FREEZE-FRACTURE ARCHITECTURE AND POLYPEPTIDE COMPOSITION OF THYLAKOID MEMBRANES FROM EUPLOID RICINUS CELLS

M. P. TIMKO*, R. E. TRIEMER AND A. C. VASCONCELOS
Department of Botany and Bureau of Biological Research, Rutgers University, New Brunswick, N.J. 08903, U.S.A.

SUMMARY

The freeze-fracture architecture and polypeptide composition of thylakoid membranes of euploid cells of Ricinus communis L. were examined. Electron microscopic examination of the chloroplasts of 1N, 2N and 4N cells revealed little variation in the size of chloroplasts, lamellar structure and internal organization of plastids, despite increases in plastid numbers per cell observed to accompany the increase in nuclear ploidy. Thylakoid membranes from euploid cells were also similar in their freeze-fracture morphology. Two basic types of intramembranous particles were observed on the fracture faces of thylakoid membranes of euploid cells. The endoplasmic fracture (EF) face of experimentally unstacked thylakoid membranes of 1N, 2N and 4N cells contain 2 size categories of particles (115–121 Å and 164–166 Å), whereas the protoplasmic fracture (PF) face of these membranes contain a single size category of particles (85–88 Å). The distribution and size of the EF- and PF-face particles were found to be similar among membranes from cells of the 3 ploidy levels. Analysis of the polypeptide composition of thylakoid membranes from 1N, 2N and 4N cells revealed no difference in the relative proportion of the constituent polypeptides of these membranes. The possible factors involved in the regulation of the development of thylakoid structure and composition in the presence of altered nuclear genome size are discussed.

INTRODUCTION

The use of freeze-fracture/freeze-etch electron microscopy has permitted the correlation of observed structural entities of the chloroplast thylakoid with the functional components of the photosynthetic apparatus. Analysis of freeze-fractured thylakoid membranes reveals 2 basic types of intramembranous particles (IMP) embedded in these membranes, each differing in cleaving behaviour, as well as in average size and distribution along the membrane (Anderson, 1975; Staehelin, 1975). The larger of the IMP are found on the endoplasmic fracture (EF) face, primarily in stacked regions of the membrane, whereas the smaller IMP are located in both stacked and unstacked membrane regions and appear on the protoplasmic fracture (PF) face (Goodenough & Staehelin, 1971; Staehelin, 1976; Staehelin, Armond & Miller, 1976).

The nature and physiological role of these particles, and their relation to the membrane on the whole, are still speculative. A number of excellent discussions of

* Present address: Institute for Photobiology, Brandeis University, Waltham, MA 02154, U.S.A.
the relationships between freeze-fracture morphology, organization of the photosynthetic apparatus and physiological activity have been published (Anderson, 1975; Arntzen et al. 1976; Arntzen, 1978). It is now generally accepted that the IMP exposed on the PF and EF faces of freeze-fractured thylakoid membranes correspond to the photosystem I (PSI) and photosystem II (PSII) reaction centre complexes and their associated light-harvesting antenna pigments, respectively (Anderson, 1975; Armond, Stacheilin & Arntzen, 1977). Such interpretations are in agreement with data for the asymmetric distribution of the EF and PF-face particles between the 2 complementary fracture faces of thylakoid membranes, and data from biochemical and immunological studies of sidedness of the distribution of the PSI and PSII complexes and the proposed vectoral aspects of the photochemical reactions (cf. Stacheilin et al. 1976).

Data reported in the literature (Chua & Bennoun, 1975; Chua & Gillham, 1977; Gillham, Boynton & Chua, 1978) suggest that the polypeptides associated with the reaction centre activities of PSI and PSII are the products of the synthesis of chloroplast protein, and probably encoded by chloroplast genes. Some investigators have implicated the involvement of nuclear-encoded factors in the development of these activities as well (Chua, Matlin & Bennoun, 1975; Chua & Gillham, 1977). On the other hand, genetic and biochemical analyses (Kung, Thornber & Wildman, 1972; Thornber, Markwell & Reinman, 1979) suggest that the formation of the apoprotein(s) of the light-harvesting chlorophyll a/b-protein (LHCP) complex is controlled by genes located in the nuclear DNA.

