Nucleotide sequence of the region between the 18S rRNA sequence and the 28S rRNA sequence of rat ribosomal DNA

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ABSTRACT

The DNA sequence of the intragenic region of the rat 45S ribosomal RNA precursor was determined. This sequence contains 2282 nucleotides and extends from the conserved EcoR I site near the 3' terminus of 18S rRNA to 69 nucleotides downstream of the 5' terminus of 28S rRNA. The sequences corresponding to 18S and 5.8S rRNA were identified by comparison with previously published data. The 5' terminus of rat 28S rRNA was identified by S1 nuclease protection and reverse transcriptase elongation assays. The internal transcribed spacers were found to be 1066 and 765 nucleotides long and had little homology with those of Xenopus and yeast. Regions of sequence homology between rat and Xenopus were found at the junctions of the internal transcribed spacers with 18S, 5.8S and 28S rRNA. These homologies suggest that these sequences may function as recognition sites for the processing of the ribosomal precursor RNA.

INTRODUCTION

Ribosomal RNA is synthesized as a 45S preribosomal precursor and processed to yield the 18S, 5.8S and 28S ribosomal RNA molecules (1.2). The fidelity of formation of these products implies that there is a stringent requirement for the recognition of the termini of the preserved portions of the initial transcript. The nucleotide sequences of these processing points may provide the information required by these reactions in a manner analogous to that which has been hypothesized for the excision and ligation events involved in the processing of mRNA precursors (3). То evaluate processing sites, a portion of a cloned rat ribosomal DNA repeat was sequenced from the 3' terminus of 18S RNA to the 5' terminus of 28S RNA. This region contains four processing We have used the terms of Hall and Maden (4) to define sites. the spacer between the 18S and 5.8S RNA sequences and the spacer between the 5.8 and 28S RNA sequences as ITS1 and ITS2, the first and second internal transcribed spacers, respectively.

The portion of the rat rDNA sequenced was examined for regions of interest and was compared with the available analogous portions of the <u>Xenopus</u> (4), yeast (5,6,7), <u>Bombyx</u> (8) and <u>Lytechninus</u> (9, and Hindenach and Stafford, personal communication).

MATERIALS AND METHODS

p59 is a fragment of ChR-B4 (10) bordered by EcoR I and BamHI sites, cloned in the vector pBR322. This subclone has been characterized by both restriction endonuclease digestion and Southern hybridization analysis and was shown to contain the 3' end of 18S RNA, 5.8S RNA and the 5' end of 28S rRNA (10). Starting at the EcoR I site near the 3' end of 18S rRNA the nucleotide sequence was determined by the method of Maxam and Gilbert (11). The 5' end of 28S rRNA was defined both by S1 endonuclease mapping (12) using a BstN1-Hinf I fragment labeled at the Hinf I site and by reverse transcriptase elongation (13) using a Sau 3A-Hinf I fragment labeled at the Hinf I site (Fig. 1). The sequence was analyzed by using the computer program of Korn and Queen (14).

RESULTS

Sequence determination. The DNA sequenced contained the 3' end of 18S, ITSI, 5.8S, ITSII and the 5' end of 28 rRNA coding regions and the sequencing strategy is shown in Fig. 1. The 2282 nucleotides of the sequence are shown in Fig. 2. The regions of mature ribosomal RNAs are indicated as boxed regions. Approximately 93% of the sequence was confirmed by sequencing both The sequence between nucleotides 1604 and 1750 was constrands. firmed by sequencing twice in the same direction and had no ambi-All of the restriction sites used in obtaining the guities. sequence were confirmed by sequencing through these sites. There were some ambiguities when fragments with runs of "G" and "GA" These are indicated with wavy lines in Fig. 2. were sequenced. In these regions only the number of repeated nucleotides varied in different sequencing runs. Thus the number of "G"s starting at nucleotide 675 was found to be 8 or 9; the sequence at 1811 could be G4TG50r G4TG6; and the run of 11 "GA" residues ending



Figure 1 The structural organization of the rat ribosomal DNA repeat. The expanded portion of the map describes the subcloned EcoR I- Bam HI fragment that contains the intragenic region. The restriction sites that are relevant for the sequence determination only are indicated. Arrows indicate the direction and extent of the sequence obtained starting from a restriction site. * represents the two fragments used in the S1 mapping and reverse transcriptase elongation assay.

at nucleotide 1906 could be 10. The exact numbers of these nucleotides could not be confirmed from the complimentary strand sequence.

