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Euploidy in *Ricinus*[1]

EUPLOIDY EFFECTS ON PHOTOSYNTHETIC ACTIVITY AND CONTENT OF CHLOROPHYLL-PROTEINS

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ABSTRACT

The effects of nuclear genome duplication on the chlorophyll-protein content and photochemical activity of chloroplasts, and photosynthetic rates in leaf tissue, have been evaluated in haploid, diploid, and tetraploid individuals of the castor bean, *Ricinus communis* L. Analysis of this euploid series revealed that both photosystem II (2,6-dichlorophenolindophenol reduction) and photosystem I oxygen uptake (*N,N,N',N'*-tetramethyl-*p*-phenylenediamine to methyl viologen) decrease in plastids isolated from cells with increasingly larger nuclear complement sizes. Photosynthetic O_2-evolution and $^{14}CO_2$-fixation rates in leaf tissue from haploid, diploid, and tetraploid individuals were also found to decrease with the increase in size of the nuclear genome. Six chlorophyll-protein complexes, in addition to a zone of detergent complexed free pigment, were resolved from sodium dodecyl sulfate-solubilized thylakoid membranes from cells of all three ploidy levels. In addition to the P700-chlorophyll *a*-protein complex and the light-harvesting chlorophyll *a/b*-protein complex, four minor complexes were revealed, two containing only chlorophyll *a* and two containing both chlorophyll *a* and *b*. The relative distribution of chlorophyll among the resolved chlorophyll-protein complexes and free pigment was found to be similar for all three ploidy levels.

With the demonstrations that the chloroplast, while possessing the component necessary for autonomy (*i.e.* DNA, DNA and RNA polymerases, and protein synthesis machinery), depends upon a cooperation with the nucleocytoplasmic system in its developmental and biosynthetic activities (9), a new dimension has been added to polyploidy research. To what extent nuclear genome duplication is capable of altering the developmental and biosynthetic activities of chloroplasts is not known.

Previous studies have examined the effects of nuclear polyploidization on chloroplast size and number per cell (4), DNA content (5, 29), and ultrastructure (7). Evidence has also been reported in the literature (7, 8, 22, 23) which suggests that nuclear genome duplication may be involved in the alteration of some photosynthetic and photorespiratory parameters of cells and organisms. Recently, altered nuclear genomic constitution has been correlated with alterations in the efficiency and/or turnover of the photosynthetic light reactions (15, 18). It is not known whether such alteration in plastid function is the consequence of altered chloroplast structure or composition.

The use of different electrophoretic systems (25) to separate both anionic and nonionic detergent-treated thylakoid membrane preparations consistently allowed the identification of at least three Chl-containing zones: CPI,[3] or the P700 Chl *a*-protein complex; CPII, or LHCP; and a zone of free Chl complexed with detergent at the electrophoretic front. Using improved conditions for membrane solubilization and electrophoretic fractionation, numerous workers (*cf.* 25) have been able to identify additional minor Chl-containing zones with electrophoretic mobilities and spectral characteristics different from those of CPI and LHCP. Of the new Chl-containing zones described, two contain both Chl *a* and *b*, and have absorption spectra similar to LHCP (1, 11, 12). These two Chl-protein complexes are thought to be trimeric and dimeric forms of LHCP, and have been designated LHCP[1] and LHCP[2], respectively (1). A third Chl-protein complex, first described by Hayden and Hopkins (10), and subsequently described by other researchers (1, 11, 28) contains primarily Chl *a*. This complex, designated CPa (1), is thought to represent the PSII reaction center complex (10, 14, 28).

Analysis of Chl and photosynthetic mutants of higher plants and algae, which have been shown to alter the content of Chl-protein complexes, has been useful in understanding the biogenesis of the individual complexes. In general, mutant analysis of this type has demonstrated that both nuclear genes and extranuclear factors, presumably genes located in the chloroplast DNA, are involved in, or influence the development of the Chl-protein complexes (19, 20).

An alternative to the mutational approach for the genetic analysis of photosynthesis is the use of nuclear polyploidization. As part of a continuing investigation to explore further the relationship between nuclear genome duplication and chloroplast development and biosynthetic activity, we have examined the Chl-protein content and photosynthetic activity of euploid cells of the castor bean, *Ricinus communis* L. The results of these analyses and the regulation of chloroplast development and biosynthetic activity in the presence of altered nuclear genome size are discussed.

MATERIALS AND METHODS

An euploid series of the castor bean, *R. communis* L., consisting of haploid (1N), diploid (2N), and tetraploid (4N) individuals was

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[3] Abbreviations: CPI, chlorophyll-protein complex I; CPII, chlorophyll-protein complex II; LHCP, light-harvesting chlorophyll *a/b* protein complex; TMPD, *N,N,N',N'*-tetramethyl-*p*-phenylenediamine, DCPIP, 2,6-dichlorophenolindophenol; methyl viologen, 1,1'-dimethyl-4,4'-bipyridinium dichloride.

used in this investigation (26).

