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PUTATIVE TERMINATION SITES FOR rDNA TRANSCRIPTION IN RICE

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Summary : A clone bearing a 9.8 kb insert DNA containing the rDNA unit was identified by screening an EcoR1 library of rice DNA in λ Charon 4 phage with [^{32}P]-rRNAs. The S1 nuclease mapping of the rDNA-precursor rRNA hybrids showed the presence of two transcription termini on the rDNA. They were mapped at positions 616 and 620 nucleotides downstream to the end of the 25S rRNA gene. The 18 nucleotide sequence, where the transcription terminates on the rDNA in rice and mice are homologous albeit in the reverse orientation.

In eukaryotes, the 18S, 5 8S, and 25S rRNA genes are encoded in multiple copies and are transcribed as a large precursor which is processed to give the mature rRNAs (1,2). The rDNA transcription units are organized into long tandem repeats with the coding regions separated by nontranscribed spacers (3). In rice, the length of these major repeat units is 7.6 to 8.8 kb (4-6). These variations are due to the presence of differing number of tandem repeats within the nontranscribed spacer. The termination of rDNA transcription in plants has been shown to be very near to the end of the 25S rRNA gene (7,8). We report here that there are two transcription termination sites for the precursor rRNA at 616 and 620 nucleotides downstream to the end of the 25S rRNA gene.

MATERIALS AND METHODS

Isolation of rice DNA

DNA from the 48 hr germinated rice (Oryza sativa L.,IR-20) embryos was isolated according to the method of Walbot and Goldberg (9). The spooled DNA obtained after ethanol precipitation

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was dissolved in 10 mM Tris-HCl, 1 mM EDTA, pH 7.4, and further purified by gel filtration on a Biogel A5m column.

Isolation of rRNAs and nuclear RNA

Ribosomes from the 48 hr germinated rice embryo were isolated and the rRNAs were extracted and purified according to the method of Rubin (10). The 5.8S, 18S and 25S rRNAs were separated by electrophoresis on a 7M urea-6% polyacrylamide gel and electroeluted.

Nuclei from the 48 hr germinated rice embryo were isolated (11), lysed in 4 volumes of 20 mM Tris-HCl, pH 8.0, 20 mM EDTA and 0.5% sodium dodecyl sulfate, deproteinized with phenol and the nucleic acids were precipitated with ethanol. The DNA was removed by digestion with DNase I (10 µg/ml) at 37°C for 30 min, and the RNA was deproteinized with phenol, precipitated with ethanol, vacuum dried and dissolved in 20 mM Tris-HCl pH 7.5. The nuclear RNA contains the precursor rRNA along with the processed mature rRNAs.

Isolation of a clone containing a rDNA unit

A genomic library obtained by the cloning of a partial EcoRI digest of rice DNA in the λ Charon 4 phage (12) was screened with 5'[^{32}P] labelled rice rRNA by the procedure of Benton and Davis (13). Six out of the 5000 plaques screened lighted up and the most prominent one was plaque purified and studied.

S1 Nuclease mapping

S1 Nuclease mapping was done according to the method of Harrington and Chikaraishi (14). The 3'[^{32}P] labelled DNA fragments (15-30 ng) were hybridized with 5 µg of the total nuclear RNA in 10 µl of 80% formamide, 0.4 M NaCl, 40 mM piperazine-N,N'-bis(2-ethanesulphonic acid), 0.1 mM EDTA at 60°C overnight. It was diluted 30-60 fold with SI nuclease buffer (28 mM NaCl, 30mM sodium acetate, pH 4.5, 4.5 mM $ZnCl_2$, 50 mM NaF), containing 20 µg/ml denatured calf thymus DNA and digested with S1 nuclease (200 u/ml). The reaction was terminated by the addition of 1/6th volume of 2.5 M ammonium acetate, 50 mM EDTA, extracted with phenol-chloroform, and the DNA-rRNA hybrid was precipitated with ethanol. The protected DNA fragments were analysed by electrophoresis either on a 1.5% agarose or on 8 M urea-7% polyacrylamide gel.

