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The nucleotide sequence of the putative transcription initiation site of a cloned ribosomal RNA gene of the mouse

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ABSTRACT

Approximately one kilobase pairs surrounding and upstream the transcription initiation site of a cloned ribosomal DNA (rDNA) of the mouse were sequenced. The putative transcription initiation site was determined by two independent methods: one nuclease S_1 protection and the other reverse transcriptase elongation mapping using isolated 45S ribosomal RNA precursor (45S RNA) and appropriate restriction fragments of rDNA. Both methods gave an identical result; 45S RNA had a structure starting from ACTCTTAG---.

Characteristically, mouse rDNA had many T clusters ($\geq$5) upstream the initiation site, the longest being 21 consecutive T's. A pentadecanucleotide, TGCCTCCCGAGTGCA, appeared twice within 260 nucleotides upstream the putative initiation site. No such characteristic sequences were found downstream this site. Little similarity was found in the upstream of the transcription initiation site between the mouse, Xenopus laevis and Saccharomyces cerevisiae rDNA.

INTRODUCTION

In eukaryotic cells, ribosomal RNA genes are repeated tandemly in the order of hundreds, one unit being composed of alternating gene and non-transcribed spacer regions (1-3).

The structure and function of ribosomal RNA genes are of special interest because they have different features than either messenger RNA genes, 5S RNA or tRNA genes. First, ribosomal RNA genes are transcribed by RNA polymerase I (or A), a special class of RNA polymerase concentrated in the nucleolus (4-6). Second, these genes appear to be under different control than other genes mentioned above as seen by the different temporal pattern of activation during development (7-10) as well as in hormon action (11-13). Different sensitivity to protein synthesis inhibition was also noted between these RNA species (14-19). However, whether some such regulation mechanism as the stringent control which

exists in Escherichia coli is operating in mammalian cells or not is yet to be established.

For the purpose of studying these aspects of gene regulation, we have cloned a 14.9 kb EcoRI fragment of mouse rDNA (20-22), which is supposed to contain the initiation region of 45S RNA and demonstrated that 45S RNA starts at a site approximately 4.0 kb upstream from the 5' end of the 18S RNA coding region (23).

Until recently, the only ribosomal RNA gene whose transcription initiation site is known at the nucleotide sequence level is that of Xenopus laevis (24,25). Nucleotide sequence between 5S RNA and 18S RNA of Saccharomyces cerevisiae was extensively determined but without information on the initiation site of 35S rRNA precursor (26). Recently, this initiation site has been determined by the analysis of the precursor molecule (27,28). Very little similarity, however, appears to exist in the nucleotide sequences preceding transcription initiation site between Xenopus and Saccharomyces.

Availability of the first mammalian —— and the third eukaryotic sequence surrounding the transcription initiation site of rRNA should permit one to compare and examine further sequences which might be significant for the regulation of ribosomal RNA genes.

In this study, we determined the nucleotide sequence surrounding and upstream the putative initiation site of a cloned ribosomal RNA gene of the mouse. The DNA site encoding the 5' end of 45S RNA was determined unequivocally by S_1 nuclease mapping and by reverse transcriptase extension method.

Several interesting features in the nucleotide sequence near the transcription initiation site of rDNA have been disclosed.

MATERIALS AND METHODS

Preparation of Recombinant Plasmid and DNA Restriction Fragments. The recombinant plasmid DNAs used were pMrEL-I and pMrSL-II. pMrEL-I carries the 14.9 kb EcoRI fragment within ribosomal DNA in pBR 322 (20,23). pMrSL-II was obtained by ligation of the 3.2 kb Sal I fragment within the 14.9 kb EcoRI fragment to pBR 322 digested with Sal I. The recombinant plasmids were propagated in E. coli χ1776.

The cloning of these recombinant DNA molecules and all the subsequent experiments involving recombinant DNA have been carried out according to the Guidelines issued by Japanese Government.

Plasmid DNA was prepared according to the described methods (29). After plasmid DNA was digested with restriction enzymes under recommended conditions, DNA fragments were separated by electrophoresis on 1% agarose gels or 5-10% polyacrylamide gels and extracted from agarose gels as described by Tabak and Flavell (30) or from polyacrylamide gels as described by Maxam and Gilbert (31).

