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Differential expression of six light-harvesting chlorophyll a/b binding protein genes in maize leaf cell types

(cDNA cloning/multigene family/light induction/bundle sheath cells/mesophyll cells)

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ABSTRACT **Bundle sheath chloroplasts of maize leaves contain about one-fourth as much light-harvesting chlorophyll a/b binding protein of photosystem II (LHCP-II) as do mesophyll chloroplasts. We have determined that this difference is, in part, the result of differential expression of different LHCP-II genes. We have prepared and partially characterized cDNA clones specific for six LHCP-II genes of maize. Transcripts of these six LHCP-II genes are present at vastly different levels and account for about 95% of total LHCP-II mRNAs in bundle sheath and mesophyll cells of illuminated dark-grown maize leaves. Three genes are preferentially expressed in mesophyll cells, and their mRNAs constitute about 54% of the total LHCP-II transcripts in greening (24 hr) maize leaves. Two genes are expressed equally in bundle sheath and mesophyll cells. Most interestingly, the RNA of one gene that contributes about 8% of the total LHCP-II transcripts in leaves greening for 24 hr is present at a much higher level in bundle sheath than in mesophyll cells. Moreover, immunoblot analysis of maize thylakoids reveals at least five sizes of LHCP-II; these also differ from one another in their relative abundance in bundle sheath and mesophyll cells of developing maize leaves.**

The light-harvesting chlorophyll a/b binding proteins (LHCPs) are the major components of the antennae complexes that capture and transfer light energy to the reaction centers of photosystem I (PSI) and photosystem II (PSII). The LHCPs of PSII (LHCP-II) make up >50% of the chlorophyll-containing polypeptides of thylakoid membranes isolated from chloroplasts (1). They have been shown to be encoded by multigene families in pea, petunia, barley, duckweed, wheat, and tomato (2–6). Furthermore, light has been demonstrated to regulate LHCP-II gene expression in plant leaves (7–14). Moreover, in maize, the accumulation of LHCP-II mRNA is coordinated with carotenoid synthesis and plastid development (15, 16).

Maize is a C_4 plant; its leaves contain two distinct photosynthetic cell types, bundle sheath cells and mesophyll cells (BSC and MC). Agranal chloroplasts in BSC are poor in PSII in relation to their activity in PSI (17, 18) and contain less LHCP-II and other PSII polypeptides (19–22) than are present in the granal chloroplasts of MC. In addition, transcripts of the LHCP-II gene have been reported to be rare or absent in BSC (21, 22). However, relatively little is known concerning the number of different LHCP-II polypeptides and genes in maize or whether differences in the abundance of LHCP-II in MC and BSC is the result of quantitatively different expression of the same gene or genes or whether different LHCP genes are expressed in the two cell types.

We have identified six members of the LHCP-II gene family that are actively expressed in maize leaves. To investigate the cell-type-specific expression of each of these six LHCP-II genes in developing maize leaves, shortened cDNA clones that show no cross-hybridization to each other have been used to probe genomic DNA and RNA blots. Immunoblot analyses with antibodies to LHCP-II were performed also to try to elucidate the relationship between mRNA and protein-accumulation patterns of LHCP-II genes.

MATERIALS AND METHODS

Plant Material and Growth. Maize seedlings (*Zea mays*: $FR9^{cms} \times FR37$; Illinois Foundation Seeds, Champaign, IL) were grown in the greenhouse or in a darkroom before greening as described (23).

Antibody to Maize LHCP-II. The total thylakoid membranes of maize were isolated as described (24), dissolved in 50 mM Tris·HCl, pH 7.5/2% $NaDodSO_4$/5% 2-mercaptoethanol, and fractionated on a $NaDodSO_4$/polyacrylamide gel (10% acrylamide) at 4°C in the dark. The green band at the 26- to 29-kDa region was excised, and protein was eluted electrophoretically in dialysis bags at 100 V for 1 hr at 4°C in the dark with 50 mM Tris glycine buffer, pH 8.3/0.1% $NaDodSO_4$. The supernatant was concentrated in Centricon filters (Amicon). Two hundred micrograms of LHCP-II in 2 ml of phosphate-buffered saline (0.9% NaCl/20 mM sodium phosphate, pH 7.2) was mixed with an equal volume of Freund's complete adjuvant and injected into a rabbit. The serum was collected 5 weeks later and analyzed by immunoprecipitation and immunoblotting.

