Plant Physiol. (1985) 78, 399–404
0032-0889/85/78/0399/06/$01.00/0

Photosynthetic Gene Expression and Cellular Differentiation in Developing Maize Leaves[1]

Received for publication September 24, 1984 and in revised form January 28, 1985

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ABSTRACT

We have exploited the positional gradient of cellular differentiation in *Zea mays* leaves to study the accumulation of mRNAs encoding subunits of the two CO_2-fixing enzymes and the major chlorophyll-binding protein. These three proteins are differentially compartmentalized in the two photosynthetically active cell types of the leaf. Previous studies have shown that accumulation of the two carboxylases commences 2 to 4 cm from the base of the leaf (Mayfield SP, WC Taylor Planta 161: 481–486) at a position where bundle sheath and mesophyll cells show morphological evidence of maturation. The light-harvesting chlorophyll *a/b* protein accumulates progressively from the leaf base, as does its mRNA, in spite of its localization in mesophyll cells after cellular differentiation occurs. While small quantities of phosphoenolpyruvate carboxylase mRNA are detectable in the basal region of the leaf, significant mRNA accumulation is coincident with that of the polypeptide at 4 to 6 cm from the leaf base, the region where bundle sheath and mesophyll cells exhibit fully differentiated morphologies. mRNAs encoding the small and large subunits of ribulose 1,5-bisphosphate carboxylase accumulate to significant levels before bundle sheath cells are fully differentiated and before their polypeptides are detectable. Cytological examination indicates that this is the position at which the maturation of intermediate vascular bundles is first evident. Cytosolically localized small subunit mRNA and chloroplast-localized large subunit mRNA are complexed with polyribosomes at all positions of the leaf.

In leaves of graminaceous plants, cell divisions occur primarily in the basal meristem, older cells being displaced by younger cells below them. This process results in a positional gradient of cell ages, with younger, less differentiated cells at the base and older, more differentiated cells toward the tip of each leaf. This developmental gradient has been utilized to examine various aspects of leaf development in barley (28), wheat (6), and oats (26). Similar studies have also been carried out in maize (3, 14, 20, 29, 30) where the simple pattern of leaf development is more complicated due to chloroplastic and cellular dimorphism. While both wheat and barley leaves contain mesophyll cells with mature chloroplasts exhibiting extensive thylakoid membrane stacking, called grana, maize leaves possess two major photosynthetic cell types; bundle sheath cells, containing agranal plastids, and mesophyll cells. These bundle sheath cells also differentiate basipetally and synchronously, or nearly so, with the small, intermediate leaf bundles (7, 25).

The two cell types in maize functionally cooperate in the multi-step scheme of CO_2 fixation called C4 photosynthesis. PEPCase[3], the enzyme responsible for initially fixing the atmospheric CO_2 in maize, is found only in mesophyll cells (22). The four-carbon acid resulting from this reaction is shuttled to bundle sheath cells where it is decarboxylated, supplying CO_2 to carry out the reductive pentose phosphate cycle, the initial product of which is a three-carbon compound, as in C3 plants (4). RuBPCase is found only in the chloroplasts of bundle sheath cells (12). This compartmentalization of the carboxylases confers photosynthetic advantage to C4 plants under conditions of high temperature and light intensity (11). In addition to these enzymic differences between the mesophyll and bundle sheath cells of C4 plants, there are differences in the chloroplast membrane proteins of the two cell types. Membrane proteins from maize mesophyll cell chloroplasts are essentially identical to those of several C3 plants, containing polypeptides responsible for the light-harvesting and energy-transduction reactions of photosynthesis (2). The bundle sheath cell chloroplasts, however, contain little if any of the PSII protein complex and its associated LHCP (1; Schuster G, I Ohad, B Martineau, WC Taylor, unpublished).

We have outlined the patterns of mRNA accumulation for four of the compartmentalized polypeptides related to C4 photosynthesis by using hybridization with DNA probes to measure accumulation of RuBPCase (LSu and SSu), PEPCase, and LHCP mRNAs in cells of increasing age and stage of differentiation. We compare our patterns of mRNA accumulation for these proteins to the patterns of their polypeptide accumulation established by Mayfield and Taylor (17) in a similar study. We have monitored leaf morphological development by using a cytological marker to indicate fully differentiated vascular bundles, again taking advantage of the gradient of cellular development in the young leaves we have chosen to study.

