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The complete nucleotide sequence of the rat 18S ribosomal RNA gene and comparison with the respective yeast and frog genes

Richard Torczynski, Arthur P.Bollon and Motohiro Fuke

Department of Molecular Genetics, Wadley Institutes of Molecular Medicine, 900 Harry Hines Boulevard, Dallas, TX 75235, USA

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ABSTRACT

The complete nucleotide sequence of the rat 18S ribosomal RNA gene has been determined. A comparison of the rat 18S ribosomal RNA gene sequence with the known sequences of yeast and frog revealed three conserved (stable) regions, two unstable regions, and three large inserts. (A,T) → (G,C) changes were more frequent than (G,C) → (A,T) changes for three comparisons (yeast → frog, frog → rat, and yeast → rat). GC pairs were inserted preferentially over AT pairs for the same three comparisons. These two factors contribute to the progressively higher GC content of 18S ribosomal RNA of yeast, frog, and rat.

INTRODUCTION

The ribosomal RNA (rRNA) genes are essential genes existing in all life forms from bacteria to man and they have been subjected to extensive analysis. Nucleotide sequence comparison of rRNA genes of different organisms should provide insight into evolutionary trends. There are two high molecular weight RNA components (18S rRNA and 25-28S rRNA) in eukaryotic ribosomes, and 18S rRNA sequences are known to be more highly conserved than the other high molecular weight rRNA sequences (1, 2).

Complete nucleotide sequences of 18S rRNA genes of *Saccharomyces cerevisiae* (3) and *Xenopus laevis* (4) have been reported recently. The present study reveals the first complete nucleotide sequence of a mammalian 18S rRNA gene (rDNA) and analyzes the rat sequence compared with the yeast and frog sequences.

MATERIALS AND METHODS

The characterization of rat rDNA cloned in lambda bacteriophages and the recloning of the rDNA in plasmid pBR322 were previously described (5). Plasmid pDF40 was used for sequencing the 3'-terminal region of the rat 18S rDNA (6) and plasmid pDF15 was used for sequencing the 5'-terminal region

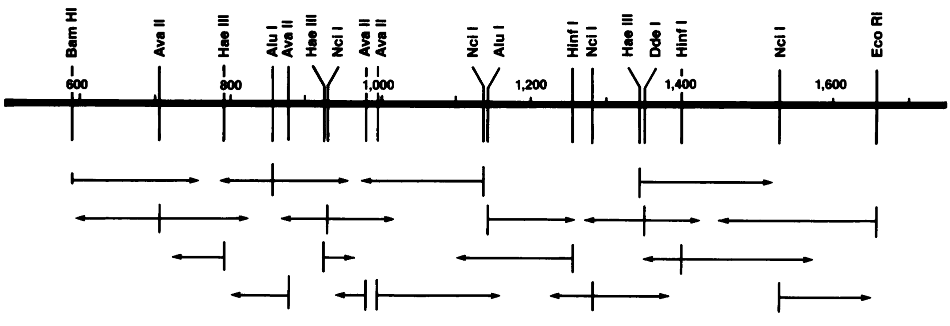

Figure 1. Strategy of sequencing the central portion of the rat 18S rDNA. Numbers reflect the distances from the 5'-terminus. Only the restriction sites that were utilized for sequencing are shown. Arrows indicate the directions of sequencing.

(7). Plasmid pDF8, which contains the central region of the rat 18S rDNA, was used to complete the sequencing of the 18S rDNA. Restriction endonucleases were purchased from BRL (Rockville, MD). DNA digestions with restriction endonucleases and gel-electrophoresis were performed as described previously (8, 9). DNA was sequenced by the method developed by Maxam and Gilbert (10) with minor modifications. In addition to the 5'-terminal labeling, in some cases, the 3'-termini of the fragments digested with restriction enzymes were labeled with α-^{32}P-dNTP and reverse transcriptase. The strategy employed for sequencing the central portion of the rat 18S rDNA is represented in Fig. 1.

