Light-regulated Gene Expression during Maize Leaf Development

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ABSTRACT We have established schedules of expression during maize leaf development in light and darkness for the messenger RNAs (mRNAs) and polypeptides for ribulose 1,5-bisphosphate carboxylase (RuBPCase) subunits, phosphoenolpyruvate carboxylase (PEPCase), and the light-harvesting chlorophyll *a*/*b*–binding protein (LHCP). Levels of mRNAs were measured by hybridization with cloned probes, and proteins were measured by immunodetection on protein gel blots. The initial synthesis in leaves of all four mRNAs follows a light-independent schedule; illumination influences only the level to which each mRNA accumulates. The synthesis of RuBPCase small and large subunits and of PEPCase polypeptides also follows a light-independent schedule which is modified quantitatively by light. However, the accumulation of LHCP polypeptides absolutely requires illumination. The accumulation of each protein closely follows the accumulation of its mRNA during growth in light. Higher ratios of PEPCase and RuBPCase protein to mRNA occur during dark growth.

Light is a regulator of many plant developmental processes. Dark-grown higher plants are incapable of photosynthesis, since they lack chlorophyll and many of the polypeptides associated with light-harvesting and light-driven electron transport. Several plant species also lack ribulose 1,5-bisphosphate carboxylase (RuBPCase, E.C.4.1.1.39),[1] a primary enzyme in carbon fixation, when grown without light (1–3). Illumination of dark-grown plants leads to the stepwise reorganization of plastid membranes, to the activation of many pre-existing enzymes, and to the appearance of chlorophyll and of previously lacking polypeptides and photosynthetic activities (1, 4–6).

The expression of both the nuclear and chloroplast genomes is required to complete light-regulated leaf development. Many thylakoid membrane polypeptides, including those associated with the two photosystem reaction centers, are plastid-encoded (3, 7–9). In maize, the light-harvesting chlorophyll *a*/*b*–binding protein (LHCP) associated with photosystem II, and phosphoenolpyruvate carboxylase (PEPCase, E.C.4.1.1.31), which catalyzes a first step in carbon fixation in C-4 type plants, are nucleus-encoded. RuBPCase, which catalyzes a later step in C-4 type carbon fixation, consists of plastid-encoded large subunits (LSu) and nucleus-encoded small subunits (SSu; for review, see reference 3). Both SSu and LHCP are translated in the cytoplasm as precursors, and transported into the chloroplast before assembly into their respective structures. In the leaves of mature C-4 type plants, RuBPCase is found exclusively in the chloroplasts of bundle sheath cells which surround leaf vascular tissue, while PEPCase is found in the cytoplasm of mesophyll cells which surround stomata (10–13).

Several groups have recently shown that the increases in RuBPCase (14–16), LHCP (17–19), and PEPCase (20, 21) that are observed during normal development and that follow illumination of dark-grown plants are due in part to increases in levels of the corresponding messenger RNAs (mRNAs). The fact that red illumination can cause similar increases in mRNA level for LHCP (22, 23) and RuBPCase SSu (23) suggests that the red-sensitive photoreceptor phytochrome is a mediator of light effects on transcription or mRNA turnover. LHCP accumulates neither under intermittent white illumination, which permits chlorophyll *a* but not chlorophyll *b* accumulation, nor in the chlorophyll *b*–less barley chlorina f2 mutant (18, 19, 22, 24). In both cases, LHCP mRNA accumulates. This suggests that additional mechanisms mon-

[1] *Abbreviations used in this paper:* cDNA, complementary DNA; LHCP, light-harvesting chlorophyll *a*/*b*–binding protein; LSu, large subunit; mRNA, messenger RNA; PEPCase, phosphoenolpyruvate carboxylase; RuBPCase, ribulose 1,5-bisphosphate carboxylase; SSu, small subunit.

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itor pigment accumulation and act upon LHCP accumulation at the levels of translation or protein turnover (25–27). The nature of light effects on the synthesis of PEPCase mRNA and protein has not been characterized, although light does affect maize PEPCase mRNA levels (21).