MATERIALS AND METHODS

Plant Material. Seeds from an inbred line of *Zea mays* (B73, a gift of Pioneer Hi-Bred International, Johnston, IA) were planted in a 2:1 mixture of soil and sand, covered with 2 cm of vermiculite, and germinated and grown in a growth chamber. Periods of 16 h light (4×10^4 lux) and 8 h dark, at 25°C, were used. Plants were harvested 10 to 14 d after germination when the third leaf to emerge from the coleoptile was 12 to 16 cm long. A third leaf of this age has not yet developed a ligule, the structure which delineates the leaf blade from its sheath. The coleoptile and first and second leaves were removed from each plant. The third leaf was cut at points 1, 2, 4, 6, and 8 cm from the leaf base. The six resulting leaf segments, representing populations of cells of the same relative age and stage of differentiation, were separated and stored in liquid N_2.

[1] Supported by a grant from the Competitive Research Grants Office of the U. S. Department of Agriculture (82-CRCC-1-1083) to W.C.T.
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[3] Abbreviations: PEPcase, phosphoenolpyruvate carboxylase; RuBPCase, ribulose 1,5-bisphosphate carboxylase; LSu, large subunit; SSu, small subunit; LHCP, light-harvesting Chl protein.

Measurement of Leaf RNAs. RNA isolation was carried out using the protocol of Schmidt *et al.* (24) except that the initial buffer added to each of the six frozen tissue samples, which had been finely ground in a chilled coffee grinder, consisted of 25 mM Tris (pH 7.5), 100 mM NaCl, 7.5 mM EDTA, 5.0% SDS, and 0.2 mg/ml Proteinase K (E. Merck, Darmstadt, Germany). Polyadenylated RNA (poly(A) RNA) was isolated from total RNA by two cycles of fractionation on oligo(dT) cellulose columns and ethanol precipitation. The columns were washed under stringent conditions (0.25 M NaCl at room temperature) before elution. Four μg poly(A) RNA samples were separated on 1.0% agarose gels in the presence of formaldehyde (15) and transferred to nitrocellulose. The blotted RNAs were then hybridized with nick-translated cDDNA probes for RuBPCase SSu (pBT76; T Nelson, WC Taylor, J Fitchen, E Bell, and L McIntosh, unpublished), PEPCase (pPC2; Harpster and Taylor, submitted), or LHCP (pM7; 18) or a cloned fragment of maize chloroplast DNA containing RuBPCase LSu (pZmc37; 5) in 5 × SSPE (1 × SSPE consists of 0.18 mM NaCl, 10 mM sodium phosphate buffer [pH 7.0], 1.0 mM EDTA), 50% deionized formamide, 0.002% Ficoll, 0.002% BSA, 0.002% PVP, 0.4 mg/ml polyadenosine, 0.2% SDS, for 36 h at 42°C. After hybridization, RNA blots were washed once with hybridization solution for 1 h and then two times in 2 × SSC (1 × SSC consists of 0.15 M NaCl, 15 mM sodium citrate) at 50°C before being exposed to film. For some experiments, RNA filters previously probed were washed free of radioactive label by washing them briefly in 0.2 N NaOH at room temperature, and then rehybridizing with a second clone probe.

Estimates of the amounts of RNA represented on an autoradiograph were made by comparison with serial dilutions of 17S *Phaseolus vulgaris* rRNA which had been electrophoresed and transferred to nitrocellulose blots as described above. The rRNA blots were hybridized with a genomic clone containing a single copy of *P. vulgaris* 17S rRNA (λBR1-4, Fragoso and Taylor, unpublished) which had been nick-translated to the same specific activity as the experimental probe. A comparison of band densities on rRNA control and experimental autoradiographs provided estimates for the amount of RNA present in each band. Because the accumulation of each mRNA varied significantly from base to tip segments, densitometric measurements were made of autoradiographs exposed for various lengths of time to ensure accurate estimates of mRNAs found less abundantly in the leaf's basal region.

Analysis of Leaf Polyribosomes. Polyribosomes were isolated from leaf base (0–4 cm) and tip (7 cm to tip) samples using the protocol of Goldberg *et al.* (9) with the following modifications. The frozen tissue was initially ground to a fine powder in a dry ice-chilled coffee grinder before being suspended in extraction buffer. Pelleted polysomes (0.2-mg samples) were loaded onto 5 ml 15 to 60% sucrose gradients and separated by centrifugation at 27,000 rpm in an AH650 rotor for 75 min at 2°C. Fractions (0.25 ml) were collected directly into phenol buffered with one-tenth volume 3 M NaAc (pH 6.2) and extracted once before being ethanol precipitated. Selected fractions were resuspended in 25 μl 0.1% SDS. Two- to 5-μl aliquots were spotted onto nitrocellulose which had been pretreated by briefly soaking in sterile water and 20 × SSC, followed by drying under a heat lamp. Filters were baked, hybridized, and autoradiographed under the same conditions used for blots of gel-fractionated RNA described above.

