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Structural characteristics of thylakoid membranes of *Arabidopsis* mutants deficient in lipid fatty acid desaturation

Nelly M. Tsvetkova [a,1], Anthony P.R. Brain [b], Peter J. Quinn [a,*]

[a] *Department of Biochemistry, Division of Life Sciences, King's College London, Campden Hill, London W8 7AH, UK*
[b] *Electron Microscopy Unit, King's College London, Campden Hill, London W8 7AH, UK*

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Abstract

The ultrastructure of thylakoid membranes from *Arabidopsis thaliana* wild-type, JB67 and LK3 fatty acid desaturation deficient mutants was studied by thin-section and freeze-fracture electron microscopy. There was a decrease in the amount of the appressed and non-appressed membranes in JB67 and LK3 *Arabidopsis* mutants when compared to the wild type, resulting in a reduction in the length of photosynthetic membrane per plastid. The results from freeze-fracture showed a decrease in size and a marked increase in packing density of membrane-associated particles on the exo- and endoplasmic fracture faces of the mutants. In addition, areas of the appressed membranes of the mutants contained particles in regular arrays under conditions where no such arrays were observed in wild-type thylakoid membranes. These observations suggest, that the decreased level of lipid fatty acid unsaturation affects the ability of the lipid matrix to mediate the assembly of chloroplast membrane components. The role of polyunsaturated membrane lipids is considered in terms of their ability to promote functional oligomeric assemblies of components of the photosynthetic apparatus.

Key words: Photosynthetic membrane; Chloroplast ultrastructure; Membrane structure; Polyunsaturated lipid; Mutation; Fatty acid desaturation; (*Arabidopsis*)

1. Introduction

The membrane lipids of the chloroplasts of higher plants are characterized by high proportions of polyunsaturated fatty acids. In some plant species, such as *Vicia faba*, trienoic fatty acids may comprise 80% or more of the total fatty acyl residues of chloroplast membrane lipids [1]. Our original approach to investigate the role of polyunsaturated membrane lipids in photosynthetic functions was to saturate the double bonds in situ using homogeneous catalytic hydrogenation [2]. With the isolation of mutants of *Arabidopsis* defective in desaturation of chloroplast lipid fatty acids [3–8] the question can be addressed directly without the problems associated with the introduction of transition metal catalysts.

The JB67 mutant of *Arabidopsis* is characterized by an increased proportion of palmitic acid (16:0) and a corresponding decrease in the unsaturated 16-carbon fatty acids of the thylakoid membrane lipids [3,4]. Quantitative analysis of the fatty acid composition of the individual lipids shows a pattern consistent with a defect in the activity of chloroplast $\omega - 9$ fatty acid desaturase. This enzyme is responsible for the introduction of a double bond into the $\omega - 9$ position of the fatty acyl residues of monogalactosyldiacylglycerol (MGDG). The defect is a result of a single nuclear mutation at a locus designated fad B [3,4]. The reduced level of unsaturation of the 16-carbon acyl chains has been shown to result in a slight decrease in CO_2 fixation and reduction in the rate of photosynthetic electron transport, but there is an increased thermal stability of the photosynthetic apparatus at elevated temperatures [3].

Another *Arabidopsis* mutant, LK3, is deficient in the activity of $\omega - 6$ desaturase responsible for introducing double bonds into 16-carbon and 18-carbon

* Corresponding author. Fax: +44 71 9375690.
[1] On leave.

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fatty acids of MGDG and digalactosydiacylglycerol (DGDG). As a result of the fad C mutation, palmitoleic (16:1) and oleic (18:1) fatty acyl residues dominate the composition of these lipid classes [5,6]. Biochemical studies of the mutant have shown that the reduced level of lipid unsaturation is associated with changes in protein and chlorophyll content of the thylakoid membranes and a reduction in photosynthetic electron transport rates [5,6]. Like the JB67 mutant, the photosynthetic electron transport of LK3 *Arabidopsis* mutant is more heat-stable compared to the wild type [6].

While the effect of reduced levels of lipid unsaturation on chloroplast functions have been well characterized, less information is available regarding the ultrastructure of chloroplast membranes of these mutants. Previous studies of chloroplasts have shown that stacked and unstacked membrane regions exhibit distinctly different structural organization [9–12]. Freeze-fracture electron microscopy has been used to reveal differences in the size and distribution of membrane-associated particles in both exoplasmic (E) and protoplasmic (P) membrane surfaces fractured laterally in stacked and unstacked regions of the thylakoids [13]. Biochemical studies are consistent with the lateral heterogeneity in the distribution of chlorophyll-protein complexes of Photosystem I and Photosystem II with Photosystem II localized in the appressed regions of the grana and Photosystem I confined predominantly to the non-appressed grana-end membranes and margines and stroma lamellae [12].