Enzymes. EcoRI was purified by the procedure described by Yoshimori (32); Alu I, Hae II, Hae III, Hha I, Hinf I, Hpa II, Kpn I, Xho I and Pst I were purchased from Bethesda Research Laboratories; Bst Nl was purchased from New England Biolabs; Sal I and Sma I were purchased from Takara Syuzo. T_4 polynucleotide Kinase as purchased from P-L Biochemicals. S_1 nuclease was a gift from Dr. M. Sakai. T_4 ligase and reverse transcriptase were gifts from Dr. M. Takanami and Dr. J. Beard, respectively. Bacterial alkaline phosphatase was purchased from Worthington. The [γ-^{32}P]ATP (>5000 Ci/mmol) was purchased from New England Nuclear.

DNA Sequencing. DNA was sequenced as described by Maxam and Gilbert (31).

45S RNA Preparation. 45S RNA was prepared from Ehrlich ascites cells as described previously (33-35).

Mapping of Restriction Sites. Cleavage sites by restriction enzymes were determined by single and double digestions and essentially according to the procedure of Smith and Birnstiel (36) by using 3.5% polyacrylamide gel instead of agarose gel.

S_1 Nuclease Protection Mapping. 5' end-labeled double-stranded DNA (50 ng) was hybridized with 45S RNA (1.5 μg) in 20 μl of 70% formamide, 0.5 M NaCl, 1 mM EDTA, 0.04 M Pipes-NaOH (pH 6.8) at 56°C for 2 h after treatment at 90°C for 3 min. The solution containing the hybrid was diluted 20 fold with S_1 buffer (30 mM sodium acetate pH 4.5, 0.02 mM $ZnSO_4$, 0.2 M NaCl) and treated with S_1 nuclease at 37°C for 1 hr followed by ethanol precipitation (37,38). The precipitates were electrophoresed on

a 5% polyacrylamide gel.

5' end-labeled single-stranded DNA (50 ng) was hybridized with 45S RNA (1.5 μg) in 20 μl of 50% formamide, 0.72 M NaCl, 1 mM EDTA, 60 mM Pipes NaOH (pH 6.8) 0.1% sodium dodecylsulfate at 56°C for 2 hr after heating at 90°C for 3 min. The solution was diluted 20 fold with S_1 buffer and treated with S_1 nuclease for 1 h. The ethanol precipitates were subjected to 20% polyacrylamide -7 M urea gel electrophoresis after alkali denaturation.

Reverse Transcriptase Extension Mapping. The 5' end-labeled single-stranded DNA (10 ng) was hybridized with 45S RNA (1.5 μg) under the conditions described above and then precipitated with ethanol. The precipitates were dissolved in 30 μl of 0.1 mM dNTP, 0.05 M Tris-HCl (pH 8.3), 0.1 M KCl, 0.01 M $MgCl_2$, 0.01 M 2-mercaptethanol and treated with reverse transcriptase at 40°C for 30 min (24,39). The extended DNA was then phenol extracted and precipitated with ethanol. The DNA was denatured in 0.05 M NaOH, 0.5 mM EDTA and electrophoresed on 20% polyacrylamide-7 M urea sequencing gel.

RESULTS

DNA Sequence of the Transcription Initiation Region of Ribosomal RNA Gene. A map of the EcoRI cleavage sites within the mouse ribosomal DNA is shown in Figure 1-A. This was deduced from the results of Southern blotting hybridization of the total mouse DNA with various cloned fragments of rDNA, which will be reported elsewhere (Kominami et al. in preparation). We have cloned a 14.9 kb EcoRI fragment and roughly located the DNA region encoding the 5' end of 45S RNA within the 3.2 kb Sal I fragment by the method of S_1 nuclease protection mapping (Figure 1-B,C; 20,23).

For the purpose of determining the initiation site of the ribosomal RNA precursor, 45S RNA, at the nucleotide level and of characterizing this region in more detail, the 3.2 kb Sal I fragment was subcloned into plasmid pBR 322 (Data not shown), propagated and was subjected to the digestions by various restriction enzymes. The positions of the cleavage sites in the DNA were determined by either the double digestion with two enzymes or by

