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A CHLOROPLAST MEMBRANE LACKING PHOTOSYSTEM II

THYLAKOID STACKING IN THE ABSENCE OF THE PHOTOSYSTEM II PARTICLE

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Summary

The polypeptide composition and membrane structure of a variegated mutant of tobacco have been investigated. The pale green mutant leaf regions contain chloroplasts in which the amount of membrane stacking has been reduced (although not totally eliminated). The mutant membranes are almost totally deficient in Photosystem II when compared to wild-type chloroplast membranes, but still show near-normal levels of Photosystem I activity. The pattern of membrane polypeptides separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis shows several differences between mutant and wild-type membranes, although the major chlorophyll-protein complexes described in many other plant species are present in both mutant and wild-type samples. Freeze-fracture analysis of the internal structure of these photosynthetic membranes shows that the Photosystem II-deficient membranes lack the characteristic large particle associated with the E fracture face of the thylakoid. These membranes also lack a tetramer-like particle visible on the inner (ES) surface of the membrane. The other characteristics of the photosynthetic membrane, including the small particles observed on the P fracture faces in both stacked and unstacked regions, and the characteristic changes in the background matrix of the E fracture face which accompany thylakoid stacking, are unaltered in the mutant. From these and other observations we conclude that the large (EF and ES) particle represents an amalgam of many components comprising the Photosystem II reaction complex, that the absence of one or more of its components may prevent the structure from assembling, and that in its absence, Photosystem II activity cannot be observed.

Abbreviation: DCIP, dichlorophenol indophenol.

Introduction

The structures visualized by freeze-fracturing and other techniques for electron microscopy within the photosynthetic membranes of higher plants have generated a great deal of speculation as to their actual identity and their relationship to the light reaction of photosynthesis. A recent review by Staehelin and his associates [1] suggested a model for the membrane where the two general classes of structures ('large' particles on the E fracture face: and 'small' particles on the P fracture face) corresponded to Photosystem I and Photosystem II reaction center complexes, surrounded by differential complements of 'light-harvesting protein-pigment complex'. Recent reports of photosynthetic membrane systems lacking this light-harvesting complex have noted a reduction in diameter of the EF particle, an observation consistent with the Staehelin et al. model [1--3].

One of the consistent findings of workers investigating the organization of the photosynthetic membrane system has been the concentration of large (EF) particles in regions where the chloroplast membranes are stacked together to form the grana so characteristic of higher plant chloroplasts. Several groups have documented the migration of these particles into stacked regions when artificially unstacked membranes are restacked [4—6], and several models for the macromolecular processes associated with thylakoid membrane stacking have argued for the intimate involvement of the large particle [1,2,4,7]. We report here on our studies of a Photosystem II-deficient mutant of a higher plant chloroplast showing that thylakoid membrane stacking can occur in the virtual absence of this large particle, and yielding new insights as to the nature of the Photosystem II reaction center in the thylakoid membrane.

Materials and Methods

The tobacco plants used were planted in vermiculite, grown under natural lighting in a greenhouse, and watered with modified Hoagland's solution. The NC 95 variegated mutant was kindly provided by Professor Peter Homann of Florida State University.

Mutant chloroplasts were isolated from the pale green sections of young (less than 2 weeks old) variegated leaves of plants of various ages, while the control chloroplasts were taken from fully greened leaves of wild-type plants. The leaf sections were cut into small strips which were then ground in isolation buffer (300 mM NaCl, 2 mM $MgCl_2$, 50 mM Tricine/NaOH, pH 7.5) at high speed in a blender for 15 s, or with a mortar and pestle for 30 s. After filtering through eight layers of cheesecloth, the solution was centrifuged at $300 \times g$ for 3 min, and the supernatant centrifuged at $3000 \times g$ for 10 min. The resulting pellet was resuspended in the isolation buffer and pelleted again at $3000 \times g$. The pellet was then resuspended in a low-salt buffer (2 mM $MgCl_2$, 5 mM Tricine/NaOH, pH 7.5) to remove chloroplast outer membranes, and centrifuged at $6000 \times g$ for 10 min to pellet the thylakoid membranes.

Photosystem assays were carried out on the supernatant from the $300 \times g$ spin (above procedure). First, chlorophyll determinations were made from 80% acetone extracts of the material, using Arnon's equations for calculating chloro-

phyll *a*, chlorophyll *b*, and total chlorophyll concentrations [28]. Samples were then diluted to equal chlorophyll concentrations and added to the reaction mixtures for the Photosystem I and Photosystem II assays. The Photosystem I assay measured methyl viologen reduction from dichlorophenol indophenol (DCIP) and ascorbate, as monitored by oxygen consumption with a Clark oxygen electrode [8]. The reaction mixture contained 1 mM methyl viologen, 1 mM NaN_3, 5 mM NH_4Cl, 500 μM DCIP, 5 mM sodium ascorbate, 5 mM $MgCl_2$, 10 mM NaCl, 30 μM 3-(3,4-dichlorophenyl)-1-dimethyl urea (DCMU), and 1 mM Tricine/NaOH (pH 7.0). The Photosystem II assay used H_2O as an electron donor while measuring DCIP reduction by monitoring absorbance at 620 nm [8]. The reaction mixture contained 10 mM NaCl, 45 μM DCIP, and 50 mM sodium phosphate buffer (pH 6.8). Saturating light intensity was used in both assays.

SDS-polyacrylamide gel electrophoresis was performed according to the methods of Laemmli [9]. Samples were prepared by the method of Apel et al. [10]. The solubilized membrane protein samples were either used fresh or stored frozen at −80°C; storage seemed to have no deleterious effect on the samples. The 8.5 cm long separating gel was 10% acrylamide, and the gels were run at 20 mA constant current at 25°C. The polypeptides were visualized by Coomassie blue staining.

Intact leaf fragments from wild-type and mutant leaves were examined in thin section by electron microscopy. Leaf fragments were cut into small pieces before fixation in 3% glutaraldehyde in 100 mM sodium phosphate buffer (pH 7.5) for 1 h. Following several rinses with the phosphate buffer, the leaves were post-fixed in 2% osmium tetroxide for 30 min, rinsed and dehydrated through a graded series of acetone concentrations. The dehydrated material was embedded in Spurr's resin, cured overnight, thin sectioned, and stained with lead citrate and uranyl acetate.

The isolated thylakoid membranes were also prepared for freeze-fracturing and deep-etching. Samples to be deep-etched were suspended in the low-salt buffer and quick-frozen in liquid freon before transfer to liquid nitrogen for storage. Samples to be freeze-fractured were infiltrated over 1 h time with glycerol as a cryoprotectant to a final concentration of 25% (v/v) in the low-salt buffer; the membranes were recovered by centrifuging at 12 000 × *g* for 10 min, and were frozen as indicated above. The freeze-fracturing was performed as described by Moor and Mühlethaler [11]; freeze-fracturing was performed at −110°C, while deep-etching was done for 1 min following the final cut at −100°C. Replicas were cleaned with commercial laundry chlorine bleach, and mounted on copper grids for electron microscopy.

