Halobacterium volcanii tRNAs
IDENTIFICATION OF 41 tRNAs COVERING ALL AMINO ACIDS, AND THE SEQUENCES OF 33 CLASS I tRNAs

Ramesh Gupta
From the Department of Genetics and Development, University of Illinois, Urbana, Illinois 61801

Transfer RNAs of Halobacterium volcanii, an archaebacterium, were separated by two-dimensional gel electrophoresis and sequenced by a combination of methods. A total of 41 tRNAs, at least one for each amino acid, were identified. These are five tRNAs for Leu, four for Gly, three each for Ala, Arg, Pro, and Ser, two each for Glu, Ile, Lys, Met (initiator and non-initiator), Thr, and Val, and one each for the remaining eight amino acids. In eucaryotes, only Leu and Ser tRNAs are class II (large extra arm). The sequences of the 33 class I tRNAs, for the remaining 18 amino acids, are presented here. These cover at least 44 codons out of a possible 49 codons for the 18 amino acids. Although these archaebacterial tRNAs follow general tRNA patterns, they are in detail distinct from both eucaryotic and eubacterial tRNAs. Moreover, the initiator tRNA is unique in having a 5'-triphosphorylated end.

Archaeabacteria were first recognized as a primary kingdom, distinct from both eubacteria and eucaryotes, in 1977 (1). Since then, numerous molecular characteristics have established the uniqueness of archaeabacteria as a separate phylogenetic category. The archaeabacteria are now known to include methanogens, the extreme halophiles, and thermococci (2).

Before the start of the present work, there were a few indications that tRNAs of archaeabacteria are, to some extent, distinct from eubacterial tRNAs. Initiation of protein synthesis in Halobacterium cutirubrum was known to be by a Met-tRNA^Met, not by fMet-tRNA^Met (3). The 5' base of H. cutirubrum tRNA^Met pairs with the fifth base from the 3' end (3). Both of these characters are eucaryotic and not eubacterial. The modification pattern of archaeabacterial tRNAs is somewhat distinct from that in both eubacteria and eucaryotes (4–5). Most notably, the nearly universal sequence TWC, characteristic of almost all eubacterial and eucaryotic tRNAs, is differently modified in archaeabacteria which have no T (1–2, 4–6).

Sequences of nearly complete sets of tRNAs of eubacteria and eucaryotes (Escherichia coli, yeasts, and mammals) are now available (7). To make a genuine comparison among tRNAs of the three kingdoms, sequences of a set of tRNAs from at least one of the archaeobacterial representatives is needed. Among archaeabacterial tRNAs, sequences for tRNA^Met of Thermoplasma acidophilum (8), initiators of T. acidophilum, Halococcus morrhuae, and Sulfolobus acidocaldarius (9), and tRNA^Ala and tRNA^Asp of H. cutirubrum (10) have so far been reported. The present paper reports sequences of an extensive set of tRNAs from one archaeobacterium, the extreme halophile, Halobacterium volcanii.

The transfer RNAs of H. volcanii have been separated by two-dimensional gels and their sequences determined by a combination of methods. A total of 41 tRNAs, at least one for each of the 20 amino acids, have been identified. The sequences for 33 of these, which are specific for a total of 18 amino acids (all being class I tRNAs), are presented here. In some respects, these sequences are similar to tRNAs of eubacteria, in others to those of eucaryotes, while in yet other ways they are quite distinct. This supports the original contention that archaeabacteria are a unique grouping, no more related to eubacteria than to eucaryotes.

MATERIALS AND METHODS AND RESULTS AND DISCUSSION

Autoradiographs of representative gels showing two-dimensional separation of H. volcanii tRNAs are presented in Fig. 1. Fig. 2 is a drawing, indicating the approximate positions of