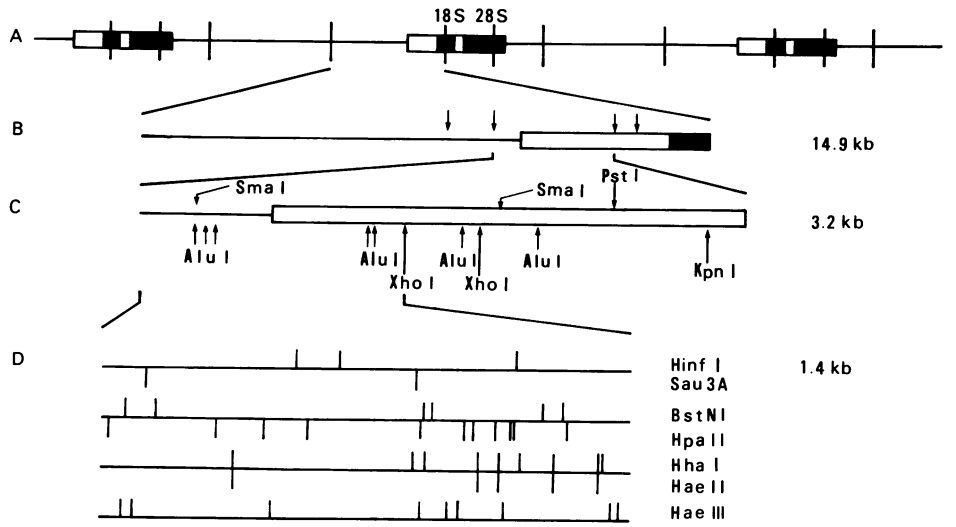


Fig. 1. Restriction map of rDNA. A: The horizontal line with boxes represents rDNA and the vertical lines show the EcoRI sites on rDNA. The filled boxes marked as 18S and 28S indicate the genes coding for 18S and 28S rRNA, respectively. The open boxes indicate the transcribed spacer region of 45S RNA. The line connecting boxes represents the non-transcribed spacer. B: The cloned 14.9 kb EcoRI fragment within rDNA. The open and filled boxes indicate 5' regions of 45S and 18S RNA, respectively. The horizontal line indicates the non-transcribed spacer region. The arrows show the Sal I cleavage sites. C: The 3.2 kb Sal I fragment within the cloned 14.9 kb EcoRI fragment. The horizontal line and the open box indicate the non-transcribed spacer and 45S RNA coding region, respectively. The arrows show the cutting sites by the indicated restriction enzyme. D: The 1.4 kb Sal I - Xho I fragment containing the starting site of 45S RNA. Cutting sites with various restriction enzymes are shown by the vertical lines.

the partial digestion of a 5'-terminally labeled fragment. With these enzymes, we could not find regular cutting arrangements in this spacer region except with Alu I enzyme, which cleaved three times at the left part of the 3.2 kb Sal I fragment.

Since the 5' end of 45S RNA was supposed to be located about 2.5 kb upstream from the right end of the 3.2 kb Sal I fragment (23), the left-hand 1.4 kb fragment produced by Xho I cleavage of the 3.2 kb Sal I fragment was mapped in more detail for the purpose of DNA sequencing (Figure 1-C). The rightward region of

this Xho I cutting site which was inside the transcribed region was cut at more sites by the enzymes, Hpa II, Hha I and Hae III than the left part which contained the non-transcribed region, suggesting a higher GC content of the transcribed region (Figure 1-D).

The region encoding the 5' terminus of 45S RNA was determined by S_1 nuclease protection mapping using the 470 nucleotide Hinf I fragment which was the second largest fragment within the 1.4 kb Sal I - Xho I, locating at the second from the right (Figure 1-D). The length of the DNA fragment protected from S_1 nuclease digestion should correspond to the distance from the nucleotide encoding the 5' end of the 45S RNA to the 5' end of the hybridized DNA. The Hinf I fragment was labeled with ^{32}P at 5' termini, hybridized to 45S RNA under the conditions of R-loop formation, treated with S_1 nuclease and analysed by polyacrylamide gel electrophoresis. As shown in Figure 2 the appearance of a fragment approximately 300 nucleotide long depended on S_1 nuclease treatment. The result indicates that the 5' terminus of 45S RNA starts at about 300 nucleotides upstream from the rightmost Hinf I site within the 1.4 kb Sal I - Xho I fragment (Figure 1-D).