Preparation of Leaf Tissue for Light Microscopy. Leaves were cut into six segments as described above and fixed immediately in freshly prepared 1.0% chromic acid, 7.0% glacial acetic acid, and 30% neutral formalin. Leaf segments were then dehydrated through a *t*-butyl alcohol series and embedded in paraffin. Sections (10 μm thick) were cut on a Spencer '280' microtome. Sections were mounted on gelatin-coated slides (Haupt's adhesive, 13) and stained using 0.5% Haematoxylin, 0.5% Safranin, and 1% fast green. Slides were viewed and photographed using bright field optics or bright field optics in conjunction with polarizing filters (Zeiss). The number of cells in each of the six segments was estimated by counting the nuclei in a transverse section of known volume and multiplying by the ratio of the section to segment length.

RESULTS

Third foliage maize leaves were chosen for our cytological and molecular analyses for two reasons. Unlike the first two leaves, third leaves have a shape and pattern of development which is more typical of the other leaves of the plant. Also, at this early stage of seedling development in this inbred line, third leaves have not yet acquired a ligule, a structure of epidermal origin which separates the leaf sheath from the blade. Consequently, the entire leaf can be treated as a single, simple gradient from base to tip. Second leaves contain a ligule at this stage.

We marked vascular bundle differentiation by using polarized light to indicate the mature, differentiated state of xylem vessels. The secondary walls of mature xylem tissue are birefringent under such conditions (8). Because lateral vascular bundles de-

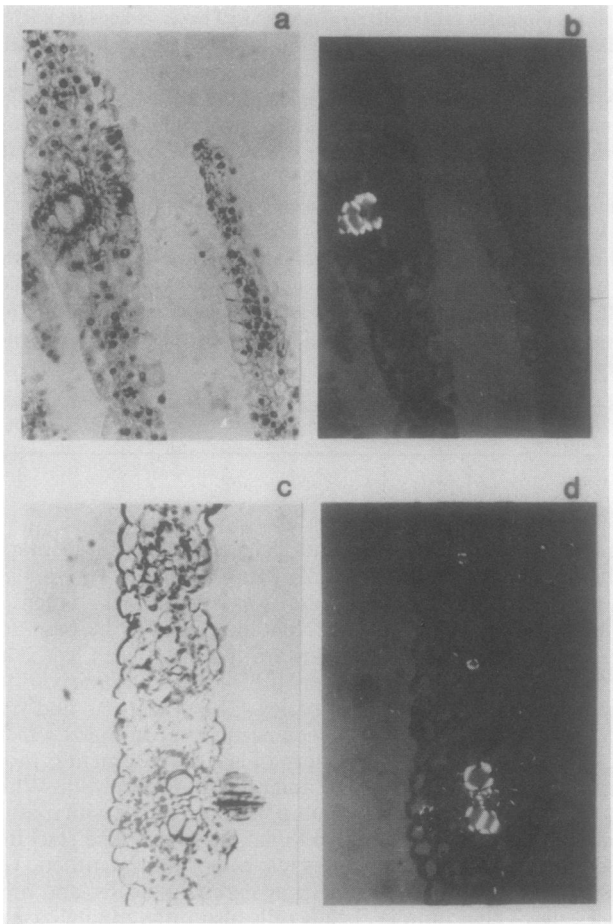

FIG. 1. Transverse sections through 12- to 16-cm long leaves. Photomicrographs a and b are of the same leaf section, 0.5 cm from the leaf base; c and d are of a different section, 5.0 cm from the base. Photomicrographs a and c were taken using bright field optics; b and d were taken using the same in conjunction with polarizing filters (Zeiss). Under these conditions, the birefringence of mature vascular tissue is evident. The photomicrographs are magnified approximately 200×.

velop very early in maize leaf development (7, 25), we would anticipate detecting them as fully differentiated xylem vessels from below the leaf node to the tip of these young, third leaves. As expected, these lateral vascular bundles are clearly evident in both the basal and mid-leaf regions (Fig. 1). There is, however, no visible evidence of differentiated intermediate vascular bundles in the leaf's basal region (Fig. 1b) because of the basipetal, relatively late, pattern of development of these bundles (7, 25). The first sign of mature intermediate vascular bundles is observed in transverse sections cut 3.0 cm from the leaf base, while many mature intermediate vascular bundles are visible between the more prominent major veins at 5.0 cm from the base (Fig. 1d).

