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Organ-specific transcripts of different size and abundance derive from the same pyruvate, orthophosphate dikinase gene in maize

(C_4 photosynthesis/chloroplast transit peptide/gene expression/gene structure/upstream sequence)

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ABSTRACT **Analyses of genomic DNA and clones indicate that the pyruvate, orthophosphate dikinase (PPDK; ATP: pyruvate,orthophosphate phosphotransferase, EC 2.7.9.1) gene family of maize (*Zea mays* L. subsp. *mays*, line B73) contains two members. Restriction site and DNA sequence comparisons between PPDK genomic and leaf cDNA clones have revealed which gene encodes the isozyme involved in C_4 photosynthesis. The region flanking the 5′ end of this gene contains two 30-base-pair (bp) repetitive elements that may be involved in its light-regulated expression. Sequence analysis of genomic and leaf cDNA clones has also shown that the entire 7.3-kDa PPDK chloroplast transit peptide is encoded in the 436-bp first exon. Northern blot experiments with probes specific for the first exon and the 3′ end of the gene showed that the smaller PPDK transcripts in roots and etiolated leaves [3.0 kilobases (kb) vs. the 3.5-kb green leaf transcript] lack the sequence encoding the chloroplast transit peptide. In addition, results from cDNA library screens have confirmed that the root transcript is ≈50-fold less abundant than the green leaf transcript. Finally, sequence comparisons among cDNA clones from green leaves and roots and genomic clones representing both members of the PPDK gene family demonstrate that the green leaf transcript encoding the C_4 isozyme and the root transcript are derived from the same gene.**

The enzyme pyruvate, orthophosphate dikinase (PPDK; ATP:pyruvate,orthophosphate phosphotransferase, EC 2.7.9.1) is known primarily for the role it plays in C_4 photosynthesis, which is the generation of phospho*enol*pyruvate (PEP) from pyruvate. PEP is a substrate for phospho*enol*-pyruvate carboxylase [orthophosphate:oxaloacetate carboxylase (phosphorylating), EC 4.1.1.31], which fixes atmospheric carbon dioxide in leaf mesophyll cells of C_4 plants (1, 2). While most studies of PPDK have focused on the form of the enzyme found in leaves of C_4 plants, PPDK is also known to occur in other parts of C_4 as well as C_3 plants (3–6).

In leaves of the C_4 plant maize (*Zea mays* L. subsp. *mays*), the PPDK isozyme involved in C_4 photosynthesis is derived from a cytoplasmically synthesized 102.7-kDa polypeptide precursor (7) that includes a transit peptide facilitating chloroplast uptake (8). Using direct amino acid and DNA sequencing, Matsuoka *et al.* (7) have established sizes of 95.4 and 7.3 kDa for the PPDK mature polypeptide and chloroplast transit peptide, respectively.

In maize an abundant, light-inducible mRNA 3.5 kilobases (kb) long encodes the leaf form of PPDK involved in C_4 photosynthesis (9, 10). Smaller (3.0 kb) and much less abundant PPDK transcripts occur in etiolated leaves and roots (10). The available evidence indicates that the maize PPDK gene family may contain as few as two members, and most or all of the green leaf mRNA is probably encoded by just one of the members. It is not clear which gene encodes the transcripts in etiolated leaves and roots (10).

Here we further characterize the maize PPDK gene family and provide an analysis of the 5′ end of the gene encoding the C_4 photosynthetic isozyme, including the sequence of the chloroplast transit peptide and 5′ flanking region.* In addition, we present data that account for the size difference between the PPDK transcripts in green leaves and roots, and we identify the gene encoding the root transcript.

MATERIALS AND METHODS

Growth and Harvesting of Plant Material. Maize (*Z. mays* L. subsp. *mays*) plants were grown from both inbred (B73) and hybrid (Golden Jubilee) lines of seed. DNA and the poly(A)$^+$ RNAs used for cDNA library construction were extracted from B73 plants. The poly(A)$^+$ RNAs used for the blot shown in Fig. 4 were obtained from hybrid (Golden Jubilee) plants. Green leaves and roots were harvested from 10- to 14-day-old and 2-month-old plants, respectively, that were grown in a room illuminated with high intensity mercury vapor lights (16-hr light/8-hr dark). Etiolated leaves, fully emerged from the coleoptile, were obtained from 14-day-old plants that were germinated, grown, and harvested in complete darkness.

