Cloning and determination of the transcription termination site of ribosomal RNA gene of the mouse

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ABSTRACT

An Eco RI 6.6 kb DNA fragment containing the 3'-end of 28S ribosomal RNA gene of the mouse was detected by Southern blot hybridization, and cloned in a λ-phage vector. The site of transcription termination and the processed 3'-end of 28S RNA were determined on the cloned fragment and the surrounding nucleotide sequence determined.

The 3'-terminal nucleotides of mouse 28S RNA are similar to those of yeast, *Drosophila* and *Xenopus* although the homology was lost drastically beyond the 3'-end of 28S RNA. 45S precursor RNA terminated at 30 nucleotides downstream from the 3'-end of 28S RNA gene. A structure of a dyad symmetry with a loop was found immediately prior to the termination site of 45S RNA. The rDNA termination site thus shares some common features with termination sites recognized by other RNA polymerases.

INTRODUCTION

In the mouse, as in all higher eukaryotes, the ribosomal RNA (rRNA) is synthesized as a large precursor molecule containing the 18S, 5.8S and 28S RNA sequences and then processed into individual species through a series of cleavage steps (1-3). The multiple genes coding for rRNA are arranged in tandemly repeating units on several chromosomes. Restriction enzyme *Eco* RI recognizes two cleavage sites on the rRNA gene region of the mouse, one site being located in the middle of 18S RNA, and the other near the 3'-terminal region of the 28S RNA (4-6). Two *Eco* RI-fragments of rRNA, 15 kb and 6.6 kb in length, have been detected by Southern blot hybridization technique and cloned (7-11). A 15 kb fragment hybridizing only with 18S RNA contains the initiation site of transcription, the nucleotide sequence of which have been determined recently in our laboratory (12). A 6.6 kb fragment hybridizable with 18S and 28S RNAs contains a
half of the 18S sequence and most of the 28S RNA sequence. These cloned fragments provide a useful tool for studies of the initiation and maturation process and the control of transcription of ribosomal RNA genes. The DNA fragment containing the termination region, however, is neither well defined nor cloned so far.

To investigate the structure of the transcription termination site of rRNA gene as well as to analyze the 3'-terminal processing of the primary transcript, 45S RNA, isolation of a gene fragment containing the 3'-end of 28S RNA and that of 45S RNA is essential.

In the present work, a DNA fragment containing the termination region of rRNA gene was cut out by Eco RI and cloned in a vector, λgtWES·λB. The clone was characterized by restriction enzyme cleavage, blot hybridization analysis and R-loop technique. Then, the nucleotide sequence surrounding the termination site was determined and the 3'-termini of 28S and 45S RNAs were located on the DNA sequence.

MATERIALS AND METHODS
Materials. Restriction endonucleases were obtained from Bethesda Research Laboratories and Takara biomedicals (Kyoto, Japan), [α-32P]dCTP, [α-32P]dATP, and [γ-32P]ATP from Amersham Searle. DNA polymerase I and T4-polynucleotide-kinase were obtained from Boehringer Mannheim. DNA ligase was purchased from New England Biolabs. Alkaline phosphatase was obtained from Worthington. T4-RNA ligase was purchased from P-L Biochemicals.

Preparation of DNA and RNA. DNA was prepared from C3H/He mouse livers according to the method of M. Gross-Bellard et al. (13).

Highly purified nucleoli were prepared by a sonication procedure described previously (14). RNA was extracted by a hot sodium dodecyl sulfate-phenol method and nucleolar 45S RNA was purified by repeated sucrose density gradient centrifugations (15).

Ribosomal 28S RNA was prepared from ddy mice after isolation of 60S ribosomal subunits with EDTA.

For labeling of 28S RNA with 32P, 28S RNA was partially hydrolyzed with a final concentration of 0.05M Na2CO3, at 60°C for
10 min. After neutralization with 1M acetic acid, 28S RNA was precipitated with 2 vol. of ethanol. The fragments of RNA (1 μg) were dissolved in 20 μl of 10 mM Tris-HCl, pH 7.6, 10 mM MgCl₂, and 1 mM dithiothreitol, and incubated at 37°C for 1 h with [α-³²P]ATP (5000 Ci/m mole) and Tα-polynucleotide-kinase. The specific activity was 1-5 x 10⁷ cpm/μg RNA. Nick-translation of the DNA with [α-³²P]dCTP was performed according to Maniatis et al. (16).

