TRANSFER RNA: Structure, Properties, and Recognition

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Edited by

Paul R. Schimmel
Massachusetts Institute of Technology

Dieter Söll
Yale University

John N. Abelson
University of California, San Diego

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Preface

During recent years, tRNA has been the subject of intense investigation for several reasons. First, it plays a central role in protein synthesis and it is a major challenge to sort out the complexities and diversity of interactions it undergoes in this process. Second, it is a relatively small, single-stranded RNA (approximate m.w. of 25,000) that can be analyzed by structural methods, including sequencing and X-ray diffraction, that are often not feasible with larger, more complex RNA molecules. In this regard, it is hoped that analysis of the structural features of tRNA will be helpful in gaining insight into the structural organization of other RNA molecules, such as rRNAs and mRNAs. Third, there are a number of reactions in which tRNAs are specifically recognized by proteins so that, with a well-characterized nucleic acid structure available, these systems are excellent for exploring the molecular basis of specific protein–nucleic acid interactions. Fourth, it is now well established that tRNAs play a role in the regulation of gene expression and in a number of other cellular processes, an observation that points to the striking versatility of the molecule. And finally, because they are relatively well characterized and because of their obvious biological importance, tRNAs have become popular for studies of gene organization, cloning, and biosynthesis.

In this volume, we treat tRNA structure, properties, and recognition. There are six sections that cover these subjects: primary structure and sequencing of tRNAs, crystal structure analysis of tRNAs, tRNA structure and dynamics in solution, aminoacyl-tRNA synthetases, recognition of tRNAs by proteins, and tRNA interactions with ribosomes. These areas are the foundations of the tRNA field. For example, our knowledge of primary structures of tRNAs, and of the crystal structure of a particular tRNA, has provided a concrete framework within which we may rationalize

experimental data and pose new questions. This structural information has also enabled us to understand in considerable depth, particularly through the use of nuclear magnetic resonance, the behavior of tRNA in solution and to design experiments that explore subtle conformational rearrangements that may have physiological consequences. These studies of isolated tRNA molecules are, of course, a prerequisite for approaching the more difficult questions of how tRNAs react with proteins and participate in protein synthesis. Here we consider the aminoacyl-tRNA synthetases, which are the most prominent class of enzymes that utilize tRNA as a substrate; the recognition of tRNAs by specific, well-defined proteins; and the interactions of tRNAs with ribosomes.

The purpose of the volume is to summarize major facts and concepts, rather than to communicate the latest preliminary, unpublished observations. With this in mind, a large number of summary figures and tables are included in some of the articles. Also, many of the articles have included extensive bibliographies that are invaluable reference sources. In addition, a special feature of this volume are the four appendices that give the primary structures of over 100 tRNAs, structural information on modified nucleotides found in tRNAs, chromatographic mobilities of modified nucleotides, and a tabulation of the characteristics of aminoacyl-tRNA synthetases. These appendices, and many of the articles, are intended to be used as a primary reference or handbook for the fundamental aspects of tRNA.

Transfer RNA: Biological Aspects, the second portion of the Transfer RNA set, summarizes current knowledge in the areas of tRNA biosynthesis, organization of tRNA genes, suppression and coding, and involvement of tRNA in regulatory processes. The volume concisely summarizes and organizes this material to give an overall perspective of ongoing research and to set the stage for future investigations.

The chapters for both texts were solicited by the editors from individuals who delivered invited lectures at the August 1978 Cold Spring Harbor Laboratory meeting on tRNA. Many of the chapters have been updated to early 1979. We wish to thank our colleagues for their help and suggestions.

In the organization of the meeting, we were helped enormously by Gladys Kist and Winifred Modzeleski of the Cold Spring Harbor Laboratory Meetings Office. This volume was made possible only through the conscientious and skillful editorial help of Mary-Teresa Halpin, Annette Zaninovic, and Nancy Ford, Director of Publications. We are also indebted to Jim Watson for his enthusiastic advice and counsel.

