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FUNCTIONAL AND STRUCTURAL ORGANIZATION OF CHLOROPHYLL IN THE DEVELOPING PHOTOSYNTHETIC MEMBRANES OF *EUGLENA GRACILIS* Z

IV. LIGHT-HARVESTING PROPERTIES OF SYSTEM II PHOTOSYNTHETIC UNITS AND THYLAKOID ULTRASTRUCTURE DURING GREENING UNDER INTERMITTENT LIGHT

G. DUBERTRET and M. LEFORT-TRAN

with technical assistance of M. POUPHILE and F. AMBARD-BRETTEVILLE

Laboratoire de Cytophysiologie de la Photosynthése, C.N.R.S., 91190 Gif-sur-Yvette (France)

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Summary

Dark-grown, non-dividing *Euglena gracilis* Z cells were exposed for 100 h to intermittent light (15 s every 15 min darkness) and were then transferred to continuous light. During chloroplast differentiation, the development of light-harvesting and trapping properties of Photosystem II was analyzed mainly with fluorescence induction measurements in the presence of 3-(3,4-dichlorophenyl)-1,1-dimethylurea and was associated with observations on ultrastructural organisation of developing thylakoids using thin section and freeze-fracture methods.

Results showed that: (a) the synthesis of chlorophyll *b* and probably that of the light-harvesting chlorophyll *a/b*-protein complex was more reduced by intermittent light than the formation of active system II reaction centers; (b) the size of the overall photosynthetic units, i.e. the number of chlorophyll molecules per O_2 molecule evolved under a regime of repetitive saturating short flashes was reduced by 2—3 compared to those developed under continuous light; (c) the lack of chlorophyll induced by intermittent light affected more

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Abbreviations: DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; PS, Photosystem; EF, exoplasmic fracture faces; PF, protoplasmic fracture faces; Chl, chlorophyll; LHCP, light-harvesting chlorophyll *a/b* protein complex.

specifically the size of light-harvesting antennae of system II units, the optical cross-section of which was reduced by 3—4; (d) energy transfers did not occur between these small system II units in spite of high concentrations of PS II reaction centers and of a high trapping efficiency of the absorbed energy; (e) thylakoids developed under intermittent light were not stacked; (f) particles on exoplasmic fracture faces were significantly smaller than those developed under continuous light; (g) rapid synthesis of chlorophyll (Chl *a* and Chl *b*) upon exposure to continuous light of cells first greened under intermittent light are concomittant with rapid recovery of light-harvesting properties and structural characteristics of thylakoids developed under continuous light.

These structural and functional observations are consistent with the hypothesis that system II units are organized in the photosynthetic membrane as individual and discrete entities, the morphological expression of which would correspond to exoplasmic fracture face particles. They also support the model whereby energy transfers between physically connected system II units could occur across the partition between exoplasmic fracture face particles brought into contact in stacked regions.

Introduction

The definition of the basic photosynthetic units, system I or system II, by the overall chlorophyll to reaction center ratio does not allow us to specify their structural organization in the photosynthetic membrane. They could a priori exist as non-individual entities according to a statistical model whereby reaction centers would be dispersed in a light-harvesting chlorophyll continuum, or on the other hand they could correspond to structural, individual entities connected to each other in the membrane or not so connected.

The absence of photochemical activity of the morphologically defined particles associated with the outer surface of thylakoids [1], initially presented by Park and Biggins [2] as the 'Quantasomes', led Park and Pfeifhofer [3] to propose that the particles embedded within the membrane and revealed by freeze-fracture techniques could correspond to the morphological expression of photosynthetic units. Such an hypothesis appears to be supported by the photochemical activity and the pigment composition of the particles isolated by Wessels [4] from detergent solubilized thylakoids, the size of which corresponds to that of these freeze-fractured particles. Experiments by mechanical [5] or detergent [6] fractionation of chloroplast lamellae then would suggest the correlation of Photosystem I and Photosystem II activity with small particles (80 Å) on protoplasmic fracture faces (PF) and large particles (170 Å) on exoplasmic faces (EF), respectively. This conclusion has been strongly supported by the recent observations of Henriques and Park [7], and of Armond et al. [8] who demonstrate a close correlation between the size of the large EF particles and the amount of light-harvesting chlorophyll *a/b* protein complex.

Park and Biggins [2] calculated that a lamellar subunit containing about 230 chlorophylls would be expected to have a diameter of 135—170 Å. On the basis of this calculation, each of the large EF particles would correspond to only one system II photosynthetic unit. The problem arises then as to how energy trans-

fers, which require distances smaller than 50 Å, can occur between system II units [9], since most of these large EF particles appear to be isolated from each other, even in partitions where they are concentrated.

In this report, analysis of greening of *Euglena gracilis* Z under intermittent light shows that morphological and functional approaches based on energy-transfer characterization converge in (a) demonstrating the individual, structural organization of system II photosynthetic units, and in (b) supporting the hypothesis that energy transfers between light harvesting antennae depend on the general relative organization of photosynthetic membranes within the chloroplast.

Material and Methods

Cell culture and preparation

Dark-grown [10], etiolated *Euglena gracilis* Z Klebs strain Pringsheim (Cambridge culture collection No. 1224-5 D) were greened under the same non-dividing conditions as already described by Stern et al. [11]. The dark grown culture was divided into two; one subculture was exposed to an intermittent light (15 s light every 15 min darkness) provided by two banks of 100 W incandescent lamps placed at 30 cm on opposite sides of the culture flask; the second subculture, taken as control, was exposed to a 1200 lux white light provided by banks of daylight fluorescent tubes. Samples were taken as a function of time in both cultures and were characterized for cell numbers and chlorophyll concentration. Cell suspensions equivalent to 5 μg chlorophyll/ml were prepared for oxygen and fluorescence measurements.

Some structural analyses were done on cells grown in the light in a pH 3.5 medium containing 17 mM glutamate and 14.9 mM malate as utilisable organic source [12].

