Genomics 96 (2010) 181–190

Contents lists available at ScienceDirect

Genomics

journal homepage: www.elsevier.com/locate/ygeno

Chromosomal conservation and sequence diversity of ribosomal RNA genes of two distant *Oryza* species

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ARTICLE INFO

Article history:
Received 29 November 2009
Accepted 18 May 2010
Available online 24 May 2010

Keywords:
Genome specific
IGS
45 S rDNA
Subrepeat
Rice

ABSTRACT

Contrary to the chromosomal polymorphism of 45 S ribosomal genes (45 S rDNA) loci in other *Oryza* species, each of *Oryza australiensis* and *Oryza brachyantha* has only one 45 S rDNA locus at the most conserved position of 45 S rDNAs in *Oryza*. *O. australiensis* and *O. brachyantha* are known phylogenetically distant and have extremely different genome sizes among diploid *Oryza* species. This study reveals that the sequences and organizations of intergenic spacer (IGS) for 45 S rDNA of both *O. australiensis* and *O. brachyantha* are different from other *Oryza* species. The IGS of *O. australiensis* contains 13 tandem repeats and only one transcriptional initiation site, while there are four tandem repeats and three transcriptional initiation sites in the IGS of *O. brachyantha*. Our results suggest different evolution processes of orthologous rDNA loci in the genus *Oryza*. Here we also demonstrate an efficient strategy to study locus-specific IGS before whole genome sequences data are available.

Introduction

The transcribed region of a 45 S ribosomal RNA gene (rDNA) contains sequences encoding 16(–18)S, 5.8 S, and 25(–28)S rRNA and two internal transcribed spacers (ITS1 and ITS2). In higher eukaryotes, highly repeated 45 S rDNA units are arranged in tandem at one or several chromosomal loci. An intergenic spacer (IGS) separates two adjacent rDNAs in the tandem arrays and consists of a nontranscribed region (NTS) flanked with two external transcribed spacers (ETS). Contrary to the high conservation in the coding regions, IGSs vary in both length and sequence among closely related species [1,2]. Like other highly repeated gene families, rDNA repeats undergo a process of concerted evolution, homogenizing the sequences of each unit in an array. Therefore, units within a species are more similar to each other than to those in related species [3,4]. However, IGS variants are common in a genome with multiple rDNA loci because the occurrence of homogenization across chromosomes is infrequent [5,6].

Highly similar and repetitive sequences such as rDNAs usually present a technical challenge in an automatic assembly. A lack of genomic sequence information hampers the progress of identifying the rDNA variants (v-rDNA) with computational and bioinformatic methods. To date, only one mouse rDNA transcription unit has been sequenced in its entirety [7] and in GenBank Release 163, December 2007. Methods other than whole genome sequencing are applied to study v-rDNAs. In human, rDNA variant on a specific chromosome was cloned after PCR amplification from a rodent–human somatic cell hybrid containing that chromosome [8]. In mouse, considerable restriction fragment length polymorphisms (RFLP) and a variable number of repeats in the nontranscribed spacer were detected in rDNA units [9]. Seven mouse v-rDNAs on different chromosome loci were cloned based on RFLPs, showing unequal activities in different cell types [10]. In *Arabidopsis thaliana*, rDNAs clustered in two nucleolar organizing regions (NORs) on chromosome 2 (*NOR2*) and chromosome 4 (*NOR4*). Both could be differentiated by RFLP [11].