Immunoblot Analysis. Total maize thylakoid or PSII particles without oxygen-evolving polypeptides (PSII-OE) (J.-Y.S., R. T. Sayre, and L.B., in preparation) were dissolved in Laemmli sample-loading buffer (25) at a $NaDodSO_4$-to-chlorophyll ratio of 20:1 and boiled for 2 min before loading. The protein samples of BSC and MC were extracted by boiling with sample-loading buffer at a $NaDodSO_4$-to-chlorophyll ratio of 200:1 and at a dilute protein concentration (about 0.2 mg/ml). The BSC and MC preparations were made as described (23), except that the starting material for preparation of BSC was the residue of digested leaves after release of MC. $NaDodSO_4$/polyacrylamide gel (12.5% acrylamide) electrophoresis of proteins (25) was carried out with a lower ratio of acrylamide-to-methylenebisacrylamide (30:0.4) and at a constant current of 20 mA at room temperature. The method of Towbin *et al.* (26) was used to transfer proteins to nitrocellulose except that 0.1% $NaDodSO_4$ was included in the transfer buffer.

Bound antibodies were located either by using horseradish peroxidase-conjugated goat second antibody (Bio-Rad) or alkaline phosphatase-conjugated goat second antibody

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Abbreviations: LHCP-II, light-harvesting chlorophyll a/b binding protein of photosystem II; BSC, bundle sheath cells; MC, mesophyll cells; PSI and PSII, photosystems I and II; PSII-OE, photosystem II particles without the three extrinsic oxygen-evolving polypeptides; bp, base pair(s).

(Promega Biotec, Madison, WI) as described by the manufacturer.

Methods for cDNA Cloning, Hybrid Selection, and Immunoprecipitation. Poly(A)$^+$ mRNA of maize leaves enriched for LHCP-II mRNAs by sucrose gradient fractionation was used to synthesize cDNAs. The first- and second-strand cDNAs were synthesized as described (27), except that the two reactions were carried out in the same tube sequentially by simply diluting the 10 μl of the first reaction to 100 μl with a mixture for the second reaction. The double-stranded cDNAs were tailed with dGTP as described (27) and inserted into the *Sac* I site of the pUC13 plasmid tailed with dCTP. MC1061 competent *Escherichia coli* cells were used for transformation (28). Differential screening with LHCP-II mRNA enriched by sucrose gradient fractionation and MC-specific mRNA was done by the colony-hybridization method of Maas (29). Hybrid selection was done as described (30). Immunoprecipitation was with 10 μl of *in vitro* translation products (23) and 20 μl of LHCP-II antibody in 200 μl of phosphate-buffered saline as described elsewhere (31).

Construction of Specific Probes. LHCP-II cDNA clones containing inserts longer than 200 base pairs (bp) were subjected to BAL-31 deletion to generate short specific probes for each gene. One microgram of each clone was linearized by either *Bam*HI or *Eco*RI to use in making deletions from both orientations of the inserts. The BAL-31 deletion was carried out in 100-μl of reaction buffer as described (28) with 1 μg of linearized plasmid DNA and 1 unit of BAL-31 (Bethesda Research Laboratories) at 30°C for 5–15 min to delete 150–450 bp. Phage T4 DNA polymerase was used to blunt the ends, and nonphosphorylated *Bam*HI or *Eco*RI linkers were ligated to them (32) to regenerate the deleted sites.