Paul R. Schimmel
Dieter Söll
John N. Abelson

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Primary Structures and Sequencing of tRNAs

Recent Developments in tRNA Sequencing Methods as Applied to Analyses of Mitochondrial tRNAs

Uttam L. RajBhandary, Joyce E. Heckman, Samuel Yin, and Birgit Alzner-DeWeerd
Department of Biology
Massachusetts Institute of Technology
Cambridge, Massachusetts 02139

Eric Ackerman
Department of Biochemistry and Biophysics
University of Chicago
Chicago, Illinois 60637

With the extensive literature that has already accumulated on tRNAs, it may not be evident that this is a relatively new area of research. Discovered only about 22 years ago (Hoagland et al. 1957), following the prediction of the existence of molecules with similar properties by Crick, the first sequence of a tRNA and indeed of any nucleic acid was published only in 1965 (Holley et al. 1965). Since then the sequence of at least 120 different tRNAs from a variety of biological sources have been established (Sprinzl et al. 1978). The two factors that have contributed most to this rapid progress in tRNA sequencing have been the development (1) of column chromatographic and gel electrophoretic methods (Gillam et al. 1967; Cherayil and Bock 1965; Pearson et al. 1971; Ikemura and Dahlberg 1973) suitable for purification of tRNAs and (2) of rapid sequencing methods requiring only very small amounts of tRNAs. Thus, whereas the first sequence analysis of a tRNA, which utilized spectrophotometric procedures for identification of nucleotides (Holley 1968), required several hundred milligrams to a gram of purified tRNA and several years of effort, methods currently available enable one to sequence a tRNA with just a few micrograms (2–10 μg) within a few weeks.

The first few tRNAs to be sequenced were all from yeast (Holley et al. 1965; Zachau et al. 1966; Madison et al. 1966; RajBhandary et al. 1967b; Baev et al. 1967). To a large extent this was a reflection of yeast being a relatively inexpensive and convenient source for the large amounts of tRNAs necessary then for sequencing. With the development of the two-dimensional electrophoretic method for the rapid separation of oligonucleotides and a rapid method for sequencing them (Sanger et al. 1965), tRNA sequence analysis could be carried out on material uni-

formly labeled with ^{32}P and most of the early applications of this method were in fact on *Escherichia coli* tRNAs that could be easily labeled in vivo with ^{32}P (Goodman et al. 1968). More recent developments have focused on methods involving the use of in vitro ^{32}P labeling for sequencing tRNAs that are available only in small quantity and cannot be labeled efficiently in vivo (Silberklang et al. 1979). This paper describes the basic principles behind such a method (details are available in a review to be published soon by Silberklang et al. [1979] and are not described here) and discusses some surprising features in the four *Neurospora crassa* mitochondrial tRNAs (mttRNAs) that have been sequenced using this method. Randerath et al. (this volume) describe a method utilizing ^{3}H labeling for the analysis and identification of modified nucleosides and for tRNA sequencing. This latter method is based on the specific labeling of 2′,3′-diol end groups in nucleosides, oligonucleotides, and RNAs (RajBhandary et al. 1967a; RajBhandary 1968) by treatment of the RNA with sodium periodate followed by reduction with [^{3}H]borohydride.

With the availability of so many tRNA sequences it is now possible to compare these sequences and to look for the overall structural features and sequences that have been conserved in tRNAs. This had led to the finding that the cloverleaf structure originally proposed by Holley et al. (1965) as one of the possible secondary structures for tRNA is a feature common to all tRNAs sequenced to date (Rich and RajBhandary 1976). In addition, tRNAs contain several "invariant" and "semiinvariant" residues located in the same relative position in all tRNAs. Largely through the work of Nishimura and coworkers (see Nishimura 1978), a substantial number of modified nucleosides present in tRNA have now been characterized. A summary of the overall general features of tRNA structures, including a list of the modified nucleosides, their occurrence in various tRNAs, and their location at specific sites within tRNAs, is provided by Dirheimer et al. (this volume). These workers also describe their recent work on yeast mttRNAs. Nishimura (this volume) describes recent results on some modified nucleosides, their characterization, and their biosynthesis.

USE OF IN VITRO ^{32}P LABELING FOR SEQUENCING tRNAs

The basic procedure used in our laboratory for the sequence analysis of a nonradioactive tRNA using in vitro ^{32}P labeling is summarized in Table 1 and consists of four steps. These are:

1. Analysis of modified nucleotide content of the tRNA.
2. Analysis of oligonucleotides present in complete T1 and pancreatic RNase digests.