Location of the mature ribosomal RNA regions in the sequence. The terminal fifty nucleotides of the 3' end of 18S rRNA of rat were determined by Azad and Deacon (15). This sequence agreed with nucleotides 177-227 of the sequence presented in Fig. 2. It was concluded that nucleotides 1 through 227 constitute the 3' end of 18S rRNA. Comparison with the published sequence of rat 5.8S rRNA (16) showed nucleotides 1294 through 1449 to be the corresponding rDNA sequence. The location of 28S rRNA was first determined by comparison of the sequence of the rat rDNA with the Xenopus rDNA sequence (4).

The 5' terminal nucleotides of 28S rRNA were determined by S_1 nuclease mapping (12) and reverse transcriptase extension (13). The fragments used for this purpose were BstNI-Hinf I fragment or Sau 3A-Hinf I fragment both labeled at the Hinf I site (shown in Fig. 1). The sequence of Bst NI-Hinf I fragment which is complimentary to the 28S rRNA is shown in Fig. 3, as well as the results of S1 mapping and reverse transcriptase extension. From the se-

GAATTCCC	10	20	30	40	50	60	70
	Agtaagtgc	GGGTCATAAG	CTTGCGTTGA	TTAAGTCCC1	IGCCCTTTGT/	NCACACCGCCC	GTC
GCTACTAC	BO	90	100	110	120	130	140
	CGATTGGAT	GGTTTAGTGA	GGCCCTCGGA	TCGGCCCCCC	CGGGGTCGCCC	CACGGCCCTGG	CGG
AGCGCTGA	50	160	170	180	190	200	210
	Gaagacgtc	GAACTTGACT	Atctagagga	Agtaaaagto	GTAACAGGT	TTCCGTAGGTG	AAC
23	20	230	240	250	260	270	280
CTGCGGAA	Ggatcatta	Acggagaagg	CCGAGGGGGG	TCGTGCCCG1	CCCCTCTTG	GCCTGTGTGA	GTG
TTCCTCCT	90	300	310	320	330	340	350
	TCTCGCCGG	Gaggcgcgtc	CCCGGGTGGG	TCCCCGTGT	CCGGCGTCCT	GCGTCGTGGC	:GCG
3	60	370	380	390	400	410	420
Тоссотос	CCGGCCGGC	GGAGGGGGT1	TCGGGACAGG	TGTGCGGTG(GCAGGGTGTC	GGGTCTGGTCC	GCC
4	30	440	450	460	470	480	490
GCGGGACC	тсстссбтт	TTCTCGCTCT	TCCCTCGACG	CCTCCGTGC	GCCCGCCTCC	GCCTCCGCCGC	:TGC
5	00	510	520	530	540	550	560 ITS I
CGTCGGCA	GCGAGGGCG	TTCTGCCTC1	TTTCCCGACCG	GCTCCGTGA	ICTCGTGTGC	Accgggggtgg	
5	70	580	590	600	610	620	630
GTGATCTC	TCCGGGCGG	TGGCCGGGAG	CGCGCTCGCTC	TCCTCGAGG	CGCCTGCCGC	ICCTCCCCCGC	:CGC
6	40	650	660	670	680	690	700
GGCTCTCC	GGCACTGAC	GGTGCCGGTC	GCTCCGCCGC	GTGCGGT <u>GG</u>	GGGGGGGTGT	GTGTGCCGGTG	3CCG
7	10	720	730	740	750	760	770
CGGGTGGG	CTCGCGCGC	cccgccgcg1	ГССТТСТБББСС	CTCCGTGTC	CGCCCCGCTC	CTGTCCGGGT	ACCT
GCTCTCGC	80	790	800	810	820	830	840
	GTTCCGGCG	CGGAGGTTTC	CGCGACCCCGG	GGGGGGTCGC	CCTACCGTCC	CCGGGGTCGGG	3GGG
