

Genomic and cDNA clones for maize phosphoenolpyruvate carboxylase and pyruvate, orthophosphate dikinase: Expression of different gene-family members in leaves and roots

(photosynthesis/enzymes/light-regulation/tissue specificity)

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Contributed by James Bonner, December 18, 1985

ABSTRACT We have isolated cDNA clones for the maize leaf enzymes phosphoenolpyruvate (*P-ePrv*) carboxylase [orthophosphate:oxaloacetate carboxy-lyase (phosphorylating) EC 4.1.1.31] and pyruvate, orthophosphate (*Prv,P_i*) dikinase (ATP:pyruvate, orthophosphate phosphotransferase, EC 2.7.9.1) by exploiting the light-inducibility and large size of the mRNAs (3.5 kilobases) that encode the two enzymes. The clones were identified by hybrid-selection and immunoprecipitation assays. From a maize genomic library, two different types of genomic clones were screened with both the *P-ePrv* carboxylase and the *Prv,P_i* dikinase cDNA clones. Information from these genomic clones and genome blots indicates that the *P-ePrv* carboxylase gene family has at least three members and the *Prv,P_i* dikinase family at least two. Transcripts for both enzymes were detected in green leaves, etiolated leaves, and roots. The results show that the *P-ePrv* carboxylase mRNAs in green leaves and roots are encoded by different genes. Whereas the *P-ePrv* carboxylase mRNAs in all three tissues appear to be the same size, the *Prv,P_i* dikinase mRNA in green leaves is about 0.5 kilobases longer than the *Prv,P_i* dikinase mRNAs in etiolated leaves and roots. It is possible that all these *Prv,P_i* dikinase transcripts are encoded by one gene, and the size differences may correspond to the presence or absence of a sequence encoding a chloroplast transit peptide.

The enzymes phosphoenolpyruvate (*P-ePrv*) carboxylase [orthophosphate:oxaloacetate carboxy-lyase (phosphorylating), EC 4.1.1.31] and pyruvate, orthophosphate (*Prv,P_i*) dikinase (ATP:pyruvate, orthophosphate phosphotransferase, EC 2.7.9.1) play important roles in C_4 and crassulacean acid metabolism photosynthesis. *P-ePrv* carboxylase is responsible for the fixation of atmospheric CO_2 , while *Prv,P_i* dikinase produces the substrate phosphoenolpyruvate for *P-ePrv* carboxylase (1). In green leaves of the C_4 plant maize (*Zea mays*), *P-ePrv* carboxylase is located in the cytoplasm of mesophyll cells (2). *Prv,P_i* dikinase is found primarily in the chloroplasts of mesophyll cells (3–5), although some is also detectable in bundle-sheath cells (6). The former enzyme has a subunit molecular mass of 100–103 kDa and has been estimated to comprise 8–15% of the total leaf soluble protein (7–9). The latter enzyme has a subunit molecular mass of 94–97 kDa and makes up 2–10% of the total leaf soluble protein (10–12). Maximal accumulation of both of these enzymes and their mRNAs is light-dependent (1, 8, 11, 13–15). In addition, *P-ePrv* carboxylase has been found in other maize tissues, including etiolated leaves and roots (8, 14–16), and *Prv,P_i* dikinase has been detected in maize seeds (17) and etiolated leaves (13). However, these forms of the enzymes have received much less study.

Here we describe the isolation and partial characterization of *P-ePrv* carboxylase and *Prv,P_i* dikinase cDNA and genomic clones from maize. Our results indicate that each enzyme is encoded by a small number of genes that exhibit differential expression in leaves and roots.

MATERIALS AND METHODS

Growth and Harvesting of Plant Material. Maize (*Zea mays*) plants were grown from both inbred (B73) and hybrid (Golden Jubilee) varieties of seed. DNA and the poly(A)⁺ RNA used to construct the leaf cDNA library were extracted from B73 plants. All other RNAs were obtained from Golden Jubilee. Green leaves and roots were harvested from 10- to 14-day-old and 60-day-old plants, respectively, that had been grown in a room illuminated with high-intensity mercury-vapor lamps (16 hr light/8 hr dark). Etiolated leaves, fully emerged from the coleoptile, were obtained from 14-day-old plants that had been germinated, grown, and harvested in complete darkness.