Southern Blot Hybridization for DNA Fragments Containing rRNA Genes. The digested DNA was run on 0.8-1.0% agarose gels at 0.4 V/cm in a buffer containing 40 mM Tris-acetate, pH 7.8, 5 mM sodium acetate, 2 mM EDTA and 0.5 μg/ml ethidium bromide. A length standard of λDNA digested with Hind III was always included in the gels. After the gels were photographed, the DNA was transferred to nitrocellulose filters following the method of Southern (4). The filters were baked in an oven for 3 h at 80°C.

When ³²P-labeled 28S RNA was used as a probe, hybridization was carried out in a solution of 50% formamide, 0.72 M NaCl, 0.04 M Pipes-NaOH, pH 6.8, 1 mM EDTA, 0.01% SDS. 20 μg/ml denatured E.coli DNA, 0.02% each of Ficoll, bovine serum albumin, and polyvinylpyrrolidone were added in the cases of DNA probe.

The filters were presoaked in hybridization solutions at 37°C for 2 h, drained and incubated for 20 h at 37°C in 0.4 ml of a hybridization solution containing about 3 x 10⁶ cpm of probes. The filters were removed, washed extensively with 400 ml of 0.1 x SSC and 0.1% SDS at 67°C, dried and then autoradiographed at -40°C with intensifying screens.

Preparation and Screening of the Recombinant Phage and Plasmid

Bacteriophage λgtWES:λB, an EK-2 vector, was propagated in an Escherichia coli strain DP50/supF. Introduction of DNA into DP50/supF cells by an in vitro packaging technique was carried out according to Blattner el al. (17). Cloning experiments were performed in a P2 facility according to the Guidelines set by Japanese Government. 1 μg of λgtWES arms were ligated with 0.2 μg DNA in a volume of 0.1 ml at 4°C for 16 h.

About 2,000 plaques on each L-broth-supplemented agar plate (Bacto-trypton (10g), yeast extract (5g), NaCl (5g), agar (15g) and distilled water (1L) were transferred to Millipore filters.
essentially according to Benton and Davis (18). Filters were hybridized with ^3P-28S RNA and nick-translated 6.6 kb rDNA under the conditions described above, and autoradiographed. Positive plaques containing rDNA were propagated as described by Tiemeier et al. (11), from which DNA was extracted. To re-clone the fragments obtained above, recombinant DNA was digested with Eco RI and ligated with plasmid pBR322 with Eco RI and alkaline phosphatase. Transformation of X1776 was done according to Enea et al. (19).

R-loop Formation and Electronmicroscopy. The formation of R-loop between 28S rRNA and recombinant DNA digested with Eco RI was carried out essentially as described by Thomas et al. (20,21). One μg of Eco RI fragments was incubated with 1 μg of 28S rRNA in 50 μl of 70% formamide, 0.04 M Pipes-NaOH, pH 6.8, 0.001 M EDTA, 0.05 M NaCl at 56°C for 2 h. The mixture was diluted 20 fold with the same buffer containing 91 μg/ml cytochrome C and spread onto a hypophase of 10% formamide, 0.1 mM EDTA, 0.01 M Tris-HCl, pH 8.0. The DNA-RNA hybrid was picked up on parlodion-coated grids, stained with 5 x 10^-5 M uranyl acetate, coated with carbon, and shadowed with platinum/paladium. The preparations were viewed with a Hitachi HU-12 electronmicroscope at a magnification of 10,000: 1.

Recovery of DNA from Agarose Gels. The gels were crushed with a syringe and incubated in a buffer containing 20 mM Tris-HCl, pH 7.6, 0.2 M NaCl and 2 mM EDTA with shaking overnight. The solution was extracted with phenol and DNA in the aqueous layer was precipitated with ethanol. In some cases, extraction of DNA from agarose gels was effected by the method of Tabak and Flavell (22).

