5S-rRNA genes in rice embryos

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Abstract

The 5S-rRNA from the ungerminated and 48-h-germinated rice embryos differs from the wheat, rye and maize by two nucleotides. The 48-h-germinated embryos contain another species of 5S-rRNA which differs by 3 nucleotides from the ungerminated embryos, thereby showing the expression of two 5S-rRNA genes during germination. The 5S-rRNA genes are present in tandem repeats of a 0.3-kb sequence with some length heterogeneity in the rice genome. The 5S-rRNA gene that was sequenced is identical to that of wheat and maize, except for two nucleotides, C and T, which are interchanged at positions 107 and 117. The insert of continuous 5S-rRNA gene in pBR322 was transcribed in vitro much more efficiently than the discontinuous gene. There was no homology between the 184-bp spacer sequence of 5S-rRNA genes in rice and other systems except the presence of the oligo(T) transcription terminator sequence.

Introduction

Nucleotide sequence analysis of 5S-rRNA from a wide variety of eukaryotes has revealed a highly conserved primary structure [9]. To follow the expression of 5S-rRNA genes in rice embryos, the nucleotide sequence of 5S-rRNAs isolated from dormant and germinated embryos were determined. In most eukaryotes, the genes for 5S-rRNAs are organized as separate clusters of tandem repeats consisting of a highly conserved gene sequence with a variable non-transcribed spacer [14]. Even though a wealth of information is available on the structure and expression of 5S-rRNA genes of lower eukaryotes and animals, relatively little is known on higher plants with the exception of wheat, rye, flax, tobacco, maize and yellow lupin [1, 2, 5, 12, 16, 22]. To study the structure and regulation of expression of 5S-rRNA genes in rice embryos, we have cloned, sequenced and transcribed in vitro a BamHI repeat unit of 0.3-kb DNA carrying a 5S-rRNA gene.

Materials and methods

Preparation of rice DNA

DNA from the germinated rice (Oryza sativa L., IR-20) embryos was isolated according to Walbot and Goldberg [33]. The spooled DNA obtained after ethanol precipitation was dissolved in 10 mM Tris-HCl, 1 mM ethylenediaminetetraacetate, pH 7.4, and further purified by gel filtration on a Biogel A5m column.

Purification of 5S- and 5.8S-rRNAs and tRNA

Ribosomes from 48-h-germinated rice embryos were isolated and the 5S- and 5.8S-rRNAs were extracted and purified according to the method of Rubin [23]. Total tRNA was isolated from the cytosolic RNA according to the method of Gestaland et al. [11], separated by electrophoresis on 10% polyacrylamide gel
containing 7 M urea using tRNA from yeast as internal marker, and eluted.

**Enzyme digestion**

Restriction endonuclease digestions of DNA were done as described in the supplier’s manual.

**Southern hybridization**

DNA fragments were separated by agarose gel electrophoresis and transferred onto nitrocellulose filter according to Southern [28]. Baking at 80°C for 6–8 h was done to increase the retention of DNA fragments smaller than 0.5 kb [25]. DNA was labelled with [α-32P]dATP by nick-translation and used as probes for hybridizations. Hybridizations were carried out in 50% (v/v) formamide, 5 x SSC (1 x SSC is 0.15 M NaCl, 15 mM sodium citrate, pH 7) and 0.1% sodium dodecyl sulphate (SDS) as described by Maniatis et al. [17]. Filters were washed twice at room temperature with 2 x SSC, twice at 50°C with 1 x SSC and at 65°C with 0.5 x SSC and 0.1% SDS and subjected to autoradiography at −70°C for 24 h.

**Cloning**

*BamHI* digested rice DNA was separated on 0.75% agarose gel and DNA fragments of size smaller than 1 kb were eluted and cloned in plasmid pBR322 at the *BamHI* site [29]. Such small-size DNA fragments may carry 5S-rRNA genes as *BamHI* fragments of DNA from wheat [5], rye [1] and maize [16] of less than 0.5 kb contained the 5S-rRNA gene. *E. coli* HB 101 was transformed and the recombinant clones were screened for 5S-rRNA genes by the method of Grunstein and Wallis [13] using (5'-32P)5S-rRNA. The plasmid bearing the 0.3-kb insert was labelled as pIR5S-201, which produced two fragments of size 4.36 and 0.3 kb on digestion with *BamHI*.

Plasmid pBR322 DNA was digested with *BamHI* and the ends were filled by using Klenow fragment of *E. coli* DNA polymerase I. The 0.3-kb insert DNA which has a single site for *AluI* was digested with the enzyme and ligated using T4 phage DNA ligase. The cohesive end ligation is at least 100 times more efficient than blunt end ligation [17]. The end-to-end ligated *AluI* fragments of the 0.3-kb DNA were inserted at the filled *BamHI* site of pBR322 by blunt end ligation. Transformants obtained from such molecules were screened by the analysis after digestion with *BamHI*, which yielded only a linearised molecule of size 4.66 kb and was labelled as pIR5S-202.

**RNA sequencing**

The 32P-end-labelled RNA was sequenced by the enzymic method according to the supplier’s manual (P.L. Biochemicals, Inc.). The G, A, U + C and A + U specific cleavages were done with RNase T1, *B. cereus*, U2 and Phy M respectively [7] by incubating the 5’-end-labelled RNA with 1 unit of the enzyme in the presence of carrier yeast tRNA at 55°C for 10 min. For limited hydrolysis, the 32P-RNA was incubated with 50 mM Na2CO3/NaHCO3 buffer at pH 9.0 in the presence of yeast RNA at 90°C for 6 min. The samples were quick-chilled in ice, the loading buffer was added and analysed by 8 M urea−20% polyacrylamide gel electrophoresis using 50 mM Tris−50 mM boric acid−1 mM ethylenediaminetetraacetate, pH 8.3. At the end of the run, the gel was transferred onto a plastic sheet, covered with Saran wrap and subjected to autoradiography at −70°C.

**DNA sequencing**

Plasmid DNA was prepared from the clone following the procedure of Birnboim and Doly [3]. The insert 0.3-kb DNA was prepared, labelled at the 5’ ends, restricted with the single cut enzyme, *AluI*, separated by 12% polyacrylamide gel electrophoresis, isolated and sequenced according to the method of Maxam and Gilbert [19]. Alternatively the insert DNA was cloned in phage M13mp19 RF DNA at the