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The nucleotide sequence of 5.8S rRNA from the posterior silk gland of the silkworm *Philosamia cynthia ricini*

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ABSTRACT

The nucleotide sequence of 5.8S rRNA from the Chinese silkworm, Philosamia cynthia ricini has been determined by gel sequencing and mobility shift methods. The complete primary structure is pAAACCAUUACCCUGGACGGUGGAUCACUUG GCUCGCGGGUCGAUGAAGAACGCAGUUAACUGCGCGUCAUAGUGUGAACUGmCAGGACACAUUUGAACAUCGAC AUUUCGAACGCACAUUGCGGUCCGUGGAGACACAUCCAGGACCACUCCUGUCUGAGGGCCGAUUAA$_{OH}$. This is one of the largest known 5.8S rRNAs. As compared to Bombyx 5.8S rRNA, it is two nucleotides longer; two nucleotides near the 5'end and two nucleotides near the 3'end are different, and Ψ_{61} of the Bombyx RNA sequence is an unmodified U in Philosamia RNA. The secondary structure of Philosamia 5.8S rRNA may differ from the Bombyx RNA structure by three additional base pairs at the 5'/3' ends.

INTRODUCTION

Eukaryotic ribosomes contain, in their large subunit, a 5.8S rRNA which is hydrogen bonded to 28S rRNA (1-4). Concerning this interaction it is of special interest, that the 28S rRNA species from insects, except aphids, (5) and Vicia faba (6) contain a hidden break. Among the three insect 5.8S rRNAs that have been sequenced, two are from Diptera (Drosophila melanogaster and Sciara coprophila). Both rRNA species are quite exceptional as they are split into two pieces, m5.8S and 2S rRNA, which are non-covalently held together by base pairing (7, 8). The 5.8S rRNA from Bombyx mori, however, is a single, 167 nucleotides long molecule (9). We have studied the 5.8S rRNA of the closely related silkworm Philosamia cynthia ricini in order to identify more general features of this insect rRNA species.

MATERIALS AND METHODS

Posterior silk glands of 5th instar larvae of Philosamia cynthia ricini were phenolyzed and the aqueous phase was made 1.5 M with NaCl. The soluble RNAs were precipitated with ethanol and fractionated by DEAE-cellulose column chromatography. Final purification of 5.8S rRNA was achieved by twodimensio-

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nal polyacrylamide gel electrophoresis (10).

After 5'-dephosphorylation, the RNA was 5'- or 3'-[^{32}P]-labeled according to published procedures (10, 11). 5'- and 3'-terminal sequences were established by the mobility shift method (12). Internal sequences were determined by rapid gel techniques after degradation with base specific ribonucleases (13). To check the sequence thus obtained, 5.8S rRNA was partially digested with RNase T_1, followed by 5'-labeling. The isolated fragments (10) were sequenced on gels and by mobility shift analysis. Nucleotide composition of 5.8S rRNA was determined as described (12) by RNase T_2 digestion, 5'-[^{32}P]-labeling and 3'-dephosphorylation with nuclease P1, followed by twodimensional thinlayer chromatography in presence of unlabeled authentic 5'-nucleotides, and autoradiography.

RESULTS AND DISCUSSION

Fig. 1 shows the terminal nucleotide sequences of *Philosamia cynthia ricini* 5.8S rRNA. It is obvious that the pAAAUG... sequence at the 5'-end of *Bombyx* 5.8S rRNA is replaced by a pAAACC... sequence in *Philosamia* RNA. The 3'-terminal sequence of *Philosamia* 5.8S rRNA ends on ...AUUAA$_{OH}$, whereas the corresponding *Bombyx* RNA sequence...GCU$_{OH}$ differs in two nucleotides and is two nucleotides shorter. There is also one difference in modification: Ψ_{61} in the *Bombyx* RNA sequence is an unmodified U in *Philosamia* RNA.

For comparison of this primary structure with other 5.8S rRNAs we refer to Fujiwara et al. (9), who compare the *Bombyx* sequence with those of six published 5.8S rRNAs. It is interesting to note that *Philosamia* 5.8S rRNA contains four GAAC tetranucleotide sequences between positions 48 and 112. One of these is part of an extended region, UGAAGAACG (nucleotides 44-52 in *Philosomia* RNA), that is conserved in 21 of 24 sequenced 5.8S rRNAs (9, 16-19). As pointed out by Darlix and Rochaix (15), this tetranucleotide sequence theoretically could interact with loop IV in eukaryotic tRNAs. As *Bombyx* 5.8S rRNA (9), the *Philosamia* 5.8S rRNA sequence can be arranged in a secondary structure which to a certain extent fits the model of Nazar et al. (14) (Fig. 2). The most striking difference between HeLa cell 5.8S rRNA secondary structure as proposed by Nazar et al. (14) and the structure of insect 5.8S rRNA is the drastically reduced stability of the 3'-proximal hairpin (nucleotides 119-147 in Fig. 2). This is due to the high abundancy of G:U base pairs and the presence of only short base paired segment, separated by odd base pairs. Fujiwara et al. (9) give ΔG values of -33.2 and -2.2 kcal for the helix structure of HeLa and insect rRNA, respectively.