RESULTS AND DISCUSSION

By screening an EcoRI genomic library of rice DNA in λ Charon 4 phage with [^{32}P]-rRNAs, a clone bearing a 9.8 kb insert containing the rDNA unit of rice was obtained. The low frequency of detection showed that the 9.8 kb unit is a minor repeat. The 8.8 kb repeat unit is the major one, occurring about 1060 times in the diploid genome of rice variety IR20 (6). The variation in the sizes of the rDNA units is due to

the differences in the length of the nontranscribed spacer region. Such variations in the length of the rDNA units is a common feature in plants (4).

A 3.3 kb EcoRI-BamHI fragment from the BamHI digest of the 9.8 kb DNA, hybridized only with 25S rRNA and contained the 3' end nucleotide sequence of the 25S rRNA gene, (data not shown). This fragment was analysed to determine the transcription termination site on the rDNA. The 9.8 kb insert DNA was restricted with HindIII (Fig.1) and the DNA fragments were separated by 1% agarose gel electrophoresis and electroeluted. The 3'[^{32}P]labelled 0.88 kb EcoRI-HindIII, 0.25 kb HindIII and 1.9 kb HindIII fragments were hybridized with the precursor rRNA, digested with S1 nuclease and the DNA-RNA hybrids were analysed by electrophoresis on a 1.5% agarose gel (Fig.2). The signals at 0.88 kb and 0.25 kb showed that these fragments were fully protected against S1 nuclease digestion, while the 1.9 kb HindIII fragment was not protected. In addition to the 0.88 kb fragment, a fragment of 0.47 kb was protected which may be due to the hybridization of the mature 25S rRNA present in the RNA preparation. The 0.25 kb HindIII fragment was fully protected, indicating that the precursor rRNA ends within this fragment. When the protected portion of the 0.25 kb HindIII fragment was analysed on a sequencing gel, two fragments of the size 248 and 244 nucleotides were seen (Fig.3) which may represent the two 3' termini of the precursor rRNA.

The 0.88 kb EcoRI-HindIII and the 1.9 kb HindIII fragments were cloned in M13mp18 and the 0.25 kb HindIII fragment was cloned in M13mp19 (16) and transformed into E.coli MV1190.

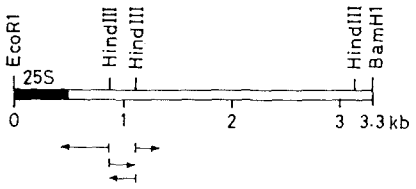

Fig.1: Restriction sites on the 3.3 kb fragment and the sequencing strategy. The 3.3 kb EcoRI-BamHI fragment of the 9.8 kb DNA bearing the rDNA was restricted with HindIII, separated by 1.5% agarose gel electrophoresis and the 25S rRNA gene was located by hybridization with labelled 25S rRNA probe. The restriction fragments were cloned in M13mp18 or M13mp19 and sequenced to the extent and in the directions indicated.

