Plant Physiol. (1979) 64, 690–694
0032-0889/79/64/0690/05/$00.50/0

Effect of Nuclear Mutation in Maize on Photosynthetic Activity and Content of Chlorophyll-Protein Complexes[1]

Received for publication January 18, 1979 and in revised form May 29, 1979

C. DONALD MILES, JOHN P. MARKWELL[2], AND J. PHILIP THORNBER[2]
Division of Biological Science, University of Missouri, Columbia, Missouri 65211

ABSTRACT

A number of new nuclear mutants have been isolated from maize by selection for high chlorophyll (Chl) fluorescence. These mutants show reduced rates of photosynthesis and/or are deficient in Chl. Electrophoretic examination of wild type thylakoid membranes revealed five Chl-protein complexes, two containing only Chl *a* and three containing Chl *a* and Chl *b*. A class of nonviable, photosystem I-deficient mutants was found to be lacking one (A-1) of the two Chl *a*-protein complexes. A second class of nonviable, photosystem I-lacking mutants was found to be missing not only this A-1 complex but also one or more of the three Chl *a* and *b*-containing, light-harvesting Chl-protein complexes. Viable mutants were obtained which appeared to have lost just one of the Chl *b*-containing complexes, whereas a second class of viable mutants was missing all three of the Chl *b*-complexes. The results confirm that the A-1 band is associated with the P700-Chl *a*-protein complex characterized previously. The data also indicate the existence of structurally different forms of the light-harvesting Chl *a*- and *b*-containing complexes. The results also show a lower molecular weight band (A-2) containing primarily Chl *a* and which appears to be required for viability.

A variety of Chl-deficient and photosynthetic mutants of higher plants, having an altered content of Chl-protein complexes, has been described (1, 6, 11, 16, 17, 28, 35, 36). During previous studies only two such complexes could be electrophoretically resolved from extracts of thylakoid membranes solubilized by SDS. The larger complex, termed the P700-Chl *a*-protein (P700-CP)[3] (34), contains those pigment molecules associated with the photochemically active center of PSI. The second complex contains both Chl *a* and Chl *b* and is called the light-harvesting Chl *a*/*b*-protein (LHCP) (34).

Previous mutant analyses have established a correlation between the loss of P700-CP with loss of reaction center (P700) activity, PSI electron transport and photophosphorylation (8, 10, 16); such mutants are obviously lethal. With one exception (6), these mutants were the result of extranuclear or plastome mutations (6, 11, 17, 36). A second group of Chl-deficient mutants exhibit a severe reduction or loss of LHCP, and with one exception (1) these mutations are all nuclear genes (14, 21, 28, 35), which correlates well with the observation of Kung *et al.* (19) of the Mendelian inheritance of a gene coding for LHCP in *Nicotiana*. Mutants lacking LHCP have been most useful in establishing the light-harvesting function of this complex since such plants continue photosynthesis at high rates even in the complete absence of the complex (35). Although most reported mutations eliminating P700-CP are extranuclear (presumably in the plastid DNA) and most mutations affecting LHCP are nuclear, occasional observations of the reverse situation indicate a role for both the nuclear and the plastid DNAs in the synthesis and assembly of both complexes into the membrane as previously suggested (4).

Recently, improved methods for solubilization and electrophoretic fractionation have resolved additional Chl-protein complexes (2, 13, 15, 18, 23, 33). Two of these complexes have both Chl *a* and Chl *b*, and one is a Chl *a*-containing complex which has been postulated to contain the pigments of the photochemical reaction center of PSII (15, 37). The possibility now exists to identify mutants missing one or more of these newly resolved complexes. In this manner the function of these newly described complexes may be deduced. For example, using a mutant lacking the primary PSII reaction, it should be possible to correlate the presence of the PSII reaction center with the newly resolved Chl *a*-containing complex if the above supposition is correct.

This report will describe the occurrence of Chl-protein complexes in several nuclear mutants of maize which have not been previously examined for Chl-protein content. Phenotypic changes in some of these mutants include an alteration of Chl content and a decrease in photosynthetic activity (24). The correlation between certain Chl-protein complexes and photochemical activity will be confirmed, and questions will be raised concerning the suggested homology between the Chl *a*- and *b*-containing complexes.