Isolation and Labeling of Nucleic Acids. Total maize DNA was extracted from etiolated leaves (11), phage DNAs were prepared according to Maniatis *et al.* (12), and plasmid DNAs were prepared by the protocol of Norgard (13). For the Northern blot shown in Fig. 4, total RNA was extracted by the guanidinium thiocyanate procedure (14), and the poly(A)$^+$ fraction was isolated by one pass over oligo(dT)-cellulose (15). DNA probes were labeled by nick-translation (12).

Isolation of Genomic and cDNA Clones. The isolation and identification of cDNA and genomic clones for maize PPDK has been described (10). For the work described here, cDNA libraries for leaves and roots were constructed by the method of Gubler and Hoffman (16). For the leaf library, double-stranded cDNA was inserted into the *Pst* I site of pBR322 (17). A library of ≈2000 clones was screened (18) with a fragment from PPDK genomic clone H2λ21 that contains most of the gene. From this library, 10 PPDK cDNA clones were identified and isolated, one of which was the nearly full-length clone pPPDK2 (3.3-kb insert). For the root library, double-stranded cDNA was ligated into the *Eco*RI site of vector λgt10 (19). A library of ≈10,000 clones was screened (20) with pPPDK2; one clone with a 0.5-kb insert was obtained and was designated λPPDK-root.

Blot Hybridization Procedures. DNA fragments were transferred from agarose gels to nitrocellulose and hybridized with DNA probes as described by Klessig and Berry (21). For the Northern blot shown in Fig. 4, RNAs were transferred to Zeta-Probe membrane (Bio-Rad) from an agarose/formalde-

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Abbreviation: PPDK, pyruvate, orthophosphate dikinase.

*The sequences reported in this paper have been deposited in the GenBank data base (accession nos. M32081 and X14927–14929).

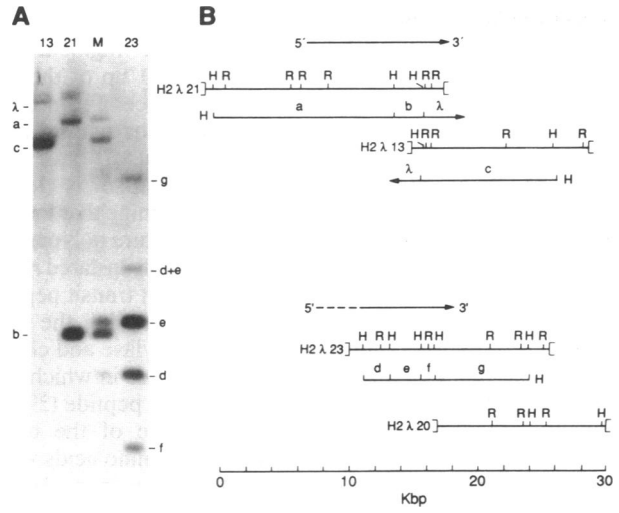

FIG. 1. Characterization of the maize PPDK gene family. (*A*) Restriction fragments from 1 ng of genomic clones H2λ13, H2λ21, and H2λ23 (lanes 13, 21, and 23) were compared to fragments from 10 μg of total maize DNA (lane M) to verify the gene family size. The DNAs were digested with *Hin*dIII and subjected to Southern blot analysis using the nearly full-length cDNA clone pPPDK2 as the probe. The data in *A* and *B* show the correspondence between *Hin*dIII fragments detectable in maize total DNA and those in the genomic clones (a–g). The fragment labeled d+e is a partial digestion product. Fragment sizes were determined by comparison with λ size markers. Hybridizable fragments from the genomic clones that contain vector arms and insert sequences are designated λ. The 7.5-kbp *Hin*dIII fragment in lane M of *A* (corresponding to fragment g in H2λ23) is more easily visible in a blot published by Hudspeth *et al.* (10). (*B*) Arrows marked 5′ to 3′ indicate the orientation and extension of the PPDK genes in the two overlapping pairs of clones, H2λ13/H2λ21 and H2λ20/H2λ23. H, *Hin*dIII; R, *Eco*RI.

hyde gel (12). DNA and RNA blots were hybridized at 42°C in a solution containing 50% (vol/vol) formamide, and final washes were carried out at 52°C in 0.2× SSC (30 mM NaCl/3 mM sodium citrate)/0.1% (wt/vol) *N*-lauroylsarcosine according to Klessig and Berry (21).

