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The Additional Guanylate at the 5′ Terminus of *Escherichia coli* $tRNA^{His}$ Is the Result of Unusual Processing by RNase P

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In eucaryotes the 5′-terminal guanylate moiety of mature $tRNA^{His}$ is added posttranscriptionally. To determine whether the same mechanism occurs in procaryotes, we processed in vitro-derived *Escherichia coli* $tRNA^{His}$ precursors to mature tRNA, either in *E. coli* extracts or by using pure M1-RNA, the catalytic component of RNase P. The results show that the extra guanylate at the 5′ end of mature *E. coli* $tRNA^{His}$ is encoded in the gene and is found in tRNA as the result of an unusual cleavage by RNase P.

tRNAs from procaryotic origins are transcribed as precursors containing 5′- and 3′-flanking sequences. The additional sequences are removed in a series of endo- and exonucleolytic steps to yield mature tRNAs (4, 5, 8). The 5′ leader sequences of precursor tRNAs are removed in a single endonucleolytic cleavage by RNase P to generate the 5′ termini of mature tRNAs (2). The enzyme shows great specificity in recognizing the correct site for cleavage in its different substrates, which do not show nucleotide sequence homology. Presumably, the enzyme recognizes a common tertiary structure in the precursor tRNA molecules (3).

All $tRNA^{His}$s sequenced to date contain an extra nucleotide at the 5′ end, as compared with other species of tRNA (19). The extra guanylate at the 5′ end of *Drosophila melanogaster* $tRNA^{His}$ and *Schizosaccharomyces pombe* $tRNA^{His}$ has been shown to result from the posttranscriptional addition of that nucleotide by a specific tRNA guanylyl transferase (7; L. Cooley, O. Orellana, and D. Söll, submitted for publication). This addition occurs after cleavage of the 5′-flanking sequence of the precursor by an RNase P-like activity (7). As in *D. melanogaster* and *S. pombe*, the $tRNA^{His}$ genes of mice, humans, and *S. cerevisiae* do not encode the extra guanylate (20).

We wanted to know whether a mechanism involving posttranscriptional addition is also responsible for the formation of the 5′ end of procaryotic $tRNA^{His}$. As the gene sequence shows (13), the extra guanylate present at the 5′ end of *E. coli* $tRNA^{His}$ is potentially encoded in the gene. We have determined the origin of the extra guanylate in this tRNA by in vitro processing experiments with precursor $tRNA^{His}$ obtained by in vitro transcription of a cloned $tRNA^{His}$ gene (13). We show that the presence of the extra guanylate results from an unusual cleavage of precursor $tRNA^{His}$ by *E. coli* RNase P. Our data suggest that the tertiary structure of precursor $tRNA^{His}$ allows the enzyme to cleave at this particular site.

MATERIALS AND METHODS

General materials and methods. Enzymes and chemicals were obtained from commercial sources and used as specified by the suppliers. DNA was prepared by standard methods. The DNA sequence was determined by the method of Maxam and Gilbert (14). M1-RNA was a kind gift of S. Altman (10, 11).

Construction of ptHis. An *E. coli* $tRNA^{His}$ gene is found in a tetrameric tRNA gene operon contained on a 1.9-kilobase *Bam*HI-*Hin*dIII DNA fragment in plasmid pLC25-25 (13). This $tRNA^{His}$ gene was subcloned as a 168-base-pair *Hha*I fragment (see Fig. 1), after the addition of *Eco*RI linkers, into the *Eco*RI site of the pBR322-derived plasmid pJG-7 (kindly donated by J. Galloway) between the *E. coli trp* promoter and the spot 42 [*rho*] independent terminator. This new plasmid was designated ptHis.