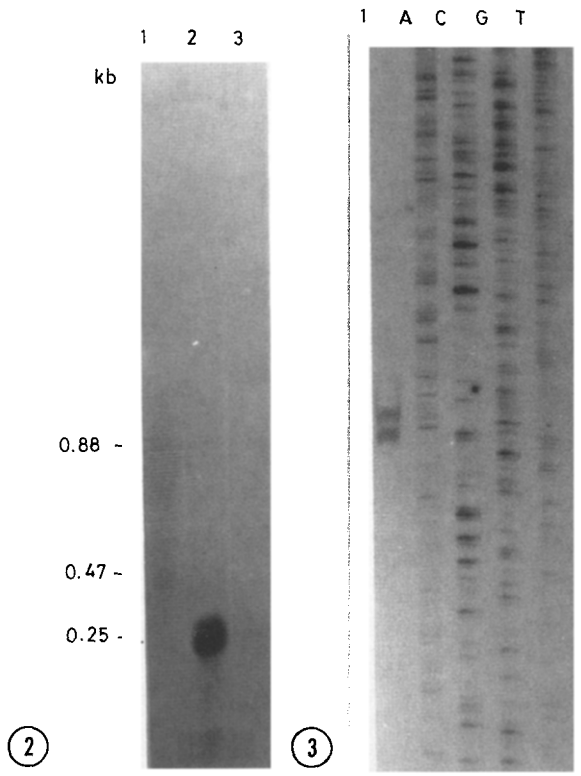

Fig.2: S1 Nuclease mapping of the rDNA-nuclear rRNA hybrids. The 3'[^{32}P] end labelled 0.88kb EcoRI-HindIII, 0.25kb and 1.9kb HindIII fragments (Fig.1) were hybridized with the nuclear rRNA, digested with S1 nuclease and analysed by electrophoresis on a 1.5% agarose gel. The radioactive bands were detected by autoradiography and their sizes were determined using Øx174 HaeIII DNA fragments as molecular size markers. Hybrid from: Lane 1, 0.88kb; 2,0.25kb and 3, 1.9kb DNA fragments.

Fig.3: Size of the protected fragment of the 0.25kb HindIII fragment-nuclear rRNA hybrid. The 3'[^{32}P] end labelled 0.25kb DNA was hybridized with the nuclear RNA, digested with S1 nuclease and analysed on a sequencing gel, where the same DNA fragment was sequenced by the dideoxy chain termination method. Lane 1: protected DNA fragment.

The phage was isolated, the DNA was prepared and sequenced using the universal primer and [α-^{32}P]dATP by the dideoxy chain termination method (17) in the directions indicated (Fig.1). A sequence of 884 nucleotides including the terminal 35 nucleotides of the 25S rRNA gene and 849 nucleotides of the 3' flanking region were obtained (Fig.4). The 3'flanking region is characterized by the presence of 3 long imperfect repeats from 285 to 488, 534 to 745, and the 752nd nucleotide onwards. The last 98 nucleotides seem to be part of the third repeat. The presence of long repeats in the nontranscribed spacer is a feature common

Fig.4: Nucleotide sequence of the 3'end and flanking region of the 25S rRNA gene. The cloned DNA fragments (Fig.1) were sequenced by the dideoxy chain termination method using the universal primer and [α ^{32}P]dATP. The sequence of nucleotides were read from the ladder sequences. The nucleotides are numbered from the start of the 3'-flanking region. Arrows indicate the S1 signals. The deleted C and T from the published 25S rRNA gene sequence are indicated. The long repeats are underlined and the putative transcription termination signal is boxed. The complementary sequences in the 25S rRNA gene and the flanking region are overlined.

to maize (7), wheat, (18) Raphanus sativus (8), Vicia faba (19), pea (20) and cucumber (21) rDNAs. The nucleotides at the near end of the 2nd long repeat are complementary to the 3'end of the 25S rRNA gene.

The 3'end of the 25S rRNA gene of the rice variety IR20 was homologous to the 25S rRNA gene sequence of rice variety Mangetsumochi (22) except for the deletions of a C and a T at positions 11 and 22 respectively upstream to the end of the 25S rRNA gene. The fully protected 0.47 and 0.88 kb DNA -nuclear rRNA hybrids (Fig.2) indicated the extent of protection up to the end of the 25S rRNA gene and the precursor rRNA transcription termination site on the rDNA, respectively. From the sizes of the protected 0.25 kb DNA-precursor rRNA hybrids (Fig.3), the two 3'termini of the precursor rRNA were mapped on the rDNA at positions 616 and 620 from the 3' end of the

25S rRNA gene (Fig.4). The sequence from the positions 611 to 628 bears extensive homology in the reverse orientation to the 18 nucleotide repetitive motif AGGTCGACCAG$^{AT}_{TA}$NTCCG which is a signal for the transcription termination of rDNA in mice (24). The 18 base pair motif in the rice DNA is at the middle of the second repeat and spans the junction of the 2 long stems (data not shown), generated by the computer analyses of the 3' flanking region (23). The analogous 18 base pair motif is not present in the other two repeats. Thus the 18 nucleotide motif may represent the signal for termination of rDNA transcription in rice. The 3' terminus of the precursor rRNA in maize and Raphanus sativus was found to be very near the 3' end of the 25S rRNA gene (7,8). In mice the polymerase seems to halt at the 18 nucleotide long conserved motif located at 565 nucleotides downstream to the 3' end of the 28S rRNA gene (7,8). In Xenopus it does so at a position 235 nucleotides downstream to the 3' end of the 28S rRNA (26). It has been shown in both these cases, that transcription proceeds till it reaches a similar signal adjacent to the initiation site, and this signal has been shown to be a part of the promotor for rDNA transcription. In the case of rat, the SI nuclease mapping studies have shown multiple 3' termini for the precursor rRNA among which two coincided with the probable termination site in vivo (27). In rice, all the rDNA transcription may end around this termination signal or traverse the nontranscribed spacer before terminating adjascent to the site of initiation of transcription.

