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Plant Gene Register

Cloning and Characterization of an Anaerobically Induced cDNA Encoding Glucose-6-Phosphate Isomerase from Maize[1]

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Anaerobic stress in maize (*Zea mays* L.) leads to a drastic alteration in gene expression and results in the selective synthesis of a small number of polypeptides named anaerobic proteins (Sachs et al., 1980). Other plants studied showed similar responses when subjected to an anaerobic environment (Russell et al., 1990). To date, the majority of the anaerobic proteins that have been identified were found to be enzymes involved in the glycolytic pathway or Glc-P metabolism. Recently, however, Peschke and Sachs (1994) reported two anaerobically induced transcripts that do not encode enzymes involved in Glc metabolism and apparently serve other roles in the response of maize to flooding stress. The expression of the anaerobic proteins has been proposed to be regulated at the levels of both transcription and translation (Sachs et al., 1980; Hake et al., 1985).

GPI (EC 5.3.1.9) is an essential glycolytic enzyme that catalyzes the reversible isomerization of Glc-6-P and Fru-6-P, a reaction that also immediately precedes Suc biosynthesis in plants. This enzyme has also been called phosphohexose isomerase and phophoglucose isomerase. Two isozyme forms of GPI, one localized in the cytosol and the other in the plastid, have been reported in other plants and may also exist in maize (Weeden and Gottlieb, 1982; Kelley and Freeling, 1984). In general, plants examined contain a single gene for GPI-C, with the exception of *Clarkia*, which has two copies, resulting from gene duplication within that species (Weeden and Gottlieb, 1979). Three forms of GPI have been reported in maize (Salamini et al., 1972). GPI-I encoded by the *phi1* gene is expressed in both the devel-

Table I. *Characteristics of a cDNA sequence encoding GPI-C in Z. mays L.*

Organism:
Zea mays L. (B73 inbred).
Gene:
phi1 (*phosphohexose isomerase1*; Goodman et al., 1980).
Gene Product and Function:
GPI-C; (EC 5.3.1.9); reversible isomerization of Fru-6-P and Glc-6-P in the glycolytic pathway. Previously identified as ANP55 (Sachs et al., 1980; Kelley and Freeling, 1984).
Clone Type:
cDNA, complete open reading frame.
Source:
cDNA library in a λ-UniZap vector constructed from poly(A)$^+$ RNA of *Z. mays* (B73) roots subjected to 6 h of anaerobic treatment.
Techniques:
cDNA screening using a heterologous GPI-C probe from Arabidopsis (Thomas et al., 1993) under low-stringency hybridization conditions; phagemid in vivo excision; double-stranded plasmid dideoxy sequencing; northern and southern analyses.
Method of Identification:
Homology of deduced amino acid sequence to those of other GPI-C genes; about 75% sequence identity with those of *Arabidopsis thaliana* and *Clarkia lewisii* (Thomas et al., 1993). Restriction fragment length polymorphism mapping locates the gene encoding this cDNA to *phi1* (on chromosome 1L; E. Coe and G. Davis, personal communication).
Gene Copy Number:
Genomic southern blot analysis indicate a low copy number.
Expression Characteristics:
A 2.1-kb single transcript was detected in roots. Transcript levels are induced in roots by anaerobic stress.
Features of cDNA Structure:
Total length of 2046 bp, with an open reading frame encoding a polypeptide of 567 amino acids with a predicted molecular mass of 62.2 kD.

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Abbreviations: GPI, Glc-6-P isomerase; GPI-C, cytosolic form of GPI.

oping endosperm and embryo. In contrast, GPI-II is expressed in only the developing endosperm, whereas GPI-III is found in only the developing embryo. The enzymatic activity of GPI has been reported to be induced under anaerobic stress in maize (Kelley and Freeling, 1984). Using electrophoretically distinguishable alleles of GPI and antibodies against spinach GPI-C, Kelley and Freeling (1984) identified ANP55 in maize as an isozyme of GPI-C encoded by the *phi1* gene.

To analyze the source of the multiple GPI isozymes and to study their anaerobic regulation, we isolated 25 clones from a cDNA library constructed from maize roots treated anaerobically for 6 h (Table I). The GPI clones were isolated using a heterologous cDNA probe encoding GPI-C from Arabidopsis (generously provided by Les Gottieb; Thomas et al., 1993). The clone with the longest insert (2.1 kb) had an open reading frame encoding a protein of 567 amino acid residues with high sequence identity to GPI sequences from other species. Northern blot analysis using the full-length cDNA probe detected a dramatic induction of transcript levels in maize roots upon anaerobic treatment. Using this cDNA as a probe in restriction fragment length polymorphism mapping showed that its gene is located on maize chromosome IL, within a couple of map units of where the *phi1* gene is expected to be localized, strongly indicating that the GPI cDNA is encoded by this gene (E. Coe and G. Davis; personal communication). The *phi1* gene was previously characterized and mapped using allozymes (Goodman et al., 1980).

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