In maize, PEPCase, RuBPCase (LSu and SSu), and LHCP polypeptides increase during development in light from low or undetectable levels to become the most abundant leaf proteins (28, 29). Since the three resulting activities must act in concert for efficient leaf function, we examined in detail the schedule of expression of mRNA and protein for each, as well as the relative influence of light on the expression of each. We have quantitatively compared changes in the four polypeptides by a sensitive immunodetection method, starting with time points prior to leaf emergence, and we have compared changes in levels of the four mRNAs using hybridization with cloned complementary DNA (cDNA) and chloroplast DNA probes. The use of hybridization probes to directly measure RNA levels avoids the need for interpretation of mRNA translational activities in exogenous in vitro systems. We show that protein levels directly reflect mRNA levels for LSu, PEPCase, and LHCP polypeptides during light growth, but that SSu mRNA is present at high levels 2–3 d before SSu protein appears. This early SSu mRNA is translatable in vitro. During dark growth, RuBPCase and PEPCase accumulate to nearly normal levels, while their corresponding mRNA levels are disproportionately low. LHCP mRNA is transcribed on schedule in total darkness, although at levels several 100-fold lower than in light growth. LHCP polypeptide is only detectable after illumination. Thus, the schedule of expression and accumulation of RuBPCase, PEPCase, and LHCP mRNAs during maize leaf development is determined by a light-independent developmental program; light only increases the final amounts that accumulate. The schedule of protein accumulation is light-independent for RuBPCase and PEPCase, while LHCP accumulation is strictly light-dependent.

MATERIALS AND METHODS

Growth Conditions: A highly inbred line of *Zea mays* (Pioneer B73) was used for all studies. Seeds were planted in dry sterile soil, covered with 2 cm of vermiculite, and germinated and grown in a 25°C growth chamber either in total darkness or under 4×10^4 lux of fluorescent light. Dark-grown plants were maintained and harvested without safelights to avoid low fluence light effects (30).

Preparation of Cloned cDNA Copies of PEPCase, RuBPCase (SSu), and LHCP mRNAs: The second leaves after the coleoptile were harvested from plants grown for 7 d in total darkness plus 24–48 h under constant illumination (4×10^4 lux). Total RNA was prepared according to Schmidt et al. (31), except initial homogenization was achieved by grinding frozen tissue into a powder in dry ice and thawing to room temperature in the presence of 1 ml/g tissue of 4 M guanidinium thiocyanate (32). Polyadenylated RNA was prepared by fractionation of total RNA on columns of oligo-dT cellulose (33), followed by ethanol precipitation. Double-stranded cDNA copies of total leaf mRNA were prepared according to Efstradiatis and Villa-Komaroff (34) and inserted at the Pst I site of vector pBR322 (35). Approximately 1,000 amp^s, tet^r colonies were picked to arrays, and nitrocellulose colony replicas were prepared (36). Colonies whose replicas hybridized under standard hybridization conditions (37) to RNA enriched by sucrose gradient sedimentation for LHCP and PEPCase mRNA were selected for further study. LHCP and PEPCase cDNA clones were identified from this group by in vitro translation in rabbit reticulocyte lysates (Amersham Corp., Arlington Heights, IL) of leaf mRNA hybrid-selected by cDNA-containing plasmids that were covalently fixed to Sephacryl S-1000 (Pharmacia Inc., Piscataway, NJ) (38). Individual in vitro translation products of hybrid-selected mRNAs were confirmed to be LHCP precursor or PEPCase subunits by immunoprecipitation with the appropriate antisera (39). Maize RuBPCase SSu clones were identified by hybridization of replicas to a previously identified wheat RuBPCase SSu clone which was the gift of Dr. Steven Smith (Commonwealth Scientific and Industrial Research Organisation, Canberra, Australia). Maize RuBPCase SSu cDNA clones were confirmed by translation of hybrid-selected RNA and immunoprecipitation as described above. The RuBPCase SSu, PEPCase, and LHCP clones used in these studies contained cDNA insertions of 960, 500, and 1100 base pairs (bp), respectively, and will be described elsewhere. An Eco RI fragment containing only the LSu gene was subcloned into pBR322 from the maize chloroplast fragment (Bam 9) cloned in pZmc37, which was the gift of John Bedbrook (Advanced Genetic Sciences, Berkeley, CA) (40). For use as probes in studies described here, the hybrid plasmids were labeled with ^{32}P-deoxynucleoside triphosphates (Amersham Corp.) by nick-translation with *E. coli* DNA polymerase holoenzyme (41) to equivalent specific activities (4.0×10^7 cpm/μg) and desalted by gel filtration on Sephadex G-75.