1 Portions of this paper (including "Materials and Methods," parts of "Results and Discussion," Fig. 5, and additional references) are presented in miniprint at the end of this paper. Miniprint is easily read with the aid of a standard magnifying glass. Full size photocopies are available from the Journal of Biological Chemistry, 9650 Rockville Pike, Bethesda, MD 20814. Request Document No. 84 M-546, cite the authors, and include a check or money order for $5.60 per set of photocopies. Full size photocopies are also included in the microfilm edition of the Journal that is available from Waverly Press. Reproductive fingerprint and gel sequencing data have been deposited in the J. Biol. Chem. Repository for scientific data. They are available as JBC Document Number 84 M-546 B, in the form of 1 microfiche(s) or 4 pages. Orders for supplementary material should specify the title, author(s) and reference to this paper and the JBC Document number, the form desired (microfiche or full size photocopy) and the number of copies desired. Orders should be addressed to The Journal of Biological Chemistry, 9650 Rockville Pike, Bethesda, MD 20814 and must be accompanied by remittance to the order of the Journal in the amount of $2.50 for microfiche or $1.60 per set of full size photocopies. The complete document is included in the microfilm edition of the Journal that is available from Waverly Press.
the various tRNAs in two-dimensional gels. There are 40 to 50 distinct spots in such patterns; however, they vary considerably in intensity. Some tRNAs always occur as two spots, the difference between them being only a single modified residue. The proportional amounts of tRNAs for Glu and Asp (and their amides) are exceptionally high. This might be expected, as the proteins of extreme halophiles are relatively acidic (3, 11).

The set of tRNAs for an individual amino acid were 3′-end labeled (following protection from periodate oxidation by aminoacylation) and separated by two-dimensional gels. Representative gels of some of these reactions are presented in Fig. 3. Sequence determination of all of these 3′-end labeled tRNAs (except one, discussed later) showed anticodon sequences which agreed with the normal codon usage for the corresponding amino acid. Two spots for the exact same tRNA were sometimes seen in these gels.

The sequences of the tRNAs are presented in standard "cloverleaf" form in Fig. 4. In that the nucleosides T and D are absent in *H. volcanii* and (most) other archaeabacteria (5), the names "common arm" and "GG arm" (an invariant GG doublet occurs at positions 18 and 19) will be used here instead of "TW arm" and "D arm."

### tRNAs for Various Amino Acids

**Alanine tRNAs**—Three Ala tRNAs have been sequenced. They in principle cover all Ala codons. The three are very similar in sequence. tRNA\(^{\text{AA}}\) is a minor species, whose corresponding gene (minus the 3′-CCA) has been found in a cloned segment of *H. volcanii* DNA, located in the spacer region between the 16 and 23 S rRNA genes\(^1\) (12). The reported sequence of tRNA\(^{\text{AA}}\) for *H. cutirubrum* (10) has the same anticodon as tRNA\(^{\text{AA}}\) of *H. volcanii* and differs from it only at 4 positions.

**Arginine tRNAs**—Three Arg tRNAs were located and sequenced. Of these, tRNA\(^{\text{R}}\) was the largest in amount and tRNA\(^{\text{AN}}\), the least. These three tRNAs can cover all Arg codons except the AGR codons. (The fact that *Halobacterium halobium* bacteriorhodopsin gene does not contain AGR codons (13) suggests that the missing tRNAs may occur in trace amounts.) In all two-dimensional gels of uniformly labeled tRNAs, tRNA\(^{\text{AN}}\) appeared as two spots (spots 5 and 5A, Fig. 2), one having W\(_{\text{AS}}\), the other U\(_{\text{AS}}\). In the two-dimensional gel pattern of tRNAs 3′-end labeled after aminoacylation by arginine (see Fig. 3), both tRNA\(^{\text{AK}}\) and tRNA\(^{\text{AN}}\) show double spots. Strangely, no sequence differences were detected between the two spots in either case. W\(_{\text{AS}}\) was present in both spots of tRNA\(^{\text{AK}}\), indicating this one to be the functional form. tRNA\(^{\text{AN}}\) shows hardly any sequence similarity to the other two Arg tRNAs, although the latter two are similar to each other. However, tRNA\(^{\text{AN}}\) bears a striking resemblance to tRNA\(^{\text{AN}}\); they are identical in the GG arm and are very similar elsewhere except for the acceptor stem.

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\(^3\) R. Gupta and C. R. Woese, manuscript in preparation.
Fig. 3. Autoradiographs of two-dimensional polyacrylamide gel separation of 3'-end labeled *H. volcanii* tRNAs, specific for a single amino acid. Electrophoresis in first dimension (right to left); second dimension (top to bottom). A, aminoacylation by methionine; i, initiator tRNA, ni, tRNA^Met^ (noninitiator). B, composite gel, showing aminoacylation by arginine (left half) and a control where no external amino acid was added (right half). 1, tRNA^Met^; 2, tRNA^Met^; 3, tRNA^Met^.