8	50	860	870	880	890	900	910
GCGGTGGG	GCCCGTAGO	GACGTCGGTC	CGTCCGCGGGGA	GGGCTTCCC	GGTCCCTCCC	CGCGTCCGGCC	3CTC
9	20	930	940	950	960	970	980
CCTCAGAC	TCAGCGGCG	CACCCCTCTC	SCCGCCGGCGC	TTCCCGAGG	CGGCGGTTCG	GGGGGGGCGTG1	rcgc
GAGCCCCC	90 1	000	1010 1	020	1030	1040	1050
	TCTGGGCGC	CCGAGGGGT	rcccggcccg a	GCGCCTGGC	TTGTCCCGCC	CGTTGGCGGGG	2000
10	60 1	070	1080 1	090	1100	1110 1	1 1 2 0
CGCCGGGT	GCCGGGTTC	GTGGCGTCCC	CGTGTCCCGTG	TGTCCCGCC	TTTCCGTCTC	CGACCCTGTC1	F T T T
11	30 1	140	1150 1	160	1170	1180 1	1 1 9 0
TTTTTTTA	TTTTCTTAC	GTGTGCCGT1	TTCGTTTCGCG	CTGGCCGGC	CTGAGGCGGA	GCCCCCTCGCC	CGTC
12	00 1	210	1220 1	230	1240	1250	1260
CGTCGGGC	CCCTCCCA1	CCCGCGCAG	GAGGGGGGGGGG	GGGGGGCGCT	TGTTGCGGTC	GTCAGCACCCC	CGTG
12	70	280	1290 1	300	1310	1320	1330
TGCGGAGT	TCCGCTCAC	ACCTCAGAT	AACCGTACGAC	TCTTAGCGG	TGGATCACTC	GGCTCGTGCG	FCGA 5.85
13	40	350	1360 1	370	1380	1390	1400
TGAAGAAC	GCAGCTAGO	TGCGAGAAT	TAATGTGAATT	GCAGGACAC	Attgatcatc	Gacacttcgai	ACGC
14	10	420	1430 1	440	1450	1460	1470
ACTTGCGG	CCCCGGGTT	CCTCCCGGGG	SCTACGCCTGT	CTGAGCGTC	GCTTGACGAT	CAATCGCCGC	CCCC
14	80	490	1500 1	510	1520	1530	1540
CTTGCGGT	TTCGCCGCC	GCGGGGGGGG	ATCGCGTCGCG	CGGCTGGGA	GTCTGCTCGC	Agggcccgct	CCCC
15	50	560	1570 1	580	1590	1600	1610
GGGGCCCT	CCGTCTCC	GAAGTTCCG	Acgtgggcggg	GTCCGGCGT	TCGCCCGCGC	CCCGTGGGTC	CGCG
16	20	630	1640 1	650	1660	1670	1680
CGGGCTCG	TCCTCCCT	CCCGCCGTC	GTCGTGCGGCC	GGGCGGCCC	TCCTCGCGGC	TCCTTCCCCG	CGCG
16	90	700	1710 1	720	1730	1740	1750
CTCCGCCG	CTCCCGCT	CCGTCCTCG	CCCGCTCTCCC	CCGCCGTGG	Gacgcgtcgg	CGGGGCCGGT	CGGG
17 TGCTGGGC	60 GCGGAGAG	770 GGGGGGGAAA	1780 1 GTCGCGTCGCC	790 GGGGTCGTT	1800 CGCCGCGCCT	1810 CCGGTGGGGT	175 II 1820 GGGG
18	30	840	1850 1	860	1870	1880	1890
GGCCGGGA	.cccccc <u>cc</u>	CGGGGGCCGG	CGCGTCCGCCC	CCGCCGCGC	GCCCGCCCGG	TGCGTGCGGA	GAGA
19		910	1920 1	930	1940	1950	1960
GAGAGAGA		CCGCGAGGG	TCGTTCGGGGG	GGCCGTACC	GCGCCCGCGG	GGCCGGTCTC	GCGT
19	70	980	1990	2000	2010	2020	2030
CCCTCTCC	TCCCCCGC	CCCTCTACC	GGGGCCGACCC	CTCCGGCGC	CGGCCCGCGG	CGCCGCGGCG	TCCG
20	40	050	2060 2	070	2080	2090	2100
TGCGCCGA	CGCGACTTO	CCCCCGGGA	TGTGGGGCCGG	CCGGCGAAG	Gagaggtcgc	GGGCTCCGGC	CTGG
21	10	120	2130 2	140	2150	2160	2170
CTCCCCCG	GCGCCCTCC	CCCCCGTCGT	CCCGTCGTTC1	CGCTCTCGC	TCTCCTCTCC	TCTCCTCTCC	TTCC
21	80	190	2200	210	2220	2230	2240
GTCGCCCG		ACCTCTCCT	CCTTCTCCTCC	TCTGGACCG	CGACCTCAGA	TCAGACGTGG	CGAC 285
22 CCGCTGAA	50 TTTAAGCA	260 Attagtcag	2270 2 CGGAGGAAAA	280 3444CT			