As an alternative to the mutational approach in the study of chloroplast development and biosynthetic activity, we have utilized nuclear polyploidization as an experimental tool. We have previously examined the photosynthetic activity of this series and we found evidence suggesting that nuclear polyploidization alters some photosynthetic parameters at the cellular and organellar level (Timko & Vasconcelos, 1981). This study was undertaken in order to ascertain whether the changes observed in the photosynthetic activity arose from altered chloroplast structure. We present here the results of our analysis of the freeze-fracture architecture and polypeptide composition of thylakoid membranes from euploid cells of Ricinus.

MATERIALS AND METHODS

Plant materials

A euploid series of the castor bean, Ricinus communis L., consisting of haploid (1N), diploid (2N) and tetraploid (4N) individuals was examined. This euploid series is of synthetic origin. The 2N and 4N clones were derived from a haploid individual following treatment with colchicine. A description of the series, culture conditions and selection of experimental material has been published (Timko, Vasconcelos & Fairbrothers, 1980).

Chloroplast isolation and purification on Ludox gradients

Crude chloroplasts were isolated by a modification of the procedure of Ireland, De Luca & Dennis (1979). Petioles and major veins were removed from the leaves and 20 g of the remaining leaf material was homogenized in a chilled Waring blender containing 100 ml of isolation buffer (IB) containing: 0.4 M-sorbitol, 45 mM-MES(2-(N-morpholino)-ethanesulphonic acid),
Composition of thylakoid membranes

pH 6.4, 10 mM NaCl, 2 mM Na₂EDTA, 2 mM isoascorbate, 1 mM MgCl₂, 1 mM MnCl₂, 0.5 mM KH₂PO₄, 1 mM-dithiothreitol and 1 mM-glutathione. The homogenate was squeezed through 4 layers of Miracloth, CHICO PEE Mills, Inc., Milltown N.J. U.S.A. filtered through 4 layers of nylon mesh (20 µm pore-size), and centrifuged (3000 g, 30 s). The supernatant was discarded and the resulting pellet was washed with 4 vol. of fresh isolation buffer and centrifuged as above. The crude chloroplast pellet was used for electron microscopic analysis.

Chloroplasts used for electrophoretic examination were further purified by sedimentation through silica sol gradients. Linear gradients of 32 ml were generated from 20% to 80% (v/v) with 14 ml of the isolation buffer and contained polyethylene glycol, Ficoll (Pharmacia Fine Ch., Uppsala, Sweden) and bovine serum albumin at final concentrations of 5%, 2.5% and 0.1% (w/v), respectively. Crude chloroplast preparations were centrifuged (9000 g, 15 min) into the gradients on a Damon/IEC B20-A centrifuge equipped with a model 940 swinging-bucket rotor. Following centrifugation, the band corresponding to intact chloroplasts was carefully removed from the gradients and washed twice with fresh isolation buffer.

Cell volumes and chloroplast numbers

Cell volumes were determined for palisade-layer parenchyma cells using Nomarski optics as described by Timko et al. (1980). Estimates of the number of chloroplasts per cell were obtained using the methods described by Wildman, Jope & Atchinson (1974). A total of 250 palisade parenchyma cells were examined in each observation, and experiments were repeated a minimum of 4 times for all determinations.

Electron microscopy

Freshly harvested leaves were selected and cut into pieces 1 mm³. Tissue was fixed for 2 h in 2% (v/v) glutaraldehyde in 0.1 M phosphate buffer (pH 6.8), rinsed 3 times in buffer (15 min each change), and post-fixed in 2% (w/v) OsO₄ in the same buffer. Samples were rinsed briefly in deionized water, dehydrated in a graded ethanol series and transferred to acetone. Fixation and dehydration were carried out in the dark at 4 °C. Tissue was infiltrated with Epon, embedded, and polymerized at 60 °C.