Isolation and Labeling of Nucleic Acids. Total maize DNA was extracted from etiolated leaves (18), phage DNAs were isolated according to Maniatis *et al.* (19), and plasmid DNAs were prepared by the protocol of Norgard (20). Total RNA was extracted from maize tissues by the guanidinium thiocyanate procedure (21), and the poly(A)⁺ fraction was isolated by one pass over oligo(dT)-cellulose (19). Oligo(dT)-primed cDNA probes were prepared according to the procedure of Maniatis *et al.* (19), and other DNA probes were labeled by nick-translation (22).

Construction and Screening of cDNA and Genomic Libraries. Double-stranded DNA copies of total poly(A)⁺ RNA from green maize leaves were synthesized according to the protocol of Efstratiadis *et al.* (23) and inserted into the *Pst* I site of pBR322 (24). The library was screened with various cDNA probes by the colony-hybridization method (25). To construct a genomic library, total maize DNA was partially digested with *Mbo* I and fragments of 15–20 kilobase pairs (kbp) were ligated to *Bam*HI-digested vector arms from EMBL3 (26). The recombinant phage were plate-amplified (27) and then screened by plaque hybridization (28).

Hybrid-Selection and Immunoprecipitation Procedures. Plasmid DNAs used for hybrid selection were bound to nitrocellulose disks (0.5 cm) as described by Kafatos *et al.* (29). Hybridization and washing of the disks and elution of bound RNA were performed according to Maniatis *et al.* (19), except the hybridization temperature was adjusted to 45°C. For each hybrid-selection, 25 μ g of total poly(A)⁺ RNA from green leaves was hybridized at a concentration of 500 μ g/ml

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Abbreviations: *P-ePrv* carboxylase, phosphoenolpyruvate carboxylase; *Prv,P_i* dikinase, pyruvate, orthophosphate dikinase; kb, kilobase(s); bp, base pair(s).

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to two disks, each containing 20 μg of the same plasmid DNA. The hybridized RNA that was eluted from the filters was translated in rabbit reticulocyte lysate (Promega Biotec, Madison, WI) in the presence of [^{35}S]methionine and [^3H]leucine.

Immunoprecipitation of *in vitro* translation products with *Staphylococcus aureus* Cowan 1 strain cells (IgG-sorb; The Enzyme Center, Boston) was performed as described by Cullen and Schwartz (30). Antibodies against maize *P-ePrv* carboxylase (obtained from Sigma) were prepared by Antibodies Inc. (Davis, CA). Maize Prv, P_i dikinase antiserum was a gift of T. Sugiyama (Nagoya University, Chikusa, Nagoya, Japan).

Proteins were analyzed by NaDodSO₄/polyacrylamide gel electrophoresis in 5–15% gradient slab gels run at 3 V/cm for 16 hr (31). The gels were treated with EN³HANCE (New England Nuclear), dried, and exposed for 16–72 hr to x-ray film at -70°C with an intensifying screen.

Gel Blot Hybridization Procedures. DNA fragments were transferred from agarose gels to nitrocellulose or Zeta-Probe membranes (Bio-Rad) and hybridized with DNA probes as described by Klessig and Berry (32). RNAs were transferred to nitrocellulose from agarose/formaldehyde gels as described by Maniatis *et al.* (19). To ensure that equal amounts of different poly(A)⁺ RNAs were loaded in gel lanes, the concentrations were quantified with a poly(^3H JU) hybridization assay (33). Unless otherwise indicated, 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 30 mM NaCl/3 mM sodium citrate/0.1% (wt/vol) *N*-lauroylsarcosine (32).