The aim of the present study is to examine the effect of reduced lipid fatty acid unsaturation on the structure and organization of thylakoid membranes of JB67 and LK3 *Arabidopsis* mutants. The investigation was undertaken using thin-section and freeze-fracture electron microscopy. The results showed a decrease in the length of the appressed and non-appressed membranes in JB67 and LK3 *Arabidopsis* mutants when compared to the wild type. There was a reduction in size and a considerable increase in packing density of membrane-associated particles, suggesting that the decreased level of lipid unsaturation affects the assembly of chloroplast membrane components.

2. Materials and methods

Plant growth conditions. Seeds of the wild-type and JB67 and LK3 *Arabidopsis* mutants were obtained from the Nottingham Arabidopsis Stock Centre through the courtesy of Dr. M. Anderson. The mutants were isolated from Columbia wild-type of *Arabidopsis thaliana* (L.) Heynh. as previously described [3,5].

The plants were grown at 22°C with 75% relative humidity and an alternating night and day cycle. The growth medium consisted of a mixture of perlite, vermiculite and sphagnum in proportion 1:1:1 and the plants were irrigated with mineral nutrients as described in [14].

Isolation of chloroplasts. Leaves were harvested at the rosette stage and chilled on ice for 5 min. Chloroplasts were isolated by grinding leaves in a medium consisting of 20 mM Tricine buffer (pH 8.4), 10 mM $NaHCO_3$, 5 mM EDTA, 5 mM EGTA, 0.3 M sorbitol, 0.1% (w/v) bovine serum albumin [15]. The homogenate was filtered through miracloth (Calbiochem) and centrifuged 5 min at $3000 \times g$. The pellet was washed with cold medium containing 20 mM Hepes (pH 7.6), 10 mM $NaHCO_3$, 0.3 M sorbitol, 5 mM $MgCl_2$, 2.5 mM EDTA and 0.1% (w/v) bovine serum albumin.

Electron microscopy. For preparation of thin sections, leaves from 3-week-old plants were fixed in 4% glutaraldehyde buffered to pH 7.4 with 100 mM sodium cacodylate. After dehydration and embedding thin sections were cut with a glass knife and stained with uranyl acetate and lead citrate.

For freeze-fracture electron microscopy, freshly prepared chloroplast suspensions were equilibrated and thermally quenched from 20°C in a jet of liquid nitrogen and subsequently fractured at −150°C in a Polaron freeze-fracture device. Replicas were prepared by shadowing with platinum and carbon and cleaned with bleach. Thin sections and freeze-fracture replicas were examined under a Philips 301G electron microscope.

Quantitative measurements of membrane profiles on electron micrographs were performed on sections of at least 20 chloroplasts each from wild-type, JB67 and LK3 *Arabidopsis* mutants. Granal, stromal, appressed and non-appressed membrane assignments were made as described previously [6]. The size distributions of membrane-associated particles were determined from high magnification electron micrographs (×220 000) of replicas showing large areas of appropriate fracture face. Particle diameter was measured at right angles to the direction of shadowing. Particle densities were also determined from the same micrographs. Each measurement was performed on at least 10 separate areas of fracture face corresponding to an area of between 0.05 and 0.1 μm^2 of membrane in each case. Measurements were made independently by three operators and the means (± standard deviations) were calculated from the pooled results.

3. Results

Thin sections of chloroplasts of the wild-type and JB67 and LK3 *Arabidopsis* mutants are presented in Fig. 1. Morphometric analysis of the chloroplasts shows