Fig. 4. Sequences of 33 class I tRNAs of *H. volcanii*, covering 18 amino acids, are presented in standard cloverleaf form. The Cold Spring Harbor numbering system (7) is used. Dotted lines indicate alternate pairing possibilities. Residues in parenthesis are the alternate ones occurring at that position, and are indicated only if these are invariably observed in a tRNA, either as a mixture in one spot or in distinct spots.
Asparagine tRNA—Although no Asn tRNA was detected by aminoacylation experiments, a tRNA (from uniformly and 3'-end labeled total tRNA gels) with the correct anticodon to be $tRNA^{Asn}$ was sequenced. The failure to charge this tRNA (with any amino acid) could be explained in any of several ways.

Aspartic Acid tRNA—The single $tRNA^{Asp}$ found always occurred as two spots in uniformly labeled tRNA gels (spots 10 and 10A, Fig. 2), one showing $m_1\Psi_{34}$, the other $\Psi_{34}$. Which form is functional could not be determined, since neither modification ($\Psi$ or $m_1\Psi$) shows a band in sequencing gels.

Cysteine tRNA—Only one $tRNA^{Cys}$ was observed. It is unique in having six bases in the extra arm and rare in having only two pairs in the GG stem (7).

Glutamic Acid tRNAs—Two Glu isoacceptors were identified. Their sequences are nearly identical in all regions except the anticodon arm.

Glutamine tRNA—The sequence of one tRNA, recovered from both uniformly and 3'-end labeled total tRNA gels, showed the Gln anticodon, but no such tRNA was detected by aminoacylation with Gln. However, the tRNA in question can be charged by Glu. It is reported for $H$. cuttirubrum (14) that $tRNA^{Gln}$ is aminoacylated by Glu and amidation of Glu-$tRNA^{Gln}$ produces Gln-$tRNA^{Gln}$. This would appear to be the case for $H$. volcanii as well. The same phenomenon has been observed in some Gram-positive eubacteria (15, 16).

Glycine tRNAs—Four Gly tRNAs were observed, which cover all Gly codons. Two of these ($tRNA^{Glu}$ and $tRNA^{Gln}$) occur in low amounts. The two major species ($tRNA^{Glu}$ and $tRNA^{Gln}$) are not separated even by “third-dimension” gels. They are identical except that two pairs in $tRNA^{Glu}$, $U_C2$-$A_U2$, are reversed to $U_C6$-$G_A6$, in $tRNA^{Gln}$. (This degeneracy is seen in sequencing gels by the presence of the bands in corresponding spots and in oligonucleotide catalogs by the occurrence of the sequences UCCCAGCCA and UCCCAGCGCA, and also GCGCACAAG and CGCACCAAG (RNase U₇ digestion) as well as the occurrence of CCG, ACG, and CCAG (RNase T₇, digestion) in less than unit molar amounts.) Their failure to separate seems due to the fact that the reciprocal changes occur in the middle of a stem (acceptor). All four Gly tRNAs possess related sequences.

Histidine tRNA—Only one $tRNA^{His}$ was detected. As noted above, its sequence is close to that of $tRNA^{His}$. Like all other His tRNAs sequenced (7), this one has one extra base before position 1 and so, as the corresponding eubacterial tRNAs, can form eight Watson-Crick pairs in the acceptor stem.

Isoleucine tRNAs—Two different $tRNA^{Ile}$ were observed, one of which occurs in trace amounts. These can cover all three Ile codons. The sequences of these two tRNAs show some similarity in all arms. The invariant A at position 21 is replaced by U in $tRNA^{Ile}$ (only); this can pair with A₁₁, allowing an extension of the GG stem.

Leucine tRNAs—A total of five Leu tRNAs have been
archaebacterial initiators to the identified H. cutirubrum initiator. Like all normal initiators, it can initiate synthesis of the protein in vitro. Methionine tRNAs—Only one Met tRNA was detected in H. volcanii. Proline tRNAs—Three Pro tRNAs were observed, tRNAPro being a minor species. Together they can cover all Pro codons. All have related sequences. The tRNAPro spot in gels is always large and diffuse, which could reflect undermodification of several residues. tRNAPro is unique in having $A_{52}$ instead of $Y_{52}$. Serine tRNAs—Three Ser tRNAs have been clearly identified so far (Fig. 2). All of them belong to class II. Their sequences will be presented elsewhere. A sequencing gel provided some sequence information for the existence of a fourth Ser tRNA, occurring in trace amounts. Its sequence has not yet been completed.

Thrreonine tRNAs—Two Thr tRNAs have been located and fully sequenced; the two, though related, are not especially close. They cover all Thr codons, except ACA. A third Thr tRNA occurs as trace contaminant in another tRNA spot, so
its complete sequence is very difficult to determine (and has not been).

