-U-Cp, and A-U-A-Gp; Fig. 4 shows the electrophoretic mobilities of these products.
TABLE IV
Products of ribonuclease T1 digestion of arginine tRNA synthesized in vitro (California)

Oligonucleotide numbers refer to those shown in Fig. 2. Nearest neighbors are given in brackets. Oligonucleotides generated by ribonuclease T1 digestion were analyzed by further digestion with ribonuclease A; the resulting labeled products are normalized to 1 mol unlabeled preceded by another integer. The letters NL mean that a product did not become labeled by the indicated radioactive triphosphate.

<table>
<thead>
<tr>
<th>Oligonucleotide</th>
<th>ATP</th>
<th>ATP</th>
<th>CTP</th>
<th>UTP</th>
<th>Reduced sequence</th>
</tr>
</thead>
</table>
| T1              | Gp  | Gp  | Gp  | Gp  | C-C (Gp)+Gp+Gp-
| T2              | NL  | Cp  | Atp | Atp | A-A-C-G-
| T3              | Cp  | Cp  | NL  | NL  | C-C-Gp
| T4              | Gp  | NL  | NL  | Gp  | Gp-
| T6              | Gp  | NL  | NL  | Cp  | C-C-Gp
| T7              | Cp  | Cp  | Cp  | NL  | C-C-C-Gp
| T8              | A-Cp | A-Cp | A-Cp | A-Cp | C-C-C-Gp
| T9              | A-Cp | A-Cp | A-Cp | A-Cp | C-C-C-Gp
| T10             | A-C-Gp | A-Gp | A-Gp | A-Gp | C-C-C-Gp
| T11             | Up | Up | Up | Up | C-G-Gp
| T12             | Cp  | Cp  | Up  | Up  | C-G-Gp
| T14             | Up  | NL  | Gp  | Gp  | Gp-

Fig. 6 (top). Nucleotide sequence of arginine tRNA showing the products obtained by limited digestion with ribonuclease T1 and with nuclease S1. The solid brackets at the bottom indicate the large oligonucleotides found after partial digestion of the tRNA with ribonuclease T1. The half-molecule size oligonucleotide at the extreme bottom was generated by digestion with nuclease S1; the ellipses marks at the left end of this fragment indicate that the oligonucleotide extends for a short, unknown distance beyond the region indicated by the solid bracket. The broken brackets surrounding the sequence indicate the small fragments obtained by complete digestion with ribonuclease T1 (top) and ribonuclease A (bottom). Although residues 11 to 22 from the 5' end of the molecule are not contained within any of the limited digestion products, the sequence of this region is uniquely defined by ribonuclease T1 and A products. The ribonuclease T1 product T15 contained the modified nucleotides collectively referred to as Up; although the location of Up in T15 was not determined by sequence analysis, it was provisionally placed such that its position corresponds to the first position of the anticodon by analogy with other known tRNA sequences (20). P-labeled arginine tRNA used in these experiments was isolated from bacteriophage strain T4D grown on Escherichia coli strain BN, except for the S1 analysis which was performed on arginine tRNA from a T4 strain carrying a mutation in serine, grown on E. coli strain B/5.

Fig. 7 (bottom). Comparison of partial products obtained from overlaps of ribonuclease T1 and ribonuclease A products generated after P-labeling in vivo (top) and in vitro (bottom). Data obtained from nearest neighbor analysis clearly generates more partial sequences than that obtained from in vivo analysis which depends primarily upon overlap dictated by the presence of minor nucleotides in ribonuclease T1 and A digestion products. Thus, although nearest neighbor analysis alone (in the absence of limited digestion to obtain overlapping fragments) cannot be used to uniquely determine the nucleotide sequence of the tRNA, the partial products derived from nearest neighbor data and the constraints on the sequence imposed by the cloverleaf arrangement of the molecule allow prediction of the sequence. For the position of Up, see the legend to Fig. 6.
The nucleotide sequence of tRNA^Arg synthesized by bacteriophage T4 is arranged in the cloverleaf pattern in Fig. 8. Its identity as an arginine tRNA is inferred from its anticodon, U-C-U; if the uridine in the first position of the anticodon recognizes both guanosine and adenosine in the third position of the codon, this tRNA can presumably recognize two arginine-specific codons, A-G-A and A-G-G. This assumption is consistent with the results of Scherberg and Weiss (3) who showed that binding of T4 arginyl-tRNA to ribosomes was stimulated by the trinucleotides A-G-A and A-G-G, but not by C-G-U and C-G-A, the codons most commonly recognized by Escherichia coli arginyl-tRNA. Although it is unlikely that the serine-specific codons, A-G-U and A-G-C, are also recognized by tRNA^Arg, this possibility cannot be eliminated until codon-binding studies with the purified tRNA have been performed.