Chloroplast Isolation and Purification on Ludox Gradients. Chloroplasts were isolated by a modification of the method described by Ireland *et al.* (13) as described before (27).

Determination of Photosynthetic Parameters in Chloroplasts and Leaf Tissue. The electron transporting capacity of PSI was estimated by following electron flow from TMPD to methyl viologen as described by Nielsen *et al.* (21). PSII activity was determined by following the photoreduction of DCPIP at 590 nm (3).

Photosynthetic O_2-evolution from leaf tissue was measured according to the method recommended by Bahr (personal communication). Freshly harvested leaves were rinsed with deionized H_2O and stored in a deionized water bath held over ice and illuminated by a 300-w incandescent bulb. Leaf segments (0.2–0.4 mm × 10 mm) were mechanically cut, away from conspicuous veins, from the lamina portion of leaves using a modified microtome apparatus. Leaf segments were stored until use (time not exceeding 10 min) in chilled buffer (0.3 M sorbitol, 50 mM Bicine, pH 8.0) under constant N_2 evacuation. Six leaf segments were placed in a reaction vial containing 2.9 ml of the above buffer, and the reaction initiated by the simultaneous addition of 100 μl of 0.4 M $NaHCO_3$ and illumination of the reaction chamber with white light equivalent to 2,000 $\mu E/m^2 \cdot s$. O_2 evolution was measured with a YSI model 53 O_2 monitor equipped with water-jacketed reaction chambers and maintained at 20 to 22 C. Leaf segments were collected and their Chl content determined. Photosynthetic rates were determined from the slopes of linear portions of the recorder tracings of reaction rates over time.

Photosynthetic $^{14}CO_2$-fixation was measured using the procedures described above with the following modifications. Samples of leaf segments were preilluminated in the reaction vials for 1 min prior to the initiation of the reaction. Reactions were initiated by the addition of 100 μl of 0.3 M $NaH^{14}CO_3$ (50 mCi/mmol). Reactions were allowed to proceed for 10 min and the reactions stopped by the removal of the light source and the addition of 0.3 ml of 24 N formic acid to the assay buffer to remove any unreacted $^{14}CO_2$. Chl contents of samples were determined and the total radioactivity in individual samples was measured by liquid scintillometry with ScintiVerse (Fisher Scientific Co.) as the fluor.

Chl was determined in 80% acetone containing 0.2% NH_4OH according to the method of Arnon (2).

Isolation of Chl-protein Complexes by SDS-polyacrylamide Gel Electrophoresis. Thylakoid membranes and samples for electrophoresis were prepared by the procedures outlined by Henriques and Park (11). Chloroplasts were washed with a 1 mM EDTA solution (pH 8.0) and the chloroplast membranes were pelleted at 30,000*g* for 10 min. The pelleted membranes were homogenized in a few milliliters of 0.625 M Tris-HCl, 4% β-mercaptoethanol, 10% glycerol (pH 6.8). The suspensions of membranes diluted to a final concentration of between 0.5 and 1.0 mg Chl/ml. SDS (Bio-Rad) from a stock solution (10% w/v) was added to a final weight ratio of SDS to Chl of 10:1, and the membranes were solubilized with a Potter glass homogenizer.

Polyacrylamide gel electrophoresis was performed using the discontinuous buffer system of Laemmli (17). Slab gels were cast between glass plates (14 × 18 cm) with 0.15 cm spacers, and were comprised of a 1-cm stacking gel and a 9-cm resolving gel containing 5.0 and 9.0% (w/v) polyacrylamide, respectively. Gels were run on a Hoefer Scientific Instruments model SE-500 slab gel apparatus to which a constant current of 10 and 20 mamp/slab gel was applied through the stacking and resolving gels, respectively. All gels were run in the dark at 6 C. Optimal separation of Chl-protein complexes was achieved when the total running time through the gels was between 1.5 to 2.5 h.

Samples containing equivalent amounts of total Chl in membrane preparations from 1N, 2N, and 4N cells were applied to the gels immediately following solubilization with SDS, and were coelectrophoresed in all trials to reduce variation due to run conditions. Best results were obtained when aliquots loaded on gels contained between 25 to 75 μg Chl. Following separation by SDS-polyacrylamide gel electrophoresis, Chl-containing bands were recorded by scanning tube gels at 650, 670, and 675 nm with a Gilford model 240 spectrophotometer equipped with a linear transport device. An estimated relative distribution of Chl in the gels was determined by cutting out and measuring the area under each peak on the recorder tracings using a LI-COR portable area meter model LI-3000 (Lambda Instruments Corp.), averaging the values obtained from tracings at 650, 670, and 675 nm of several representative gels from each of the three ploidy levels, and expressing the area for each peak as a percentage of the total area for all peaks.

