Specific Expression of the Chloroplast Gene for RNA Polymerase (rpoB) at an Early Stage of Leaf Development in Rice

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The rpoB gene for the β subunit of rice chloroplast RNA polymerase was found to be highly expressed in unexpanded immature leaves that contained proplastids, indicating the specific expression of rpoB at an early stage of chloroplast development. A putative transcription start site (tss) was identified, but the 5' upstream region of the tss had no sequences resembling typical --35 and --10 elements. A palindromic sequence and high AT-content were recognized.

Key words: Chloroplast development — Chloroplast promoter — Gene expression — Oryza sativa.

The transcription of genes encoded in the plastid genome relies on the activity of a plastid-specific RNA polymerase (Igloi and Kossel 1992). The plastid RNA polymerase is homologous to its bacterial counterparts and consists of at least four subunits, designated a, β, β', and β", which are encoded within the plastid genome as the single-copy genes rpoA, rpoB, rpoCl, and rpoC2, respectively (Shinozaki et al. 1986, Hudson et al. 1988, Igloi et al. 1990, Hiratsuka et al. 1989). rpoB is located in the gene cluster rpoB-rpoCl-rpoC2, while rpoA is located in a large cluster of genes that encode ribosomal proteins and infA, a gene for initiation factor 1. It was confirmed that the products of the rpo genes are functional components of the chloroplast RNA polymerase by the demonstration of the similarity between the amino-terminal sequences of the subunits of the plastid RNA polymerase and the sequences deduced from the rpo genes. However, the transcript of rpoB, probably because of its low level of expression, has not been detected by Northern hybridization in any higher plant (Kanno and Hirai 1993) with the exception of barley (Rapp et al. 1992). We report here the high-level expression of rpoB in the unexpanded immature leaves at the base of the shoot in rice. Primer extension experiments allowed determination of a putative tss for rpoB.

The "Kinmaze" variety of Japanese paddy rice was obtained from the Institute of Genetic Resources, Kyushu University. Seedlings were grown until full expansion of the third leaf under constant illumination with cool-white fluorescent light (2,000 lux) at 30°C in growth cabinets at the Biotron Institute, Kyushu University. The relative humidity was maintained at about 65%. A modified version of White's medium (Iba et al. 1991) containing 6% agar was used as the culture medium.

Total RNA was purified by the phenol/SDS method (Ausubel et al. 1987). RNA was denatured and fractionated in 1% agarose-formaldehyde gel, and was transferred to a nylon membrane (Biodyne B; Pall Ultratine Filtration, U.S.A.) as described elsewhere (Ausubel et al. 1987). Fragments of rpoB (Hiratsuka et al. 1989) and the rice gene for nuclear tRNA-Gly (CCC) (Reddy and Padayatty 1988) were amplified by the polymerase chain reaction (PCR) with the primer pairs 5'-GAATGTCCACAATACCCGAT-3' and 5'-TATAGTCTAATCCAGAGCGGT-3', and 5'-GGCATCTAAGGGTAGTGTTG-3' and 5'-CATAATGCATCAAGGTGCC-3', respectively. The PCR-amplified fragments were labeled with [α-32P]dATP by the random primer method (Feinberg and Vogelstein 1983) and used as probes for hybridization. Pre-hybridization, hybridization, and post-hybridization procedures were performed as described in the instructions supplied with the Biodyne B nylon membrane.

The primer extension experiment was performed as described by Sambrook et al. (1989). The 5'-end 32P-labeled oligonucleotide (5'-TTCTCCTTCTATGAATGGG-3'), complementary to nucleotides 320 to 341 with respect to the initiation (ATG) codon of rpoB (Hiratsuka et al. 1989), was allowed to hybridize with 150 µg of total RNA in 30 µl of hybridization buffer that contained 40 mM PIPES (pH 6.4), 1 mM EDTA, 0.4 M NaCl, and 80% formamide at 85°C for 10 min. Then the solution was cooled to 30°C and the RNA was precipitated in ethanol. The precipitate was resuspended in 20 µl of reverse transcriptase buffer that contained 50 mM Tris-HCl (pH 8.3), 75 mM KCl, 3 mM MgCl2, 10 mM DTT, 0.5 mM dNTPs, and 200 units of SuperScript reverse transcriptase (Gibco, U.S.A.), and the reaction mixture was incubated at 37°C for 2 h.