Chloroplasts were fixed by diluting the chloroplast suspensions with IB, containing 2% (v/v) glutaraldehyde and 1 mM CaCl₂. After 30 min the chloroplasts were pelleted (3000 g, 2 min) and washed twice with fresh IB. The plastids were then post-fixed for 30 min with 2% (w/v) OsO₄ in 0.1 M phosphate buffer (pH 6.4). Fixation, dehydration and embedding procedures were as above. Silver/gold sections were cut on a Sorvall MT 2B ultramicrotome, post-stained with uranyl acetate and lead citrate, and observed with a Siemens 1A electron microscope operating at 80 kV.

Freeze-fracture analysis

Chloroplasts used for freeze-fracture analysis were treated as described by Armond et al. (1977). Crude chloroplast pellets were resuspended, washed once with 50 mM-Tricine (pH 7.8), centrifuged (3000 g, 30 s), and the resulting pellet resuspended in 5 mM-Tricine (pH 7.8). Samples were then incubated at 4 °C for 30 min, followed by 10 min at 20 °C to allow for the unstacking of membranes and the intermixing of IMP from stacked and unstacked membrane regions. Glycerol was slowly added to the samples to a final concentration of 30% (v/v) over a 30 min period. The membranes were then pelleted (10000 g, 5 min). Aliquots were frozen in liquid Freon 22 and stored in liquid nitrogen. Replicas were prepared on a Balzers BAF-301 high-vacuum freeze-etch apparatus (Balzers High Vacuum Corp.), and observed.

Measurement of structural parameters

The procedures for determination of particle size and distribution were modelled after those described by Staehelin (1976). Particle distribution counts were made on 8-10 micrographs of large flat membrane regions (photographed at x 27000 and enlarged to x 100000) by imposing a transparent sheet of graph paper and counting all particles within quadrants.
Particle sizes were determined from micrographs, taken at $\times 27\,000$ and enlarged to $\times 200\,000$, and viewed through a $\times 7$ objective lens equipped with a micrometer grating. The width of a shadow of a given particle was measured over the shadowed half of the particle. Where the edge of the particle appeared fuzzy, or irregular, a minimum width was always taken. Between 750 and 850 particles were measured in large continuous areas of membrane on 8 to 10 micrographs to prepare each histogram of particle size distribution that is presented.

**Statistical considerations in freeze-fracture analysis**

Following accumulation of data on particle-size distribution, mean particle diameter ($\bar{X}$), standard deviation ($\sigma$), and standard error of the mean ($\sigma_m$) were calculated for PF- and EF-face particles. To determine whether differences in the measured parameters for particles observed in membrane preparations from $1N$, $2N$ and $4N$ cells were statistically significant, an Analysis of Variance was employed.

**Electrophoretic analysis**

Gradient purified chloroplasts were washed twice with a 1 mM-EDTA solution (pH 8.0) and centrifuged (10000 $g$, 10 min). Membranes were either solubilized immediately in the buffer described by Laemmli (1970), or heated for 2 min in a boiling-water bath immediately following solubilization. Samples applied to the gels consisted of aliquots of unheated or heated membrane preparations containing equivalent amounts of total chlorophyll, or of acetone-insoluble precipitate equivalent to that amount of total chlorophyll. Membrane preparations from all 3 ploidy levels were co-electrophoresed in all trials to reduce variation due to the conditions of the experiment.

Electrophoretic fractionation of thylakoid membrane samples was performed using the discontinuous buffer system of Laemmli (1970) modified to be consistent with the procedure for polyacrylamide-gradient gel electrophoresis as described by Chua & Bennoun (1975). Slab gels were cast between glass plates (25 cm x 35 cm) with 0.15-cm spacers, and were composed of a 1.0 to 1.5 cm stacking gel and a 20 cm resolving gel. The stacking gel contained 5.0% (w/v) acrylamide, whereas the resolving gel was made up of a linear gradient of acrylamide from 7.5% to 15% (w/v), stabilized by a 5.0% to 17.5% (w/v) sucrose gradient in the gel. Urea (4 M) was included in both the stacking and resolving gels. Slab gels were run at a constant current of 25 mA per slab gel through both the stacking and resolving gels, in the dark at 4 °C. The average length of run was between 12 and 15 h.