MATERIALS AND METHODS

Leaf material was collected from 10-day-old seedlings of maize (*Zea mays* L.) and barley (*Hordeum vulgare* L.). Plants were grown in a greenhouse with available light in Los Angeles, Calif., or in controlled environment chambers. Light was provided for 14 h at 29 C and 10-h dark at 25 C in the latter case. Light intensities of either 1,500 (low light) or 3,000 (high light) ft-c were used. Nuclear mutants were obtained by mutagenic treatment of pollen and selection of families segregating high fluorescent individuals in the M-2 generation (29). With this procedure only nuclear recessive mutants were selected.

Washed thylakoid membranes were prepared as previously described (22) in the absence of Mg^{2+}. Isolation medium contained 0.4 M mannitol, 20 mM Tricine (pH 7.8), and 10 mM NaCl. Washed thylakoid membranes were sedimented in 50 mM Tris (pH 8.0) and 1 mM EDTA. Solubilization of the membranes and polyacrylamide gel electrophoresis of the solubilized Chl-proteins were carried out as reported (23) except that the gel and reservoir buffer were chilled to 5 C immediately prior to electrophoresis. Following

[1] This work was supported by National Science Foundation Grant PCM 76-08831 to CDM, National Science Foundation Grant PCM 75-20252 to JPT, and United States Department of Agriculture Grant 5901-0410-8-0123-0 to CDM.

[2] Address: Department of Biology and Molecular Biology Institute, University of California, Los Angeles, California 90024.

[3] Abbreviations: P700-CP: P700-chlorophyll *a*-protein complex; LHCP: light-harvesting chlorophyll *a*/*b*-protein complex; MV: methylviologen; FeCN: ferricyanide; DPIP: 2,6-dichloroindophenol; DBMIB: 2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone.

electrophoresis, the gels were scanned for Chl bands at 672 nm (21) to quantitate the per cent of total Chl in the resolved Chl-protein complexes. The method used to determine the mol wt equivalence of the Chl-protein bands has been described (23).

In vivo Chl fluorescence was monitored in leaf segments held in a mask to expose an area (6 × 20 mm) of the upper leaf surface. After dark adaptation, the leaf was illuminated with a beam from a high intensity microscope lamp passed through a photographic shutter and a Corning 5-57 blue glass filter. Fluorescent emission was measured at 45° to the leaf surface through a Corning 2-64 red glass filter with an EMI 9558 photomultiplier. A Pacific Photometric Instrument's high voltage power supply and picoammeter was used. The fluorescence yield was recorded over 20 s on a Tektronix 214 storage oscilloscope.

Photosynthetic O_2 evolution was measured with a Clark electrode at light saturation using isolated chloroplasts in a previously described apparatus (27). The 5-ml reaction medium contained 0.2 M sucrose, 40 mM Tricine (pH 7.8), 30 mM NaCl, 4 mM $MgCl_2$, 5 mM NH_4Cl, 2 mM $K_3(FeCN)_6$, and 50 μg of Chl. MV reduction was monitored by O_2 uptake in a similar medium except that 0.1 mM viologen and 0.5 mM NaN_3 replaced FeCN. DPIP (80 μM) reduced by ascorbate was used as an electron donor to PSI with MV as acceptor.

PSII electron transport with DBMIB as an electron acceptor was employed as described by Miles (25). This procedure measures O_2 uptake with the Clark electrode in the presence of 10 μM DBMIB and 100 μM $MnCl_2$. The reaction mixture was as used for FeCN with DBMIB and $MnCl_2$ substituted for $K_3(FeCN)_6$.

Cyclic photophosphorylation was measured in chloroplasts by H^+ consumption with pyocyanin as a cofactor (20). The 2-ml reaction mixture contained 0.33 M sucrose, 10 mM KCl, 2 mM $MgCl_2$, 1.25 mM Na_2HPO_4, 1.25 mM K_2HPO_4, 30 μM pyocyanine, 0.3 mM ADP and 100 μg Chl. The reaction mixture was adjusted to pH 8.0 and the amount of H^+ consumed was titrated upon completion of the reaction. The relative concentration of P700 was obtained from a FeCN (5 mM)-minus-ascorbate (4 mM) difference spectrum (9) using 500 μg Chl suspended in 3 ml of 25 mM Tris (pH 7.3), 5 mM NaCl, and 2 mM $MgCl_2$ and a 1-cm path length cell. All absorption spectra were measured at room temperature in an Aminco DW-2 spectrophotometer.