Blot-Hybridization and Dot-Blot Analysis. Poly(A)$^+$ RNAs from greening (24 hr) leaves and total RNA from BSC and MC were prepared as described (23). The blot-hybridization analyses were also carried out as described elsewhere (23). For dot-blot analyses, 5 μg of each total RNA sample was denatured in 5 mM Tris·HCl, pH 7.5/2.2 M formaldehyde at 80°C for 3 min before spotting onto Nytran (Schleicher & Schuell). The hybridization and washing conditions for blot-hybridization analysis have been described (23). The specific probes used were excised insert DNAs (*Bam*HI and *Eco*RI) of six different short and shortened cDNA clones and were end-labeled (28) with [α-^{32}P]dATP and [α-^{32}P]dCTP to equal specific activity.

DNA Cross-Hybridization and Genomic Southern Blot Analysis. cDNA clone inserts were excised (by *Bam*HI and *Eco*RI) and fractionated electrophoretically on a 5% polyacrylamide gel before elution in dialysis bags by electrophoresis. Ten nanograms of each DNA insert sample was denatured in 10 μl of 2.2 M formaldehyde/1 mM EDTA at 80°C for 3 min before spotting onto Nytran. The prehybridization and hybridization were done in 4× SSPE (1× SSPE = 0.18 M NaCl/10 mM $NaH_2PO_4 \cdot H_2O$/1 mM Na_2EDTA, pH 7.4) containing 5× Denhardt's solution (0.002% Ficoll/0.002% bovine serum albumin/0.002% polyvinylpyrrolidone), 0.2% $NaDodSO_4$, and 100 μg of calf thymus DNA per ml for 2 hr and 16 hr, respectively, at 65°C. The washing was in 1× SSC (0.15 M NaCl/0.015 M Na citrate) containing 10 mM sodium pyrophosphate and 0.2% $NaDodSO_4$ for 1 hr at 68°C and then in 0.1× SSC at 68°C for 30 min.

For genomic Southern blot analyses, total maize DNA was extracted from leaves of 2-week-old seedlings (33). Maize genomic DNA (10 μg) was digested with *Bam*HI, *Bgl* II, *Eco*RI, and *Hin*dIII, respectively, for 16 hr at 37°C to obtain complete digestion. DNA samples were electrophoretically fractionated on a 0.8% agarose gel. To denature the DNA after electrophoresis, gels were treated with 0.25 M HCl for 30 min, 0.5 M NaOH for 45 min, and 1.5 M NaCl/1 M Tris·HCl, pH 7.5, for 30 min before blotting to Nytran with 10× SSC for 10 hr. The hybridization and washing conditions were as described for DNA cross-hybridization. Probes were insert DNAs nick-translated with [α-^{32}P]dATP and [α-^{32}P]dCTP (28).

RESULTS

Analysis of LHCP-II by Anti-LHCP-II Antibody. The LHCP-II of maize migrated as a broad diffuse green band to the 26- to 29-kDa region of the gel when thylakoid samples were not heated prior to $NaDodSO_4$/polyacrylamide gel electrophoresis at 4°C in the dark (data not shown). The same broad green band of LHCP-II was also seen by Metz *et al.* (24). However, when thylakoid samples were heated at 70°C for 4 min at a $LiDodSO_4$-to-chlorophyll ratio of 10:1 (24) or at 100°C for 3 min at a $NaDodSO_4$-to-chlorophyll ratio of 20:1, three major bands were resolved in the same region by Coomassie blue staining (Fig. 1, lane 1a). The same banding pattern of LHCP-II was also exhibited on electrophoresis of purified PSII particles after the removal of oxygen-evolving proteins (PSII-OE) (Fig. 1, lane 1b) prepared as described by Bricker *et al.* (34). We show in Fig. 1, lanes 2, that the LHCP-II antibody raised against the green band excised from the $NaDodSO_4$/polyacrylamide gel reacted mainly with these three major bands of LHCP-II in heated total thylakoid and PSII-OE samples.