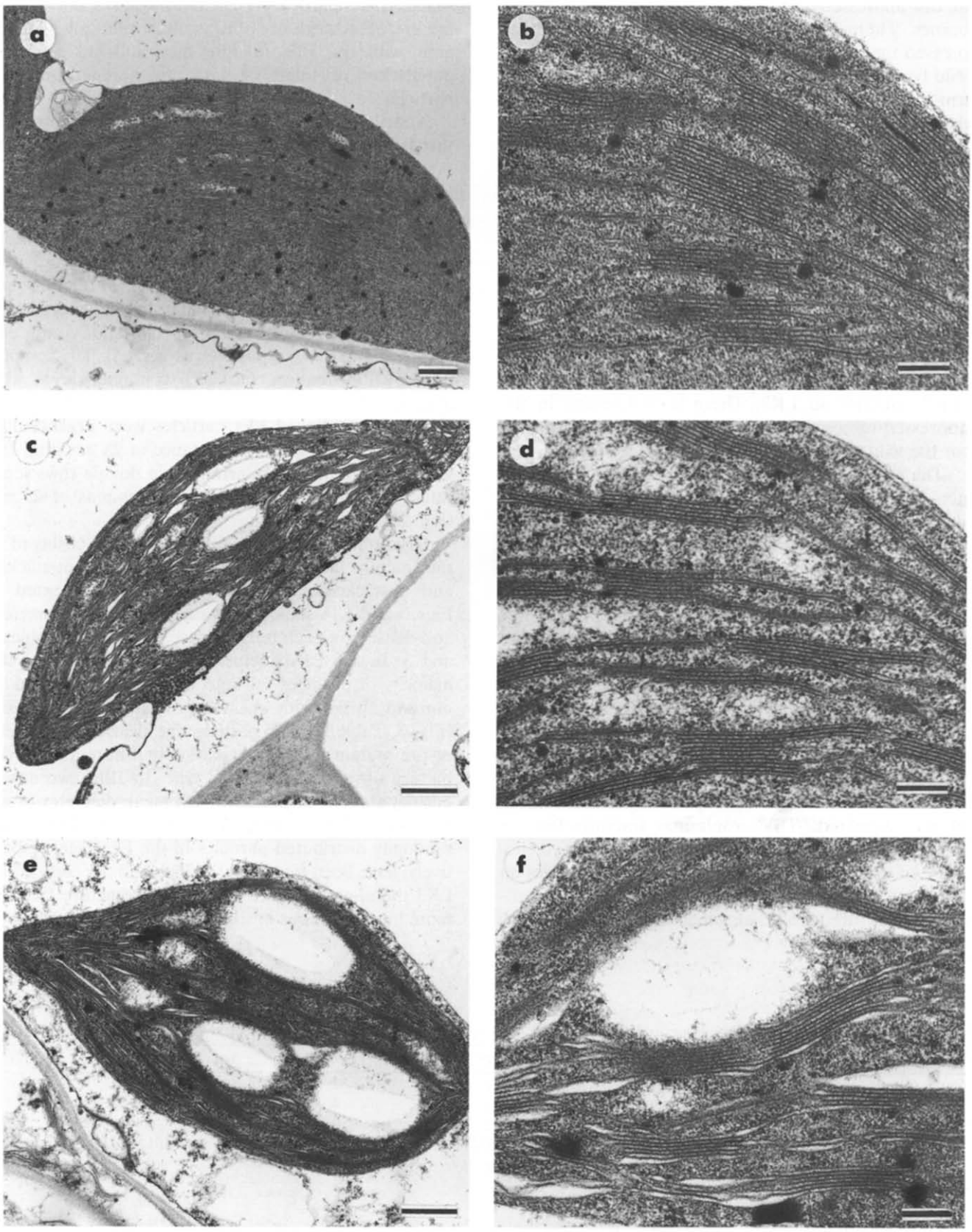

Fig. 1. Electron micrographs of thin sections of chloroplasts in leaves of wild-type (a,b), JB67 (c,d) and LK3 (e,f) *Arabidopsis* mutants deficient in fatty acid desaturation. Bar equals 0.5 μm in a, c and e, and 0.2 μm in b, d and f.

that the main effect of the mutations is on the amount of the appressed and non-appressed thylakoid membranes. There is a decrease of the length of the appressed membranes per plastid from 118.3 μm in the wild type to 64.6 μm in JB67 (45% reduction) and 71.2 μm in LK3 (40% reduction) (Table 1). This is due to a decrease in the number of thylakoids per granum (29% reduction in JB67 and 33% reduction in LK3) and a decrease in the granal width (26% in JB67 and 12% in LK3). The decrease in the granal size in LK3 is partially compensated by an increase in the number of grana per plastid.

The mutations cause a decrease in the stromal membrane length, which is 0.2 μm for the wild type and 0.17 μm and 0.16 μm for JB67 and LK3 *Arabidopsis* mutants, respectively (Table 1). This results in a reduction of the average amount of the non-appressed thylakoid membranes, which is decreased by 22% in JB67 and 21% in LK3. There is a decrease in the appressed-to-non-appressed membrane ratio from 2.5 for the wild type to 1.8 for JB67 and 1.9 for LK3.

The reduction in the amount of the appressed and non-appressed membranes in JB67 and LK3 *Arabidopsis* mutants corresponds to a 39% decrease in the total membrane length per chloroplast in JB67 and a 34% decrease in LK3 compared to the wild-type. Since there was no significant difference in the number of plastids per section for the three plants, the lower thylakoid membrane length reflects a decrease in the amount of photosynthetic membrane.