Room temperature absorption spectra of Chl-containing bands were measured in slab gel slices in a Cary 17D spectrophotometer. Comparison of absorption spectra from several recordings of representative trials were used to prepare final absorption spectra.

Fluorescence spectra at −196 C were taken in an Aminco-Bowman spectrofluorometer equipped with a R446S Hamumatsu TV photomultiplier. The slab gel slices, after being frozen in liquid N_2, were positioned for front illumination in a Dewar flask. The band pass on the excitation side was 11 and 2.7 nm on the emission side. The spectra were uncorrected for lamp output and emission grating phototube efficiency. Comparison of fluorescence emission spectra from several recordings of representative trials were used to prepare the final fluorescence spectra.

RESULTS

Photosynthetic Activities in Chloroplasts and Leaves. The photosynthetic activity of haploid, diploid, and tetraploid plants of *Ricinus communis* L. was determined by measurement of the rates of photosynthetic O_2-evolution and $^{14}CO_2$-fixation in leaf tissue (Table I).

The data show a significant decrease in the photosynthetic activity of leaf tissue per mg of Chl with the increase in nuclear ploidy level. The rate of photosynthetic O_2-evolution in leaf tissue of 1N individuals was found to be 16 and 33% greater than that observed in leaf tissue from 2N and 4N individuals, respectively. It was also found that the leaves of 1N individuals exhibit a capacity for $^{14}CO_2$-fixation 26 and 37% greater than that observed in leaf tissue from 2N and 4N individuals, respectively.

To take into account differences in leaf thickness and structure which may be present among ploidy levels, the above activities were expressed per fresh weight of leaf tissue. When the data are presented in this manner, a similar trend is observed. Both the O_2-evolving and $^{14}CO_2$-fixing capacity of leaves decreases with the increase in size of the nuclear complement.

The electron transport capacity of chloroplasts of haploid, diploid, and tetraploid cells was estimated by measurement of the PSI and PSII activities in isolated chloroplast preparations from

Table I. *Photosynthetic Activities in Leaves of Haploid, Diploid, and Tetraploid Plants of R. communis L.*

The values represent means from duplicate trials in a series of at least four separate experiments. Values presented for photosynthetic activities as a function of fresh weight of tissue were calculated using values for Chl content presented in Timko *et al.* (26).

Ploidy	O_2-evolved	$^{14}CO_2$-fixed	O_2-evolved	$^{14}CO_2$-fixed
	μmol mg⁻¹ Chl·h⁻¹	*μmol mg⁻¹ Chl·h⁻¹*	*μmol mg⁻¹ fresh wt·h⁻¹*	*μmol mg⁻¹ fresh wt·h⁻¹*
1N	164.6	121.7	0.34	0.25
2N	138.9	90.2	0.25	0.16
4N	110.1	76.7	0.22	0.15

these cells as a function of Chl content (Table II).

PSI activity was observed to decrease with the increase in size of the nuclear genome. Chloroplasts isolated from 1N cells have levels of PSI activity which are 24% higher than plastids isolated from 2N cells, and 42% higher than plastids isolated from 4N cells. PSII activity of plastids from euploid cells also decrease with the increase in size of the nuclear genome. Chloroplasts from 4N cells have PSII activity levels 44% lower than plastids isolated from 1N cells, whereas the PSII activity levels of 2N cells is observed to be 16% lower than the levels observed in plastid preparations from 1N cells. Although the ratio of PSII:PSI activity is slightly higher in chloroplasts of 2N cells than in plastids isolated from 1N and 4N cells, the observed difference is not significant.

Chl-Protein Complexes in Euploid Cells. Seven Chl-containing zones are resolved from thylakoid membranes isolated from 1N, 2N, and 4N *Ricinus* cells (Fig. 1). On the basis of their spectral characteristics (discussed below) and the occurrence of protein following staining, the resolved zones are designated in order of increasing electrophoretic mobility using the terminology of Anderson *et al.* (1) as follows: CP1a, CP1, LHCP[1], LHCP[2], CPa, LHCP[3], and FP. CP1 and LHCP[3] correspond to the P700 Chl *a*-protein complex and the light-harvesting Chl *a/b*-protein complex, respectively. These two Chl-protein complexes have been described and consistently characterized by other researchers (25). The four additional Chl-protein complexes resolved represent minor components of the photosynthetic apparatus which have been described by other researchers and are discussed in a recent review (*cf.* 25).

When aliquots of SDS-solubilized thylakoid membranes from 1N, 2N, and 4N cells, containing equivalent amounts of total Chl are subjected to co-electrophoresis under identical fractionation conditions, the resulting Chl-protein patterns are qualitatively identical. The relative distribution (Table III) of Chl among the complexes is similar for the three ploidy levels. Approximately 21% of the fractionated Chl is found in the CP1 complexes (CPa and CP1); 41 to 42% in the LHCP complexes (LHCP[1], LHCP[2], and LHCP[3]); and 5% of the total is found in CPa. Using the stated electrophoretic conditions, the amount of detergent-complexed free Chl is approximately 25% of the total. It is therefore possible to account for 90% or more of the total Chl fractionated in the above complexes and free pigment. The remaining Chl is associated with unidentified Chl-containing bands which appear as minor peaks on the recorder tracings. The obtained values appear similar to those reported by other researchers (1, 11, 25).

