*Proc. Natl. Acad. Sci. USA*
Vol. 79, pp. 6475–6479, November 1982
Biochemistry

# Post-transcriptional nucleotide addition is responsible for the formation of the 5′ terminus of histidine tRNA

(nucleotide addition/tRNA modification/*in vitro* transcription/tRNA gene sequence)

LYNN COOLEY, BERND APPEL†, AND DIETER SÖLL

Department of Molecular Biophysics and Biochemistry, Yale University, P.O. Box 6666, New Haven, Connecticut 06511

*Communicated by Joseph S. Fruton, July 30, 1982*

**ABSTRACT** **All sequenced histidine tRNAs have one additional nucleotide at the 5′ end when compared to other tRNA species. Sequence analysis of histidine tRNA genes from *Drosophila melanogaster* and *Schizosaccharomyces pombe* showed that the terminal guanylate residue of the mature tRNAs is not encoded by the genes. Analysis of the products from *in vitro* transcription of these genes in extracts from *Drosophila* Kc cells demonstrated that the 5′-terminal nucleotide present in the mature tRNA is added post-transcriptionally. The addition reaction requires ATP. A portion of the mature tRNAs are then modified at the 5′-terminal pG. Analysis of the RNA species formed during the *in vitro* maturation of the *Drosophila* histidine tRNA primary transcript uncovered the following maturation scheme: (*i*) the primary transcript is processed by RNase P at the 5′ end to form an intermediate precursor; (*ii*) the 3′-flanking sequence is endonucleolytically removed, and a guanylate moiety is added to the 5′ end to form mature-sized histidine tRNA; and (*iii*) a fraction of the 5′-terminal guanylate residues then undergoes modification. In contrast to the capping of eukaryotic mRNA, the guanylate addition to histidine tRNA results in the formation of a (3′-5′)-phosphodiester bond. There are no precedents for the post-transcriptional addition of nucleotides (in phosphodiester linkage) to the 5′ end of RNA precursors.**

Eukaryotic tRNAs are formed from precursor molecules that contain a 5′-leader sequence, a 3′-flanking sequence and, in some cases, an intervening sequence. The maturation of precursor RNAs is a multistep enzymatic process (1, 2). The 5′-leader sequence is cleaved accurately by an endonuclease activity similar to RNase P at a position seven bases upstream of the constant uridine residue (position 8 of the mature tRNA) (3, 4). After removal of the 3′-flanking nucleotides, the C-C-A terminus of mature tRNA is added, and the intervening sequence, if present, is excised (5, 6). Nucleotide modification occurs at various stages during the maturation process (7).

All histidine tRNAs of known sequence are one nucleotide longer at the 5′ end than are other tRNA species. Histidine tRNAs that have been sequenced include species from prokaryotic (8), eukaryotic (9, 10), and mitochondrial (11, 12) origin. It was not previously clear whether the additional nucleotide at the 5′ end is formed by "aberrant" RNase P processing of the tRNA precursor or by an unknown maturation reaction.

In this report we show that a novel post-transcriptional nucleotide addition is responsible for the formation of the 5′ terminus of histidine tRNAs. We have investigated the maturation *in vitro* of eukaryotic histidine tRNA, making use of cloned *Drosophila melanogaster* and *Schizosaccharomyces pombe* histidine tRNA genes. We discovered that the 5′-terminal nucleotide of histidine tRNA from *D. melanogaster* and *S. pombe* is not encoded in the respective genes but is added post-transcriptionally.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

## MATERIALS AND METHODS

**General.** Enzymes were obtained from commercial sources and used as described by the suppliers. [$\gamma$-$^{32}$P]ATP was prepared as described (13). Guanosine, guanosine 5′-[$\alpha,\beta$-methylene]triphosphate (pp[$CH_2$]pG), cGMP, and GTP were obtained commercially.

**Plasmid DNA.** A 1.1-kilobase DNA fragment (*Bam*HI/*Hin*dIII) was isolated from the *D. melanogaster* DNA insert contained in plasmid p38B10 (14). This fragment hybridized to *Drosophila* histidine tRNA and was cloned into pBR322, forming plasmid p48FHis. The plasmid pYM7.2 (15) contains a 271-base pair *S. pombe* DNA fragment (with *Hin*dIII and *Bam*HI ends) cloned into pBR322. Covalently closed circular plasmid DNA was prepared by standard methods (16). DNA sequence determination was performed by the method of Maxam and Gilbert (17).