Measurement of Leaf mRNAs: Steady-state levels of RuBPCase SSu, PEPCase, and LHCP mRNAs were measured by hybridization of the cloned cDNA probes described above to nitrocellulose blots of electrophoretically separated RNAs. Polyadenylated RNA from leaves grown under various conditions was prepared as described above and separated by size on 1.5% agarose gels in the presence of formaldehyde (42, 43). Samples contained 2 μg of polyadenylated RNA in 50% formamide, 0.5% formaldehyde, and were heated to 60°C for 2 min before electrophoresis at room temperature. The separated RNAs were blotted to nitrocellulose (44) and subjected to hybridization with 10^7 cpm of nick-translated RuBPCase SSu, PEPCase, or LHCP cDNA-containing plasmids in 5 × SSPE, 50% deionized formamide, 0.002% Ficoll, 0.002% bovine serum albumin, 0.002% polyvinylpyrrolidone, 0.2% SDS, for 36 h at 42°C. SSPE is 0.18 M NaCl, 10 mM sodium phosphate buffer, pH 7.0, 1 mM EDTA. Filters were washed three times at 42°C in 2 × SSPE, 0.2% SDS for 20 min each, blotted dry, and exposed to film. In some cases, RNA blots were hybridized with a second probe after eluting the previous probe by treatment in 0.05% sodium pyrophosphate, 0.4% Ficoll, 0.04% bovine serum albumin, 0.04% polyvinylpyrrolidone, at 60°C for 2 h and then washing in 2 × SSPE, 0.2% SDS. Amounts of individual mRNAs were estimated by comparing densitometrically the autoradiograph of the sample with serial dilutions of 17S maize rRNA electrophoresed and transferred to the same blot, but hybridized with clone prR1 labeled by nick-translation to the same specific activity. Only those exposures within the linear range of the film and of the standards were used for quantitation. Genomic clone prR1 was the gift of Lino Fragoso, (University of California, Berkeley) and contains a single copy of 17S rDNA from *Phaseolus vulgaris*. RuBPCase LSu mRNA was estimated by hybridization of similar blots of total leaf RNA with the cloned fragment of maize chloroplast DNA described above, labeled by nick-translation to the same specific activity.

Preparation of Antisera: RuBPCase holoenzyme was prepared according to Goldthwaite and Bogorad (45), LHCP was prepared by elution from polyacrylamide gels according to Chua and Blomberg (46), and PEPCase holoenzyme was prepared according to Hague and Sims (20). RuBPCase preparations contained polypeptides of 12 kd (SSu) and 55 kd (LSu), LHCP preparations contained a doublet of polypeptides of ~28 kd, and PEPCase preparations contained a single 100-kd polypeptide as determined by SDS PAGE. For rabbit antisera to each protein, 1 mg of purified protein in complete Freund's adjuvant was injected subcutaneously into 2-kg New Zealand white rabbits. After 4 wk, rabbits were injected with an additional 100 μg of protein, and 1 wk later serum was collected and stored at −70°C. Antisera were characterized and titered by preparing serial dilutions and reacting them with known amounts of purified protein fixed to cyanogen bromide paper as described below. Without further purification, serum titers were sufficient to linearly measure proteins down to 1% of their maximal levels in leaf extracts. In the procedure outlined below, this corresponds to linear measurement of 0.1–10 μg of RuBPCase and 0.02–2.0 μg of PEPCase in 20 μg total soluble leaf protein, and of 0.1–10 μg of LHCP in 20 μg total membrane fraction leaf protein.

Measurement of Leaf Proteins: Total leaf protein was prepared from the second leaves (after the coleoptile) of dark- or light-grown seedlings or from plumules if prior to leaf emergence. Leaves were homogenized with a Brinkmann Polytron (Brinkmann Instruments, Inc., Westbury, NY) in 3 ml/gm tissue of 18% sucrose, 10 mM $MgCl_2$, 100 mM Tris, pH 8.0, 40 mM 2-mercaptoethanol. The homogenate was filtered through two layers of cheesecloth and cleared by centrifugation at 10,000 *g* for 15 min. A membrane protein fraction was prepared by extraction of the pellet with 2% SDS, 6% sucrose, 40 mM 2-mercaptoethanol. The supernatant was centrifuged at 15,600 *g* for 15 min to remove remaining membrane fraction and brought to 2% SDS, 6% sucrose, before electrophoresis. Total protein concentrations were determined by the method of Lowry et al. (47). Equal amounts (40–50 μg per sample lane) of total membrane fraction or soluble fraction proteins were separated by electrophoresis on 15-cm long 7.5–15% gradient polyacrylamide SDS slab gels according to Chua (48). Separated polypeptides were electrophoretically trans-

ferred in 50 mM sodium acetate, pH 7, to cyanogen bromide paper (49), which was quenched and washed as described by Nyari et al. (50). Blots were treated with 5–20 μl of antiserum in 100 ml of wash solution (10 mM sodium phosphate buffer, pH 7.4, 0.127 M NaCl, 0.1% bovine serum albumin, 0.1% Nonidet P-40, 0.02% sodium azide) for 4 h at room temperature with rotation in heat-seal bags, followed by two 20-min washes under the same conditions but without antiserum. Washed protein blots were then treated with 0.4 μCi ^{125}I-Staphylococcal protein A (labeled with the New England Nuclear Radioiodination Kit [New England Nuclear, Boston, MA] to 90 mCi/mg) in 100 ml of wash solution for 3 h at room temperature, washed for an additional 30 min, blotted dry, and exposed to film. Amounts of individual proteins on blots were estimated by comparing densitometrically the autoradiograms of samples with those of known amounts of purified proteins on the same blots. Exposures were chosen that remained within the linear range of the film.