Following electrophoresis, the gels were fixed and stained in 50 % methanol containing 0.15% (w/v) Coomassie brilliant blue G250, and destained by repeated washes in 30 % methanol/7 % acetic acid solutions. Densitometric profiles of the polypeptide compositions of thylakoid membrane preparations were prepared using an ORTEC model 4310 densitometer equipped with an area integration function. Molecular weights of polypeptides were estimated from a plot of the molecular weight versus distance migrated, obtained with the following standard proteins (mol. wt in parenthesis): bovine serum albumin (68 x 10^3); catalase (60 x 10^3); ovalbumin (43 x 10^3); carbonic anhydrase (29 x 10^3); ribonuclease (13.7 x 10^3); and cytochrome c (11.7 x 10^3).

Chlorophyll content of the samples was determined according to Arnon (1949).

**RESULTS**

**Ultrastructure of chloroplasts**

Examination of the chloroplasts of haploid (1N), diploid (2N) and tetraploid (4N) cells of *Ricinus* by electron microscopy in intact tissue and their following isolation revealed little variation in the structure or internal organization of plastids. The number of thylakoids per granum ranged from 3 to 12, with average granas containing
Composition of thylakoid membranes

7–8 lamellae each in plastids obtained from the 3 ploidy levels. The ratio of grana to stroma lamellae was found to be similar in chloroplasts examined from 1N, 2N and 4N cells.

Chloroplast size, as estimated by light microscopy and the measurement of electron photomicrographs, revealed that plastids at each ploidy level ranged from 3.5 to 7.0 μm in length with a thickness from 1.2 to 3.0 μm. The average size of plastids did not differ significantly between the ploidy levels.

Table 1. Characteristics of haploid, diploid and tetraploid cells of Ricinus communis L.

<table>
<thead>
<tr>
<th>Ploidy</th>
<th>1N</th>
<th>2N</th>
<th>4N</th>
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<tbody>
<tr>
<td>Cell volume (μm³ × 10⁴ ± S.E.)</td>
<td>20.13 ± 0.07</td>
<td>38.82 ± 0.05</td>
<td>67.27 ± 0.06</td>
</tr>
<tr>
<td>Chloroplast no. per cell (X)</td>
<td>13.2</td>
<td>21.0</td>
<td>36.6</td>
</tr>
</tbody>
</table>

Analysis of the number of plastids per palisade parenchyma cell of 1N, 2N and 4N individuals revealed that the number of chloroplasts per cell increases with the increase in size of the nuclear genome (Table 1). The number of chloroplasts per cell increased 1.6-fold for the first doubling of the nuclear complement. Tetraploid cells exhibited a 1.8-fold increase in chloroplast number over that recognized at the diploid level.

Polypeptide composition of thylakoid membranes

Samples of EDTA-washed thylakoid membranes from 1N, 2N and 4N cells were fractionated by sodium dodecyl sulphate (SDS)/polyacrylamide-gradient gel electrophoresis. The results of a typical fractionation are presented in Fig. 1. Examination of the polypeptide composition of these membranes visually, and by comparison of their densitometric profiles, revealed no qualitative differences in the complement of constituent polypeptides resolved from thylakoid membrane preparations of cells of different ploidy levels.