In vitro transcription of the cloned $tRNA^{His}$ gene and processing of RNA transcripts. In vitro transcription of ptHis by *E. coli* RNA polymerase was not very efficient and yielded a mixture of differently sized fragments. However, since HeLa cell RNA polymerase III recognized well the internal transcription control regions in this *E. coli* gene, ptHis DNA was transcribed in a HeLa cell extract as described earlier (9). Transcription products were resolved by electrophoresis on polyacrylamide gels (17). Processing of the RNA transcripts was performed at 37°C in a reaction mixture containing 30 mM *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES)–KOH (pH 8.0), 3 mM dithiothreitol, 8 mM creatine phosphate, 100 mM KCl, 3 mM $MgCl_2$, 1 mM ATP, 0.02 mM GTP, and *E. coli* cell extract (S-100) in a final volume of 40 μl. M1-RNA-directed cleavage of RNAs was carried out for 30 min in 20 μl of a buffer containing 50 mM Tris hydrochloride (pH 7.6), 100 mM NH_4Cl, 100 mM $MgCl_2$, and M1-RNA (0.84 or 3.36 μg) at 37°C (10).

RNA analysis. 5′-End analysis of the eluted RNAs was carried out by digestion with RNase T_2 and RNase A. The hydrolysates were analyzed by polyethyleneimine-cellulose (PEI-cellulose) thin-layer chromatography. For pNp the solvent was 1 M ammonium formate (pH 3.5) (16), and for pppNp 0.75 M potassium phosphate (pH 3.5) was used. Two-dimensional oligonucleotide mapping of RNAs was carried out by standard methods (23). Analysis of pGp was performed by digestion of the nucleotide with nuclease P1 and product separation by PEI-cellulose chromatography with 0.75 M potassium phosphate (pH 3.5) as the solvent.

RESULTS

In vitro transcription of the $tRNA^{His}$ gene. An *E. coli* $tRNA^{His}$ gene has been characterized as part of a tetrameric tRNA gene operon (13). This gene (Fig. 1) was subcloned

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                -40       -30       -20       -10       1
5'-ATTCCCTTGAGCTGCGGTGGTAGTAATACCGCGTAACAAGATTTGTAGTGGTGGCTA

 10        20        30        40        50        60
TAGCTCAGTTGGTAGAGCCCTGGATTGTGATTCCAGTTGTCGTGGGTTCGAATCCCATTA

 70        80        90       100       110       120
GCCACCCCATTATTAGAAGTTGTGACAATGCGAAGGTGGCGGAATTGGTAGACGGGA-3'
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FIG. 1. DNA sequence of the noncoding strand of the tDNA insert in ptHis. The mature coding region is shown in boldface type. The numeral 1 indicates the position of the 5′ end of all nonhistidine tRNAs (according to the standard numbering system in reference 17).

and transcribed as described in Materials and Methods. Transcription of ptHis in HeLa cell extracts yielded three major RNA bands which could be resolved by polyacrylamide gel electrophoresis (Fig. 2A): RNA-1, about 450 bases long, and RNA-2 and RNA-2′, 85 and 84 bases long, respectively. 5′-Terminal analysis of the transcripts by standard methods (7) showed that transcription initiated equally well with A at position −4 and with G at position −3 in the DNA sequence (see arrows in Fig. 1). The sizes of the RNAs are consistent with these initiation sites and with the presence of two potential RNA polymerase III termination signals (18) in the DNA sequence, a TTATT sequence (underlined in Fig. 1) adjacent to the 3′ end of the mature coding sequence (giving rise to RNA-2 and RNA-2′) and a TTTTT sequence 375 base pairs downstream of the gene (giving rise to RNA-1).