RESULTS

An example of the sequencing gels is shown in Fig. 2. The region in Fig. 2 includes large T1 RNase fragment 8 (UUUUCAUUAAUCAAG) that was sequenced previously using the rat 18S rRNA (11). The total length of the rat 18S rDNA is 1869 bp and the nucleotide sequence determined is shown in Fig. 3 together with the yeast and frog sequences for comparison.

All the 19 large T1 RNase fragments, of which sequences were previously reported (11), were located in the rat 18S rDNA sequence in Fig. 3. The nucleotide sequences determined for these RNA fragments agree with the DNA sequence except for fragment 15, the sequence of which was cited from the work of other investigators who used HeLa cell 18S rRNA (12). Fragment 15 contains a hypermodified nucleoside identified as 1-methyl-3-γ-(α-amino-α-carboxypropyl) pseudouridine (13). The nucleotide sequence of fragment 15

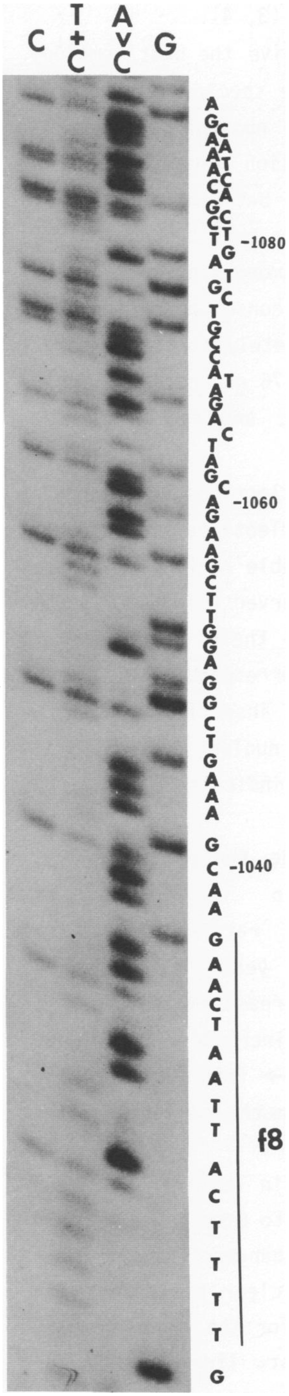

Figure 2. A typical sequencing gel. Numbers show the distances from the 5'-terminus (Fig. 3). The Ava II digested fragment was labeled with ATPγP^{32} at the 5'-termini and secondarily digested with Hae III. The electrophoresis was performed in a 6% polyacrylamide gel.

(AC^{am}ψCAACACG) is conserved among yeast, frog, and rat (3, 4).

In Fig. 3, the three sequences are arranged to give the best homology and the nucleotides that are common to all the three species are in bold letters. The whole sequence was sectioned every 10 nucleotides and the numbers of the nucleotides conserved in every section were plotted in Fig. 4. The sequence comparison was done in two ways: between frog and rat (top) and among the three species (bottom). It was known that the nucleotide sequence of the 3'-terminal region is well conserved among many species (6, 14, 15). This study shows that two additional larger conserved regions are located inside of 18S rDNA (Fig. 4). The three completely conserved large regions are: region A (nucleotides 424-499 in Fig. 3, 76 nucleotides long), region B (nucleotides 600-665, 66 nucleotides long), and region C (nucleotides 1837-1885, 49 nucleotides long).

Region U1 (nucleotides 181-357 in Fig. 3, 177 nucleotides long and 42% conserved) and Region U2 (nucleotides 690-805, 116 nucleotides long and 33% conserved) were identified as two evolutionarily unstable regions which are larger than 100 nucleotides and are less than 50% conserved (Fig. 4). Salim and Maden (4) identified four variable regions from their comparison of yeast and frog sequences. Two of the four regions correspond to U1 and U2 in this study. Unstable Region U1 contains two large insertions indicated by arrows in Fig. 4. Another large insertion, 10 nucleotides long, is located from nucleotides 1053 to 1062 in Fig. 3 and is indicated by an arrow in Fig. 4.