***In Vitro* Transcription of Cloned tRNA Genes and Analysis of RNA Products.** DNA from plasmids p48FHis and pYM7.2 was transcribed in a *Drosophila* Kc cell extract (18). The transcription products were resolved by electrophoresis on thin polyacrylamide gels (19); 5′-end analysis of the eluted RNAs was carried out by digestion with RNase T2 and RNase A. The hydrolysates were analyzed by polyethyleneimine (PEI)-cellulose thin-layer chromatography by using 1 M ammonium formate (pH 3.5) (20) or 0.75 M potassium phosphate (pH 3.5) as the solvent. Two-dimensional oligonucleotide mapping of RNA transcripts was carried out by standard methods (21). Separation in the first dimension was by electrophoresis at pH 3.5 on cellulose acetate; separation in the second dimension was by homochromatography on PEI-cellulose thin-layer plates (22).

**Guanylate-Addition Reaction.** RNA isolated from transcription reactions was reincubated at 24°C for 90 min under transcription reaction conditions (30 mM Hepes·KOH, pH 8.0/3 mM dithiothreitol/8 mM creatine phosphate/100 mM KCl/3 mM $MgCl_2$) in nucleotide-depleted *Drosophila* Kc cell extract with supplemental nucleotides or nucleoside triphosphates (0.5 mM). The reaction volume was 40 $\mu$l. Nucleotide-depleted extract was prepared by chromatography of the supernatant from centrifugation of the *Drosophila* Kc cell extract at 100,000 × *g* (S-100) on DEAE-Sephadex A-25 and CM-Sepharose 6B (D. L. Johnson, personal communication). The chromatographed extract was dialyzed against 20 mM Hepes·KOH, pH 7.9/5 mM $MgCl_2$/2 mM dithiothreitol/0.1 mM EDTA/20% glycerol/100 mM KCl and stored in liquid nitrogen. Separation of or-

Abbreviations: pp[$CH_2$]pG, guanosine 5′-[$\alpha,\beta$-methylene]triphosphate; PEI, polyethyleneimine.

† Present address: Max Planck Institut für Molekulare Genetik, Ihnestrasse 63-73, D-1000 Berlin 33, Federal Republic of Germany.

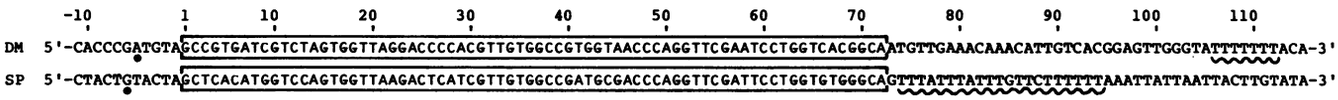


FIG. 1. DNA sequences of the noncoding strand of histidine tRNA genes from *Drosophila* (DM) and *S. pombe* (SP). The mature tRNA coding sequences are boxed. Transcription initiation sites are indicated by dots and the termination sequences by wavy lines.

thophosphate from pG was required to quantitate the addition reaction and was performed by thin-layer chromatography with Avicel cellulose (2-propanol/concentrated HCl/water, 70:15:15, vol/vol).

## RESULTS

**Histidine tRNA Gene Sequences.** The nucleotide sequence of the *Drosophila* histidine tRNA gene contained on the plasmid p48FHis was determined by standard methods (17) and is shown in Fig. 1 together with the *S. pombe* histidine tRNA gene sequence present on the plasmid pYM7.2 (15). The mature tRNA coding sequences are 75% homologous and contain no intervening sequences.