According to genetic nomenclature for maize, the mutants used in this study are designated: *hcf**E1252 etc. for high Chl fluorescent of unknown locus. Only the last four numbers will be used here. The dominant mutant OyYg-700 has been previously described (30).

RESULTS

Washed thylakoid membranes from chloroplasts of wild type and mutant maize seedlings were extracted with SDS. The soluble material, containing the Chl-protein complexes, was fractionated by electrophoresis on SDS-polyacrylamide gels (Fig. 1). As previously reported (23), the gels revealed a large (110 kdalton) Chl *a*-containing complex and three smaller (80, 60, and 46 kdalton) Chl *a*+*b*-containing complexes. These were termed A-1, AB-1, AB-2, and AB-3, respectively (23). Migrating at the ion front was a free pigment zone, termed F, containing detergent-complexed Chl. We found that by cooling the gel and reservoir buffer to 5 C immediately prior to electrophoresis at room temperature, a fifth Chl-protein complex termed A-2 could be consistently observed migrating just above the AB-3 band. This Chl *a*-containing complex is a bluer green than the green of AB-2 or AB-3. Its spectrum reveals a red absorption maximum of 670.5 nm (Fig. 2) and suggests the absence of Chl *b* (lack of a peak of 650 nm). The mol wt equivalence of the A-2 band was determined to be approximately 50,000. This complex could be the same as one described as band A (15) or complex IV (12) or CPa (2) by other groups.

A series of highly fluorescent mutants of maize, resulting from

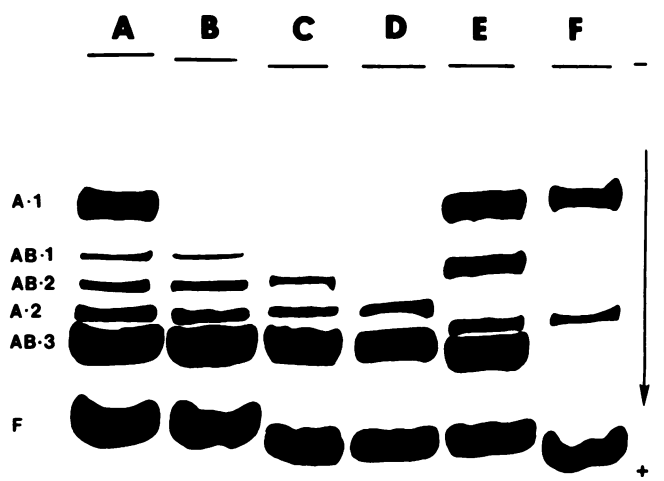

FIG. 1. Diagram drawn from photographs of electrophoretic separation of Chl containing bands extracted from maize thylakoid membranes with SDS. A: wild type; B: mutant 1480; C: mutant 1490; D: mutant 1482B; E: mutant 1483; F: OyYg-700.

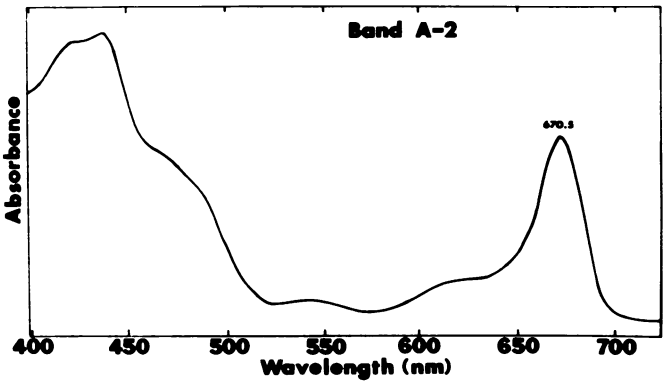

FIG. 2. Room temperature absorption spectrum of the A-2 band cut from electrophoretic gel.