RESULTS

Isolation and Partial Characterization of cDNA and Genomic Clones. Three different criteria were used to select potential *P-ePrv* carboxylase and Prv, P_i dikinase clones from a 1000-member maize leaf cDNA library: (i) greater reactivity to a cDNA probe made from green leaf poly(A)⁺ RNA than to a cDNA probe made from etiolated leaf poly(A)⁺ RNA, (ii) a positive reaction to a cDNA probe made from green leaf

poly(A)⁺ RNA enriched for *P-ePrv* carboxylase and Prv, P_i dikinase mRNA by size fractionation, and (iii) a blot-hybridization reaction with a green leaf mRNA of adequate size to encode the *P-ePrv* carboxylase and Prv, P_i dikinase polypeptides [≥ 3 kilobases (kb)].

Two cDNA clones, designated pH1 and pH2, were isolated through this selection process. Preliminary hybrid-selection assays indicated that pH1 was a *P-ePrv* carboxylase clone and pH2 was possibly a Prv, P_i dikinase clone. On the basis of these results, we screened a maize genomic library with both clones. Analysis of the isolated genomic clones showed that pH1 hybridizes to a set of clones that is different from the set to which pH2 hybridizes. In addition, we found that two different types of genomic clones had been screened with each cDNA clone. These genomic clones can be distinguished by their different restriction endonuclease maps (Fig. 1) and their different intensities of hybridization with each cDNA clone. Fig. 2 shows that pH1 hybridizes more intensely with H1 λ 14 than it does with H1 λ 21, whereas pH2 hybridizes more intensely with H2 λ 13 than it does with H2 λ 23. These differences in hybridization are probably due to differences in base sequence homology and, if so, indicate that H1 λ 14 and H2 λ 13 have greater homology with the respective cDNA clones than do H1 λ 21 and H2 λ 23. In addition, Fig. 2 shows that four *Hind*III fragments in the maize genome hybridize to pH1, of which two are accounted for by *Hind*III fragments in H1 λ 14 and H1 λ 21. The number of genomic *Hind*III fragments observed hybridizing to pH2 is two, and these correspond to *Hind*III fragments in H2 λ 13 and H2 λ 23. The genomic clone reconstructions in Fig. 2 indicate that the corresponding genomic fragments are present in 1–2 copies per haploid genome.

Clone Identification by Hybrid-Selection and Immunoprecipitation Assays. In order to increase the length of sequence that could hybridize to mRNA, genomic subclones pH1 λ 14-13 and pH2 λ 13-4 were subsequently used for additional hybrid-selection experiments. Genomic subclone pH1 λ 14-13 contains a 13-kbp insert that hybridizes to the pH1 cDNA clone, and genomic subclone pH2 λ 13-4 contains a 4-kbp insert that hybridizes to the pH2 cDNA clone (Fig. 1).