Tryptophan tRNA—One Trp tRNA has been identified. It contains eleven modified residues. So far this tRNA is unique among those reported in having Um28 (7).

Tyrosine tRNA—A single tRNA Tyr was detected. It belongs to class I (having a short extra arm), which is the case for eucaryotic Tyr tRNAs also.

Valine tRNAs—Two Val tRNAs having anticodons GAC and CAC were observed in the gels. They cover all Val codons except 9 positions (exclusive of the modifications and anticodon sequence).

Two Val tRNAs have been reported for *H. cutirubrum* (10). They have the same two anticodons (GAC and CAC) as do the present Val tRNAs. Strangely, the two *H. cutirubrum* examples do not differ from each other except for a single (wobble) position in the anticodon. In basic sequence, *H. volcanii* tRNAVal and tRNAVal differ from this common *H. cutirubrum* tRNAVal sequence at 4 and 9 positions, respectively. Of these, there are only two positions where both of the *H. volcanii* tRNAs differ from the *H. cutirubrum* common sequence. The modification m4C is observed at position 48 in *H. volcanii* Val tRNAs, but reported at position 51 in their *H. cutirubrum* counterparts. None of the 41 *H. volcanii* tRNAs so far sequenced show a modification at position 9, but one *H. cutirubrum* tRNAVal is reported to be modified at this position. It is unusual that there are so many differences between analogous tRNAs of two closely related species, and that in one of the species, the sequences of the isoacceptors are extremely conserved, while in the other, they have diverged.

General Features of *H. volcanii* tRNAs and Their Comparison with Other tRNAs

The *H. volcanii* initiator resembles most closely other archaebacterial initiators (9). All archaebacterial initiators resemble their eucaryotic counterparts in having a terminal, A1-U20, pair (eubacteria have a noncanonical C1-A20 pair) and the base A20 (Dzo in eubacteria). However, they resemble eubacterial initiators in having an unmodified A37, some form of U at position 54, and U30 (eucaryotic initiators have t6A37, A54, and A60). Archaebacterial initiators are unique in having a G11-C24 pair (C11-G23 in eucaryotes, A11-U20 in eubacteria). Other than the initiators, tRNAPhe has also been sequenced in many organisms (7). All eubacterial tRNAPhe have D20 and U45, while the eucaryotic versions have G20 and G46. *H. volcanii* tRNAPhe resembles the latter in having G20, but the former in having U45.

As mentioned above, in *H. volcanii*, as in eubacteria, the residue before position 1 in tRNAVal pairs with the residue at position 73 (eucaryotes have a G-A juxtaposition instead). On the other hand, *H. volcanii* tRNAVal, as in eucaryotes, has a short extra arm (eubacteria have a large arm). In general,
Trp tRNAs show many modifications, more so in the eukaryotes; this is also the case in *H. volcanii*. (Interestingly, for a stretch of 15 residues in the anticodon arm (positions 28 through 42), the sequence and its modifications for *H. volcanii* tRNA*<sub>Trp</sub>* are identical to that of Trp tRNAs of chicken cells and bovine liver (7), with the exception that the *H. volcanii* tRNA has Um<sub>39</sub> while the other two have m<sub>2</sub>G<sub>39</sub>.)

Archaebacterial tRNAs have certain characteristics so far not reported for other tRNAs. All have Cm rather than C at position 56 (2, 6–7, 9). The occurrences of m<sub>1</sub>I at position 57 (18) and m<sub>2</sub>G at position 10 are also unique to archaebacteria. The modifications m<sub>1</sub>Ψ, which occurs at position 54 (19), and possibly X (a probable G derivative), observed at position 15, are also archaebacteria specific. Only archaebacteria modify the residue at position 15.

A preliminary characterization of the modification patterns in tRNAs of ten archaebacterial species, including *H. volcanii* (5), showed that the nucleosides T and m<sub>7</sub>G are absent from...
all, and D is absent from all but one. It was also reported (5) that the archaeabacterial tRNAs contain a number of methylated residues previously considered to be characteristic of eucaryotic tRNAs.

Cys tRNA and the two Glu tRNAs in H. volcanii have 11 residues in the loop of the GG arm; the α and β variable regions of this loop have their maximum sizes, 5 and 4 residues, respectively. In other organisms, a maximum of 10 residues has been reported in this loop.