RESULTS

RuBPCase, PEPCase, and LHCP Polypeptides and mRNAs Accumulate Rapidly at the Time of Maize Leaf Emergence during Growth in Light

To establish a schedule of protein accumulation for maize RuBPCase subunits, PEPCase, and LHCP, we sampled second leaves from plants grown from germination under continuous illumination at 25°C for various times. Under these growth conditions, leaves emerge from the coleoptile at 4–5 d after germination. Total soluble or membrane fraction proteins were isolated, separated electrophoretically, and each of the four polypeptides was measured immunologically as described in Materials and Methods. Individual antibody probes were used to quantitate each polypeptide relative to standards on protein blots of SDS polyacrylamide gels. Fig. 1 shows that large changes in the levels of all four polypeptides occur between 3 d (before leaf emergence) and 7 d (after emergence). RuBPCase antiserum decorates bands in blots of 7-d total leaf protein of 55 kd (LSu) and 12–14 kd (SSu, Fig 1 *b*), PEPCase antiserum decorates a 100-kd band (Fig. 1 *d*), and LHCP antiserum decorates a doublet of bands of 27–28 kd which will be treated as a single species here (Fig. 1 *f*). The kinetics of accumulation in light of each polypeptide are shown in Fig. 2, *A–D* (open circles). All four polypeptides begin accumulation at 4–6 d of growth, concurrent with leaf emergence. Accumulation of each continues to 7–9 d after germination, at which time SSu, LSu, and PEPCase represent 6, 19, and 10%, respectively, of total soluble leaf protein by weight. At the same time, LHCP represents 15% of total membrane fraction leaf protein.

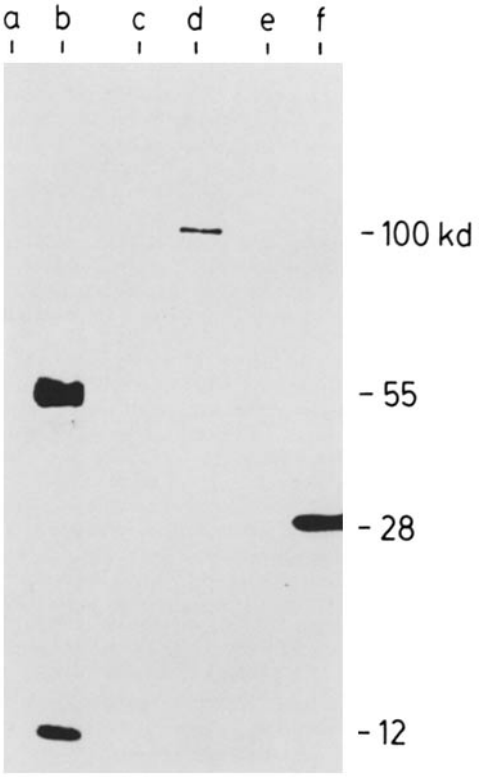

FIGURE 1 Immunological detection of RuBPCase, PEPCase, and LHCP during leaf development. Total soluble protein (lanes *a–d*) and total membrane protein (lanes e and *f*) fractions were prepared from light-grown maize second leaves at 3 d after germination (*a*, *c*, and e) or at 7 d after germination (*b*, *d*, and *f*). Equal amounts were separated on SDS polyacrylamide slab gels and blotted to cyanogen bromide paper as described in Materials and Methods. Blots were decorated with RuBPCase (*a* and *b*), PEPCase (c and *d*), or LHCP (e and *f*) antisera, treated with ^{125}I-Staphylococcal protein A, and subjected to autoradiography as described.