Accumulation of mRNAs during leaf development was measured by hybridization of cloned cDNA probes to poly(A) RNA isolated from each of the six leaf segments. While a gradient of increasing mRNA accumulation from leaf base to tip exists for all four mRNAs examined, three distinct patterns of mRNA accumulation are revealed. RuBPCase LSu and SSu mRNAs are both present (in small but detectable quantities) in poly(A) RNA isolated from the basal (0–1 cm) portion of the leaf. A significant increase in SSu and LSu mRNA accumulation is observed in the leaf segment 1 to 2 cm from the leaf base, but dramatic increases in the accumulation of these mRNAs do not occur until 2 to 4 cm from the leaf base (Fig. 2, b and c). PEPCase mRNA exhibits a pattern of accumulation similar to that for RuBPCase mRNAs in Figure 2, a and b. The relative quantity of PEPCase mRNA, however, appears to decrease at 2 to 4 cm from the base and does not increase significantly until 4 to 6 cm from the leaf base (Fig. 2, b–d). LHCP mRNA accumulation increases earlier in leaf development than do those of the carboxylase mRNAs and without points of dramatic increase or decrease in accumulation.

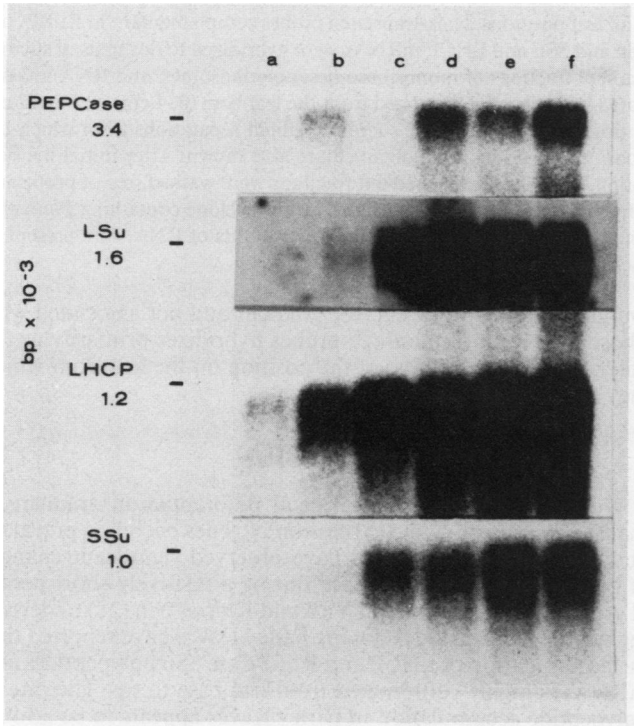

FIG. 2. mRNA accumulation as a function of leaf position. Composite of blots of gel-fractioned poly(A) RNA isolated from each of the six segments and hybridized with nick-translated probes complementary to the mRNAs for PEPCase, RuBPCase LSu, SSu, and LHCP. Equal amounts of poly(A) RNA are loaded into each lane. The intensity of hybridization provides a measure of the relative amount of each mRNA with increasing distance from the leaf base. Because of differences in the specific activities of the hybridization probes used, absolute comparisons of mRNA steady state levels between probes on this blot cannot be made.

It should be noted that the RuBPCase LSu probe hybridized to nitrocellulose blots of poly(A) RNA (isolated using oligo(dT) cellulose Type 3; Collaborative Research, Lexington, MA) as well as any of the cDNA probes, although it is generally assumed that chloroplast-encoded transcripts are not polyadenylated. There are no AT-rich sequences within the amino acid coding region of the LSu structural gene (19). There are, however, two oligo(A) sequences in the region 3′ to the coding region that could be responsible for the observed binding to oligo(dT) cellulose. Twenty-seven of the thirty-eight bases immediately following the ochre codon, including a series of nine sequential bases, are adenines. A series of twelve sequential adenines is located at a position approximately 110 bases 3′ to the ochre stop codon. Three possible eukaryotic polyadenylation consensus sequences are also observed in the 3′ region of the gene. One, a sequence of six bases that immediately follows the translation stop codon, matches the AATAAA consensus sequence perfectly. Two others, mismatched by one base each, are located approximately 30 and 40 bases 3′ to the stop codon, respectively (19). To our knowledge, the 3′ end of the maize RuBPCase LSu transcript has not been determined.

The amounts of total and poly(A) RNA per cell were estimated from the RNA yields of each leaf segment sample in Table I. When measured per g (wet weight) of tissue, RNA yields tend to be lower in more differentiated regions, especially for fractions of poly (A) RNA. When considered on a per cell basis, however, RNA yields for total RNA and poly(A) RNA increase more than 10- and 4-fold, respectively, between the leaf's basal region and the tip. Estimates, based on data presented in Table I, indicate that the number of LSu, SSu, and LHCP mRNA molecules per cell increase from 10 to 15 at the leaf base to greater than 1,000 to 10,000 at the leaf tip. Estimates of PEPCase mRNA molecules per cell increased approximately 10-fold over the same distance. These estimates do not take into account the fact that all of the mRNAs under examination are likely to be confined to one or the other of the leaf's major photosynthetic cell types.

