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The structure of the 5 S ribosomal RNA of a member of the phylum of green non-sulfur bacteria and relatives

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The 5 S rRNA sequence was determined for the bacterium *Herpetosiphon* strain Senghas Wie 2. It is the first 5 S RNA sequence reported for a member of the eubacterial phylum defined by green non-sulfur bacteria. The sequence fits into a consensus secondary structure model for eubacterial 5 S RNA. At four positions, the sequence shows substitutions with respect to strongly conserved nucleotides found in other hitherto examined eubacterial 5 S RNAs.

Green nonsulfur bacteria; 5 S rRNA; Nucleotide sequence; Secondary structure; Molecular evolution

1. INTRODUCTION

A division of the eubacterial primary kingdom into 10 phyla has been proposed on the basis of 16 S rRNA oligonucleotide catalogs of over 400 species [1] and on the basis of complete 16 S rRNA sequences of 12 species [2–5]. The molecular systematics thus obtained differs substantially from the bacterial classification presented in the 8th edition of Bergey's manual [6]. It seems worthwhile to have independent confirmation of the bacterial systematics based on the structure of another universally occurring molecule such as 5 S rRNA. Although 5 S RNA sequences are known for 109 eubacteria [7], the distribution of the examined species over the 10 proposed phyla [1] is very uneven, as illustrated in table 1. Here, we present the first 5 S RNA sequence determined for a bacterium belonging to the green non-sulfur bacteria and relatives. The organism concerned, strain Wie 2 [8], though not validly named as yet,

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Table 1

Distribution of published 5 S RNA sequences over 10 eubacterial phyla

Phylum[a]	Number of species examined[b]
Gram-positive bacteria	34
Purple photosynthetic bacteria and relatives	67
Sulfate respirers and relatives	0
Spirochetes and relatives	0
Bacteroides, Flavobacteria, Cytophagas and relatives	0
Cyanobacteria	4
Green sulfur bacteria	0
Green non-sulfur bacteria and relatives	0
Radio-resistant bacteria and relatives	4
Planctomyces and relatives	0

[a] As defined in [1]

[b] The number of published sequences outnumbers the numbers of examined species due to sequence heterogeneity reported for several species. Most of the sequences are compiled in [7]. Sequences from chloroplast and mitochondrial 5 S RNAs are not included

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will hereafter be referred to as *Herpetosiphon* strain Senghas Wie 2, in agreement with [1,9].

2. MATERIALS AND METHODS

Approx. 0.25 g lyophilized cells of *Herpetosiphon* strain Senghas Wie 2 were suspended in 50 mM Tris-chloride, 10 mM $MgCl_2$ (pH 7.6) and ground in a mortar with 0.5 g Al_2O_3. Ribosomal RNA was obtained by phenol extraction and the 5 S RNA fraction was isolated by polyacrylamide gel electrophoresis [10]. The yield was about 130 A_{260} units rRNA, from which 0.72 A_{260} units 5 S RNA were obtained. Labeling of the 5 S RNA at the 3′-terminus by ligation of [5′-^{32}P]pCp followed by electrophoresis on 8% polyacrylamide gels revealed chain length heterogeneity. Two bands were cut out and sequenced separately. Peattie's partial chemical degradation method [11] and partial nuclease digestion [12] of the 3′-labeled material, followed by electrophoresis on 8% polyacrylamide gels kept at a constant temperature of 65°C, made it possible to elucidate the entire sequence.