nuclear mutations, show the absence of the A-1 complex (*e.g.* strain 1480, Fig. 1). Such mutants are lethal upon exhaustion of endosperm reserve carbohydrates (*i.e.* after about 14 days). Characteristics of this class are presented in Table I. Both the amount of Chl per gram of tissue (data not shown) and the Chl *a*/*b* ratios of these chloroplasts are lower than for the wild type; this indicates a preferential loss of Chl *a* from the mutants which correlated well with the loss of the Chl *a*-containing A-1 complex. Little or no P700 was detected in any of these mutants. A corresponding decrease of PSI electron transport activity (DPIP → MV) occurred. The low rates of electron transport observed in mutant preparation could be due to harvesting a wild type leaf among the mutant's leaves or to problems in background electron flux with ascorbate (31). Cyclic photophosphorylation was similarly diminished. Three of these mutants retained reasonable rates of PSII activity (H_2O → FeCN and H_2O → DBMIB) while it was severely depleted or zero in the other two. All mutants still contained the other four Chl-protein complexes.

Relative fluorescence yield of all mutants was increased markedly (Table II) as compared to normal green plants. Induction kinetics of Chl fluorescence in dark-adapted leaves of the five mutants (Fig. 3) indicated loss of the P to S shift; this shift is thought to be characteristic of the presence of PSI activity in a leaf and the change of chloroplasts from state 1 to state 2 (32). Mutant 1480, one of the mutants with no detectable PSII electron transport, shows fluorescence kinetics with no variable yield changes, probably indicating the absence of any oxidation of the PSII primary acceptor.

Table I. *Photosynthetic Characteristics of Lethal Maize Mutants Missing the P700-Chl a Protein, A-1*

Mutant	Chl *a/b*	A-1[a]	P700[b]	Electron Transport DPIP → MV	Electron Transport H_2O → FeCN	Electron Transport H_2O → DBMIB	Cyclic ATP Synthesis
				μeq/mg Chl·h			*μmol/mg Chl·h*
1277	2.7	0	0	14	11	0	0
1278	2.6	0	0	29	42	35	11
1480	1.9	1	0	37	0	4	13
1481	1.8	6	14	57	96	136	53
1489B	2.1	0	0	27	64	189	
Wild type	2.7	29	128	206	104	160	163

[a] Per cent total Chl *a*.
[b] *A* change at 702 nm × 10^3/ml·mg Chl.

Table II. *Relative Fluorescence Yield of Maize Mutant Leaves*

Mutant *hcf**E	Fluorescence Yield
1231	1.5
1252	3.0
1266	2.5
1277	3.6
1278	5.0
1480	3.0
1481	3.5
1482A	2.6
1482B	4.1
1483	2.1
1489B	4.0
1490	4.2
Wild type	1.0

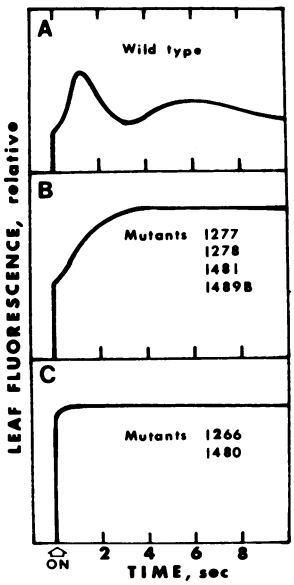

FIG. 3. Fluorescence induction kinetics of whole leaves of normal maize and photosynthesis mutants. A: wild type; B: mutants missing band A-1; C: mutants missing band A-1 or A-1 and A+B complexes.

A second series of lethal, Chl-deficient and highly fluorescent mutants was isolated. They are yellow-green and can be differentiated from the first series in that they lack one or more of the Chl *a*+*b*-containing complexes as well as the A-1 complex (*e.g.* mutants 1490 and 1482B, Fig. 1). Mutant 1490 was found to have no A-1 or AB-1 complexes; the AB-2 complex is present, though much reduced in concentration. Mutants 1266 and 1482B are missing both AB-1 and AB-2 in addition to the A-1 complex. All three mutants retain the A-2 and AB-3 complexes. Fluorescence kinetics of whole leaves (Fig. 4) indicates an inhibition of electron transport through PSI but the leaves of 1482B and 1490 retain some variable fluorescence. Available electron transport data (Table III) support the notion that PSI is inhibited and an active PSII are present in these mutants.