Polypeptide subunits of RuBPCase and PEPCase are first detectable 2 to 4 cm from the base of similar-sized third leaves of maize, and significantly increase in quantity 6 to 8 cm from the base. LHCP polypeptides, however, accumulate at earlier stages of leaf development, reaching their maximum quantity approximately 6 to 8 cm from the leaf base (17). Figure 3 presents a comparison of the accumulation of the four polypeptides and their mRNAs as a function of leaf position. Relative accumulation is expressed as the percentage of the maximum density recorded in scanning autoradiographs of the respective RNA or

Table I. *RNA Yields from Leaf Segments*

Distance from Base	Yield of Total RNA[a]	Yield of poly(A) RNA[a]	No. of Cells[b]	Total RNA Content	poly(A) RNA Content
cm	*mg/g wet wt*	*μg/g wet wt*	*cells/g wet wt × 10⁸*	*pg/cell*	*fg/cell*
0–1	1.7	10.0	3.2	5.3	3.1
1–2	1.1	8.0	1.7	6.4	4.7
2–4	1.6	6.0	1.01	15.8	5.9
4–6	1.1	2.7	0.32	34.3	8.4
6–8	1.0	na	0.33	30.3	na
8+	1.2	2.6	0.19	63.1	13.7

[a] Data from one isolation, but typical. [b] Number of cells estimated by counting nuclei in transverse sections of known volume. Cell numbers represent an average over a 1 or 2 cm length.

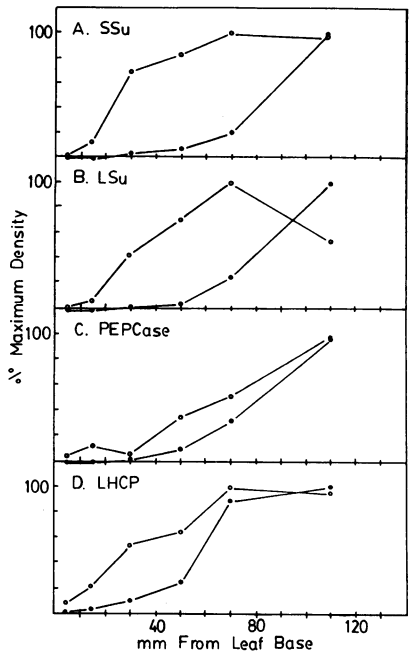

FIG. 3. mRNA accumulation (O) compared with protein accumulation (●) for RubPCase (SSu and LSu), PEPCase, and LHCP. RNA and protein are isolated from leaf tissue gathered in the same harvest. For purposes of comparison, data are expressed as the percentage maximum density recorded in scanning autoradiographs of the respective RNA (Fig. 2) or protein blot (17) with a densitometer. The measurements of PEPCase mRNA accumulation are an average of two independent determinations.

protein blot with a densitometer.

The patterns of RuBPCase LSu and SSu mRNA accumulation differ significantly from the accumulation patterns of their respective polypeptides (Fig. 3, a and b). Significant amounts of mRNA coding for both enzyme subunits are present in the basal 2 cm of the leaf, yet no carboxylase enzyme can be detected in this region despite the sensitive immunological method used for that purpose (17). The lower limit of polypeptide detection for the antisera probes used in this comparison has been estimated to be less than 1% of leaf tip values (17). If there are any PEPCase, LSu, or SSu polypeptides in the basal 2 cm of the leaf, their quantities are less than 1% of the amount present in the distal half of the leaf. Over 60% of the maximum observed levels of both LSu and SSu mRNAs are found before the polypeptides are detected. Low levels of PEPCase mRNA are also present in the basal region of the leaf where PEPCase polypeptides are not detectable. However, from the leaf segment in which PEPCase polypeptides are first detected, 2 to 4 cm from the leaf base, to the leaf tip, PEPCase mRNA and protein accumulate with similar profiles (Fig. 3c). A lack of coordination between mRNA and protein accumulation also exists for LHCP, although not to the same extent as for the RuBPCase subunits (Fig. 3d) and may be related to the control of Chl pigments over LHCP polypeptide accumulation (10).