Heated membrane preparations show a greater number of resolved polypeptide bands than unheated preparations (42 versus 34 polypeptides, respectively) at the 3 ploidy levels. Based upon our previous examination of the chlorophyll–protein complexes of these membranes (Timko & Vasconcelos, 1981) it was possible to identify 3 of the chlorophyll-containing bands on gels of unheated membrane samples: chlorophyll a–protein complex I (CP1), light-harvesting chlorophyll a/b–protein complex (LHCP), and a minor chlorophyll-a-containing complex (RCPsII), which we believe represents the reaction centre complex associated with PS II. In unheated membrane preparations CP1 migrates at a mol. wt of approximately 100–110 (× 10³), whereas the apoprotein of this complex, visible only in heated membrane preparations, has an apparent mol. wt of 68 × 10³. The LHCP complex of euploid Ricinus cells contains 3 constituent polypeptides of apparent mol. wts 28, 25 and 23 × 10³. A
single polypeptide moiety of mol. wt $46 \times 10^3$ is associated with the minor chlorophyll-$a$-containing complex, RCPSII.

The content of protein in each band on the densitometric profiles was determined from the amount of bound stain by calculating the area under each peak on the profile. Comparison of the relative area (data not shown) corresponding to individual peaks on the profiles revealed the existence of little significant variation in the relative proportions of the individual polypeptide components, regardless of whether unheated or heated membrane preparations from the 3 ploidy levels were used for comparison.

**Freeze-fracture analysis of thylakoid membranes**

Two distinctly different types of membrane subunits can be distinguished on the thylakoid membranes of euploid *Ricinus* cells (Figs. 2–4). We have designated these fracture faces as EF and PF (Branton *et al.* 1975), corresponding to the endoplasmic and protoplasmic fracture faces, respectively. The freeze-fracture appearance of thylakoid membranes from euploid *Ricinus* cells is similar to that reported for mature thylakoids from other species (Goodenough & Staehelin, 1971; Staehelin,
Composition of thylakoid membranes

Fig. 1. Densitometric profiles of thylakoid membrane polypeptides resolved by SDS/polyacrylamide gradient gel electrophoresis of heated (b) and unheated (A) membrane samples from haploid, diploid and tetraploid cells. All samples shown contained 50 μl total chlorophyll. CPI, chlorophyll a-protein complex; apo CPI, apoprotein CPI; LHCP, light-harvesting chlorophyll a/b-protein complex; RCPSII, reaction centre PSII.

The EF face of the thylakoid membranes of euploid cells is characterized by a population of relatively large, widely spaced particles. In contrast, the PF face reveals the presence of tightly packed, smaller particles, which are more numerous than those observed on the EF face. Comparison of thylakoid membranes isolated from 1N, 2N and 4N cells revealed no detectable differences in the general freeze-fracture morphology or cleaving behaviour of membranes. To determine whether there are differences in the number of IMP within thylakoid membranes of 1N, 2N and 4N cells, particle density measurements were made of the EF- and PF-face particles of mature thylakoids from cells of the 3 ploidy levels. To obtain particle density values that would average out the differences in particle distribution between stacked and unstacked membrane regions (Ojakian & Satir, 1974; Staehelin, 1976; Armond et al. 1977), we have experimentally unstacked the thylakoid membranes by washing them in solutions containing low cation concentrations.
Composition of thylakoid membranes

The freeze-fracture appearance of experimentally unstacked thylakoid membranes of euploid *Ricinus* cells are illustrated in Figs. 2–4. It is evident from our observations of these membranes that the particles from stacked and unstacked regions have become evenly distributed along their respective membrane surfaces as a result of the low-salt treatment. Particle density measurements, taken on electron photomicrographs of similar quality to those presented, show that there is little variation in the density of particles on PF and EF faces among membranes prepared from cells of different ploidy level (Table 2). The ratio of PF to EF-face particles in thylakoid membranes of 1N, 2N and 4N cells was also found to be similar. The possible changes in particle size on PF and EF faces as a result of nuclear polyploidization have also been evaluated. Histograms of particle diameters for PF and

<table>
<thead>
<tr>
<th>Ploidy</th>
<th>EF fracture face (particles/m^2 ± σ_m)</th>
<th>PF fracture face (particles/m^2 ± σ_m)</th>
<th>Ratio of PF to EF particles</th>
</tr>
</thead>
<tbody>
<tr>
<td>1N</td>
<td>946 ± 22</td>
<td>3250 ± 60</td>
<td>3.4</td>
</tr>
<tr>
<td>2N</td>
<td>938 ± 25</td>
<td>3388 ± 68</td>
<td>3.6</td>
</tr>
<tr>
<td>4N</td>
<td>978 ± 35</td>
<td>3518 ± 81</td>
<td>3.6</td>
</tr>
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See Table 3 for definitions of m and σ_m.