Figure 2

Sequence of the intragenic region of rat ribosomal DNA repeat. Boxed regions designate the 18S, 5.8S and 28S coding regions. The sequence shown is that of the noncoding strand of the DNA. The wavy lines indicate portions of the sequence where the number of nucleotides varied slightly from one sequence determination to the next. Maximum number of nucleotides determined are indicated in such instances. The arrow in 5.8S rDNA sequence indicates the location of the GC dinucleotide reported in the RNA sequence (16). The horizontal lines indicate the regions in the ITS that were greater than 75% homologous with regions of the ITS of Xenopus.



Figure 3

1

Determination of the 5' terminus of rat 28S rRNA. A Bst NI - Hinf I fragment (nucleotides 2103-2295) or Sau 3A - Hinf I fragment (nucleotides 2230-2295), both labeled at the Hinf I site using polynucleotide kinase were used in these experiments (see Fig. 1 for location of these fragments). Lane 1. Sau 3A - Hinf I fragment extended with reverse transcriptase after hybridization to 28S rRNA. Lane 2. Sau 3A - Hinf I fragment. Lane 3. Bst NI - Hinf I fragment hibridized to 28S and digested with S1 nuclease. Maxam and Gilbert sequence ladder of Bst NI - Hinf I fragment (GACT). This sequence corresponds to the coding strand of DNA. The non-coding strand sequence is written below the autoradiograph. *, indicates the 5' terminus of 28S rRNA. quence specificity of the restriction enzyme Sau 3A (GATC); the Hinf-Sau 3A fragment labeled at its Hinf site has the 3' end sequence terminating with CCACGTCT (Fig. 3; sequence written alongside the ladders). But this fragment when electrophoresed alongside of sequence ladders lines up between G and A, hence it moved $1\frac{1}{2}$ nucleotides slower (Fig. 3, Lane 2). This phenomenon has been described by Sollner-Webb and Reeder (25). Thus it was concluded after a $1\frac{1}{2}$ nucleotide correction that the reverse transcriptase extended fragment (Fig. 3, Lane 1) terminated at the last G in the sequence TCGCG. Hence the 5' end of 28S rRNA has the sequence CGCGA; locating the 5' end at nucleotide 2215 in the sequence shown in Fig. 2.