3'-End Labeling of RNA and DNA. RNA molecules were labeled at their 3'-termini with T,RNA ligase and ^3pCp under conditions derived from those of Bruse and Uhlenbeck (23). ^3pCp was made by incubation of cytosine monophosphate, with[γ-32P]ATP (5000 Ci/m mole) and polynucleotide kinase. Reaction was done in 10 μl of 50 mM Tris-HCl, pH 8.3, 5 mM dithiothreitol, 5 mM MgCl₂, 50 μM ATP, and 10 μg/ml gelation at 0°C overnight.

DNA was labeled at the 3'-end by a nick-translation procedure producing largely full-length DNA (16). DNA was digested
with \textit{Ava} I in 50 \mu l of 20 mM Tris-HCl, pH 7.4, 30 mM NaCl, and 10 mM MgCl\textsubscript{2}, and further incubated at 15°C for 1 h after addition of \textit{a-\textsuperscript{32}P}dATP and 2 units \textit{E.coli} DNA polymerase I.

The rRNAs labeled at the 3'-end (3 \mu g) were hybridized with 3 \mu g of the cloned DNA fragments bound to nitrocellulose filters in the 50% formamide buffer described above at 37°C for 15 h. After RNase treatment, the hybridized RNA fragments were eluted with 0.05 M Tris-HCl, pH 7.6, 1 mM EDTA and 0.1% SDS at 100°C. The purified RNA was digested with RNase T\textsubscript{1} and subjected to PEI-chromatography.

\textit{S}_{1}-Nuclease Protection Mapping.} 3'-end-labeled RNA was hybridized with RNA fragments in 25 \mu l 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 was diluted 20 fold with a cold \textit{S}_{1}-buffer (30 mM sodium acetate, pH 4.6, 0.02 mM ZnSO\textsubscript{4}, and 0.2 M NaCl) containing 10 \mu g of denatured \textit{E.coli} DNA and treated with \textit{S}_{1}-nuclease at 37°C for 1 h. After phenol extraction, the protected fragments were precipitated with ethanol. These fragments were electrophoresed on a 5% polyacrylamide gel (24).

3'-end-labeled DNA was hybridized with 28S RNA and 45S RNA in 20 \mu l of 70% formamide, 1.0 M NaCl, 1 mM EDTA and 0.04 M Pipes-NaOH, pH 6.8, at 40°C and 46°C, respectively. \textit{S}_{1}-nuclease treatment was done as described above. The protected fragments were subjected to 15% polyacrylamide-7M urea gel electrophoresis after alkali denaturation.

DNA Sequencing. DNA was sequenced as described by Maxam and Gilbert (25).

\textbf{RESULTS}

Detection of the Sequence Containing the 3'-end of 28S RNA.

Digestion of mouse DNA with the restriction enzyme \textit{Eco} RI yields a wide range of fragment sizes, as shown in Fig. 1a. Transfer of these fragments to nitrocellulose filters and hybridization to \textit{\textsuperscript{32}P}-28S rRNA identifies one band of 6.6 kb rDNA fragment (Fig. 1b). This is essentially compatible with the restriction mapping described by Southern (4) and Cory and Adams (6). This band at least contains the 6.6 kb rDNA fragment
Fig. 1. Hybridization of $^{32}$P-23S rRNA to restriction fragments of C3H/He mouse DNA. 10 μg of DNA was digested with Eco RI and electrophoresed on an 1% agarose gel. The region containing DNA fragments of about 6.6 kb was cut out and the DNA extracted from the gel. These 6.6 kb fragments were electrophoresed again in parallel. Ethidium bromide stained gels showing, a) Eco RI-digested total DNA (middle) and 6.6 kb fragments (right). Size markers (left) are Eco RI-digested λ fragments. b) Autoradiograph of the filter hybridized with $^{32}$P-28S rRNA. c) 6.6 kb fragments were further-digested with either Bam HI or Bgl II and subjected to blot hybridization with $^{32}$P-28S RNA (c) or $^{32}$P-nick-translated internal 5.6 kb rDNA fragment (d). e) Ethidium bromide staining of the cloned internal 6.6 kb DNA (11) digested with either Bam HI (left) or Bgl II (right).

isolated by Tiemeier et al. (11), containing both the 18S and 28S rRNA sequences separated by the internal transcribed spacer (termed internal 6.6 kb fragment). Another fragment described by Cory and Adams containing the 3'-end of 28S gene and a part of the non-transcribed spacer could not be found on the autoradiogram. Grummt et al. and also Arnheim and Southern described a similar finding (5,8).

