Expression of Amyloplast and Chloroplast DNA in Suspension-Cultured Cells of Sycamore (*Acer pseudoplatanus* L.)[1]

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ABSTRACT

Green mutant cells of sycamore (*Acer pseudoplatanus* L.), which had been selected by mutagenic treatment of the white wild type, grow photoheterotrophically in auxin-depleted culture medium. In contrast to the wild-type cells, mutant cells exhibit photosynthetic O_2-evolution activity during their growth coincident with increases of (a) chlorophyll, (b) protein, and (c) ribulose-1,5-bisphosphate (RuBP) carboxylase activity. Functionally competent chloroplasts were isolated from the green cells. Mechanism(s) governing gene expression of amyloplast DNA in the heterotrophically grown white cells were compared with those of the chloroplast DNA isolated from the mutant cells. We have demonstrated in both amyloplast and chloroplast DNAs the presence of sequences homologous to the maize chloroplast genes for photosynthesis, including the large subunit of ribulose 1,5-bisphosphate carboxylase/oxygenase (RuBisCO) (*rbcL*), the 32 kDa Q_B protein (PG32) (*psbA*), the apoprotein of P700 (*psaA*) and subunits of CF_1 (*atpA, atpB,* and *atpE*). However, employing either enzyme assays or immunological techniques, RuBisCO and CF_1 cannot be detected in the white wild type cells. Northern blot hybridization of the RNA from the white cells showed high levels of transcripts for the 16S rRNA gene and low level of transcripts for *psbA*; based on comparison with results obtained using the green mutant cells, we propose that the amyloplast genome is mostly inactive except for the 16S rRNA gene and *psbA* which is presumably regulated at the transcriptional level.

In view of the fact that amyloplasts are the sites of starch synthesis in storage organs such as seeds and roots (21), it is imperative to examine the structure and function of the amyloplast genome and to elucidate the regulatory mechanism(s) which control its expression (10). It is frequently postulated that amyloplasts and chloroplasts are ontogenically related (9), although neither the functional nor the structural nature of the former organelle has been substantively characterized in comparison with that of the latter which has been studied by numerous investigators employing molecular biological techniques (7). One can surmise that there may exist closely related genetic machineries in these two different types of plastids, and in the case of potato the essentially identical restriction patterns were obtained between chloroplast (leaf) and amyloplast (root) DNAs (23). It is well recognized that upon illumination, proplastids or etioplasts in some plant tissues such as potato tuber and etiolated wheat seedlings are transformed into the Chl-containing chloroplasts. Many biochemical investigations have been performed with this system (6, 7, 22). In recent years, extensive research has been undertaken to elucidate the molecular mechanism(s) of development and differentiation of another class of plastid, chromoplasts, during the period of tomato fruit growth and maturation. In these studies, the mode of plastid gene expression during fruit development has been examined in comparison with that operating in chloroplasts (2, 3, 19, 20).

The suspension-cultured cells of sycamore (*Acer pseudoplatanus* L.) originally derived from nonphotosynthetic cambium cells, are heterotrophic and contain mainly one type of differentiated plastid (amyloplasts), but they are unable to transform to the photosynthetically active cells upon illumination. Since the structure and the function of amyloplasts and chloroplasts are clearly distinct, mechanism(s) underlying the expression of the amyloplast genome and its regulation are evidently of importance and value to determine the nature of genes specific to this unique organelle. The presence in the amyloplast DNA of several homologous sequences to genes for photosynthesis *e.g.,* *rbcL* (large subunit of RuBisCO),[4] *psbA* (32kDa Q_B protein, PG32), *atpA* (α-subunit of CF_1), *atpB* (β-subunit of CF_1), *atpE* (ϵ-subunit of CF_1), and *psaA* (apoprotein of P700), and 16S rDNA, was previously reported (15). However, in a subsequent investigation (16), it was found that most of the amyloplast DNA was not transcribed in the sycamore cells.

The ultimate goal of our present investigation is to elucidate the mechanism(s) governing the gene expression in amyloplasts by taking advantage of the availability of the green mutant type sycamore cells which had been selected from the white wild type cells of sycamore after chemical mutagenesis (12). Transcriptionally active chloroplast genomes isolated from the mutant cell line have been employed as the control.