<u>The sequence of internal transcribed spacers.</u> In Fig. 2, nucleotides 228-1293 were defined as the first internal transcribed spacer (ITS I) and nucleotides 1450 through 2214 as the second internal transcribed spacer (ITS II). These sequences had several interesting features. The lengths of the internal transcribed spacers regions (Table I) were more than twice those of <u>Xenopus</u> rDNA (4). The G + C content of the spacer regions is about 20% higher than that found in rat rRNAs (1).

When either the G or C content of the internal transcribed spacers is considered, the probability of occurrence of seven contiguous "G" or "C" residues is approximately 1 in 1000 nucleo-However, there are seven such "G" "stretches" and no "C" tides. stretches 7 nucleotides long. Since the length of the rat internal transcribed spacers were twice as long as those of Xenopus, it was possible that this increase in length occurred through the endoreduplication of a portion of the sequence. Hence we analyzed the sequence for direct repeats. There are about 270 direct repeats between 7 and 14 nucleotides long. This number is an overestimate because overlapping sequences were not excluded from the analysis. Except for the "G" stretches, each direct repeat occurs only once. These sequences are scattered in the ITS sequences without any apparent order. Thus it appears that the rat ITS sequence did not increase in size due to the endoreduplication of a smaller region.

There are two sequences of unique nucleotide composition. One consists of 11 "T's" starting at nucleotide 1117 and the

TABLE I

TRANSCRIBED SPACERS									
	R/ ITS I	RAT ITS I ITS II		XENOPUS UTS I ITS II					
A	5.3	5.5	12 .5	4.9					
С	37.4	43.2	42.8	53.6					
G	37.1	36.8	41.1	34.3					
т	20.1	14.6	3.6	7.2					
Length	1067bp	765bp	559	256					

NUCLEOTIDE COMPOSITION RAT AND XENOPUS INTERNAL

TRANSCRIBED SPACERS

Base composition is expressed as percent of total

ITS I or ITS II nucleotides.

other consists of 10 or 11 alternating "GA" residues starting at nucleotide 1885. Comparison of the rat sequence with the sequence of the spacer region of <u>Xenopus</u> (4) resulted in the finding that there are several 10 nucleotide long sequences and thirty 20 nucleotide long sequences with greater than 75% homology between the two sequences. There are six 25 nucleotide long sequences with greater than 75% homology. These six sequences are underlined in Fig. 2. Homologous sequences between rat and <u>Xenopus</u> are scattered with no apparent order and do not suggest that these sequences have been conserved during evolution.

DISCUSSION

<u>Sequence homologies at the 3' end of 18S rRNA</u>. The last 20 nucleotides of the small subunit ribosomal RNA, 18S rRNA, are highly conserved both in eukaryotes and prokaryotes. except that the "CCTCC" sequence implicated in prokaryotic translation is missing in the eukaryotic ribosomal RNA molecules (18). Among the last 230 nucleotides of the 3' end of 18S rRNA compared in Fig. 4, there is greater than 90% homology among eukaryotes except the sequence from nucleotide 113 to nucleotide 158. A similar result was found when yeast 18S rRNA (5,6) and <u>Xenopus</u>