A third series of nonlethal, nuclear mutants of maize (Table IV) have normal quantities of the A-1 and A-2 complex, but have lost one or more of the Chl *b*-containing complexes (see mutant 1483, Fig. 1). These mutants carry out photosynthetic CO_2 fixation at good rates (13), and exhibit lower levels of leaf fluorescence than mutants missing the A-1 complex (Table II). The kinetics of variable fluorescence observed (Fig. 4) indicates a functional PSII; this was confirmed by measurement of electron transport (Table IV). Mutants 1252, 1231, 1483, and 1482A all have specifically lost just one complex, AB-2. The dominant nuclear gene mutant OyYg-700 (30) also lacks AB-2 when grown under low light intensity; when grown under high light intensity (3,000 ft-c) the leaves synthesize little or no Chl *b*, and none of the Chl *b*-containing complexes are seen (Fig. 1). Only two Chl *a*-protein complexes, A-1 and A-2, are observed; the latter band can be seen very distinctly in extracts of this mutant as originally reported by Hayden and Hopkins (13). The Chl *a/b* ratios of the extracts varied from 10 (*e.g.* Table IV) to over 30 (13).

These last results seemed at odds with those for a photosynthetically active Chl *b*-less barley, *chlorina*-f2, which had been reported (21, 35) to contain only one Chl-protein complex. We reexamined this barley mutant and found results identical to those of OyYg-700 maize grown under high light intensity, *i.e.* both the A-1 and A-2 complexes were present (data not shown). Wild type barley contained the same Chl-protein complexes in approximately the same proportions as wild type maize.

DISCUSSION

Studies of higher plant mutants have been useful in establishing the role of Chl-proteins in the photosynthetic process (*e.g.* see 17, 35). The research on the nuclear mutants of maize reported here extends such studies.

Five examples of recessive, nuclear mutations were presented which cause the selective loss of the A-1 complex (23) and the concomitant decrease in PSI electron transport. These data support the contention (23) that this complex represents part of PSI and contains the P700 reaction center. These PSI mutants can be placed into three different groups on the basis of photosynthetic characteristics, but it is unknown if the phenotypic differences are a result of three different genes: the first group includes mutants

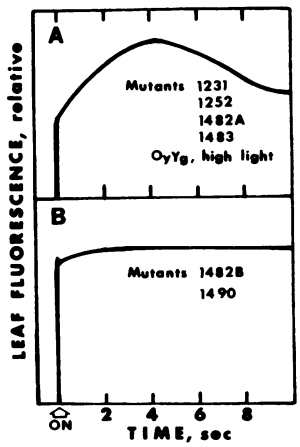

FIG. 4. Fluorescence induction kinetics of whole leaves of maize photosynthesis mutants. A: mutants missing only band AB-2; B: mutants missing A-1, AB-1, or AB-2.

Table III. *Characteristics of Yellow-Green Lethal Maize Mutants Missing Certain Chl-Proteins*

Mutant	Chl *a/b*	Complex Lost	Electron Transport			Cyclic ATP Synthesis
			DPIP → MV	H_2O → FeCN	H_2O → DBMIB	
			μeq/mg Chl·h			*μmol/mg Chl·h*
1266	1.9	A-1	4	37	643	0
		AB-1				
		AB-2				
1490	2.0	A-1	0		91	0
		AB-1				
1482B	1.2	A-1	0	54		
		AB-1				
		AB-2				

Table IV. *Characteristics of Chl-deficient, Viable Mutants of Maize Missing Chl a-b Proteins*

Mutant	Chl *a/b*	Complex Lost	Electron Transport			Cyclic ATP Synthesis
			DPIP → MV	H_2O → FeCN	H_2O → DBMIB	
			μeq/mg Chl·h			*μmol/mg Chl·h*
1252	3.6	AB-2	201	107	168	241
1231	2.7	AB-2	194	98	204	287
1483	2.7	AB-2	125	125		
1482A	4.5	AB-2	110	106		
OyYg-HL[a]	10.0	AB-1				
		AB-2				
		AB-3				
OyYg-LL	3.5	AB-2				

[a] HL, high light—3,000 ft-c; LL, 1,500 ft-c.