Processing of precursor tRNA^{His} in an *E. coli* cell extract. The different primary transcripts obtained were tested for their ability to be substrates for the *E. coli* processing enzymes. RNA-1 (Fig. 2A) was purified and incubated with an *E. coli* S-100 preparation as the source of processing enzymes under the conditions described in Materials and Methods. The processing products obtained were analyzed by polyacrylamide gel electrophoresis (Fig. 2B, lane 1). A mixture of RNA-2 and RNA-2′ was subjected to the same processing reaction, and the products obtained are shown in Fig. 2B, lane 2. Identical processing products designated RNA a, RNA b, RNA c, and RNA d (Fig. 2B) were obtained from each source of precursor tRNA^{His}. The two predominant products were RNA a and RNA c. When RNA a was reincubated under the same conditions, it was converted completely to RNA c (data not shown). Base analysis of RNase T_2 hydrolysates and fingerprint analysis were performed on all RNA species. These showed that RNA a is a partially processed intermediate that contains the 5′ leader sequence (indicated by the finding of pppAp and pppGp in the spot at the origin of Fig. 3A, lane 1; see legend to Fig. 3) but lacks some 3′-terminal sequence. RNA c was identified as mature tRNA^{His}; its G-labeled RNase A fingerprint (Fig. 3B) showed that pGpGpUp is the 5′-terminal oligonucleotide. RNA b is processed at its 5′ end and contains a 5′-terminal pGp (Fig. 3A, lane 2). The trace amount of RNA d (Fig. 2B, lane 2) contains part of the tRNA^{His} sequence but is probably a degradation product since it did not accumulate after reprocessing of RNA a (data not shown). These results indicate that most of 3′-trailer sequences are processed before 5′ endonucleolytic cleavage in precursor tRNA molecules.

The pG contained at the 5′ end of RNA c represents the extra guanylate of tRNA^{His} (see spot marked in Fig. 3B). The origin of this extra nucleotide was characterized by determining the position of the labeled phosphate(s) in pGp formed after digestion of RNA c with a mixture of RNase T_2 and RNase A. The finding that both phosphates in pGp were labeled (pG and P_i; Fig. 4) indicates that the extra guanylate was present in the primary transcript and is contained in mature tRNA as the result of unusual processing of the precursor at the 5′ end.

Processing of precursor tRNA^{His} by pure *E. coli* RNase P. To confirm with pure RNase P the results of precursor tRNA^{His} cleavage obtained with the *E. coli* cell extract, we incubated the different primary transcripts in the presence of M1-RNA, the catalytic component of RNase P (10). As an example, the processing of RNA-2 is shown in Fig. 5A. RNA-2 was converted to a smaller-sized RNA by this treatment. The 5′-terminal nucleotide of either [α-^{32}P]GTP- or [α-^{32}P]UTP-labeled RNA was analyzed after RNase T_2 digestion by PEI-cellulose chromatography (Fig. 5B). ^{32}P-labeled pGp was found in G-labeled RNAs but not in U-labeled transcripts. This result shows that M1-RNA cleaves precursor tRNA^{His} at the phosphodiester bond just

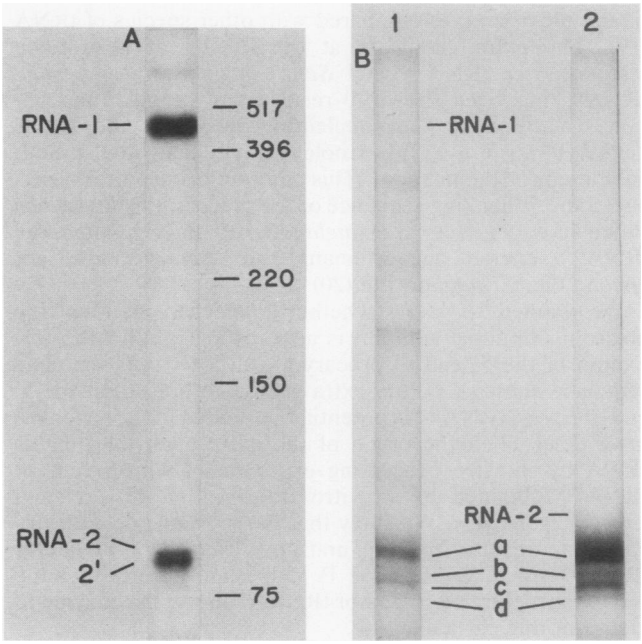

FIG. 2. In vitro transcription of the tRNA^{His} gene and processing of the primary transcripts. (A) Autoradiogram of polyacrylamide gel electrophoresis of RNAs transcribed from plasmid ptHis in a HeLa cell extract. RNA-1, RNA-2, and RNA-2′ were the primary transcripts obtained. The chain lengths of single-stranded DNA size markers are indicated. (B) Processing of the transcripts in an *E. coli* cell extract. Shown in an autoradiogram of the separation by polyacrylamide gel electrophoresis of products (a, b, c, and d) obtained from RNA-1 (lane 1) and a mixture of RNA-2 and RNA-2′ (lane 2). The migration positions of the primary transcripts are indicated.

upstream of the extra guanylate of mature tRNA (position −1 in Fig. 1), in agreement with the results of 5′ processing of the primary transcript by *E. coli* S-100. Since pure M1-RNA was used, the resulting RNA still contained the 3′-trailer sequence of RNA-2.