We now demonstrate that this 6.6 kb band contains another fragment of the same size which constitutes a different portion, a stretch containing the 3'-end of the 28S rRNA gene (termed terminal 6.6 kb fragment). DNA fragments migrated around 6.6 kb were recovered from the gels as shown in Fig. 1-a and b, and were further digested separately with Bam HI and Bgl II. The digests were electrophoresed on 1% agarose gels and subjected to Southern blot hybridization (4). Fig. 1-c and 1-d show the autoradiograms of filters hybridized with labeled 28S RNA and
the cloned internal 6.6 kb DNA, respectively.

The 6.6 kb DNA probe hybridized to 4.0 kb and 1.8 kb (doublet) fragments of the Bam H1 digest and to 4.6 kb and 2.0 kb Bgl II fragments. This pattern is identical with the cleavage pattern of the cloned 6.6 kb fragment shown in Fig. 1e.

Hybridization pattern to 28S probe showed additional fragments of 5.2 kb and 1.0 kb, and 3.8 kb in the digests of Bam H1 and Bgl II, respectively (Fig. 1c).

These findings indicate that two kinds of DNA fragments were present in the original 6.6 kb rRNA band recovered from the gel. One was the internal 6.6 kb fragment and the other was the terminal 6.6 kb fragment mentioned above.

These results are summarized in Fig. 2. One Eco RI cleavage site is present near the 3'-end of 28S RNA coding region and another Eco RI site is located about 6.6 kb downstream from that Eco RI site.

Cloning of the Terminal 6.6 kb Eco RI Fragment Encoding 3'-end of 28S RNA. The 6.6 kb DNA fragments recovered from the gels were ligated with λgtWES arms in vitro by T4-ligase, and transfected into E.coli cells as described in experimental procedures. About 5,000 recombinant phage plaques were transferred to two pairs of nitrocellulose filters and screened for the presence of 28S rRNA or the internal 6.6 kb sequences.

Seven recombinants hybridized to 28S rRNA probe, six of which hybridized to the clone internal 6.6 kb fragment (data not shown). The clone which hybridized with 28S RNA, but not with the internal 6.6 kb DNA was picked up and grown in a small scale. This recombinant phage should be a candidate of the clone con-
taining the terminal 6.6 kb fragment carrying the 3'-end of 28S RNA. DNA was extracted, digested with Eco RI, Eco RI plus Bam HI, and Eco RI plus Bgl II, and run on an agarose gel. The blotting patterns with 28S RNA suggested the presence of 3'-terminal sequence of 28S RNA (data not shown).

The Restriction Map of the Clone. The terminal 6.6 kb fragment now isolated was transferred into the plasmid pBR322 and then the recombinant plasmid was digested with various restriction enzymes. The positions of the cleavage sites in the DNA were determined by double digestion with two enzymes or by partial digestion of the terminally-labeled fragment with an enzyme. The location of 28S RNA sequences was determined by blot hybridization (data not shown). A restriction map was constructed and shown in Fig. 3. These results indicate that the cloned fragment actually covers the sequence between the two Eco RI sites, which should contain the transcription termination site of rRNA.

Electron Microscopy of the R-loop. To confirm that this cloned fragment contained a part of the 28S rRNA at one end, hybrids formed between the 28S RNA and the fragment were examined under an electron microscope.

Fig. 4 shows typical electron micrographs of hybrids formed by incubation of 28S RNA and Eco RI-digested fragment of the clone. A number of molecules with a fork at one end which was several hundred nucleotides in length were seen. The fork may be interpreted to represent an rDNA-28S RNA. Hybrid and the displaced anti-strand as illustrated in the diagram. At one end of the hybrid was seen a thread-like structure which is likely to be the unhybridized portion of 28S RNA. The lengths
Fig. 4. Electron micrographs of the R-loop formed between the 28S RNA and the cloned terminal 6.6 kb fragment digested with Eco RI. Molecules were viewed at 10,000-fold magnification in a Hitach HU-12 electron microscope. The bar indicates 0.5 nm. DNA strands are drawn with continuous lines, whereas 28S RNA molecules are traced with broken line.

of rDNA and the fork determined on electronmicrographs are consistent with the data obtained by biochemical analysis.