960 nucleotides surrounding the starting site of 45S RNA transcription were sequenced by the method of Maxam and Gilbert (31). The sequencing strategy is shown in Figure 3-A. Most of the sequences were confirmed by determining in two opposite directions or more than twice in the same direction. The nucleotide sequence of the strand corresponding to 45S RNA is displayed in 5' to 3' direction in Figure 3-B. The transcription proceeds to the right. The non-transcribed region between nucleotide -400 and -1 was higher in A + T content than the transcribed region between +1 and +100. The former was 51% as compared with 37% of the latter. There were eight regions (-769 to -765, -561 to -557, -423 to -419, -326 to -312, -239 to -235, -233 to -226, -224 to -204 and -125 to -118) of T clusters longer than 5 nucleotides; the longest being 21 nucleotides (-224 to -204). The sequence of 15 nucleotides TGCCTCCCGAGTGCA was repeated twice (-258 to -244 and -141 to -127), each followed by a long T cluster (Figure 3-B).

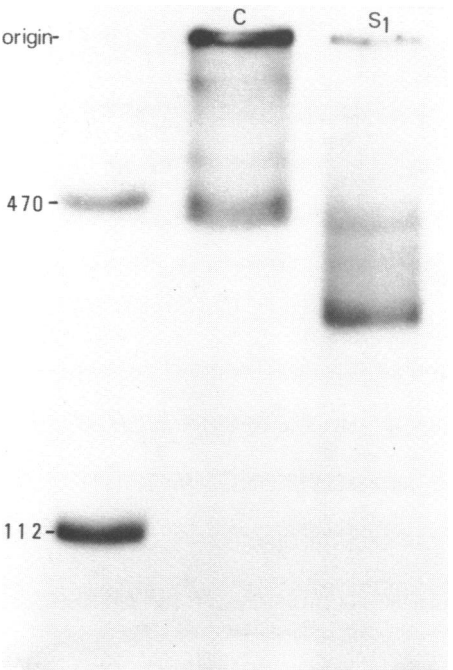
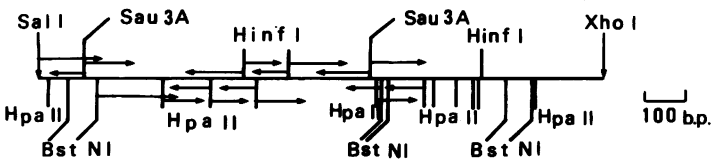


Fig. 2. A rough sizing of the fragment protected by 45S RNA. 5' terminally labeled Hinf I fragment was hybridized with 45S RNA under the conditions of R-loop formation and the hybrid was treated with S_1 nuclease. The protected fragment was electrophoresed on 5% polyacrylamide gel. The size marker, the hybrid not treated with S_1 nuclease (C) and the hybrid treated with S_1 (S_1) were run in the left, the middle and the right column, respectively.

Determination of the 5' End of 45S RNA on rDNA by S_1 Nuclease Protection Mapping. To locate the DNA site encoding the 5' end of the 45S RNA precisely at the nucleotide level, we first used the S_1 nuclease protection mapping with a comigrating Maxam-Gilbert ladder (31). The Hinf I - Hpa II fragment (-191 to +25) was labeled with ^{32}P at the 5' termini and the strands were separated by polyacrylamide gel electrophoresis after alkaline denaturation. The coding strand was hybridized to 45S RNA which had been purified from Ehrlich ascites cells and digested with S_1 nuclease.