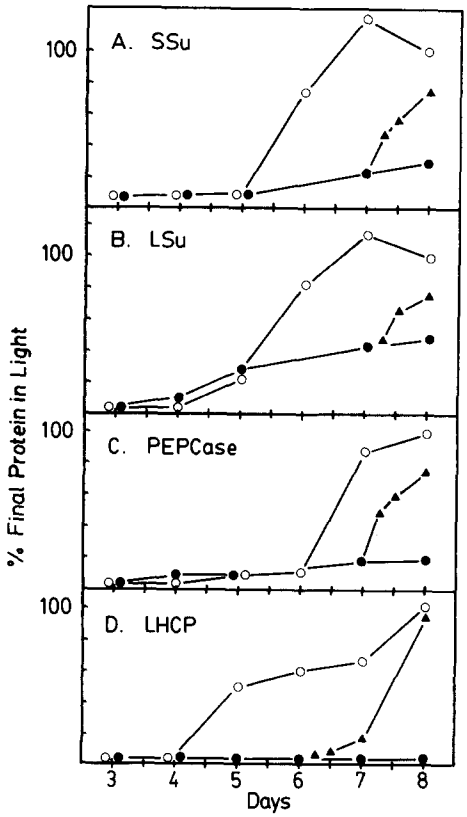

FIGURE 2 Accumulation of maize leaf polypeptides during light and dark growth. Total proteins were prepared from leaves grown for the indicated times after germination in constant light (○) or constant darkness (●). At 6 or 7 d, half of the dark grown seedlings were illuminated and protein fractions were prepared (▲). Amounts of RuBPCase SSU (*A*), LSu (*B*), PEPCase (*C*), and LHCP (*D*) were measured as described in Materials and Methods and are expressed relative to the levels found in leaves grown 8 d in constant light.

To establish a similar schedule of expression in light growth for the mRNAs encoding RuBPCase LSu and SSu, PEPCase, and LHCP, we isolated total polyadenylated RNA or total RNA from leaves grown under the same conditions described above and measured individual mRNAs by hybridization with cloned probes. Cloned cDNA probes were constructed as described in Materials and Methods and used to quantitate RuBPCase, PEPCase, and LHCP mRNAs in total polyadenylated RNA. A cloned maize chloroplast fragment containing

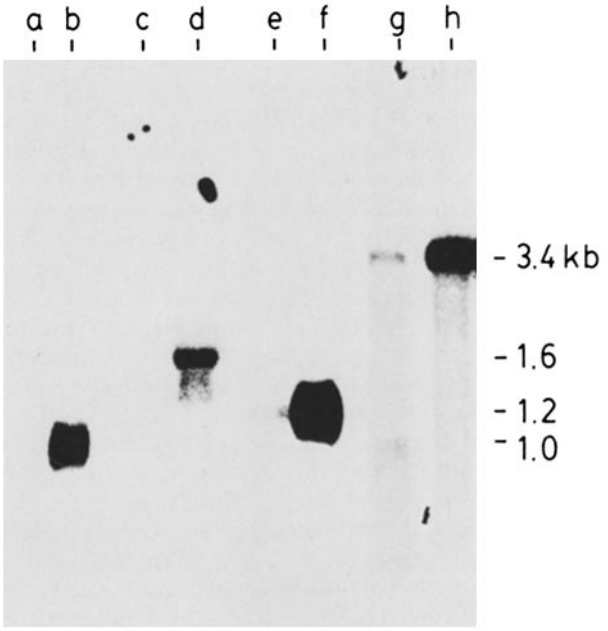

FIGURE 3 Hybridization detection of mRNA for SSu, LSu, LHCP, and PEPCase during maize leaf development. Polyadenylated RNA was prepared according to Materials and Methods from second leaves of plants grown 5 d (*a*, *c*, *e*, and *g*) or 9 d (*b*, *d*, *f*, and *h*) under constant illumination. Equal amounts of RNA were separated by size and hybridized with ^{32}P-labeled cDNA clones for SSu (*a* and *b*), LHCP (*e* and *f*), or PEPCase (*g* and *h*), or with a labeled cloned chloroplast DNA fragment with the LSu gene (*c* and *d*), all as described in Materials and Methods. (*kb*, kilobase pairs.)

the LSu gene was used to quantitate RuBPCase mRNA in total leaf RNA. Fig. 3, *b*, *f*, and *h* shows that hybridization of nitrocellulose blots of polyadenylated leaf RNA from 7-d leaves with RuBPCase SSu, LHCP, and PEPCase cDNA clones detects RNA species of ~1.0, 1.2, and 3.4 kilobase pairs (kb), respectively. The width of the detected RNA bands is probably due to variation in the length of polyadenylated tails on each species. The same RNA species are absent or decreased in leaves grown without illumination (Fig. 3, *a*, *e*, and *g*). Fig. 3, *c* and *d* show that the 1.7-kb LSu mRNA detected in total RNA by a cloned chloroplast DNA probe follows the same pattern of accumulation. The kinetics of accumulation in light of all four RNA species are shown in Fig. 4, *a–d* (open circles). As is the case with the corresponding polypeptides (Fig. 2, *a–d*), all four mRNAs increase beginning at or just prior to leaf emergence until at least 9 d after germination at which time SSu, PEPCase, and LHCP mRNAs represent ~1.0, 0.05, and 0.5% of total polyadenylated RNA in the leaf. SSu mRNA is remarkable in that it accumulates to maximal levels 2–3 d before LSu mRNA and protein and before SSu protein. LSu mRNA is present at ~90% the level of SSu mRNA on a molar basis at 9 d.