S\textsubscript{nuclease} Protection Mapping. Although the cloned plasmid DNA was shown to contain the 3'-'end of 28S RNA, it is of particular interest to map the 3'-termini of the 45S precursor RNA and the 28S RNA on the clone. The S\textsubscript{nuclease} protection mapping (24) was used to determine this.

45S and 28S RNAs were labeled with "\textsuperscript{32}P"-Cp at the 3'-termini by means of T\textsubscript{4}RNA ligase and hybridized with the Eco RI-digest of the cloned plasmid under the conditions of R-loop formation. The mixture was digested with S\textsubscript{nuclease} and the resultant DNA-RNA hybrid was sized by gel electrophoresis (Fig. 5). The length of the fragment protected from S\textsubscript{nuclease} digestion should correspond to the distance from the Eco RI end to the nucleotide encoding the 3'-end of 45S or 28S RNA, depending upon the RNA used for hybridization.

Upon treatment with S\textsubscript{nuclease}, the hybrids protected by 45S and 28S RNA were 580 and 550 nucleotides in length, respec-
Fig. 5. $S_1$-nuclease protection mapping of the 3'-ends of the 28S and 45S RNA. The 28S and 45S RNAs were 3' terminally labeled with T4-RNA ligase and $^3$H-pCP, hybridized to the cloned terminal 6.6 kb fragment digested with Eco RI, and treated with $S_1$-nuclease. The protected fragments were electrophoresed on a 5% polyacrylamide gel. The gel was dried and autoradiographed. a) Only 45S RNA was incubated omitting the DNA fragment, followed by treatment with 5U of $S_1$-nuclease. b) Incubated with 45S RNA, and treated with 5U $S_1$-nuclease. c) Incubated with 45S RNA, and treated with 50U $S_1$-nuclease. d) Incubated with 28S RNA, and treated with 50U $S_1$-nuclease. The protection of these fragments depended on the presence of the cloned fragment in hybridization mixture.

These results indicated that the site of transcription termination was located around the Sma I site of this clone. DNA Sequence of the Transcription Termination Region. The DNA sequence of 300 base pairs around the Sma I site in the clone was determined by the method of Maxam and Gilbert (25). The sequencing strategy and the deduced sequence of the non-coding strand are shown in Fig. 6-a and b. The sequence is numbered negatively from the 3'-end of 28S RNA gene toward upstream, transcription proceeding to the right (see below).

The location of the termini of 45S and 28S RNA was mapped on the DNA sequence. The Sau 3A fragment containing the Sma I site was nick-translated with [$\alpha$-$^3$P]dATP (see Fig. 6-a) and the Sau 3A/Sma I fragment was isolated. This fragment was hybridized to 28S and 45S RNA and subjected to $S_1$-nuclease treatment. The protected fragments were sized by 15% polyacrylamide-7M urea gel electrophoresis in parallel with G-cleavage products of the end-labeled original fragment (Fig. 7). When the rRNA was omitted from the hybridization mixture, no protected fragment was found.

The results thus indicated that the 3'-end of 28S RNA was
Fig. 6. The structure of rDNA termination site and comparison with *Xenopus* rDNA. a) Restriction enzyme cleavage sites in the 1.3 kb EcoRI-SalI region in the terminal 6.6 kb fragment of the mouse ribosomal RNA gene. Sequence analysis was carried out in the direction of the arrows. S, H, and A show Sau3A, HinfI, and Aval sites, respectively. b) The sequence of the non-coding strand surrounding the transcription termination site of the mouse ribosomal RNA gene. The nucleotide encoding 3'-end of 28S RNA is numbered position 1. The corresponding *Xenopus laevis* sequence determined by Sollner-Webb and Reeder (30) is shown together. Only the bases that are different are printed. Dashes show the deletion of a nucleotide. The two arms of the Dashes show the deletion of a nucleotide. The two arms of the dyad symmetric regions are indicated by the inverted arrows.