Size measurements of the freeze-fracture face particles were made by enlarging the electron micrographs to 200 000X and using a 7X ocular micrometer to measure the diameters of the particles perpendicular to the direction of shadowing.

Particle densities were determined by counting the number of particles in each type of fracture face region on these electron micrographs, then dividing this number by the area (in μm^2) covered by the particular face. The areas were determined by two methods: (1) weighing of cut-out tracings of the regions, or (2) counting the number of squares in a standard transparent grid superimposed

on the micrograph.

Statistical analyses of the particle densities and size distributions were done using the student's two-tailed *t*-test.

Results

The general structure of the chloroplast, pigment composition, and photosynthetic characteristics of the NC 95 variegated mutant of tobacco were described by Schmid and his coworkers [12—14]. Their studies indicated that the variegated plants possessed two types of chloroplast: a type in the green regions of the leaves which was indistinguishable from wild-type chloroplasts with respect to pigmentation, photosynthetic activity, and structure; and a pigment-deficient chloroplast with unusual structure in the pale green leaf regions. The pigment composition and photosynthetic activity of these mutant chloroplasts is shown in Table I and Fig. 1.

The general structural organization of chloroplast from the green and pale green portions of the NC 95 variegated tobacco plant can be seen in Fig. 2. In contrast to the wild-type chloroplast, the mutant plastid shows a great reduc-

TABLE I

PHOTOSYNTHETIC ACTIVITIES IN WILD-TYPE AND NC 95 VAR. CHLOROPLASTS

Data are from a representative experiment. CO_2 fixation is expressed as μM $^{14}CO_2$ fixed/mg Chl per h (data from Homann and Schmid [13]). Chl, chlorophyll.

Plant	Chl *a*/Chl *b*	Chl/protein	CO_2 fixation	Photosystem I *	Photosystem II **
Wild-type tobacco	3.3	0.220	170 ± 50	15.6	3.49
NC 95 var. mutant	4.2	0.122	23 ± 18	22.8	0.035

* $\Delta\mu$mol O_2/μg Chl per min; methyl viologen reduction from DCPIP/ascorbate, monitored by oxygen consumption.

** Δnmol DCIP/μg Chl per min; DCIP reduction with H_2O as an electron donor, monitored by absorbance at 620 nm.

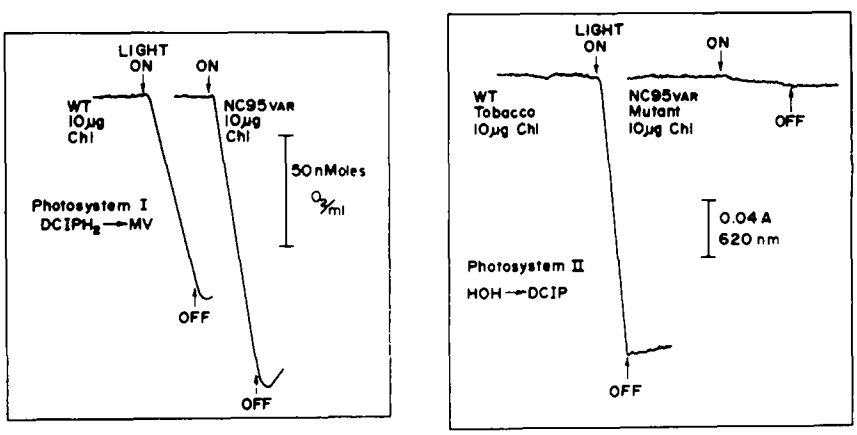
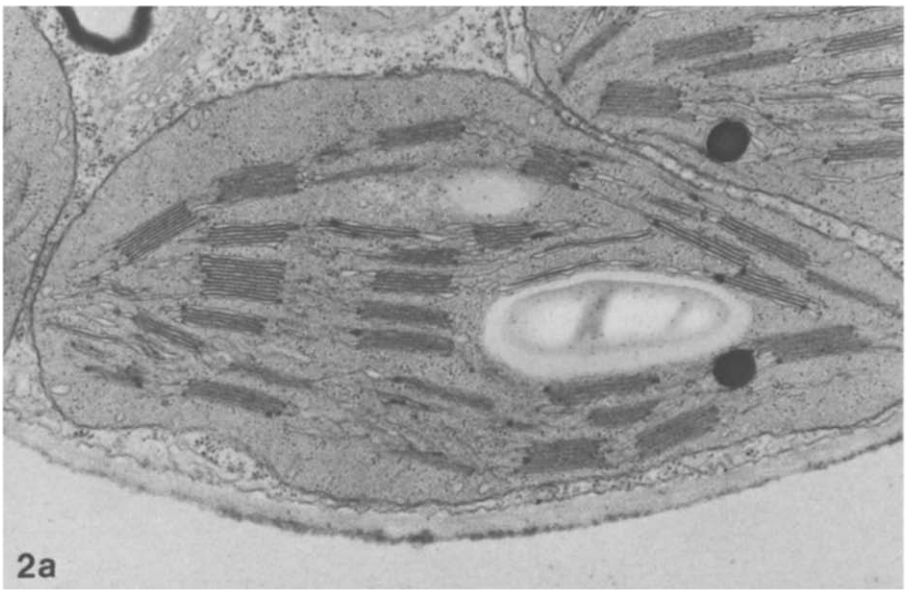


Fig. 1. Photosystem I (*left*) and II (right) measurements of mutant and wild-type tobacco thylakoids. Reaction conditions are given in text.

tion in the amount of membrane stacking, and the grana which dominate the profile of the wild-type organelle are almost completely lacking from the mutant chloroplast. Nevertheless, in contrast to many earlier reports [12—14], we were able to observe small amounts of membrane stacking in virtually every chloroplast we examined. In most cases, these took the form of small grana of five or six thylakoid sacs, as shown in Fig. 2. Membrane stacking, therefore,