MATERIALS AND METHODS

Suspension Culture of Sycamore Cells. White wild-type sycamore cells were grown in the liquid medium as previously described by Bligny (4). Mutant green sycamore cells which were originally selected by Lescure (12) and maintained in the CNRS

[4] Abbreviations: RuBP, ribulose-1,5-biophosphate; RuBisCO, ribulose-1,5-bisphosphate carboxylase/oxygenase; CF_1, coupling factor of chloroplasts; PG32, 32 kDa Q_B protein (Figs. 5 and 6).
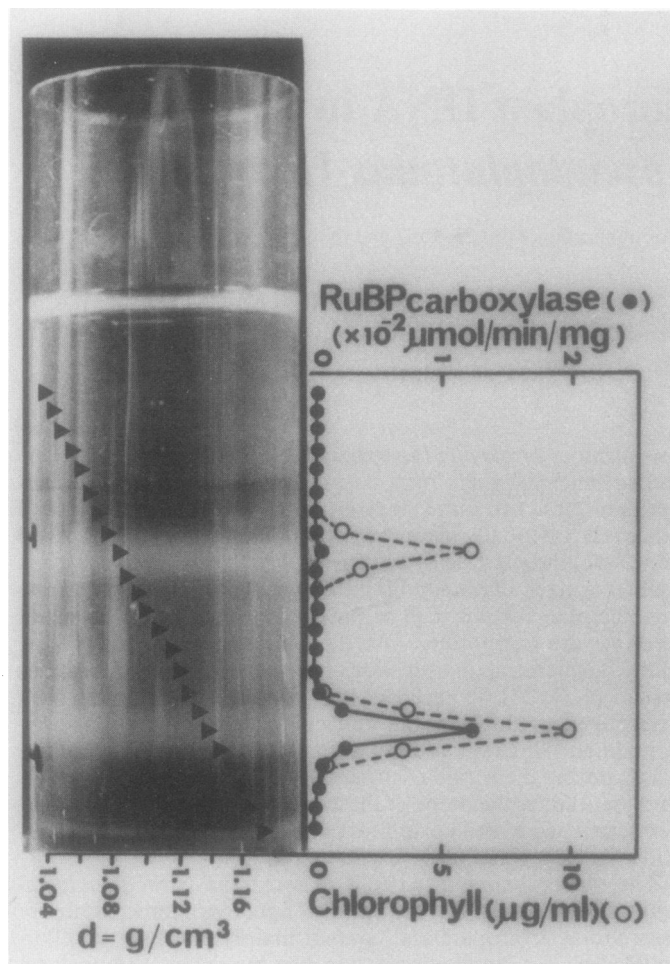
FIG. 1. Ioslation profile of intact chloroplasts from green mutant cells of sycamore. Chloroplasts of the green mutant cells were isolated from the ruptured sycamore protoplasts by the linear gradient of Percoll according to the method of Takabe *et al.* (29). The Chl content and RuBPcarboxylase activity were measured as described in text.

laboratories of Marseille (France) were provided to us by Dr. Péaud-Lenoël. They were grown photoheterotrophically in the culture-medium depleted of 2,4-D according to the description of Lescure (12). They were cultured under the continuous illumination of fluorescent light (2000 lux, 25°C).

Enzyme Assay. About 2 g (fresh weight) of sycamore cells, freshly harvested at the exponential growth stage, were suspended in approximately 2 ml of sonicating buffer consisting of 50 mM HEPES in KOH (pH 7.5, 4°C), 5 mM DTT, 1 mM EDTA, and 0.3 M KCl. The suspension was sonicated twice at 200 W for 10 min at 0°C in a Kubota Insonator Model 200M, and then centrifuged at 94,000g for 1 h. The supernatant was fractionated with $(NH_4)_2SO_4$ solution and the 25 to 50% saturation precipitate was dissolved in a minimal volume of the sonicating buffer and passed through a Sephadex G-25 column, which was preequilibrated with the sonicating buffer as described above. RuBP carboxylase (EC 4.1.1.39) activity was measured as described by Nishimura *et al* (18).

Analytical Methods. Chl content in the sycamore cells was determined according to Arnon (1) after extraction in 80% acetone. Protein content was determined by the spectrophotometric method of Lowry *et al.* (14) using BSA as a standard.

Immunological Blotting Analysis of Proteins. The crude extract of sycamore cells after sonication was first subjected to SDS-PAGE (10–20% linear gradient) and electrophoretically trans-
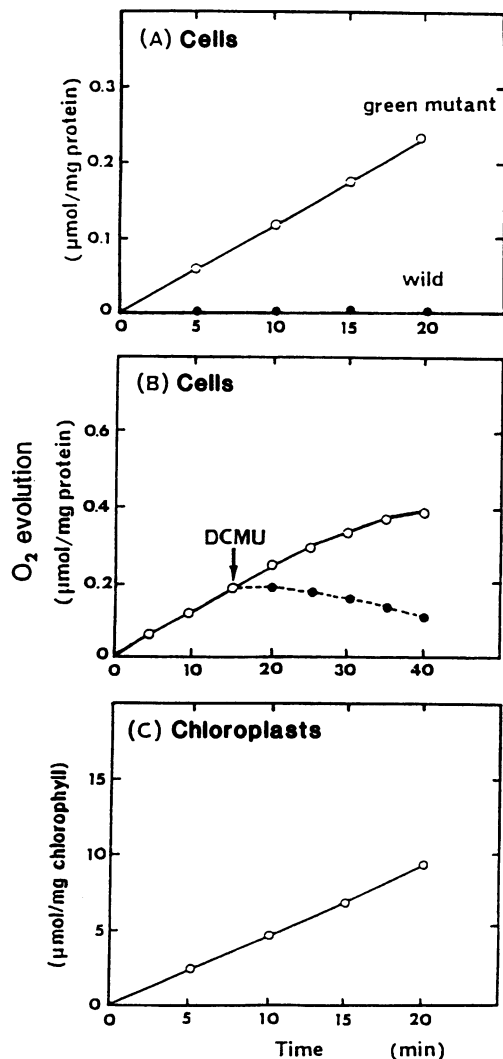

