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Short communication

The 5S ribosomal RNA gene from the early diverging protozoa *Trichomonas vaginalis*☆

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Trichomonas vaginalis is a parasitic amitochondriate protozoa, etiologic agent of human trichomonosis. Phylogenetic analyses based on rRNA and actin sequences place this organism among the earlier eukaryotic branches [1,2]. This proposal is consistent with other trichomonad ribosomal features that are reminiscent of prokaryotes [3].

The typical eukaryotic ribosome is composed of ribosomal RNA (rRNA) and proteins, organized into a large subunit (LSU, 60S) and a small subunit (SSU, 40S). The rRNA in the SSU is 18S, while the LSU contains the 28S, 5.8S and 5S-type rRNAs. The 18S, 5.8S and 28S rRNAs are generally all encoded in a ribosomal main transcription unit and are transcribed by RNA polymerase I. In contrast, the 5S rRNA is transcribed by RNA polymerase III and is not generally linked to the rDNA unit. The 5S rRNA is found in almost every organism with the exception of some plant, fungi and protist mitochondria [4]. The function of the 5S rRNA has not been entirely elucidated but it has important roles in translational fidelity, ribosome biogenesis, peptidil-transferase activity and LSU stability [5].

In trichomonads, the sedimentation coefficient of ribosomes is 70S and the ribosomal SSU and 5.8S RNAs are smaller than in a typical eukaryote, thus trichomonads ribosomes have features of eubacterial ribosomes [3]. How far this eubacteria-like nature of trichomonads ribosomes extends is not yet known. The most recent studies on the trichomonad ribosome have focused on the rDNA unit [3,6] and little is known about structural similarity of the various rRNAs or their transcriptional signals. Of special interest is the analysis of the genomic organization and type of RNA polymerase involved in transcription of each of the rRNA species.

The aim of this work was to characterize the *T. vaginalis* 5S rRNA gene to determine its relatedness, in nucleotide sequence and in potential folding, to eukaryotic and bacterial 5S rRNAs. We also aimed at finding whether the 5S rRNA gene possessed a potential RNA polymerase III promoter. The latter was of particular interest because although RNA polymerase I and RNA polymerase II promoters for *T. vaginalis* have been documented [6,7], no RNA polymerase III promoter has yet been identified in this early diverging organism.

Our initial but unsuccessful approach to identify the 5S rRNA gene in *T. vaginalis* involved biochemical and molecular biology techniques [8]. During this time, the partially assembled sequence for the *T. vaginalis* genome became accessible at the *T. vaginalis genome sequence project* (The Institute for Genomic Research, www.tigr.org/tdb/e2k1/tvg). An in silico search for the 5S rRNA gene was then carried out using the Blast program incorporated in the web page. Various 5S rRNA sequences from prokaryotic and eukaryotic organisms were used as probes [9]. Blast search parameters were modified to reduce stringency. In this way about 30 contigs of the *T. vaginalis* genome were identified which contained potential 5S rRNA related sequences. Among these, two different repeated sequences (of 334 and 335 bases each) were identified and selected for further in silico analysis. In each repeat sequence a putative 5S rRNA coding region of 118 bp within a conserved region of 234 bp

Abbreviations: b, base; bp, base pairs; ICR, internal control region; LSU, ribosomal large subunit; rDNA, ribosomal DNA; rRNA, ribosomal RNA; S, Svedberg sedimentation units; sse, start site element; upe, upstream promoter element

☆ *Note:* Nucleotide sequence data reported in this paper are available in the GenBank™ database under the accession number DQ029070.

