HOMOLOGIES IN EUKARYOTIC 5.8S RIBOSOMAL RNA

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The primary nucleotide sequence of Novikoff hepatoma ascites cell 5.8S rRNA (also known as 5.5 or 7S RNA) has been determined to be:

p(C)-G-A-C-U-C-U-U-A-G-C-G-G-U(m)-G-G-A-U-C-A-C-U-C-G-
(U-G-U-C-G-C-G)-G-C-U-C-G-A-U-G-A-A-G-A-A-C-G-C-A-G-C-
G-C-U-A-G-C-(ψ)-G-C-G-A-G-A-A-U-U-A-A-U-G-ψ-G-A-A-U-U-
Gm-C-A-G-G-A-C-A-C-A-U-U-G-A-U-C-A-U-C-G-A-C-A-C-U-U-C-
G-A-A-C-G-C-A-C-U-U-G-C-G-G-C-C-C-C-G-G-G-U-U-C-C-U-C-
C-C-G-G-G-G-C-U-A-C-G-C-C-U-G-U-C-U-G-A-G-C-G-U-C-G-C-U.

This sequence is 75% homologous with the primary nucleotide sequence of yeast 5.8S rRNA and 100% homologous with oligonucleotide marker fragments from HeLa cell RNA. In contrast, only limited homology is evident with oligonucleotides from 5.8S RNA of several flowering plants and many of the characteristic fragments differ.

INTRODUCTION

The larger ribosomal subunit of eukaryotic cells contains two low molecular weight RNA species, 5S and 5.8S rRNA (1-6) in equimolar ratios to the 28S rRNA molecule; the 5.8S rRNA is hydrogen bonded to the high molecular weight component (2-6). The primary nucleotide sequence of yeast (Saccharomyces cerevidiae) 5.8S rRNA has been determined (7-8), but only preliminary studies have been reported on 5.8S RNA from higher organisms. These include flowering plants (9-10), HeLa cell (11), wheat embryo (12) and Novikoff ascites hepatoma (13-15) RNA.

The present report describes the tentative primary nucleotide sequence of Novikoff hepatoma 5.8S rRNA and its sequence

homologies with other 5.8S rRNA molecules. Oligonucleotide comparisons between 5.8S rRNA from different species of flowering plant (10) have previously suggested a highly conserved sequence during their evolution. This report indicates that as much as 75% homology exists between such widely divergent species as yeast and man.

MATERIALS AND METHODS

Novikoff hepatoma ascites cells were labeled in vitro with ^{32}P orthophosphate (16) and the RNA was extracted from either whole cells or ribosomes with sodium dodecylsulfatephenol at 65°C (6). Low molecular weight RNA was separated on 10-40% sucrose density gradients and 5.8S rRNA fractionated by electrophoresis on 10-12% polyacrylamide gel slabs (14).

Complete pancreatic or T_1 RNase digests; 1:20 w/w, enzyme: substrate (17), were fractionated by two-dimensional electrophoresis or column chromatography as previously described (14) and the oligonucleotide sequences were deduced by the methods of Sanger and Brownlee (17). Overlapping oligonucleotide fragments obtained by partial digestion at 0°C with pancreatic, T_1 or U2 RNase, 1:500-10,000 (w/w), enzyme:substrate (7,17), were separated by two-dimensional electrophoresis on 14-20% polyacrylamide gel slabs (18). Their sequences were deduced by analysis of their complete pancreatic and T_1 RNase digestion products (17). Modified nucleotides were detected and characterized as previously reported (14).

RESULTS

The primary nucleotide sequence of Novikoff ascites hepatoma 5.8S rRNA (Figure 1) was deduced by overlapping fragments obtained from complete pancreatic or T_1 RNase digests (13-15)