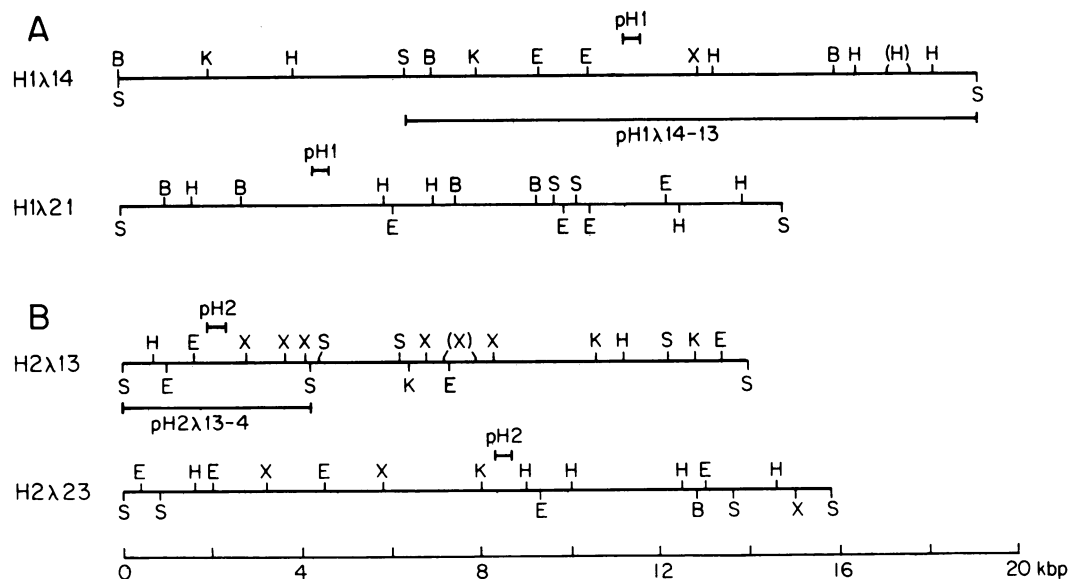


FIG. 1. Representative examples of the two types of genomic clones that were screened with both the pH1 (A; *P-ePrv* carboxylase) and pH2 (B; Prv, P_i dikinase) cDNA clones. The cDNA-clone inserts are shown above the genomic-clone restriction endonuclease fragments to which each hybridizes. The genomic subclones used for hybrid selection and as RNA blot probes (pH1 λ 14-13 and pH2 λ 13-4) are indicated directly below the maps of H1 λ 14 and H2 λ 13. Restriction sites: E, *Eco*RI; H, *Hind*III; S, *Sal* I; B, *Bam*HI; K, *Kpn* I; X, *Xho* I. Sites enclosed in parentheses map to one of the two positions indicated.

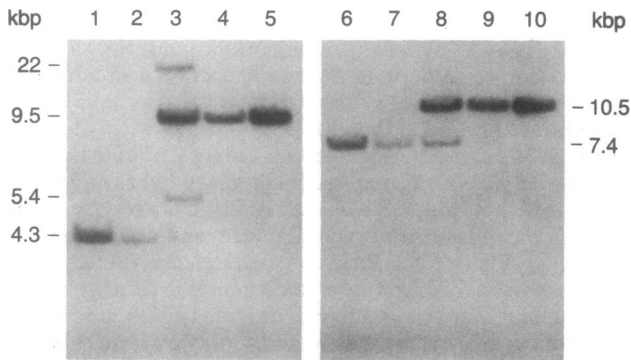


FIG. 2. Representation of fragments homologous to the pH1 (*P-ePrv* carboxylase) and pH2 (Prv, P_i dikinase) cDNA clones in the maize genome, showing the correspondence between fragments in the genome and in genomic clones screened with pH1 and pH2. All DNAs were digested with *Hind*III, subjected to electrophoresis in 1.0% agarose, and transferred to Zeta-Probe membrane. Lanes 3 and 8 contain total maize DNA (10 μ g), and the other lanes contain one- and three-copy reconstructions (75 pg and 225 pg) of H1 λ 21 (lanes 2 and 1), H1 λ 14 (lanes 4 and 5), H2 λ 23 (lanes 7 and 6), and H2 λ 13 (lanes 9 and 10). Lanes 1–5 were hybridized with pH1, and lanes 6–10, with pH2.

Fig. 3 shows the data used to identify pH1 as a *P-ePrv* carboxylase clone and pH2 as a Prv, P_i dikinase clone. The mRNA selected by pH1 λ 14-13 encodes a polypeptide that comigrates with the 103-kDa *P-ePrv* carboxylase marker (lane 2). This is the size of both the *in vitro*- and *in vivo*-synthesized protein subunit (8, 14). The 103-kDa polypeptide in lane 2 is also immunoprecipitated by *P-ePrv* carboxylase antibody (lane 5).

The mRNA selected by pH2 λ 13-4 codes for a 110-kDa polypeptide (Fig. 3, lane 3), which is the size of the Prv, P_i