RuBPCase SSu and LSu Polypeptides, but Not their mRNAs, Accumulate during Dark Growth of Maize

To determine the effect of light upon the schedules of RuBPCase protein and mRNA levels, we grew plants under conditions identical to those described above, except in total darkness (including sample collection). After 7 d, half of the plants were continuously illuminated with white light and the remaining half maintained in darkness for an additional 2 d (Figs. 2, *a* and *b* and 4, *a* and *b*, solid circles and triangles). Leaf emergence began also at 4–5 d in dark-grown plants. RuBPCase subunits and their mRNAs were measured at the indicated times. Fig. 2, *a* and *b* show that SSu and LSu polypeptides begin accumulating at the same time in darkness as they do in light, although to lower levels. At 8 d after germination, dark-grown leaves contain levels of SSu and LSu 31 and 45% of that found after 8-d growth in light. Illumination of dark-grown plants at 7 d causes approximately twofold and 1.5-fold increases in RuBPCase SSu and LSu accumulation. In contrast, Fig. 4, *a–b* shows that levels of SSu and LSu mRNAs are considerably lower in dark-grown plants than in light-grown controls (0.5 and 13% at 9 d) and increase rapidly with illumination to nearly normal levels. The disproportionately high levels of LSu and SSu protein relative to mRNA levels in dark-grown plants is striking and is currently under investigation.

PEPCase Polypeptide, but Not mRNA, Accumulates during Dark Growth of Maize

PEPCase protein and mRNA were monitored under conditions identical to those in the preceding section. Fig. 2*c* shows that the kinetics of accumulation of PEPCase protein are indistinguishable in light and dark growth for the first 6 d after germination. Illumination increases the level of PEPCase

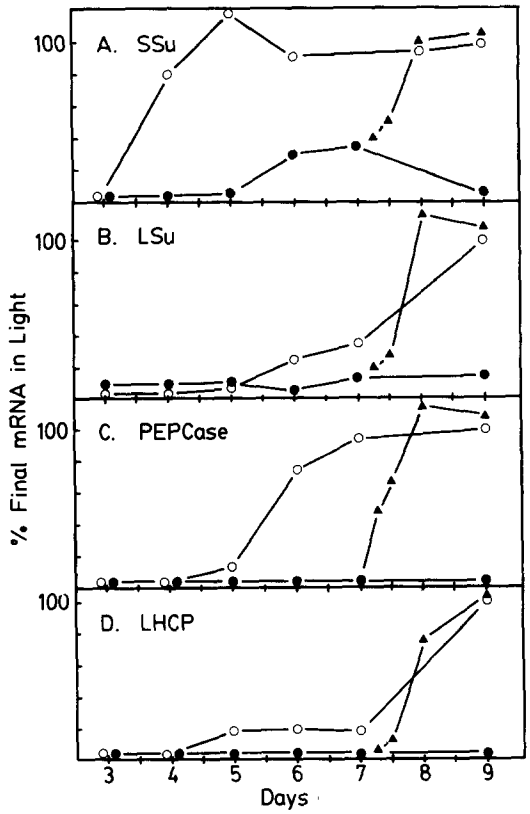

FIGURE 4 Accumulation of maize leaf mRNA during light growth and dark growth. Polyadenylated RNA was prepared from leaves grown after germination for the indicated times in constant light (○) or constant darkness (●). At 7 d, half of the dark-grown plants were illuminated and RNA was prepared (▲). Amounts of SSu, LSu, PEPCase, and LHCP mRNA were measured as described in Materials and Methods and are expressed relative to the levels found in leaves grown 9 d in constant light.

after 6 d, and by 8 d the level is fivefold above the level in dark-grown plants. Fig. 4*c* shows that substantial amounts of PEPCase mRNA are detectable only after illumination of 7-d dark-grown plants. PEPCase mRNA does not accumulate in darkness even after 9 d of growth. Since the low PEPCase mRNA level present in dark-grown plants appears capable of directing the accumulation of a disproportionately high level of PEPCase protein, we are currently investigating PEPCase turnover under light and dark conditions.