The size and the location of the DNA segment protected by

A



B

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                -800
GACCAGTTGTTCCTTTGAGGTCCGGTTCTTTTCGTTATGGGGTCATTTTTGGGCCACCTCCACA
-750                                                        -700
GGTATGACTTCCAGGTATTCTCTGTGGCCTGTCACTTTCCTCCCTGTCTCTTTTATGCTTGTGA
                                          -650
TCTTTTCTATCTGTTCCTATTGGACCTGGAGATAGGTACTGACACGCTGTCCTTTCCCTATTAA
                     -600
CACTAAAGGACACTATAAGAGACCCTTTCGATTTAAGGCTGTTTTGCTGTCCAGCCTATTCTTT
      -550                                                  -500
TTACTGGCTTGGGTCTGTCGCGGTGCCTGAAGCTGTCCCCGAGCCACGCTTCCTGCTTTCCCGG
                                                -450
GCTTGCTGCTTGCGTGTGCTTGCTGTGGGCAGCTTGTGACAACTGGGCGCTGTGACTTTGCGTG
                                    -400
TCAGACGTTTTTCCCGATTTCCCCGAGGTGTCGTTGTCACATCTCCTGTCCCGGTTGGAATGGT
               -350
GGAGCCAGCTGTGGTTGAGGGCCGCCTTATTTCGGCTCACTTTTTTTTTTTTTTTCCTCTTGGA
-300                                                        -250
GTCCCGAACCTCCGCTCTTTTCTCTTCCCGGTCTTTCTTCCACATGCCTCCCGAGTGCATTTCT
                                          -200
TTTTGTTTTTTTTCTTTTTTTTTTTTTTTTTTTTTTGGGGAGGTGGAGAGTCCCGAGTACTTCAC
                          -150
TCCTGTCTGCGGTGTCCAAGTGTTCATCCCACGTGCCTCCCGAGTGCACTTTTTTTTTGTGGCAG
        -100                                                    -50
TCGCTCGTTGTGTTCTCTTGTTCTGTGTCTGCCCGTATCAGTAACTGTGCTTGCCCCGCGTGTA
                                                1
AGACATTCCTATCTCGCTTGTTTCTCCCGATTGCGCGTCGTTGCTCACTCTTAGATCGATGTGG
                                    50
TGCTCCGGAGTTCTCTTCGGGCCAGGGCCAAGCCGCGCCAGGCGAGGGACGGACATTCATGGCG
                     100
AATGGCGGCCGCTCTTCTCGTTCTGCCAGCGGGCCCTCGTCTCTCCACCCCATCCGTCTGCCGG
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Fig. 3. DNA sequence of the region surrounding the starting site of 45S RNA. The nucleotide sequence of the non-coding strand is displayed in 5' to 3' direction. 5' terminus of 45S RNA starts at nucleotide 1 and proceeds to the right. The numbers denote the distance from this starting nucleotide. The strategy of DNA sequencing is shown in the upper part of the figure. The horizontal arrows indicate the direction and range of the sequence read.

45S RNA was determined by running it in Maxam-Gilbert gel in parallel with chemical G cleavage products of the same fragment as that used for hybridization (Figure 5-A). As the nucleotide sequence of the fragment is known, the G cleavage ladder provided sufficient information for the alignment of the S_1 resistant DNA with the corresponding sequence. The fragments generated by the chemical reactions are known to migrate $1\frac{1}{2}$ nucleotides faster than the corresponding fragments generated by S_1 nuclease digestion (24) since the chemical reactions leave a 3' phosphate group while S_1 nuclease leaves a 3' hydroxyl group and the chemical reactions eliminate the 3' terminal modified nucleotide. When the S_1 resistant fragment was compared with the G ladder, the fragment was found to have the size of 25 nucleotides starting from a site numbered as 1 in the sequence shown in Figure 3, as ACTC---.

Figure 5-B shows that when the Hpa II - Hpa II fragment (+24 to +145) was hybridized with 45S RNA and then treated with S_1 nuclease, this fragment was completely protected indicating that the initiation site was located upstream of this fragment as expected by the previous experiment.

Determination of the 5' end of 45S RNA on rDNA by Reverse Transcriptase Extension Mapping. To confirm the above conclusion, we took another approach; i.e. reverse transcriptase extension mapping (24,39). In this experiment, 45S RNA was hybridized with Sau 3A - Bst NI (+12 to +42) fragment and the latter was extended by reverse transcriptase towards 5' end of 45S RNA. The size of the elongated products were determined by comigrating with the G-ladder of Hinf I - Bst NI (-188 to +42) fragment labeled at Bst NI site. Figure 5 (lane 3) shows that the product was extended exactly up to the nucleotide 1 determined by the previous S_1 nuclease mapping (see Figure 4). No longer products were detected. The bands of shorter fragments may probably be due to either the presence of 45S RNA having shorter 5' end or to the premature termination of reverse transcriptase or both. As a sort of internal standard and also to confirm the results of Figure 4, another S_1 nuclease mapping was carried out using Hinf I - Bst NI (-188 to +42) fragment. Comigration with the G ladder together with the reverse transcriptase extension products shows clearly that the majority of the fragments protected had