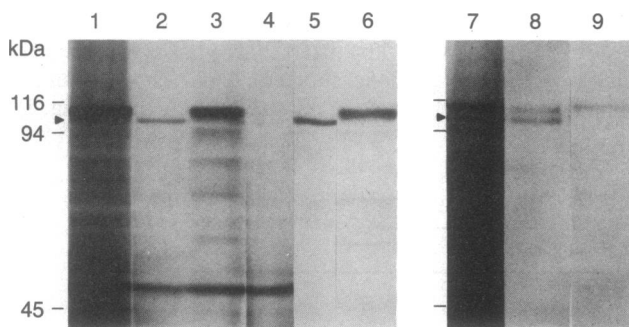


FIG. 3. Identification of clones containing *P-ePrv* carboxylase and Prv, P_i dikinase sequences by hybrid-selection and immunoprecipitation. The hybrid-selections were done with total poly(A)⁺ RNA from green maize leaves and filter-bound genomic subclone DNAs that contain inserts that react with the pH1 cDNA clone (pH1 λ 14-13), or the pH2 cDNA clone (pH2 λ 13-4). Lanes 1–4: products of *in vitro* translations of total green leaf poly(A)⁺ RNA (lane 1) and of mRNA selected by pH1 λ 14-13 (lane 2), pH2 λ 13-4 (lane 3), and pBR322 (lane 4). Lane 5: immunoprecipitate obtained with *P-ePrv* carboxylase antibody, from a sample equivalent to that in lane 2. Lane 6: immunoprecipitate obtained with Prv, P_i dikinase antibody, from a sample equivalent to that in lane 3. Characterization of the antibodies is shown in lanes 7–9. Lane 7: *in vitro* translation products from total green leaf poly(A)⁺ RNA. Lane 8: immunoprecipitate from a sample equivalent to that in lane 7, with *P-ePrv* carboxylase antibody (some Prv, P_i dikinase was also immunoprecipitated, probably because the *P-ePrv* carboxylase used to elicit the antibody was contaminated with Prv, P_i dikinase). Lane 9: immunoprecipitate from a sample equivalent to that in lane 7, with Prv, P_i dikinase antibody. Molecular mass markers are indicated at left in kDa. The arrowhead shows the position of a 103-kDa *P-ePrv* carboxylase marker.

dikinase subunit precursor synthesized *in vitro* (11, 34). The size difference between the *in vitro* translation product (110 kDa) and the 94- to 97-kDa subunit found *in vivo* is probably accounted for by a transit peptide which is removed during the transport of Prv, P_i dikinase from the cytoplasm into the chloroplasts of mesophyll cells (5, 11, 34). Immunoprecipitation of the 110-kDa polypeptide by Prv, P_i dikinase antibody is shown in lane 6. Characterization of the *P-ePrv* carboxylase and Prv, P_i dikinase antibodies is shown in lanes 7–9. From these results, we conclude that pH1 is a *P-ePrv* carboxylase cDNA clone and pH2 a Prv, P_i dikinase cDNA clone.

***P-ePrv* Carboxylase and Prv, P_i Dikinase mRNA Expression in Maize Leaves and Roots.** When the *P-ePrv* carboxylase cDNA clone (pH1) is used to probe a blot of electrophoretically fractionated poly(A)⁺ RNA from green leaves, an intense reaction with an mRNA of about 3.5 kb is observed. There is no detectable reaction of pH1 with any transcripts in poly(A)⁺ RNA from etiolated leaves or roots (Fig. 4A). These results corroborate a previous report (15) that showed that maximal accumulation of one or more forms of 3.5-kb *P-ePrv* carboxylase mRNA is induced by light in maize leaves.

When the genomic subclone pH1 λ 14-13 is used to probe the same RNAs, a similar pattern of hybridization to that observed with the pH1 probe is obtained, except very weak reactions can also be detected to 3.5-kb transcripts in the etiolated leaf and root RNAs (Fig. 4B). The greater sensitivity obtained with the pH1 λ 14-13 probe is due at least in part to the fact that it contains a substantial amount of a *P-ePrv* carboxylase gene coding region (\approx 3 kbp; unpublished data), whereas the hybridizable sequence in pH1 is much smaller (400 bp).

In contrast to pH1 and pH1 λ 14-13, the genomic clone H1 λ 21 exhibits a very different pattern of hybridization to the leaf and root RNAs (Fig. 4C). H1 λ 21 reacts most strongly with the root mRNA, while exhibiting weaker and approximately equal reactions with the green and etiolated leaf mRNAs. Clearly, the *P-ePrv* carboxylase mRNAs in green leaves and roots are encoded by different genes that differ substantially in base sequence. Further, these results provide evidence that pH1 λ 14-13 contains genomic sequences that encode the light-inducible mRNA in green leaves, and that H1 λ 21 contains sequences encoding root mRNA. The data in Fig. 4B and C also suggest that the mRNA in etiolated leaves may be encoded by a third type of *P-ePrv* carboxylase gene.