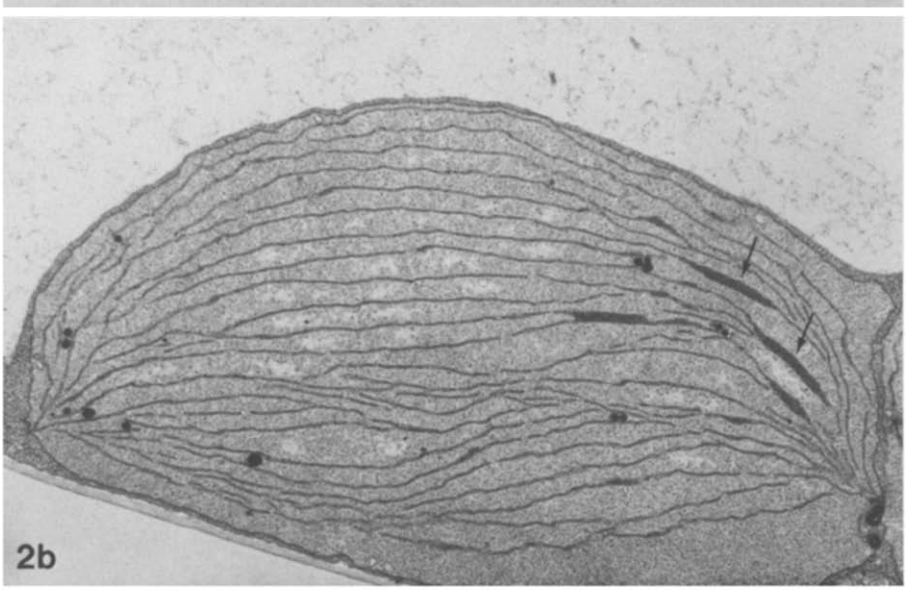


Fig. 2. (a) Thin section of a wild-type tobacco chloropast, showing the membrane development and extensive grana stacking chracteristic of higher plant photosynthetic membrane. Magnification: 20 000×. (b) Thin section of a mutant chloroplast from a variegated leaf region of the strain MC 95 var. The membranes are largely unstacked, although in contrast to some earlier reports, distinct regions of membrane stacking are observed (arrows). Magnification: 20 000×.

persists in the mutant chloroplasts, but is substantially reduced when compared to wild-type chloroplast membranes.

We investigated the polypeptide composition of the mutant chloroplast membranes in an effort to determine whether specific biochemical differences in membrane composition might be related to the photosynthetic and structural alterations in the mutant membranes. Membrane polypeptides of mutant and wild-type chloroplasts as separated by SDS-polyacrylamide gel electrophoresis are shown in Fig. 3. The biochemical alterations in the mutant chloroplast membranes are complex, and involve changes in the positions and densities of several of the bands in the gel pattern.

Bands with apparent molecular weights of 125 000 and 46 000 are missing or greatly depleted in the mutant, while a band at 50 000 daltons is strongly enhanced in the mutant relative to the wild type. In the early stages of electrophoresis, both high molecular weight and low molecular weight (CP II) chlorophyll-protein complexes are visible in the mutant and wild-type samples. CP II is clearly present in both samples in the completed gel (see Fig. 3), as is the 67 000 dalton polypeptide which results from chlorophyll-protein complex I breakdown in this and other species (Cushman, R.A., unpublished data). The

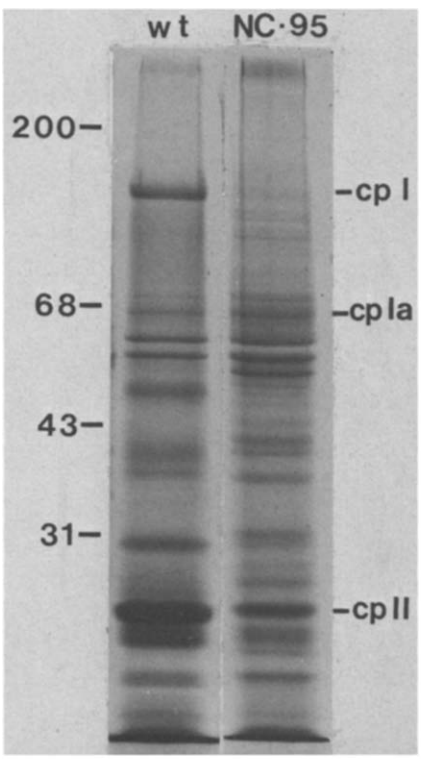


Fig. 3. SDS-polyacrylamide gel electrophoresis of thylakoid membrane polypeptide from mutant (NC 95) and wild-type (WT) chloroplasts. Indicated are the positions of molecular weight standards (myosin, 200 000; bovine serum albumin, 68 000; ovalbumin, 43 000; and DNAase I, 31 000), and the following protein bands: chlorophyll protein complex I (CP I), its apoprotein (CP Ia), and chlorophyll-protein complex II (CP II).

125 000 dalton chlorophyll-protein complex I band, however, is not seen in the completed gel under these conditions of electrophoresis in the mutant, although the presence of a 67 000 dalton polypeptide associated with strong Photosystem I activity (Table I) suggests that a functional Photosystem I reaction center is present in the mutant.

Changes in the polypeptide composition of the photosynthetic membrane have been shown in a number of cases to be accompanied by changes in the internal structure of the membrane [2,5,15] and we have compared the structure of mutant and wild-type membranes in an effort to determine if structural changes were associated with this mutant. The internal structure of wild-type tobacco thylakoid membranes as revealed by membrane splitting during freeze-fracturing is shown in Fig. 4. As seen in other and higher plant chloroplast membranes, four fracture faces are observed in the membranes, two derived from the splitting of the thylakoid membrane in stacked regions, and two from membrane splitting in unstacked regions. These four fracture faces show basically the same distributions of large and small particles on various faces as has been extensively described for other chloroplast membrane systems (see Staehelin et al. [1] for a review of these studies). This pattern of internal membrane organization is virtually identical with the chloroplast membranes of

Fig. 4. Freeze-fracture of wild-type chloroplast. The four faces characteristic of higher plant chloroplast membranes are clearly visible. EFs and PFs faces are derived from membrane splitting in stacked regions, while EFu and PFu faces are formed by membrane splitting in unstacked region. Note the characteristic large particles clearly visible on the EFs face. Magnification: 100 000X.

other plants which have been studied, here and elsewhere.

Fig. 5 shows freeze-fracture membranes from a chloroplast isolated from the pale green region of the tobacco leaf. Surprisingly, the large (approx. 140 Å) particles normally visible on the E fracture face, and so characteristic of the photosynthetic membrane, are virtually absent from the membranes of the mutant!

Despite the absence of large particles on the EFs fracture face of the mutant, these membranes still form four fracture faces, two derived from the splitting of membranes in stacked regions, and two from membranes in unstacked regions. Stacked regions of mutant thylakoid membranes can be recognized in three ways: (1) the presence of closely appressed E and P fracture faces, indicating the apposition of two thylakoids; (2) the presence of an extended (approx. 1 μm) flattened region, characteristic of membrane contact in grana; (3) a change in the background matrix of the E fracture face, from the pock-marked appearance of unstacked regions to the smooth and uniform face characteristic of stacked regions (see Figs. 7 and 8).