FIG. 2. Photosynthetic activities of green mutant sycamore cells. (A) Light-dependent O_2-evolution of the green mutant cells (10-day) comparing to the white wild type cells (5-d). O_2 evoltuion of sycamore cells was measured in a vessel of Hansatech-oxygen electrode. (B) Inhibitory effect of DCMU on the light-dependent O_2 evolution by the green mutant cells. Experimental condition were same as those shown in (A), except 10 μM DCMU was added at 15 min. (C) Light-dependent O_2 evolution of intact chloroplasts isolated from green mutant cells of sycamore. Intact chloroplasts of green mutant cells (see Fig. 1) were applied to the vessel for measuring the of light-dependent O_2 evolution.

ferred to nitrocellulose filter (S&S PH79, 0.1 μM) according to the method of Lin and Kasamatsu (13). Subunits of RuBisCO and CF_1 were detected by the specific rabbit antibodies against spinach enzymes prepared by us. Bands were detected using the horseradish peroxidase-conjugated anti-rabbit goat IgG and 4-chloro-1-naphthol according to the instruction manual from Bio-Rad with washing buffer containing dry milk (11).

Photosynthetic O_2 Evolution. Photosynthetic O_2 evolution of cells and intact chloroplasts were measured using a Hansatech-oxygen electrode basically following the procedures reported by Takabe *et al.* (29). Illumination was provided by a white tungsten lamp (200 W). Unless otherwise indicated, light intensity was 30,000 lux (14 mW/cm^2 of 400–750 nm) which can saturate the photosynthetic reaction chamber under the condition presently employed. Photosynthetic O_2 evolution was inhibited by adding DCMU at the final concentration of 10 μM according to Etienne (8).
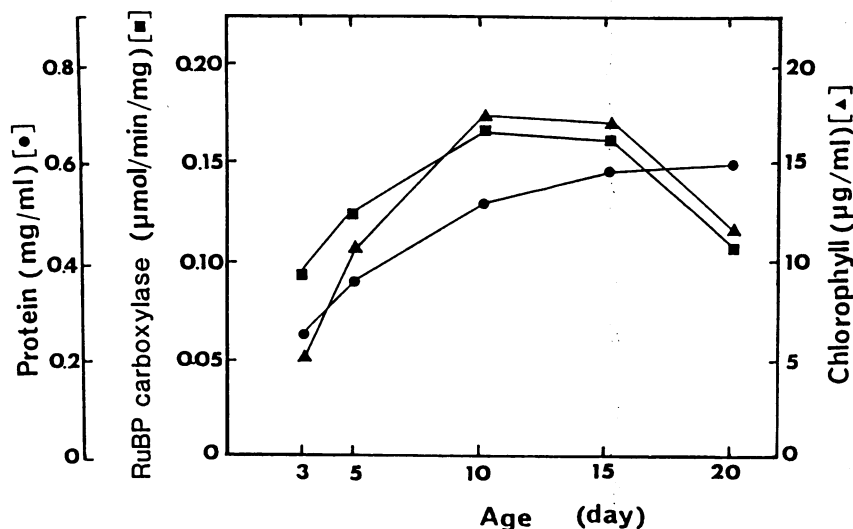
FIG. 3. Time-sequential analysis of protein, Chl and RuBisCO activities of green mutant sycamore cells. At the selected time intervals of the growth stage, the cells were harvested for the following analyses: total protein (●), chl (▲) and RuBisCO activities (■) as explained in the text.

Amyloplasts and Chloroplasts Preparation. Protoplasts were prepared following the method as described previously by Macherel *et al.* (15) using the freshly harvested white sycamore cells at the exponential growth stage (5-d). Amyloplasts and mitochondria free from other organelles were prepared according to the procedure reported by Macherel *et al.* (16).