Like the *P-ePrv* carboxylase cDNA clone (pH1), the Prv, P_i dikinase cDNA clone (pH2) also reacts intensely with a

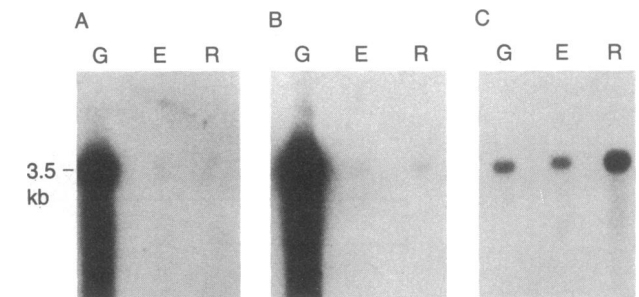


FIG. 4. Blot-hybridization analysis of *P-ePrv* carboxylase mRNA expression in maize leaves and roots. Poly(A)⁺ RNA (1 μ g) from green leaves (lanes G), etiolated leaves (lanes E), and roots (lanes R) was subjected to electrophoresis in a 1.0% agarose/formaldehyde gel, transferred to nitrocellulose, and hybridized with *P-ePrv* carboxylase probes: cDNA clone pH1 (A), genomic subclone pH1 λ 14-13 (B), and genomic clone H1 λ 21 (C). Maize leaf rRNAs were used as size markers (35) for estimation of mRNA length. Autoradiography with one intensifying screen was done for 60 hr (A) or 22 hr (B and C).

3.5-kb mRNA in green leaves (Fig. 5A). pH2 also reacts weakly with larger transcripts in green leaves, which may be precursors of the 3.5-kb species. Whereas pH1 does not react detectably with etiolated leaf and root RNA, pH2 does hybridize weakly to transcripts in both of these tissues (Fig. 5A). The intensity of these reactions is not enhanced by reducing the hybridization and wash temperatures by 12°C (data not shown). Therefore, these weak signals are probably due to the low abundance of the Prv,P_i dikinase transcripts in these tissues and not due to sequence divergence. These results are consistent with an earlier study (11) and indicate that one or more forms of Prv,P_i dikinase mRNA in green leaves are induced by light to become much more abundant than the Prv,P_i dikinase transcripts in etiolated leaves and roots.

When the genomic clones pH2λ13-4 and H2λ23 are used to probe the two types of leaf poly(A)⁺ RNA and root poly(A)⁺ RNA, a pattern of hybridization similar to that of the pH2 probe is observed (Figs. 5B and C). Compared to pH2λ13-4, H2λ23 reacts less intensely with the Prv,P_i dikinase transcripts in all three tissues (on a longer exposure, reaction with the etiolated leaf and root Prv,P_i dikinase transcripts by H2λ23 becomes clearly visible). Because the specific activities of the two probes were very similar, this difference is probably due to H2λ23 having less homology with these mRNAs than does pH2λ13-4. Thus, it is possible that the Prv,P_i dikinase transcripts detected in all three tissues are encoded by the gene represented by pH2λ13-4. The function of the Prv,P_i dikinase gene sequence in H2λ23 is unclear; two possibilities are that it (i) codes for an mRNA that is expressed in a tissue or cell type not analyzed here or (ii) contains a pseudogene that has diverged from the sequence of the leaf and root transcripts.