located at 37 nucleotides downstream from the Sau3A site and further the transcription of 45S RNA terminated 30 nucleotides downstream from the 3'-end of 28S RNA, although S-mapping technique used could not locate the 3'-end more accurately than a few nucleotides. This is consistent with the results obtained with the 3'-end labeled 28S and 45S RNA probes described in
Fig. 7. Termination site located by S-nuclease protection mapping. A 165 bp Sau 3A spanning the Sma I site was labeled at the 3'-end with nick-translation and cut with Sma I. A 91 bp Sau 3A-Sma I fragment was hybridized to 4S, 28S and 45S RNA and subjected to S-nuclease treatment. The protected fragments were denatured and fractionated on a 15% acrylamide gel in parallel with the G-cleavage products of this DNA fragment. The region of the autoradiogram shown has been aligned in part with the anti-sequence of the protected coding strand. The protected fragments moved as if one nucleotide shorter than the corresponding G-cleavage products probably because of the indentation by S-nuclease digestion under these conditions. Therefore, the written sequence is shifted by one nucleotide from the ladder.

Fig. 5.

The previous nucleotide analysis had shown that the 3'-end of 28S RNA was PyGUoh (26), which was confirmed by the analysis of 3'-end labeled 28S RNA with "pCp and RNA ligase (Fig. 8). Together with this, the 3'-terminus of 28S RNA was mapped at nucleotide -1 in this sequence (see Fig. 6).

The 3'-terminal nucleotides of 45S RNA exhibited small heterogeneity, although most of the nucleotides were uridine monophosphate (Fig. 8). 45S RNA was labeled at the 3'-end with 5'-"pCp T4-RNA ligase. In order to purify the labeled terminal fragments from any degraded products, the RNA was hybridized with the 1.0 kb EcoRI/Bam H1 fragment bound to nitrocellulose filters (see Fig. 3). After RNAase digestions, the hybridized RNA was eluted by boiling and digested with RNase T1. Majority of the labeled nucleotides comigrated with uridine monophosphate by PEI-chromatography. Unless the labeled RNA was purified by filter hybridization, the labeled nucleotides yielded four kinds of nucleotides (data not shown). It remains unclear,
Fig. 8. Analysis of 3'-terminal nucleotides of 28S and 45S RNA. The 28S and 45S RNA molecules were labeled at their 3'-termini with T^RNA ligase and "pCp. The labeled nucleotides present in ribosomal RNA were purified by hybridization to the fragment containing the termination site. The hybrid molecules were denatured by boiling and digested with RNase T_2. The enzymatically released, #P-labeled 3'-terminal nucleotides were identified by chromatography on a PEI-cellulose sheet with 1M ammonium formate, pH 3.5, followed by autoradiography.

however, what the minor nucleotides, released from the hybridized RNA, are originated from. It may be derived from degradation products or heterogeneous termini. We conclude from these data that the terminus of the 45S RNA is present at or close to the nucleotide +30, which is located within a few nucleotide downstream from the dyad symmetry element found in this region (Fig. 6-b).

DISCUSSION

In the present study, we cloned a mouse rDNA fragment containing a termination region and mapped the 3'-termini of 28S and 45S RNA on the DNA sequence.

Previous workers had constructed restriction enzyme maps on repeated rRNA genes of the mouse with the aid of Southern blot hybridization (4,5,6). Although EcoRI was shown to cut both in the middle of the 18S RNA gene and somewhere near the 3'-end of 28S RNA gene, the second site was rather ambiguous. Southern, Arnheim and Southern, and Grummt et al. (4,5,8) described no extra bands hybridizing with 28S RNA and inferred that the second site is almost at the 3'-end of the 28S RNA gene. Cory and Adams, on the contrary, detected a second band with a smaller size (6.5 kb) than the internal fragment (7.1 kb), thus concluding that the second site was within the 28S RNA gene region and the third site was located 6.5 kb downstream from the
second site. Our blotting pattern of Eco RI digests did not reveal the third band that was hybridizable with 28S RNA probe. Further restriction analysis, however, indicated clearly the presence of another 6.6 kb fragment containing the terminal region of 28S RNA (Fig. 1).