LHCP mRNA and Protein Do Not Accumulate during Dark Growth of Maize

In a series of experiments analogous to those described above, the kinetics of accumulation of LHCP mRNA and protein were measured during dark growth. Fig. 2*d* shows that LHCP protein is undetectable after 9-d dark growth, but is induced within 24 h of illumination of 7-d seedlings. Similarly, Fig. 4*d* shows that maize LHCP mRNA is present before illumination at <1% of its final level, whether monitored by hybridization as shown or by in vitro translation and immunoprecipitation (data not shown). The accumulation of LHCP mRNA after illumination precedes protein accumulation by ~6 h.

DISCUSSION

In this study, we have directly measured the appearance during development of the mRNAs and polypeptides that contribute to three of the major proteins in maize leaves—RuBPCase, PEPCase, and LHCP. We show that during growth in light, RuBPCase SSu and LSu, PEPCase, and LHCP polypeptides and mRNAs accumulate rapidly beginning at the time of leaf emergence from the coleoptile. We show that the accumulation of mRNAs for the two carboxylases is more sensitive to illumination than is protein accumulation. During growth in darkness for 8–9 d, leaves accumulate up to 50% of normal levels of RuBPCase and 20% normal levels of PEPCase. In contrast, at 9 d the same leaves contain only 13% of the normal level of LSu mRNA, while PEPCase and SSu mRNAs are present at <0.5% of normal levels. LHCP protein is undetectable during dark growth, although LHCP mRNA is detectable at very low levels. Illumination of dark-grown leaves leads to rapid induction of all four mRNAs and polypeptides to near normal levels within 24–48 h.

Several other studies have followed the appearances in higher plants of LSu and SSu polypeptides or of RuBPCase activity during light or dark development of leaves or cotyledons or during greening of dark-grown leaves (16, 51–55). RuBPCase is extremely low or undetectable during dark growth of certain plant species, such as pea, while others species accumulate nearly normal levels. In cucumber cotyledons (55), for example, it has been demonstrated that RuBPCase subunits accumulate during growth in darkness to levels 100% of normal at 3 d and 30% at 6 d after germination. It is unclear to what extent cotyledon growth relates to the development of leaves. However, the protein measurements of our studies in maize leaves follow this pattern of punctual RuBPCase expression in darkness. Further, our measurements did not distinguish any significant difference in the kinetics of accumulation of LSu and SSu polypeptides during normal development or during greening, in agreement with studies of normally developing wheat leaves by Dean and Leech (52).

It is of particular interest to compare the schedules of accumulation of RuBPCase and PEPCase in maize and other C-4 type plants, since the participation of both enzymes is a distinguishing feature of C-4 type carbon fixation (56, 57). The relative appearances of the two enzymes may influence whether the leaf follows a C-3 or C-4 type scheme of carbon fixation at any given stage of development and may thus be a factor limiting overall efficiency of photosynthesis. It has been proposed that pea seed development may include early C-4 type photosynthetic stages in addition to later C-3 type stages (58). Kobayashi et al. (54) showed that carbon fixation and PEPCase activity in greening maize leaves increase to maximal levels by ~24 h after illumination of 5-d dark-grown seedlings, while RuBPCase is maximal by 12 h. Similarly, we show maximal levels of RuBPCase polypeptides accumulate ~24 h before PEPCase during development in continuous light. We observe accumulation of RuBPCase and PEPCase protein in parallel during greening of 7-d dark-grown leaves which already contain 30 and 20% of normal levels of RuBPCase and PEPCase, in basic agreement with activity measurements of Kobayashi et al. (54). Any differences between the activity measurements of Kobayashi et al. (54) and our protein measurements for PEPCase and RuBPCase accumulation in light and dark tissue may be due to the influence of light on the activities of the two carboxylases, independent of its influence on their syntheses (6).

The appearance of RuBPCase significantly before PEPCase during normal development suggests that leaf cells at earlier stages of development may utilize C-3 type carbon fixation. This is not in agreement with studies of RuBPCase and PEPCase in maize leaves of various ages (29) which showed that the two activities are similarly low in young tissue, although it is possible that the three tissue ages chosen in that study did not include the period of imbalance found in our study and observed in greening tissue by others (54).

The accumulation of mRNAs for RuBPCase LSu and for PEPCase precedes the accumulation of the corresponding polypeptides by 12–24 h during normal development. This is supported by similar studies of protein and mRNAs from successive maize leaf sections containing cells of increasing age (B. Martineau and W. Taylor, manuscript in preparation). During dark growth, however, accumulation of PEPCase mRNA is depressed below the limits of detection in this study (<0.5% normal levels), even after 9-d growth in darkness. Previous reports of the induction by light of maize PEPCase mRNA, measured by in vitro translation (21), showed that the mRNA undergoes at least three- to fourfold increase after illumination of dark tissue. We measure at least 200-fold increases after a similar shift. This is remarkable in light of the considerable level of PEPCase protein (20% of normal level) present in dark-grown tissue of the same age. Patterns of accumulation of LSu mRNA vary among plants. Large amounts accumulate in dark-grown cucumber coytledons measured by in vitro translation (55), but LSu mRNA is present only after illumination of pea seedlings (15). We show that maize LSu mRNA is present at 13% of normal levels after 8 d unless leaves are illuminated, while nearly 50% of normal LSu protein levels accumulate in the dark. The low levels of LSu and PEPCase mRNAs present in dark-grown leaves must be efficiently used to generate the levels of protein observed. It is unlikely that the appearance of both polypeptides is the consequence of conversion of preexisting protein from forms not recognized by our probes to those that are