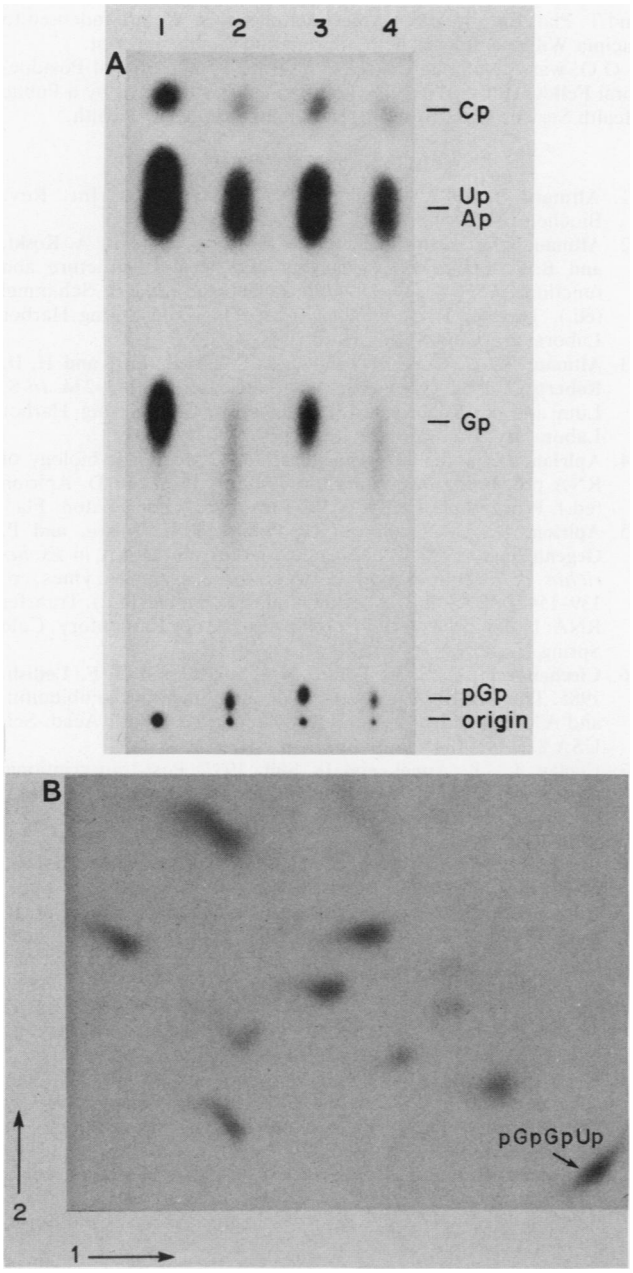

FIG. 3. Analysis of processed products. (A) Autoradiogram of the products obtained after RNase T_2 digestion of RNA a (lane 1), RNA b (lane 2), RNA c (lane 3), and RNA d (lane 4) and resolved by PEI-cellulose thin-layer chromatography. [α-^{32}P]GTP was used as the source of radiolabel. Further thin-layer chromatography of the spot at the origin revealed equal mixtures of pppAp and pppGp in lane 1. The reason for the slight difference in mobility of the pGp spot in lane 3 is not known. (B) RNase A fingerprint of RNA c. Shown is an autoradiogram of the two-dimensional chromatographic resolution of [α-^{32}P]GTP-labeled oligonucleotides. The 5′-terminal oligonucleotide pGpGpUp is indicated.