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REFERENCES

1. Long,E.O. and David,T.B. (1980) Annu. Rev. Biochem. 49, 724-764.
2. Perry,R.P. (1976) Annu.Rev.Biochem. 45, 605-629.
3. Miller,O.L., Jr. and Beatty,B.R. (1969) Science, 164, 955-957.
4. Takaiwa,F., Oono,K. and Sugiura,M. (1984) Nucleic Acids Res. 12, 5441-5448.

5. Hariharan,N. and Padayatty,J.D. (1987) Indian J. Biochem. Biophys. 24, 157-164.
6. Reddy,P.S. and Padayatty,J.D. (1987) Indian J. Biochem. Biophys. 24, 293-295.
7. Toloczyki,C. and Feix,G. (1986) Nucelic Acids Res. 14, 4969-4985.
8. Delcasso-Tremousaygue,D., Grillet,F., Panabieres,F., Ananiev,E.D., and Delseny,M. (1988) Eur.J.Biochem. 172, 767-776.
9. Walbot,V. and Goldberg,R (1979) In Nucleic Acids in Plants (T.C. Hall and J.W. Davies, Eds.), Part I, pp.3-40. CRC Press, Florida.
10. Rubin,G.M. (1975) In Methods in Cell Biology (D.M. Prescott, Ed.), Vol.XII, pp.45-64, Academic Press, New York.
11. VanLoon, L.C., Trewavas,A. and Chapman,K.S.R. (1975) Plant Physiol. 55, 288-292.
12. Blattner,F.R., Williams,B.G., Blechl,A.E., Thompson,K.D, Fabor,H.E., Furlong,L.A., Grunwald,D.J., Kiefer,D.O., Moore,D.D., Schumm,J.W., Sheldon,E.L. and Smithies,O. (1977) Science, 196, 161-169.
13. Benton,W.D. and Davis,R.W. (1977) Science, 196, 180-182.
14. Harrington,C.A. and Chikaraishi,D.M. (1983) Nucleic Acids Res. 11, 3317-3332.
15. Roger,S.O. and Bendich,A.J. (1987) Plant-Mol.Biol. 9, 509-520.
16. Messing,J. (1983) Methods Enzymol, 101, 20-78.
17. Sanger,F., Coulson,A.R., Barrek,B.G., Smith,A.J.H. and Roe,B.A. (1980) J.Mol.Biol. 143, 161-178.
18. Appels,R. and Dvorak,J. (1982), Theor.Appl.Genet. 63, 337-348.
19. Yakuro,K., Kato,A., and Tanifuji,S. (1984) Mol.Gen.Genet. 193, 400-405.
20. Jorgenson,R.A, Cuellar,R.E., Thompson,W.F., and Kavanagh,T.A. (1987) Plant Mol.Biol. 8, 3-12.
21. Ganal,M., Torres,R. and Hemleben,V. (1988) Mol.Gen.Genet. 212, 548-554.
22. Takaiwa,F., Oono,K., Iida,Y. and Sugiura,M. (1985) Gene. 37, 255-259.
23. Zuker,M. and Stiegler,P. (1981) Nucleic Acids Res. 9, 133-148.
24. Grummt,I, Rosenbauer,H., Niedermeyer,I., Maier,U. and Ohrlein,A. (1986) Cell, 45, 837-846.
25. Grummt,I., Kuhn,A., Bartsch,I and Rosenbauer,H. (1986) Cell, 47, 901-911.
26. McStay,B. and Reeder,R.H. (1986) Cell, 47, 913-920.
27. Meiva,T., Kominami,R., Yoshikura,H., Sudo,K. and Muramatsu,M. (1987) Nucleic Acids Res. 15, 2043-2059.