Protoplasts of mutant green cells, harvested at the exponential growth stage (10-d), were prepared basically following the procedure described by Macherel *et al.* (15), except for the 45-min incubation with 2% (w/v) Cellulase Onozuka R10 (Yakult Co. Ltd., Japan), 0.2% (w/v) Pectolyase Y23 (Seishin Co. Ltd., Japan), and 0.2% (w/v) Macerozyme R10 (Yakult Co. Ltd., Japan). The resulting protoplasts were then ruptured by passage through a syringe and chloroplasts were prepared according to the procedure of Sugiura *et al.* (28) for DNA extraction and Takabe *et al.* (29) for the purpose of assaying photosynthetic activities.

DNA and Cellular RNA Preparation. Amyloplast DNA and mitochondrial DNA were prepared as described by Macherel *et al.* (16). Chloroplast DNA was prepared according to Sugiura *et al.* (28) after a slight modification by a second centrifugation in a CsCl gradient containing bisbenzimide Hoeschst 33258 (Sigma) as an intercalating agent for the double strand DNA. The extraction of total cellular RNA was performed following the procedure reported by Macherel *et al.* (16).

Characterization of DNA and RNA. DNA was labeled radioactively with $[\alpha\text{-}^{32}P]$-dCTP *in vitro* by using the Klenow fragment of the *Escherichia coli* DNA polymerase I and primers of Oligolabelling Kit (Pharmacia). The digestion of DNA by restriction endonucleases and subsequent agarose gel electrophoresis of the digested fragments were performed using the conventional techniques. Purified cellular RNA was denatured by glyoxal and electrophoresed on agarose gel run in 10 mM Na phosphate (pH 7.0). The transfer of nucleic acids to GeneScreen (New England Nuclear) membranes and the hybridization conditions for Northern and Southern blot analyses were carried out according to the instruction manual of New England Nuclear (24). The radioactive bands on GeneScreen were detected by radioautography.

Plasmids containing the maize chloroplast genes; *rbcL* (large subunit of RuBisCO), *psbA* (32 kDa Q$_B$ protein,PG32), *atpA* (α-subunit of CF$_1$), *atpB* (β-subunit of CF$_1$), *atpE* (ϵ-subunit of CF$_1$), *psaA* (apoprotein of P700), and 16S rDNA were kindly provided to us by Dr. Bogorad (5).

RESULTS

The suspension-cultured cells of green mutant sycamore grow photoheterotrophically in the culture medium lacking 2,4-D and

containing 2% sucrose as a carbon source. The isolation profile of intact chloroplasts from these cells, employing the linear Percoll gradient centrifugation, is shown in Figure 1. Under phase contrast light-microscope, typical refractile appearance of chloroplasts, having an intact envelope, was observed. It will be noted that the size of intact chloroplasts isolated from green mutant sycamore cells is much smaller (diameter = 1 μm) than those isolated from spinach (diameter = 8 μm) (photographs not shown).

The unique property of the green mutant cells of sycamore, in contrast to the white wild-type cells, is its photosynthetic competency. As shown in Figure 2A, they sustain the light-dependent O$_2$ evolution and this activity was shown to be totally suppressed by the addition of DCMU (Fig. 2B). The intact chloroplasts isolated from green mutant cells can also sustain the light-dependent O$_2$ evolution (Fig. 2C).

We have measured the Chl and protein contents and RuBP carboxylase activity during the relatively slow growth period under the continuous illumination with a fluorescent lamp. The results of time-sequential analyses are presented in Figure 3. There is a nearly parallel increase of RuBP carboxylase activity and Chl content up to the 10-d stage of growth. As presented in Figure 4A, employing the immunoblotting analysis, we have been unable to detect the presence of RuBisCO and CF$_1$ in the wild-type cells (lanes 2 and 5). In contrast, potentially high signals of RuBisCO large (A) and small (B) subunits, as well as α-, β-, γ-, δ-, and ϵ- subunits of CF$_1$ were detected in the green mutant cells (lanes 3 and 6). Essentially identical profiles were obtained with spinach leaf extracts (lanes 1 and 4). Using the total extracts of green mutant cells at different growth stages, we have been able to demonstrate a prominent increase with culture age in the amount of RuBisCO and CF$_1$ by the immunoblotting analysis (Fig. 4B).