Table II. *Activities of Photosystems in Isolated Chloroplasts of Haploid, Diploid, and Tetraploid Cells*

The values presented in the table represent means from duplicate trials in a series of at least four separate experiments.

Ploidy	PSII Activity (Photoreduction of DCPIP)	PSI Activity (O_2 Consumption)	PSII/PSI
	$\mu mol\ mg^{-1}\ Chl \cdot h^{-1}$		ratio
1N	118.9	211.6	0.56
2N	99.8	161.3	0.62
4N	66.8	123.6	0.54

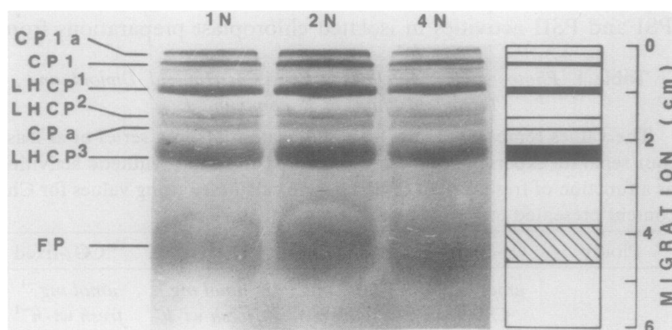

FIG. 1. Photographic representation of Chl-protein complexes resolved by electrophoretic fractionation on slab gels from SDS (SDS: Chl, 10:1) solubilized membranes from euploid *Ricinus* cells.

Room temperature absorption spectra of gel slices containing the Chl-protein complexes resolved from thylakoid membrane preparations of 2N cells are shown in Figure 2. These spectra are typical for Chl-proteins resolved from membrane preparations of all three ploidy levels. The experimental conditions employed did not allow recognition of absolute quantitative differences in absorbance. No qualitative differences are found in the absorption spectra among individual Chl-protein complexes resolved from preparations of thylakoid membranes of cells of different ploidy levels.

The absorption spectra of gel slices containing CP1a (not shown) and CP1 show a similar red maximum for Chl *a* at 673 nm. CPI spectra from euploid *Ricinus* cells are similar to those previously reported for the P700 Chl *a*-protein complex of other plants (25). CPa, which we observed to contain primarily Chl *a*, as indicated by a single absorption maximum at 670 nm, has a spectrum similar to those reported for CPa by Anderson *et al.* (1), complex A (11), and complex IV (12).

The spectra for *Ricinus* LHCP[3], with maxima at 668 and 653 nm, are similar to those previously reported for this complex isolated from other organisms (25). The appearance of the 653 nm peak, characteristic of Chl *b* absorption, demonstrates the increased amount of Chl *b* relative to Chl *a* in this complex. LHCP[1] and LHCP[2] have absorption spectra similar to that of LHCP[3] with maxima at 671 and 653 nm, but show a slight reduction of Chl *b* relative to Chl *a*. A reduction of Chl *b* content in the oligomeric forms of LHCP was also recorded by Henriques and Park (11).

Fluorescence emission spectra at −196 C of gel slices containing Chl-protein complexes from thylakoid membrane preparations of 2N cells are shown in Figure 3. Inasmuch as it was not possible to determine the exact amount of Chl in each of the individual complexes prior to fluorescence measurement and Chl concentrations varied from preparation to preparation, no attempt was made to quantify differences observed among the Chl-protein complexes during measurement of fluorescence emission spectra. The spectra presented are therefore strictly qualitative in nature. On this basis, no differences were recognized among the Chl-protein complexes resolved from thylakoid membrane preparations from cells of different ploidy level. The CP1 complex exhibits two fluorescence bands at 684 and 727 nm. The relative proportions of the bands varied in different CPI preparations within each ploidy level and among ploidy levels. The difference in ratio of the two bands is probably due in part to reabsorption of fluorescence as a result of Chl content of the gel slices and to technical problems involved in the freezing of the samples. The second Chl *a*-protein CPa, exhibits a fluorescence emission peak at 678 nm. LHCP[1], LHCP[2], and LHCP[3] all exhibited very similar fluorescence emission spectra with single maxima at 684, 680, and 682 nm, respectively. The fluorescence emission spectra observed for euploid *Ricinus* cells are similar to those reported for other species (25).