In the genus *Oryza*, 45 S rDNA loci are polymorphic in both number and location. From one to four rDNA loci have been detected on *Oryza* chromosomes by fluorescence in situ hybridization (FISH) analyses [12]. *Oryza australiensis* (EE) as well as *Oryza brachyantha* (FF) has only one 45 S rDNA locus at the end of the short arm of chromosome 9 (9 S), which is the most conserved position of the 45 S rDNA locus in the genus *Oryza* [12,13]. The length polymorphism of IGSs in the genus *Oryza* has been well documented [14–17]. The integrated rDNA array at the end of chromosome 9 S in *Oryza sativa* ssp. *japonica* cv. Nipponbare were analyzed [18] soon after the completion of the whole rice genome sequencing project [19]. Three types of DNAs ranging from 7928 bp to 8934 bp long were contained in this region. The length heterogeneity in these three types of rDNAs is due to the fact that they contain different numbers of a 254-bp subrepeat in their IGS [18]. Such 254-bp subrepeats in IGS were detected in most of the *Oryza* genome except for EE and CCDD [21]. The existence of 254-bp subrepeats in the FF genome was unknown at that time. However, a fragment of 61 bp downstream of those subrepeats in IGS of AA genome was found also to be also specific to the FF genome [15].

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doi:10.1016/j.ygeno.2010.05.005

Table 1
Accessions of *Oryza* species used in this study.

Number[a]	*Species* (accession number)	Genome
1	*O. sativa, ssp. japonica,* cv. Nipponbare	AA
2	*O. sativa, ssp. indica,* cv. Dular	AA
3	*O. sativa, ssp, japonica,* cv. TNG 67	AA
4	*O. sativa, ssp. indica,* cv. IR36	AA
5	*O. glaberrima* (W0025)	AA
6	*O. rufipogon* (W0107)	AA
7	*O. rufipogon* (W1623)	AA
8	*O. australiensis* (W0008)	EE
9	*O. punctata* (W1593)	BB
10	*O. punctata* (W1577)	BB
11	*O. punctata* (W1564)	BBCC
12	*O. minuta* (W0045)	BBCC
13	*O. officinalis* (W0567)	CC
14	*O. gradiglumis* (W0613)	CCDD
15	*O. latifolia* (W0019)	CCDD
16	*O. brachyantha* (101232)	FF

[a] Each accession in the figures is represented by the number denoted here.

In this study, PCR profiles and genomic southern analyses show that the organizations of IGS of *O. australiensis* and *O. brachyantha* differ from those of other *Oryza* genomes. We cloned and characterized the IGS fragments amplified from *O. australiensis* and *O. brachyantha*, respectively. The results show that both *O. australiensis* and *O. brachyantha have* IGS that are extremely genome specific, although their rDNA loci are at a conserved chromosomal position in the genus *Oryza*. Together with the results presented in the present paper and the variations in the number and position of rDNA loci reported previously [12], the genome-specific IGS in *O. brachyantha* and in *O. australiensis* suggest different evolution processes of rDNAs from other *Oryza* species.

Materials and methods

Plant materials

Sixteen accessions representing 11 *Oryza* species were used in this study (Table 1). In the following figures, each accession is represented with a number identical to that in Table 1. The original seeds of wild species of rice, except for those of *O. brachyantha* (FF), were kindly provided by the National Institute of Genetics, Japan (http://www.shigen.nig.ac.jp/rice/oryzabase/wild/coreCollection.jsp). The International Rice Genebank at the International Rice Research Institute (IRRI) in the Philippines kindly provided the seeds of *O. brachyantha*. Plants were grown at the experimental field of the Institute of Plant and Microbial Biology, Academia Sinica, Taipei, Taiwan. Genomic DNA samples were extracted from young leaves using DNeasy Plant Mini Kit (Qiagen, Oslo, Norway).

PCR analysis

Appropriate primers were used in the PCR to amplify fragments of 18 S rDNA, IGS, and subrepeats in the IGS from genomic DNA of accessions (Fig. 1A). The primers for 18 S rDNA are P1 (5′-CGAACTGTGAAACTGCGAATGGC-3′) and P2 (5′-TAGGAGCGACGGGCGGTGTG-3′) [18]. The primers for the IGS region are P3 (5′-TTGCTGCCACGATCCACTGAG-3′) and P4 (5′-CTACTGGCAGGATCAACCAGG-3′), which are designed according to the sequences at the 3′ end of 18 S rDNA and the complementary sequence at the 5′ end of 25 S rDNA [20]. The primers for the IGS subrepeat are P5 (5′-CTCGCCCCAGCTCCCGAG-3′) and P6 (5′-GGCTACGTGCCCGAACAC-3′) [18].