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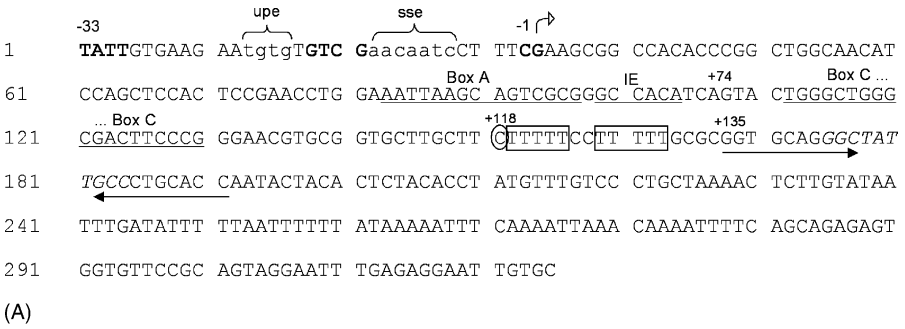
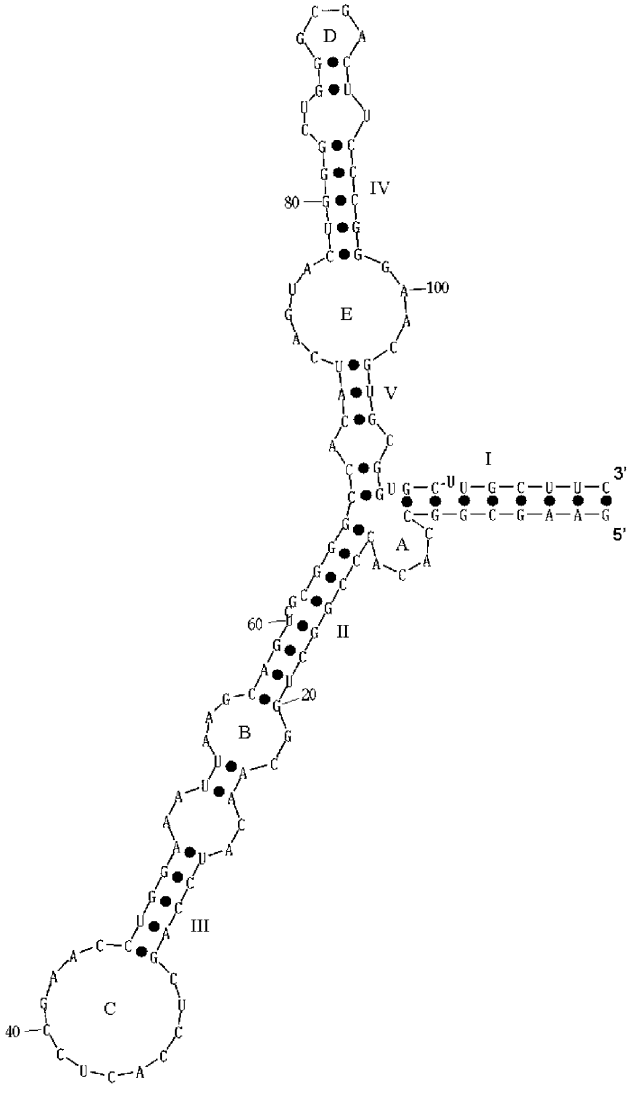


(A)



(B)

Fig. 1. (A) *T. vaginalis* 5S rRNA gene and putative RNA polymerase III promoter elements within the coding region. The non-template chain is shown. The three conserved upstream regions of 5S rRNA genes are denoted in bold (an A + T rich element at position −32, G + C rich region at −13 and a C at −1). The proposed first transcribed base (G) is marked with an empty-headed arrow and the putative last nucleotide of the mature molecule is shown circled (C in position +118). Small caps correspond to sequences similar to the *Saccharomyces cerevisiae* upstream promoter element (upe: 5′-TGCG-3′) and start site element (sse: 5′-AACTATC-3′) [11]. Underlined sequences correspond to the putative RNA polymerase III type I internal control region (ICR): Box A [5′-N(G/C)(C/T)(C/T)AANCNNNNNNN-3′], intermediate element [IE: 5′-(C/G)NN(G/A)(G/A)N-3′] and Box C [5′-NNG(G/A)TGGGNG(T/A)CCN(C/T)NNG-3′]. The –AGUA– motif characteristic of eukaryotic 5S rRNA corresponds to positions +74 to +77. Empty boxes reckon T-runs as putative termination sequences. Black-headed arrows indicate a palindromic sequence and, within this and in italics, a sequence similar to the nuclear factor 1 (NF1) binding site (5′-YTGGCANNNTGCCAR-3′) [10]. The first 234 bases were conserved between the two *T. vaginalis* 5S rRNA genes identified, while the remaining 101 bases were minimally 61% identical (not shown). (B) Predicted mature *T. vaginalis* 5S rRNA secondary structure. Helixes are denoted by roman numerals and loops are named by capital letters. dG of the secondary structure = −33.1 kcal/mol. The sequence was folded with Zuker's *mfold* software (http://www.bioinfo.rpi.edu./applications/mfold/old/rna).