Table 1 summarizes base composition data of the three 18S rRNA's. The total number of nucleotides increases in the order of yeast ⟶ frog ⟶ rat, and the difference between rat and frog (44 nucleotides) is larger than the difference between yeast and frog (36 nucleotides). The numbers of U and A in 18S rRNA decrease in the order of yeast ⟶ frog ⟶ rat and the numbers of C and G increase. Hence, GC content in 18S rRNA increases in the order of yeast ⟶ frog ⟶ rat. The GC content difference between rat and frog (1.8%) is much smaller than the difference between yeast and frog (8.8%).

A more detailed analysis of base changes is shown in Table 2. From the data in Fig. 3, the number of base changes from A or T to G or C was counted (line [a] in Table 2) and the number of opposite base changes from G or C to A or T was also counted (line [b]). There is a clear preference for (A,T) ⟶ (G,C) changes over (G,C) ⟶ (A,T) changes for the three comparisons (yeast ⟶ frog, frog ⟶ rat, and yeast ⟶ rat, line [c]). Four

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           -10       -1 1                  20                            40                            60                            80
Yeast      TTAAGATAGT TATCTGGTTGATCCTGCCAGTAGTCATATGCTTGTCTCAAAGATTAAGCCATGCATGTCTAAGTATAAGCAATTTATACAGTGAAACTGC
Frog       GAAAGGTGGC TACCTGGTTGATCCTGCCAGTAG-CATATGCTTGTCTCAAAGATTAAGCCATGCACGTGTAAGTACGCACGGCCGGTACAGTGAAACTGC
Rat        TCGCGCTCCT TACCTGGTTGATCCTGCCAGTAG-CATATGCTTGTCTCAAAGATTAAGCCATGCATGTCTAAGTACGCACGGCCGGTACAGTGAAACTGC

                100                 120                 140                 160                 180
Yeast      GAATGGCTCATTAAATCAGTTATCGTTTATTTGA---TAGTTCC-TTTACTACATGGTATAACCGTGGTAATTCTAGAGCTAATACATGCTTAAAATCTC
Frog       GAATGGCTCATTAAATCAGTTATGGTTCCTTTGA---TCGCTCCATCTGTTACTTGG-ATAACTGTGGTAATTCTAGAGCTAATACATGCCGACGAGCGC
Rat        GAATGGCTCATTAAATCAGTTATGGTTCCTTTGTCGCTCGCTCC-TCTCCTACTTGG-ATAACTGTGGTAATTCTAGAGCTAATACATGCCGACGGGCGC

                200                 220                 240                 260                 280
Yeast      -GACCC---TT----T----GGAAGAGATGTATTTATTAGATAAAAAATCAAT---GTC-------T-----T------------------CGGAC-TC
Frog       TGACCCCC-----------AGGGATGCGTGCATTTATCAGACCAAAA-CCAATCCGG--GGCCCCC----GC---G-CC-----------CCGG-CCGC
Rat        TGACCCCCCTTCCCGTGGGGGGGACGCGTGCATTTATCAGATCAAAA-CCAACCCGGTCAGCCCCCTCCCGCTCCGGCCGGGGTCGGGCGCCGGACGGC

                300                 320                 340                 360                 380
Yeast      TTTGATGATTCATAATAACTTTTCG--AATCGCATGGCCTT-GTGCTGGCGATGGTTCATTCAAATTTCTGCCCTATCAACTTTCGATGGTAGGATAGTG
Frog       TTTGGTGACTCTAGATAACCTCGGGCCGATCGCACGTCC-CCGTGACGGCGACGATACATTCGGATGTCTGCCCTATCAACTTTCGATGGTACTTTCTGC
Rat        TTTGGTGACTCTAGATAACCTCGGGCCGATCGCACGCCCTCCGTGGCGGCGACGACCCATTCGAACGTCTGCCCTATCAACTTTCGATGGTAGTCGCCGT