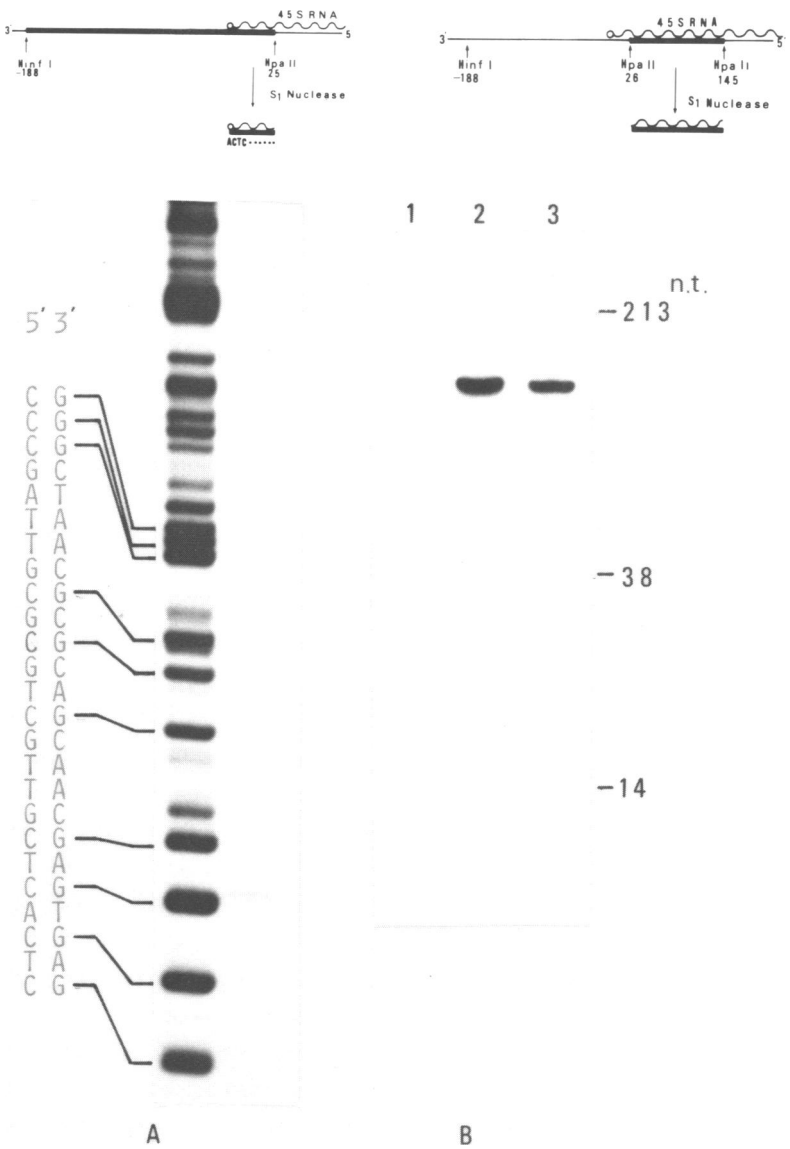

Fig. 4. Determination of the 5' end of 45S RNA on rDNA by S_1 nuclease mapping. (A) The Hinf I - Hpa II fragment (-191 to +25) was labeled with polynucleotide kinase and γ- ^{32}P-ATP. The 5' end-labeled coding strand (-188 to +25) obtained by strand separation was hybridized with 45S RNA and digested with 10 U of S_1 nuclease. The protected material was treated with alkali and was electrophoresed in 20% polyacrylamide -7 M urea gel in parallel with G cleavage products of the same fragment as that used

for hybridization. The nucleotide sequences of the non-coding and the coding strands are shown to the left of the gel. (B) S_1 nuclease protection mapping of the Hpa II - Hpa II fragment (+26 to +145) as a control. The Hpa II - Hpa II fragment (+24 to +145) was labeled with polynucleotide kinase and γ-^{32}P-ATP. The 5' end-labeled coding strand (+26 to +145) obtained by strand separation was hybridized with 45S RNA and digested with 10 U of S_1 nuclease. After alkaline denaturation, the protected material was run on 20% polyacrylamide -7 M urea gel (lane 2). The mock hybridization with yeast RNA and S nuclease treatment was done (lane 1) and the hybrid with 45S RNA was run without S nuclease treatment (lane 3).

exactly the same sequence as determined by the other two experiments, starting at nucleotide 1 (Figure 5, lane 2). These experiments together confirm the previous conclusion that the 45S RNA had the 5' end at nucleotide 1.