Oxygen and fluorescence measurements

As previously described [10], (a) measurements of oxygen evolution under saturating continuous light ($8.5 \cdot 10^5$ erg $\cdot$ cm^{-2} $\cdot$ s^{-1}) or under a regime of repetitive, saturating short flashes were performed at 25°C with an YSI oxygen electrode and (b) fluorescence induction was recorded at room temperature and in the presence of $2 \cdot 10^{-5}$ M DCMU in order to isolate the primary light-harvesting and trapping reactions of Photosystem II from the electron flow towards Photosystem I. Principles of interpretation of the measured parameters are detailed in another report [10] but we can summarize them as follows: fluorescence induction area was utilized for estimation of active system II reaction center concentrations. The reciprocal of half-rise time of variable fluorescence was taken as a relative measure of the optical cross-section of light-harvesting antennae of system II reaction centers. The exponential or sigmoidal shape of induction curves was interpreted as reflecting the absence or the occurrence of energy transfers between system II units. Variation in F_v/F_{max} ratio can be interpreted as reflecting change either in the proportion of unorganized chlorophylls, or in the concentration of active system II reaction centers on well-organized chlorophylls or also in the concentration of permanent quenching traps, depending on the rise-time and the shape of induction curve.

Preparations for electron microscopy

Thin sections of whole cells. Peletted cells were kept for 2 h at room temperature with 4% glutaraldehyde in 0.1 M phosphate buffer (pH 6.8), washed and post-fixed for 2 h at 4°C with 1% OsO_4 in the same buffer. Samples were dehydrated in increasing concentrations of ethanol and were embedded in Epon-Araldite.

Freeze-fracturing of isolated chloroplasts. For plastid isolation, cells were concentrated in a 0.04 M Tricine buffer (pH 7.6), 0.2 M sucrose and 10 mM NaCl and passed through a French press at 2000 lbs · $inch^{-2}$. Unbroken cells and debris were removed by a 3 min centrifugation at 300 × *g* and the supernatant was then centrifuged at 5000 × *g* for 10 min. The pellet, greatly enriched in chloroplasts, was suspended in minimum volume of same buffer without sucrose, plus 30% glycerol. Samples were deposited on appropriate gold discs, rapidly frozen in liquid Freon 22 then stored in liquid N_2. Freeze-fracturing and platinum-carbon shadowing were performed at −100°C with a Balzers BA 360 M apparatus and the replicas were observed with a Hitachi HU 12 A electron microscope.

Quantitative analysis of micrographs. Particles sizes and distributions on EF fracture faces of thylakoids were determined for each greening condition on several micrographs enlarged to 200 000× from the same initial magnification, with an image analyzer Kontron MOP AM 02.

Results

1. Chlorophyll synthesis and formation of active system II reaction centers

Chlorophyll and system II reaction center accumulation in cells greened under continuous light and in control exposed to continuous light are compared in Fig. 1. The control exhibits the already described delay (about 10 h) in the formation of active system II reaction centers compared to chlorophyll synthesis and its persistence till the end of greening [10]. By comparison, intermittent light does not seem to markedly affect lag phases in chlorophyll synthesis and reaction center formation, but appears to reduce the accumulation rate of these two components. However, this effect is much more pronounced on chlorophyll synthesis and the relative concentrations of chlorophyll and system II reaction centers reached at the end of greening under such conditions represent 15—20% and 30—40%, respectively, of those measured in fully greened control cells. This preferential reduction of chlorophyll concentration could result from a selective inhibition of the synthesis of chlorophyll *b* and probably of the light-harvesting chlorophyll *a/b* protein complex [13], since Chl *a*/Chl *b* ratios are very high in cells greened under intermittent light (over 18), contrary to those measured in control cells (about 5—7).

Exposure of cells greened under intermittent light to continuous light induces immediate synthesis of chlorophyll with a rate similar to that observed in control cells, till a final concentration close to that found in fully greened cells is reached. Simultaneously, rapid synthesis of chlorophyll *b* entails restoration in about 8 h of normal value (about 5—7) for Chl *a*/Chl *b* ratio. Rapid formation of new active system II reaction centers follows chlorophyll synthesis with a constant delay introduced by a lag of about 10 h after exposure of cells to continuous light.

TABLE I

COMPARISON OF SOME PHOTOSYNTHETIC PARAMETERS AT DIFFERENT STAGES OF GREENING OF *EUGLENA* CELLS EXPOSED TO INTERMITTENT OR CONTINUOUS LIGHT

C.L., continuous light; I.L., intermittent light.

Unit		Greening conditions			
		100 h C.L.	100 h I.L.	175 h C.L.	100 h I.L. +75 h C.L.
Chlorophyll	$\mu g/10^6$ cells	13	2.3	15	12
PS II R.C.	number/μg Chl *	$38 \cdot 10^4$	$75 \cdot 10^4$	$38 \cdot 10^4$	$45 \cdot 10^4$
	relative number/Chl **	940	1955	985	965
Oxygen	μmol O_2/h/mg Chl ***	130	375	100	210
	μmol O_2/h/10^{14} PS II R.C. *	36	46	26	46
	O_2/h/PS II R.C. (relative units) **	36	43	24	53

* Numbers of PS II reaction centers (R.C.) are quantitatively calculated from oxygen measurements under a regime of repetitive saturating short flashes.

** The relative number of PS II reaction centers is estimated from determinations of fluorescence induction areas.

*** Measurements are performed under saturating continuous light ($8 \cdot 10^5$ erg $\cdot$ $s^{-1} \cdot cm^{-2}$).

Reaction centers, measured with fluorescence induction areas in the presence of DCMU, are definitely associated with functional electron donor chains (water splitting system) and with functional primary electron acceptors (quencher Q). However, this method does not permit us to ascertain whether these reaction centers are functionally connected with complete electron transfer chains and are efficient in the overall photosynthetic reaction. Concentrations of such system II reaction centers were determined by measuring oxygen evolution under a regime of repetitive saturating short flashes, according to Emerson and Arnold [14]. Table I shows that the two methods used provide similar results during greening and, consequently, that all reaction centers measured by fluorescence method are functionally connected to complete electron transport chains.