The semi-invariant Y1-R4 pair is replaced by an R11-Y24 pair in nine H. volcanii tRNAs (seven are G-C and two A-U). Six of those with a G11-C24 pair (Arg, Gin, His, Met, Pro, and Phe) tRNAs show a stronger alternate pairing to the standard form in the stem of the GG arm, i.e. if G11 pairs with Y24 instead of C24, and so on (shown as dotted lines in the sequences of these tRNAs in Fig. 4). The same is the case with the H. morrhuae initiator (9).

The anticodon stem (five pairs) of eubacterial and eucaryotic tRNAs can occasionally be extended by an additional pair on one or both sides. In H. volcanii tRNAs, such extensions are frequent. Of the 33 tRNAs reported here, 12 have Watson-Crick pairs at position 26-44 and 9 have them at position 12-23. The occurrence of these pairs in nine positions in H. volcanii tRNAs is remarkably high when compared to other tRNAs.

The most frequent sequences in the common arm loop of H. volcanii tRNAs are m^ΨV^ΨCmm^IAAU and m^ΨV^ΨCm-GAAU. m^Ψ occupies the same position in these tRNAs as T does in most other tRNAs. These two nucleosides have similar pairing properties and orientation of methyl groups relative to ribose and the polynucleotide chain, which may indicate an evolutionary convergence of structures at position 54 (20).

A more detailed comparative analysis of archaeabacterial tRNA sequences (among themselves and with those of eubacteria and eucaryotes) will be published later.

The sequences of H. volcanii tRNAs presented here indicate that, of the possible 49 codons for the 18 amino acids (that have class I tRNAs), at least 44 are covered. The codons not covered are two AGR (Arg), GU(A) Val, CAA (Gln), and ACA (Thr) (but the last one would probably be covered by the unsequenced Thr tRNA mentioned above). The tRNAs for the remaining four codons, if present, probably occur in trace amounts.

CONCLUSION

H. volcanii tRNAs fit the generalized tRNA secondary structure derived from eubacterial and eucaryotic sequences. As discussed elsewhere (20), they also contain the equivalent, tertiary base pairs suggested by Kim (21). These indicate that the basic reactions in which tRNA is involved are essentially similar in all three kingdoms. In spite of this similarity in basic organization of tRNAs in the three kingdoms, the H. volcanii tRNAs are as different in specific details from the tRNAs of the other two groups as those tRNAs are from one another. At present it is difficult to say whether all specific characteristics of H. volcanii tRNAs are general archaeabacterial features or whether some are confined to the extreme halophiles.

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Additional references on p. 9471.
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MATERIALS AND METHODS
Analyses and Chemicals
Phosphate-free Dowex 1, Nae A, spore phosphodiesterase, snake venom phosphodiesterase, and bacterial alkaline phosphatase were purchased from Bio-Rad; and nucleotides and dialyzed water from Sigma.

Tryptic digests of tRNAs were performed in the presence of 100 mm NaCl, 10 mm Tris-Cl (pH 7.5), 0.1 mm MgCl2, 0.1 mm Na3PO4, 0.1 mm EDTA, pH 8.0, and commercial lysine deaminase. The enzyme was incubated for 2 h at 37°C. In a separate experiment, a similar digestion was carried out with RNase T1, T2, and A (Sigma) in the presence of 10 mm Tris-Cl, 1 mm EDTA, pH 8.0. The mixture was then heat-denatured at 95°C for 5 min and allowed to cool. The tRNAs were then precipitated, and the supernatant was analyzed by gel electrophoresis.

The RNA was isolated by the phenol extraction and ethanol precipitation method. The RNA was then treated with RNase A (1000 units/nl) and DNase I (1000 units/nl), and precipitated with ethanol. The RNA was then dissolved in a small volume of water and electrophoresed on a 1% agarose gel.

The RNA was then electrophoretically transferred to Whatman 3MM paper and stained with ethidium bromide. The RNA was then extracted with phenol:chloroform:isoamyl alcohol (25:24:1) and precipitated with ethanol.

The RNA was then electrophoresed on a 5% polyacrylamide gel in 1X TBE buffer and stained with ethidium bromide. The RNA was then extracted with phenol:chloroform:isoamyl alcohol (25:24:1) and precipitated with ethanol.

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Halobacterium volcanii tRNAs

RESULTS AND DISCUSSIONS

Aminoacylation of tRNAs

Aminoacylation of tRNAs with 14C-labelled amino acids, phenylalanine, and proline indicated that the general conditions of aminoacylation of E. coli tRNAs are suitable for H. volcanii, even though the two species require quite different concentrations of salts for optimal growth.