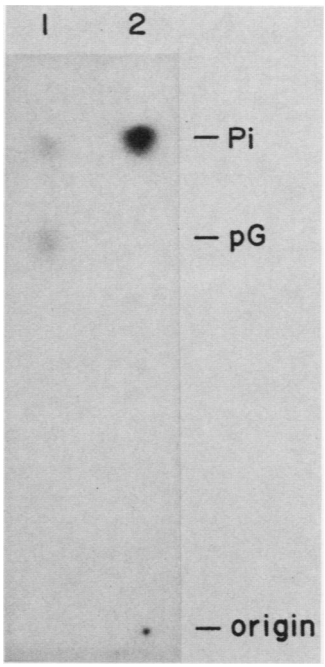

FIG. 4. Characterization of the 5′-terminal nucleotide of RNA c. Shown is an autoradiogram of nuclease P1 digestion products of purified pGp (lane 1) separated by PEI-cellulose thin-layer chromatography. The $^{32}P_i$ marker is shown in lane 2.

DISCUSSION

Encoded terminal guanylate of *E. coli* tRNA^{His}. The work reported here shows that the 5′-terminal guanylate of *E. coli* tRNA^{His} is present in the primary transcript and is maintained in mature tRNA through an "inaccurate" cleavage event by RNase P. We do not think that this unusual cleavage results from the fact that the precursor was derived by transcription with a eucaryotic polymerase, since the *E. coli* cell extracts processed tRNA^{His} primary transcripts, which vary significantly in the lengths of their 3′-trailer sequences, to mature tRNA.

Structural considerations for RNase P cleavage. Processing by RNase P has been described as a highly specific reaction (3). No special sequence requirement in the 5′-flanking regions of tRNA genes has been found for correct precursor tRNA cleavage by RNase P. Several precursor tRNA^{Asp}s with 5′ leaders of various lengths, obtained from a thermosensitive RNase P mutant, are correctly processed in vitro by RNase P (1). *E. coli* tRNA^{His} has the potential to form a base pair between the extra guanylate and the C at position 73. Such a base pair could conceivably interfere with RNase P processing. However, in the case of precursor tRNA^{Tyr}, the U at position −1 can pair with the A at position 73, but the RNA is processed normally (i.e., there is no additional U at the 5′ end of this tRNA^{Tyr} [1]). In contrast, bacteriophage T5 tRNA^{His} does possess an extra U at its 5′ end which can base pair with the A at position 73 (19); it is likely that this mature tRNA is also a product of inaccurate RNase P cleavage. We think that the tertiary structure of *E. coli* precursor tRNA^{His}, and not a specific flanking sequence, is important for the altered cleavage specificity of RNase P. M1-RNA cleaves *D. melanogaster* precursor tRNA^{His} at the normal site (position 1 of mature tRNA, data not shown). The sequences of *E. coli* tRNA^{His} and *D. melanogaster*