While striking changes could be seen on the E fracture faces of the mutant, there appeared to be few if any changes in the P fracture faces (see Figs. 4—6, 9, 10). In order to be sure that a slight alteration in one of these fracture faces

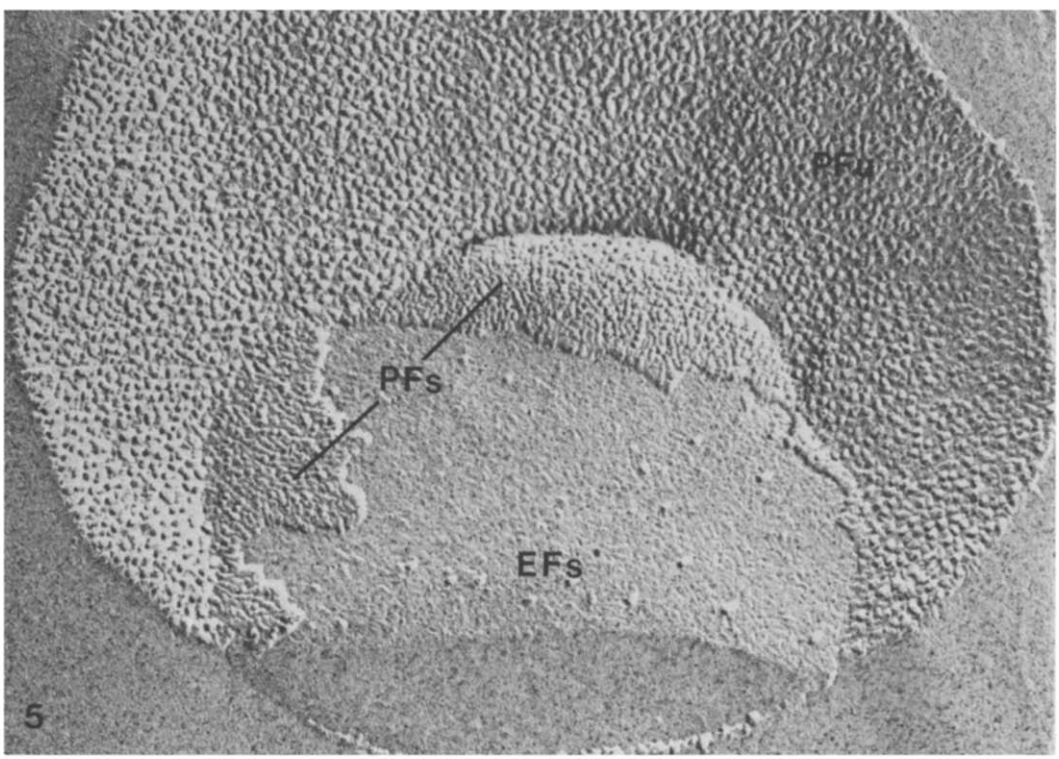


Fig. 5. Freeze-fracture of a mutant (NC 95) chloroplast, deficient in Photosystem II activity. Although the two P fracture faces visible are indistinguishable from those of the wild-type membrane (Fig. 4), the EFs fracture face, in contrast to its wild-type counterpart, is almost totally devoid of large particles. Magnification: 100 000×.

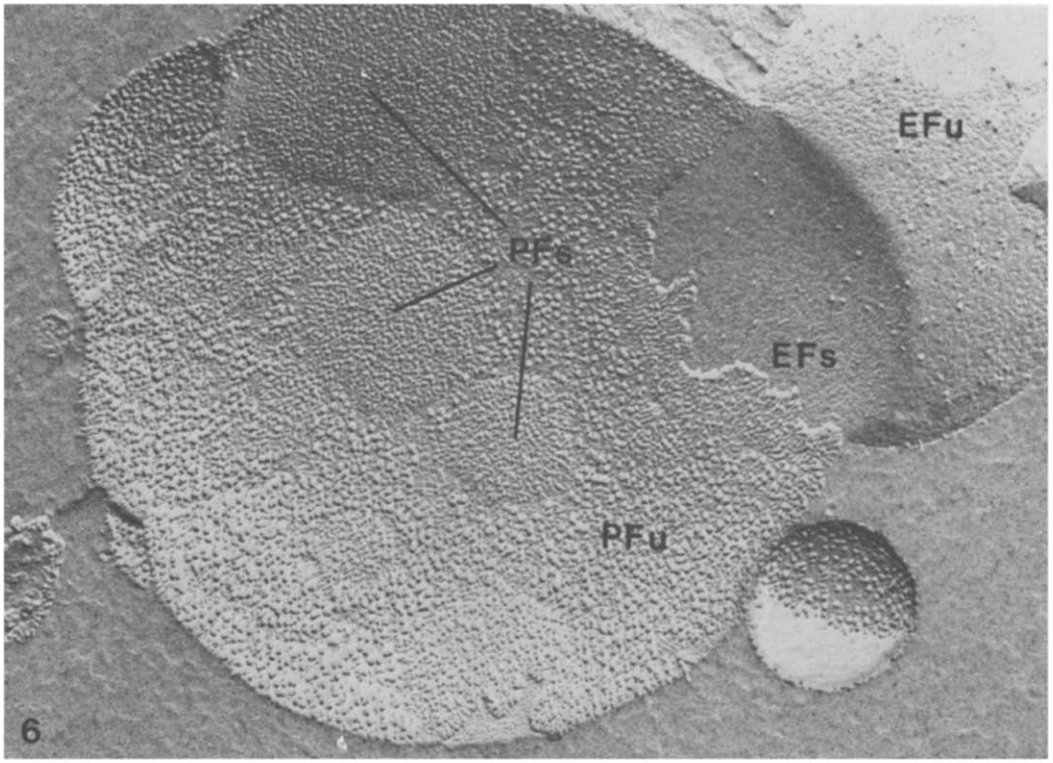
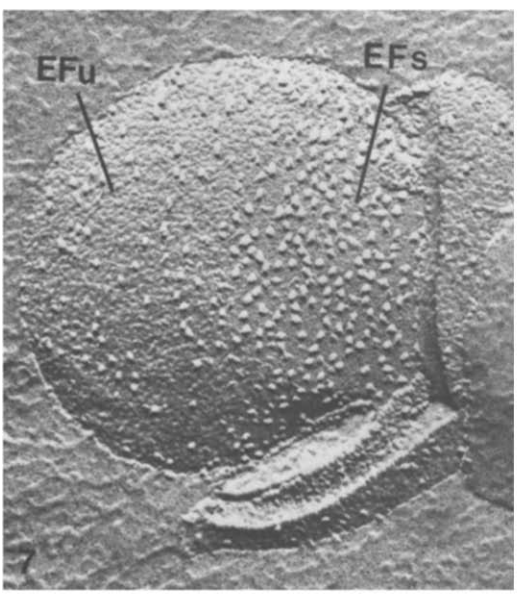


Fig. 6. Freeze-fracture of the Photosystem II-deficient mutant. Here all four fracture faces are visible, and distinct stacked (PFs) regions can be observed in the P fracture face. Magnification: 75 000X.