2. Evolution of the size of photosynthetic units

The photosynthetic unit can be defined by the overall ratio of total chlorophylls to system II reaction centers as estimated by fluorescence (relative determinations) or oxygen (quantitative determinations) measurements. We have seen in a previous paper [10] that when cells are greened under continuous light, the initial delay in system II reaction center formation compared to chlorophyll synthesis and the parallel accumulation of these two components are responsible for a decrease of this ratio during greening till a low steady-state value is attained in fully greened cells (Fig. 2A). The preferential reduction of chlorophyll synthesis by intermittent light leads to an important reduction of this chlorophyll/reaction center ratio; after a slight decrease during first stages of greening, the size of the overall system II photosynthetic unit rapidly stabilized at values 2- to 3-times smaller than that measured in fully greened cells (Fig. 2A). The immediate synthesis of chlorophyll *a* and *b* upon exposure to continuous light and the delayed formation of reaction centers are responsible

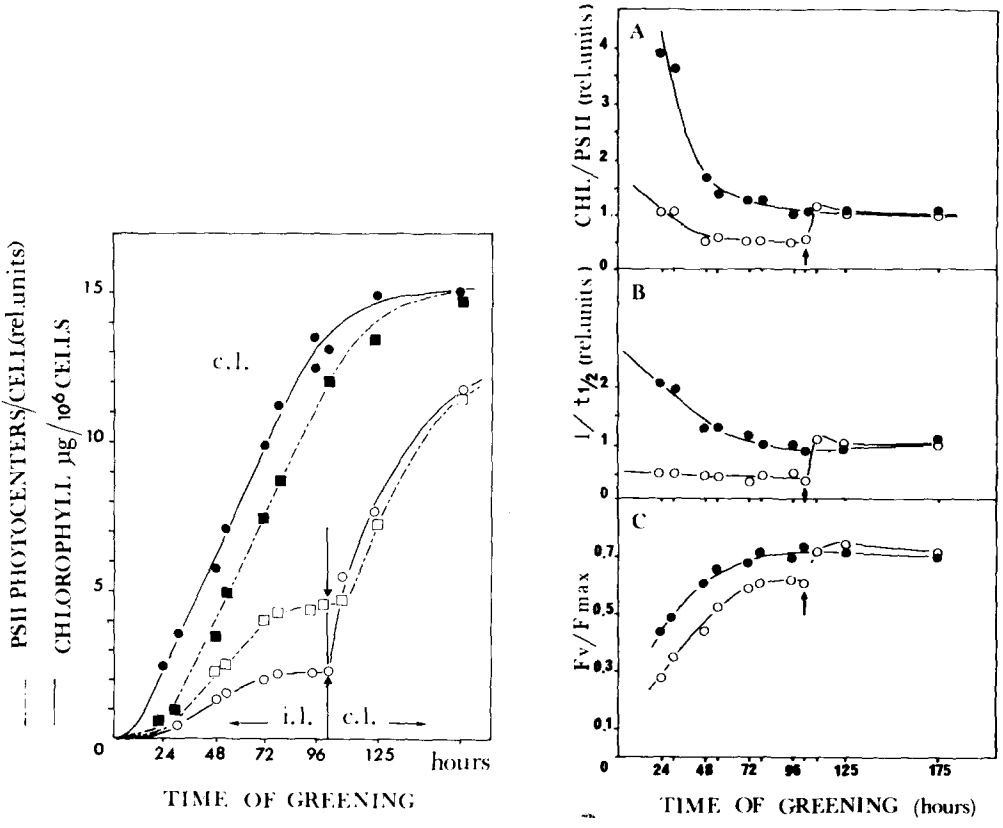

Fig. 1. Time-courses of chlorophyll (———) and system II reaction center (· — · — ·) accumulation in control cells (●, ■) greened under continuous light (C.L., 1200 lux) and in cells first greened under intermittent light (I.L.), then transferred to continuous light (○, □).

Fig. 2. Changes during greening under continuous light (●) or under intermittent light (○) of: (A) the size of the overall photosynthetic unit (total chlorophyll/PS II reaction center); (B) the size of the optical cross-section of system II light harvesting atennae ($1/t_{1/2}$); (C) the trapping efficiency by PS II reaction centers of energy absorbed by chlorophylls capable of fluorescence (F_v/F_{max}).

for a rapid increase of this ratio to the same value as that found for photosynthetic units of fully greened control.

Table II shows that similar results are obtained when system II reaction center concentrations are quantitatively determined with oxygen measurements under flashing light. The size of photosynthetic unit developed under intermittent light is 800 chlorophylls for each O_2 molecule evolved instead of 1600 chlorophylls per O_2 molecule when developed under continuous light.

However, these definitions give only a statistical meaning to photosynthetic units, which are then best physiologically defined by the number of chlorophylls functionally connected to each reaction centers. Relative estimations of the size of the optical cross-section of light-harvesting antennae associated to system II reaction centers are possible by measuring the rate of closure of these reaction centers by light, in the presence of DCMU. The evolutions of the reciprocal of half-rise time ($1/t_{1/2}$) of variable fluorescence (which express the size

TABLE II

EVOLUTION OF THE SIZE OF THE OVERALL PHOTOSYNTHETIC UNITS AND OF SYSTEM II LIGHT HARVESTING ANTENNAE AT DIFFERENT STAGES OF GREENING UNDER CONTINUOUS OR INTERMITTENT LIGHT

C.L. continuous light; I.L., intermittent light.

Units		Greening conditions			
		100 h C.L.	100 h I.L.	175 h C.L.	100 h I.L. +75 h C.L.
Chlorophyll	$\mu g/10^6$ cells	13	2.3	15	12
Size of overal photosynthetic units	Chl molecules/O_2 molecule *	1560	810	1560	1320
	Chl/PS II reaction center **	210	95	190	190
Size of PS II light harvesting antennae	$1/t_{1/2}$ (relative units) ***	180	7.0	200	210

* Calculated from oxygen measurements under a regime of repetitive saturating short flashes (quantitative values).

** Estimated from fluorescence induction area (relative values).

*** Half-rise times of variable fluorescence ($1/t_{1/2}$) are measured on induction curves.

of system II units) in cells greened under continuous or intermittent light are compared in Fig. 2B. By contrast with the already described decrease of the optical cross-section of system II reaction centers formed under continuous light [10], the size of system II photosynthetic units is almost stable throughout greening under intermittent light at values 3- to 4-times smaller than that measured in fully greened control. Effects of the reduced chlorophyll synthesis induced by intermittent light thus appear to be more marked in system II light harvesting antennae than on overall photosynthetic units, which appear to be only 2- to 3-times smaller than in fully greened cells (Table II). This is not surprising, since chlorophyll *b*, which is absent in cells greened under intermittent light, is known to be more specifically connected with Photosystem II.