This T4 tRNA^Arg possesses two unusual features discernible upon examination of the cloverleaf arrangement of the molecule. The Gp-Up base-pair in the anticodon stem is unique in that psu,+ op is an inefficient suppressor, a fact which may reflect a detrimental effect of the tRNA (21). Also in the recent isolation of a T4 U-G-A suppressor associated with tRNA^Arg, designated psu,+op, provides the opportunity to study the function of this tRNA (22).

**DISCUSSION**

The nucleotide sequence of tRNA^Arg synthesized by bacteriophage T4 is arranged in the cloverleaf pattern in Fig. 8. Its identity as an arginine tRNA is inferred from its anticodon, U-C-U; if the uridine in the first position of the anticodon recognizes both guanosine and adenosine in the third position of the codon, this tRNA can presumably recognize two arginine-specific codons, A-G-A and A-G-G. This assumption is consistent with the results of Scherberg and Weiss (3) who showed that binding of T4 arginyl-tRNA to ribosomes was stimulated by the trinucleotides A-G-A and A-G-G, but not by C-G-U and C-G-A, the codons most commonly recognized by Escherichia coli arginyl-tRNA. Although it is unlikely that the serine-specific codons, A-G-U and A-G-C, are also recognized by tRNA^Arg, this possibility cannot be eliminated until codon-binding studies with the purified tRNA have been performed.

This T4 tRNA^Arg possesses two unusual features discernible upon examination of the cloverleaf arrangement of the molecule. The Gp-Up base-pair in the anticodon stem is unique in that a Gp-Up pairing has never before been observed at this position in any sequenced tRNA molecule (21). Also in the recent isolation of a T4 U-G-A suppressor associated with tRNA^Arg, designated psu,+op, provides the opportunity to study the function of this tRNA (22). One observation which has emerged from such studies is that psu,+op is an inefficient suppressor, a fact which may reflect a detrimental effect of

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**Table V**

Some products of ribonuclease A digestion of tRNA^Arg synthesized in vitro (California)

| Oligonucleotide numbers refer to those shown in Fig. 3. Nearest neighbors are given in brackets (see Table IV). Ribonuclease A digestion of tRNA labeled in vitro generates a heterogeneous spectrum of small products, which complicates sequence analysis. Therefore, analysis of the smaller digestion products was not performed, although all digestion products derived from tRNA^Arg were present. The sequences of products used to deduce the final structure are shown in Fig. 6.

**Sequence Analysis of tRNA^Arg Synthesized in Vitro**

Fig. 2C is a ribonuclease T1 oligonucleotide map of the tRNA^Arg synthesized in vitro with an input label of [α-32P]GTP. The RNA is pure and its fingerprint pattern is the same as that for RNA synthesized in vivo (Fig. 2B). The nucleotide sequences of all the digestion products of ribonuclease T1, except for T15, can be deduced from the data shown in Table IV. Larger products of digestion with ribonuclease A are shown in Table V; because digestion of RNA synthesized in vitro generates a heterogeneous spectrum of small products, which complicates sequence analysis, analysis of these products was not performed. However, all products generated by digestion of in vitro synthesized RNA were also present in the digest of RNA synthesized in vitro.

Although analysis of partial digestion products of the RNA synthesized in vitro was not performed, the sequence could be predicted, though not uniquely determined, from the nearest neighbor data which provide strong constraints on the final sequence (Fig. 7).

A comparison of the two procedures used to determine the nucleotide sequence of this RNA reveals the power of the in vitro labeling technique in the analysis of nucleotide sequences. In the in vitro system, the use of each of the four labeled triphosphate substrates separately makes it possible to obtain valuable nearest neighbor sequence information which simplifies the problem of sequencing oligonucleotide products. As demonstrated in Fig. 7, the nearest neighbor data also generate a set of uniquely defined partial products which cannot be derived from simple overlaps of ribonuclease A and T1 products of digestion of RNA synthesized in vitro. In the absence of a partial digest of the RNA, the nearest

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**Fig. 8.** Nucleotide sequence of arginine tRNA arranged in the cloverleaf structure. For the position of Up, see the legend to Fig. 6.
these unusual structural features upon the function of tRNAArg.

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