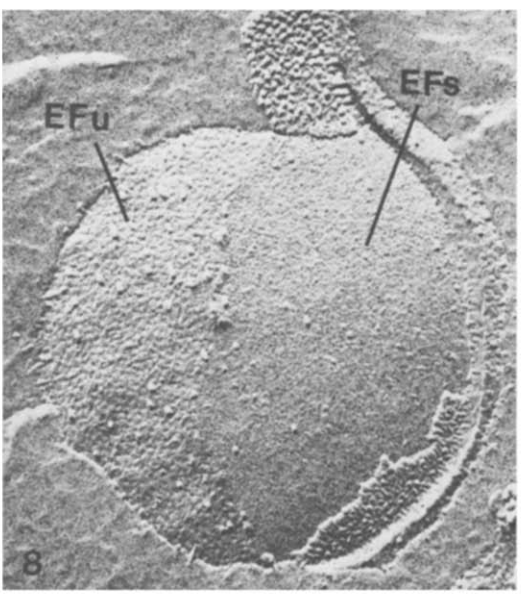
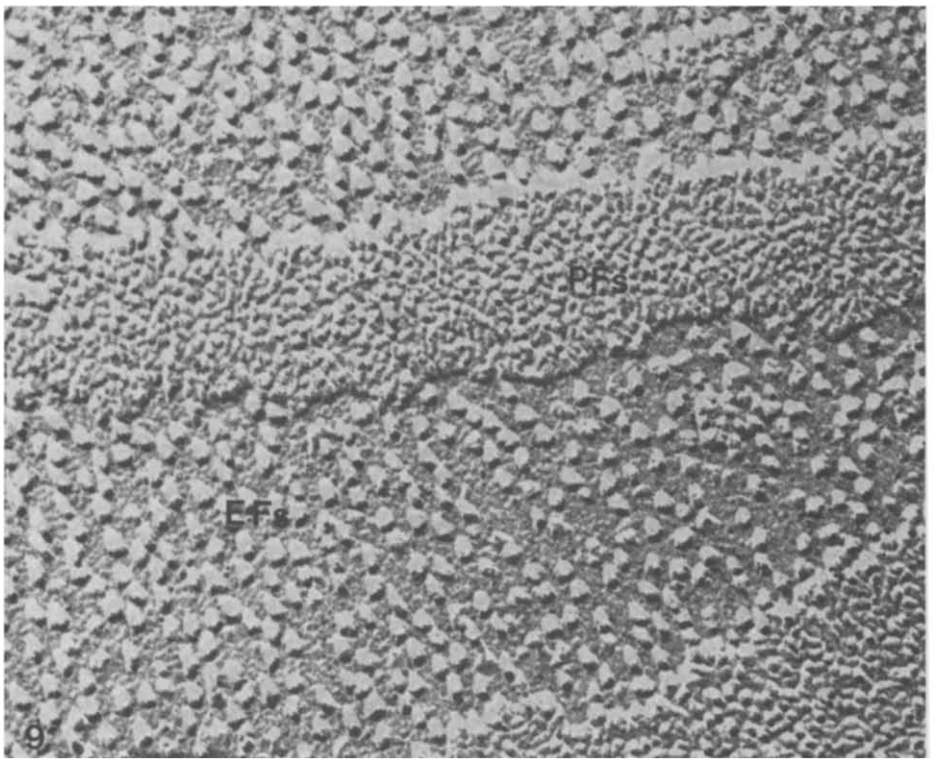
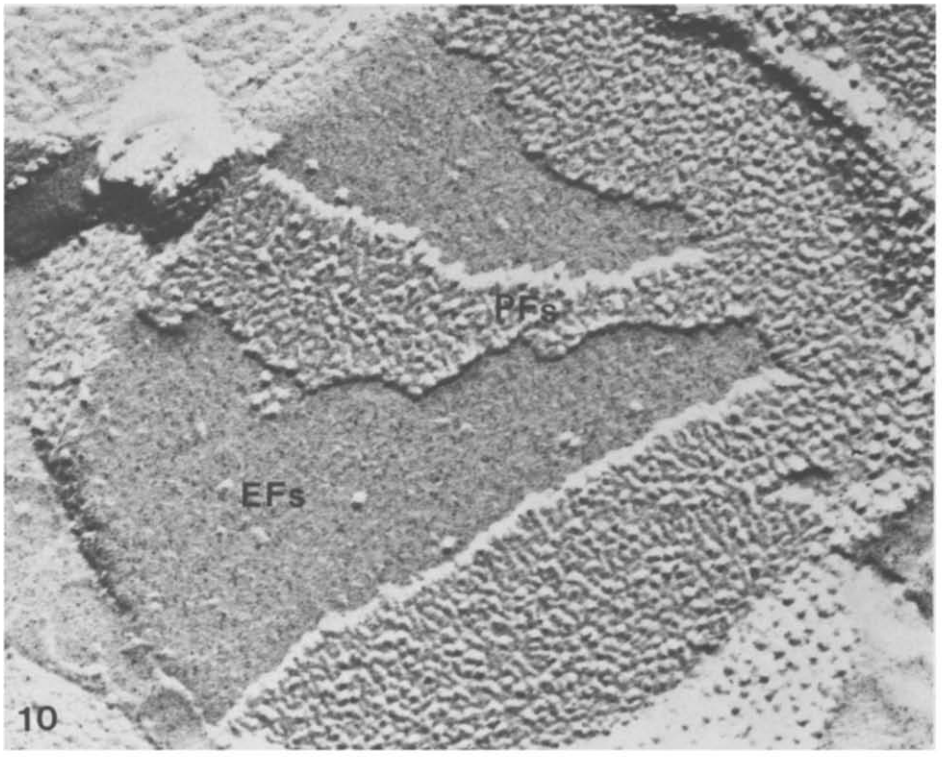


Fig. 7. Small portion of a wild-type membrane showing the transition from a stacked region (EFs) to an unstacked region (EFu). Large particles are concentrated in the stacked region, and the background matrix of the stacked region is much smoother than that of the pockmarked unstacked region. Magnification: 75 000X.

Fig. 8. A membrane region showing the E face transition from a stacked to an unstacked region, similar to Fig. 7, but taken from a sample of the Photosystem II-deficient mutant. Although the change in membrane background matrix is still observed, no large particles are observed on the EFs face. Magnification: 75 000X.





Figs. 9 and 10. High magnification views of stacked regions from freeze-fractured samples of wild-type and mutant thylakoid membranes, respectively. Magnification: 150 000×.