DNA Sequence Analysis. DNA sequence analysis was performed by both the dideoxynucleotide chain-termination method (22) and the chemical degradation method (23). For the sequence determined by the dideoxynucleotide chain-termination method, universal M13 oligonucleotide primers were used to initiate synthesis, and overlapping M13 clones generated by BAL-31 digestion served as templates (24, 25). Regions of sequence ambiguity were resequenced with Sequenase and dITP in place of dGTP (United States Biochemical). All of the reported sequences were derived from analysis of both strands, with the exception of ≈100 base pairs (bp) immediately downstream from the *Sca* I site shown in Fig. 2.

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      ScaI
-804  agtactgaaagcaatctaaagacatggaggtggaaggcctgacgtagatagagaagatgc
-744  tcttagctttcattgtctttcttttgtagtcatctgatttacctctctcgtttatacact
-684  ggtttttaaacactccttaacttttcaaattgtctctttctttaccctagactagataa
-624  ttttaatggtgattttgctaatgtggcgccatgttagatagaggtaaaatgaactagtta
-564  aaagctcagagtgataaatcaggctctcaaaaattcataaactgtttttaaatatccaa
-504  atatttttacatggaaaataataaaatttagtttagtattaaaaaattcagttgaatata
-444  gttttgtcttcaaaaattatgaaactgatcttaattatttttccttaaaaccgtgctcta
-384  tctttgatgtctagtttgagacgattatataatttttttttgtgcttaactacgacgagct
-324  gaagtacgtagaaatactagtggagtcgtgccgcgtgtgcctgtagccactcgtacgcta
-264  cagcccaagcgctagagcccaagaggccggaggtggaagcgtcgcggcactatagccac
-204  tcgccgcaagagcccaagaggccggagctgaaggatgagggtctgggtgttcacgaattg
-144  cctggaggcaggaggctcgtcgtccggagccacaggcgtggagacgtccgggataaggtg
-84   agcagccgctgcgatagggcgcgtgtgaaccccgtcgcgccccacggatggtataagaa
                    +1
-24   taaaggcattccgcgtgcaggattCACCCGTTCGCCTCTCACCTTTTCGCTGTACTCACT
                          SacI
37   CGCCACACACACCCCCTCTCCAGCTCCGTTGGAGCTCCGGACAGCAGCAGGCGCGGGGCG
                                NdeI
97   GTCACGTAGTAAGCAGCTCTCGGCTCCCTCTCCCCTTGCTCCATATGATCGTGCAACCCA
157  TCGAGCTACGCGCGTGGACTGCCTTCCCTGGGTCGGCGCAGGAGGGGATCGGAAGGATGG
                                                          M
217  CGGCGTCGGTTTCCAGGGCCATCTGCGTTCAGAAGCCGGGCTCAAAATGCACCAGGGACA
     A  A  S  V  S  R  A  T  C  V  Q  K  P  G  S  K  C  T  R  N
277  GGGAAGCGACCTCCTTCGCCCGCCGATCGGTCGCAGCGCCGAGGCCCCCGCACGCCAAAG
     R  E  A  T  S  F  A  R  R  S  V  A  A  P  R  P  P  H  A  K
337  CCGCCGGCGTCATCCGCTCCGACTCCGGCGCGGGACGGGGCCAGCATTGCTCGCCGCTGA
     A  A  G  V  I  R  S  N  S  G  A  G  R  G  Q  H  C  S  P  L
                                   ↓
397  GGGCCGTCGTTGACGCCGCGCCGATACAGACGACCAAAAAGgtatcccttgcagctctta
     R  A  V  V  N  A  A  P  I  Q  T  T  K  K
           EcoRI
457  gaaactgaattc
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FIG. 2. Sequence analysis of the 5′ end of the PPDK gene encoding the isozyme involved in C_4 photosynthesis, plus 804 bp of 5′ flanking DNA. To obtain this sequence, ≈500 bp of cDNA clone pPPDK2 and all of the 1.2-kb *Eco*RI/*Sca* I fragment from genomic clone H2λ21 (see Fig. 4) were analyzed. The gene sequence includes the chloroplast transit peptide (contained entirely in the first exon) and its deduced amino acid sequence (indicated by one-letter code). Intron and 5′ flanking sequence are represented by lowercase letters, while exon sequence is shown in capital letters. Restriction sites pertinent to the data in Figs. 3 and 4 are indicated. Direct repeats with a high degree of similarity are boxed. The presumptive TATA box, transcription start site (+1), and two start codons are underlined. Also underlined is a GAG sequence at positions 198–200 that corresponds to the position of a stop codon, TAG, determined by Matsuoka *et al.* (7). Arrow indicates the position at which the PPDK polypeptide precursor is cleaved between a glutamine (Q; amino acid 71) and a threonine (T; ref. 7).