The RNA blot results shown in Fig. 5 also suggest that Prv,P_i dikinase transcripts exist in etiolated leaves and roots that may be smaller than the 3.5-kb mRNA in green leaves. (Actually two transcripts can be detected in lanes E of Fig. 5A and B, one that is about 3.5 kb long and one that is smaller.) As a consequence, the blot hybridizations in Fig. 6 were carried out to verify whether or not size differences exist among the *P-ePrv* carboxylase and Prv,P_i dikinase messages in leaves and roots. The *P-ePrv* carboxylase genomic subclone (pH1λ14-13) and the Prv,P_i dikinase cDNA clone (pH2) were used to probe blots in which a reduced amount of green leaf poly(A)⁺ RNA (25 ng) was mixed with 1 μg of root poly(A)⁺ RNA, and the two were electrophoresed together in the same lane. In adjacent lanes, 1 μg of root and etiolated leaf poly(A)⁺ RNA and 25 ng of green leaf poly(A)⁺ were electrophoresed separately (Fig. 6). No size differences were detected among the different *P-ePrv* car-

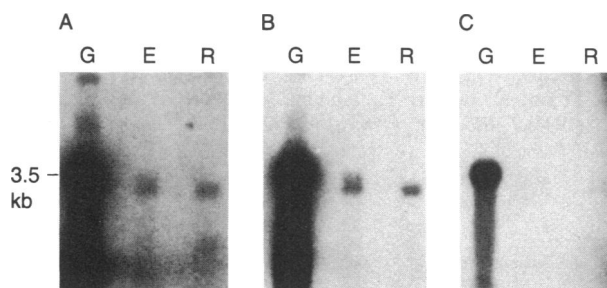


FIG. 5. Blot-hybridization analysis of Prv,P_i dikinase mRNA expression in maize leaves and roots. Poly(A)⁺ RNA (1 μg) from green leaves (lanes G), etiolated leaves (lanes E), and roots (lanes R) was blotted as indicated in the legend to Fig. 4 and hybridized with three different Prv,P_i dikinase probes: cDNA clone pH2 (A), genomic subclone pH2λ13-4 (B), and genomic clone H2λ23 (C). Size markers were the same as for Fig. 4, and film exposure times were again 60 hr (A) or 22 hr (B and C).

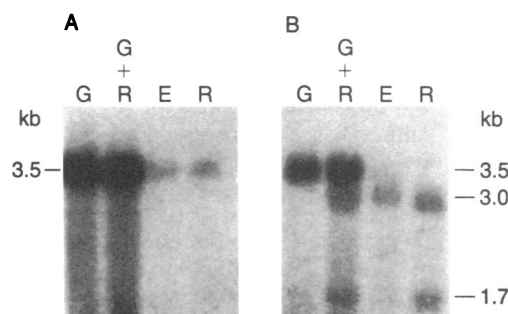


FIG. 6. Blot-hybridization analysis of *P-ePrv* carboxylase and Prv,P_i dikinase mRNA sizes in maize leaves and roots. Poly(A)⁺ RNA from green leaves (25 ng), etiolated leaves (1 μg), and roots (1 μg) was subjected to electrophoresis in a 0.8% agarose/formaldehyde gel, transferred to nitrocellulose, and probed with the *P-ePrv* carboxylase genomic subclone pH1λ14-13 (A) and the Prv,P_i dikinase cDNA clone pH2 (B). The poly(A)⁺ RNAs loaded in each lane were as follows: green leaf (G), green leaf plus root (G+R), etiolated leaf (E), and root (R). Size markers were the same as in Fig. 4.

boxylase transcripts (Fig. 6A), but the results clearly show that etiolated leaves and roots contain Prv,P_i dikinase transcripts ≈0.5 kb shorter than green leaf mRNA (3.0 kb vs. 3.5 kb; Fig. 6B). In the lanes containing root poly(A)⁺ RNA, an unidentified transcript too small (≈1.7 kb) to encode any known form of Prv,P_i dikinase is also visible.

The RNA blot in Fig. 6B also shows that the Prv,P_i dikinase mRNA in green leaves is at least 40-fold more abundant [per μg of poly(A)⁺ RNA] than the root and etiolated leaf transcripts. This is concluded because the green leaf mRNA produced the strongest signal, yet 1/40th as much green leaf poly(A)⁺ RNA was probed compared to the other two RNAs (25 ng vs. 1 μg).