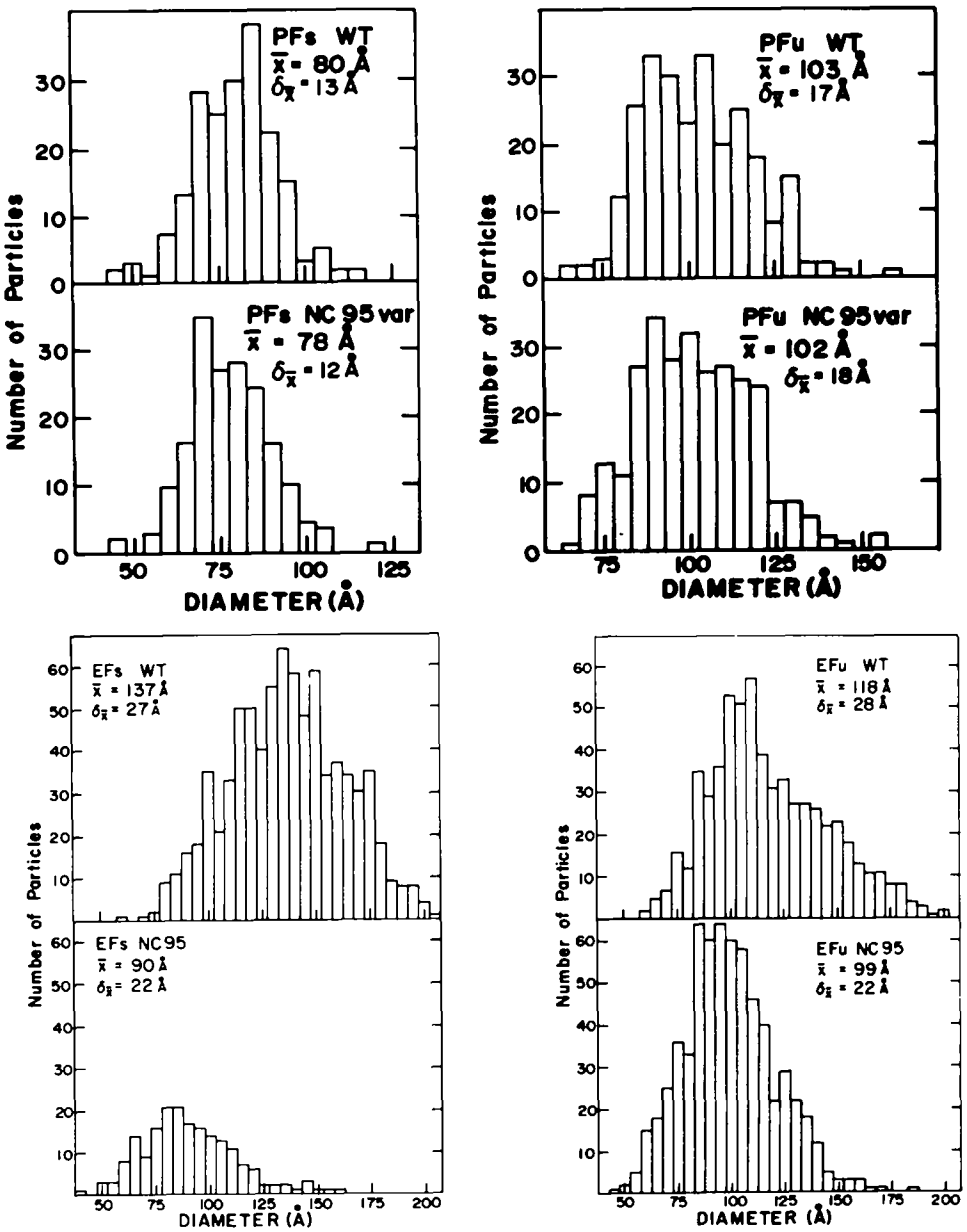


Fig. 11. Particle size histograms made from measurements on all four fracture face types in the mutant and wild-type thylakoid membranes. Note the changes in the E faces of the mutant, in contrast to the similarities between the P faces of the mutant and wild type.

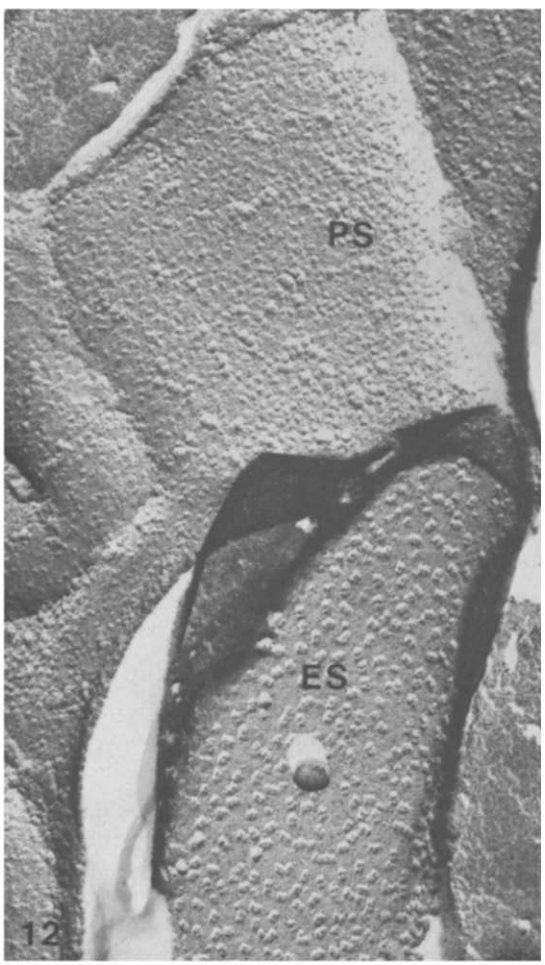
did not escape our attention, we carefully analyzed the size distributions and densities of the particles visible on all of the fracture faces of the wild-type and mutant thylakoid membranes. The particle size distribution data are shown in Fig. 11, and these, with the density determinations, are summarized in Table II.

The data in Table II support the observation that the E faces of the mutant are greatly altered while the P faces are virtually identical to those of the wild-

TABLE II

FREEZE-FRACTURE PARTICLE SIZE AND DENSITY MEANS FROM WILD-TYPE AND NC 95 VAR. FACES

Face	Plant	Number of particles measured	Particle size ($\bar{x} \pm \delta\bar{x}$) ((Å))	Significant difference (P)	Number of areas counted	Particle density (particle/rm^2) ($\bar{x} \pm \delta\bar{x}$)	Significant difference (P)
EFs	WT	789	137 ± 27	<0.01	7	1558 ± 212	<0.01
	NC 95	193	90 ± 22		11	190 ± 56	
EFu	WT	610	118 ± 28	<0.01	5	640 ± 47	<0.01
	NC 95	647	99 ± 22		7	346 ± 83	
PFs	WT	197	80 ± 13	>0.05	9	6569 ± 645	>0.05
	NC 95	180	78 ± 12		7	7225 ± 748	
PFu	WT	256	103 ± 17	>0.05	11	5411 ± 462	>0.05
	NC 95	281	102 ± 18		6	5076 ± 476	