***In Vitro* Transcription of Histidine tRNA Genes.** Efficient transcription of the cloned histidine tRNA genes was obtained in an extract from *Drosophila* Kc cells (18). RNA transcripts formed were resolved by polyacrylamide gel electrophoresis (Fig. 2). From a calculation of the length of the transcripts and from 5'-end analysis (see below), the following conclusions (confirmed by two-dimensional chromatography) were reached. The primary transcript of the *Drosophila* gene initiates with pppA at position −5 in the gene sequence (Fig. 1) and has a length consistent with termination at the site indicated in Fig. 1. The primary transcript contains both 5'- and 3'-flanking sequences. The 5'-flanking nucleotides found in the primary transcript were removed in the *Drosophila* intermediate precursor RNA. The mature-sized tRNA is formed after endonucleolytic removal of the 3'-flanking nucleotides from the intermediate precursor. These intact, 3'-flanking sequence fragments are noted in Fig. 2. The array of primary transcripts transcribed from the *S. pombe* clone correspond in length to RNAs (initiated at nucleotide −6 in the gene) that have terminated at different sites in the termination region (see Fig. 1).

**5'-End Analysis of RNA Transcription Products.** RNA transcripts were eluted from gel slices and digested with RNase T2 and RNase A. The nucleoside 5'-triphosphate 3'-monophosphates liberated from the 5' terminus of the primary transcript were identified by thin-layer chromatography. By using transcripts formed in the presence of different nucleoside [$\alpha$-$^{32}$P]triphosphates, it was possible to determine the exact RNA initiation site based on the known DNA sequence (Fig. 1). The *Drosophila* tRNA gene primary transcript initiates with pppA (at position −5), and the *S. pombe* RNA, with pppG (at position −6). The 5'-terminal nucleotides of processed RNAs (intermediate precursor and mature-sized tRNA) were also identified

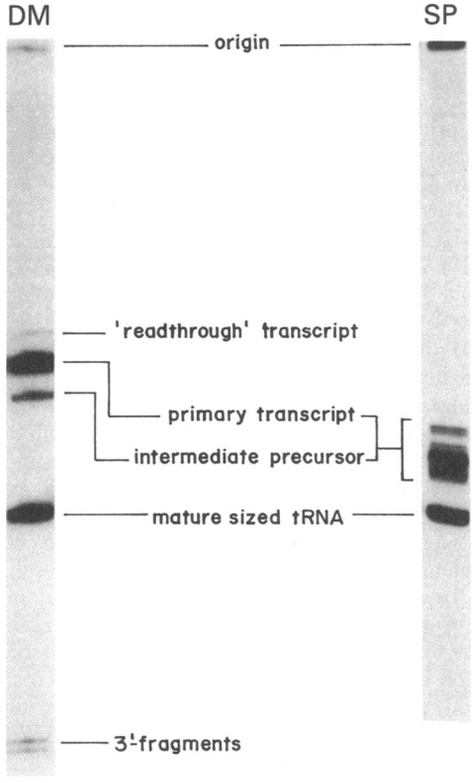


FIG. 2. *In vitro* transcription of histidine tRNA genes in *Drosophila* Kc cell extract. Autoradiograms of gel electrophoretic separations of RNA transcribed from plasmids p48FHis (*Drosophila* clone, DM) and pYM7.2 (*S. pombe* clone, *SP*). [$\alpha$-$^{32}$P]GTP was used as the source of radiolabel.

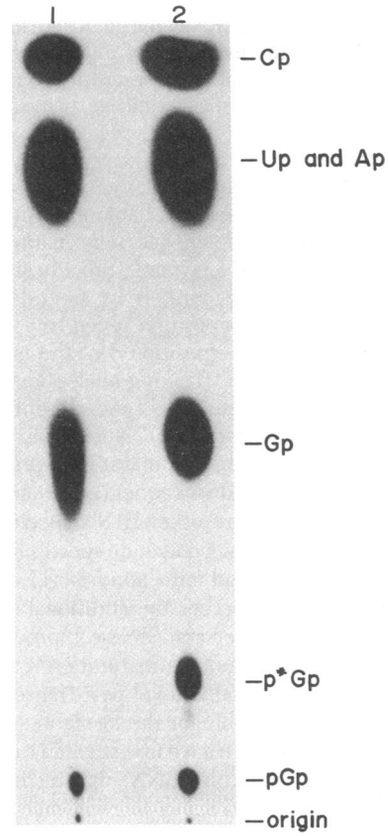


FIG. 3. Characterization of the 5' end of RNA transcribed from p48FHis DNA. Autoradiogram of RNA digestion products separated by PEI-cellulose thin-layer chromatography. Intermediate precursor (lane 1) and mature-sized tRNA (lane 2) were digested with RNase T2 and RNase A.