The ultrastructure of thylakoid membranes of the wild type and the two mutants was examined by freeze-fracture electron microscopy and the results are presented in Fig. 2. Fig. 2 a shows an overall view of a fracture face exposing exoplasmic and protoplasmic membrane surfaces in stacked and unstacked regions of the thylakoid. The exoplasmic fracture face in stacked thylakoids (EFs) is the most distinctive, because of its population of large well spaced particles on a relatively smooth background matrix. The protoplasmic fracture face (PFs), which is complementary to the EFs, appears to have a dense population of smaller-sized particles. The exoplasmic fracture face in the unstacked regions (EFu) is often seen to be continuous with the EFs fracture face and has a sparse population of relatively large membrane-associated particles.

A striking feature of the fracture faces of stacked thylakoids in JB67 and LK3 *Arabidopsis* mutants is the presence of aligned membrane-associated particles together with regions with randomly distributed particles (Fig. 2c,g). Aligned particles have not been observed in wild-type thylakoids prepared using the procedure described in Materials and methods. In JB67 and LK3 mutants the aligned particles on the EFs fracture face were organized in a rectangular lattice with repeat distance along the two axes of 19 nm and 23 nm, and 18 nm and 23 nm, respectively (Fig. 2e,i). The complementary PFs fracture face also contained areas with aligned and randomly distributed particles (Fig. 2b,f). In JB67 the aligned PFs particles were organized in single rows separated by a distance of 23 nm (Fig. 2b), while in LK3 they were arranged in double rows separated by equally spaced single depressions of 25 nm (Fig. 2f).

Histograms of particle size distribution observed in the fracture faces of the wild-type and mutant stacked and unstacked membrane regions are presented in Figs. 3 and 4. A summary of average particle diameters and density on each fracture face are given in Tables 2 and 3. In the EFs fracture face of the wild-type the majority of particles fall into a size range between 10 nm and 16 nm with an average diameter of 13.2 nm (Fig. 3, Table 2). The particle size distribution in EFs of the mutant plants was markedly different from the pattern observed in the wild type. In JB67 two different populations of particles with mean diameter of 8.9 nm and 12.3 nm corresponding to the aligned and randomly distributed particles in the EFs face, respectively, have been found (Fig. 3, Table 2). By contrast, in LK3 the aligned particles in the EFs fracture face are more heterogeneous in size and fall into a size category

Table 1
Morphomeric analysis of chloroplasts from *Arabidopsis thaliana* wild-type, JB67 and LK3 mutants deficient in lipid fatty acid desaturation

Measurement	Wild type	JB67	LK3
Grana/plastid	58 ± 6.2	52 ± 7.1	64 ± 7.2
Thylakoids/granum	5.8 ± 2.1	4.1 ± 1.8	3.9 ± 2.1
Granal width (μm)	0.5 ± 0.09	0.37 ± 0.08	0.44 ± 0.07
Stromal thylakoids/plastid	97.7 ± 23.5	94.3 ± 13.1	101 ± 11.5
Stromal thylakoid length (μm)	0.21 ± 0.07	0.17 ± 0.08	0.16 ± 0.03
Appressed thylakoids/plastid (μm)	118.3	64.6	71.2
Non-appressed thylakoids/plastid (μm)	47.4	36.9	37.5
Total thylakoids (μm)	165.7	101.5	108.7
Appressed/non-appressed thylakoids	2.50	1.75	1.89
Average cross-sectional area of chloroplast (μm^2)	11.7 ± 2.0	11.2 ± 2.7	9.8 ± 3.9