3. RESULTS AND DISCUSSION

The nucleotide sequence derived for the 5 S RNA of *Herpetosiphon* strain Senghas Wie 2 is presented in fig.1 in the form of a secondary structure model. A consensus secondary structure for eubacterial 5 S RNAs has been derived in [13]. The main difference between the model of fig.1 and the consensus model is that helix B is extended by several base pairs at the expense of internal loop I_1, and this helix is assumed to bear two bulges instead of one. It has been shown [14] that such a model can be devised for most eubacterial 5 S RNAs, as well as for archaebacterial 5 S RNAs, but not for eukaryotic 5 S RNAs. In the present case the additional bulge postulated on the 5′-proximal strand of helix B could occupy 4 alternative positions and possibly be subject to bulge migration [15]. Alternative base pairing opportunities that exist in most 5 S RNA structures in loops I_1 and H_1 are indicated on the model. It has been postulated [15,16] that alternative base pairing in area I_1-C may result in a universal secondary structure equilibrium in 5 S RNA. However, in *Herpetosiphon* strain Senghas Wie 2 5 S RNA the

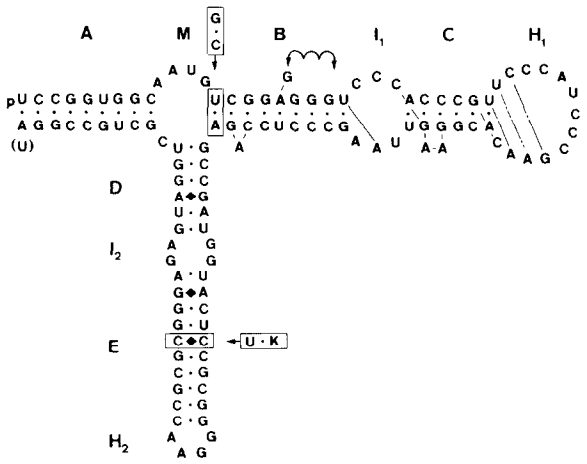

Fig.1. Secondary structure model for the 5 S RNA of *Herpetosiphon* strain Senghas Wie 2. Helices are labeled A–E, loops are labeled M (multibranched), I_1, I_2 (internal), and H_1, H_2 (hairpin). The sequence shows length heterogeneity at the 3′-terminus, with the U residue between parentheses present in submolar amounts. Odd base pairs (pairs other than G·C, A·U and G·U) are indicated by a lozenge replacing the conventional dot. Lines connecting bases within loops I_1 and H_1 reflect potential base pairing that may lead to equilibrium structures described elsewhere [15,16]. The possible movement of a bulge alongside helix B, indicated by a two-headed arrow, is described as bulge migration in [15]. The positions enclosed in boxes contain nucleotides different from those conserved at the 90% level in the consensus model for eubacterial 5 S RNA [13]. The nucleotides usually present at these positions are shown in the boxes fitted with an arrow (K = U or G).

base pairs that would be involved in such an alternative structure are not adjacent, which casts some doubt on the aforementioned hypothesis.

An inventory has been made [13] of bases conserved at the 90% level in eubacterial 5 S RNAs. The following exceptions to this pattern of conservation are found in the 5 S RNA of *Herpetosiphon* strain Senghas Wie 2. The ultimate base pair of helix B adjoining loop M is 5′-U·A-3′ instead of 5′-G·C-3′. The 6th base pair of helix E counting from loop H_2 is C-C instead of 5′-G·U-3′ or U-U. In both respects the sequence is unique among hitherto examined eubacterial 5 S RNAs.

On the basis of 16 S rRNA oligonucleotide catalogs [1,9] the genus *Herpetosiphon* was placed in a bacterial phylum named green non-sulfur

bacteria, which also comprises species belonging to the genera *Chloroflexus* and *Thermomicrobium*. This view is in contradiction with the classification used in Bergey's manual [6], where *Herpetosiphon* is considered as a genus of the family Cytophagaceae. Preliminary comparisons of phylogenetic trees based on 5 S RNA sequences [17,18] with the bacterial phylogeny based on 16 S RNA catalogs [1] show points of resemblance, but also points of conflict. A fully fledged comparison will only be possible after 5 S RNA sequences have been examined for a sufficient number of species from each of the phyla (table 1) proposed on the basis of 16 S RNA catalogs.

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