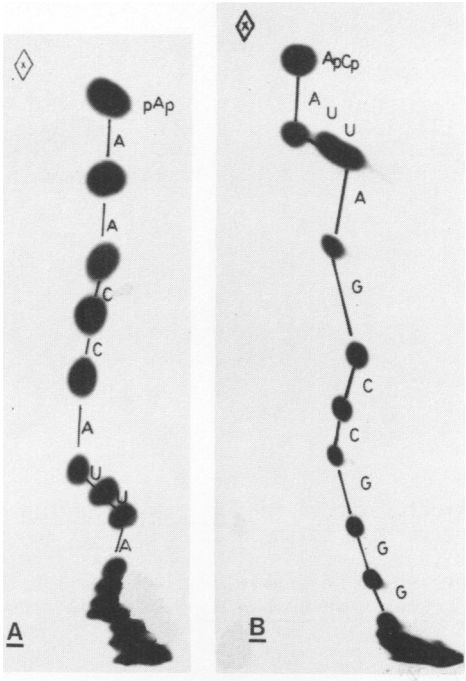

Fig. 1. Nucleotide sequences at the ends of Philosamia 5.8S rRNA. (A) 5'-[^{32}P]-labeled 5.8S rRNA was partially digested with alkali (20) and the resulting fragments were separated in two dimensions by electrophoresis and homochromatography (12). Read the sequence in 5'-3' direction from top to bottom. X = Xylene cyanol marker dye. (B) Nucleotide sequence at the 3' end of Philosamia 5.8S rRNA. 3'-[^{32}P]-labeled RNA, i.e. with 5'[^{32}P]-pCp added at its 3'-terminus, was treated as described above. Read the sequence in 5'-3' direction from bottom to top.

According to Pace et al. (4) the exceptionally stable hairpin in HeLa 5.8S rRNA is an important element in the stabilization of the 5.8S-28S rRNA junction complex. The 28S rRNA of insects, however, is split into two pieces (5). The nature of the junction complex formed here might be different, and this change could depend on a reduced stability of this 3'-proximal hairpin. This conclusion is supported by the fact that Drosophila, Bombyx, Philosamia and Vicia faba 5.8S rRNAs, with a split 28S rRNA counterpart, have such a hairpin with odd base pairs. Its validity is ruled out, however, by the finding that (a) 5.8S rRNAs of several other species also bear this structural element: Chlamydomonas, Acanthamoeba, Artemia (19) and (b) the diptera Sciara coprophila RNA structure contains an uninterrupted sequence of 9 G:C and A:U base pairs. The major difference between Bombyx mori (9) and

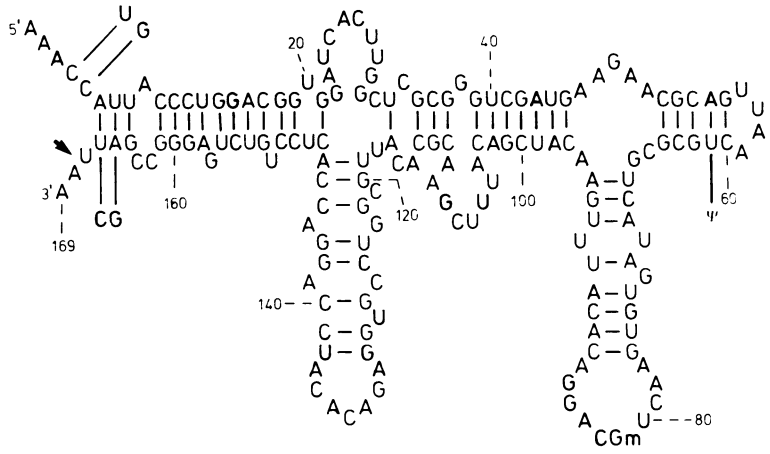

Fig. 2. Secondary structure model for Philosamia cynthia ricini 5.8S rRNA. The model was drawn according to the proposed Bombyx RNA structure, however, we want to point out that several isolated base pairs (e.g., G_{21}:C_{148} and U_{33}:A_{117} are not expected to be stable. Nucleotides outside the sequence are those occurring in Bombyx mori RNA. The arrow indicates the 3'end of Bombyx 5.8S rRNA.

Philosamia cynthia ricini 5.8S rRNA secondary structures can be seen in the stem formed by the 5' and 3' ends: The outermost three base pairs present in the Philosamia RNA sequence are absent in the Bombyx RNA structure (9). Consequently, the Philosamia 5.8S rRNA is slightly different from the Bombyx RNA sequence as far as size, nucleotide sequence near the 5' and 3' ends, modification of nucleotide No. 61 and secondary structure at the 5'/3' ends are concerned. These differences, however, should not be used to discuss the phylogenetic relations among Philosamia and Bombyx since they are too small and since 5.8S rRNA sequences do not reliably reflect phylogenetic relations as noticed by Darlix and Rochaix (15).

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