Identification of LHCP-II Genes. More than 60 putative LHCP-II cDNA clones were obtained originally by differential screening with size-enriched LHCP-II and MC poly(A)$^+$ mRNAs. *In vitro* translation products of RNAs selected by hybridization from leaf poly(A)$^+$ mRNA by 12 of these candidate LHCP-II cDNA clones are shown in Fig. 2*a*. The sets of hybrid-selected mRNAs were translated into different numbers and sizes of polypeptides depending on the insert length. The long-insert clones, such as 8-17 (750 bp), M14 (750 bp), and 8-22 (730 bp), hybrid-selected RNAs that directed the synthesis of at least 3–4 polypeptides, whereas clones with short inserts, such as 8-21 (200 bp) and 8-27 (150 bp), hybrid-selected mRNAs encoding a single polypeptide of unique size. There are six different sizes of polypeptides (range ≈ 30–33 kDa) synthesized by RNAs selected by the clones. Immunoprecipitation results showed that all 12 of these clones indeed encoded LHCP-II (Fig. 2*b*).

To further establish the relationships among different clones encoding different sizes of LHCP-II polypeptides *in vitro*, cross-hybridization experiments with isolated inserts of 12 LHCP-II clones were carried out as shown in Fig. 3. At least six different genes were represented among these 12 LHCP-II clones examined. In Figs. 2 and 3, the clones are identified by numbers 1–12. Among these 1 [clone 8-11 (250 bp)] and 2 [clone 8-15 (420 bp)], 4 [clone 11-12 (550 bp)] and 5 [pmc6 (500 bp)], 6 [8-20 (270 bp)] and 8 [8-27 (150 bp)], and 11 [13-7 (250 bp)] and 10 [12-5 (400 bp)] were closely related to one another and different from the rest; clones designated 3 (8-22), 7 (8-21), 9 (8-17), and 12 (M14) hybridized strongly

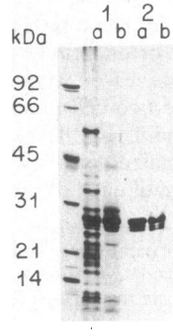

FIG. 1. Analysis of LHCP-II and its antibody in maize. Five microliters of total thylakoids of maize (chlorophyll, 2 mg/ml) (lane 1a) and 5 μl of maize PSII-OE (chlorophyll, 2 mg/ml) (lane 1b) were fractionated on a $NaDodSO_4$/polyacrylamide gel and stained with Coomassie blue. A duplicate set of samples was run in the same gel; proteins were blotted onto nitrocellulose and probed with LHCP-II antibody (lanes 2).

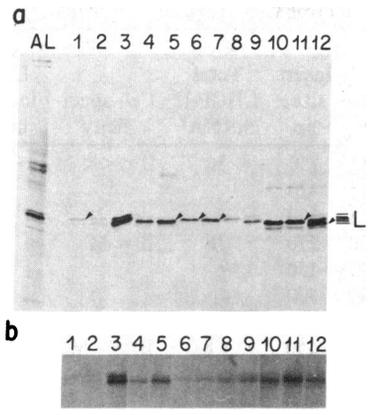

FIG. 2. Identification of LHCP-II cDNA clones. (*a*) *In vitro* translation products of RNAs hybrid-selected by 12 putative LHCP-II cDNA clones were analyzed in parallel. Arrows indicate the positions of the six different-sized LHCP-II precursors. *In vitro* translation products of maize poly(A)$^+$ mRNA (lane A) and immunoprecipitation products of LHCP-II antibody (lane L) are shown as standards. (*b*) All 12 cDNA clones encoded polypeptides that were immunoprecipitated by LHCP-II antibody. The 12 cDNA clones were 8-11 (lane 1), 8-15 (lane 2), 8-22 (lane 3), 11-12 (lane 4), pmc6 (lane 5), 8-20 (lane 6), 8-21 (lane 7), 8-27 (lane 8), 8-17 (lane 9), 12-5 (lane 10), 13-7 (lane 11), and M14 (lane 12). (Also, see Table 1.)

only to themselves. Therefore, clones 8-11, pmc6, 8-21, 8-27, 13-7, M14, 8-17, and 8-22 were chosen for further analysis.

