Plant Gene Register

Molecular Cloning of the Gene (SodCc1) that Encodes a Cytosolic Copper/Zinc-Superoxide Dismutase from Rice (Oryza sativa L.)

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SOD (superoxide:superoxide oxidoreductase; EC 1.15.1.1) is considered a crucial component in biological defense against oxidative stress (Bowler et al., 1992). In higher plants, there are three known forms of the enzyme: Cu/Zn-, Mn-, and Fe-SODs. These SOD activities are typically associated with specific subcellular locations such as mitochondria (Mn-SOD), plastids (Cu/Zn-SOD and/or Fe-SOD), and the cytosol (Cu/Zn-SOD) and display a differential expression pattern in response to environmental and chemical stresses (Perl-Treves and Galun, 1991; Tsang et al., 1991). We have isolated SOD cDNA clones for a mitochondrial Mn protein and two cytosolic Cu/Zn isozymes from a rice (Oryza sativa L. cv Nipponbare) cDNA library prepared from developing seeds (Sakamoto et al., 1992a, 1993). Two nuclear genes (SodCc1 and SodCc2),3 which corresponded to the cytosolic Cu/Zn-SOD cDNAs, were also obtained and the exon/intron organization of the SodCc2 gene was characterized (Sakamoto et al., 1992b). To our knowledge, this is the only complete analysis of a plant Cu/Zn-SOD gene structure. We present the identification of the gene, SodCc1, that codes for an another cytosolic Cu/Zn-SOD in rice (Table I).

Isolation of the genomic λ clone (gSOD27) containing the SodCc1 gene was described previously (Sakamoto et al., 1992b). Sequence analysis of the structural region revealed that the SodCc1 transcribed region spans a chromosomal region over 3 kb long and is segmented by seven introns, the first of which appeared in the 5′ transcribed but untranslated region. The borders between exons and introns were in full agreement with the GT-AG rule. The exon/intron structure was identical between the SodCc1 and the SodCc2 gene encodings.

Table I. Characteristics of the rice SodCc1 gene

| Organism: | Oryza sativa L. (rice) cv Nipponbare. |
| Location in Genome: | Nuclear genome, chromosome location not known. |
| Gene Designation: | SodCc1. |
| Gene Product: | Cytosolic Cu/Zn-SOD (rice Cu/Zn-SOD II; EC 1.15.1.1). |
| Function: | Disproportionation of the superoxide anion radical into hydrogen peroxide and molecular oxygen. |
| Source: | Genomic library constructed in λEMBL3 after partial restriction of rice germ nuclear DNA with Sau3AI. |
| Techniques: | A genomic λEMBL3 library was screened with the radiolabeled cDNA (RSODB) that encodes a rice cytosolic Cu/Zn-SOD (Sakamoto et al., 1992a). Restriction fragments containing the SodCc1 gene from the λ clone gSOD27 was recloned into the plasmid Bluescript SK+ vector and sequenced by the dideoxy chain termination method on double-stranded DNA. |
| Method of Identification: | Complete agreement of the nucleotide sequences between the SodCc1 exons and the cognate RSODA cDNA (Sakamoto et al., 1992a). |

Features of Gene Structure:

Transcribed region stretched over a 3-kb genomic sequence. Six introns were identified in the SodCc1-coding region, and an additional intron occurred in the 5′ untranslated region.

Gene Copy Number:

Genomic Southern blot and analyses of cytosolic Cu/Zn-SOD clones showed that the rice genome has an additional gene (SodCc2) coding for cytosolic Cu/Zn-SOD (Sakamoto et al., 1992a, 1992b).

Expression Characteristics:

SodCc1 transcripts were detected on northern blots of total RNAs from leaves, roots, seeds, and calli of rice. The SodCc1 promoter is activated by the treatment with thiol compounds in rice protoplasts.

Subcellular Location:

Cytosol.

Abbreviations: SOD, superoxide dismutase; SodCc, gene encoding cytosolic copper/zinc-superoxide dismutase.

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3 Two rice genes, SodCc1 and SodCc2, which encode cytosolic Cu/Zn-SODs, were originally designated sodA and sodB, respectively, but have been renamed according to the recommendation of the Commission of Plant Gene Nomenclature (1993).

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larger in the SodCc1 gene. In each gene, the longest intron was the first one (5' intron), occurring in the 5' untranslated leader. Since the tobacco SodCc gene also contained a 5' intron at a position similar to those in rice SodCc genes (Hérouart et al., 1993), the presence of an intron in the 5' noncoding region is likely to be a common structural feature in plant SodCc genes. This speculation is supported by the fact that 5' noncoding sequences surrounding the splice junction of the 5' intron were significantly conserved throughout plant SodCc cDNA sequences thus far available, despite the fact that no amino acids are encoded by the sequences. If we assume the ubiquitous existence of an intron in this region in plant SodCc genes, it seems possible that conserved nucleotide residues on the 5' noncoding region are involved in the processing of the intron from the precursor transcripts and function as cis-acting elements for the maturation of the transcripts.

The SodCc1 gene is expressed in seeds, calli, roots, and leaves and the steady-state transcript level is elevated by superoxide-generating conditions. Regulation of rice SodCc1 and SodCc2 expression was investigated by the use of SodCc promoter-reporter GUS fusions in rice protoplasts. In transient GUS assays, two SodCc promoters differentially responded to the phytohormone ABA (A. Sakamoto, T. Okumura, H. Kaminaka, and K. Tanaka, unpublished data). In agreement with an earlier report of the tobacco SodCc promoter (Hérouart et al., 1993), the transcriptional activity of the rice SodCc1 promoter was stimulated by the application of thiol molecules in transient assays. The 5' intron in the tobacco SodCc gene has been shown to confer neither a positive nor a negative effect on the expression of the reporter GUS gene in transgenic tobacco (Hérouart et al., 1993). On the contrary, the rice SodCc1 5' intron drastically enhanced the reporter gene expression, especially under the transcriptional control of strong promoters such as the cauliflower mosaic 35S sequence.

NOTE ADDED IN PROOF


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