DISCUSSION

We have demonstrated that the maize enzymes *P-ePrv* carboxylase and Prv,P_i dikinase are encoded by small multigene families with members that exhibit differential expression in green leaves, etiolated leaves, and roots. Until all the sequences that hybridize with *P-ePrv* carboxylase and Prv,P_i dikinase coding-region probes are cloned, it will not be possible to specify the exact number of genes in each family. Simply estimating from the number of genomic *Hind*III fragments observed to hybridize to each cDNA clone (Fig. 2), we can place a lower limit on the number of *P-ePrv* carboxylase genes at three or four and on the number of Prv,P_i dikinase genes at two. In the case of *P-ePrv* carboxylase, it is clear that different genes encode the mRNAs in green leaves and roots and that these genes differ substantially in base sequence (Fig. 4). This substantial sequence divergence among *P-ePrv* carboxylase gene-family members may explain a previous report of protein accumulation in the absence of detectable mRNA (15); the detectability of different *P-ePrv* carboxylase mRNAs is clearly probe-dependent. The data in Fig. 4 also suggest that the mRNA in etiolated leaves may be transcribed from a third member of the *P-ePrv* carboxylase gene family.

In the case of Prv,P_i dikinase, it is possible that one gene or two very similar genes (represented by genomic clone H2λ13) encode the mRNAs detected in green leaves, etiolated leaves, and roots. The genomic-clone and genome-blot information (Figs. 1 and 2) provide evidence for only one other type of Prv,P_i dikinase gene (represented by H2λ23). This gene appears to have diverged substantially from the leaf and root transcripts, and it may encode an mRNA expressed in a tissue not analyzed here or it may be a pseudogene. If one gene does encode all the Prv,P_i dikinase transcripts in leaves

and roots, different transcription start or stop sites and/or differential RNA processing would be required to account for the different sizes of the green leaf mRNA (3.5 kb) versus etiolated leaf and root mRNAs (3.0 kb).

The Prv, P_i dikinase transcript-size differences are interesting in the context of known differences in the size of the Prv, P_i dikinase polypeptide precursors in photosynthetic versus nonphotosynthetic tissues (34). Chloroplast uptake studies and comparisons between *in vitro* translation products and mature subunits found *in vivo* provide evidence that the precursors in maize and wheat green leaves have a transit peptide, about 16 kDa in size, that the precursors in maize and wheat seeds lack (5, 11, 34). The 0.5-kb size difference we observe between Prv, P_i dikinase transcripts in photosynthetic and nonphotosynthetic tissues corresponds closely to the amount of RNA required to encode 16 kDa of polypeptide. These mRNA size differences may therefore be accounted for by the presence or absence of the sequence that encodes the transit peptide.

Light is required for maximal accumulation of the *P-ePrv* carboxylase and Prv, P_i dikinase isozymes involved in C_4 photosynthesis (1, 8, 13), and as shown here and in other studies (11, 14, 15), maximal accumulation of *P-ePrv* carboxylase and Prv, P_i dikinase leaf mRNA is also light-dependent. Because the genomic subclones pH1 λ 14-13 and pH2 λ 13-4 hybridize very strongly to the light-inducible leaf messages, it is possible that the genomic clones from which they were derived contain the genes that encode the C_4 -photosynthetic forms of these enzymes. Additional studies will be needed to confirm the identification of these clones and to determine the structure and function of the remaining members of the *P-ePrv* carboxylase and Prv, P_i dikinase gene families.

Note Added in Proof. We have recently derived amino acid sequence information from DNA sequence data obtained from the *P-ePrv* carboxylase clone H1 λ 14. This information indicates that there are several regions 12–26 amino acids long in which the maize enzyme encoded in this clone is 70–90% homologous to *Escherichia coli P-ePrv* carboxylase (36).

We thank Dr. Tatsuo Sugiyama for antiserum against maize leaf Prv, P_i dikinase and Dr. Hans Lehrach for EMBL phage vectors and host strains. Drs. Richard Yenofsky, Constantin Flytzanis, and David Anderson provided valuable information on various procedures. We thank Drs. Robert Goldberg, Richard Yenofsky, and David Anderson for very helpful criticism of the work while in progress and of the manuscript. Mary Strother and Elizabeth Hanson assisted in the manuscript preparation. This work was supported by Eli Lilly and Company (Indianapolis, IN) and the J. G. Boswell Company (Los Angeles).

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