by thin-layer chromatography (Fig. 3). The *Drosophila* intermediate precursor RNAs have pGp at the 5′ end, as do those from *S. pombe* (data not shown), whereas the mature-sized RNA is heterogeneous, containing either pGp or a modified pGp at the 5′ end. The nature of the modification is not known.

**Two-Dimensional Oligonucleotide Mapping of Processed RNAs.** Intermediate precursor and mature-sized *Drosophila* tRNA and mature-sized *S. pombe* tRNA were digested with RNase A and subjected to two-dimensional oligonucleotide mapping (Fig. 4). Secondary analysis identified the oligonucleotides containing the 5′ ends of RNA transcripts. These oligonucleotides and their sequences are indicated in Fig. 4. The 5′-terminal sequence of the *Drosophila* intermediate precursor is pGpCp, which corresponds to positions 1 and 2 of the DNA sequence (Fig. 1). We conclude that the intermediate precursor RNA is an RNase P cleavage product of the primary transcript. The two-dimensional maps of the mature-sized RNA of *Drosophila* and *S. pombe* showed three 5′-terminal oligonucleotides (see Fig. 4 *B* and *C*). Approximately 5% of the molecules contained pGpCp at the 5′ end, the same 5′ end as in the intermediate precursor. The remaining molecules had a pGpGpCp terminus, and approximately 70% of these contained a modified 5′ pG. This analysis demonstrates that a pG moiety is added to the 5′ end of the intermediate precursor and that, in our extract, a portion of the histidine tRNA population is then modified at the 5′ pG.

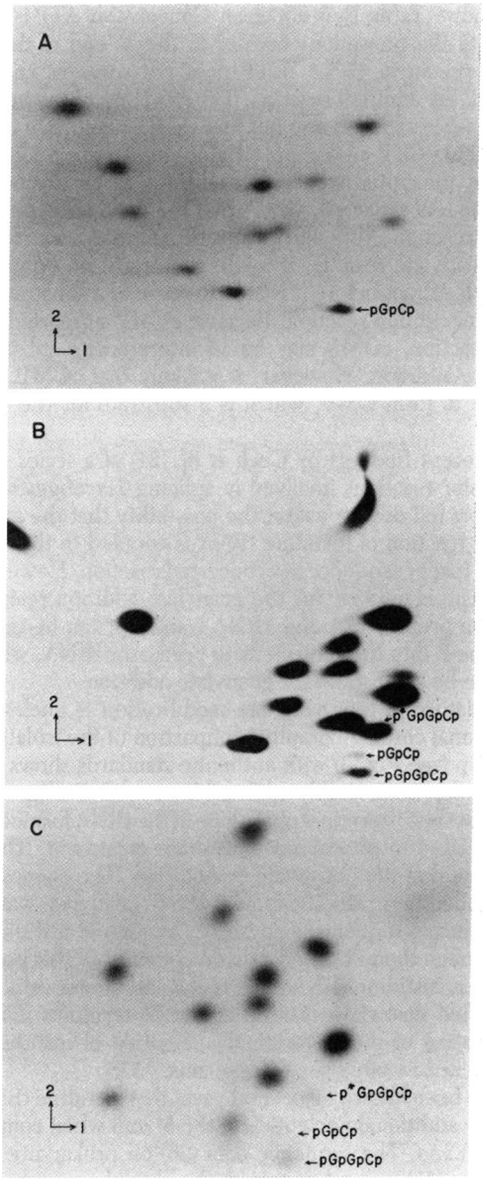


FIG. 4. Two-dimensional chromatographic analysis of RNase A digests of transcription products. (*A*) *Drosophila* intermediate precursor RNA labeled with [$\alpha$-$^{32}$P]CTP; (*B*) *Drosophila* mature-sized tRNA labeled with [$\alpha$-$^{32}$P]GTP. (*C*) *S. pombe* mature-sized tRNA labeled with [$\alpha$-$^{32}$P]GTP.