                400                 420                 440                 460                 480
Yeast      GCCTACCATGGTTTCAACGGGTAACGGGGAATAAGGGTTCGATTCCGGAGAGGGAGCCTGAGAAACGGCTACCACATCCAAGGAAGGCAGCAGGCGCGCA
Frog       GCCTACCATGGTGACCACGGGTAACGGGGAATCAGGGTTCGATTCCGGAGAGGGAGCCTGAGAAACGGCTACCACATCCAAGGAAGGCAGCAGGCGCGCA
Rat        GCCTACCATGGTGACCACGGGTAACGGGGAATCAGGGTTCGATTCCGGAGAGGGAGCCTGAGAAACGGCTACCACATCCAAGGAAGGCAGCAGGCGCGCA

                500                 520                 540                 560                 580
Yeast      AATTACCCAATCCTAATTCAGGGAGGTAGTGACAATAAATAACGATACAGGGCCCATTCG-GGTCTTGTAATTGGAATGAGTACAATGTAAATACCTTAA
Frog       AATTACCCACTCCCGACGCGGGGAGGTAGTGACGAAAAATAACAATACAGGACTCTTTCGAGGCCCTGTAATTGGAATGAGTACACTTTAAATCCTTTAA
Rat        AATTACCCACTCCCGACCCGGGGAGGTAGTGACGAAAAATAACAATACAGGACTCTTTCGAGGCCCTGTAATTGGAATGAGTCCACTTTAAATCCTTTAA

                600                 620                 640                 660                 680
Yeast      CGAGGAACAATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGGTAATTCCAGCTCCAATAGCGTATATTAAAGTTGTTGCAGTTAAAAAGCTCGTAGTTGA
Frog       CGAGGATCTATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGGTAATTCCAGCTCCAATAGCGTATATTAAAGTTGCTGCAGTTAAAAAGCTCGTAGTTGG
Rat        CGAGGATCCATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGGTAATTCCAGCTCCAATAGCGTATATTAAAGTTGCTGCAGTTAAAAAGCTCGTAGTTGG

                700                 720                 740                 760                 780
Yeast      ACTTTGGGCCC-----GGTTGGCCGGTCCGATTTTTTCGTGTAC-TGGATTTCCAACGGGGCCTTTCCTTCTGGCTAACCTTGAGTCCTTGTGGCTCTTG
Frog       ATCTTGGGATCGAGCTGGCGGTCCGCCGCGAGGCG-GCTACCGCCTGTCCCAGCCCCT-G-CCTCTCGGCGCCTCCCCGATGCTCTTGACTGAGTGTCCC
Rat        ATCTTGGGAGCGGGCGGGCGGTCCGCCGCGAGGCGAGTCACCGCCCGTCCCCGCCCCTTG-CCTCTCGGCGCCCCCTCGATGCTCTTAGCTGAGTGTCCC
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                 800                 820                 840                 860                 880
Yeast    GCGAACCAGGAC--TTTTACTTTGAAAAAATTAGAGTGTTCAAAGCAGGC--GTATTGCTCGAATATATTAGCATGGAATAATAGAATAGGACGTTTGGT
Frog     G-GGGGCCCGAAGCGTTTACTTTGAAAAAATTAGAGTGTTCCAAGCAGGCC-GCGTCGCCTGGATACTTCAGCTAGGAATAATGGAATAGGAC-TCCGGT
Rat      GCGGGGCCCGAAGCGTTTACTTTGAAAAAATTAGAGTGTTCAAAGCAGGCCCGAGCCGCCTAGATACCGCAGCTAGGAATAATGGAATAGGAC-CGCGGT