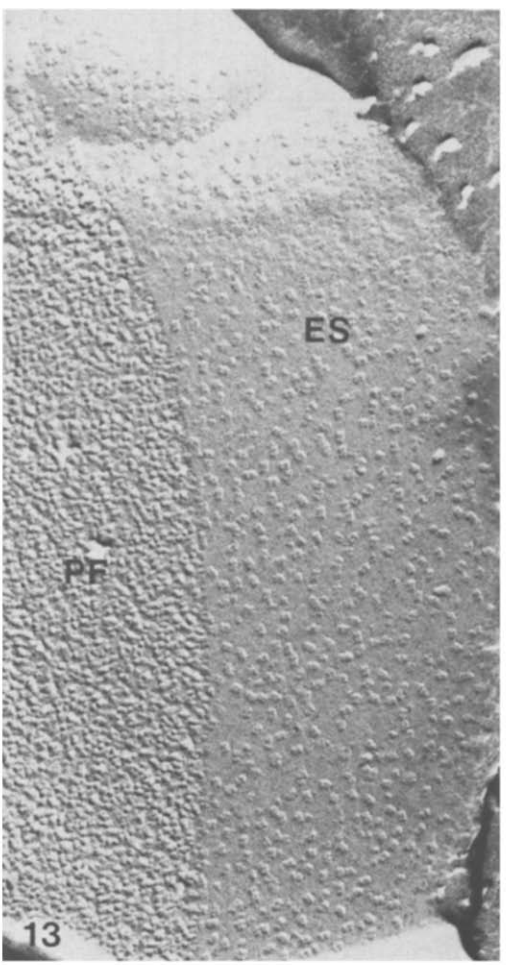
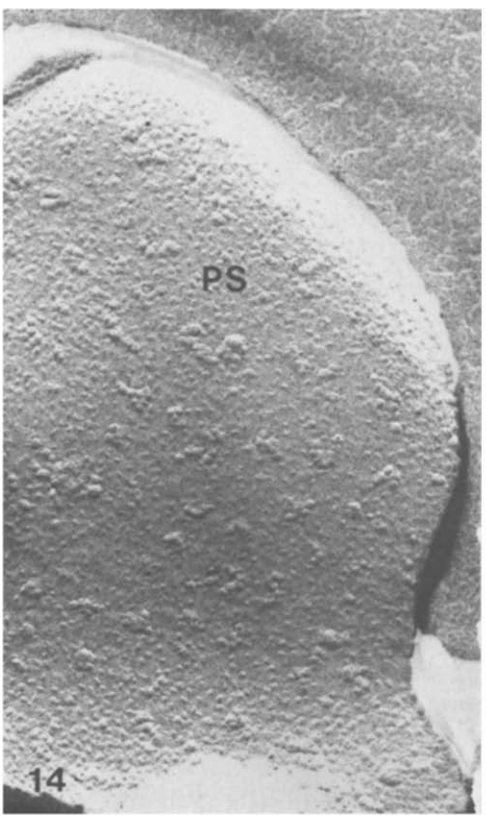


Fig. 12. Inner (ES) and outer (PS) surfaces of the thylakoid membrane of wild-type chloroplasts exposed by deep-etching. The outer (PS) surface is covered with a mixed population of large (approx. 120 Å) and small (approx. 80 Å) particles. The inner surface shows a population of particle with a tetrameric appearance. Magnification: 94 000×.

Fig. 13. Fractured (PF) and etched (ES) surfaces formed from a single wild-type membrane. In such samples, ES and PF views of the membrane are always adjacent, as shown. Magnification: 94 000×.

type control. The particles of both the unstacked and stacked E faces of the mutant were significantly smaller and less densely packed than their counterparts on the respective wild-type faces. In addition, contrary to the wild-type E faces, where the EFs particles are larger and packed more densely than those on the EFu faces, the mutant EFs particles are both smaller and less densely packed than the particles on the mutant EFu faces. In contrast to these differences between the various E faces, there are no statistically significant differences between the particle size distributions or densities when the mutant P faces are compared with the P faces of the wild type.

Because a previous study with a chlorophyll-deficient mutant [2] had shown significant alterations visible at the surface of the thylakoid membrane, and because an earlier study indicated that the tetrameric particles visible at the inner surface of the membrane were the same structures as the large EFs particles [16], we also studied the outer surfaces of the photosynthetic mem-



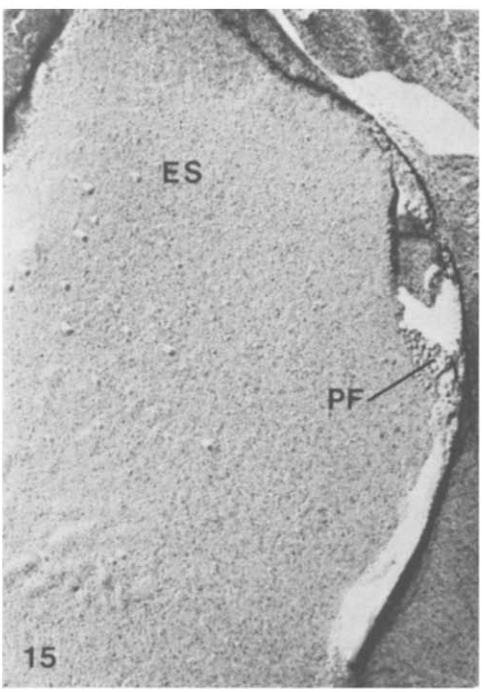


Fig. 14. Etched outer (PS) surface of a mutant thylakoid membrane. No differences are observed with respect to wild-type membrane on this surface. Magnification: 94 000X.

Fig. 15. Etched inner (ES) surface of a thylakoid membrane from a Photosystem II-deficient thylakoid membrane of the NC 95 var. mutant. Because of the striking absence of the inner surface particles so clearly observed in wild-type membranes, we have used the presence of a small PF region to identify this as an E surface. Magnification: 94 000X.

branes of these plastids. Figs. 12 and 13 show the appearance of the inner and outer surfaces of the tobacco thylakoid membrane, exposed by etching away frozen buffer from the surfaces of isolated chloroplasts. The outer surface shows a diverse population of large particles and small particles. (In another species, the large particles have been shown to be coupling factor molecules [17].) The inner surface of the membrane is covered with a population of polymeric structures, which in many cases can be seen clearly to be tetrameric. Fig. 13 shows a fracture face adjacent to this etched surface, illustrating how, for purposes of identification, we may expect to find P fracture faces and E etched surfaces adjacent to each other.

The outer surface of the mutant thylakoids, as shown in Fig. 14, is identical to that of the wild-type membranes. However, the inner surface of the membrane seems to lack the particles so characteristic of the E surface of the wild-type membranes. Fig. 15 shows such a surface; it is identifiable by the presence of an adjacent P fracture face, is virtually smooth, and lacks the typical ES particles. At low shadowing angles some surface structure to these membranes is apparent; however, the differences from wild-type membranes are obvious. The membranes of the NC 95 variegated mutant, therefore, lack the inner (ES) surface particles as well as the large (EF) particles visible on the fracture faces of the photosynthetic membrane.