DISCUSSION

The possible effects of nuclear genome duplication on the photosynthetic activity and content of Chl-proteins have been evaluated in haploid, diploid, tetraploid individuals of the castor bean, *R. communis* L. Evidence has been provided suggesting that polyploidization of the *Ricinus* nuclear genome is capable of altering some photosynthetic parameters at the cellular and orga-

Table III. *Distribution of Chl Among the Chl-protein Complexes in Thylakoids from Euploid Ricinus Cells*

Ploidy	Chl *a* / Chl *b*	CPIa	% Pigment in Complexes								
			CPI	LHCP[1]	LHCP[2]	CPa	LHCP[3]	FP	Others	CPIs	LHCPs
1N	2.37	4.3	17.5	12.9	6.5	5.3	22.4	25.2	3.4	21.8	41.8
2N	2.56	4.7	17.0	13.2	6.4	5.5	21.8	25.3	3.0	21.7	41.4
4N	2.43	4.4	17.3	12.4	6.9	5.2	22.7	24.8	3.8	21.7	42.0

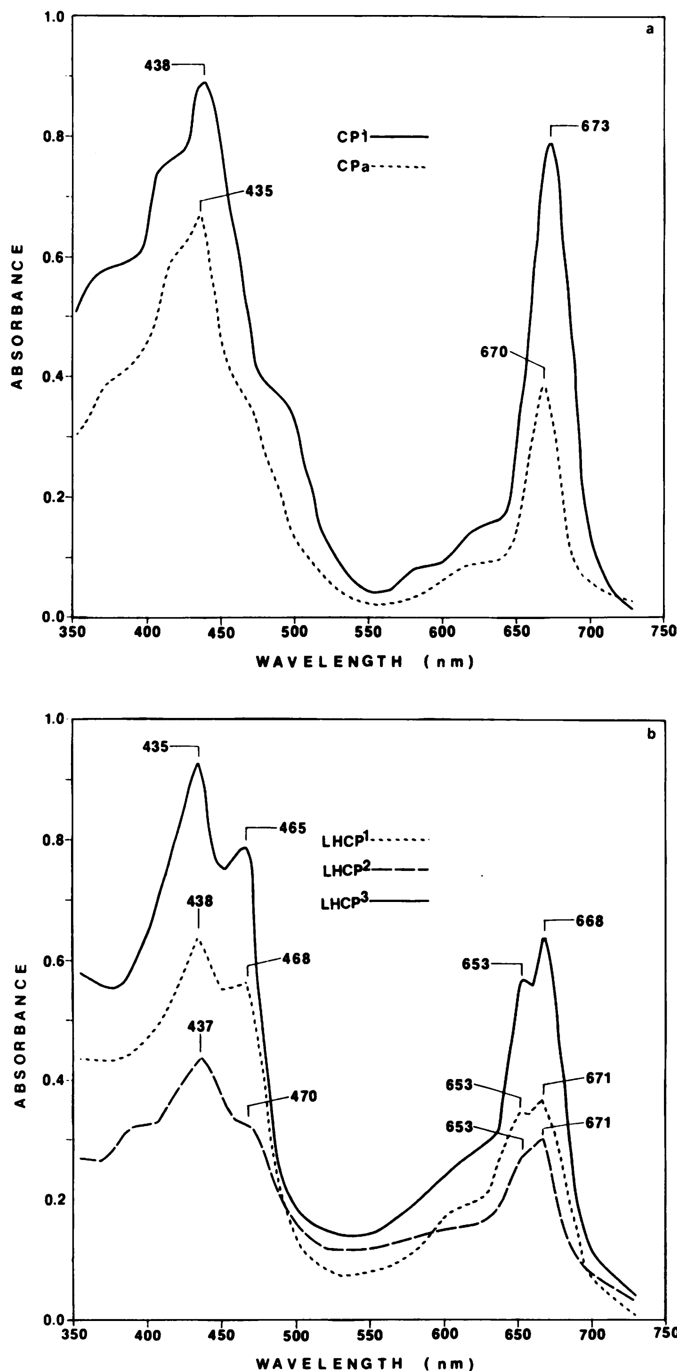

FIG. 2. Room temperature absorption spectra of gel slices containing Chl-protein complexes resolved by polyacrylamide gel electrophoresis of SDS-solubilized thylakoid membranes of diploid cells. a, CP1 and CPa; b, LHCP[1], LHCP[2], and LHCP[3].

nellar level without substantially altering the composition of the photosynthetic lamellae.