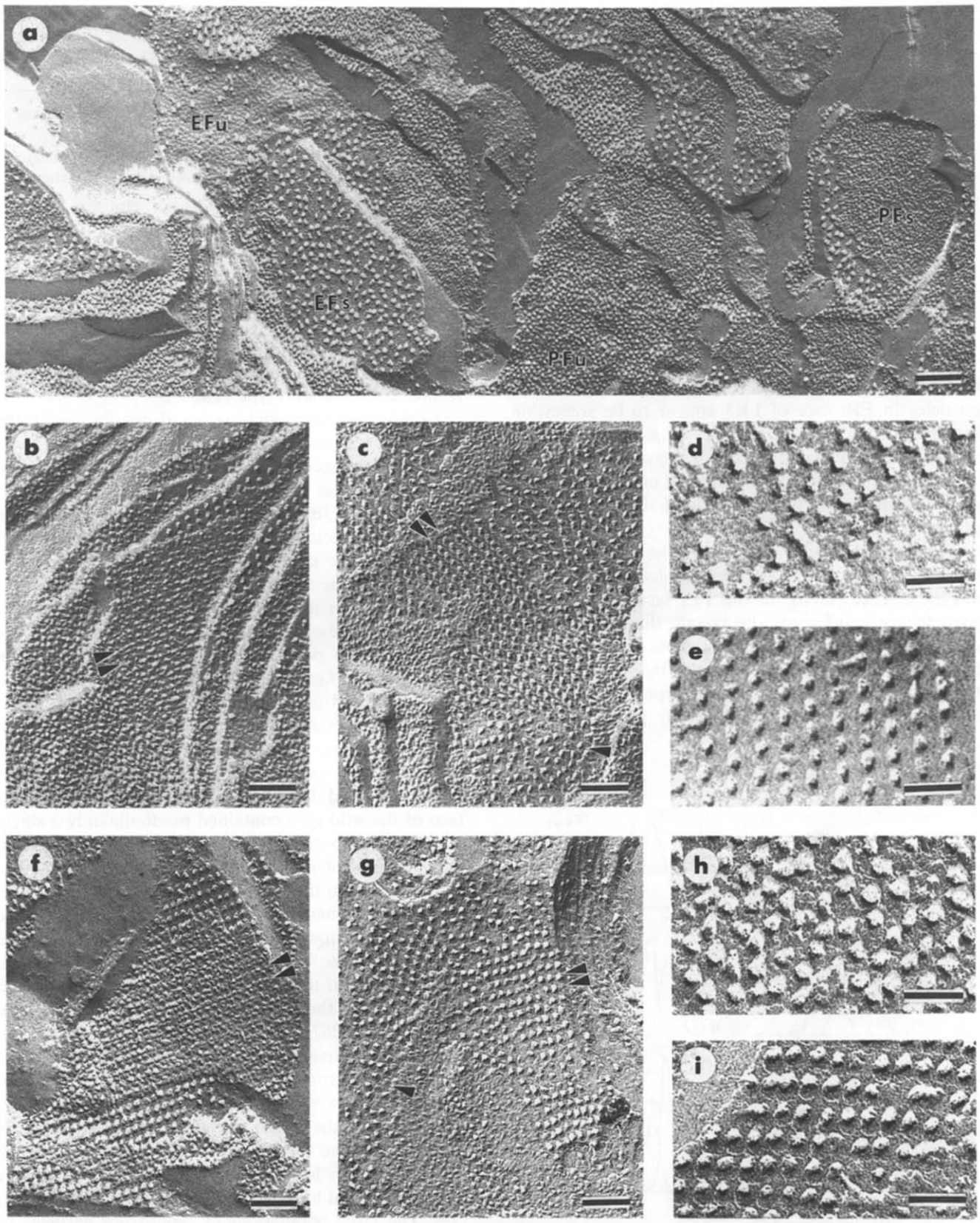

Fig. 2. Electron micrographs of freeze-fracture replicas of thylakoid membranes from *Arabidopsis thaliana* wild type showing EFs, PFs, EFu and PFu fracture faces (a); replicas of thylakoid membranes from JB67 *Arabidopsis* mutant showing aligned particles in PFs (b), aligned and randomly distributed particles in EFs face (c), enlarged areas with randomly distributed (d) and aligned (e) particles in EFs face; replicas of thylakoid membranes from LK3 *Arabidopsis* mutant showing particles in PFs face organized in double rows (f), aligned and randomly distributed particles in EFs face (g), enlarged areas of randomly distributed (h) and aligned (i) particles in EFs face. Bar equals 0.1 μm in a, b, c, f and g, and 0.05 μm in d, e, h and i.

Table 2
Average diameter of particles on the fracture faces of thylakoids of *Arabidopsis thaliana* wild-type, JB67 and LK3 mutants deficient in lipid fatty acid desaturation

Fracture face	Average diameter (nm) (±S.D.)		
	wild type	JB67	LK3
EFs	13.2±2.3	8.9±1.2 aligned 12.3±1.9 random	8.0±1.6 aligned 10.8±1.2 random
PFs	7.6±2.7	7.2±0.9 aligned 6.7±2.4 random	7.5±0.8 aligned 7.4±2.1 random
EFu	8.8±3.1	8.9±2.5	8.3±1.9
PFu	9.8±2.5	7.4±2.4	7.6±1.5

Table 3
Packing densities of particles on the fracture faces of thylakoids of *Arabidopsis thaliana* wild-type, JB67 and LK3 mutants deficient in lipid fatty acid desaturation

Fracture face	Particle density (particles/μm^2) (±S.D.)		
	wild type	JB67	LK3
EFs	1527± 70	1469± 49 random 2262± 5 aligned	1281± 78 random 1818± 10 aligned
PFs	6234±163	7583±184 random 4374± 43 aligned	7229±239 random 3586± 32 aligned
EFu	381± 64	449± 23	459± 28
PFu	4219± 98	6975±217	6062± 49

varying from 5 nm to 10 nm with an average diameter of 9.3 nm. Similarly to JB67, the randomly distributed particles in EFs face of LK3 appear to be somewhat larger than the aligned particles (Fig. 3, Table 2). It should be noted that the sizes of the aligned particles were not influenced by any preferred orientation of the particles with respect to the angle of shadowing of the platinum replica.