**Nucleotide Requirements for Guanylate Addition to Histidine tRNA.** The nucleotide requirements for addition of a guanylate residue were investigated by reincubation of purified *Drosophila* primary transcript RNA (formed in the presence of [$\alpha$-$^{32}$P]GTP) in a nucleoside triphosphate-depleted *Drosophila* extract. The reactions were supplemented with guanosine derivatives (Fig. 5). Additional radioactivity was not included in these reactions. Processing of the primary transcript by RNase P without guanylate addition results in the 5′-terminal sequence $\overset{*}{p}$GpCp ..., where the radioactive phosphate is at the 5′ terminus. Guanylate addition will form pG$\overset{*}{p}$GpCp ..., where the labeled phosphate is located internally.

After incubation of purified intermediate precursor RNA (data not shown) or of primary transcript RNA in the extract, mature-sized tRNA was resolved by polyacrylamide gel electrophoresis (Fig. 5). The mature-sized tRNAs have slightly different mobilities, possibly due to incomplete C-C-A addition at the 3′ terminus. After RNase T2 digestion of the mature-sized tRNA, the 5′-terminal nucleotides were isolated. To distinguish between the two possible 5′ termini (pG$\overset{*}{p}$ and $\overset{*}{p}$Gp), the pGp residues arising from the 5′ termini were digested with nuclease P1 to hydrolyze the 3′ phosphate. The pG and orthophosphate formed were separated as described. Occurrence of [$^{32}$P]orthophosphate indicates that guanylate addition has occurred in the mature-sized tRNA (pG$\overset{*}{p}$GpCp ...), whereas the presence of [$^{32}$P]GMP ($\overset{*}{p}$G) indicates that addition has not occurred.

After autoradiography, the amount of radioactivity in the

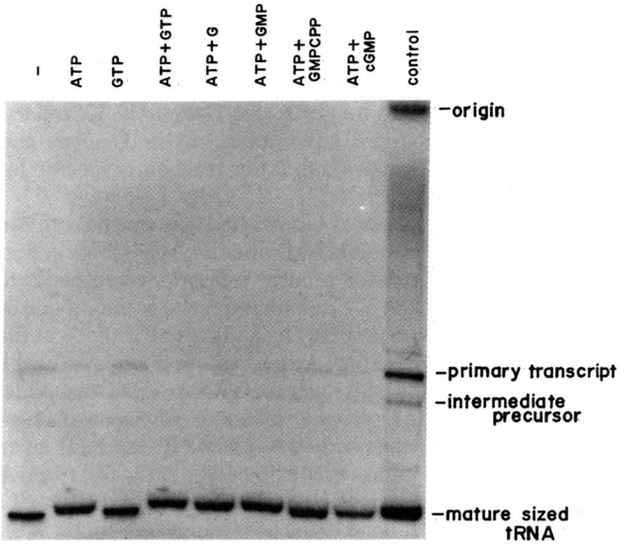


FIG. 5. Autoradiogram of gel electrophoretic separation of RNA processing products. *Drosophila* histidine tRNA primary transcript labeled with [$\alpha$-$^{32}$P]GTP was processed in nucleoside triphosphate-depleted *Drosophila* Kc cell extract. Supplements to the extract are indicated at the top of each lane. The control lane contains products of a *Drosophila* histidine tRNA gene transcription reaction as size markers (in base pairs). The 5′-terminal nucleotides of the RNAs were determined as described.

Table 1. Analysis of guanylate addition reaction in nucleoside triphosphate-depleted *Drosophila* extract supplemented with various compounds

| Supplements | % addition |
|---|---|
| None | 9 |
| ATP | 12 |
| GTP | 10 |
| ATP + GTP | 61 |
| ATP + GMP | 66 |
| ATP + guanosine | 46 |
| ATP + pp[$CH_2$]pG | 17 |
| ATP + cGMP | 49 |

phosphate and pG spots was quantitated. Percentage of addition of pG was calculated as the amount of phosphate divided by the amount of phosphate plus pG. The results of this experiment (Table 1) demonstrate that the addition reaction requires ATP (presumably as an energy source) and a guanosine substrate.