Primer-Extension and Nuclease S1 Protection Assays. Mapping of the transcription start site by primer-extension and nuclease S1 protection assays was carried out as described by Yenofsky *et al.* (26) except for the following changes: Poly(A)$^+$ RNA (5 μg) from green maize (B73) leaves was used instead of total RNA, 500 units of nuclease S1 was used, and the primer-extension assay was incubated at 37°C for 90 min. The DNA fragments used for each experiment are described in Fig. 3. Size analysis of the products was carried out on an 8% denaturing polyacrylamide gel and their lengths were determined by comparison with a known DNA sequence ladder loaded on the same gel.

RESULTS AND DISCUSSION

Further Characterization of the Maize PPDK Gene Family. We have previously reported that the maize PPDK gene family may contain as few as two members (10). Wright *et al.* (27) have also obtained evidence for only two PPDK gene family members in maize, and they mapped their positions to chromosomes six and eight. Since our previous report (10), we have analyzed additional genomic clones and obtained more genome blot data, which also confirm our earlier estimate of the gene family size. Fig. 1 shows the maps of two pairs of overlapping genomic clones representing the two PPDK genes. Clones H2λ13 and H2λ23 have been described (10), while H2λ21 and H2λ20 are newly discovered isolates. The location and orientation of the PPDK genes within these clones were determined by hybridizing strand-specific probes to maize leaf RNA (28) and the nearly full-length cDNA clone pPPDK2 to genomic clone restriction fragments (see Fig. 1).

The data in Fig. 1 also show that all the *Hin*dIII restriction fragments in genomic DNA that hybridize to pPPDK2 can be aligned with *Hin*dIII fragments in the genomic clones. We have also determined that all of the *Eco*RI, *Bam*HI, and *Xho* I restriction fragments in genomic DNA that hybridize to pPPDK2 can be aligned with fragments in the genomic clones or are consistent with their maps (data not shown). Based on these and previously reported results (10, 27), we conclude that the PPDK gene family in maize has two members, which are represented by the overlapping clones H2λ13/H2λ21 and H2λ20/H2λ23.

To investigate whether only one or both genes serve as templates for the abundant, light-inducible mRNA that encodes the PPDK in green leaves (9, 10), we mapped 11 PPDK cDNA clones isolated from two leaf libraries. No differences were found among the clones in the location of 15 restriction sites (28), suggesting that the abundant PPDK mRNA in green leaves is probably composed of only one species. In addition, the cDNA clone maps are consistent with the map of H2λ21 but not the map of H2λ23. Thus, we conclude that the PPDK isozyme involved in C_4 photosynthesis is encoded by the gene in H2λ21. This conclusion is confirmed by sequence data presented later (see Fig. 5).

Sequence Analysis of the Chloroplast Transit Peptide and the 5′ Flanking Region of the PPDK Gene Encoding the C_4 Isozyme. Previous studies have shown the maize PPDK gene encoding the C_4 isozyme is light regulated (9, 10) and includes sequences encoding a chloroplast transit peptide (7–9). To further characterize the transit peptide and begin the search for cis-acting light-regulatory elements, we sequenced a 500-bp region of a nearly full-length leaf cDNA clone (pPPDK2) that corresponds to the 5′ end of the mRNA, and all of a 1.2-kb *Eco*RI/*Sca* I fragment from genomic clone H2λ21. The latter fragment was chosen for analysis because blot hybridization data indicated that it contains the 5′ end of the gene (data not shown).