To clarify what step(s) of plastid gene expression are suppressed in the wild-type white cells, we attempted to analyze the levels of transcripts of *rbcL*, *psbA*, *atpA*, *atpB*, *atpE*, and *psaA* in both wild and mutant cells. Prior to performing these experiments, we must be certain that the gene probes specifically hybridize with the transcripts of plastid origin and not with those of the mitochondrial DNA. This precaution is considered to be particularly important, because of the widespread cross-homologies found between the two organellar genomes in several plant species (27). Thus, the homology between amyloplast and mitochondrial DNAs was examined at first. The DNAs of amyloplasts and mitochondria were isolated from the white cells and subjected to the Southern blot hybridization using the above gene
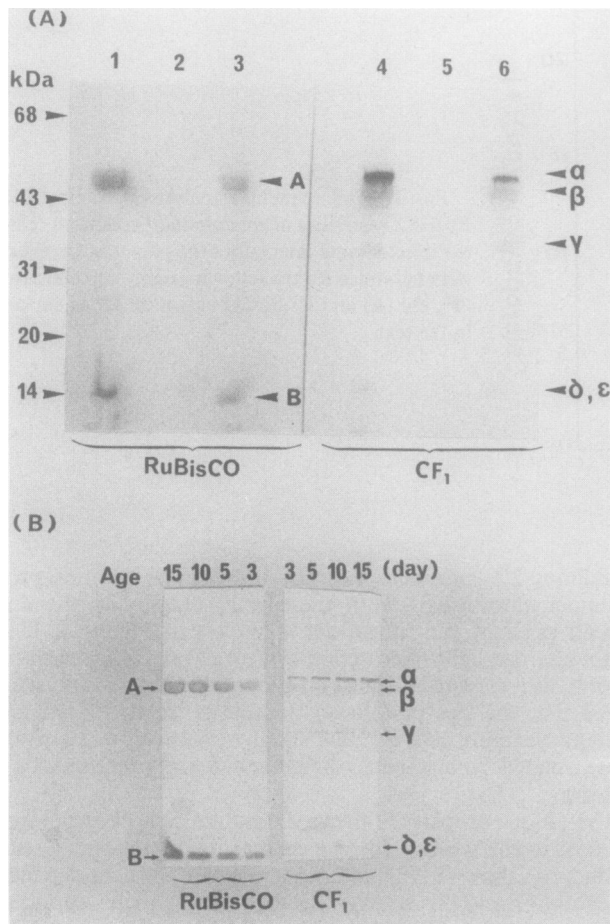
FIG. 4. Immunoblotting analysis of RuBisCO ind CF₁ in sycamore cells. (A) Blotting analysis of RuBisCO (left) and CF₁ (right) among wild-type (lane 2 and 5), green mutant (lane 3 and 6) and spinach leaf (lane 1 and 4) extracts. At the exponential phase of growth (10-day with green mutant and 5-d with wild type), 5 ml of cells were harvested and suspended in 1 ml of the sonicating buffer; 20 μl of the sonicated materials were then subjected to the SDS-PAGE. The immunoblotting analysis of RuBisCO and CF₁ was carried out as explained in the text. Crude extract from spinach was used as control. Lane 2, 5: crude extract from wild type; lane 3, 6: crude extract from green mutant; lane 1, 4: crude extract from spinach leaf. Mol wt estimated by the standard markers are shown on the left. (B) Immunoblotting analysis of RuBisCO and CF₁ during development of green mutant cells. Green mutant cells harvested at 3, 5, 10, and 15 d of growth were treated in the same way as that shown in (A), followed by the immunoblotting analysis for RuBisCO (left) and CF₁ (right).

