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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 pACCAACGGCCAUACCACGUUGAA GU ACCCAGUCUCGUCAGAUCCUGGAAGUCACACAACGUCGGGCCCGGUCAGUACUUGGAUGG UG ACCGCCUGGGAACACCGGGUGCUGUUGGCAU$_{OH}$.

INTRODUCTION

The availability of gel sequencing techniques applicable to small RN molecules[1-5] is resulting in a rapid growth of the collection of known 5 S rRNA primary structures[6]. These data can be used in phylogenetic studies[7,8], and in attempts do 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 Ochoa[13] and ribosomal RNA was obtained from these by phenol extraction[14]. 5 S RNA was isolated by gel filtration on Sephadex G 100 as described by Monier[15], except that the column was not recycled. It was further purified by electrophoresis on slab gels[16] 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 Wachter[5] and of Tanaka, Dyer and Brownlee[4], both of which are two-dimensional adaptations of Stanley and Vassilenko's procedure[2], and Peattie's[3] chemical degradation method. RNA labeled at the 3'-terminus by [^{32}P] pCp ligation was also used for a confirmation of the terminal sequence by a moving spot analysis[17]. 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 H_3PO_4 adjusted to pH 2.2 with NH_3, 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 succesfull 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 method[3] 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 [^{32}P] 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 reported[20] as $GGGCGC_{69}$, but later corrected[21] to $GCGGGC_{69}$. The recent determination of the 5 S RNA gene sequence[18] 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

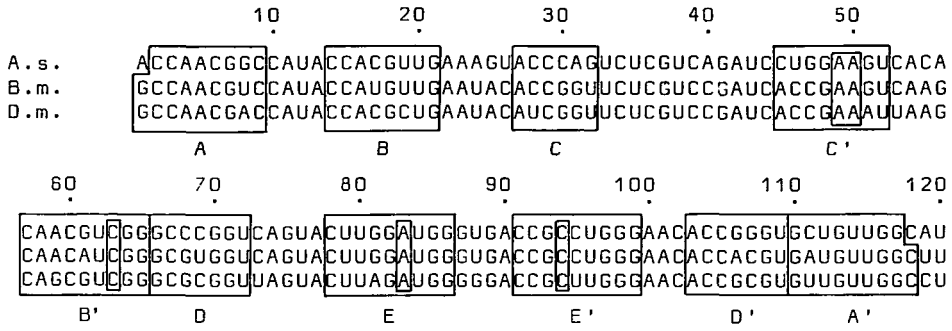

Fig. 1. Conservation of 5 S RNA primary and secondary structure within the phylum arthropods (A.s., Artemia salina; B.m., Bombyx mori; D.m., Drosophila melanogaster). The five double-stranded areas of our secondary structure model are indicated by the boxes A-A', B-B' etc.. Nested boxes delimit bulges and small interior loops within these areas.

Drosophila in 28 positions and with that of the silkworm in 21 positions. The two insect sequences show 16 differences. Among the vertebrates, the mean divergence between two organisms belonging to different classes (fish, amphibia, reptiles, birds, and mammals have been investigated) is limited to 9 positions.

A common base pairing scheme can be applied to the three arthropod sequences, as indicated by the boxes in Fig. 1. The resulting secondary structure model is illustrated with the Artemia 5 S RNA in Fig. 2. There now seems to be a consensus among the majority of investigators that there is a

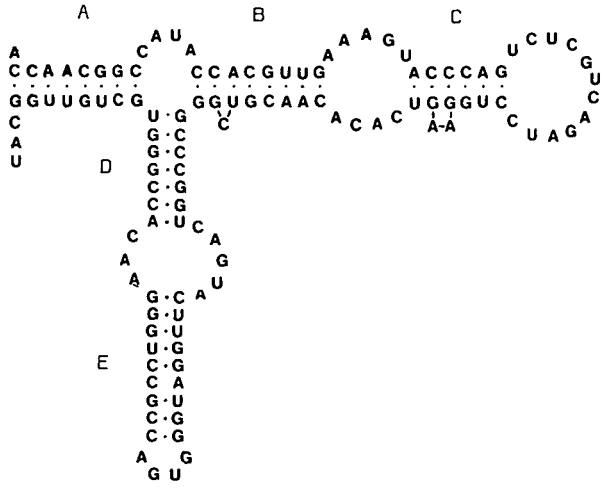

Fig. 2. Secondary structure model for Artemia 5 S RNA.

basic difference between the secondary structures of bacterial and eukaryotic 5 S RNAs, the former having four helical regions, the latter five[9-12]. 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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