Discussion

Recent models for the organization of the photosynthetic membrane have attempted to take into account, in one way or another, the observations that freeze-fractured chloroplast membranes show two general types of fracture faces (E and P), each covered with a characteristic type of particle [1,18]. When these observations were combined with fractionation studies of the membrane system, it was suggested that the large (EF) particles might be associated with Photosystem II, and the smaller (PF) particles with Photosystem I [19]. More recently, studies on mutant chloroplasts [2] and chloroplasts greened under intermittent light [3] have suggested that the large particles observed on E fracture faces are 'core' particles containing the Photosystem II reaction center, and, in most cases, surrounded by several molecules of a light-harvesting chlorophyll-protein complex associated with Photosystem II. These reports appear to rule out the suggestion [20] that the particles might be the chlorophyll-protein complex itself, since in each case large numbers of EF particles were observed in the absence of this chlorophyll-protein complex. Our correlations of structure and function in the photosynthetic membrane of the NC 95 mutant provide additional support for the 'core' model of photosynthetic membrane structure [1,2,18].

The overall structure of the mutant chloroplast membrane system is essentially the same as reported earlier by Homann and Schmid [13], with the modification that we found the formation of small stacked regions in the plastid to be a common occurrence, while they did not observe these small grana frequently. It is possible that this difference may be related in part to seasonal and geographic differences in growing the plants.

The basic alteration seen in freeze-fractured preparations of mutant chloro-

plast membranes is the almost total absence of the large EF particle so characteristic of the photosynthetic membanes of green plants. Since the mutant tissue displays near-normal rates for Photosystem I reactions, but is almost completely lacking in Photosystem II (approximately 5% of normal rates, see Table I and Fig. 1), it seems likely that the absence of large particles is correlated in some way with lack of Photosystem II reaction centers in the membrane. The most straightforward conclusion is that the mutant membranes fail to organize a Photosystem II reaction center, and the morphological manifestation of this failure is the loss of large EF particles.

The loss of EF particles is not merely a consequence of reduced membrane stacking in the mutant. The bundle sheath chloroplasts of maize and several other C4 plants are, like the NC 95 var. mutant, severely reduced in membrane stacking, almost to the point of being completely agranal [21]. However, freeze-fracture studies of these chloroplasts [22] have shown that they contain large numbers of EF particles; and, in regions where limited membrane stacking does occur, distinct concentrations of large EFs particles are visible. The agranal chloroplasts of certain plants, therefore, are distinctly different in internal organization from the thylakoids of the mutant chloroplasts reported here. It is in principal possible that the Photosystem II reaction center could be correlated with some other component of the membrane system found in stacked regions. Our measurements of thin sections of both types of chloroplasts indicate that about 70% of the membrane surface was in stacked regions in wild-type chloroplasts, while in mutant chloroplasts only 10% of the membrane surface was in stacked regions. However, the persistence of Photosystem II activity in chloroplasts with reduced stacking (such as the bundle sheath system mentioned above) argues against such an explanation.

Two chloroplast types have been reported by other workers which also lack the large EF particle [15,23]; each of these mutants is deficient in Photosystem II. These observations, when considered with structural evidence that the migration of the large particles during membrane stacking parallels the concentration of Photosystem II in stacked regions of the membrane [6] and with studies of other mutant and developing systems where loss of a Photosystem II-associated pigment-protein complex caused alterations in the structure of the large particle, form a convincing case that this particle is indeed a manifestation of the Photosystem II reaction complex in the thylakoid membrane.

A major point of several careful analyses of the fracture face patterns observed in the photosynthetic membrane has been that the actual appearance of P and E fracture faces derives in part from the fact that they are roughly complementary to each other, being derived from the splitting of a single membrane during freeze-fracture [4,5,7]. In other words, several workers have proposed that the appearance of the PFs face is due, in part, to the fact that the large EFs particles were pulled out of it during the fracturing process. If this were indeed the case, we might expect to observe substantial changes in the PFs faces of our Photosystem II-deficient mutant due to the absence of the large particles. Instead, the PFs faces that are observed are virtually indistinguishable from their wild-type counterparts (see Figs. 9 and 10, and also Figs. 4—6), a conclusion which is supported by direct examination of the micrographs, and by the statistical analysis presented in Table II. Therefore, com-

plete complementarity is not observed during freeze-fracturing of the photosynthetic membrane. The structural appearance of the PFs face seems to be due largely to the structure of one external half of the thylakoid membrane, and not to the presence or absence of certain structures on the opposite (EFs) fracture face.

Differences in polypeptide composition between the mutant and wild-type thylakoid are complex (see Fig. 3). It does not seem possible, therefore, to associate a specific polypeptide with the structural changes seen in the mutant. However, it is significant that the mutant thylakoid membranes contain both the high (CP I) and low (CP II) molecular weight chlorophyll-protein complexes described by Thornber [24,25]. The apoproteins of both these complexes are clearly visible in Fig. 3. The presence of CP I, which is associated with an active Photosystem I [25] is not unexpected but the presence of CP II, a light-harvesting complex associated with Photosystem II is surprising. The arguments for the association of this complex with the Photosystem II particle [2,3] have been noted. How does this complex exist in the membrane when the core structure with which it is normally associated is missing? Arntzen and his associates [3,18] have made a strong argument that the CP II complex is the moving force behind the cation-mediated association of the thylakoid membranes to form grana stacks. The small stacked regions which are so evident in freeze-fractured preparations of the mutant chloroplasts may be the result of membrane stacking maintained by CP II. The presence of significant amounts of this complex in the mutant membrane does not seem to be sufficient to form an observable structure on the E face in the stacked regions; however, CP II seems, therefore, to be capable of causing membrane stacking in some regions without the presence of Photosystem II core particle. This raises questions about the three-dimensional structure of this chlorophyll-protein complex in the thylakoid, which at the present time we cannot answer.

Perhaps the most intriguing question concerning the Photosystem II core particle is the manner in which its structure and assembly are controlled by the organelle. Although a definitive genetic analysis of the NC 95 var. mutant has not been carried out, it seems likely (because it is a variegated mutant, without a consistent tissue pattern of variegation) that NC 95 var., like other variegated mutants [26], is caused by an alteration in the chloroplast genome. So there is a suggestion that transcription, translation and processing of some components of the Photosystem II core particle may occur within the chloroplast. Recent experiments with inhibitors of protein synthesis on 70 S ribosomes [27] have also suggested that the assembly of the particle may require components synthesized within the chloroplast. In contrast, a nuclear mutant of barley has also been shown to lack the Photosystem II core particle [23], indicating that the actual synthesis and assembly of this structure within the membrane may require the cooperation of both cellular genomes.

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