Genomic Southern Blot Analysis of Different LHCP-II Genes. It has been shown that the coding regions of different LHCP-II genes of petunia are highly conserved, while the 3′ or 5′ nontranslated regions of the transcripts are highly divergent (3, 4, 6). To study the expression of different members of the maize LHCP-II gene family (or families), we constructed shortened cDNA clones by deletion with BAL-31 toward either the 3′ or 5′ end of the inserts and used these as specific probes. The specificity of each clone was confirmed by genomic Southern blot analysis. Probes prepared from clones such as M14 and 8-17, carrying long inserts (750 bp) that extended through most of the coding region, showed at most 12 bands when genomic DNA was digested with *Bgl* II (Fig. 4). Fewer hybridizing bands were detected when genomic DNA was digested with *Bam*HI, *Eco*RI, or *Hin*dIII. The short or shortened clones Δ8-11 (200 bp), Δpmc6 (250 bp), 8-21, 8-27, Δ13-7 (each 180 bp), and ΔM14 (230 bp) appear to carry only a divergent region, as each clone hybridized to a single band that corresponded to one of the genomic bands revealed by hybridization with long-insert clones carrying the conserved region (Fig. 4). Thus, probes were identified for 6 of the perhaps 12 members of the maize LHCP-II gene family. These six LHCP-II genes are herein designated as *cab*-m1 (Δ8-11), *cab*-m2 (8-27), *cab*-m3 (8-21), *cab*-m4 (Δpmc6), *cab*-m5 (Δ13-7), and *cab*-m6 (ΔM14) (*cab*-m = gene for chlorophyll a/b binding protein of maize). They are numbered sequentially

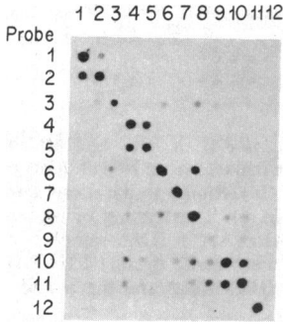

FIG. 3. Cross-hybridization of LHCP-II cDNA clones. Inserts of 12 LHCP-II cDNA clones, identified by the same numbers as in Fig. 2, were isolated and spotted onto nitrocellulose. Probes were the nick-translated insert DNAs isolated from the 12 LHCP-II cDNA clones.

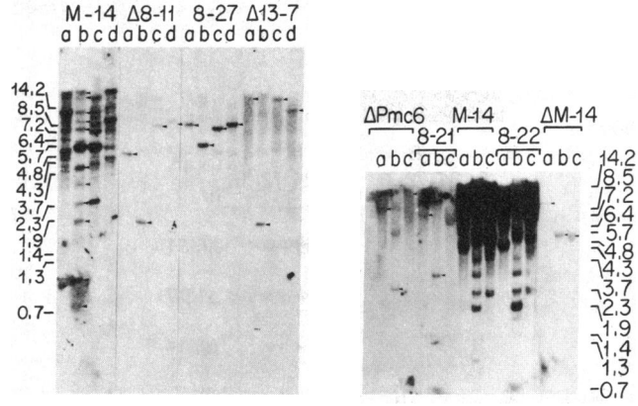

FIG. 4. Genomic Southern blot analysis of LHCP-II cDNA clones. Maize genomic DNA (10 μg) samples isolated from leaves were digested with *Bam*HI (lanes a), *Bgl* II (lanes b), *Eco*RI (lanes c), or *Hin*dIII (lanes d), fractionated on 0.8% agarose gels, and blotted onto Nytran. Probes were nick-translated insert DNAs isolated from eight different LHCP-II cDNA clones as indicated. Arrows indicated the location of the band or bands revealed by each probe. Sizes are shown in kilobase pairs.

according to the sizes of the precursor proteins for which they code (sizes are shown in Fig. 2*a*).

Expression Levels of Six LHCP-II Genes. Transcripts of the six LHCP-II genes accumulated to different levels in maize leaves during 24 hr of greening. As seen in Fig. 5, *cab*-m1 (Δ8-11) was the most highly expressed gene and constituted about 30% of the LHCP-II mRNA in 24-hr-greening leaves; *cab*-m2 (8-27) and *cab*-m5 (Δ13-7) each contributed about 18% and 20%; and *cab*-m3 (8-21), *cab*-m4 (Δpmc6), and *cab*-m6 (ΔM14) contributed 15%, 8%, and 4%, respectively. The longer probes, M14 and 8-22, were used to estimate the total LHCP-II mRNA. Approximately 95% of the total LHCP-II mRNA was transcribed from the six genes studied.

