Plant Gene Register

Characterization of cDNAs Encoding Small GTP-Binding Proteins from Maize

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To date, more than 100 genes encoding small GTP-binding proteins (21–25 kD) have been identified from various eukaryotic organisms, including mammals, insects, yeasts, and higher plants (Hall, 1990; Bourne et al., 1991; Terryn et al., 1993). These proteins, which contain highly homologous regions that have been conserved during evolution, are conveniently classified into three major groups; ras, rho, and rab/ypt families (Kahn et al., 1992). Although the physiological functions of these proteins are not fully understood, increasing evidence suggests that whereas the ras-related proteins are involved in the signal transduction pathways, the rab/ypt-related proteins are associated with intracellular transport and cell growth (Bednarek and Raikhel, 1992).

From the plant kingdom, about 30 small GTP-binding protein genes have been identified in Arabidopsis thaliana, rice, tobacco, maize, pea, and others (Terryn et al., 1993). Here, we report the isolation and characterization of two new cDNAs, mgp1 and mgp2, that encode small GTP-binding proteins in maize (Table 1). The mgp1 sequence of 941 bp contains a 633-bp open reading frame that encodes a protein of 211 amino acids with a calculated M, of 22,400. The mgp2 sequence consists of 769 bp with a 648-bp open reading frame encoding a protein of 216 amino acids and a calculated M, of 23,800. Both proteins contain four conserved regions necessary for GTP binding and an effector loop that interacts with specific effector proteins such as GAP. The mgp1 and mgp2 proteins show the highest sequence similarities with rab11 and the marine ray ora3 proteins followed by various rab/ypt family proteins. This indicates that the mgp1 and mgp2 proteins belong to the rab/ypt subfamily, although they are rather distantly related to the only other maize small GTP-binding proteins, YPTm1 and YPTm2, reported to date (Palme et al., 1992).

Northern hybridization analyses revealed that, although the mgp1 and mgp2 mRNAs were detected in all tissues examined, the highest transcript levels of mgp1 and mgp2 were in the tassel and ear tissues, respectively. The mRNA levels of mgp1 and mgp2 were also examined during the various developmental stages of the plant at 14, 24, 50, 70, and 90 d after emergence. Both mgp1 and mgp2 mRNAs were detected at all developmental stages, with maximum levels at between 50 and 70 d for mgp1 and at 70 d for mgp2. However, transcript levels of mgp1 and mgp2 did not vary among tall, dwarf, and tilling phenotypes of maize plants. This is in contrast to our previous report that the mRNA level of a rice rpg1 gene was reduced in 5-azacytidine-induced dwarf rice, which had suggested that some

Table 1. Characteristics of cDNAs encoding small GTP-binding proteins from maize

<table>
<thead>
<tr>
<th>Organism:</th>
<th>Zea mays (inbred line, Daggin).</th>
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<tr>
<td>Source:</td>
<td>Atg10 cDNA library constructed from poly(A)⁺ RNA of 14-d-old green seedlings.</td>
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<td>Method of Isolation:</td>
<td>Hybridization screening of the cDNA library with a 350-bp probe DNA fragment amplified by PCR using primers designed based on conserved regions of a rice rpg1 cDNA (Sano and Youssefian, 1991).</td>
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<td>Sequencing Strategy:</td>
<td>Double-stranded plasmid sequencing of both strands by the dideoxynucleotide chain termination method using a Sequenase version 2.0 kit (manual) and synthetic primers with an Applied Biosystems (Foster City, CA) automated sequencer.</td>
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<td>Structural Features of cDNAs:</td>
<td>mgp1 cDNA consists of 941 bp with an initiation codon at position 76 and a termination codon at 709. mgp2 cDNA consists of 769 bp with an initiation codon at position 88 and a termination codon at 736. Both cDNAs lack poly(A) tails.</td>
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<tr>
<td>Structural Features of Proteins:</td>
<td>The mgp1 protein consists of 211 amino acids, and the predicted M, is 22,400. The mgp2 protein consists of 216 amino acids with a predicted Mr of 23,800. The proteins are 76% similar at the amino acid level and both proteins contain two phosphate-binding sites (VGKS, DTAG), two guanine-binding sites (GNKAD, ETSA), and the effector loop (TIGVEF). The C-terminal isoprenylation site contains two cysteine residues (CCS, CCSD). Amino acid sequence similarity (%) of mgp1 and mgp2 with: rab11 (71, 46), ara (65, 53), YPTm1 (46, 43), YPTm2 (44, 46), rpg1 (66, 61). Amino acid sequences were aligned using GENETYX program (Tokyo).</td>
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Antibodies: Not produced.

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small GTP-binding proteins may be involved in plant dwarfism (Sano and Youssefian, 1991).

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The GenBank accession numbers for the sequences reported in this article are D31905 (mgp1) and D31906 (mgp2).

LITERATURE CITED