Thylakoid membranes of euploid *Ricinus* cells have been found to contain the two major Chl-proteins, CP1 and LHCP[3], described by others (25). Additionally, it was possible to resolve four minor Chl-proteins, two of which contain Chl *a* and two containing both Chl *a* and *b*. The CP1 and LHCP[3] of euploid *Ricinus* cells appear to have spectral characteristics similar to those previously reported by other researchers (1, 11, 25). Of the minor Chl-protein complexes resolved, the two Chl *a*/*b*-protein complexes, LHCP[1] and LHCP[2], have spectroscopical properties similar to that of LHCP[3] and probably represent oligomeric forms (trimer and dimer, respectively) of this complex. This finding is in agreement with previous reports of these complexes in other species (1, 11, 12, 20). The complex termed CP1a in the present investigation is similar to the CP1a described by Anderson *et al.* (1) and most likely represents an oligomeric form of the P700 Chl *a*-protein complex, CP1. The other minor Chl *a*-containing complex isolated from *Ricinus* thylakoid preparations, CPa, appears to have spectral characteristics similar to CPa (1), complex A (11), and complex IV (10). This complex has been suggested as representing the PSII reaction center complex (14, 28). Based upon the relative electrophoretic mobility of this complex under our electrophoretic conditions, and its spectroscopical similarity to reported Chl *a*-containing complexes of comparable nature, it is possible that this complex represents the PSII reaction center complex of the *Ricinus* photosynthetic apparatus.

Our data demonstrate that both the electron transport capacity in isolated chloroplasts and the photosynthetic activity in leaves decrease in response to the increase in the size of the *Ricinus* nuclear genome. Recently, Leto *et al.* (18) and Krueger and Miles (15) have reported increases in electron transport capacity with increased nuclear complement size. Factors responsible for the observed reduction of electron transport capacity in plastids of euploid *Ricinus* cells are not presently known.

The observed decrease in electron transport capacity with increased nuclear ploidy level may reflect a decrease in the numbers of PSI and PSII reaction center complexes in the thylakoid membranes of plastids from cells with increasingly larger nuclear genome sizes. It is also possible that although the numbers of PSI and PSII reaction center complexes may be similar among thylakoid membranes from euploid cells, the number of functional reaction centers is not. The observed decrease in electron transport capacity with increased nuclear ploidy level may reflect a difference in the size of the photosynthetic unit in thylakoids of 1N, 2N, and 4N cells. This is suggested by the fact that equivalent amounts of Chl are less efficient in the capture and/or transfer of light energy equivalents to the reaction center trap molecules in thylakoids of 4N cells compared to those of 2N and 1N cells. Since nuclear genome duplication may substantially alter the cytoplasmic environment in which the plastid must function, the balance between NADP-reduction and photophosphorylation and the metabolic utilization of ATP and NADPH may be altered in a manner which adversely affects the efficient working of the electron transport chain.

Reports on the effects of altered nuclear ploidy level on photosynthetic rates in leaves and organisms vary (7, 8, 22–24). Altered expression of genes for ribulose-1,5-bis-P carboxylase activity and/or some enzymes involved in photorespiratory processes may be responsible in part for the observed reduction in photosynthetic rates in leaves of *Ricinus* euploids in response to