EF-face particles are presented in Figs. 5 and 6 (and Tables 3 and 4), respectively. The histograms presented in Fig. 5 (and Table 3) demonstrate that the PF face of experimentally unstacked thylakoid membranes of euploid *Ricinus* contain a single size class of particle, with a mean diameter of 85–88 Å. The slight decrease in mean particle diameter observed to accompany increased nuclear ploidy level is not significant (F = 0.05). The histograms of particle diameters for EF-face particles (Fig. 6 and Table 4) reveal the presence of two distinct size classes of particle on EF faces of experimentally unstacked *Ricinus* thylakoids. The smaller class (< 140 Å) has a mean diameter between 115 and 121 Å, and the larger class (> 140 Å) has a mean value for particle diameter between 164 and 166 Å. The mean diameter for particles > 140 Å is not significantly different (F = 0.05) among ploidy levels, whereas the mean diameter of the < 140 Å particles shows a slight, but significant increase with the increase in nuclear ploidy level.

A comparison of the distribution of numbers of particles in the 2 size classes on EF faces shows that there is an increase in the numbers of particles in size classes

Figs. 2–4. Fracture faces of experimentally unstacked thylakoid membranes of euploid *Ricinus* cells. EF, endoplasmic fracture face; PF, protoplasmic fracture face.

Fig. 2. Experimentally unstacked thylakoid membranes from haploid cells, × 61 600.
Fig. 3. Experimentally unstacked thylakoid membranes from diploid cells, × 57 000.
Fig. 4. Experimentally unstacked thylakoid membranes from tetraploid cells, × 59 000.
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> 140 Å, and a corresponding reduction in the proportion of particles in size classes < 140 Å in progressing from the 1N to 4N nuclear complement size. The mean diameter of > 140 Å size class of particles is not significantly different among ploidy levels, whereas the mean diameter of the < 140 Å particles is significantly different (F = 0.05) when 1N and 4N values are compared. The mean diameter for all EF-face particles in thylakoid membranes of euploid cells shows an increase of approximately 10 Å when 1N and 4N values are compared. This increase is significant (F = 0.05) only when 1N and 4N values are compared.

![Histograms demonstrating particle-size distributions of PF-face particles of experimentally unstacked thylakoid membranes of 1N, 2N and 4N cells.](image)

**Table 3. PF-particle size distribution**

<table>
<thead>
<tr>
<th></th>
<th>1N</th>
<th>2N</th>
<th>4N</th>
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<tr>
<td>(\eta)</td>
<td>794.0</td>
<td>800.0</td>
<td>801.0</td>
</tr>
<tr>
<td>(\bar{X})</td>
<td>88.2 Å</td>
<td>86.1 Å</td>
<td>85.4 Å</td>
</tr>
<tr>
<td>(\sigma)</td>
<td>22.2 Å</td>
<td>24.5 Å</td>
<td>23.6 Å</td>
</tr>
<tr>
<td>(\sigma_m)</td>
<td>0.8 Å</td>
<td>0.9 Å</td>
<td>0.8 Å</td>
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* \(\eta\), number of particles; \(\bar{X}\), mean diameter; \(\sigma\), standard deviation; \(\sigma_m\), standard error of the mean; \(m\), mean.