DISCUSSION

In this paper, we reported a relatively long nucleotide sequence surrounding and preceding the putative transcription initiation site of mouse ribosomal RNA gene. The location encoding the 5' end of 45S RNA was determined unequivocally at the nucleotide level. Whether this is truly the site of transcription initiation depends on whether 45S RNA used in this study was the primary transcript of rDNA. In the mouse, however, the bulk of 45S RNA has only monophosphate on the 5' terminus when isolated (34) thus making the demonstration of the primary transcript rather difficult. We tried to detect the presence of di- or triphosphate by using vaccinia capping enzyme and S-adenosyl-[methyl-^{3}H]methionine (40). It was found, however, that only less than 0.1% of the total 5' termini of 45S RNA could be capped (Data not shown). Furthermore, we used a permealized cell system in which nucleoside triphosphates could be incorporated and the initiation with RNA polymerase I was demonstrated to occur (Mishima et al., in preparation). In this system, however, we could not detect any labeling of 45S RNA with β-^{32}P-ATP. We interpret this to mean that some enzymatic activity exists in the nucleolus of these cells which cleaves off γ and β phosphates from the 5' end of the primary transcript. Therefore we are inclined to think that the facts that most of 45S RNA had the same

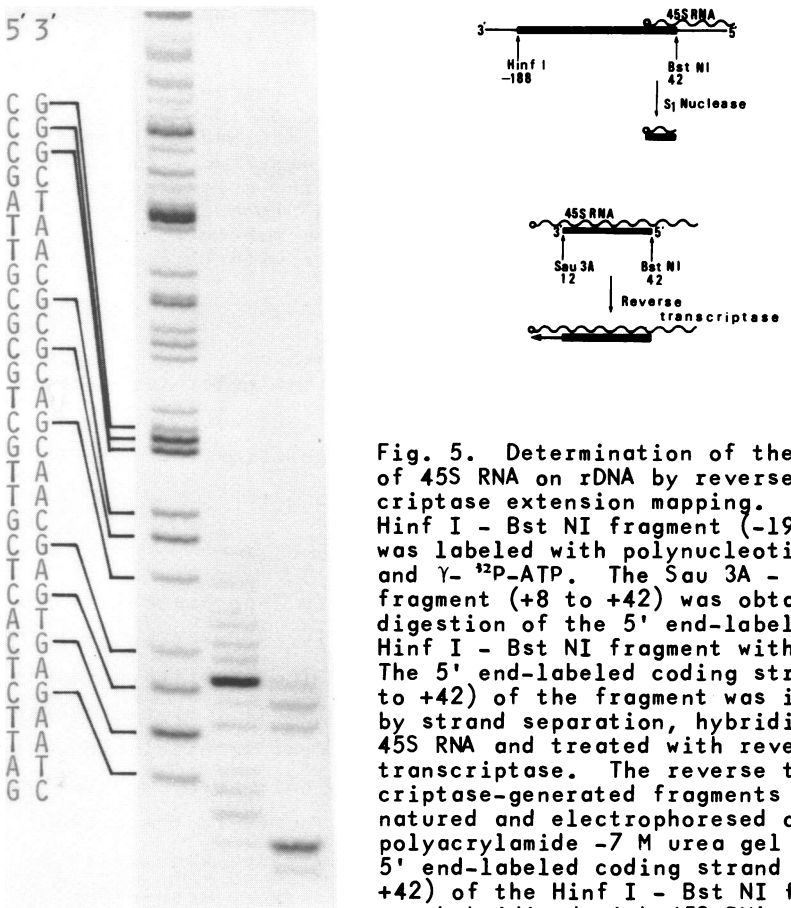

Fig. 5. Determination of the 5' end of 45S RNA on rDNA by reverse transcriptase extension mapping. The Hinf I - Bst NI fragment (-191 to +42) was labeled with polynucleotide kinase and γ-^{32}P-ATP. The Sau 3A - Bst NI fragment (+8 to +42) was obtained by digestion of the 5' end-labeled Hinf I - Bst NI fragment with Sau 3A. The 5' end-labeled coding strand (+12 to +42) of the fragment was isolated by strand separation, hybridized to 45S RNA and treated with reverse transcriptase. The reverse transcriptase-generated fragments were denatured and electrophoresed on a 20% polyacrylamide -7 M urea gel (lane 3). 5' end-labeled coding strand (-188 to +42) of the Hinf I - Bst NI fragment was hybridized with 45S RNA and digested with S_1 nuclease. The protected fragments were run parallelly (lane 2). G cleavage products of the 5'-labeled coding strand of the Hinf I - Bst NI fragment were run at the same time on lane 1.

terminus (Figure 4 and 5, lane 2) and that no longer molecules were detected as the template of reverse transcriptase indicate that 45S RNA starts transcription at this point, although the possibility of very rapid trimming at the 5' end of the primary transcript and the accumulation of this 45S RNA molecule as a relatively stable intermidiate cannot be ruled out completely.