probes prepared from the maize chloroplasts (Fig. 5). Except for 16S rDNA, the gene probes were shown to hybridize only with amyloplast DNA, indicating that the levels of transcripts of the photosynthetic genes in the plastids can be accurately estimated by this experimental system. In order to examine the levels of transcripts for the photosynthetic genes in the amyloplasts, total cellular RNAs were prepared from both white and green cells. They were then electrophoresed on an agarose gel after denaturing with glyoxal, and finally subjected to the hybridization analysis (Fig. 6). In these experiments, the same amount of cellular RNA was applied to each lane. As shown in the figure, the transcripts for rbcL, atpA, atpB, atpE, and psaA are barely detectable in the wild white cells. However, in addition to the significant expression of psbA in amyloplasts we have found that almost the same levels of 16S rRNA were observed in both the green mutant and the white cells. Overall results indicate that
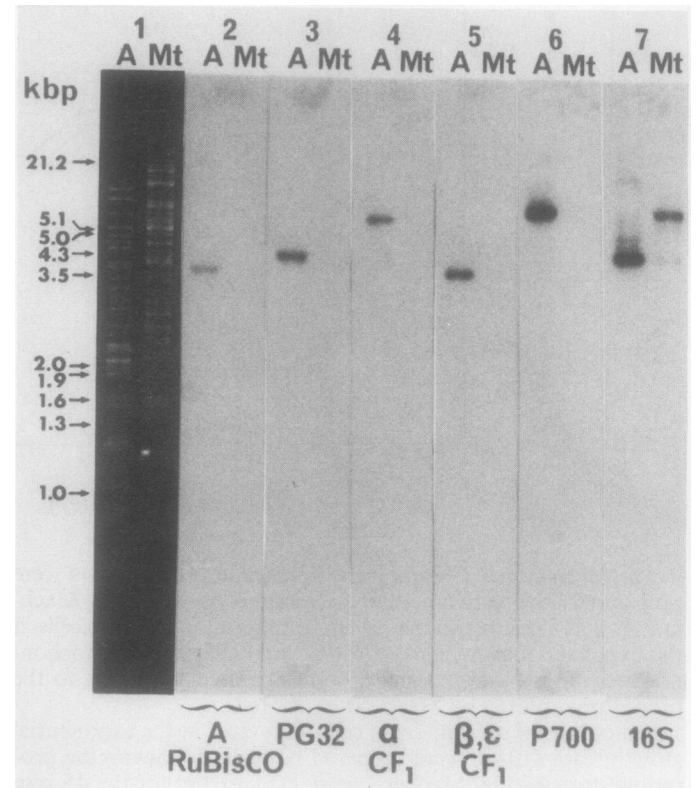
FIG. 5. Southern blot hybridization of amyloplast DNA (A) and mitochondrial DNA (Mt) isolated from wild type cells with chloroplast gene probe. Basic experimental procedures employed were the same as previously reported (15). Agarose gel (0.7%) electrophoretic pattern of amyloplast DNA (1 μg) and mitochondrial DNA (1 μg) digested by EcoRI (lane 1). Autoradiographs of Southern hybridization with probes of maize chloroplast gene were shwon as follows. Lane 2, RuBisCO large (A) subunit; lane 3, 32-kDa Q_B protein (PG32); lane 4, α-subunit of CF₁; lane 5, β- and ε-subunits of CF₁; lane 6, apoprotein of P700; and lane 7, 16S rRNA. Mol wt are shown at the left in kilobase pairs (kbp).

the low magnitude of the expression of the amyloplast genome in the heterotrophically grown white sycamore cells is presumably due to regulation at the level of transcription.

DISCUSSION

There are several potentially useful systems to study the mechanism(s) of gene expression of differently differentiated plastids. It is well recognized that upon illumination, proplastids or etioplasts in some plant tissues such as potato tuber and etiolated wheat seedlings are transformed to the chloroplasts (6). Another feasible system is the developmental transformation of chloroplasts to chromoplasts during the step of ripening of tomato fruits and intensive research has been carried out with these plastids at the molecular level (19, 20).

Our investigation dealing with the examination of the gene expression of the amyloplast DNA in the cultured sycamore cells is greatly facilitated by the availability of the green mutant type cells, growing photoheterotrophically yet sustaining photosynthetic activities. The transcriptionally active chloroplasts genome isolated from the latter cells has served as control throughout this study. Although the usefulness of green plant mutants has been often emphasized for photosynthesis research (17), it should be recognized that most of them are defective in a single or multiple sets of genes for photosynthesis. In contrast, the green mutant type sycamore cells presently used have acquired the total photosynthetic ability, accompanying the formation of the
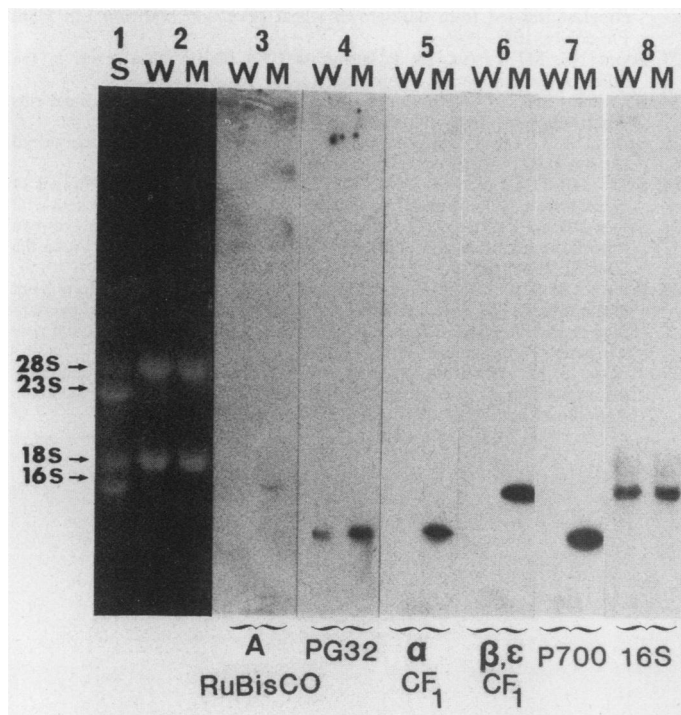
FIG. 6. Northern blot hybridization of total cellular RNA isolated from wild type (W) and mutant (M) cells with chloroplast gene probes. Agarose gel (1.2%) electrophoretic pattern of purified total cellular RNA (10 μg) of wild type and mutant cells (lane 2). Autoradiographs of Northern hybridization with probes of maize chloroplast genes were shown as follows. Lane 3, RuBisCo large (A) subunit; lane 4, 32-kDa Q_B protein (PG32); lane 5, α-subunit of CF_1; lane 6, β- and ε-subunits of CF_1, lane 7, apoprotein of P700; and lane 8, 16S rRNA. Mol wt markers 28S and 18S rRNA from HeLa cells and 23S and 16S rRNA from E. coli are indicated at the left (lane 1).