**DISCUSSION**

The possible effects of altered nuclear complement size on the ultrastructure of chloroplasts, and the freeze-fracture morphology and polypeptide composition of thylakoid membranes, have been evaluated in a euploid series of the castor bean, *R. communis* L. Examination of chloroplasts from haploid (1N), diploid (2N) and tetraploid (4N) cells by transmission electron microscopy revealed little variation in the ultrastructure of plastids or in the organization of the photosynthetic lamellae,
Composition of thylakoid membranes

Fig. 6. Histograms demonstrating particle-size distributions of EF-face particles of experimentally unstacked thylakoid membranes of 1N, 2N and 4N cells. The measured percentages of particles less than and greater than 140 Å are indicated by the arrows.

Table 4. EF-particle size distribution

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<tbody>
<tr>
<td>&lt;140 Å</td>
<td>&gt;140 Å</td>
<td></td>
<td></td>
</tr>
<tr>
<td>η (%)</td>
<td>61.6</td>
<td>38.4</td>
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Table 4: EF-particle size distribution

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Despite increases in chloroplast number per cell that were observed to accompany increased nuclear ploidy. Further examination of the thylakoid membranes of plastids from euploid cells by the freeze-fracture technique has demonstrated that these membranes contain the 2 basic types of IMP characteristic of mature chloroplast thylakoids (Goodenough & Staehelin, 1971; Staehelin, 1975; Anderson, 1975; Arntzen, 1978). Thylakoid membranes of euploid cells possess EF faces containing large, widely spaced particles, whereas the PF faces of these membranes contain
smaller particles, which are more numerous than those on the EF face. The distribution of EF- and PF-face particles on thylakoid membranes from 1N, 2N and 4N cells was found to be similar. The distribution of IMP on thylakoid membranes of euploid Ricinus cells is similar to that observed in other species (Ojakian & Satir, 1974; Staehelin, 1976; Staehelin et al. 1976; Armond et al. 1977).

Analysis of the size distribution of particles on PF and EF faces of experimentally unstacked thylakoid membranes revealed the presence of a single size category of particles (85-88 Å) on the PF face, and 2 size categories of particles (115-121 Å and 164-166 Å) on the EF face. The mean diameters for EF- and PF-face particles on experimentally unstacked thylakoid membranes reported in the present investigation are similar to those reported for thylakoid membranes in other species (Ojakian & Satir, 1974; Staehelin, 1976; Staehelin et al. 1976).

Comparison of the distribution of particle size on PF faces of experimentally unstacked thylakoid membranes from euploid cells of different ploidy levels has shown that the mean particle diameters for these particles are not significantly different. The mean diameters for all EF-face particles, and for EF-face particles in size classes < 140 Å are significantly different when values from 1N and 4N cell preparations are compared. Our analysis has shown that the increase in size for all EF-face particles, observed to accompany increased nuclear ploidy level, results from the increase in size of the particles in size classes < 140 Å, and from the increased proportion of particles in size classes > 140 Å. The increase in EF-face particle size observed to accompany increased ploidy level may also have arisen as a result of the procedures used to randomize particles from stacked and unstacked membrane regions. Staehelin (1976) has presented evidence that suggests that during the experimental unstacking of thylakoid membranes there is a destabilization of the association between PSII core complexes and LHCP. Such a destabilization allows for the free exchange of LHCP molecules among PSII reaction centres, and is capable of resulting in alterations in the proportions of numbers of particles found in size classes < 140 Å and > 140 Å, when measured and calculated values for particle-size distribution on experimentally unstacked membranes are compared. Our finding that the proportions of particles in size classes < 140 Å and > 140 Å differ among ploidy levels may reflect minor variations in the degree of LHCP exchange among PSII reaction centre complexes as a result of experimental unstacking. Given the magnitude of the difference between 1N and 4N cell preparations in mean particle diameter for all EF-face particles, and EF-face particles in size classes < 140 Å (10 and 6 Å, respectively), and the possibility that the ability to exchange LHCP may differ among PSII core complexes in thylakoids from cells of different ploidy levels, we believe that the observed differences in EF-face particle size among ploidy levels reflect alterations in membrane architecture as a result of the experimental unstacking, and not true differences between the ploidy levels.