1278, 1481, and 1489B which have a reasonably active PSII. The second group, represented by mutant 1277, shows variable fluorescence indicating an available PSII acceptor pool, but has little PSII activity. The third group (mutant 1480) has no detectable PSII activity. It seems reasonable that these three groups probably represent the mutational loss or alteration of three separate genes, although genetic evidence for this is not yet available.

Another group of recessive nuclear mutants (Table III) have lost one or more of the Chl *a+b*-containing complexes (23) in addition to the A-1 complex. This would indicate two different genes which can coordinately affect the synthesis of the A-1 complex as well as the Chl *a+b*-containing complexes.

Previously described PSI-less mutants (6, 8, 10, 11, 16, 17, 36) were nonnuclear mutations. The ones described here and elsewhere (6) are nuclear mutants. This difference is probably best explained by the difference in selection procedure for mutants. Those in maize were selected on the basis of high Chl fluorescence (26) whereas most others were selected by Chl deficiency.

It is not surprising that both mutation types were observed since it is clear that both nuclear and nonnuclear genes in some way code for the A-1 complex (7). The results here provide the first possible indication of a coordinate regulation for the A-1 complex and the Chl *a+b*-containing complexes. The nature of the regulation and its physiological or biochemical significance is not yet understood.

The *in vivo* fluorescence kinetics of the PSI-deficient mutants are identical to those reported for other PSI mutants (8) in that they lack the P to S decline. This could indicate a lack of excitation energy transfer (5) from PSII to PSI (*i.e.* an absence of state 1-state 2 transitions) which would be anticipated if A-1 is absent and if A-1 represents PSI. The above photochemical, electrophoretic, and fluorescence data support a previous suggestion (23) that the A-1 band is equivalent to the previously characterized P700-CP.

We have also reported the resolution of the Chl *a*-containing A-2 band. The function of this complex is uncertain at this time. It may prove to be identical to a Chl *a*-containing complex, migrating at approximately the same position, which has been postulated to contain the PSII reaction center (15, 37). Our data would support this idea.

Examination of the viable mutants which have lost one or more of the Chl *b*-containing complexes appeared to reveal two distinct types of mutation. One type is genetically recessive and causes the loss of only the AB-2 complex (mutants 1252, 1231, 1483, and 1482A—Table IV). The other type is dominant and is represented by the OyYg strain, which when grown under light intensities of 3,000 ft-c loses all three Chl *b*-containing complexes; growth under low light intensity (1,500 ft-c) resulted in the absence of AB-2 only. When these two mutant types are compared with the other mutants described in Tables I and III, it would appear that there are as many as four different genes involved in the synthesis of the Chl *b*-containing complexes, although no firm genetic data are yet available.

Mutants lacking Chl *b* and the LHCP have now been reexamined by the recently improved fractionation system for Chl-protein complexes. We confirm here the earlier results that mutants lacking Chl *b* are deficient in all of the Chl *b*-containing complexes and no new complexes are evident. Apparently none of the Chl *b*-containing complexes we have resolved are absolutely required for photosynthetic function. However, one original, and potentially far reaching, observation was made: genetic mutations can cause the selective disappearance of one or two of the three Chl *b*-containing complexes. Other groups (12, 15, 33) have suggested that the larger Chl *a/b* complexes (*e.g.* AB-1, AB-2) were multimeric forms (dimeric and trimeric) of the smallest. Our data seem to indicate a mutation could eliminate the multimeric form without changing the monomer by this interpretation. The specific loss of AB-1 in mutant 1490, the loss of AB-2 in mutants 1252, 1231, 1483, and 1482A, and the loss of both complexes in mutants 1266 and 1482B could argue against the simple multimer theory. It is difficult to envision a mutational modification that would prevent formation of the dimeric form while permitting stable association of the trimer. It is possible that the AB-1 and AB-2 are composed of some complex-specific, additional component associated with a fundamental Chl *a+b* complex, presumably the AB-3. The additional subunit could itself be a different Chl-protein complex or a colorless protein as has been described for the green alga *Acetabularia* (3). In either case, both the AB-1 and AB-2 should share some polypeptides in common with the AB-3 complex as observed (15, 37). If structurally different forms of the light-harvesting Chl *a+b* complexes proves to be correct, it seems likely that the different complexes will eventually be proven to have functional differences as well.