The lack of coordination between mRNA and protein accumulation for RuBPCase (LSu and SSu) and LHCP is not due to failure of the respective mRNAs to form polysomal complexes. Polyribosomal fractions obtained from both the tip, where RuBPCase LSu, SSu, and LHCP polypeptide accumulation is considerable (7 cm to tip), and the base of the leaf where little or no LSu and SSu polypeptides can be detected (0–4 cm), contain mRNAs encoding these proteins (Fig. 4). Nick-translated probes complementary to RuBPCase LSu and SSu and LHCP mRNAs were hybridized to monoribosomes, polyribosomes, and RNAs

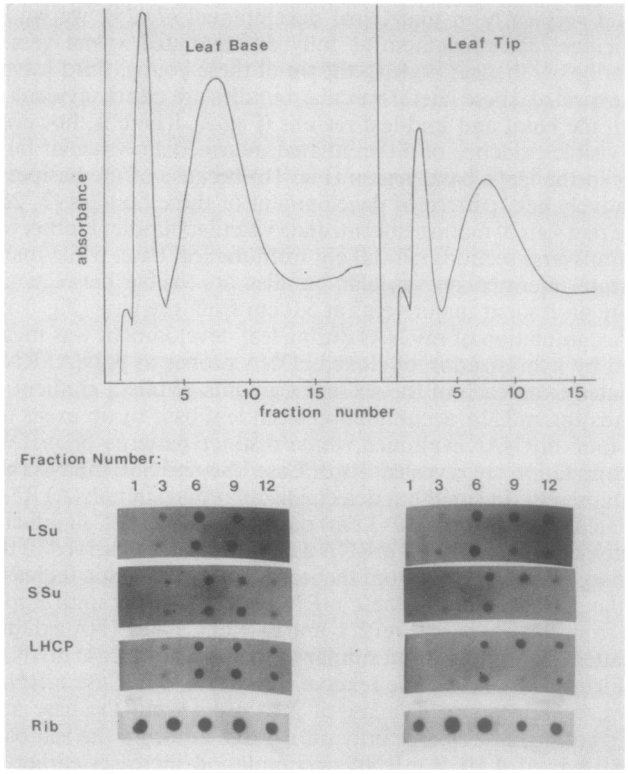

FIG. 4. LSu, SSu, and LHCP mRNAs are complexed with polysomes at all leaf positions. Nick-translated probes complementary to RuBPCase LSu and SSu and LHCP mRNAs were hybridized to dot blots of sucrose gradient fractions of monoribosomes, polyribosomes, and RNA unassociated with ribosomes isolated from the leaf base (0–4 cm) and tip (7 cm to tip). The profiles of the sucrose gradient separations from which the ribosomal fractions were obtained are also shown. After initial hybridization, blots of sucrose gradient fractions were washed free of probe and rehybridized with a nick-translated genomic clone containing *Phaseolus vulgaris* rDNA to ensure that similar amounts of RNA were present in each fraction examined.

found at the top of the sucrose gradient and not associated with ribosomes. Each of the three probes hybridizes primarily to the polyribosomes, regardless of the position on the leaf from which they are isolated.

DISCUSSION

We have exploited the basipetal differentiation gradient of maize leaves to study the expression of genes encoding prevalent photosynthetic proteins and have observed significant changes in gene expression taking place during a relatively short period of growth and development. Viro and Kloppstech (28) undertaking similar studies of developing barley leaves have reported that the accumulation of LHCP and RuBPCase SSu polypeptides and mRNAs is highly coordinated from leaf base to tip. The rate of polypeptide accumulation in barley leaves appears to be a function of the amount of translatable mRNAs encoding those polypeptides. By contrast, our investigations have revealed that the patterns of accumulation of RuBPCase LSu and SSu and LHCP polypeptides do not parallel those of their respective mRNAs in developing maize leaves (Fig. 3, a, b, d). Also, RuBPCase is found at the base of barley (28) and wheat (6) leaves. The absence of any RuBPCase polypeptides in the basal 2 cm of maize leaves (17) while the mRNAs that encode them are present suggests that some posttranscriptional control directs the expression of these proteins. RuBPCase polypeptide accumulation in maize is

not simply a function of mRNA accumulation.

mRNAs coding for RuBPCase LSu and SSu and LHCP are associated with polyribosomes isolated from the maize leaf base. The absence of polypeptides encoded by these mRNAs in this leaf region must be due to translational inhibition or to rapid protein turnover. Posttranslational regulation has been reported for the SSu of RuBPCase in *Chlamydomonas reinhardi* (23). These authors find that while RuBPCase SSu continues to be cytoplasmically synthesized when LSu synthesis is blocked by inhibition of organellar protein synthesis, it is rapidly degraded soon after transport into the chloroplast. A constant stoichiometric ratio of the two subunits results.