G A A T T C C C A G T A A G T G C G G G T C A T A A G C T T G C G T T G A T T A A G Rat X.laevis TTCCCAGTAAGTGCGGGTCATAAGCTCGCGTTGATTAAG T T C C T A G T A A G C G C A A G T C A T C A G C T T G C G T T G A T T A C G S. cervisiae TTCC-AGTAAGCGCGAGTCATAAGCTCGCGTTGATTACG B.mori TCCCTGCCCTTTGTA C A C A C C G C C C G T C G C T A C T A C C G A T T G G A T G G T C C C T G C C C T T G T A C A C C G C C C G T C G C T A C T A C C G A T T G G A T G G т С С С Т G С С Т Т Т G Т А С С А С С G С С G Т С G С Т А G Т А С С СА Т Т G А А Т G G TCCCTGCCCGTTGTAACCACA-CGCCCGTCGCTACTACCGATTGAATGA 113 T T T A G T G A G G C C C T C G G A T C G G C C C C C C G G G G T C G C C C A C G G C C C T G G C T T T A G T G A G G T C C T C G G A T C G G C C C C C C G G G G T C G G C C A C G G C C C T G G C CTTAGTGAGGCCTCAGGATCTGCTTAAGAAGGGGGG-CAACT-CCATCTC 158 G G A G C G C T G A G A A G A C G T C G A A C T T G A C T A T C T A G A G G A A G T A A A A G T C G G A G C G C C G A G A A G A C A T C A A A C T T G A C T A T C T A G A G G A A G T A A A A G T C -- A GAGCGG A G A A TT TGAC A A A C T T GGT C A TT TGG A G G A ACT A A A A G T C CGTT - - - GGGAAGTTACCAAACTTGATCATTTAGAGGAAGTAAAAGTC G T A A C A G G T T T C C G T A G G T G A A C C T G C G G A A G G A T C A T T A G T A A C A G G T T T C C G T A G G T G A A C C T G C G G A A G G A T C A T T A G T A A C A G G T T T C C G T A G G T G A A C C T G C G G A A G G A T C A T T A G T A A C A G G T T T C C G T A G G T – A A C C T G C G G A A G G A T C A T T A

Figure 4 Comparison of the 3' terminal sequences of 18S rDNA from rat, X.laevis (4), S.cerevisiae (5), and B.mori (8). Homologous sequences are boxed. The variable region lies between nucleotides 113 and 158.

18S rRNA were compared (4). From nucleotide 113 through nucleotide 158, the sequence of eukaryotic 18S rRNAs diverge from each other. In this variable region, rat and yeast have only 50% homology. The rat and <u>Xenopus</u> sequences are 92% homologous in this variable region, demonstrating that this region reflects the relative evolutionary distances between these organisms (26).

5.8S rDNA. The 5.8S rDNA sequence agreed well with the published 5.8S rRNA sequence (16) except that a GC dinucleotide reported in the RNA sequence was not found in the rDNA sequence. The location of this GC dinucleotide in Figures 2 and 5 is indicated by an arrow. Fig. 5 is an autoradiograph of a sequencing gel of this region of 5.8S rDNA. This same discrepancy was observed when <u>Xenopus</u> rDNA was sequenced (4,17). Rat 5.8S rRNA separates into two isomeric bands on 7 M urea gels (19). It is

possible that there are two different 5.8S rRNAs and that one sequence contains this GC dinucleotide, or there is an error in the 5.8S rRNA sequence.

<u>28S rDNA</u>. The 5' end of rat 28S rRNA has been reported to be heterogeneous (20). We were able to define the 5' end as one of two nucleotides in the rDNA sequence, nucleotide 2214 or 2215 (Fig. 3). The sequence of the first 70 nucleotides at the 5' end of rat 28S rRNA is highly homologous (95%) with <u>Xenopus</u>. Yeast and <u>Xenopus</u> are only 80% homologous in this region (7). The overall sequence homology between the mature rRNAs (the 3' end of 18S, 5.8S and 5' end of 28S rRNAs) of rat and <u>Xenopus</u> are more extensive (95-98%) than that of rat and yeast (75-80%) or yeast and

GACT 3' ACACATT AATTGCAGG 23 AATGTG . CGAATT 203 -AGCTG -CGCAGC -TGAAGAA r^{GCGTCGA} CTCGGCT 5

Figure 5 Nucleotide sequence of a portion of the 5.8S rDNA. А Hinf I fragment labeled at the 5' ends was cut with Dde I and the fragment corresponding to the non-coding strand of 5.8S rDNA was sequenced. The sequence written along side of the sequence ladders corresponds to the sequence between nucleotides 1314 to 1379 as shown in Fig. 2. The arrow indicates the position where the 'GC' dinucleotide reported to be present in 5.8S rRNA (16) would be found.