                 900                 920                 940                 960                 980
Yeast    TCTATTTTGTTGGTTTCTAGGACCATCGTAATGATTAATAGGGACGGTCGGGGGCATCGGTATTCAATTG-TCGAGGTGAAATTCTTGGATTTATTGAAG
Frog     TCTATTTTGTTGGTTTTCGGAACTGGGGCCATGATTAAGAGGGACGGCCGGGGGCATTCGTATTGTGCCGCTAGAGGTGAAATTCTTGGACCGGCGCAAG
Rat      TCTATTTTGTTGGTTTTCGGAACTGAGGCCATGATTAAGAGGGACGGCCGGGGGCATTCGTATTGCGCCGCTAGAGGTGAAATTCTTGGACCGGCGCAAG

                 1000                1020                1040                1060                1080
Yeast    ACTAACTACTGCGAAAGCGTTTGCCAAGGACGTTTTCGTTAATCAAGAACGAAAGTTGAGGG----------ATCTGATACCGTCGTAGTCTTAACCATA
Frog     ACGAACCAAAGCGAAAGCATTTGCCAAGAATGTTTTCATTAATCAAGAACGAAAGTCGGAGGTTCGAAGACGATCAGATACCGTCGTAGTTCCGACCATA
Rat      ACGGACCAGAGCGAAAGCATTTGCCAAGAATGTTTTCATTAATCAAGAACGAAAGTCGGAGGTTCGAAGACGATCAGATACCGTCGTAGTTCCGACCATA

                 1100                1120                1140                1160                1180
Yeast    AACTATGCCGACTAG--ATCGGGTGGTGTTTTTTTAATGACCCACTCGGTACCTTACGAGAAATCAAAGTCTTTGGGTTCTGGGGGGAGTATGGTCGCAA
Frog     AACGATGCCGACTAGCGATCCGGCGGCGTTATTCCCATGACCCGCCGAGCAGCTTCCGGGAAACCAAAGTCTTTGGGTTCCGGGGGGAGTATGGTTGCAA
Rat      AACGATGCCGACTGGCGATGCGGCGGCGTTATTCCCATGACCCGCCGGGCAGCTTCCGGGAAACCAAAGTCTTTGGGTTCCGGGGGGAGTATGGTTGCAA

                 1200                1220                1240                1260                1280
Yeast    AGGCTGAAACTTAAAGGAATTGACGGAAGGGCACCACTAGGAGTGGAGCCTGCGGCT-AATTTGACTCAACACGGGGAAACTCACCAGGTCCAGACACAA
Frog     AG-CTGAAACTTAAAGGAATTGACGGAAGGGCACCACCAGGAGTGGAGCCTGCGGCTTAATTTGACTCAACACGGGAAACCTCACCCGGCCCGGACACGG
Rat      AG-CTGAAACTTAAAGGAATTGACGGAAGGGCACCACCAGGAGTGG-GCCTGCGGCTTAATTTGACTCAACACGGGAAACCTCACCCGGCCCGGACACGG

                 1300                1320                1340                1360                1380
Yeast    TAAGGATTGACAGATTGAGAGCTCTTTCTTGATTTTGTGGGTGGTGGTGCATGGCCGTTTCTCAGTTGGTGGAGTGATTTGTCTGCTTAATTGCGATAAC
Frog     AAAGGATTGACAGATTGATAGCTCTTTCTCGATTCTGTGGGTGGTGGTGCATGGCCGT-TCTTAGTTGGTGGAGCGATTTGTCTGGTTAATTCCGATAAC
Rat      ACAGGATTGACAGATTGATAGCTCTTTCTCGATTCCGTGGGTGGTGGTGCATGGCCGT-TCTTAGTTGGTGGAGCGATTTGTCTGGTTAATTCCGATAAC

                 1400                1420                1440                1460                1480
Yeast    GAACGAGAC--CTT-AACCTA-CTAAATAGTGGTGCTAGCATTTGCTGGTTATCC----ACTTCTTAGAGGGACTATCGGT-TTCAAGCCGATGGAAGTT
Frog     GAACGAGACTCCTCCATGCTAACTAGTTACGCGACCCCCG-GCGGTCGGC-GTCC---AACTTCTTAGAGGGACAAGTGGCGTTCA-GCCACACGAGATC
Rat      GAACGAGACTCTGGCATGCTAACTAGTTACGCGACCCCCGAGCGGTCGGC-GTCCCCCAACTTCTTAGAGGGACAAGTGGCGTTCA-GCCACCCGAGATT