Table 1 Basic steps in the sequence analysis of nonradioactive tRNA using in vitro ^{32}P labeling

Analysis of modified nucleotide content
1. Complete digestion of tRNA with T2 RNase to Np.
2. 5′-^{32}P labeling of Np to yield ^{32}pNp.
3. Conversion of ^{32}pNp to ^{32}pN using the 3′-phosphatase activity present in nuclease P1.
4. Identification of ^{32}pN by two-dimensional thin-layer chromatography.

Analysis of oligonucleotides present in T1 or pancreatic RNase digests
1. Complete digestion of tRNA with T1 or pancreatic RNase.
2. Removal of 3′-terminal phosphomonoester group by treatment with alkaline phosphatase.
3. 5′-^{32}P labeling of oligonucleotides.
4. Separation of 5′-^{32}P-labeled oligonucleotides by two-dimensional electrophoresis.
5. Sequencing of 5′-^{32}P-labeled oligonucleotides:
   a. 5′-end group analysis.
   b. Partial digestion with snake venom phosphodiesterase and/or nuclease P1 and analysis by two-dimensional homochromatography.

Alignment of these oligonucleotides (above) by isolation and sequencing of large oligonucleotide fragments
1. Controlled digestion with T1 or pancreatic RNase or specific chemical cleavage.
2. 5′-^{32}P labeling of the large fragments.
3. Separation of the 5′-^{32}P-labeled large fragments by two-dimensional gel electrophoresis and their sequencing as below.

Direct sequence analysis of ^{32}P end-labeled tRNAs or large fragments
1. End group labeling of tRNAs:
   a. 5′ end using polynucleotide kinase and [γ-^{32}P]ATP.
   b. 3′ end using tRNA nucleotidyl transferase and [α-^{32}P]ATP.
2. Partial digestion of end-labeled tRNAs with nuclease P1 and analysis by two-dimensional homochromatography.
3. Partial digestion with specific enzymes and analysis by polyacrylamide gel electrophoresis.

3. Alignment of oligonucleotides present in these complete digests into a unique sequence by isolation and sequencing of large oligonucleotide fragments.
4. Direct sequence analysis of 5′- or 3′-end-labeled [^{32}P]tRNAs.

In most instances, steps 1, 2, and 4 provide the information necessary for the derivation of the total sequence. In such cases, step 3, which requires relatively larger amounts (50 μg or so) of tRNAs, is dispensed with.

Szekely and Sanger (1969) demonstrated that oligonucleotides with a free 5′-OH end that are present in digests of nonradioactive nucleic acids could be labeled to high specific activity using T4 polynucleotide kinase and [γ-^{32}P]ATP. With certain modifications and improvements, we have used this approach for labeling with ^{32}P the 5′ ends of both mononucleotides present in complete tRNA hydrolysates (Silberklang et al. 1977b) and oligonucleotides present in T1 or pancreatic RNase digests of tRNA (Simsek et al. 1973). The 5′-^{32}P-labeled mononucleotides (see Table 1) are identified by two-dimensional thin-layer chromatography and can, therefore, be used for analysis of the modified nucleotide content of a tRNA or of an oligonucleotide (Silberklang et al. 1979). The 5′-^{32}P-labeled oligonucleotides are separated by fingerprinting (Fig. 1) (Sanger et al. 1965). The labeled oligonucleotides are then recovered and partially digested with snake venom phosphodiesterase and/or nuclease P1, a relatively nonspecific endonuclease from *Penicillium citrinum*. These partial digestion products are separated by two-dimensional homochromatography (Sanger et al. 1973) and the sequence of the oligonucleotide in question is deduced (Fig. 2) from the characteristic mobility shifts resulting from the successive removal of nucleotides from the 3′ end (Gillum et al. 1975; Silberklang et al. 1977b). All the oligonucleotides present in complete T1 RNase and pancreatic RNase digests of an RNA can, therefore, be sequenced. Using oligonucleotides present in tRNAs of known sequence as