Each 25-μL reaction mixture contained 25 ng of total genomic DNA, 200 μM of each dNTP, 0.1 μM of each primer, 1× PCR buffer

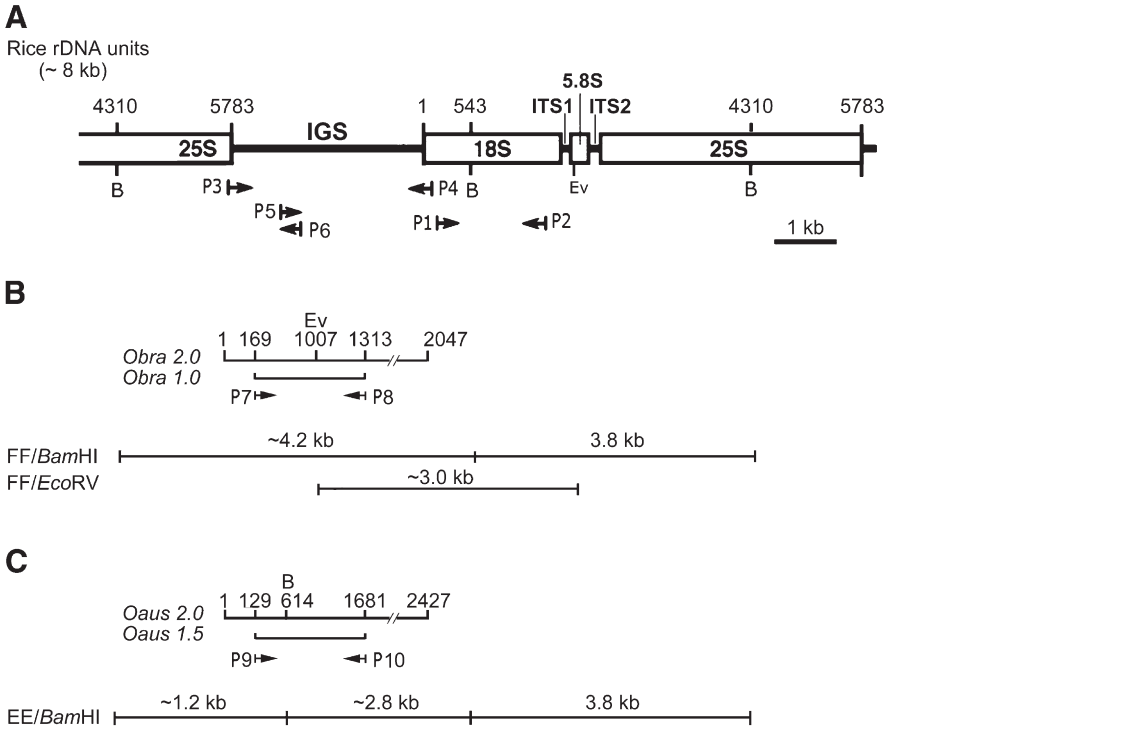

Fig. 1. Diagrammatic representation of the 18–5.8–26 S rDNA unit in rice and the corresponding positions of the fragments cloned in this study. (A) Diagrammatic representation of the 18–5.8–26 S rDNA unit in rice modified from Fujisawa et al. [18]. The restriction sites for *Bam*HI (B) and *Eco*RV (Ev) are indicated. The positions of primer pairs are indicated: P1–P2 for 18 S DNA, P3–P4 for the intergenic spacer (IGS), and P5–P6 for the subrepeats in the IGS. The numbers indicate the nucleotide positions. (B, C) Schematic representation of the IGS fragments cloned from *Oryza brachyantha* (B) and *Oryza australiensis* (C) in this study. The positions of primer pairs, P7–P8 and P9–P10, are indicated. The sizes and corresponding positions of restricted fragments detected in southern hybridization.