The aligned particle domains on the EFs face in the mutant plants appear to be complementary to the aligned particle areas on the PFs face, although the particle size is different. The average diameter of particles in the PFs face is significantly less than in the EFs face and the PFs particle diameter is, in turn, less in the mutants compared to the wild type (Table 2). This is also reflected in the significantly greater particle density in the PFs regions of the mutant thylakoids compared to the wild type (Table 3).

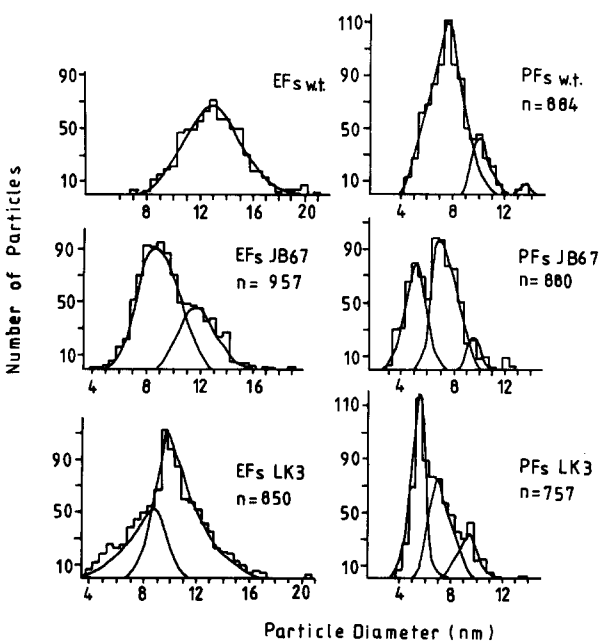

Fig. 3. Histograms of the size distribution of membrane-associated particles in the stacked exoplasmic (EFs) and protoplasmic (PFs) fracture faces of thylakoids from the wild-type, JB67 and LK3 *Arabidopsis* mutants deficient in fatty acid desaturation.

Comparison of particle size and distribution in the unstacked regions of the thylakoid membrane also revealed differences between the wild-type, JB67 and LK3 *Arabidopsis* mutants, but no significant differences between JB67 and LK3. Thus, the density of the membrane-associated particles in EFu and PFu faces is greater and the mean diameter is smaller in the mutants than in the wild type (Tables 2 and 3).

A detailed analysis of the histograms of particle size distribution presented in Figs. 3 and 4 indicate, that more than one population of particles are present in each fracture face. The individual populations have been identified as shown in Figs. 3 and 4 and their mean diameters are presented in Table 4. There were clear differences in the particle size distribution in the stacked and unstacked membrane regions of the wild-type, JB67 and LK3 *Arabidopsis* mutants. The EFs face of the wild type contained predominantly a single population of particles with mean diameter of 13.2 nm and particles of similar size and random distribution were present on the EFs faces of JB67 mutant. In LK3 mutant the randomly spread EFs particles were smaller-sized than those in JB67 and the wild-type *Arabidopsis*. The aligned particles in JB67 and LK3, however, appear to be smaller, than those with random distribution on the same fracture face (Table 4). In the PFs face of JB67 and LK3 three different size categories are observed (Fig. 3, Table 4). In both mutants the aligned particles fall into a single population of particles with a mean diameter of about 7 nm and coincide with the major population in wild-type PFs face (Fig. 3). The two populations with diameters of 4.9 nm and 9.7 nm for JB67 and 5.5 nm and 9.7 nm for LK3 correspond to the randomly distributed PFs particles. The large populations of small-sized particles of mean diameter of about 5 nm have not been identified in the wild-type PFs face. Statistical analysis of the size distribution by *t*-test revealed a significant difference between the wild-type PFs particles and the small-sized

Table 4
Average particle diameters of individual populations of particles resolved as shown in the histograms (Figs. 3 and 4)