                 1500                1520                1540                1560                1580
Yeast    TGAGGCAATAACAGGTCTGTGATGCCCTTAGAACGTTCTGGGCCGCACGCGCGCTACACTGA-CGGAGCCAGCGAGTCT--AACCTTGGCCGAGAGGTCT
Frog     -GAG-CAATAACAGGTCTGTGATGCCCTTAGA-TGTCCGGGGCTGCACGCGCGCTACACTGAACGGATC-AGCGTGTGTCTACCCTGCGCCGACAGGTGC
Rat      -GAG-CAATAACAGGTCTGTGATGCCCTTAGA-TGTCCGGGGCTGCACGCGCGCTACACTGACTGGCTC-AGCGTGTGCCTACCCTACGCCGGCAGGCGC
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                  1600                1620                1640                1660                1680
                   '         '         '         '         '         '         '         '         '         '
Yeast     TGGTAATCTTGTGAAACTCCGTCGTGCTGGGGATAGAGCATTGTAATTATTGCTCTTCAACGAGGAATTCCTAGTAAGCGCAAGTCATCAGCTTGCGTTG
Frog      GGGTAACCCGCTGAACCCCGTTCGTGATAGGGATCGGGGATTGCAATTATTTCCCATGAACGAGGAATTCCCAGTAAGTGCGGGTCATAAGCTCGCGTTG
Rat       GGGTAACCCGTTGAACCCCATTCGTGATGGGGATCGGGGATTGCAATTATTCCCCATGAACGAGGAATTCCCAGTAAGTGCGGGTCATAAGCTTGCGTTG

                  1700                1720                1740                1760                1780
                   '         '         '         '         '         '         '         '         '
Yeast     ATTACGTCCCTGCCCTTTGTACACACCGCCCGTCGCTAGTACCGATTGAATGGCTTAGTGAGGCCTCAGGATCTGCTTAGAGAAGGG-GG-CAAC-TCCA
Frog      ATTAAGTCCCTGCCCTTTGTACACACCGCCCGTCGCTACTACCGATTGGATGGTTTAGTGAGGTCCTCGGATCGGCCCCGCCGGGGTCGGCC-ACGGCCC
Rat       ATTAAGTCCCTGCCCTTTGTACACACCGCCCGTCGCTACTACCGATTGGATGGTTTAGTGAGGCCCTCGGATCGGCCCCGCCGGGGTCGGCCCACGGCCT

                  1800                1820                1840                1860                1880
                   '         '         '         '         '         '         '         '         '
Yeast     TCTC--AGAGCGGAGAATTTGGACAAACTTGGTCATTTGGAGGAACTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATTA AAAGA
Frog      TGGCGGAGCGCCGAGAAGACGATCAAACTTGACTATCTAGAGGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATTA ACGAG
Rat       TG-CGGAGCGCTGAGAAGACGGTCGAACTTGACTATCTAGAGGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATTA ACGGA
                                                                                                              '
                                                                                              3'-terminus