The differences in gene expression between wheat or barley and maize are probably due to the organization of maize leaves for C4 photosynthesis. PEPCase is uniquely utilized in maize and not in the leaves of wheat and barley (C3 plants) for photosynthesis. The pattern of accumulation, as a function of cell age, of PEPCase and its mRNA differs significantly from those of the other proteins measured in this study. For example, the accumulation of PEPCase mRNA does not increase monotonically from base to tip; instead, a small decrease in its accumulation is noted at 2 to 4 cm from the base. Because the relative quantities of LSu, SSu, and LHCP mRNA increase dramatically at this position, a constant quantity of PEPCase mRNA may be effectively diluted in a given poly(A) RNA sample. Another possible explanation for this consistent decrease in PEPCase mRNA is raised by the discovery that multiple forms of PEPCase exist in maize (27). M. Harpster and W. C. Taylor (unpublished) identified at least three forms of PEPCase mRNA which are 5 to 10% different in sequence. Our preliminary results indicate that the decrease in PEPCase mRNA observed between 2 and 4 cm from the leaf base is coincident with a shift in expression between isozyme mRNAs. We may be measuring the accumulation of different mRNA molecules of the same molecular weight in Figure 2. Also, between 2 and 4 cm from the base, where this decrease in steady state mRNA level is observed, and the leaf tip, PEPCase mRNA accumulation is closely paralleled by that of its protein suggesting that regulation of the genes encoding this enzyme may be primarily transcriptional.

In separate studies, Esau (7) and Sharman (25) have indicated that maize bundle sheath cells develop synchronously, or nearly so, with the intermediate vascular bundles they surround. In fact, Esau (7) states that maize bundle sheaths 'obviously develop as integral parts of the vascular bundle'. We have followed the differentiation of the latter using the birefringence of mature xylem vessels as a marker. Our data indicate that the first visible sign of fully differentiated intermediate xylem vessels appears at approximately 3 cm from the base of 12- to 16-cm-long third foliage leaves of maize; intermediate vascular bundles are well established by 5 cm from the leaf base (Fig. 1d). Direct proof that the photosynthetically active cell types themselves are differentiating in this region of maize leaves of the same length and age has been reported by Leech, Rumsby, and Thomson (14). Using EM, these workers have found that in leaf sections taken 4 to 6 cm from the leaf base, differentition into agranal bundle sheath plastids and mesophyll chloroplasts with extensive thylakoid membrane stacks has taken place (14). RuBPCase LSu and SSu and PEPCase polypeptides are first detected 2 to 4 cm from the leaf base (17); we report a dramatic increase in the accumulation of LSu and SSu mRNAs in the same region (Fig. 3, a–c). In the next leaf segment, where bundle sheath and mesophyll chloroplasts have differentiated, signficant amounts of RuBPCase LSu, SSu, and PEPCase polypeptides have accumulated and a dramatic increase in PEPCase mRNA accumulation has occurred. Clearly, this region, 2 to 6 cm from the base of these young leaves, is important for both the regulation of genes and the differentiation of a major cell type involved in C4 photosynthesis.

RuBPCase LSu mRNAs are confined to bundle sheath cells in mature maize leaves (16). We find measureable accumulation of LSu and SSu mRNAs occurring before morphologically observable bundle sheath differentiation has been completed (14). Whether RuBPCase mRNA accumulation at the leaf base is due to low level expression in the entire population of relatively undifferentiated cells at the base or to higher levels of expression in cells predetermined to become bundle sheath cells remains to be seen.

The surges in gene expression resulting in products associated with C4 carbon fixation, and the coincident appearance of new vascular tissue anticipatory of the anatomy essential to this photosynthetic scheme, are not necessarily interdependent events. The present descriptive study suggests, however, that the differentiation of bundle sheath cells may be linked with the regulation of genes specifically expressed in these specialized cells.

Acknowledgments—We are indebted to Dr. Donald Kaplan for extensive training and advice in cytological techniques and analysis and for numerous discussions on leaf development. We also thank A. Otsuda for the use of microscope facilities; S. Mayfield for helpful discussions; and D. Dunlavey, M. Freeling, S. Hake, J. Yamaguchi, and T. Nelson for critical readings of the manuscript.