	Particle diameter (nm) (±S.D.)			
	EFs	PFs	EFu	PFu
Wild type	13.2 ± 2.3	7.6 ± 1.6	6.3 ± 0.6	9.6 ± 1.6
		10.3 ± 0.7	8.9 ± 0.9	11.3 ± 1.1
			11.2 ± 0.5	13.1 ± 0.9
			12.5 ± 0.5	
JB67	* 8.9 ± 1.2 aligned	** 4.9 ± 0.8 random	6.3 ± 1.0	** 5.5 ± 0.8
	12.3 ± 1.9 random	7.2 ± 0.9 aligned	8.8 ± 0.9	7.5 ± 0.6
		9.7 ± 0.6 random	11.4 ± 0.6	9.7 ± 1.0
LK3	8.0 ± 1.6 aligned	** 5.5 ± 0.6 random	6.5 ± 0.8	6.8 ± 0.8
	10.8 ± 2.1 random	7.5 ± 0.8 aligned	9.3 ± 0.6	9.2 ± 0.9
		9.7 ± 0.6 random	11.3 ± 0.9	

t-test has been used to compare the average diameters of the mutants and the wild type.
* $P < 0.05$, ** $P < 0.01$.

particle populations on the same fracture face of the mutants (Table 4).

Particles observed in the EFu face of the wild type and the two mutants are described by three categories of sizes with average diameter of about 6 nm, 9 nm and 11 nm, respectively (Fig. 4, Table 4). The proportion of particles in each size category, however, is different with a higher proportion of the smaller-sized particles in JB67 and particularly in LK3 compared to the wild type. The particle distribution in PFu face is more complex. A population of particles with average diameter of about 9 nm is present in PFu face of the three plants (Fig. 4, Table 4). However, the two populations with larger mean diameters in the wild type ($d = 11.3$ nm and $d = 13.1$ nm) were not represented in either of the mutants. By contrast, the mutants contained predominantly particles with diameters of about 6 nm, which were not observed in the wild-type PFu face (Fig. 4, Table 4).

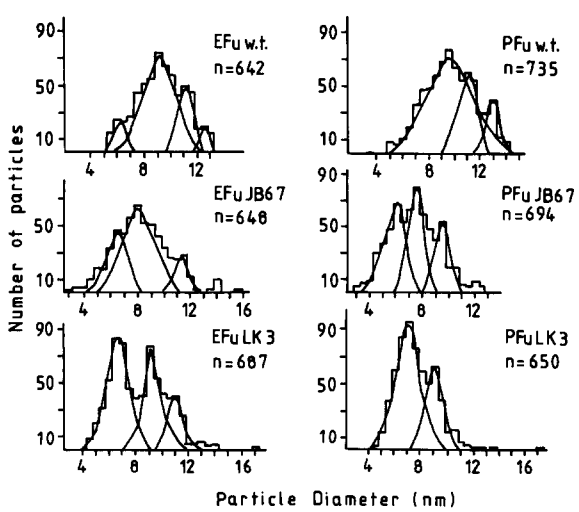

Fig. 4. Histograms of the size distribution of membrane-associated particles in the unstacked exoplasmic (EFu) and protoplasmic (PFu) fracture faces of thylakoids from *Arabidopsis thaliana* wild-type, JB67 and LK3 mutants deficient in fatty acid unsaturation.

4. Discussion

The present paper examines differences in the ultrastructure of thylakoid membranes from *Arabidopsis thaliana* wild-type, JB67 and LK3 mutants deficient in lipid fatty acid desaturation. Despite the large changes in the fatty acid pattern of these mutants, they were not visually distinguishable from the wild type under standard growth conditions. The results show that the reduction in lipid acyl chain polyunsaturation has a marked effect on the chloroplast ultrastructure. A decrease in the amount of the appressed and non-appressed thylakoid membranes in JB67 and LK3 *Arabidopsis* mutants compared to the wild-type resulting in an overall decrease in the length of photosynthetic membrane is consistent with a previous report on the relative amounts of the appressed membranes in LK3 *Arabidopsis* mutant compared with the wild-type [6]. However, these studies revealed no difference in the stromal thylakoid length of LK3 mutant and the wild type. This inconsistency with the observations in the present study may be related to differences in growth conditions, although they were identical for both plants in this study. The present results are also in variance with data reported by Kunst et al. [4] who found no obvious differences in chloroplast ultrastructure between the wild-type and JB67 *Arabidopsis* mutant.

The ultrastructural features of freeze-fractured thylakoid membranes of *Arabidopsis* are consistent with those reported for thylakoids of a variety of other higher plants including spinach [17–19], maize [20], lettuce [21], pea [22–25] and barley [26]. Most of these measurements have been performed on replicas produced by unidirectional shadowing. However, similar

data are obtained using rotary shadowing method [27]. The present results reveal marked differences between the structural features of the wild-type, JB67 and LK3 *Arabidopsis* mutants. One of the consistent observations recorded in the analysis of particle sizes is that all four fracture faces in JB67 and LK3 mutants contain populations of particles that are on average smaller, than those observed in wild-type thylakoids. Moreover, the size distribution of these particles is in ranges that have not been reported previously (Figs. 3, 4, Table 4). Smaller particle sizes are invariably associated with an increase in the packing density of the particles on the fracture faces (Table 3).