Yeast     AATTTAATAATTTTGAAAATGGATTTTTTTGTTTT
Frog      ACCCCCCTCACCCGGAGAGAGGGAAGGCGCCCGCC
Rat       GAAGGCCGAGGGGGGTCGTCGTCCCGTCCCTCTTG
                   '         '         '
                  1900                1920
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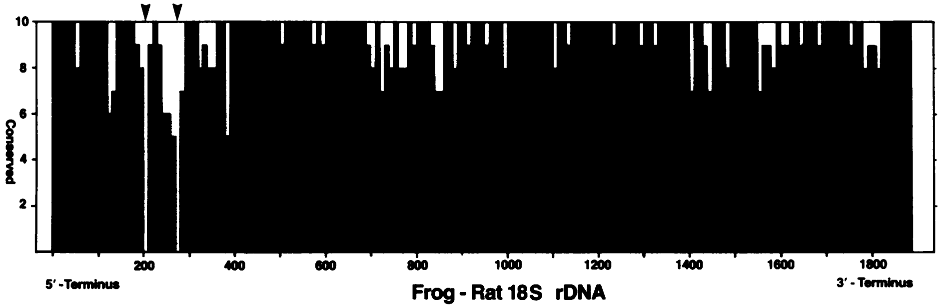
Figure 3. Nucleotide sequence of Sprague-Dawley rat 18S rDNA and adjacent transcribed spacers with sequences of yeast *Saccharomyces cerevisiae* (3, 28, 29) and frog *Xenopus laevis* (4, 30, 31) 18S rDNA and adjacent spacers. Bases were numbered from the 5'-terminus and the upstream transcribed spacer region was numbered with negative numbers. The 3'-terminus was at nucleotide 1885 (the total length of the rat 18S rDNA was 1869 bp without dashes in this figure) and the adjoining spacer region was numbered under the sequences. The three sequences were arranged to give the best homology and dashes were added to fill spaces. But, slight ambiguities remained. For example, locations of T bases of yeast sequence or locations of G and C bases of frog sequence between nucleotides 250 and 280 (Fig. 3) were arbitrary. Bases which were common to the three species were typed with bold letters. Four portions, in which six or more consecutive A or T bases of yeast sequence changed mainly to G or C bases for frog and rat sequences, were shown with frames.



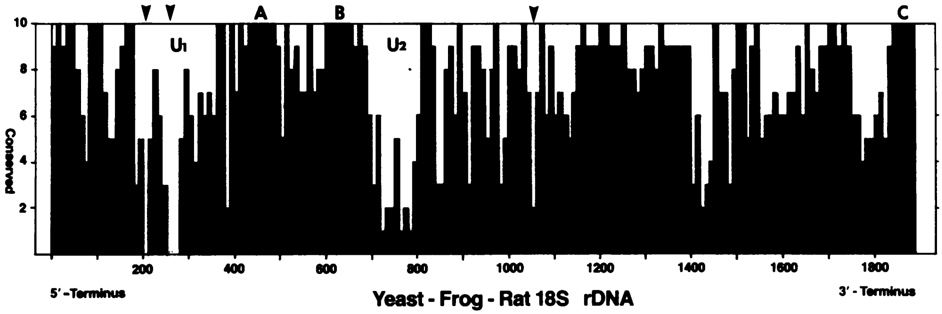


Figure 4. Conserved sequence analysis of 18S rDNA between frog and rat (top) and among the three species, yeast, frog, and rat (bottom). Sequences in Fig. 3 were sectioned every 10 nucleotides from the 5'-terminus and the numbers of the conserved nucleotides were plotted along the sequence. The 3'-terminal 5 nucleotides, conserved completely, were plotted as fully conserved with a half width. Three long completely conserved regions (A, B, and C) were marked. Arrows show positions of large (10 nucleotides or more) inserts. U1 and U2 are two unstable regions.

Table 1. 18S rRNA base composition data

	No. of nucleotides	U	A	C	G	%G+C
Yeast	1789	509	475	347	458	45.0
Difference	36	98	43	120	57	8.8
Frog	1825	411	432	467	515	53.8
Difference	44	3	11	30	28	1.8
Rat	1869	408	421	497	543	55.6
Total Difference[1]	80	101	54	150	85	10.6

[1] Total difference is the difference between yeast and rat.