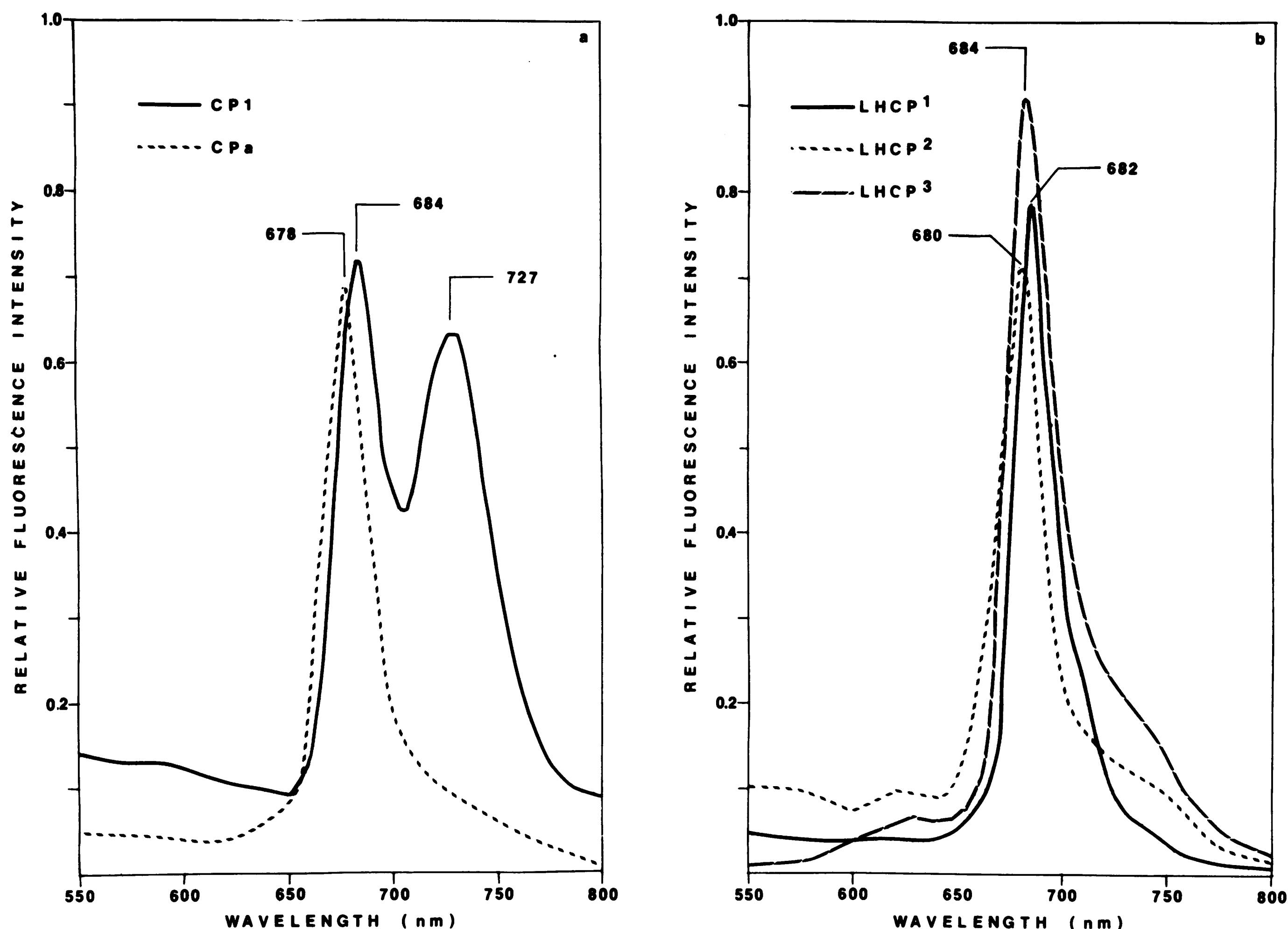

FIG. 3. Fluorescence emission spectra at −196 C of gel slices containing Chl-protein complexes resolved by polyacrylamide gel electrophoresis of SDS-solubilized thylakoid membranes of diploid cells. Excitation was at 435 nm. a, CP1 and CPa; b, $LHCP^1$, $LHCP^2$, and $LHCP^3$.

increased nuclear complement size. Such factors have been implicated in examinations of ploidy effects on photosynthetic activities by other researchers (22, 23). Decreased photosynthetic rates in leaves of *Ricinus* euploids with increased ploidy may also be attributed to a decline in the availability of cofactors necessary for the dark reactions of photosynthesis as a result of the observed reduction in electron transport capacity. Such alterations in cofactor availability will invariably affect the activity levels of enzymes involved in the intermediary steps in these reactions, and may serve as the rate-limiting step in the regulation of photosynthetic activity in the presence of nuclear polyploidization. Inbreeding effects, resulting in the altered anatomical structure of leaves and other organs, associated with colchicine, or similarly artificially induced nuclear polyploidization has also been correlated with decreased photosynthetic activity with increased nuclear complement size (8, 24). It is possible that the observed decreases in both the light and dark reactions of photosynthesis reported in this investigation are characteristic of this experimental *Ricinus* euploid series, and not of those polyploid series found in other species of plants in which hybridization as well as genome duplication have been involved in their origin.

The finding that there exists little difference in the Chl-protein content of thylakoid membranes of cells of different ploidy level raises questions as to the possible regulatory mechanism active in chloroplast development in cells with altered genome size. It is well established that many of the polypeptides of chloroplast thylakoids have their genetic origin in the nuclear DNA and are the products of cytoribosomal translation (9). Evidence presented in the literature (16, 25) demonstrate that among the nuclear encoded thylakoid polypeptides are the constituent polypeptides of the LHCP complex. Chua and his colleagues (6, 9) have also presented data which indicate the possible involvement of nuclear encoded factors in the insertion of CP1 into developing thylakoids.

It is possible that while chloroplasts from cells of different ploidy level are equivalent in their potential for development, factors resulting from the polyploidization of the nuclear genome may influence developmental processes, causing changes in plastid structure, composition, and/or biosynthetic activity. Thus, the polyploidized cell may be faced with the choice of either altering individual plastid structure and composition to contend with potential increased gene expression of either nuclear or chloroplast genome origin, or producing more but identical replicas of the existing model plastid. Our data suggest that any increased synthesis of components involved in the formation of the photosynthetic lamellae, regardless of their genetic origin, are met with a selective uptake and/or assembly into the developing thylakoid membrane. Identical copies of the original prototype membrane are formed, rather than an increased addition of components to the existing membrane. The findings of the present study allow speculation that despite a dependency of the chloroplast on a cooperation with the nucleocytoplasmic system in processes related to lamellar formation, the mechanism responsible for the overall coordination of chloroplast development is under the control of the chloroplast, rather than the nuclear genome. The

mechanism(s) by which such regulation is accomplished remains in the realm of speculation.