LITERATURE CITED

1. ANDERSON JM 1980 Chlorophyll-protein complexes of higher plant thylakoids: distribution, stoichiometry and organization in the photosynthetic unit. FEBS Lett 117: 327–331
2. ANDERSON JM, RP LEVINE 1974 Membrane polypeptides of some higher plant chloroplasts. Biochim Biophys Acta 333: 378–387
3. BAKER NR, RM LEECH 1977 Development of photosystem I and photosystem II activities in leaves of light-grown maize (*Zea mays*). Plant Physiol 60: 640–644
4. CALVIN M, JA BASSHAM 1962 The Photosynthesis of Carbon Componds. Benjamin, New York
5. COEN DM, JR BEDBROOK, L BOGORAD, A RICH 1977 Maize chloroplast DNA fragment encoding the large subunit of ribulose bisphosphate carboxylase. Proc Natl Acad Sci USA 74: 5487–5491
6. DEAN C, RM LEECH 1982 Genome expression during normal leaf development. Plant Physiol 69: 904–910
7. ESAU K 1943 Ontogeny of the vascular bundle in *Zea mays*. Hilgardia 15: 327–368
8. GOFFINET MC, PR LARSON 1981 Structural changes in *Populus deltiodes* terminal buds and in the vascular transition zone of the stems during dormancy induction. Am J Bot 68: 118–129
9. GOLDBERG RB, GA GALAU, RJ BRITTEN, EH DAVIDSON 1973 Nonrepetitive DNA sequence representation in sea urchin embryo messenger RNA. Proc Natl Acad Sci USA 70: 3516–3520
10. HARPSTER MH, SP MAYFIELD, WC TAYLOR 1984 Effects of pigment-deficient mutants on the accumulation of photosynthetic proteins in maize. Plant Molec Biol. 3: 59–74
11. HATCH MD 1978 Regulation of enzymes in C4 photosynthesis. Curr Top Cell Regul 14: 1–27
12. HUBER SC, TC HALL, GE EDWARDS 1976 Differential location of fraction I protein between chloroplast types. Plant Physiol 57: 730–733
13. JOHANSEN DA 1940 Plant Microtechnique. McGraw-Hill, New York, p 20
14. LEECH RM, MG RUMSBY, WW THOMSON 1973 Plastid differentiation, acyl lipid, and fatty acid changes in developing green maize leaves. Plant Physiol 52: 240–245
15. LEHRACH H, D DIAMOND, JM WOZNEY, H BOEDTKER 1977 RNA molecular weight determinations by gel electrophoresis under denaturing conditions, a critical evaluation. Biochemistry 16: 4740–4751
16. LINK G, DM COEN, L BOGORAD 1978 Differential expression of the gene for the large subunit of ribulose bisphosphate carboxylase in maize leaf cell types. Cell 15: 725–731
17. MAYFIELD SP, WC TAYLOR 1984 The appearance of photosynthetic proteins in developing maize leaves. Planta 161: 481–486
18. MAYFIELD SP, WC TAYLOR 1984 Carotenoid-deficient maize seedlings fail to accumulate light-harvesting chlorophyll a/b binding protein (LHCP) mRNA. Eur J Biochem 144: 79–84
19. MCINTOSH L, C POULSON, L BOGORAD 1980 Chloroplast gene sequence for the large subunit of ribulose bisphosphate carboxylase of maize. Nature 288: 556–560
20. MIRANDA V, NR BAKER, SP LONG 1981 Anatomical variation along the length of the *Zea mays* leaf in relation to photosynthesis. New Phytol 88: 595–605
21. PERCHOROWICZ JT, M GIBBS 1980 Carbon dioxide fixation and related properties in sections of the developing green maize leaf. Plant Physiol 65: 802–809

22. PERROT-RECHENMANN C, J VIDAL, J BRULFER, A BURLET, P GADAL 1982 A comparative immunocytochemical localization study of phosphoenolpyruvate carboxylase in leaves of higher plants. Planta 155: 24–30
23. SCHMIDT GW, ML MISHKIND 1983 Rapid degradation of unassembled ribulose 1,5-bisphosphate carboxylase small subunits in chloroplasts. Proc Natl Acad Sci USA 80: 2632–2636
24. SCHMIDT GW, SG BARTLETT, AR GROSSMAN, AR CASHMORE, N-H CHUA 1981 Biosynthetic pathways of two polypeptide subunits of the light-harvesting chlorophyll *a/b* protein complex. J Cell Biol 91: 468–478
25. SHARMAN BC 1942 Developmental anatomy of the shoot of *Zea mays* L. Ann Bot 6: 245–282
26. TAYLOR JA, RO MACKENDER 1977 Plastid development in the first leaf of *Avena sativa* L. Plant Physiol 59: 5–10
27. TING IP, CB OSMOND 1973 Multiple forms of plant phosphoenolpyruvate carboxylase associated with different metabolic pathways. Plant Physiol 51: 448–453
28. VIRO M, K KLOPPSTECH 1980 Differential expression of the genes for ribulose-1,5-bisphosphate carboxylase and light-harvesting chlorophyll *a/b* protein in the developing barley leaf. Planta 150: 41–45
29. WILLIAMS LE, RA KENNEDY 1978 Photosynthetic carbon metabolism during leaf ontogeny in *Zea mays* L.: enzyme studies. Planta 142: 269–274
30. WILLIAMS LE, RA KENNEDY 1978 Relationship between early photosynthetic products, photorespiration, and stage of leaf development in *Zea mays*. Z Pflanzenphysiol 81: 314–322