The DNA and deduced amino acid sequence of the chloroplast transit peptide and 804 bp of sequence flanking the 5′ end of the gene are shown in Fig. 2. The location of the transcription start site, obtained from nuclease S1 protection and primer-extension assays (Fig. 3), is also indicated in Fig. 2. The data show that the 1.2-kb *Eco*RI/*Sca* I fragment from H2λ21 contains the 436-bp first exon and 31 bp of the first intron. The remainder is 5′ flanking sequence. From direct amino acid sequence of the N terminus of the mature polypeptide and cDNA sequence, Matsuoka *et al.* (7) have identified the site at which the polypeptide precursor is cleaved (Fig. 2). This information allowed us to determine that the first exon encodes only four amino acids of the mature polypeptide. The remaining 424 bp consist of a 211-bp 5′ untranslated region plus 213 bp that encode the entire chloroplast transit peptide. It is noteworthy that other plant genes encoding the small subunit of ribulose-1,5-bisphosphate carboxylase and chlorophyll *a*/*b* binding protein also have a structure in which their first exon encodes mostly chloroplast transit peptide (29–32). Finally, the deduced amino acid sequence of the transit peptide indicates that it is composed of 71 amino acids with a predicted molecular mass of 7338 Da. For a more detailed analysis of the PPDK chloroplast transit peptide, see Glackin (28) and Matsuoka *et al.* (7).

It is worth noting that there are two start codons in the first exon that are in the same reading frame (Fig. 2). The first occurs 72 bp upstream of the one designated by Matsuoka *et al.* (7) as the functional start codon. We accept their assignment, which is based on the fact that their sequence contains a stop codon (TAG) in the reading frame established by the first start codon. However, our sequence does not contain this stop codon (we read GAG instead of TAG; see Fig. 2). Therefore, it is possible that the first start codon is functional in line B73, although it is the case that the sequence surrounding the second start codon is much more similar to the consensus sequence (AACAATGGC) proposed for translation initiation in plants (33).

In the 5′ flanking region there is a sequence 32 bp upstream of the transcription initiation site that resembles the consen-

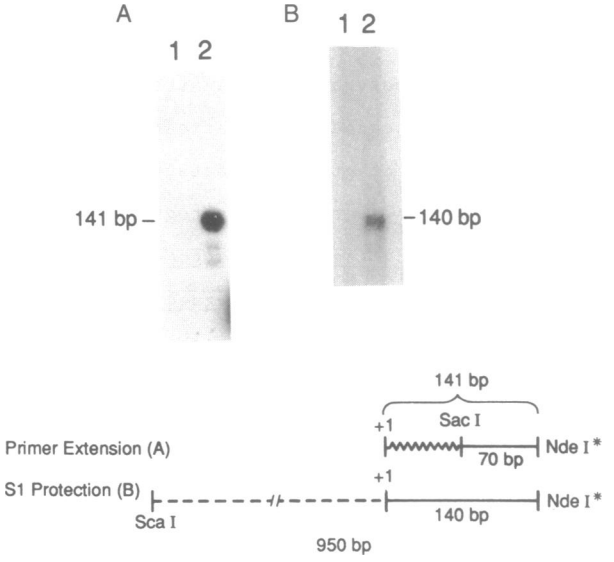

FIG. 3. Identification of the 5′ end of the PPDK gene encoding the C_4 isozyme by primer-extension (*A*) and nuclease S1 protection (*B*) analyses. The control in each case was 5 μg of yeast tRNA (lanes 1), while 5 μg of leaf poly(A)$^+$ RNA was used for the experimental samples (lanes 2). The end-labeled restriction fragments used for the experiments are shown below. The fragment used for the primer-extension experiment was a 70-bp *Sac* I/*Nde* I fragment, and for the nuclease S1 protection experiment a 950-bp *Sca* I/*Nde* I fragment was used. Both fragments were derived from genomic clone H2λ21 (see Fig. 2), and in each case the *Nde* I site was kinase labeled (*). The primer-extended DNA is indicated by a jagged line, the nuclease S1 protected fragment is indicated by a solid line, and the nuclease S1 digested DNA is indicated by a dashed line.