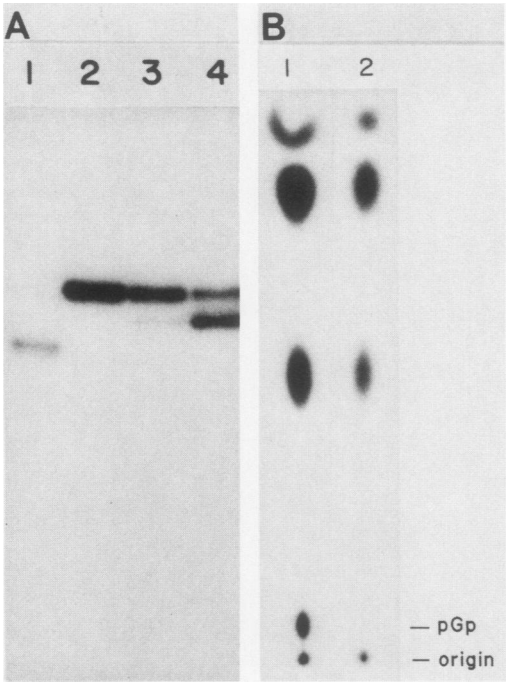

FIG. 5. Processing of primary transcripts by M1-RNA and 5′-terminal analysis of the products. (A) Autoradiogram of the processing products obtained from RNA-2 incubated in the absence (lane 2) or in the presence of 0.84 μg (lane 3) or 3.36 μg (lane 4) of M1-RNA in a final volume of 20 μl. Reaction conditions were as described in Materials and Methods. The $tRNA^{His}$ size marker is shown in lane 1. (B) Autoradiogram of RNase T_2 digests of the M1-RNA-cleaved RNA products resolved by PEI-cellulose thin-layer chromatography. Lanes: 1, RNA labeled with [α-^{32}P]GTP; 2, RNA labeled with [α-^{32}P]UTP.

$tRNA^{His}$ show very little homology. Thus, studies with mutationally altered precursors $tRNA^{His}$ may reveal new aspects of RNA recognition by RNase P.

Are all procaryotic $tRNA^{His}$s processed in the same fashion? All known procaryotic $tRNA^{His}$ sequences (derived from RNA or gene data), from *E. coli*, *Salmonella typhimurium*, and *Bacillus subtilis* (20), have the guanylate potentially encoded. In addition, there is a C at position 73 in these tRNAs. This base may very well be paired to the extra guanylate in the precursor structure. This feature may reflect a special property of precursors $tRNA^{His}$ that allows RNase P to cleave at the unusual site.

Significance of the terminal guanylate in $tRNA^{His}$. It is interesting that two different mechanisms are used by *E. coli* and by eucaryotic cells to maintain the extra base at the 5′ ends of their respective $tRNA^{His}$s. The maintenance of the extra guanylate in both procaryotic and eucaryotic organisms suggests that the structure at the 5′ end is important for an unknown function of $tRNA^{His}$. Although it has not been tested, there is little reason to believe that this additional guanylate is necessary for aminoacylation or protein synthesis. For instance, tRNA-like structures such as the 3′-terminal part of some viral RNAs can be aminoacylated (12). tRNAs have been implicated in regulatory roles. The *divE* mutation, a mutation affecting cell division, has been localized in a $tRNA^{Ser}$ gene (21). Similarly, the *dnaY* mutation has been found in a $tRNA^{Arg}$ gene (15). $tRNA^{His}$ is known to participate in the regulation of the *E. coli his* operon (reviewed in reference 22). $tRNA^{His}$ may also be involved in the regulation of mammalian proteolysis, as it is an essential component of the ubiquitin- and ATP-dependent proteolytic system (6). It is possible that the additional guanylate at the 5′ terminus of $tRNA^{His}$ is crucial for these or other as-yet-unknown roles of $tRNA^{His}$ in cell metabolism.