By cloning a DNA fragment which hybridized to 28S RNA but not to internal 6.6 kb DNA, we confirmed the presence of this fragment. The size (6.6 kb) was almost the same as the internal Eco RI fragment containing both 18S and 28S RNA genes. We then showed that it contained the 3'-termini of 28S RNA and of 45S RNA by the methods of R-loop, S1-nuclease protection mapping and DNA sequencing.

Although this cloned DNA is not fully proved to be transcriptionally functional, this DNA has not only the same size as major rDNA Eco RI fragment containing the termination site as revealed by Southern blots but also can form hybrids with both 28S and 45S RNAs in the stringent conditions of R-loop formation, indicating that a high degree of homology exists between this DNA and rRNA. Therefore, we regard it likely that this clone has been derived from functional rDNA.

In other mammalian species, it is not certain that Eco RI cuts at a similar site on the 28S rRNA gene to that of the mouse (4,27).

Eco RI digestion of human and rat DNAs yielded two fragments hybridizing to 28S RNA, one of which was detected with the terminal 6.6 kb clone of the mouse as probe (data not shown). It indicates that the Eco RI site on the 28S RNA gene as well as that on the 18S gene is well conserved among these species.

The 3'-terminus of 28S RNA was located by S1-nuclease protection mapping at position -1, whose sequence being --GGTTTGToh (non-coding strand). Yeast Saccharomyces carlsbergensis and Drosophila melanogaster have the 28S RNA 3'-termini. --GATTGToh and --GATTGCGoh, respectively (28,29). Although Xenopus laevis 28S RNA was suggested to have the 3'-terminus --CCAAGCoh, the gene has a sequence --TTTTGToh immediately after that sequence (30). Considering the similarity between the mouse and yeast, Xenopus 28S RNA might also have the similar sequence at the 3'-terminus.
The transcription termination site or the 3'-terminal sequence of the 45S RNA was mapped on the RNA sequence by DNA sequence analysis and S1-nuclease protection methods. The 45S RNA terminated 30 nucleotides downstream from the 3'-end of the 28S RNA coding sequence. Since the 3'-terminus of 45S RNA showed uridine monophosphate in majority, we tentatively conclude that the rRNA gene transcription terminates at or very near nucleotide 30. The possibility that the primary transcript terminates at further downstream could not be ruled out completely, since demonstration of the 45S RNA being the primary transcript is impossible in any way at the present time. Termination of rRNA transcription may not be stringent but take place at one of the several nucleotides near this region. Similar pattern of termination has been reported for prokaryotic genes (31,32,33). In yeast, the rRNA precursor, 37S RNA, terminates at 7 nucleotides beyond the 3'-end of mature 26S RNA (28). However, no similarity in sequence was found around this region between yeast, *Xenopus*, *Drosophila melanogaster* and mouse. In our data, a hairpin structure may be formed just before the termination site of 45S RNA using the dyad symmetry consisting of 6 consecutive GC pairs. This structure shares some features with previously known termination sites of eukaryotic 5S RNAs (34), adenovirus VA RNA (35), sea urchin histone mRNAs (36) and of some prokaryotic mRNAs (31,37), namely, the presence of a GC rich hyphenated symmetry some nucleotides prior to termination. One difference is that a string of T residues usually present immediately before termination site is not found anywhere near our termination site. As for eukaryotic rRNA genes, two species of yeast (28,38) and a toad *Xenopus laevis* (30) have been analyzed with respect to the sequence of transcription termination site. In *Xenopus*, a dyad symmetry involving just four bases is found several nucleotides upstream the 3'-end of 28S RNA (30). Although some rather extensive dyad symmetry was pointed out beyond the terminatin sites of yeast (28), its relative position and the structural feature are far different from those discussed above. Further accumulation of the data concerning the transcription termination site of rRNA genes of eukaryotes is required to discuss the specific signals of termi-
Comparison of the nucleotide sequences near the 3'-terminal region of 28S RNA between mouse and *Xenopus* reveals that those sequences are more than 80% homologous. A relatively short region (-128 to -137) was found where the sequence were quite different from each other. Immediately beyond the 3'-end of 28S RNA, the homology was lost drastically, suggesting the functional importance of the 3'-end of 28S RNA in the ribosome.

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