Communication

Differential Expression of Three Plastidial Sigma Factors, OsSIG1, OsSIG2A, and OsSIG2B, during Leaf Development in Rice

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We isolated and characterized two rice nuclear genes, OsSIG2A and OsSIG2B, encoding the putative σ-factor of the plastid RNA polymerase. Deduced protein sequences predicted a plastid-localizing signal in the N-terminus and subsequent polypeptides similar to known SIG2 proteins. Gene expression analysis revealed that the OsSIG2A transcript is more abundant than the OsSIG2B transcript in all tissues tested and that both rice SIG2s are expressed from earlier stages of leaf development than that in the case of OsSIG1. These results indicate differential expression of SIG genes in leaf morphogenesis, suggesting the existence of tissue- and stage-specific functions of SIG proteins for transcriptional regulation of chloroplast genes in plant development.

Key words: sigma factor; plastid-encoded RNA polymerase; chloroplast

Transcriptional regulation in plastids is governed by two distinct RNA polymerases, the plastid-encoded RNA polymerase (PEP) and the nuclear-encoded RNA polymerase (NEP).1,2 PEP is a eubacteria-type σ-factor dependent RNA polymerase consisting of multiple subunits. As defined, subunits of the PEP core-enzyme are encoded on the plastidial DNA,3 but genes encoding σ-factor, a regulatory subunit of the eubacterial RNA polymerase, is missing from the plastid DNA. We and other groups have previously determined that σ-factor is encoded on the nuclear genome.4,5 To date, six σ-factor genes (SIG1, SIG2, SIG3, SIG4, SIG5, and SIG6) encoding σ-factors have been identified from Arabidopsis thaliana,6,7 and homologs of each Arabidopsis SIG gene have also been identified from other land plants.8–10 Among these SIG families, functional evidence of Arabidopsis SIG2 for chloroplast gene expression during the early stage of leaf development has been demonstrated by characterizing a gene-disrupted mutant.10 It has also been reported that a monocotyledonous plant, maize, possesses two SIG2 homologous genes, ZmSIG2a and ZmSIG2b,9 but the roles of these two maize SIG2 genes have yet to be understood fully. It is known that leaf development in monocotyledonous and dicotyledonous plants is totally different in terms of morphology. To investigate SIG2 functions in early stages of leaf development in monocotyledonous plants, in this study we isolated two rice (Oryza sativa) genes homologous to known SIG2 and characterized their gene expressions.

Rice SIG2 genes were isolated by plaque hybridization from the rice cDNA library as described previously.7 In order to prepare a screening probe, we prepared internal segments of Arabidopsis SIG2 (SigB) cDNA10 by PCR with oligonucleotide primers AtSIG2F (5’-GGCAACGTTACATCATGCGTACC-3’) and AtSIG2R (5’-GCTTGCTTGTACGTGACCC3’). Further, by searching the rice full-length cDNA collection of MAFF (http://cdna01.dna.affrc.go.jp/cDNA/) full-length clones, ID-1427 and ID-2985, were identified, corresponding to two cDNAs that we isolated. It was observed, however, that the nucleotide sequence of the identified full-length clone of ID1427 contained excess nucleotides in its ORF. In order to rectify this problem, we re-determined the sequence of the ORF region for 3 independently isolated clones corresponding to ID1427. As expected, all the analyzed cDNA encoded ID1427 type ORF without the additional nucleotide residues that are found in ID1427 sequence data. Based on this analysis, we confirmed that those sequences as correct cDNA sequences, and we designated them OsSIG2A (accession no AB095094) and OsSIG2B (AB095095) respectively.
Fig. 1. Comparison of Amino Acid Sequences of Plant Sigma Factors.