In Figure 6, several nucleotides immediately upstream the putative initiation point (nucleotide 1) of this rDNA were com-

	Cap ↓	references
SV40 16S late gene	ACTGACACACAT TTCAGAGGTTAT	45;46
Adenovirus major late gene	GTTCGTCCTCAC	47
Rabbit β globin	GCTGCTGCTTAC	Efstratiadis et al.; quoted from ref. 50
Mouse α globin	GGTCCAAGACAC	48
Mouse β globin minor	GTTGCTTCTTAC	49
Mouse β globin major	GTTGCTCCTCAC	50
Mouse immunoglobulin λ light chain	CCTGCTGCTGAC	51; quoted from ref. 50
	Initiation ↓	
Mouse rDNA	GTCGTTGCTCAC	this paper
Xenopus laevis rDNA	GCAGGAAGGTAG	24;25
Saccharomyces cerevisiae rDNA	TGGAGTACAAGT GAGGTACTTCAT	28

Fig. 6. Nucleotide sequences of transcription initiation sites of eukaryotic genes. The sequence of the region surrounding the transcription initiation site of mouse rDNA is compared with the sequences of the regions surrounding the capping sites of SV 40 16S late gene, adenovirus major late gene, rabbit β-globin gene, mouse α-globin gene, mouse β-globin minor gene, mouse β-globin major gene and mouse immunoglobulin light chain gene. Corresponding regions of Xenopus laevis and Saccharomyces cerevisiae rDNAs are also shown. Underlined are the nucleotides coincident with those of mouse rDNA sequence.

pared with various known sequences immediately before the transcription initiation site (or capping site) of mammalian mRNA. Some similarity may be seen among these regions, although calculation of the coincidence rate may be meaningless with this large variance. It must be remembered, however, that mRNA genes and rRNA genes are transcribed by different RNA polymerases, designated II (or B) and I (or A), respectively. The similarity of the sequence in spite of the different polymerases involved may indicate the existence of some common recognition signals between them. It may be noted in this connection that at least one of

the smaller subunits appears to be common between RNA polymerase I and II according to gel electrophoresis (4). In Figure 6, corresponding sequences of rDNA of Xenopus laevis and Saccharomyces cerevisiae are shown together. Surprisingly, the similarity in this region is much less between these rDNAs than between mouse rDNA and mammalian mRNA genes. It may be pointed out that many of the known eukaryotic rRNA genes appear to start from A regidue; e.g. Saccharomyces cerevisiae (27,28) Saccharomyces carlsbergensis (41), Tetrahymena pyriformis (42), Dictyostelium discoideum (43), Xenpous laevis (40) and the mouse reported herein. As is discussed below, the sequence similarity between mouse and Xenopus rDNA appears very low over the relatively long region upstream the transcription initiation site.

A few interesting characteristics of mouse rDNA was noted upstream the putative transcription initiation region. One is the presence of abundant T clusters. Within 769 nucleotides upstream the putative initiation site, there were eight T clusters consisting of more than 5 T's. The longest cluster was consecutive 21 T's, but this is connected by a C to the next T clusters having 8 T's, which is again connected by a G to the next 5 T's. Thus, from -204 to -243, the sequence consists of 40 T's interrupted by only 2 C's and 1 G. Within 100 nucleotides from this region, another 15 consecutive T's appear. This unusual T clusters were not present in the corresponding region of rDNA of Xenopus laevis (24,25,44) and Saccharomyces cerevisiae (28). Whether or not this is a special characteristics of the mouse rDNA remains to be seen. Another interesting feature of this region is the twice occurrence of a pentadecanucleotide, TGCCTCCCGAGTGCA within an interval of about 100 nucleotides. The first one appears at position -258, right upstream the aforementioned longest T cluster, the second appearing at position -141 followed by a C and a relatively long T cluster. Although neither functional nor structural significance is clear, this sequence must have arisen by some gene duplication mechanism.

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