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LITERATURE CITED

1. Anderson JM, JC Waldron, SW Thorne 1978 Chlorophyll-protein complexes of spinach and barley thylakoids. FEBS Lett 92: 227–233
2. Arnon DI 1949 Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris.* Plant Physiol 24: 1–15
3. Baker NR, RM Leech 1977 Development of photosystem I and photosystem II activities in leaves of light-grown maize (*Zea mays* L.). Plant Physiol 60: 640–644
4. Butterfass T 1973 Control of plastid division by means of nuclear DNA amount. Protoplasma 76: 167–195
5. Cattolico RA 1978 Variation in plastid number. Effect on chloroplast and nuclear deoxyribonucleic acid complement in the unicellular alga *Olisthodiscus luteus.* Plant Physiol 62: 558–562
6. Chua N-H, K Matlin, P Bennoun 1975 A chlorophyll-protein complex lacking in photosystem I mutants of *Chlamydomonas reinhardtii.* J Cell Biol 67: 361–377
7. De Maggio AE, DA Stetler 1971 Polyploidy and gene dosage effects in chloroplasts of fern gametophytes. Exp Cell Res 67: 287–294
8. Frydrych J 1971 Photosynthetische Aktivatat diploider und tetraploider Formen von *Brassica oleracea* var. gongyloides. Photosynthetica 5: 38–43
9. Gillham NW, JE Boynton, N-H Chua 1978 Genetic control of chloroplast proteins. Curr Top Bioenerget 8: 211–260
10. Hayden DB, WG Hopkins 1977 A second distinct chlorophyll *a*-protein complex in maize mesophyll chloroplasts. Can J Bot 55: 2525–2529
11. Henriques F, RB Park 1978 Spectral characterization of five chlorophyll-protein complexes. Plant Physiol 62: 856–860
12. 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
13. Ireland RJ, V De Luca, DT Dennis 1979 Isozymes of pyruvate kinase in etioplasts and chloroplasts. Plant Physiol 63: 903–907
14. Klein SM, LP Vernon 1974 Polypeptide composition of photosynthetic membranes from *Chlamydomonas reinhardi* and *Anabaena variabilis.* Plant Physiol 53: 777–778
15. Krueger RW, D Miles 1980 The effects of ploidy on electron transport and photophosphorylation in isolated chloroplasts of fescue. Plant Physiol 65: S-9
16. Kung S-D, JP Thornber, SC Wildman 1972 Nuclear DNA codes for the photosystem II chlorophyll-protein of chloroplast membranes. FEBS Lett 24: 185–188
17. Laemmli UK 1970 Cleavage of structural proteins during the assembly of the head of bacteriophage T_4. Nature 227: 680–685
18. Leto K, J Beckett, CJ Arntzen 1979 Effect of nuclear gene dosage on photosynthetic light reactions. Plant Physiol 63: S-160
19. 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
20. Miles CD, JP Markwell, JP Thornber 1979 Effect of nuclear mutation in maize on photosynthetic activity and content of chlorophyll-protein complexes. Plant Physiol 64: 690–694
21. Nielsen NC, RM Smillie, KW Henningsen, D von Wettstein, CS French 1979 Composition and function of thylakoid membranes from grana-rich and grana-deficient chloroplast mutants of barley. Plant Physiol 63: 174–182
22. Randall DD, CJ Nelson, KH Asay 1977 Ribulose bisphosphate carboxylase. Altered genetic expression in tall fescue. Plant Physiol 59: 38–41
23. Rathnam CKM, R Chollet 1980 Photosynthetic and photorespiratory carbon metabolism in mesophyll protoplasts and chloroplasts isolated from isogenic diploid and tetraploid cultivars of rye grass (*Lolium perenne* L.) Plant Physiol 65: 489–494
24. Setter TL, LE Schrader, ET Bingham 1978 Carbon dioxide exchange rates, transpiration, and leaf characters in genetically equivalent ploidy levels of alfalfa. Crop Sci 18: 327–332
25. Thornber JP, JP Markwell, S Reinman 1979 Plant chlorophyll-protein complexes: recent advances. Photochem Photobiol 29: 1205–1216
26. Timko MP, AC Vasconcelos, DE Fairbrothers 1980 Euploidy in *Ricinus.* I. Euploidy and gene dosage effects on cellular proteins. Biochem Genet 18: 171–183
27. Timko MP, RE Triemer, AC Vasconcelos 1980 Biochemical and electron microscopic analysis of the effects of nuclear genome duplication on chloroplast development and biosynthetic activity in euploid *Ricinus* cells. Proceedings of the 5th International Photosynthesis Congress Kassandra-Halkidiki, Greece. In press
28. Wessels JCS, MT Borchert 1978 Polypeptide profiles of chlorophyll-protein complexes and thylakoid membranes of spinach chloroplasts. Biochim Biophys Acta 503: 78–93
29. Whiteway MS, RW Lee 1977 Chloroplast DNA content increases with nuclear ploidy in *Chlamydomonas.* Molec Gen Genet 157: 11–15