When cells greened under such conditions are exposed to continuous light, the observed rapid chlorophyll synthesis contributes to restore the normal size of system II units measured in fully greened cells (Fig. 2B).

3. Evolution of fluorescence induction characteristics

The evolution of fluorescence induction kinetics in cells greened under intermittent light appreciably differs from that already described in cells greened under continuous light. The slight increase of reaction centers to chlorophyll ratio is still responsible for an increase of F_v/F_{max} ratio (Figs. 2C and 3). However, contrary to what is observed in control cells, this evolution is not accompanied by any important increase of half-rise time of variable fluorescence (Figs. 2B and 3) nor by any significant change in the exponential shape of induction curves (Fig. 3). It is important to notice that even at the end of greening under intermittent light, induction curves are still exponential in spite of (a) high concentration of system II reaction centers per chlorophyll unit (fluorescence and oxygen measurements, Table I), (b) values of F_v/F_{max} ratios as high as 0.65 and (c) long half-rise times of F_v.

The rapid chlorophyll synthesis (Chl *a* and Chl *b*), occurring during the lag in

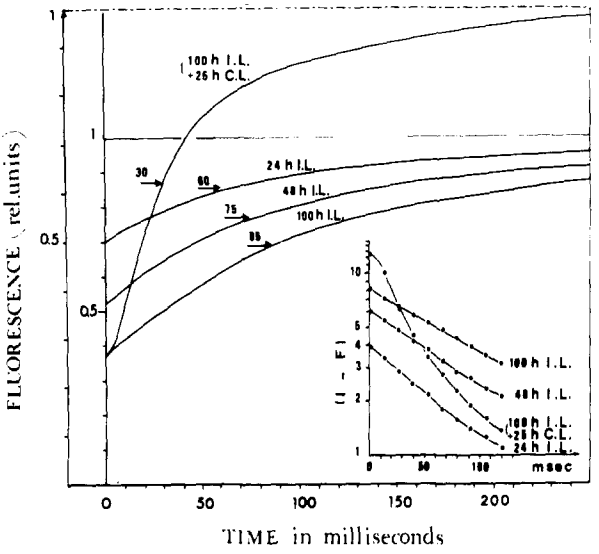

Fig. 3. Evolution of fluorescence parameters during greening of *Euglena* under intermittent light and after transfer to continuous light; fluorescence induction curves then recover characteristics of fully greened control cell. Insert: log plot of $1 - F$. Cells greened for 100 h under intermittent light display an exponential rise of variable fluorescence in spite of (a) high concentration of system II reaction centers per chlorophyll (large area above fluorescence induction curve), (b) very small optical cross-sections of system II light harvesting antennae (long half-rise time) and (c) a good trapping efficiency of the absorbed energy ($F_v/F_{max} = 0.63$). Rapid chlorophyll synthesis upon exposure of cells to continuous light is concomitant with (a) an increase of the size of system II light harvesting antenna (decrease $t_{1/2}$), (b) an increase of the amplitude of variable fluorescence and (c) a change of induction curve from exponential to sigmoidal.

reaction center formation (Fig. 1) in cells transferred to continuous light, is accompanied by a rapid restoration of characteristics of fully greened control: (a) Chl *a*/Chl *b* ratio decreases to about 7, (b) reactions center to chlorophyll ratio decreases to normal values (Table I), (c) half-rise-times of fluorescence decrease by 2—3 (Figs. 2B and 3), (d) F_v/F_{max} increases to about 0.70—0.75 (Figs. 2B and 3) as a consequence of a slight increase of F_v (Fig. 3) and (e) the shape of induction curves becomes sigmoidal (Fig. 3).

The chlorophylls synthesized during the first hours of continuous illumination thus restore normal light-harvesting and trapping properties of pre-existent Photosystem II units. The subsequent formation of new chlorophylls and active system II reaction centers does not markedly change fluorescence characteristics (Fig. 2) and thus seems to participate in the formation of new system II units similar to those of fully greened cells.

4. Ultrastructure

Non-dividing cells in which steady state concentrations of chlorophyll have been attained after about 4 days of greening under intermittent or continuous light and green cells mixotrophically grown in the light for several generations are compared here. Chloroplasts present in fully greened cells (8—10 μg Chl/10^6 cells, Chl *a*/Chl *b* ≈ 5—7) or in green cells grown in the light (5—6 μg Chl/10^6 cells, Chl *a*/Chl *b* ≈ 7—9) are elongated and bigger than the rather ovoid ones developed under intermittent light (2—3 μg Chl/10^6 cells, Chl *a*/Chl *b* >