The absence of appreciable amounts of containing tRNAs in aminoacyl-tRNA synthetase preparations was determined by setting up two sets of three different aminoacylation reactions each (using the above mentioned three 14C-amino acids) with no external RNA added, but with H. volcanii in one set. There was no significant difference observed for the preparations of comparable reactions of the two sets (data not presented), indicating that the tRNAs of H. volcanii, whose three-dimensional structures are distantly related to those of E. coli on

isoelectric focusing (three tRNAs or from published studies (18)). Fig. 5 shows the mobilities in the two TLC systems of all H. volcanii produced nucleotides and dinucleotides encountered in sequences of H. volcanii tRNAs.

Detection and Characterization of Modified Nucleotides

H. volcanii tRNAs of individual uniformly 14C-labelled tRNAs were resolved by two-dimensional TLC in two systems. Different tRNAs differed only in the solvent used for the second dimension. In general, nucleotides completely resolved in one system, separate in the other system. Identification of modified nucleotides in oligonucleotides was done by TLC on "multidimensional plates" after appropriate digestion. The modified nucleotides were identified by retention characteristics and by chroomotographic cochromatographies (elution of total RNA or from published studies (18)). Fig. 5 shows the mobilities in the two TLC systems of all H. volcanii produced nucleotides (and dinucleotides) encountered in sequences of H. volcanii tRNAs.

Preparation of Aminoacyl-tRNA Synthetase and Aminoacylation Reaction

The procedure for preparing crude aminoacyl-tRNA synthetases and the conditions for aminoacyl-tRNA formation were the same as used for E. coli (134). A typical aminoacylation reaction mixture contained E. coli RNA polymerase, 10 nM Tris-Cl, pH 7.5, 1 mM MgCl2, 50 mM sodium acetate, pH 4.5, 10 mM ATP, 100 mM KCl, 1 mM MnCl2, 200 nM 14C-amino acid (total about 0.5 μCi), 14C-tyrosyl-tRNA synthetase, and the mixture was incubated at 37°C for 20 min. The reaction mixture was then incubated at 0°C for 30 min. The reaction was terminated by adding in order: 2 ml of ice cold solution containing 100-fold excess of cold amino acid corresponding to the 14C-amino acid used, 0.1 μl of 1 M guanidine hydrochloride, and 100 ml of 4 M ice cold trichloroacetic acid. It was filtered through a glass fiber filter (grade 934, Moore Angell). The filter was washed, dried, and counted by standard procedures. The following enzyme of H. volcanii tRNA synthetases, indicating that different nucleotide residues are present at position 34 (residue 34).

The nucleic acid to A was different in the two systems, and the nucleic acid to B was different in the two systems.

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General Sequencing Procedures

Individual uniformly $^{32}$P-labelled tRNAs eluted from gels were used to generate $\text{RNAse T}_1$, $\text{A}$, and sometimes $\text{U}_2$ (termed glyoxal blocked C residues) catalogs, as well as the two-dimensional TLC patterns. These $\text{RNAse T}_2$ patterns contain numerous partial digestion products and so are not completely reproducible; these, however, provide useful additional information. In nearly all cases, a tRNA sequence can be unambiguously deduced from these three catalogs, and in all such cases the catalog sequence was confirmed by information obtained from sequencing gels.

The individual 3'-end labelled tRNAs used for sequencing gels were isolated from two-dimensional gels of either total 3'-end labelled tRNA or selected 3'-end labelled tRNAs, which had been protected from periodate oxidation by aminoacylation. The entire sequence of D. tRNA could not be determined from sequencing gels alone, as these gels showed several artifacts. Bands in the G lanes were light or absent if G occurred in a double stranded region or was modified. The 5'-stem region of the common and anticodon arms showed serious band compressions, double bands, and other apurinic bands, especially if adjacent G-C pairs are present in the stem. Occasionally, inversions of bands for positions near the 5'-end of a stem are seen. Identification of modified nucleosides in these gels is difficult. $\text{T}$, $\text{A}$, $\text{U}$, $\text{G}$, and $\text{C}$ show gaps in all lanes. While $\text{G}$ is indistinguishable from C (in the C lane), so is $\text{A}$ from $\text{G}$. As mentioned before, in sequencing gels, distorted positions of bands are seen for the residues near X. However, the information obtained from sequencing gels, in conjunction with just the $\text{RNAse T}_2$ and A catalogs is almost always sufficient to produce a unique sequence.

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