Identification of cDNA encoding cytochrome c oxidase subunit 5c (COX5c) from rice: comparison of its expression with nuclear-encoded and mitochondrial-encoded COX genes

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Little is presently known about the nuclear-encoded genes for cytochrome c oxidase (COX) in higher plants. In rice, only the nuclear-encoded COX5b gene has been reported. To understand the relationship between the expression of nuclear-encoded and mitochondrial-encoded COX genes in rice, we first characterized a cDNA encoding one of the other nuclear COX genes, COX5c, which encodes 63 amino acids. The deduced amino acid sequence of COX5c from rice was highly homologous to that from sweet potato. Genomic Southern hybridization indicated that the rice COX5c subunit is encoded by a single copy of the COX5c gene. Furthermore, we compared the expression patterns of the nuclear-encoded COX5c and COX5b genes with the expression pattern of the mitochondrial-encoded COX1 gene among several organs by Northern blot analysis. The results suggested that regulatory systems of expression between the nuclear-encoded and the mitochondrial-encoded COX genes are different among different organs in rice.

The cytochrome respiratory pathway in mitochondria basically consists of NADH: ubiquinone oxidoreductase (complex I), succinate:ubiquinone oxidoreductase (complex II), ubiquinol:ferricytochrome c oxidoreductase (complex III), cytochrome c and cytochrome c oxidase (complex IV). These components act in sequence to accept reducing equivalents from NADH or FADH2 and transfer them through oxidation-reduction reactions to O2. The terminal oxidase that converts O2 to H2O is cytochrome c oxidase (COX). The oxidation-reduction reactions between NADH or FADH2 and cytochrome c are reversible and almost in equilibrium, whereas the oxidation-reduction reaction from cytochrome c and oxygen, which is catalyzed by COX, is irreversible. Therefore, these characteristics indicate that COX plays an important role in regulating the cytochrome pathway (reviewed by Poyton and McEwen, 1996).

The COX of higher plants is composed of at least ten subunits (Jänsch et al., 1996). The largest three subunits (COX1, COX2 and COX3) are encoded by mitochondrial genes, while the remaining subunits are encoded by nuclear genes. Although the mitochondrial-encoded COX1, COX2 and COX3 subunits are essential for catalysis of COX, the three subunits are not sufficient for its catalysis. In Saccharomyces cerevisiae, it was reported that deletion of the nuclear-encoded subunits such as COX4, COX5a, COX5b and COX6 led to the complete loss of cytochrome c oxidase activity (reviewed by Poyton and McEwen, 1996). This evidence indicates that the nuclear-encoded COX subunits as well as the mitochondrial-encoded COX subunits are important for the activity of COX. Because COX is composed of equimolar amounts of each subunit, it is assumed that expression coordinated between the nuclear-encoded COX genes and the mitochondrial-encoded COX genes is required for full activity of cytochrome c oxidase.

The mitochondrial-encoded COX genes (COX1, COX2 and COX3) have been characterized in several plant species, including rice, in detail (Kao et al., 1984; Bailey-Serres et al., 1986; Kadowaki et al., 1989; Kaleikau et al., 1990). In contrast, there is less information about the nuclear-encoded COX genes in higher plants. To our knowledge, only two nuclear-encoded COX genes have been described: the COX5b gene from rice (Kadowaki et
al., 1996) and the COX5c gene from sweet potato (Nakagawa et al., 1987, 1990). It is known that the sequences of COX5b and COX5c in higher plants are completely different.

In order to understand the relationship between the expression of the mitochondrial-encoded and the nuclear-encoded COX genes in rice (Oryza sativa L. cv Nipponbare), we selected the COXI gene encoded in mitochondrial DNA (mtDNA) and the COX5b and COX5c genes encoded in nuclear DNA as probes for a Northern blot analysis. The genomic clone containing the COXI gene was used from our clone bank of rice mtDNA (Iwahashi et al., 1992). The nuclear-encoded COX5b gene from rice has already been identified by Kadowaki et al. (1996). To date, however, there is no information regarding the rice COX5c gene. In order to obtain some information about the rice COX5c subunit, in the present study, we searched for rice expressed sequence-tag (EST) clones that are homologous to sweet potato COX5c gene. A database search showed that the amino acid sequence of the sweet potato COX5c protein has significant homology to the putative protein encoded by the EST clone R0095 from rice (Nipponbare) roots. The EST clone R0095 from rice roots was provided by the Rice Genome Research Program of the National Institute of Agrobiological Resources. We completely determined nucleotide sequence of the cDNA clone R0095 in the direction of the sense and antisense strands using an automatic DNA sequencer (model 373S; Perkin Elmer, USA). There was a 533-bp insert (accession number AB027123) that encoded a complete 189-bp open reading frame (ORF) in the R0095 clone (Fig. 1A). The amino