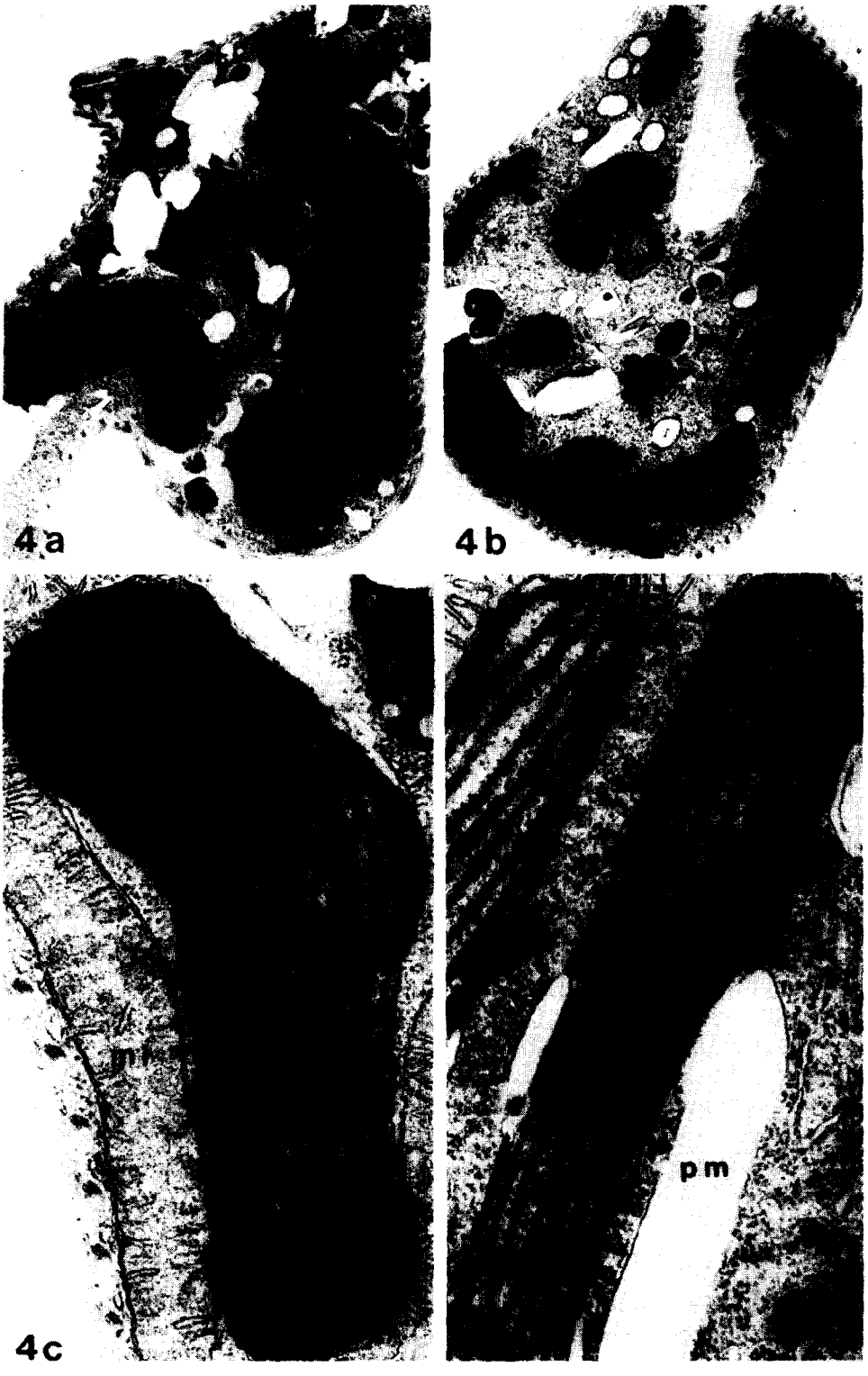

Fig. 4. Thin section of *Euglena* cells. m.i., mitochondria. (a) Cells greened for 4 days under intermittent light. Chloroplasts are ovoid and appear densely packed with numerous thylakoids. (b) Cells are fully greened under continuous light and contain elongated chloroplasts. (c) Chloroplasts developed under intermittent light. Thylakoids (t) are isolated, (single arrows), grouped by pairs (double arrows) or organized as granalike structures (g.l) at the opposite ends of the chloroplast (chl). (d) Chloroplast fully differentiated under continuous light. Long thylakoids stretch the full length of the chloroplast and appear to be appressed by two or three. Paramylon granules (pm) are located outside the chloroplast.

TABLE III

COMPARISON OF STRUCTURAL CHARACTERISTICS IN RELATION WITH CHLOROPHYLL CONTENT OF *EUGLENA* CELLS GREENED UNDER CONTINUOUS OR INTERMITTENT LIGHT, OR MIXOTROPHICALLY GROWN IN THE LIGHT

C.L., continuous light; I.L., intermittent light.

	Non dividing cells greened for		Green cells mixotrophically grown in the light (log phase)
	72 h C.L.	100 h I.L.	
Chlorophyll ($\mu g/10^6$ cells)	8—10	2—3	5—6
Chl *a*/Chl *b*	5—7	>18	7—9
EF particles (density/μm^2)	750	880	973
Average diameter of EF particles (Å)	135—(155)	55—(70)—90	95

18) (Table III, Fig. 4). These chloroplasts contain numerous thylakoids (up to 20 per chloroplast), isolated, associated by pairs, (Fig. 5c) or grouped in grana-like structures (Fig. 5b). However, those thylakoids developed under intermittent light remain isolated from each other (Fig. 5b and c) and it is exceptional to observe between them a dark line characteristic of the partition between stacked thylakoids. On the other hand, chloroplasts developed under continuous light exhibit thylakoids stacked by two or three and between which typical partitions can beobserved (Fig. 5a). Transversally freeze-fractured chloroplasts (Figs. 6a and 7a) display the same differences in the general organisation of the chloroplast lamellar system. These are associated with changes in the membrane structure, namely in the size and distribution of particles in the exoplasmic fractured faces (EF) of thylakoids. Morphological results are summarized in Table II in relation with the chlorophyll content of the analysed cells. Thylakoids developed under intermittent light are not stacked. They will, therefore, exhibit only two types of fracture face, one protoplasmic (PF) and one exoplasmic (EF) (Fig. 6b). This is in contrast to thylakoids developed under continuous light, which exhibit four different fractured faces according to their stacked or unstacked organization (Fig. 7b) [15].

Measurements of particle density on fractured faces of thylakoids provide similar values for cells greened under intermittent light or continuous light or for cells mixotrophically grown in the light (880, 750, 973 EF particles/μm^2, respectively, Table III).

In contrast, particle size distribution on the same E fractured faces appears to be much more dependent on greening or culture conditions (Fig. 8). Most of the stacked EF particles in cells greened under continuous light (Fig. 8A) (Chl *a*/Chl *b* = 5—7) exhibit a diameter close to 130 Å, similar to that measured by Miller and Staehelin [16] and by Ophir and Ben-Shaul [17], markedly smaller than that observed for stacked EF particles of higher plants (around 160 Å). When cells are greened under intermittent light (Fig. 8c, Chl *a*/Chl *b* > 18), the histogram of particle size is markedly shifted to lower values, and most of EF particles exhibit sizes ranging from 55 to 90 Å with a peak at about 70 Å. The shape of this histrogram suggests some heterogeneity in particle size distribution, which could result from the existence on this E fracture face of at least two classes of particle with peaks around 55 Å and 90 Å, and eventually

Fig. 5. Details of thylakoid organization. p, paramylon; t, thylakoids. (a) Thylakoids of chloroplasts developed under continuous light are tightly suppressed by at least two, as shown by the dark line (partition) visible between two adjacent thylakoids and corresponding to stacked regions. (b) Thylakoids in grana-like structures exhibited by chloroplasts developed under intermittent light are isolated from each other. (c) Long thylakoids in chloroplasts developed under intermittent light are often grouped by pairs but are not appressed and remain isolated from each other. They appear to be appressed in very limited regions (arrows).