Another distinguishing characteristic of the particles in the EFs face of the mutants is that, in part, they are arranged in a rectangular lattice and they are smaller than those randomly distributed on the EFs face (Fig. 2d,e, Table 2). Particle arrays have been observed in wild-type and chlorina-f2 barley mutant [26,27] and spinach [28,29]. Their presence is considered as an atypical configuration induced during chloroplast isolation. It has been found that certain buffers, such as Hepes, Tricine, Mes and Pipes were effective in inducing array formation in wild-type and chlorina-f2 barley mutant, whilst such arrays were not observed when phosphate buffer was used in chloroplast isolation [26]. We found that all these buffers including phosphate buffer, were effective in inducing particle arrays in chloroplasts of JB67 and LK3 *Arabidopsis* mutants (Tsvetkova, N., Apostolova, E., unpublished data).

The particles in some regions of the PFs face in the mutants were also arrayed in rows complementary to the aligned particles observed in EFs faces (Fig. 2b,f). The ratio of particle density in PFs and EFs arrays was approximately 2:1 for *Arabidopsis* mutants. This compares to a value of 1:3 reported about chlorina-f2 barley mutant [26].

While the factors responsible for inducing particle arrays are not yet known, the ratio of particles in complementary fracture faces suggests that the decreased level of lipid unsaturation results in changes in the association of membrane chloroplast components. This, together with the occurrence of smaller-sized particles in the mutant membranes and the pronounced increase in packing density on both exo- and protoplasmic fracture faces suggest that the reduced number of lipid double bonds affects the ability of the lipid matrix to provide the assembly between the components of the thylakoid membranes.

There is still debate regarding the precise nature of the membrane-associated particles observed in freeze-fracture replicas of thylakoid membranes. It is generally accepted, that the large particles found on the EFs and EFu fracture faces are morphological equivalents of the pigment-protein complexes of PSII [19,23,31]. Immunochemical and electron microscopy studies of chloroplasts of chlorophyll-*b* deficient maize mutant revealed that the light-harvesting antennae of PSII exist in two distinct subpopulations (bound and peripheral forms) [32]. Support for this view comes from biochemical and image processing analyses of particle arrays in spinach chloroplast membranes [33]. The PSII core complex, along with its bound LHCII antennae, partitions with the exoplasmic membrane leaflet during freeze-fracture giving rise to EFs and EFu particles. In contrast, the peripheral LHCII-complexes partition exclusively with the protoplasmic membrane leaflet as PFs and PFu particles [31,32,34]. This conclusion is also consistent with studies of particle distribution in mutants of barley [35] and maize [36] lacking PSI activity. These studies revealed a marked decrease in particle number normally found on the PFu face, but little or no changes in the size and packing densities of particles in the PFs face, supporting the concept, that the appressed regions are mainly associated with PSII, while those associated with PSI complexes are largely restricted to the non-appressed regions of the membrane. In addition to the particles associated with the chlorophyll-protein complexes of PSI, the PFu face is thought to contain particles associated with the cytochrome b_6/f complex and CF_0-CF_1 complex of the coupling factor, but the assignment of specific groups of particles to these complexes has not been demonstrated.

Biochemical studies of JB67 and LK3 *Arabidopsis* mutants showed a reduction in chlorophyll content per unit leaf fresh weight (15% for JB67 and 18% for LK3) and a slight decrease in the amount of LHCP associated with PSII compared to the wild-type [4,6]. In addition, there was an increase in 77 K fluorescence at 685 nm (corresponding to LHCP associated with PSII) in the two mutants [4,6]. This increase has been interpreted as a result of partial dissociation of LHCP from PSII. Support for this concept comes from experiments in which the degree of lipid unsaturation in thylakoid membranes was reduced by homogeneous catalytic hydrogenation [16]. Fluorescence studies of hydrogenated pea chloroplasts showed, that the fatty acid composition of MGDG markedly affects the supramolecular structure of PSII complex [16]. Highly unsaturated MGDG were particularly effective in restoring energy transfer between LHCP and the reaction centre of PSII, indicating that the extent of membrane lipid unsaturation has a crucial role in the functional organization of PSII complex. This conclusion is also consistent with reconstitution studies showing that, in presence of polyunsaturated MGDG, the chlorophyll-protein complexes reassemble into supramolecular structures similar to those in intact thylakoid membranes [37]. The freeze-fracture data on thylakoid membrane structure of *Arabidopsis* mutants obtained in this study support the notion that polyunsaturated membrane

lipids are required to induce functional associations of components of the photosynthetic apparatus.

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