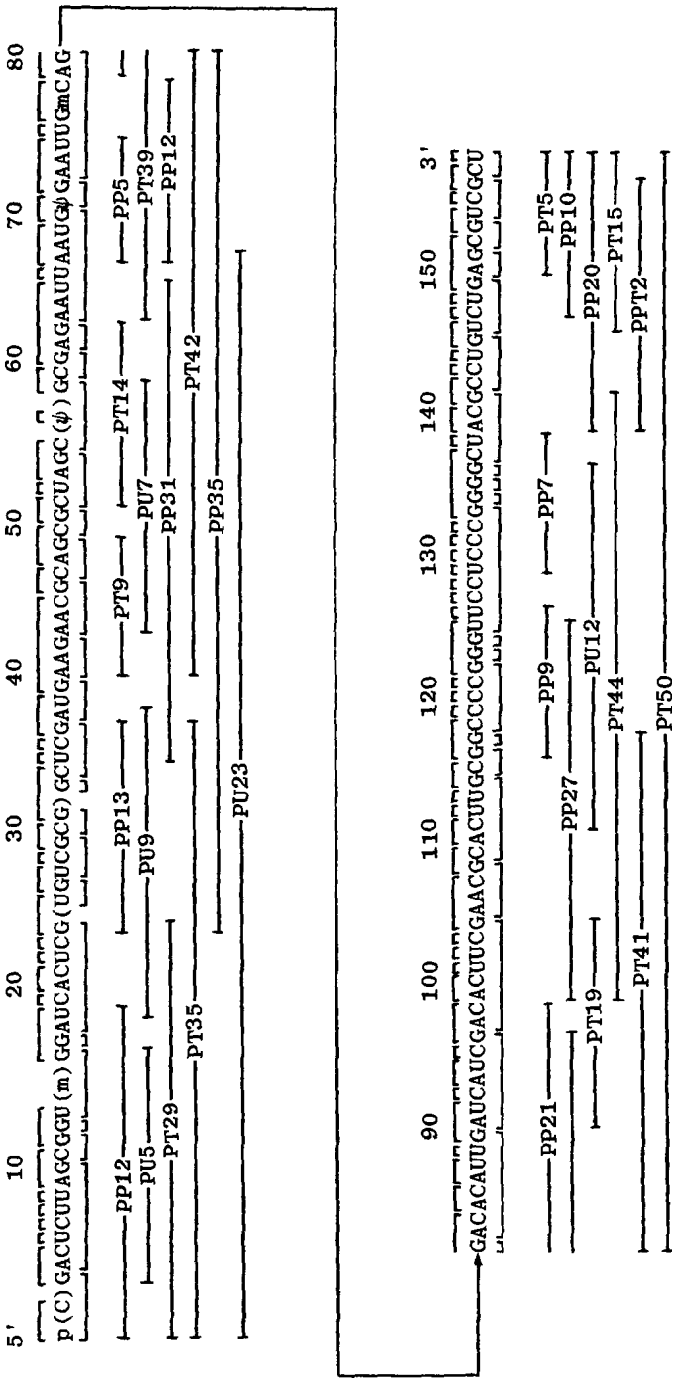

Figure 1 The primary nucleotide sequence of Novikoff ascites hepatoma 5.8S rRNA. Products of complete pancreatic RNase digestion are designated by square brackets above the sequence; the T_1 RNase digestion products are designated by brackets below the sequence. Fragments from limited pancreatic, T_1 or U_2 RNase digestion are numbered in order of increasing chain length with the prescript PP, PT and PU, respectively.

and partial pancreatic, T_1 or U_2 digests. This RNA molecule contains 158 nucleotide residues, including four modified nucleotides. Two of the sequences containing modifications, A-A-U-U-Gm-C-A-G (residues 74-80) and ψ-Gp (residues 71-72), are present in molar amounts. A second alkali stable dinucleotide, Um-Gp (residues 15-16), is only found in about 30% of the molecules (14) and the oligonucleotide C-U-Gp is modified to C-ψ-Gp (residues 57-59) in about 50% of the molecules as was reported for HeLa cell 5.8S rRNA (11). Although the 3' terminus, $-U_{OH}$ of hepatoma 5.8S rRNA is homogeneous and not phosphorylated, the 5' terminus is heterogeneous; the major termini are pCp and pGp (14). At this time only the sequence between nucleotides 25 and 31 remains uncertain (Figure 1).

Figure 2 compares the nucleotide sequence of Novikoff hepatoma 5.8S rRNA to that of yeast (7,8) and to the sequences of published oligonucleotide fragments from 5.8S rRNA of flowering plants (9,10) and HeLa cells (11). In Figure 2 the shaded areas indicate homologous sequences between the hepatoma and yeast. Since the actual positions of oligonucleotides from HeLa and bean cell RNA are unknown, their positions are suggested based upon the best homologies possible. In each case the proposed sequences are identical to the hepatoma RNA.

The homology between hepatoma and yeast 5.8S rRNA is striking. Both molecules are 158 nucleotides long; 102 of the 158 base residues are in identical sequences. A total of 116 nucleotides or about 75% of the total sequence can be directly superimposed overall. Furthermore, several of the changes retain a common feature, e.g., Cp is replaced by the alternate pyrimidine Up in nine positions, Ap is replaced by the alternate purine Gp in seven positions and the doublet G-Gp replaces