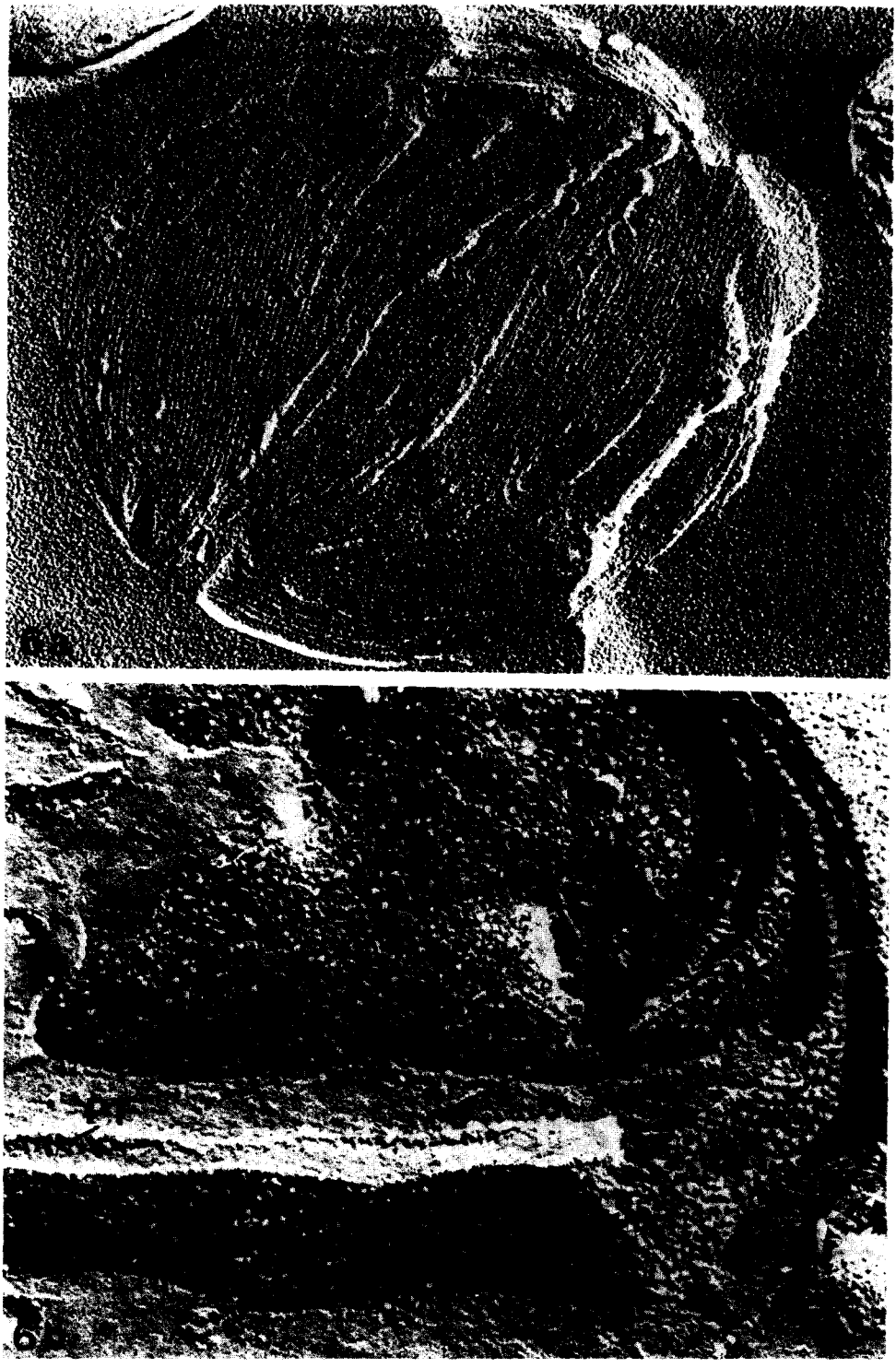

Fig. 6. Freeze-fracture of chloroplasts developed under intermittent light. (a) Transverse freeze-fracture of a whole chloroplast showing thylakoids (t) isolated from each others (compare with Fig. 5b). (b) Details of exoplasmic (EF) and protoplasmic (PF) fracture faces of these unstacked thylakoids.

Fig. 7. Freeze-fracture of chloroplasts developed under continuous light. (a) Transverse freeze-fracture of a whole chloroplast showing thylakoids (t) stacked by two or more (compare with Fig. 5a). (b) Detail of stacked thylakoids exhibiting four fracture-faces, EFs and PFs in stacked regions, EFu and PFu in unstacked regions.