Differential Expression of Six LHCP-II Genes in BSC and MC of Developing Maize Leaves. By blot-hybridization and dot-blot analyses with probes specific for six LHCP-II genes, we found that *cab*-m1 (Δ8-11), the most highly expressed gene of the family in greening leaves, and *cab*-m6 (ΔM14) are both strongly induced by light in MC, but their mRNAs were barely detectable in BSC. Other genes were induced to relatively lower levels in BSC or MC (Fig. 6). Transcripts of *cab*-m5 (Δ13-7) were more abundant in MC than in BSC and were induced by light in both cell types. Transcripts of *cab*-m2 (8-27) and *cab*-m3 (8-21) were about equally abundant

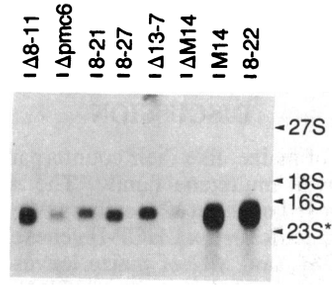

FIG. 5. Relative mRNA levels of different LHCP-II genes in greening (24 hr) maize leaves determined by blot hybridization. One microgram of maize poly(A)$^+$ mRNA isolated from 24-hr-greening leaves was fractionated on formaldehyde-agarose gels (1% agarose) and blotted onto Nytran. Probes were insert DNAs isolated from eight different LHCP-II cDNA clones as indicated, end-labeled to equal specific activity. The clones M14 and 8-22 carrying long inserts were used to estimate the overall expression of LHCP-II genes. The star (23S*) signifies the position of the common breakdown product of 23S rRNA of plastids.

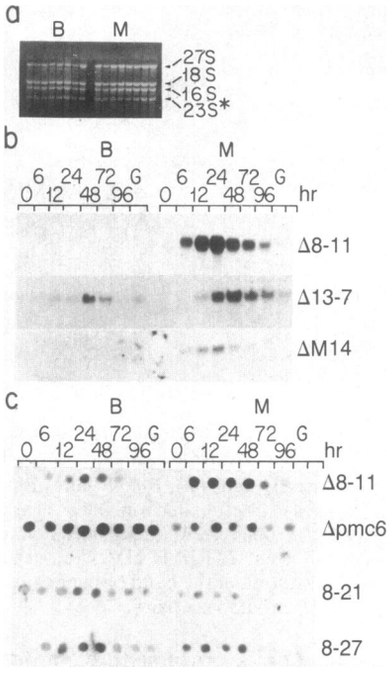

FIG. 6. Cell-specific expression of different LHCP-II genes in BSC and MC of developing maize leaves. Total RNA (20 μg) isolated from BSC (B) and MC (M) of etiolated (0 hr), greening (6, 12, 24, 48, 72, and 96 hr), and green (lane G) maize leaves were fractionated on formaldehyde-agarose gels (1% agarose) and blotted onto nitrocellulose. (*a*) Four rRNA bands were seen under UV light after staining with ethidium bromide. (*b*) RNA blots were probed with nick-translated insert DNA of clones Δ8-11, Δ13-7, and ΔM14. (*c*) Aliquots (5 μg) of the same set of RNA samples were spotted onto Nytran for dot-blot analysis. Probes were nick-translated insert DNAs of clones as indicated.

and induced by light slightly in BSC and MC. *Cab*-m4 (Δpmc6) was preferentially expressed in BSC, and the transcripts were induced by light slightly in both cell types. The expression behavior of these six LHCP-II genes of maize and properties of their cDNA probes are summarized in Table 1.