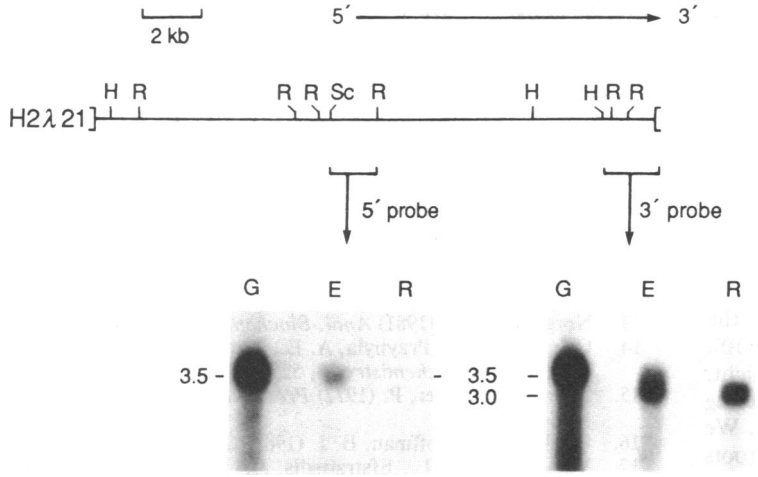

FIG. 4. Northern blot experiment showing that the smaller (3.0 kb) PPDK transcripts in etiolated leaves and roots lack the sequence encoding the chloroplast transit peptide. A blot containing 0.5 μg of poly(A)$^+$ RNA from maize green leaves (G) and 5 μg of poly(A)$^+$ RNA from etiolated leaves (E) and roots (R) was first probed with a 1.2-kbp *Sca* I/*Eco*RI fragment from H2λ21 that contains 5′ flanking DNA and the entire sequence of the chloroplast transit peptide (5′ probe; see Fig. 2). The blot was strip-washed at 90°C with 0.1× SSC/0.1% SDS and then reprobed with a 1.8-kbp *Hin*dIII/*Sal* I fragment (the *Sal* I site is in the polylinker of the λEMBL3 arm) that contains ≈1 kb of coding sequence from the 3′ end of the PPDK gene in H2λ21 (3′ probe). Horizontal arrow indicates the orientation and length of this gene. H, *Hin*dIII; R, *Eco*RI; Sc, *Sca* I (only the *Sca* I site used to isolate the 5′ probe is shown). Transcript sizes are indicated in kb, as determined by comparison with DNA fragments of a known size (not shown).

sus TATA box sequence (TATAAGA; see Fig. 2). However, there are no sequences resembling the consensus CAAT box sequence (34). The most noteworthy features in the 5′ flanking region are two very similar 30-bp direct repeats at positions −200 and −255 (Fig. 2). Although no significant sequence similarities were found between the PPDK gene upstream region and that of several other light-regulated genes from both dicots and monocots (30, 35–38), the arrangement, location, and size of these repetitive sequences resemble repeated elements that are important in the organ-specific and light-dependent expression of a pea gene encoding the small subunit of ribulose-1,5-bisphosphate carboxylase (36).

The Smaller PPDK Transcripts in Roots and Etiolated Leaves Lack Most or All of the Chloroplast Transit Peptide Sequence. We have previously shown (10) that PPDK transcripts exist in maize roots and etiolated leaves that are ≈0.5 kb smaller than the green leaf transcript (3.0 vs. 3.5 kb). A reasonable explanation for this size difference is that the sequence encoding the chloroplast transit peptide is absent in organs not conducting the photosynthesis. While Aoyagi and co-workers have published strong indirect evidence supporting this hypothesis (4, 5, 39), it has never been directly tested.

To conduct a direct test, a Northern blot containing RNA from green leaves, etiolated leaves, and roots was first probed with the 1.2-kb *Eco*RI/*Sca* I fragment from H2λ21, which contains the transit peptide-encoding first exon and 5′ flanking sequence (see Figs. 2 and 4). After hybridization with this transit peptide probe (5′ probe), the blot was strip-washed and then rehybridized with a fragment from

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                                                                    45
H2λ21          CTGCAGCTCAGGTGCTTGTCTGA  GGCTGCCTCCTCGTTGGCAACC
H2λ13          ----------------------  ----------------------
pPPDK2(LEAF)   ----------------------  ----------------------
λPPDK-ROOT     ----------------------  ----------------------
H2λ23          ----------------------GG--------------------***

                                                                    90
H2λ21           GGATTGCCTGCTGCTGGTGGATGTGGTGAT   CAACAGTATTAT
H2λ13           -----------------------------   ------------
pPPDK2(LEAF)    -----------------------------   ------------
λPPDK-ROOT      -----------------------------   ------------
H2λ23           *****--------T-----C--C-------TAAT--T-C--C---

                                                                   135
H2λ21           TACAGAGCC  ATGCTATGTGAACATTACTAGTAGCAGTGCTCAT
H2λ13           ---------  --------------------------------
pPPDK2(LEAF)    ---------  --------------------------------
λPPDK-ROOT      ---------  --------------------------------
H2λ23           G--------AT-----C------G-G--T---------C------