(A) Alignment of the predicted amino acid sequences of OsSIG2A and OsSIG2B with those of other SIG proteins. The sequences, corresponding to the region from 1.2 to the carboxyl terminus, are encoded by the following genes: OsSIG2A and OsSIG2B (this study); ZmSIG2A, and ZmSIG2B, Zea mays SIG2A (GenBank accession no. AF099110) and SIG2B (AF099111); AtSIG2, A. thaliana SIG2 (AB004293); SaSIG2, mustard (Sinapis alba) sig2 (SAL276656). Hyphens indicate gaps introduced to optimize alignment. Residues shared among these sequences are shaded, and residue numbers are shown on the left. (B) Phylogenetic relations among higher plant sigma subunits. The amino acid sequences analyzed are: rice OsSIG1 (product of Os-sigA, accession no. AB005290), OsSIG2A, and OsSIG2B; Arabidopsis AtSIG1 (SIG1, D89993), AtSIG2, AtSIG3 (SIG3, D89994), AtSIG4 (SIG4, AB021119), AtSIG5 (SIG5, AB021120), and AtSIG6 (SIG6, AB029916); Nicotiana tabacum NtSIG1A (SIG1A, AB023571) and NtSIG1B (SIG1B, AB023572); maize ZmSIG1a (Sig1, AF058708), ZmSIG1b (Sig2, AF058709), ZmSIG2A, ZmSIG2B, ZmSIG3 (SIG3, AY091464), and ZmSIG6 (SIG6/Sig3, AF099112); mustard SaSIG1 (SIG1, Y15899), SaSIG2 (SIG2, AF276656), and SaSIG3 (SIG3, AJ276657); Triticum aestivum TaSIG1 (SIG1, AJ132658); Sorghum bicolor SbSIG1 (SIG1, Y14276). The sequences, corresponding to the region from 1.2 to the carboxyl terminus (A), were aligned and the phylogenetic tree was constructed from evolutionary distance data derived by the neighbor-joining method.12) The bootstrap procedure was sampled 1,000 times with replacement by CLUSTAL W.12) The bar indicates a distance corresponding to 10 changes per 100 amino acid positions.
ChloroP software\(^{11}\) predicted chloroplast targeting signal sequences in N-termini of the deduced amino acid sequences encoded by both OsSIG2 genes. Putative mature OsSIG2A and OsSIG2B shared 75% identity with respect to amino acid sequence, and exhibited overall similarity with other SIG2 proteins (Fig. 1A). These isolated sequences were aligned with other \(\sigma\)-factor genes identified from higher plants, and a phylogenetic tree was constructed with Clustal W software.\(^{12}\) As shown in Fig. 1B, some of the genes form families such as the SIG1-, SIG2-, and SIG3-groups, and both the SIG1- and the SIG2-groups form sub-clusters of dicotyledonous and monocotyledonous groups. The monocotyledonous SIG2 family is further classified into two groups, SIG2A and SIG2B, both in rice and in maize.

To examine the tissue- and light-dependent modes of expression of three rice SIG genes, OsSIG1 (formerly OsSigA),\(^7\) OsSIG2A and OsSIG2B, RNA gel blot hybridization was carried out. Digoxigenin (DIG)-labeled antisense RNA probes were used for hybridization as described previously.\(^7\) As shown in Fig. 2, both OsSIG2A and OsSIG2B were detected in green shoots but not in roots, and this tissue specificity was similar to that in the case of OsSIG1. Expression of all three genes was induced by light illumination. The sizes of their transcripts were estimated approximately to be 2.2-kb for OsSIG2A and 2.1-kb for OsSIG2B, and their signal intensity indicates that OsSIG2A was more abundantly expressed than OsSIG2B in all samples tested. Notably, OsSIG2A transcripts were clearly detectable even in etiolated leaves in which signals of OsSIG1 and OsSIG2B were hardly detected. Therefore, it seems that OsSIG2A plays major roles as a SIG2-type \(\sigma\)-factor in rice.

Next, to clarify tissue specific expression of three SIG genes, \textit{in situ} hybridization analysis was performed with the same DIG-labeled antisense RNA probes used in RNA blot hybridization analysis, as described above. As shown in Fig. 3, it can be seen that two rice SIG2 genes are expressed from early stages of leaf development (Fig. 3B, 3C, 3E, and 3F). In contrast, OsSIG1 is expressed maximally in the leaf tip region, which represents a relatively late stage of leaf development (Fig. 3A, and 3D). Images observed under natural light (Fig. 3G) and fluorescent light (Fig. 3H) showed that the tissues accumulating both OsSIG2 transcripts are the pre-mature leaves inside the leaf sheath and that they contain chlorophyll or its precursors abundantly. Although \textit{in situ} hybridization is not a quantitative method for gene expression analysis, it can be surmised that in earlier stages of leaf development, OsSIG2A and
OsSIG2B are fully expressed whereas OsSIG1 has not reached the maximum expression level.

Phylogenetic clusters of SIG families among the evolutionally distant plants shown in Fig. 1B suggest that each SIG family conserves not only structural similarity based on amino acid sequence but also functional similarity as transcriptional initiator. We have demonstrated in this study that two rice SIG2 gene products showed high similarity with SIG2 proteins of other higher plants (Fig. 1A). Expression analysis revealed that both rice SIG2 genes are expressed similarly in the tissues studied (Fig. 3) and that OsSIG2A is more abundantly accumulated than OsSIG2B (Fig. 2), suggesting that OsSIG2A has a more significant role as the SIG2 gene than that of OsSIG2B in rice. In situ hybridization analysis of horizontally cut rice seedling specimens clearly demonstrated that expression of SIG2 genes occurs at an early stage of leaf development (Fig. 3), which suggests the functional significance of SIG2 proteins in chloroplasts maturation during leaf development. Our results therefore coincide with suggested functions of Arabidopsis SIG2 beyond the morphological differences of monocotyledonous and dicotyledonous plants. On the other hand, transcripts of OsSIG1 accumulated in the tip of the fully expanded leaf (Fig. 3), indicating significant roles of OsSIG1 in mature chloroplasts. Heterogeneity of σ-factors thus likely confers distinct functional properties to the PEP enzyme in concert with leaf development in higher plants.

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References