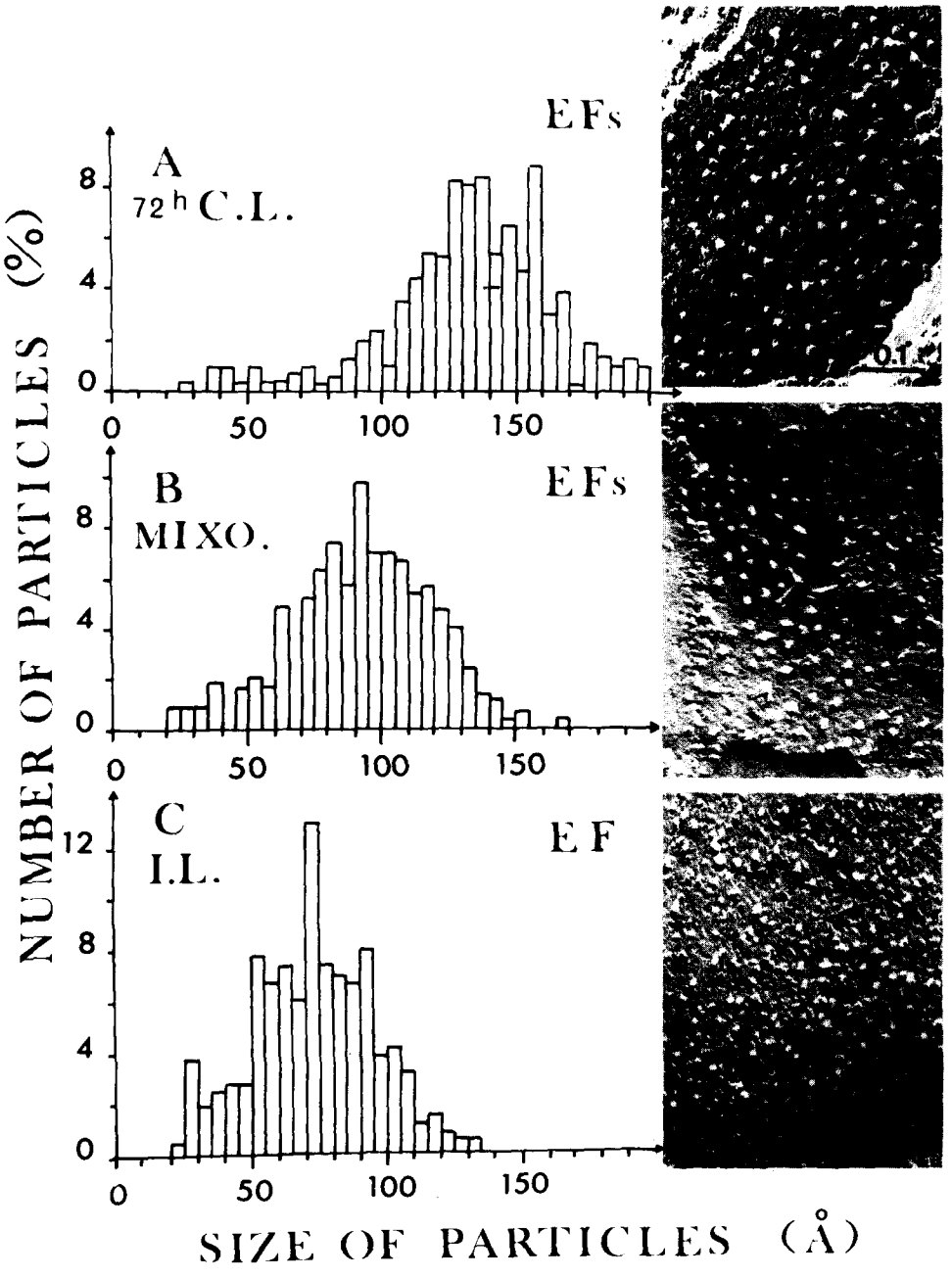

Fig. 8. Histograms of particle size from exoplasmic fractured faces of stacked (EFs) and unstacked (EF) thylakoids. (A) EFs face of stacked thylakoids developed during greening under continuous light (72 h C.L., Chl *a*/Chl *b* = 5—7). The particle density is 750 particles per μm^2. (B) EFs face of stacked thylakoids developed in green cells mixotrophically grown in the light for several generations (Chl *a*/Chl *b* = 7—9). The particle density is 973 particles per μm^2. (C) EF face of unstacked thylakoids developed under intermittent light. (100 h I.L., Chl *a*/Chl *b* > 18). The particle density is 880 particles per μm^2. Depending on experimental conditions, particle size distributions are shifted to low values compared to the control greened under continuous light. This decrease in EF particle size is visible on micrographs on the right side of the figure. It can be correlated to decreasing relative amounts of chlorophyll *b* in the analyzed cells.

a third class with a peak at 70 Å. For intermediate Chl *a*/Chl *b* ratios (about 7—9) found in cells mixotrophically grown under continuous light, Fig. 8b shows that particle sizes are distributed around an intermediate mean size of about 95 Å.

Discussion

The decrease in size of EF particles from 130 Å to about 70—80 Å when chlorophyll ratio increases from 5—7 (fully greened *Euglena* cells) to 7—9 (green cells mixotrophically grown) or to values higher than 18 (cells greened under intermittent light) confirms the direct correlation between the size of particles and the amount of LHCP observed in lettuce by Henriques and Park [7] and in pea by Armond et al. [8] and provides strong evidence in favor of the hypothesis that EF particles correspond to the morphological expression of Photosystem II units. When such comparative analyses are extended to different organisms, it appears that the minimal size of EF particles obtained when LHCP is absent is similar (ranging between 80 and 100 Å) in organisms as different as the primitive eukaryotic alga *Cyanidium* [18], *Euglena* or higher plants such as lettuce [7] or pea [8]. However, the maximal size of these particles seems to be related to the maximal amounts of LHCP synthesized by these organisms. As a matter of fact, *Euglena*, which naturally contains few LHCP, exhibits particles of 130 Å, in contrast to pea and lettuce and other organisms containing large amounts of LHCP in which the size of EF particles ranges between 150 and 170 Å. It thus seems that the general architecture of system II units is very similar in plants and algae and that it corresponds, as proposed by Staehelin [19], to the association of a basic core unit of similar size (80—100 Å) and of variable amounts of LHCP. Furthermore, analyses on *Chlamydomonas* and tobacco mutants have recently lead Olive et al. [20] and Miller and Cushman [21] to propose that these core units, which would correspond to the chlorophyll-protein complexes associated with system II reaction centers, might be required for assembly of LHCP complements as large EF particles.

At the functional level, the reduction of the relative amount of chlorophyll *b* when *Euglena* cells are greened under intermittent light is responsible for a decrease of the size of the overall photosynthetic unit, as defined according to Emerson (chlorophylls/evolved oxygen molecule), or of the size of the basic system II unit, as defined by the optical cross-section of the light harvesting antennae associated with system II reaction centers. However, the decrease in size of these units is much more marked on system II units (3- to 4-times) than on the overall photosynthetic units (only 2-times). This confirms that chlorophylls associated with LHCP are mainly involved in harvesting light for system II reaction centers. One thus can extend the correlation between the amount of chlorophyll *b* (and LHCP) and the size of EF particles to the size of the light harvesting antennae of system II units.