## DISCUSSION

We have studied the *in vitro* maturation of the 5′ end of *Drosophila* and *S. pombe* histidine tRNA, making use of *in vitro* transcription of cloned tRNA genes. Transcription of the genes initiates with a purine as is generally the case in tRNA gene transcription (23). However, the long transcribed 3′ flank of the *Drosophila* gene is an unusual feature in tRNA gene organization. Analysis of the *in vitro* transcripts (see RNAs in Fig. 2) demonstrates that the 5′-leader sequence is removed before the 3′-flanking sequence. Analysis of the 3′ fragments indicates that an endonucleolytic cleavage occurs at the 3′ end of the mature coding sequence and that transcription termination occurs preferentially at two of the seven thymidylate residues in the terminator (D. Frendewey, personal communication).

The nucleotide sequence of the *Drosophila* histidine tRNA gene predicts that the extra nucleotide at the 5′ terminus of the corresponding tRNA will be pA. However, Altwegg and Kubli (9) found the extra nucleotide to be pG. We provide experimental evidence that, at least *in vitro*, the 5′-terminal guanylate residue in *Drosophila* and *S. pombe* mature histidine tRNAs is added post-transcriptionally. Post-transcriptional guanylate addition to the 5′ end of tRNA has not been observed with any other cloned tRNA genes transcribed in *Drosophila* Kc cell extract (ref. 18; unpublished observations) or in *Xenopus* and HeLa cell extracts. It appears that the reaction is specific for histidine tRNAs.

The proposed sequence of enzymatic steps involved in the formation of the 5′ terminus of histidine tRNAs is shown in Fig. 6. The RNA sequence of the primary transcript corresponds to the DNA sequence. The 5′-flanking sequence of the precursor tRNA is removed, most probably by an RNase P activity, at the site where all other tRNAs have their mature 5′ end. Although we do not know whether this nuclease is RNase P in *Drosophila*, we have converted the *Drosophila* histidine tRNA gene primary transcript *in vitro*, using purified *S. pombe* RNase P (4), to an RNA identical to the intermediate precursor RNA. In a reaction that requires ATP, a guanosine derivative is linked through a (3′-5′)-phosphodiester bond to the 5′-terminal nucleotide of the intermediate precursor. In a fraction of the mature histidine tRNAs, this added residue undergoes modification. The addition reaction occurs after removal of the 3′-flanking nucleotides because the *Drosophila* intermediate precursor tRNA (Fig. 2) is not matured at the 5′ end (shown in Fig. 6). However, it remains to be determined whether guanylate addition precedes or follows the addition of the 3′-terminal C-C-A sequence.

The role of the ATP, which is required for addition of a guanylate residue (Table 1), is not clear. Conceivably ATP is needed to activate the phosphomonoester at the 5′ end of the intermediate precursor tRNA. GTP does not serve as an energy source for the addition reaction. The structure of the guanylate moiety that is added is also not known. The failure of the nonhydrolyzable GTP analog pp[$CH_2$]pG to support addition implies that the initial step is not addition of guanosine 5′-triphosphate. As expected, GMP (pG) is a good substrate in the addition reaction. Although guanosine serves as a source for the added guanylate residue, it might require 5′-phosphorylation in the cell extract before it is able to serve as a substrate in the guanylate addition reaction. Because cGMP supported the addition reaction, cGMP may be an intermediate substrate for guanylate addition. However, it is likely that cGMP was hydrolyzed to form GMP, which is a substrate for the addition reaction.

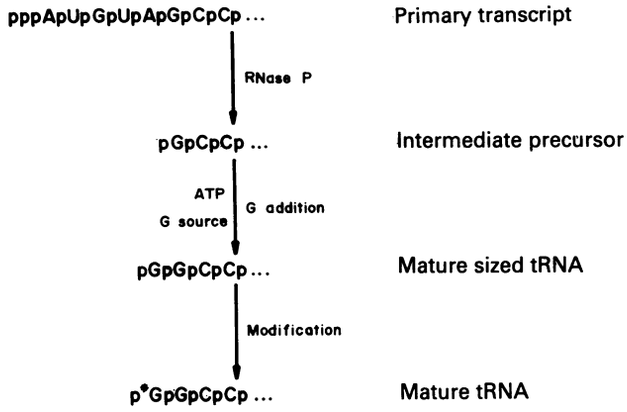


FIG. 6. Scheme of reactions involved in the maturation of the 5′ end of *Drosophila* histidine tRNA.