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LITERATURE CITED

1. **Altman, S.** 1982. Transfer RNA biosynthesis. Int. Rev. Biochem. **17**:19–44.
2. **Altman, S., E. J. Bowman, R. L. Garber, R. Kole, R. A. Koski, and B. C. Stark.** 1980. Aspects of RNase P structure and function, p. 71–82. *In* D. Söll, J. Abelson, and P. Schimmel (ed.), Transfer RNA: biological aspects. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
3. **Altman, S., C. Guerrier-Takada, H. M. Frankfort, and H. D. Robertson.** 1982. RNA-processing nucleases, p. 243–274. *In* S. Linn and R. Roberts (ed.), Nucleases. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
4. **Apirion, D., and P. Gegenheimer.** 1984. Molecular biology of RNA processing in prokaryotic cells, p. 35–62. *In* D. Apirion (ed.), Processing of RNA. CRC Press, Inc., Boca Raton, Fla.
5. **Apirion, D., B. K. Ghora, G. Plantz, T. K. Misra, and P. Gegenheimer.** 1980. Processing of rRNA and tRNA in *Escherichia coli*: cooperation between processing enzymes, p. 139–154. *In* D. Söll, J. Abelson, and P. Schimmel (ed.), Transfer RNA: biological aspects. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
6. **Ciechanover, A., S. L. Wolin, J. A. Steitz, and H. F. Lodish.** 1985. Transfer RNA is an essential component of the ubiquitin- and ATP-dependent proteolytic system. Proc. Natl. Acad. Sci. USA **82**:1341–1345.
7. **Cooley, L., B. Appel, and D. Söll.** 1982. Post-transcriptional nucleotide addition is responsible for the formation of the 5′ terminus of histidine tRNA. Proc. Natl. Acad. Sci. USA **79**:6475–6479.
8. **Deutscher, M. P.** 1984. Processing of tRNA in prokaryotes and eukaryotes. Crit. Rev. Biochem. **17**:45–71.
9. **Dingermann, T., S. Sharp, B. Appel, D. DeFranco, S. Mount, H. Reinhard, O. Pongs, and D. Söll.** 1981. Transcription of cloned tRNA and 5SRNA genes in a *Drosophila* cell free extract. Nucleic Acids Res. **9**:3907–3918.
10. **Guerrier-Takada, C., K. Gardiner, T. Marsh, N. Pace, and S. Altman.** 1983. The RNA moiety of ribonuclease P is the catalytic subunit of the enzyme. Cell **35**:849–857.
11. **Guerrier-Takada, C., W. H. McClain, and S. Altman.** 1984. Cleavage of tRNA precursors by the RNA subunit of *E. coli* ribonuclease P (M1 RNA) is influenced by 3′-proximal CCA in the substrates. Cell **38**:219–224.
12. **Haenni, A.-L., and F. Chapeville.** 1980. tRNA-like structures in viral RNA genomes, p. 539–556. *In* D. Söll, J. Abelson, and P. Schimmel (ed.), Transfer RNA: biological aspects. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
13. **Hsu, L. M., H. J. Klee, J. Zagorski, and M. J. Fournier.** 1984. Structure of an *Escherichia coli* tRNA operon containing linked genes for arginine, histidine, leucine, and proline tRNAs. J. Bacteriol. **158**:934–942.
14. **Maxam, A. M., and W. Gilbert.** 1980. Sequencing end-labeled DNA with base-specific chemical cleavages. Methods Enzymol. **65**:499–560.
15. **Mullin, D. A., G. M. Garcia, and J. R. Walker.** 1984. An *E. coli* DNA fragment 118 base pairs in length provides dnaY$^+$ complementing activity. Cell **37**:669–674.
16. **Randerath, K., R. C. Gupta, and E. Randerath.** 1980. ^{3}H and ^{32}P derivative methods for base composition and sequence analysis

of RNA. Methods Enzymol. **65**:638–680.
17. **Sanger, F., and A. R. Coulson.** 1978. The use of thin acrylamide gels for DNA sequencing. FEBS Lett. **87**:107–110.
18. **Schaack, J., S. Sharp, T. Dingermann, D. J. Burke, L. Cooley, and D. Söll.** 1984. The extent of a eukaryotic tRNA gene: 5′ and 3′-flanking sequence dependence for transcription and stable complex formation. J. Biol. Chem. **259**:1461–1467.
19. **Sprinzl, M., J. Moll, F. Meissner, and T. Hartmann.** 1985. Compilation of tRNA sequences. Nucleic Acids Res. **13**:r1–r49.
20. **Sprinzl, M., T. Vorderwülbecke, and T. Hartmann.** 1985. Compilation of sequences of tRNA genes. Nucleic Acids Res. **13**:r51–r104.
21. **Tamura, F., S. Nishimura, and M. Ohki.** 1984. The *E. coli divE* mutation, which differentially inhibits synthesis of certain proteins, is in tRNA^{Ser1}. EMBO J. **3**:1103–1107.
22. **Umbarger, H. E.** 1980. Comments on the role of aminoacyl tRNA synthetases in the regulation of amino acid biosynthesis, p. 453–467. *In* D. Söll, J. Abelson, and P. Schimmel (ed.), Transfer RNA: biological aspects. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
23. **Volckaert, G., W. MinJou, and W. Fiers.** 1976. Analysis of ^{32}P-labeled bacteriophage MS2 RNA by a mini-fingerprint procedure. Anal. Biochem. **72**:433–446.