Table 2. 18S rDNA base change data[1]

		Yeast ► Frog	Frog ► Rat	Yeast ► Rat
[a]	(A,T) → (G,C)	217	46	224
[b]	(G,C) → (A,T)	70	21	65
[c]	[a]/[b]	3.1	2.2	3.4
[d]	(G,C) insertion	46	35	81
[e]	(A,T) insertion	17	12	14
[f]	[d]/[e]	2.7	2.9	5.8
[g]	(A,T) deletion	19	2	10
[h]	(G,C) deletion	8	1	5
[i]	[g]/[h]	2.4	2	2

[1] Base changes were counted in Fig. 3.

portions of the genes where six or more consecutive A or T bases of yeast sequence are changed mostly to G or C bases are framed in Fig. 3. There is another clear preference for insertions of G or C over A or T for the three comparisons (lines [d], [e], [f]). GC rich insertions in the 5'-domain of rabbit 18S rRNA that are absent in both yeast and frog 18S rRNA were also pointed out by Lockard *et al.* (16). Though the numbers are small, there are also selective deletions of A or T over G or C (lines [g], [h], [i]).

DISCUSSION

In addition to other genetic systems that have been utilized for the analysis of evolutionary trends (17, 18), the 18S rDNA sequence is an excellent system to study evolutionary trends among various organisms. Because the complete sequence information of 18S rDNA is limited to only three species, it is not easy to discuss their evolutionary relationship. But, these sequences will serve as an introduction to a detailed phylogenetic analysis in the future. Recently, Eckenrode and Meagher (personal communication) have determined the complete 18S rDNA sequence of soybean. It is only 1807 nucleotides long and quite different from the rat sequence. The other high molecular weight rRNA, 25S rRNA for yeast and 28S rRNA for rat, is more variable than 18S rRNA and would be suitable to study evolutionary trends between closer species. Nucleotide sequences of 25S rDNA were compared between two yeasts, *S. cerevisiae* (19) and *S. carlsbergensis* (20), and 16 base differences were observed between the two species (21).

The present study shows that there are three completely conserved large regions in the 18S rRNA (Fig. 4). These conserved regions are considered to be functionally important. Possibilities of base-paired interactions between the 3'-terminal region C (Fig. 4) of 18S rRNA and 5S RNA and between the 3'-terminal region of 18S rRNA and the 5'-terminal region of many eukaryotic mRNA's have been suggested (14, 15). Functions for internal conserved regions A and B (Fig. 4) have not been suggested previously.

Two main factors are recognized which may have contributed to the evolution of 18S rRNA. The first is a preference of point mutations of (A,T) ⟶ (G,C) compared to mutations of (G,C) ⟶ (A,T). The second is preferential insertions of (G,C) over (A,T). Point mutations are more frequent in the yeast ⟶ frog comparison than in the frog ⟶ rat comparison, but insertions are introduced almost to the same degree in these two cases. The only large T1 RNase fragment found to be different between rat and human 18S rRNA is fragment 12 (ACCCCCCUUCCCG) (22). This fragment is missing in human 18S rRNA and a large portion of this rat fragment (CUUCCCG) is located in the inserted region between frog and rat, nucleotides 199-205 (Fig. 3). We can expect some difference between rat and human 18S rRNA due to insertions. Fragment 12 is also missing in chicken 18S rRNA (11).

The closer relatedness of the rat and *Xenopus* 18S rDNA sequences compared with the *Saccharomyces cerevisiae* sequence is consistent with the contrast between the organization of rat and *Xenopus* rDNA repeat units, which have the 5S and 45S rRNA information separated (23), and the *S. cerevisiae* rDNA repeat unit, which contains both the 5S and 37S rRNA information together (21, 24). Nevertheless, the 5S rDNA is not a part of the repeat unit containing the 37S rDNA precursor in *Neurospora crassa* (25) and *Schizosaccharomyces pombe* (26).

Comparing the base compositions of the 18S rRNA's, the total differences of pyrimidines between yeast and rat were about twice the total differences of purines (Table 1). In structural genes, because of wobble of the third bases of triplets, pyrimidines have more freedom for change than purines without changing the corresponding amino acids. The fact that pyrimidines changed about twice more than purines between yeast and rat might lead to a speculation that 18S rRNA functions, or functioned, as mRNA at some stage before maturation or at some evolutionary stages. But, the present mature *X. laevis* 18S rRNA is considered to be unsuitable as a template for protein synthesis (27).

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