functionally competent chloroplasts. Furthermore, since the wild and mutant type cells are not mutally interconvertible, comparative studies of gene expression in these cells stand the best chance of clarifying the regulatory mechanism(s) operating in the gene expression in amyloplasts.

The Southern blot hybridization of amyloplast DNA from white wild cells shows the presence of homologous sequences of genes for photosynthesis and 16S rDNA, in agreement with our previous results (15). In contrast, when analogous experiments were performed with mitochondrial DNA, the only positive signal observed was with the 16S rDNA probe. The results support the postulate that the sequences homologous to genes for photosynthesis are not in the mitochondrial DNA, and that rDNA, which is involved in protein synthesis, is present in the mitochondrial DNA. By examining the transcriptional products of amyloplast and mitochondrial DNAs from the wild white cells, it was shown that abundant transcripts of mitochondrial DNA exist, in agreement with our previous work (16). It is thus reasonable to conclude that the amyloplast DNA in the wild sycamore cells is not actively transcribed. Hansman et al. (10) have suggested that DNA of the chromoplast, another type of differentiated plastid, is also not active.

We next carried out the Northern blot hybridization analysis of the total cellular RNA from both wild and mutant cells; we were able to detect the transcripts of genes for photosynthesis (rbcL, psbA, psaA) and CF_1 subunits (atpA, atpB, atpE) as well as 16S rRNA in the total RNA from the green mutant cells. On the other hand, only one positive signal was detectable with the 16S rRNA in the wild white cells. The faint signal of the tran-

scripts of the genes for 32 kDa Q_B protein (PG32) (psbA) in the RNA from wild white cells was detected in this experiment by using the higher sensitive method than our previous experiment (16) for labeling the gene probes. This faint signal indicates the low but significant level of the transcripts of psbA in the wild cells compared to the mutant cells. The absence of positive signals with other genes may indicate that either they are not transcribed or the transcribed molecules are rapidly degraded.

Our results show the presence of transcripts of 16S rRNA from the amyloplast DNA, but a hybridization signal was obtained with the 18S rRNA. It has been reported that the maize mitochondria contain principally 18S rRNA rather than 16S rRNA (25), but it is known that there exist homologous sequences between maize mitochondrial and chloroplasts DNAs (26). Therefore, without knowing the exact size of sycamore mitochondrial rRNA at the present stage of investigation, we tentatively conclude that the amyloplast 16S rRNA is actively transcribed.

Overall, our present experimental results strongly suggest that there is a homology between the DNAs from amyloplasts of white wild type cells and chloroplasts of green mutant cells of sycamore. However, in view of the fact that there exists differentiated status of their expression, the regulatory mechanisms operating in the expression of the plastid genome is truly of interest for future investigations. From the results obtained, it appears that the amyloplast genome is mostly inactive except for rDNA and the gene for 32 kDa Q_B protein (PG32), and presumably it is regulated at the level of transcription.