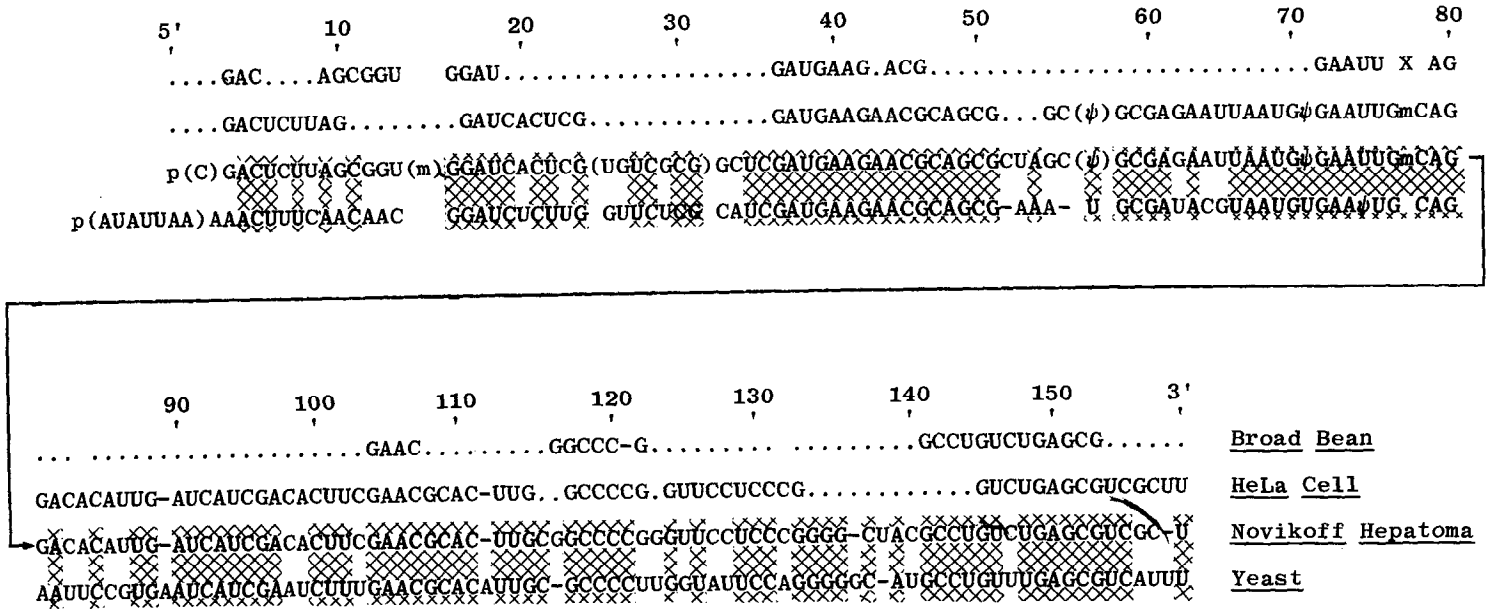

Figure 2 A comparison of complete and partial nucleotide sequences of various 5.8S rRNA species. The base residues are numbered as in Figure 1. Shaded boxes signify identical nucleotide sequences in Novikoff hepatoma and yeast (7,8) 5.8S rRNA and the dashes represent gaps in the sequences created by directly superimposing the homologous residues. The dotted lines represent unknown sequences in HeLa cell (11) and broad bean (9,10) 5.8S rRNA which join the homologous oligonucleotides.

A-Ap in yeast at nucleotides 12-13 and U-Up at nucleotides 122-123. The most interesting differences are associated with modified nucleotides and the 5' terminus. Neither pseudouridylic acid residue in the hepatoma RNA corresponds to the single residue in yeast although they are all located in the center of the molecule. As previously reported (7), both alkali stable dinucleotides are not found in yeast although the A-A-U-U-Gm-C-A-Gp (residues 73-80) sequence appears in a completely homologous unmethylated oligonucleotide A-A-U-U-G-C-A-Gp.

DISCUSSION

Among the four sequences, two common structural features present in yeast appear to be conserved; the G-A-A-Cp sequence at positions 43 to 46 and an arm formed by nucleotides 115 to 137. Although the function of these regions is unclear, Nishikawa and Takemura (19) have postulated that the G-A-A-Cp sequence may be involved in tRNA binding and Rubin has previously commented that the arm formed by nucleotides 115 to 137 is unusually stable (7). The present comparison focuses further on the importance of these structures.

The heterogeneity (8,14) and sequential differences in the 5' termini of yeast and Novikoff hepatoma 5.8S rRNA may be significant with respect to the synthesis of this rRNA. 5.8S rRNA is derived from 45S nucleolar ribosomal precursor as a cleavage product of the 32S nRNA intermediate (2,11,15). The heterogeneity indicates a relatively relaxed processing mechanism and since the terminal sequences differ markedly, cleavage from the 5' end is probably limited by the secondary structure of the RNA or hindered by ribosomal proteins rather than being sequence specific. Terminal studies on other 5.8S species may be helpful in this respect.

Although the comparisons are tentative, the homology of Novikoff hepatoma and HeLa cell 5.8S rRNA appears virtually complete. The sequences of oligonucleotide markers obtained by T_1 RNase digestion of HeLa cell RNA (11) are identical to those in the Novikoff hepatoma. The two-dimensional T_1 RNase digestion map of HeLa cell 5.8S RNA is also identical with the exception of the 3' terminus which is C-U-U_{OH} rather than C-U_{OH} in the hepatoma and the partially methylated G-G-Um-G-A-A-Up sequence which does not appear to be methylated in HeLa cells. Preliminary studies of two-dimensional fingerprints of RNase digestion products indicate that normal rat liver 5.8S rRNA may also have a virtually identical primary sequence suggesting a highly conserved sequence in mammalian cells and only about 25% change in their evolution from primitive unicellular eukaryotes.

The homology with flowering plants is considerably less when oligonucleotides from pancreatic or T_1 RNase digests are compared for 100% homology. Furthermore, many of the longer unique oligonucleotides from flowering plant 5.8S rRNA (9,10) could not be matched with either the yeast or hepatoma sequence. It appears that far greater change has occurred in the evolution of plants, although greater homology may become apparent when a complete plant 5.8S sequence is known.

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