On immunoblot analysis, five different sizes of LHCP-II (designated LHCP-II-ma to LHCP-II-me based on size) were revealed when a high $NaDodSO_4$-to-chlorophyll ratio (200:1) was used to dissolve the thylakoid membranes in a dilute protein concentration (<0.2 mg/ml) (Fig. 7). The accumulation of all LHCP-IIs was induced by light in both cell types. LHCP-II-ma and -mc were about equal in BSC and MC. The LHCP-II-mb and -mf proteins were much more abundant in MC than in BSC, whereas there was more LHCP-II-md protein in BSC than in MC.

DISCUSSION

The LHCP-IIs of maize, like their counterparts in C_3 plants, are encoded by a multigene family. The maize family is probably composed of about 12 members. We have identified cDNA clones specific for six LHCP-II genes that are actively expressed in BSC and MC of maize leaves. Four lines of evidence indicate that these six LHCP-II cDNA clones represent six specific genes. First, six different sizes of LHCP-II precursors are resolved on $NaDodSO_4$/polyacrylamide gel electrophoresis of *in vitro* translation products of RNAs hybrid-selected with different cDNA clones. Second, the inserts of these cDNA clones share little homology with one another as shown in cross-hybridization experiments. Third, each of the short or shortened clones (the latter bearing a divergent sequence after the original longer insert was partially deleted with BAL-31) hybridizes to a single band on genomic DNA blots at a position corresponding to one of the bands hybridized by clones with long inserts. Fourth, by using specific probes end-labeled to equal specific activity, we show that the levels of RNAs complementary to these six distinct LHCP-II genes are different in leaves greening for 24 hr and in BSC and MC. The sum of their expression accounts for >95% of LHCP-II RNAs detected in the 24-hr-greening leaves, even though these six cDNA clones represent half of the genes revealed by genomic Southern blotting. Other LHCP-II genes not obtained by cDNA cloning may be expressed at low levels or may be present as silent genes or pseudogenes, as demonstrated for the ribulose-bisphosphate carboxylase small subunit multigene family (35).

Table 1. Summary of LHCP-II genes in maize

Genes*	cDNA clones	Insert size, bp	% of Total LHCP-II mRNA†	Cell specificity	Light inducibility	Designation in Figs. 2/3
cab-m1	8–11	250	30	B <<< M	+++	1
	8–15	420				2
	Δ8–11	200				
cab-m2	8–20	270	18	B = M	+	6
	8–27	150				8
	8–22	730				3
cab-m3	8–21	200	15	B = M	+	7
cab-m4	11–12	550	8	B >> M	+	4
	pmc6	500				5
	Δpmc6	250				
cab-m5	8–17	750	20	B << M	++	9
	12–5	400				10
	13–7	250				11
	Δ13–7	180				
cab-m6	M14	750	4	B <<< M	+++	12
	ΔM14	230				

*These genes are numbered sequentially according to the sizes of the precursor proteins synthesized *in vitro*. The sizes range from 30 kDa to 33 kDa as shown in Fig. 2*a*.

†The poly(A)$^+$ mRNA was isolated from maize leaves illuminated for 24 hr. These values were estimated from Fig. 5.

LHCP-II is less abundant in agranal chloroplasts of BSC than in granal chloroplasts of MC (17–22), and LHCP-II mRNA was also seen to be either reduced or absent in BSC (21, 22). Our data suggest that, overall, the RNA and protein products of LHCP-II genes are more abundant in MC than in BSC. Blot-hybridization and dot-blot analyses demonstrated that three LHCP-II genes, *cab*-m1 (Δ8-11), *cab*-m5 (Δ13-7), and *cab*-m6 (ΔM14), are preferentially expressed in MC and contribute >50% of total LHCP-II mRNA detected in illu-

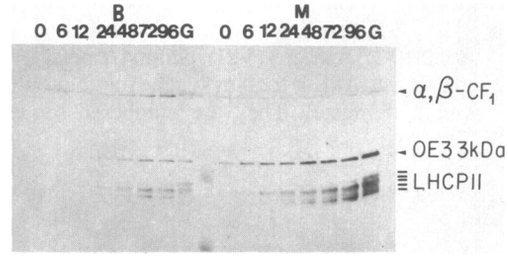

FIG. 7. Immunoblot analysis of LHCP-II in BSC and MC of developing maize leaves. Proteins were isolated from 10% aliquots of the same set of BSC (B) (*Left*) and MC (M) (*Right*) preparations used for RNA samples (as described in Fig. 6). The loading of protein samples followed proportionally the same set of RNA samples for RNA blot analysis. Antibodies used were made against LHCP-II, oxygen-evolving 33-kDa polypeptide (OE33kDa) and the α and β subunits of coupling factor (α,β-CF_1).