Fig. 1. A. Nucleotide and deduced amino acid sequences of the cDNA encoding the rice COX5c gene. The nucleotide sequence has been deposited in the DDBJ, EMBL, NCBI and GSDB DNA databases under accession number AB027123. B. Alignment of the deduced amino acid sequences of COX5c of rice (this paper) and sweet potato (Nakagawa et al. 1990). The alignments were generated by the CLUSTAL W algorithm (Thompson et al., 1994). Asterisks and dots below the amino acid sequences indicate identical amino acids and homologous amino acids, respectively.
acid sequence of the ORF indicated high homology with the predicted COX5c protein of sweet potato (Fig. 1B). Therefore, the gene that encodes this ORF was designated as the rice COX5c gene.

In sweet potato, it was reported that the COX5c gene is encoded by a single nuclear gene (Nakagawa et al., 1993). In order to determine the copy number of the rice COX5c gene, genomic Southern hybridization was carried out using the probe whose sequence corresponds to the coding region of COX5c (whose position is between nucleotide numbers 72 and 284 in Fig. 1) and the DIG DNA Labeling and Detection Kit (Roche Diagnostics, Germany). As shown in Figure 2, the probe hybridized to a unique band of ApaI-, EcoRI-, EcoRV-, XbaI- and HindIII-digests of rice total DNA. This result indicated that rice COX5c is encoded by a single copy of the COX5c gene, as in the case of the sweet potato COX5c gene.

The expression of the rice COX5c gene was examined by Northern hybridization by determining the relative steady-state mRNA amounts in different organs of rice. Northern blot hybridization was performed with the DIG DNA Labeling and Detection Kit. Using equal amounts (5 µg each) of total RNA extracted from coleoptiles, young leaves, young roots, mature leaf blades, mature leaf sheaths, young panicles and panicles after heading, a single transcript of COX5c of approximately 0.6 kb was observed (Fig. 3). Although the rice COX5c gene is transcribed in all the organs examined, signal intensities varied among the organs. Regardless of whether the seedlings were grown in the light or the dark, the steady-state levels of the COX5c transcript in the roots of 11-day-old seedlings were apparently higher than those in the leaves. In mature rice plants, the highest relative amounts of COX5c transcripts were detected in the young panicles and the panicles after heading followed by leaf blades and leaf sheaths. Interestingly, we also observed a high level of the COX5c mRNA in the coleoptiles germinated under submerged conditions.

Furthermore, to compare the expression pattern of the COX5c gene with the expression patterns of the other nuclear-encoded COX gene and the mitochondrial-encoded COX gene, we investigated the expression of the COX5b and COX1 genes from rice (Fig. 3). Using the COX5b gene as a probe, we found a single transcript of approximately 1.1 kb (Fig. 3). The expression pattern of the rice COX5b gene was very similar to that of the COX5c gene, indicating that expression of the nuclear-encoded COX genes of COX5b and COX5c are commonly regulated in the organs examined. In contrast, the amounts of transcript of the mitochondrial-encoded COX1 gene were relatively constant, compared with those of the nuclear COX5b and COX5c genes, among the organs, although higher expression of the COX1 gene was observed in the coleoptiles under the submerged condition, young panicles and panicles after heading (Fig. 3). As shown in Figure 3, two transcripts of approximately 2.0 kb and 3.2 kb were found; they may safely be assumed to be the mature transcript and the precursor transcript, respectively, of the COX1 gene.

High levels of expression during flower (panicle) development have been observed for the genes coding for mitochondrial proteins in several plant species, indicating that the flowers (panicles) have a high demand for the energy transduced in the mitochondria (Huang et al., 1994; Landschütze et al., 1995; Heiser et al., 1996; Schmidt-Bleek et al., 1997). For example, Huang et al. (1994) reported that tobacco flowers contained several times more mitochondrial proteins than did the leaves. Examples of the mitochondrial proteins are the nuclear-encoded Rieske iron-sulfur protein, the β subunit of P;F0- ATP synthase, and the mitochondrial-encoded α subunit.
Fig. 3. Northern hybridization analysis of transcripts of COX5c, COX5b and COX1. Each lane was loaded with 5 µg total RNA extracted from coleoptiles (germinated under the submerged condition), young leaves and young roots (of 11-day-old seedlings under light or dark conditions), mature leaf blades, mature leaf sheaths, young panicles (whose lengths were 7–8 cm) and panicles after heading. The sizes of the transcripts are shown by the arrows at the right. Nu and Mt indicate nuclear-encoded and mitochondrial-encoded, respectively. Equal loadings of total RNA were checked by ethidium bromide staining (EtBr-staining).

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