It has been reported that the transcript of rpoB cannot be detected in mature rice leaves (Kanno and Hirai 1993). To identify the part(s) of the rice seedling in which rpoB is expressed, we performed Northern analysis of RNA from
Fig. 1 Northern analysis of the transcript of rpoB in rice seedlings with fully expanded third leaves. The parts used for the preparation of RNA samples were the root (R), the immature leaves folded in the shoot base (I), and the mature third leaf (M). Total RNA (20 μg) was loaded in each lane. The same blots were reprobed with a rice gene for nuclear (nu) tRNA-Gly in order to verify that approximately the same amounts of total RNA has been loaded in each lane. The positions of ribosomal RNAs from E. coli are indicated for reference on the left side of the figure.

several parts of seedlings with fully expanded third leaves. An early anatomical study of rice plants indicated that the shoot base always contains unexpanded immature leaves, in which formation of basic structures is occurring (Yamazaki 1963). Strong signals were detected in the case of the RNA from these immature leaves, which were folded in the shoot base, whereas no transcript was detected in the RNA from other parts of rice plants, such as the root, or the mature third leaf (Fig. 1). The plastids in the cells of these immature leaves are at an early stage of development (proplastids) and they are not photosynthetically active (Iba et al. 1991). These observations suggest that, in rice, rpoB is expressed specifically at an early stage of the development of leaf cells, in which plastids have not yet differentiated into chloroplasts.

In barley, a 6.0-kb RNA, probably the result of cotranscription of rpoB and rpoC1, was detected by Northern analysis (Baumgartner et al. 1993). In rice, by contrast, only a 3.1-kb RNA corresponding in size to the open reading frame of rpoB was detected as a major transcript in the sample of RNA prepared from the immature leaves.

The 5′ end of rpoB mRNA was previously mapped within the trnC-rpoB spacer region by SI mapping in spinach (Hudson et al. 1988). In that study, however, the tss was not accurately determined. We determined the 5′ end of rice rpoB mRNA by primer extension using the RNA sample prepared from the shoot base (Fig. 2). The 5′ end of the longest transcript was an A residue at position −381 with respect to the initiation (ATG) codon. The 5′ ends of two shorter transcripts were T and A residues at positions −370 and −358, respectively. It was unclear whether these

Table 1 AT contents (%) of the 5′ regions upstream of the transcription start sites of rice plastid genes

<table>
<thead>
<tr>
<th>Gene</th>
<th>5′ upstream region</th>
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<tbody>
<tr>
<td></td>
<td>-1 to -50</td>
</tr>
<tr>
<td>rpoB</td>
<td>70</td>
</tr>
<tr>
<td>rbcL</td>
<td>68</td>
</tr>
<tr>
<td>atpB</td>
<td>68</td>
</tr>
</tbody>
</table>

* The tss is designated position +1.
shorter transcripts arose from the processing of precursor transcripts or from the initiation of transcription. In higher plants, promoters of plastid genes, such as rbcL, generally contain elements that resemble the −10 (TATAAT) and −35 (TTGACA) sequences of Escherichia coli upstream of the tss (Kung and Lin 1985). No sequences that closely resembled such elements were, however, found in the region upstream of the putative tss of rice rpoB. Instead, a 28-bp palindromic sequence (CAAAAAAGAACCTCT-AATTCTTTTTTG) was found from positions −41 to −68 with respect to the putative tss. Table 1 shows that the 5′ upstream region (between −51 and −150) of the putative tss of the rice rpoB also had an extremely high AT content (≈78%), as compared with corresponding regions of rice plastid genes with −10 and −35 elements, namely rbcL (Nishizawa and Hirai 1987) and atpB (Nishizawa and Hirai 1989). The AT contents of the 5′ upstream regions of these genes were almost constant (≈68%) from position −1 to position −200.

Differential early expression of rpoB in chloroplast development, as compared to the expression of other plastid genes with −10 and −35 elements, has been reported in barley (Baumgartner et al. 1993). In rice, RNAs transcribed from rbcL and psbA were found to accumulate at high levels within mature leaves but not in the shoot base that contains immature leaves, in which plastids are in an early stage of development (Iba et al. 1991). These observations suggest that the −10 and −35 elements are not necessarily essential for transcription during the early stages of chloroplast development. Differential early transcription of rpoB might be due to the action of another plastid RNA polymerase encoded in the nucleus (Siemenroth et al. 1981, Morden et al. 1991), modulation of the conformation of the DNA (Lam and Chua 1987, Thompson and Mosig 1990), or modulation of the selection of promoters by trans-acting factors (Lam et al. 1988, Zaitlin et al. 1989, Tiller et al. 1991). There could also be unidentified promoter elements required for the expression the rpoB that are necessary for the build-up of transcriptional capacity during the early phase of chloroplast development.

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