During our survey we also examined twelve other Chl-deficient mutants. All of these mutants had a full complement of the Chl-protein complexes even though reduced in specific Chl content (data not shown). We conclude that low concentrations of Chl and altered Chl *a/b* are not necessarily indicative of loss or one or more Chl-protein complexes.

Information has been gained about the site of the genes controlling the appearance of the various Chl-protein complexes, either through synthesis or regulation, and the possible coordinate regulation of the different complexes is unclear. The data also raise questions as to the relationship between the various Chl-protein complexes.

LITERATURE CITED

1. ANDERSON JM, RP LEVINE 1974 The relationship between Chl-protein complexes and chloroplast membrane polypeptides. Biochim Biophys Acta 357: 118–126
2. ANDERSON JM, JC WALDRON, SW THORNE 1978 Chlorophyll-protein complexes of spinach and barley thylakoids. FEBS Lett 92: 227–233
3. APEL K 1977 The light-harvesting chlorophyll *a/b* protein complex of the green alga *Aceta-*

bularia mediterranea. Biochim Biophys Acta 462: 390–402
4. BAR-NUN S, I OHAD 1977 Presences of polypeptides of cytoplasmic and chloroplastic origin in isolated photoactive preparations of photosystem I and II in *Chlamydomonas reinhardtii* y-1. Plant Physiol 59: 161–166
5. BONAVENTURA C, J MYERS 1969 Fluorescence and oxygen evolution from *Chlorella pyrenoidosa.* Biochim Biophys Acta 189: 366–383
6. BÖRNER T, B SCHUMANN, S KRAHNERT, M PECHAUF, FH KNOTH, R HAGEMANN 1975 Struktur und Funktion der genetischen Information in den Plastiden. XIII. Lamellar proteine bleichen Plastiden von Plastomund Genmutanten von *Hordeum* and *Lycopersicon.* Biochem Physiol Pflanzen 168: 185–193
7. CHUA N-H, NW GILLHAM 1977 The site of synthesis of the principal thylakoid membrane polypeptides in *Chlamydomonas reinhardtii.* J Cell Biol 74: 441–452
8. CHUA N-H, K MATLIN, P BENNOUN 1975 A chlorophyll-protein complex lacking in photosystem I mutants of *Chlamydomomas reinhardtii.* J Cell Biol 67: 361–377
9. GOLBECK JH, S LIEN, A SAN PIETRO 1977 Isolation and characterization of a subchloroplast particle enriched in iron-sulfur protein and P700. Arch Biochem Biophys 178: 140–150
10. GREGORY RPF, S RAPS, W BERTSCH 1971 Are specific chlorophyll-protein complexes required for photosynthesis? Biochim Biophys Acta 234: 330–334
11. HAGEMAN R, F HERRMANN, T BÖRNER 1972 The use of plastid and gene mutants of higher plants in studying the genetic control of plastid functions. *In* Y Nasyrov, Z Sestak, eds, Genetic Aspects of Photosynthesis. Dr W Junk, The Hague, pp 115–118
12. HAYDEN DB, WG HOPKINS 1976 Membrane polypeptides and chlorophyll-protein complexes of maize mesophyll chloroplasts. Can J Bot 54: 1684–1689
13. HAYDEN DB, WG HOPKINS 1977 A second distinct chlorophyll *a* protein complex in maize mesophyll chloroplasts. Can J Bot 55: 2525–2529
14. HENNINGSEN KW, NC NIELSEN, RM SMILLIE 1974 The effect of nuclear mutations on the assembly of photosynthetic membranes in barley. Portugaliae Acta Biol 14: 323–344
15. HENRIQUES F, RB PARK 1978 Characterization of three new chlorophyll-protein complexes. Biochem Biophys Res Commun 81: 1113–1118
16. HERRMANN F 1971 Genetic control of pigment-protein complexes I and Ia of the plastid mutant En:Alba-1 of *Antirrhinum majus.* FEBS Lett 19: 267–269