minated dark-grown maize leaves; two LHCP-II genes, *cab*-m2 (8-27) and *cab*-m3 (8-21), are expressed equally in both cell types; and transcripts of *cab*-m4 (Δpmc6) are more abundant in BSC than in MC. Furthermore, mRNAs of only a few LHCP genes [*cab*-m3 (8-21) and -m4 (Δpmc6)] are present in etioplasts, and the time courses of size changes in the RNA pool during greening are not identical [compare *cab*-m1 (Δ8-11) and *cab*-m5 (Δ13-7)] (Fig. 6).

Immunoblot analysis (Fig. 7) reveals five LHCP-II bands when samples are treated at a $NaDodSO_4$-to-chlorophyll ratio of about 200:1 in a dilute protein concentration (<0.2 mg/ml) instead of 20:1 and are fractionated on a low methylenebisacrylamide $NaDodSO_4$/polyacrylamide gel at constant current. More than five LHCP-II bands have been separated before when low temperature and different acrylamide concentrations were used (36). The accumulation patterns of these five LHCP-IIs correlate well with changes in LHCP-II mRNAs in BSC and MC of greening maize leaves. Two sizes of LHCP-IIs are more abundant in MC than BSC and constitute most of the LHCP-II in green maize leaves. Two faint bands are detected equally in two cell types, and one band is more distinct in BSC than in MC. However, even though we have shown the expression of six different LHCP-II genes in BSC and MC and that each codes for a precursor protein of different apparent molecular weight, the LHCP-IIs of various size detected *in vivo* could be either the products of different genes or gene products processed differently after translation, or both.

The accumulation of LHCP-II in whole maize leaves has been shown to be absolutely light-dependent while the mRNA is present at a low level in the dark and is dramatically stimulated by light (14). Our data indicate that the previously described (14) expression pattern of "the LHCP-II gene" only matches the expression of *cab*-m1 (Δ8-11) and *cab*-m6 (ΔM14) in MC of illuminated dark-grown maize seedlings; in addition, the elevated mRNA level during 24 hr of greening declines to a low steady-state level after 72 hr of illumination. The *cab*-m5 (Δ13-7) gene is also induced by light in MC but much less than are *cab*-m1 and *cab*-m6. The mRNA of *cab*-m5 is also detected in BSC. Transcripts of three other LHCP-II genes, expressed equally in two cell types or preferentially in BSC, all show moderate light-induction for mRNA accumulation. The accumulation of the protein products of different LHCP-II genes, despite the presence of small amounts of their mRNAs in the dark, is promoted by light in both cell types and stays at high levels despite the decline of the mRNA in late-greening and green maize leaves.

In green leaves, the mRNA level differences in BSC and MC are not as great as in greening leaves before 48 hr of illumination, and yet the protein levels differ by about 4-fold. We propose that the transient accumulation of LHCP-II transcripts in MC during early illumination (6–48 hr) plays a crucial role in MC-specific build-up of LHCP-II, since the steady-state levels of LHCP-II mRNAs are not dramatically different in BSC and MC. However, higher synthetic rates or lower turnover rates of LHCP-II in MC than in BSC is not ruled out.

In summary, the LHCP-IIs in maize thylakoids are the products of different members of the LHCP-II gene family. Apparently, the six members of the LHCP-II multigene family we have studied are not regulated through a common mechanism; rather, there are at least three differently regulated types (summarized in Table 1) in terms of their cell-specificity, light-inducibility, and levels of mRNA and protein accumulation. Whether different LHCP-IIs encoded by different genes and under different regulatory controls have distinct functions or are interchangeable needs further investigation.

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