
The sequence of the 5S ribosomal RNA of the crustacean *Artemia salina*

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ABSTRACT

The primary structure of the 5 S rRNA isolated from the cryptobiotic cysts of the brine shrimp *Artemia salina* is pACCAACGGCCAUACCACGUUGAAAGU ACCCAGUCUCGUCAGAUCCUGGAAGUCACACAACGUCGGGCCCGGUCAGUACUUGGAUGGGUG ACCGCCUGGGAACACCGGGUGCUGUUGGCAU_{OH}.

INTRODUCTION

The availability of gel sequencing techniques applicable to small RNA molecules¹⁻⁵ is resulting in a rapid growth of the collection of known 5 S rRNA primary structures⁶. These data can be used in phylogenetic studies^{7,8}, and in attempts to derive general secondary structure models for 5 S RNA^{7,9-12} by a search for common sequence complementarities. We report the primary structure of 5 S rRNA isolated from the cysts of the brine shrimp *Artemia salina* (Phylum arthropoda, class crustacea).

MATERIALS AND METHODS

Dry cysts of *Artemia salina* were obtained from San Francisco Bay Brand (Newark, California). The ribosomes were isolated according to Zasloff and Ochoa¹³ and ribosomal RNA was obtained from these by phenol extraction¹⁴. 5 S RNA was isolated by gel filtration on Sephadex G100 as described by Monier¹⁵, except that the column was not recycled. It was further purified by electrophoresis on slab gels¹⁶ containing 10% polyacrylamide, 7 M urea, 0.05 M Tris-borate buffer pH 8.3 and 0.001 M EDTA.

Three different gel-sequencing methods were used to derive the primary structure: the methods of Diels and De Wachter⁵ and of Tanaka, Dyer and Brownlee⁴, both of which are two-dimensional adaptations of Stanley and Vassilenko's procedure², and Peattie's³ chemical degradation method. RNA labeled at the 3'-terminus by [³²P] pCp ligation was also used for a confirmation of the terminal sequence by a moving spot analysis¹⁷. The 5'-terminal

base was determined by alkaline hydrolysis of 20 µg unlabeled RNA, followed by HPLC identification of the resulting nucleoside bisphosphate. The hydrolysate was fractionated on a 4.6 x 250 mm Partisil SAX (Reeve Angel, Clifton, N.J.) column eluted with 120 ml of a concentration gradient of H₃PO₄ adjusted to pH 2.2 with NH₃, rising from 0.01 M to 1 M in 100 min. Nucleotides are eluted in the order Cp, Ap, Up, Gp, pCp, pAp, pUp, pGp.

RESULTS

Different gel sequencing methods were used to establish the complete sequence, as summarized in Table 1. Each part of the sequence was confirmed by at least three successful experiments. In the area of positions 62 to 70, which is rich in G's and C's probably involved in base pairing, the chemical degradation method³ was preferable to the methods based on aspecific hydrolysis^{4,5} which gave poor resolution. The 3'-terminal sequence was confirmed by a moving spot analysis of a partial hydrolysate of 5 S RNA labeled at the 3'-end with [³²P] pCp. We have found it difficult to dephosphorylate the 5 S RNA without partially degrading it, hence we could not label the 5'-terminus satisfactorily. However, the 5'-terminal residue was identified unequivocally as pAp by complete alkaline hydrolysis and HPLC-analysis of unlabeled material. In Fig. 1, the sequence of *Artemia salina* 5 S RNA is compared with that of the two other arthropods thus far investigated, *Drosophila melanogaster*¹⁸ and *Bombyx mori*¹⁹.

DISCUSSION

The primary structure of *Drosophila melanogaster* 5 S rRNA was originally reported²⁰ as GGGCGC₆₉, but later corrected²¹ to GCGGGC₆₉. The recent determination of the 5 S RNA gene sequence¹⁸ and the comparison with other arthropods (Fig. 1) now make it evident that the first version was the correct one. The primary structure of *Artemia* 5 S rRNA differs with that of

Table 1. Methods used to establish the *Artemia* 5 S rRNA sequence

Method	Positions identified
Gel sequencing : Diels & De Wachter ⁵	4-25,27-65,70-117
Tanaka et al. ⁴	2-61, 70-111
Peattie ³	4-120
Other : moving spot ¹⁷	102-120
alkaline hydrolysis, HPLC	1

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basic difference between the secondary structures of bacterial and eukaryotic 5 S RNAs, the former having four helical regions, the latter five⁹⁻¹². We do not share this opinion and we present evidence elsewhere (De Wachter, Chen and Vandenberghe, manuscript submitted) that the model of Fig. 2 applies, with minor variations, to all 5 S RNAs hitherto sequenced.

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REFERENCES

1. Simoncsits, A., Brownlee, G.G., Brown, R.S., Rubin, J.R., Guilley, H. (1977) *Nature* 269, 833-836.
2. Stanley, J., Vassilenko, S. (1978) *Nature* 274, 87-89.
3. Peattie, D.A. (1979) *Proc. Nat. Acad. Sci. U.S.* 76, 1760-1764.
4. Tanaka, Y., Dyer, T.A., Brownlee, G.G. (1980) *Nucl. Acids Res.* 8, 1259-1272.
5. Diels, L., De Wachter, R. (1980) *Arch. Internat. Physiol. Biochim.* 88, B26-B27.
6. Erdmann, V.A. (1981) *Nucl. Acids Res.* 9, r25-r42.
7. Hori, H., Osawa, S. (1979) *Proc. Nat. Acad. Sci. U.S.* 76, 381-385.
8. Küntzel, H., Heidrich, M., Piechulla, B. (1981) *Nucl. Acids Res.* 9, 1451-1461.
9. Fox, G.E., Woese, C.R. (1975) *Nature* 256, 505-507.
10. Hori, H., Osawa, S., Iwabuchi, M. (1980) *Nucl. Acids Res.* 8, 5535-5539.
11. Luehrsen, K.R., Fox, G.E. (1981) *Proc. Nat. Acad. Sci. U.S.* 78, 2150-2154.
12. Garrett, R.A., Douthwaite, S., Noller, H.F. (1981) *Trends Biochem. Sci.* 6, 137-139.
13. Zasloff, M., Ochoa, S. (1971) *Proc. Nat. Acad. Sci. U.S.* 68, 3059-3063.
14. Vandenberghe, A., Nelles, L., De Wachter, R. (1980) *Anal. Biochem.* 107, 369-376.
15. Monier, R. (1971) in *Procedures in Nucleic Acid Research*, Cantoni, G.L. and Davies, D.R. Eds., Vol. 2, pp. 618-622, Harper & Row, New York.
16. De Wachter, R., Fiers, W. (1971) *Methods Enzymol.* 21, 167-178.
17. Pinck, L., Pinck, M. (1979) *FEBS Lett.* 107, 61-65.
18. Tschudi, C., Pirrotta, V. (1980) *Nucl. Acids Res.* 8, 441-451.
19. Komiya, H., Kawakami, M., Shimizu, N., Takemura, S. (1980) *Nucl. Acids Res.* 8, s119-s122.
20. Benhamou, J., Jordan, B.R. (1976) *FEBS Lett.* 62, 146-149.
21. Benhamou, J., Jourdan, R., Jordan, B.R. (1977) *J. Mol. Evol.* 9, 279-298.