17. HERRMANN FH, B SCHUMANN, T BÖRNER, T KNOTH 1976 Struktur und Funktion der genetischen Information in den Plastiden. XII. Die plastidalen Lamellarproteine der photosynthesedefekten Plastommuntante en:gil-1 ("Mrs. Pollock") und der Genmutante "Cloth of Gold" von *Pelergonium zonale* Ait. Photosynthetica 10: 164–171
18. HILLER RG, S GENGE, D PILGER 1974 Evidence for a dimer of the light-harvesting chlorophyll-protein complex. II. Plant Sci Lett 2: 239–242
19. KUNG SD, JP THORNBER, SG WILDMAN 1972 Nuclear DNA codes for the photosystem II chlorophyll-protein of chloroplast membranes. FEBS Lett 24: 185–188
20. LILLEY RMcC, DA WALKER 1973 The measurement of cyclic photophosphorylation in isolated chloroplasts by determination of hydrogen ion consumption. An evaluation of the method using titration at constant pH. Biochim Biophys Acta 314: 354–359
21. MACHOLD O, A MEISTER, H SAGROMSKY, G HØYER-HANSEN, D VON WETTSTEIN 1977 Composition of photosynthetic membranes of wild-type barley and chlorophyll *b*-less mutants. Photosynthetica 11: 200–206
22. MARKWELL JP, CD MILES, RT BOGGS, JP THORNBER 1979 Solubilization of photosystem II from higher plant chloroplasts by two zwitterionic detergents. FEBS Lett 99: 11–14
23. MARKWELL JP, S REINMAN, JP THORNBER 1978 Chlorophyll-protein complexes from higher plants: a procedure for improved stability and fractionation. Arch Biochem Biophys 190: 136–141
24. MILES CD 1975 Genetic analysis of photosynthesis. Stadler Symp 7: 135–154
25. MILES CD 1976 Manganese stimulation of oxygen consumption in chloroplasts with dibromothymoquinone. FEBS Lett 61: 251–254
26. Miles CD, DJ Daniel 1973 A rapid screening technique for photosynthetic mutants of higher plants. Plant Sci Lett 1: 237–240
27. MILES CD, DJ DANIEL 1974 Chloroplast reactions of photosynthetic mutants in *Zea mays.* Plant Physiol 53: 589–595
28. MILLER KR, GJ MILLER, KR MCINTYRE 1976 The light-harvesting chlorophyll-protein complex of photosystem II—its location in the photosynthetic membrane. J Cell Biol 71: 624–638
29. NEUFFER MG 1978 Induction of genetic variability. *In* DB Walden, ed, Maize Breeding and Genetics. John Wiley & Sons, New York, pp 579–600
30. NEUFFER MC 1973 *Yg-4* allelic to *oy* designated *OyYg.* Maize Genet Coop News Lett 47: 149–150
31. ORT DR, S IZAWA 1974 Studies on the energy-coupling sites of photophosphorylation. V. Phosphorylation efficiencies (P/e_2) associated with aerobic photooxidation of artificial electron donors. Plant Physiol 53: 370–376
32. PAPAGEORGIOU G 1975 Chlorophyll fluorescence: an intrinsic probe of photosynthesis. *In* Govindjee, ed, Bioenergetics of Photosynthesis. Academic Press, New York, pp 320–366
33. REMY R, J HOARAU, JC LECLERC 1977 Electrophoretic and spectrophotometric studies of chlorophyll-protein complexes from tobacco chloroplasts. Photochem Photobiol 26: 151–158
34. THORNBER JP, RS ALBERTE, FA HUNTER, JA SHIOZAWA, K-S KAN 1976 The organization of chlorophyll in the plant photosynthetic unit. Brookhaven Symp Biol 28: 132–148
35. THORNBER JP, JR HIGHKIN 1974 Composition of the photosynthetic apparatus of normal barley leaves and a mutant lacking chlorophyll *b.* Eur J Biochem 41: 109–116
36. VERNOTTE C, J-M BRIANTAIS, R REMY 1976 Light-harvesting pigment protein complex requirement for spill-over changes induced by cations. Plant Sci Lett 6: 135–141
37. WESSELS JSC, MT BORCHERT 1978 Polypeptide profiles of chlorophyll-protein complexes and thylakoid membranes of spinach chloroplasts. Biochim. Biophys Acta 503: 78–93