T4 BACTERIOPHAGE +RNAGly*

Stephen Stahl[†], Gary Paddock[‡] and John Abelson
Department of Chemistry
University of California, San Diego
La Jolla, California 92037

Received July 30, 1973

SUMMARY. The nucleotide sequence of T4 tRNA $^{\rm Gly}$ is: pGCGGAUAUCGUAUAAUGmG-DAUUACCUCAGACUUCCAA ψ CUGAUGAUGUGAGT ψ CGAUUCUCAUUAUCCGCUCCA-OH. The sequence is compared with the sequences of five other tRNA $^{\rm Gly}$ species.

When bacteriophage T4 infects E. coli 8 tRNAs and 2 other small stable RNA molecules are transcribed from the T4 genome (1-4). These tRNAs can be selectively labeled with ³²P following T4 infection and are easily purified by electrophoresis in a 10% acrylamide gel (5). One of the T4 tRNAs accepts glycine. We have referred to this species as band 6 in previous publications (5,6). Using the techniques developed by Sanger and his colleagues (7) we have completed the nucleotide sequence of this tRNA and it is shown in the cloverleaf configuration in Figure 1.

Bacteriophages T2 and T6 also produce a tRNA with the same electrophoretic mobility as band 6. Analyses of the pancreatic and T1 ribonuclease digestion products of T2 and T6 band 6 RNA indicate that the sequences must be identical to that of T4 $tRNA^{Gly}$.

The anticodon of T4 tRNA $^{\rm Gly}$ is UCC indicating that the tRNA recognizes ${\rm GG}_{\rm G}^{\rm A}$. Our evidence suggests that the U in the anticodon is probably modified but we have not determined the nature of the modification.

From the work of Scherberg and Weiss (3) we know that the T4 tRNA Gly is acylated by E. coli glycyl tRNA synthetase (although detailed kinetics are

 $[\]mbox{* A complete proof of the sequence of $T4$ tRNA <math display="inline">\mbox{\sc Gly}$$ will be presented elsewhere.

[†] Present address: Department of Biochemistry, University of California, Berkeley, Calif. 94720.

[‡] Present address: Department of Biology, University of California, Los Angeles, Calif. 90024.

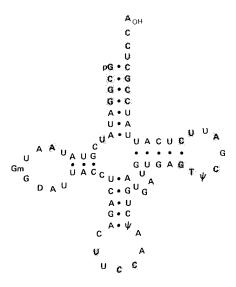


Fig. 1. Cloverleaf model of T4 tRNA Gly. The shaded residues are those which are identical in 6 tRNA Gly species (see text). In a previous publication (6) we reported a preliminary catalog of the T1 ribonuclease digestion products of T4 tRNA Gly. Further work has revealed three errors in that catalog. There is one mole each of UG and AG instead of the two previously reported and the sequence of T12 is DAUUACCUCAG as shown in the model.

not available). It is therefore of interest to compare the sequence of T4 tRNA Gly with those of other tRNA species recognized by the same enzyme. These include E. coli tRNA Gly I (8,9), E. coli tRNA Gly II (J. W. Roberts and J. Carbon, personal communication), E. coli tRNA Gly III (10), and Staphlococcus epidermidis tRNA Gly Ia, Ib (11). The latter S. epidermidis tRNAs are not active in protein synthesis but do participate in peptidoglycan synthesis (12). They are acylated with glycine by E. coli extracts (R.J. Roberts, personal communication).

In Figure 1 the nucleotides which are common to all six tRNA sequences are shaded. The most striking result of this comparison (also noted earlier by Hill et al. (9)) is that the 3'-5' stems of the molecules are identical through the fourth base pair of the stem. This region has already been implicated as a site of synthetase recognition in other tRNAs (13-16). It has previously been shown that the anticodon is important in the synthetase recognition of tRNA Gly (10.17). Thus two regions of the

molecule, the 3'-5' stem and the anticodon, are apparently involved in glycyl tRNA synthetase recognition.

The sequence of T4 tRNA Gly is most closely related to its cognate tRNA in \underline{E} . $\underline{\text{coli}}$ tRNA $_{\text{GGA/G}}^{\text{Gly II}}$ (59 out of 74 residues are identical).

Acknowledgments. We would like to thank Peter Johnson for his help, and Drs. John Carbon and Richard Roberts for communicating their unpublished results. This work was supported by a grant from the National Cancer Institute (CA 10984). J.A. is a Faculty Research Associate of the American Cancer Society (FRA-80). G.P. was supported by a USPHS Training Grant (GM-01045).

REFERENCES

- Hsu, W. T., Foft, J. W., and Weiss, S. B. (1967) Proc. Nat. Acad. Sci. USA 58, 2028-2035.
- Daniel, V., Sarid, S., and Littauer, U. Z. (1970) Science 167, 1682-
- 3. Scherberg, N., and Weiss, S. B. (1970) Proc. Nat. Acad. Sci. USA 67, 1164-1171.
- Waters, L. C., and Novelli, G. D. (1969) Proc. Nat. Acad. Sci. USA 57, 979-985.
- Pinkerton, T. C., Paddock, G., and Abelson, J. (1972) Nature New Biol. 240, 88-90.
- 6. Nierlich, D., Lamfrom, H., Sarabhai, A., and Abelson, J. (1973) Proc. Nat. Acad. Sci. USA 70, 179-182.
- 7. Barrell, B. G. (1971) Proc. Nucl. Acid Res. 2, 751-779.
- 8. Riddle, D. L., and Carbon, J. (1973) Nature New Biol. 242, 230-234.
- 9. Hill, C. W., Combriato, G., Sternhart, W., Riddle, D. L., and Carbon, J. (1973) J. Biol. Chem. 248, 4252-4262.
- 10. Squires, C., and Carbon, J. (1971) Nature New Biol. 233, 274-277.
- ll. Roberts, R. J. (1972) Nature New Biol. 237, 44-45.
- 12. Stewart, T. S., Roberts, R. J., and Strominger, J. (1971) Nature 230, 36-38.
- 13. Schulman, L., and Chambers, R. (1968) Proc. Nat. Acad. Sci. USA 61, 308-315.
- Shimura, Y., Aono, H., Ozeki, H., Sarabhai, A., Lamfrom, H., and Abelson, J. (1972) FEBS Lett. 22, 144-148.
- Hooper, M. L., Russell, R., and Smith, J. D. (1972) FEBS Lett. 22, 149-15.
- Smith, J. D., and Cellis, J. (1973) Nature New Biol. 243, 66-71. Carbon, J., and Curry, J. B. (1968) J. Mol. Biol. 38, 201-216. 16.