It is now generally accepted that the IMP exposed on the PF and EF faces of freeze-fractured thylakoids correspond to the morphological equivalent of the PSI reaction centre complex and the PSII reaction centre complex with its associated LHCP, respectively (Arntzen et al. 1976; Staehelin, 1976; Armond et al. 1977;
Composition of thylakoid membranes

Arntzen, 1978). Data have also been reported in the literature (Chua & Bennoun, 1975; Chua et al. 1975; Chua & Gillham, 1977; Gillham, 1978) that suggest that the polypeptides associated with the reaction-centre activities of PSI and PSII are the products of synthesis of chloroplast protein and are probably encoded by chloroplast genes. On the other hand, genetic and biochemical analyses (Kung et al. 1972; Thornber et al. 1979) suggest that the formation of the constituent polypeptide(s) of the LHCP complex is controlled by genes in nuclear DNA.

Data from the examination of the polypeptide composition of the thylakoid membranes of 1N, 2N and 4N cells have demonstrated that there are no qualitative or quantitative differences in the constituent polypeptides of these membranes. We have detected no difference in the relative proportions of the apoproteins of PSI (CPI), PSII (RCPSII), or LHCP in thylakoids from cells of different ploidy levels. The observation that the content of PSI apoproteins is similar among thylakoid membranes from 1N, 2N and 4N cells is consistent with the observed lack of variation in the size and distribution of the PF-face particles in these membranes. Finding that the size and distribution of PF-face particles, and the content of PSI apoproteins, are similar among membranes from euploid cells is in agreement with the suggestions in the literature that the formation of this complex is under the control of the chloroplast genome. Therefore the lack of variation we observed is what would be expected if increased nuclear complement size has no influence on chloroplast development. Similarly, our finding that the content of the PSII apoproteins, and the numbers of EF-face particles, are similar among thylakoid membranes from euploid cells is also in agreement with the postulate of chloroplast control of the development of this reaction centre. The reported formation of the constituent polypeptides of the LHCP complex on cytoplasmic ribosomes and their possible encoding in the nuclear DNA (Kung et al. 1972; Thornber et al. 1979) suggests that the observed lack of variation in the mean diameters of EF-face particles among thylakoid membranes from euploid cells may result from the differential uptake and/or incorporation of these polypeptides into the thylakoid membranes of plastids from cells of different ploidy levels. Arntzen and co-workers (Arntzen et al. 1976; Armond et al. 1977) have demonstrated that during lamellar greening there is an increase in the size of EF-face particles correlated with the insertion of the constituent polypeptides of the LHCP into the membrane. They have suggested that the observed size categories of EF-face particles represent varying amounts of LHCP associated with PSII ‘core’ complexes in the membrane. Our finding that the relative proportion of the constituent polypeptides of the LHCP among thylakoid membranes of euploid Rictinus cells is similar indicates that the uptake of the constituent polypeptides of the LHCP complex by the chloroplast and their assembly into developing membranes may be regulated by the chloroplast genome. Therefore, regardless of any possible increase in the synthesis of the constituent polypeptides of the LHCP as a result of genome duplication, chloroplasts from cells with different ploidy levels will utilize identical amounts of these polypeptides in the formation of individual reaction centres.

The data of the present investigation allow speculation that, despite a dependency of the chloroplast on cooperation with the nucleocytoplasmic system in processes
related to the formation of the thylakoid membranes, the assembly and overall development of the photosynthetic lamellae are coordinated and regulated by the chloroplast genome. We suggest that any increase in the synthesis of components involved in the formation of the individual structural entities, or development of all the photosynthetic lamellae in cells with altered nuclear genomic constitution, regardless of the genetic origin of these components, is met with a selective uptake and/or assembly of these components into the developing thylakoids. The mechanism(s) by which chloroplasts alone, or in conjunction with the nucleocytoplasmic system, regulate such activities in cells with altered nuclear complement size remains in the realm of speculation.

REFERENCES


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