                                                                   180
H2λ21           AAAAGCTACAATCCCATCTCCCTTTTTTTTCCAGTCATGTAAAAC
H2λ13           ---------------------------------------------
pPPDK2(LEAF)    ---------------------------------------------
λPPDK-ROOT      ---------------------------------------------
H2λ23           ----------G-T-----****-A----C-*---CT---------

                                                                   225
H2λ21           TTCCAAACTGCTCCATGGTTCAAAACTCTGTTCTTCAATACATCA
H2λ13           ---------------------------------------------
pPPDK2(LEAF)    ---------------------------------------------
λPPDK-ROOT      ----------------------------------]
H2λ23           ----------T-*----C--*------GA-GGT--TCG-GGTGTG

                                                                   270
H2λ21           TCAATTATCGATTATATATGTGGCAGAGGTTTTTTCTTTTCTTTC
H2λ13           -----------]
pPPDK2(LEAF)    -------------------------]
λPPDK-ROOT
H2λ23           AG-TG-GCATG-CG-TGT--A---CATT-C-GCACA--CCACC-A
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FIG. 5. Sequence comparisons showing that the same gene encodes the PPDK transcripts in green leaves and roots. About 200–270 bp of sequence, derived mostly from the 3′ untranslated regions, was obtained from each of the indicated cDNA (pPPDK2 and λPPDK-ROOT) and genomic clones (H2λ21, H2λ13, and H2λ23). The stop codons (TGA) and presumptive poly(A) addition signals are underlined. Residues in agreement with the sequence of H2λ21 are shown by dashes and mismatches are indicated by letter codes. Residues that occur only in H2λ23 are reflected by a space in the other sequences. Residues in the other sequences that are absent in H2λ23 are indicated by asterisks. Brackets denote the end of the clone or the extent to which the sequence was determined.

H2λ21 that contains the 3′ end of the gene (3′ probe). The results of this experiment (Fig. 4) show that the 5′ probe hybridizes only to the 3.5-kb transcript in green and etiolated leaves. In contrast, the 3′ probe hybridizes to the 3.0-kb transcript in etiolated leaves and roots as well as to the 3.5-kb transcript in green and etiolated leaves. Thus, this experiment confirms that the smaller PPDK transcripts lack most or all of the sequence encoding the chloroplast transit peptide (we cannot exclude the possibility that a region of the transit peptide too small to hybridize is present). These results also verify that etiolated leaves contain both transcripts, as was previously reported (10). Although full expression of the 3.5-kb transcript in leaves requires light stimulation (9, 10), clearly small amounts can accumulate in the absence of light.

The PPDK Transcripts in Green Leaves and Roots, Differing in Size and Abundance, Are Encoded by the Same Gene. We have previously reported that the PPDK transcripts in roots are at least 40-fold less abundant than those in green leaves, and yet they may be encoded by the same gene (10). To further investigate these issues, we constructed a maize root cDNA library and screened it with the leaf cDNA clone pPPDK2. Based on the number of PPDK cDNA clones obtained from our root and leaf libraries (see *Materials and Methods*), we estimate that the PPDK transcripts in roots comprise ≈0.01% of the poly(A)$^+$ RNA mass, whereas those in green leaves make up ≈0.5% of the poly(A)$^+$ RNA mass. These values are consistent with our previous estimate of the relative abundance of the PPDK transcripts in these two organs (10).

To determine which gene encodes the PPDK transcripts in roots, we sequenced a portion of the root cDNA clone (λPPDK-root) and compared it to the corresponding sequences of the leaf cDNA clone (pPPDK2) and genomic clones H2λ21, H2λ13, and H2λ23 (Fig. 5). As Fig. 5 shows, the sequences of the root and leaf cDNA clones are identical to each other and are also identical to the sequence of the gene in H2λ21/H2λ13. In contrast, the sequence of the gene in H2λ23 is quite different and exhibits numerous single base mismatches as well as several small insertions and deletions (Fig. 5). Thus, these results confirm that the PPDK transcripts in green leaves and roots, although differing substantially in both size and abundance, are transcribed from the same gene. In addition, these transcripts differ by the inclusion or exclusion of most or all of an exon. This exon, in turn, encodes a chloroplast transit peptide that has a function only in photosynthetic cells.

While we cannot presently draw any conclusions about the mechanisms generating the different PPDK transcripts, the data reported here add to a small but growing body of evidence (40, 41) that the complexities in gene expression already well-documented in animals (42) and yeast (43–45) also exist in plants.

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