<u>Xenopus</u> (75-80%), which is consistant with the taxonomic distances of these species (26).

Internal transcribed spacer sequences. The rat ITS sequences are not homologous to those of Xenopus or yeast. This was not surprising, as in 1972 Brown et al (21) showed that there was little homology between the spacer sequences of two closely related Xenopus species. There are some characteristics of the ITS sequences that are specific for the ITS of each species and which distinguish them from the remainder of the gene. For example, in rat there are very few "A's" in the spacer sequence (5%, Table I), and there are "unique" sequences such as runs of "T' residues or "GA" residues, as were described earlier. A large number of "G strings" (7 consecutive G's) are found in the rat ITS, whereas there was a preference for "C strings" in the ITS of Xenopus (4). The yeast ITS is "U" rich (7). In both rat and Xenopus, the internal transcribed spacer regions are 75-80% "GC" (Table I) which is 15-20% more "GC" than the 60% of the mature rRNAs (1). It is not known whether any of these or any other features of the sequence make the spacer sequence a recognizable region for processing. It is also possible that there is some secondary structure conserved among the various spacer sequences. The high G + C content and the length of the ITS makes it difficult to select a significant secondary structure solely on the basis of computer simulation. As there was little sequence conservation within the internal transcribed spacers, it was remarkable that the sequences flanking the 3' termini of 5.8S and 18S apparently were conserved (Fig. 6). This observation suggests that these sequences are involved in processing.

When the DNA sequence immediately beyond the 3' end of 18S rRNA of rat was compared with the corresponding rDNA sequences of various species (Fig. 6A), the first three nucleotides of the internal transcribed spacer (ITS 1), were found to be identical in rat, <u>Xenopus</u> and <u>Bombyx</u>, i.e. A-C-G. The first five nucleotides were found to be A-C-G-Pu-Pu. In rat, <u>Xenopus</u> and <u>Lytechninus</u>, there is an A-G-A or G-A-G-A within these first six nucleotides. Beyond these five nucleotides, no consensus sequence was found.

The sequence similarity beyond the 3' end of 18S rRNA could have arisen by chance. The probability that an A-C-G triplet

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a)
   Rat
                    TTAACGGAGA AGGCCGAGGGGGGTC
   <u>B</u>.mori
                    TTA ACGGGTGATGGGAAGAAA
                     T T A A C G A G A C C C C C C C T C A C C C G G A
   X.laevis
   L.variegatus
                    TTACCGAGAGACAAGCACCGCACAC
   S.carlsbergensis
                    TTA <u>A G A A A T T</u> T A A T A A T T T T G A A A
   S. cervisiae
                    TTAA<u>AAGAAATT</u>A
                    T T A T T G T A T A A T A T C C T T A C C G T T A
   D.melanogaster
   E.coli
                     T T A C C T T <u>A A A G A A</u> G C G T A C T T T G C
   CONSENSUS
                         A C G PuPu
b)
   Rat
                    CGCTCACACCTCAGATAACCGTACGA
                     A C C G A C C G A C G C G T C G G C G A G A G C T C
   X.laevis
                    GATTTTCTTTCCGAGAATTGACACAA
   L.variegatus
   S.carlsbergensis
                    T T T T C G T A A C T G G A A A T T T T A A A A T A
c)
                    G C T T G A C G A T C A A T C G C C G C C C C C C
   Rat
                    TCC GACGTCC ATCGCCCCCCCCC
   X.laevis
                    G T C G T T C A T G C C A A T C G A G G G G A T C C
   L.variegatus
                    T T T C C T T C T C A A A C A T T C T G T T T G G T
   S. carlsbergensis
d)
                    C C T T C T C C T C C T C T G G A C C G C G A
   Rat
                    C C C C C C C C C C A C G A C T C A G A
   X.laevis
   S.carlsbergensis
                    GAACAATGTTCTTAAAGTTTGAC
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Figure 6 Comparison of the sequences of the internal transcribed spacers immediately adjacent to the 18S, 5.8S and 28S rRNA sequences of rat, <u>B.mori</u>, (8), <u>X.laevis</u> (4), <u>L.-variegatus</u> (9, and Hindenach and Stafford personal communication), <u>S.carlsbergensis</u> (6), <u>S.cerevisiae</u> (5), <u>D.melanogaster</u> (24) and <u>E.coli</u> (22). The arrow indicates in a) the 3' end of 18S rRNA, b) the 5' end of 5.8S rRNA, c) the 3' end of 5.8S rRNA and in d) the 5' end of 28S rRNA. Homologous sequences are indicated either by enclosing those sequences in boxes or by single or double lines under the sequences.