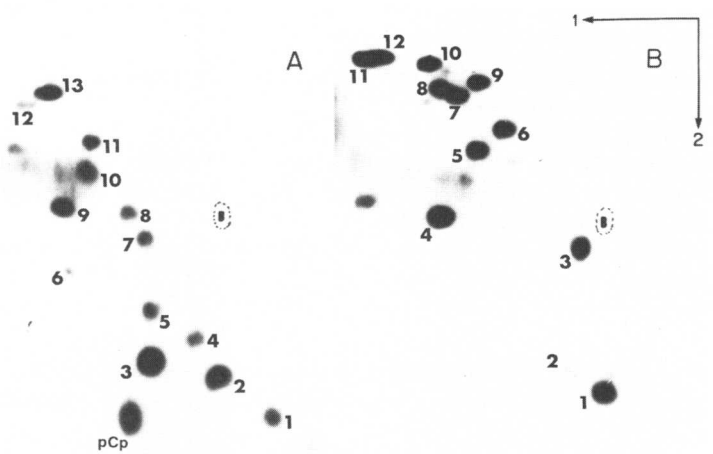

Figure 1 Fingerprints of 5′-^{32}P-labeled oligonucleotides obtained from pancreatic RNase digestion (*A*) and T1 RNase digestion (*B*) of *N. crassa* mttRNA^{fMet}. B. circled is blue dye marker. (Reprinted, with permission, from Heckman et al. 1978.)

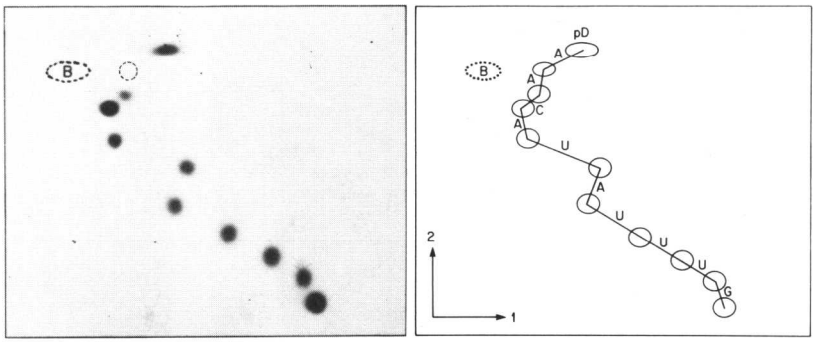

Figure 2 Autoradiograph of partial snake venom phosphodiesterase digest on spot 12 of Fig. 1B as analyzed by two-dimensional homochromatography. First dimension, electrophoresis on cellulose acetate at pH 3.5; second dimension, homochromatography. B circled is blue dye marker.

standards, we have also characterized the mobility shifts of many commonly occurring modified nucleotides, which has proven useful in recognizing these modified nucleotides within unknown sequences (Silberklang et al. 1979).

To order the shorter oligonucleotides present in complete T1 or pancreatic RNase digests into a total tRNA sequence, larger (overlapping) oligonucleotide fragments may be obtained by specific chemical cleavage of the tRNA or by limited digestion with base-specific nucleases. The use of in vitro ^{32}P labeling allows sequence analysis of such fragments on a picomole scale. In addition, using [5′-^{32}P]tRNA or [3′-^{32}P]tRNA (Silberklang et al. 1977a), we have used two methods for direct sequence analysis of large nucleotide stretches at either end of the molecule. One method involves partial digestion of [5′-^{32}P]tRNA or [3′-^{32}P]tRNA with nuclease P1, followed by two-dimensional homochromatographic separation and mobility shift analysis of the digestion products (Fig. 3) (Silberklang et al. 1977a). This method allows one to derive the sequence of 15–25 nucleotides directly from either end of a tRNA. Figure 3 shows an example of this method as applied to the 5′-terminal sequence analysis of *N. crassa* initiator mitochondrial formylmethionine tRNA (mttRNA^{fMet}).

The other method, similar in principle to the Maxam and Gilbert (1977) method for DNA sequencing, involves partial digestion of the end-labeled tRNA with base-specific nucleases followed by mapping of the base-specific cleavage sites by polyacrylamide gel electrophoresis (Donis-Keller et al. 1977; Simoncsits et al. 1977; Lockard et al. 1978). Figures 4 and 5 provide examples of this procedure as applied to 5′-^{32}P-labeled *N. crassa* mttRNA^{fMet} and mttRNA^{Tyr}, respectively. These last two methods for direct