LITERATURE CITED

1. ARNON DI 1949 Copper enzymes in isolated chloroplastss. Polyphenoloxidase in Beta vulgaris. Plant Physiol 24: 1–15
2. BATHGATE B, PW GOODENOUGH, D GRIERSON 1986 Regulation of the expression of the psbA gene in tomato fruit chloroplasts and chromoplasts. J Plant Physiol 124: 223–233
3. BATHGATE B, ME PAERTON, D GRIERSON, PW GOODENOUGH 1985 Plastid changes during the conversion of chloroplasts to chromoplasts in ripening tomatoes. Planta 165: 12–19
4. BLIGNY R 1977 Growth of suspension-cultured Acer pseudoplatanus L. cells in automatic culture units of large volume. Plant Physiol 59: 502–505
5. BOGORAD L, EJ GUBBINS, E KREBBERS, IM LARRINUA, BJ MULLIGAN, KMJ MUSHAWITCH, EA ORR, SR RODERMAL, R SCHANTZ, AA STEINMETZ, G DE VOS, YK YE 1983 Cloning and physical mapping of maize plastid genes. Methods Enzymol 97: 524–554
6. BRADBEER JW 1981 Development of photosynthetic function during chloroplast biogenesis. In MD Hatch, NK Boardman, eds, The Biochemistry of Plants, Vol 8. Academic Press, New York, pp 423–472
7. DYER TA 1984 The chloroplast genome: its nature and role in development. In NR Baker, J Barber, eds, Topics in Photosynthesis, Vol 5. Elsevier, New York, pp 23–69
8. ETIENNE AL 1974 New results on the properties of photosystem II centers blocked by 3-(3,4-dimethylurea) in their different photoactive states. Biochim Biophys Acta 333: 320–330
9. FREY-WYSSLING A, K MUHLETHALER 1965 Ultrastructure Plant Cytology. Elsevier, New York, pp 241–249
10. HANSMANN P, H FALK, K RONAI, D SITTE 1985 Structure, composition and distribution of plastid nucleodis in Narcissus pseudonarcissus. Planta 164: 459–472
11. JOHNSON DA, JW GAUTSCH, JR SPORTMAN, JH ELDER 1984 Improved technique utilizing nonfat dry milk for analysis of protein and nucleic acids transferred to nitrocellulose. Gene Anal Tech 1: 3–8
12. LESCURE AM 1969 Mutagenèse et sélection de cellules d' Acer pseudoplatanus L. cultivées in vitro. Physiol Veg 7: 237–250
13. LIN W, H KASAMATSU 1983 On the electrotransfer of polypeptides from gels to nitrocellulose membranes. Anal Biochem 128: 302–311
14. LOWRY OH, NJ ROSEBROUGH, AL FARR, RJ RANDALL 1951 Protein measurement with the folin phenol reagent. J Biol Chem 193: 265–276
15. MACHEREL D, H KOBAYASHI, T AKAZAWA, S KAWANO, T KUROIWA 1985 Amyloplast nucleoids in sycamore cells and presence in amyloplast DNA of

homologous sequences to chloroplasts genes. Biochem Biophys Res Commun 133: 140–146

16. MACHEREL D, H KOBAYASHI, E VALLE, T AKAZAWA 1986 Expression of amyloplast DNA in suspension-cultured cells of sycamore (*Acer pseudoplatanus* L.). FEBS Lett 201: 315–320

17. MILES D 1982 The use of mutations to probe photosynthesis in higher plants. *In* M Edelman, RB Hallick, N-H Chua, eds, Methods in Chloroplast Molecular Biology. Elsevier, Amsterdam, pp 75–107

18. NISHIMURA M, T TAKABE, T SUGIYAMA, T AKAZAWA 1973 Structure and function of chloroplast proteins. XIX. Dissociation of spinach leaf ribulose-1,5-diphosphate carboxylase by *p*-mercuribenzoate. J Biochem 74: 945–954

19. PIECHULLA B, KRC IMLAY, W GRUISSEM 1985 Plastid gene expression during fruit ripening in tomato. Plant Mol Biol 5: 373–384

20. PIECHULLA B, E PICHERSHY, AR CASHAEORE, W GRUISSEM 1986 Expression of nuclear and plastid genes for photosynthesis-specific proteins during tomato fruit development and ripening. Plant Mol Biol 7: 367–376

21. PREISS J 1982 Regulation of the biosynthesis and degradation of starch. Annu Rev Plant Physiol 33: 431–454

22. SANDELINS AS, E SELSTAM 1984 Localization of galactolipid biosynthesis in etioplast isolated from dark-grown wheat (*Triticum aestivum* L.). Plant Physiol 76: 1041–1046

23. SCOTT NS, MJ TYMMS, JV POSSINGHAM 1984 Plastid-DNA levels in the different tissue of potato. Planta 161: 12–19

24. SOUTHERN EM 1975 Detection of specific sequences among DNA fragments separated by gel electrophoresis. J Mol Biol 98: 503–517

25. STERN DB, TA DYER, DM LONSDALE 1982 Organization of the mitochondrial ribosomal RNA genes of maize. Nucleic Acid Res 10: 3333–3340

26. STERN DB, DM LONSDALE 1982 Mitochondrial and chloroplast genomes of maize have a 12-kilobase DNA sequence in common. Nature 299: 698–702

27. STERN DB, JB PALMER 1984 Extensive and widespread homologies between mitochondrial DNA and chloroplast DNA in plants. Proc Natl Acad Sci USA 81: 1946–1950

28. SUGIURA M, S KUZUO, N ZAITA, M KUSUDA, M KUMANO 1986 Clone bank of the tobacco (*Nicotiana tabacum*) chloroplast genome as a set of overlapping restriction endonuclease fragments: mapping of eleven ribosomal protein genes. Plant Science 44: 211–216

29. TAKABE T, M NISHIMURA, T AKAZAWA 1979 Isolation of intact chloplasts from spinach leaf by centrifugation in gradients of the modified silica percoll. Agric Biol Chem 43: 2137–2142