Fluorescence measurements performed on bean by Akoyunoglou [22] and on pea by Armond et al. [23] showed an about 6 to 8-times extension in half-rise time of variable fluorescence when these plants were greened under intermittent light. This increase in half-rise time appears to be the double of that

(3- to 4-times) observed in *Euglena* greened in similar conditions. The interpretation of this extension as reflecting a decrease of the size of sytem II units leads to the conclusion that the participation of LHCP in constituting system II light harvesting antennae is much more important in bean or pea than in *Euglena*, which is consistent with the low LHCP content in this alga. On the other hand, unit size measurements in our experimental conditions provide higher values for organisms (spinach, *Chlorella*) exhibiting low Chl *a*/Chl *b* ratios (about 3) than for fully greened *Euglena*, in which this ratio is high (5—7). These functional results thus are in agreement with the hypothesis proposed by Diner and Wollman [24] that system II light harvesting antennae are formed by the association of a basic PS II center-antenna complex, or core unit, of constant size with variable amounts of LHCP. Structural measurements of size of EF particles and functional measurements of size of system II light harvesting antennae thus lead to the same conclusion concerning the structure of system II units.

Park and Biggins [2] and more recently Williams [25] calculated that a multimolecular complex containing 230 chlorophyll molecules would be expected to have a diameter of 135—170 Å. Each of the EF particles would then correspond to only one system II unit. Since most of these particles appear dispersed on the photosynthetic membrane and isolated from each other, energy transfers between system II units should not occur. However, functional measurements show that such energy transfers do occur (for review, see Ref. 25). This supposes that system II units are tightly connected by groups of at least five units [26] or that they are organized as a chlorophyll continuum allowing free energy migration. If we could demonstrate that system II units can be isolated from each other (and thus that they effectively correspond to individual structural entities) and if we could account for a mechanism which would allow energy transfers between EF particles appearing structurally isolated, then the results obtained by the structural and functional methods could be reconciled.

Concerning the structure of system II units as individual entities, results show that the rise of variable fluorescence is exponential when *Euglena* cells are greened under intermittent light. System II reaction centers thus are functionally isolated from each other as they are during the first stages of greening of a *Chlorella* mutant under continuous light [27] and of bean leaves under intermittent light [22]. However, the determination of precise causes of this exponential rise requires us to take into account other parameters of induction curves. It is important to notice that this exponential shape is manifest in spite of (a) a high level of variable fluorescence ($F_v/F_{max} = 0.66$), (b) a high concentration of active system II reaction centers per chlorophyll (about twice as high as in fully greened controls) and (c) a small size of light harvesting antenna (half-rise time 3- to 4-times longer than in control). These characteristics exclude the possibility that this exponential rise results from too low a concentration of active system II reaction centers on chlorophylls organized in such a way as to allow free energy migration. As a matter of fact, this situation, which is found in *Euglena* during first stages of greening under continuous light, entails a high level of constant fluorescence (low F_v/F_{max} ratio) and a very short half-rise time [10]. Neither are these characteristics consistent with

the possibility that active system II reaction centers are functionally isolated by chlorophylls not yet organized in a way allowing energy migration, because these chlorophylls would be responsible for high level of constant fluorescence (low F_v/F_{max} ratio).

The simplest hypothesis to account for these characteristics of fluorescence induction curves thus appears to be that, in the special case of *Euglena*, system II units developed under intermittent light are organized as small discrete entities (short half-rise time), physically isolated from each other (exponential rise of F_v) and exhibiting a good trapping efficiency of the absorbed energy (low F_v/F_{max} ratio).

Functional and structural approaches thus concur in giving to system II units an individual structure corresponding to multimolecular complexes, whose morphological expression would correspond to EF particles. However, they remain contradictory concerning their organization in the photosynthetic membrane, since energy transfers occur between system II units, in spite of the physical isolation of EF particles in the membrane. Two possibilities thus present themselves: (1) If energy transfers occur in the plane of one membrane, one has to postulate the existence of pigments acting as conductors between these particles, either in the lipid phase of the membrane, which seems to be unlikely [28], or involved in chlorophyll-protein complexes which would remain associated with PF fractures (Ref. 29 and Wollman, F.A., Olive, J., Bennoun, P. and Recouvreur, M., personal communication). (2) Another possibility could be that energy transfers occur between EF particles belonging to two adjacent membranes, brought into contact in stacked areas, as proposed by Arntzen [30]. Direct experimental evidence is still insufficient to allow a choice between these two possibilities.

The second hypothesis is consistent with three facts: (a) As Miller has shown [31], EF particles span the membrane and thus allow direct contact with those EF particles of adjacent stacked membranes [33]. Such contacts could involve groups including more than two particles (since the density of EF particles, which is already increased in stacked areas, is doubled in the partitions), which would account for the occurrence of energy transfers among approximately five interconnected photosynthetic units. (b) Energy transfer is absent in *Euglena* greened under intermittent light; this correlates with a lack of stacking of thylakoids. (c) The experimental unstacking of thylakoids by low ionic strength is accompanied not only by the appearance of energy transfers from system II towards system I ('spillover') but also by the disappearance of energy transfers between system II units.

According to this hypothesis, the function of stacking of photosynthetic membranes thus would be to increase the quantum yield of photochemical reactions, not only by regulating energy transfers from PS II to PS I as proposed by Armond et al. [23], but also by regulating those between system II units. One has to keep in mind, however, that experimental data supporting this hypothesis are also consistent with the model proposed by Wollman et al. (Wollman, F.A., Olive, J., Bennoun, P. and Recouvreur, M., personal communication) where energy transfers would occur in the plane of the membrane through chlorophyll-protein complexes associated with PF faces; such energy conduction on PF faces could then be interrupted by the dispersion of EF

particles subsequent to destacking of photosynthetic membranes [32,33]. It must also be recognized that the two processes for energy transfer, in the plane of the membrane or across the partition are not mutually exclusive and could occur simultaneously at the level of appressed thylakoids.

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