The recent findings by Cech *et al.* (24) of a series of phosphotransfer reactions involved in splicing *Tetrahymena* rRNA precursors led us to consider the possibility that the guanylate addition reaction of histidine tRNA is coupled to the cleavage of the 5′-leader sequence in a concerted reaction. However, this mechanism is unlikely for the guanylate addition reaction because the primary histidine tRNA transcript can be converted by RNase P into the intermediate precursor tRNA, which was shown to be a substrate for guanylate addition.

The nature of the guanylate modification is unclear. Two-dimensional chromatographic comparison of the isolated modified pGp (see Fig. 3) with authentic standards shows that the modification is not simply $m^1G$, $m^2G$, or $m^7G$. Preliminary data show that the 5′-terminal phosphate of the tRNA formed *in vitro* is insensitive to phosphomonoesterase treatment. Therefore, it appears that the phosphate is modified. The guanosine may also be modified. Modification of the 5′ guanylate was not observed during sequence analysis of *Drosophila* histidine tRNA (9), although the published data do not rule out this possibility. However, histidine tRNA from HeLa and mouse cells contains a modified guanylate residue at the 5′ terminus (25). Thus, modification of the 5′-terminal nucleotide of histidine tRNA does occur *in vivo* in some organisms.

It has been known for several years that histidine tRNAs possess one additional nucleotide at the 5′ end when compared to other tRNAs. The sequence data (26) on prokaryotic and eukaryotic tRNAs reveal that, with the possible exception of bacteriophage T5-encoded histidine tRNA, the extra nucleotide is guanylic acid. The extra nucleotide is base-paired in prokaryotic and organelle tRNAs but not in eukaryotic cytoplasmic tRNAs. In addition to the gene sequences from *Drosophila* and *S. pombe* reported here, histidine tRNA genes have been se-

quenced from *Euglena* (M. J. Hollingsworth and R. B. Hallick, personal communication) and maize (27) chloroplasts, *S. cerevisiae* (28, 29) and *Neurospora* (12) mitochondria, and *E. coli* (L. M. Hsu, personal communication). In the organelle and prokaryotic histidine tRNA genes, the 5′-terminal tRNA sequence and the gene sequence correspond. It will be interesting to learn whether in these organisms, guanylate addition takes place or whether an "aberrant" RNase P cleavage gives rise to the 5′ terminus of the histidine tRNAs.

It seems reasonable to postulate that the unusual structure at the 5′ end of histidine tRNA serves a specific purpose in the cell. The *Drosophila* histidine tRNA sequenced by Altwegg and Kubli (9), which does not contain a modification of the 5′-terminal guanylate residue, was identified by aminoacylation. Therefore, it is possible both histidine tRNAs, modified and unmodified at the 5′-terminal nucleotide, are present *in vivo* and that only the tRNA unmodified at the 5′-guanylate residue is aminoacylated and functional in translation. Another function of histidine tRNA may be regulated by the extent to which the histidine tRNA population *in vivo* is modified at the extra 5′ nucleotide.

**Note Added in Proof.** Han and Harding (30) have determined the sequence of a histidine tRNA gene from mouse. The DNA sequence contains a cytidylate residue at the position that corresponds to the 5′-terminal unpaired guanylate residue present in the homologous tRNA (25).

We thank D. L. Johnson for fractionated extract and J. Schaack, S. Sharp, T. Dingermann, and D. DeFranco for many helpful comments and critical reading of the manuscript. This work was supported by grants from the National Institutes of Health and from the National Science Foundation. B.A. was a Deutsche Forschungsgemeinschaft Postdoctoral Fellow.