would be present immediately adjacent to the 3' end of 18S rRNA is 1/64, and that the same triplet would occur by random chance in three species of rDNA is $(1/64)^3$. In the consensus sequences shown in Fig. 2, the triplet ACG occurs at a frequency of 0.45. Thus, the consensus sequence appears to be a preferred or conserved sequence rather than one of random probability. Therefore, sequences similar to the consensus sequence A-C-G-Pu-Pu could be recognition sites for processing at the 3' end of 18S rRNA in higher eukaryotes. The sequences A-A-G-A-A or A-A-A found in E. coli and yeast could be involved in a similar way in prokaryotes.

The sequence of the terminal 20 nucleotides of 18S rRNA, which is more than 95% conserved in eukaryotes, could constitute a part of this recognition site for processing.

At the 5' end flanking regions of 5.8S ribosomal RNA (Fig.6B), there is very little sequence conservation.

The 3' end flanking region of rat 5.8S was compared with the sequence from <u>Xenopus</u> and sea urchin for possible homologies. In rat and Xenopus, G-A-C-G, A-T-C-G-C-C, and C-C were common sequences (boxed sequences in Fig. 6C). In rat and sea urchin, C-A-A-T-C-G is common in this region. Assuming that this homology is not a chance occurrence, at least in <u>Xenopus</u> and rat, the sequence at the 3' end of 5.8S and the corresponding flanking region could constitute a recognition sequence for processing.

The 5' end flanking regions of 28S rDNA of rat and <u>Xenopus</u> are similar (Fig. 6D). There is a pyrimidine stretch followed by G-A-C immediately 5' to the 28S coding sequences.

In yeast the 5' termini of precursor and mature rRNA, the 5.8S, 17S and 26S, share a consensus sequence (7). Similarly the 3' termini of 5.8S, 7S, 17S and 18S rRNA of yeast have a consensus sequence (6,7). Except for the conserved 3' end of 18S rRNA, the sequences at the termini of mature ribosomal RNAs of rat and yeast are different. Other consensus sequences were not found among the sequences of rat rRNA termini shown in Fig. 2.

Veldman <u>et al</u> (7) have proposed that in yeast the 3' end of 5.8S rRNA and 5' end of 26S rRNA could interact to form a secondary structure. A similar structure can be constructed for the 5' end of rat 28S rRNA and the 3' end of 5.8S rRNA:

> 5.85 $3'OH_{TCGCTGCGAGTCT-GTCCGCATCG--GGGCCCC}^{5'}$ 285 $5'pCGCGAC-CTCAGATCAGACGTGGCGACCCGCT_3,$

In the absence of further information on the processing sites that generate the secondary precursors and any evidence of definitive secondary structure interactions, our findings are consistant with the possibility that whether or not the secondary structure of the ribosomal RNA precursors are conserved, the nucleotide sequence of the processing sites are involved in generating the proper termini of the mature ribosomal RNA. The homologies in the regions which flank 18S, 5.8S and 28S rRNA are more pronounced in the comparisons of the rat and Xenopus sequences than when the other eukaryotic sequences were compared. This finding suggests that processing mechanisms may vary with phylogeny and that the higher vertebrates might share a common process.

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