recognized. Alternatively, the carboxylase levels observed in the light may be subjected to more rapid turnover than those in the dark. Work is currently in progress to distinguish the possibilities.

The accumulation of the mRNA for RuBPCase SSu precedes the appearance of SSu protein and of LSu mRNA and protein by nearly 3 d during growth in light. The reason for the early accumulation of the SSu mRNA is unclear, as is the form in which the mRNA is stored. This is unlikely to be an artifact of the measurement method, such as a disproportionate sensitivity of SSu mRNA, since at 7 d of growth, LSu mRNA is easily measured to be equimolar to SSu mRNA. It is also unlikely that an untranslatable transcript is made from one of the 5–10 maize SSu genes (T. Nelson, unpublished observation) early in development, since the early SSu mRNA is also detected by in vitro translation in rabbit reticulocyte lysates (data not shown). It has previously been shown that in 7-d wheat leaves, SSu mRNA and RuBPCase protein appear simultaneously in leaf cells of the same age, but this study did not provide quantitation of mRNA levels (59). Similarly, in pea leaves, the accumulation of RuBPCase protein appears to be directly proportional to the SSu mRNA which can be measured by in vitro translation (60). Reports on SSu mRNA light dependence in other plants range from the presence of half-normal levels during dark growth of cucumber cotyledons (55) to absolute dependence on illumination as in *Lemna* (16) and pea (15). In general, SSu mRNA is strongly light dependent in the leaves of C-3 type plants. In our study of maize, SSu mRNA accumulates to ~30% of normal levels after 7-d growth in darkness and is induced to normal levels by light with kinetics similar to those for the LSu mRNA.

The pattern of accumulation of maize LHCP protein and mRNA is similar to those in other higher plants. This study shows that maize LHCP protein is absolutely dependent on light, while LHCP mRNA is only quantitatively dependent on light. Low levels (<0.5% of final levels, corresponding to an average of 5–10 molecules per cell) of LHCP mRNA can be detected in dark-grown tissue, but levels increase at least 200-fold with illumination. Both mRNA and protein are ordinarily expressed at or just before the time of leaf emergence from the coleoptile. In barley (22) and *Lemna* (23), LHCP mRNA accumulation is known to depend on phytochrome (red light effects) while translation or stabilization of LHCP protein depends on chlorophyll biosynthesis (white light effects) (26, 27). It is likely that similar effects govern maize LHCP, although we have recently observed that some carotenoid-deficient mutants of maize have extremely low levels of LHCP mRNA as well as the expected low levels of LHCP protein, in spite of having phytochrome responses that are otherwise normal (S. Mayfield and W. Taylor, manuscript in preparation).

Expression of LSu and SSu and RuBPCase, PEPCase, and LHCP during early maize leaf development thus consists of a light-independent schedule for initial expression of all four mRNAs and of SSu, LSu, and PEPCase polypeptides, upon which are superimposed several light effects, including quantitative increases in all three carboxylase polypeptides and the appearance of LHCP polypeptides. In this pattern of leaf development, maize is distinct from most plants studied thus far, in which the expression of both RuBPCase and LHCP is light dependent. It should be noted that all experiments with "dark-grown" plants presented in this study used material grown and harvested in total darkness. Many previous studies of "dark" growth were actually of plants grown and/or harvested under "safelights" which can no longer be considered "safe" without appropriate controls (e.g., see reference 30). In addition, it should be noted that we have made no effort to look for possible differences in patterns of expression in light and dark among different varieties of maize, and have chosen a widely available inbred line (Pioneer B73) for the standard in all of our studies. The study described above necessarily averages expression of mRNAs and proteins over regions of second leaves which vary from little or no expression (at the undifferentiated leaf base) to full expression (at the fully differentiated tip). We are currently examining the expression of carboxylase and LHCP mRNAs and polypeptides in successive maize leaf sections, which contain cells synchronized in developmental age at progressively increasing stages of differentiation (Mayfield and Taylor, submitted for publication).

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