1. Altman, S. (1981) *Cell* **23**, 3–4.
2. Schmidt, O. & Söll, D. (1981) *BioScience* **31**, 34–39.
3. Kole, R. & Altman, S. (1982) in *The Enzymes*, ed. Boyer, P. D. (Academic, New York), pp. 469–483.
4. Kline, L., Nishikawa, S. & Söll, D. (1981) *J. Biol. Chem.* **256**, 5058–5063.
5. Garber, R. L. & Gage, L. P. (1979) *Cell* **18**, 817–828.
6. Abelson, J. (1979) *Annu. Rev. Biochem.* **48**, 1035–1069.
7. Melton, D. A., DeRobertis, E. M. & Cortese, R. (1980) *Nature (London)* **284**, 143–148.
8. Singer, C. E. & Smith, G. R. (1972) *J. Biol. Chem.* **247**, 2989–3000.
9. Altwegg, M. & Kubli, E. (1980) *Nucleic Acids Res.* **8**, 3259–3262.
10. Boisnard, M. & Petrissant, G. (1981) *FEBS Lett.* **129**, 180–184.
11. Sibler, A.-P., Martin, R. P. & Dirheimer, G. (1979) *FEBS Lett.* **107**, 182–186.
12. Kumar, C. C., Chang, D. C., Alzner-DeWeer, B. & Raj-Bhandary, U. L. (1982) *Nucleic Acids Res.*, in press.
13. Walseth, T. F. & Johnson, R. A. (1979) *Biochim. Biophys. Acta* **526**, 11–31.
14. Dudler, R., Egg, A. H., Kubli, E., Artavanis-Tsakonas, S., Gehring, W. J., Steward, R. & Schedl, P. (1980) *Nucleic Acids Res.* **13**, 2921–2937.
15. Gamulin, V., Mao, J., Appel, B., Yamao, F., Pearson, D. & Söll, D. (1982) *Nucleic Acids Res.*, in press.
16. Clewell, D. F. (1972) *J. Bacteriol.* **110**, 667–676.
17. Maxam, A. M. & Gilbert, W. (1980) *Methods Enzymol.* **65**, 449–560.
18. Dingermann, T., Sharp, S., Appel, B., DeFranco, D., Mount, S., Heiermann, R., Pongs, O. & Söll, D. (1981) *Nucleic Acids Res.* **9**, 3907–3918.
19. Sanger, F. & Coulson, A. R. (1978) *FEBS Lett.* **87**, 107–110.
20. Randerath, K., Gupta, R. C. & Randerath, E. (1980) *Methods Enzymol.* **65**, 638–680.
21. Volckaert, G., MinJou, W. & Fiers, W. (1976) *Anal. Biochem.* **72**, 433–446.
22. Barrell, B. G. (1972) in *Procedures in Nucleic Acids Research*, eds. Cantoni, G. L. & Davies, D. R. (Harper & Row, New York), Vol. 2, pp. 751–779.
23. Dingermann, T., Sharp, S., Schaack, J., DeFranco, D., Johnson, D. L., Cooley, L. & Söll, D. (1982) in *Gene Regulation*, UCLA Symposium on Molecular and Cellular Biology, ed. O'Malley, B. (Academic, New York), Vol. 26, in press.
24. Cech, T. R., Zaug, A. J. & Grabowski, P. J. (1981) *Cell* **27**, 487–496.
25. Rosa, M. D., Hendrick, J. P., Jr., Lerner, M. R. & Steitz, J. A. (1982) *Nucleic Acids Res.*, in press.
26. Sprinzl, M. & Gauss, D. H. (1982) *Nucleic Acids Res.* **10**, r1–r55.
27. Schwarz, Z., Jolly, S. O., Steinmetz, A. A. & Bogorad, L. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 3423–3427.
28. Bos, J. L., Osinga, K. A., VanderHorst, G. & Borst, P. (1979) *Nucleic Acids Res.* **6**, 3255–3266.
29. Berlani, R. E., Pentella, C., Macino, G. & Tsagoloff, A. (1980) *J. Bacteriol.* **141**, 1086–1097.
30. Han, J. H. & Harding, J. D. (1982) *Nucleic Acids Res.* **10**, 4891–4900.