Proc. Natl. Acad. Sci. USA
Vol. 88, pp. 8262–8266, September 1991
Biochemistry

Lateral redistribution of cytochrome b_6/f complexes along thylakoid membranes upon state transitions

(photosynthesis/maize/*Chlamydomonas reinhardtii*/light harvesting complex/immunocytochemistry)

OLIVIER VALLON*, LAURENCE BULTE†, PAOLA DAINESE‡, JACQUELINE OLIVE*, ROBERTO BASSI‡, AND FRANCIS-ANDRE WOLLMAN†§

*Institut Jacques Monod, 2 place Jussieu, 75251 Paris cedex 05, France; †Institut de Biologie Physico-chimique, 13 rue Pierre et Marie Curie, 75005 Paris, France; and ‡Dipartimento di Biologia, Università di Padova, 75 Via Trieste, 35121 Padua, Italy

Communicated by Pierre Joliot, June 10, 1991 (received for review April 16, 1991)

ABSTRACT **The cytochrome b_6/f complex operates in photosynthetic electron transfer either in linear electron flow from photosystem II to photosystem I or in cyclic flow around photosystem I. Using membrane fractionation and immunocytochemistry, we show a change in lateral distribution of cytochrome b_6/f complexes along the thylakoid membranes during state transitions. This change is seen in maize as well as in the green algae *Chlamydomonas reinhardtii*. When either of the two organisms were adapted to state II *in vivo*, the proportion of cytochrome b_6/f complexes found in the photosystem I-enriched stroma lamellae regions was significantly larger than after adaptation to state I. A similar observation was made upon state I to state II transitions done *in vitro* by illuminating, in the presence of ATP, broken maize chloroplasts prepared from dark-adapted leaves. This reorganization of the electron-transfer chain is concurrent with the change in light-energy distribution between the two photosystems, which requires lateral displacement of light-harvesting complex II. That the changes in lateral distribution of both cytochrome b_6/f and light-harvesting II complexes seen upon state transition *in vitro* similarly required addition of exogenous ATP, suggests that the change in cytochrome b_6/f organization also depends on kinase activity. The increased concentration of cytochrome b_6/f complexes in the vicinity of photosystem I in state II is discussed in terms of an increase in cyclic electron flow, thus favoring ATP production. Because transition to state II can be triggered *in vivo* by ATP depletion, we conclude that state transitions should be regarded not only as a light-adaptation mechanism but also as a rerouting of photosynthetic electron flow, enabling photosynthetic organisms to adapt to changes in the cell demand for ATP.**

State transitions are known as a regulation mechanism that controls light-energy distribution between the two photosystems working in series in organisms performing oxygenic photosynthesis (1, 2). In higher plants and green algae, state transitions are now attributed to a lateral displacement along the thylakoid membranes of the mobile fraction of a peripheral antenna complex, the light harvesting complex of photosystem II (LHCII). Upon transition from state I to state II, LHCII moves from the photosystem II (PSII)-enriched grana regions to the photosystem I (PSI)-enriched stroma lamellae (SL) regions (for review, see ref. 3). The driving force for the movement of LHCII is a reversible phosphorylation process, whereby increased electrostatic repulsion upon phosphorylation at the N terminus of several LHCII subunits leads to the migration of some phospho-LHCII out of the grana regions in the state II. Dephosphorylation of the same subunits in state I allows the return of these peripheral antenna complexes to the grana regions.

Although the changes in organization of the light-harvesting antenna upon state transitions are well documented, possible changes in the organization of the photosynthetic electron transfer chain have not been directly investigated. Cytochrome b_6/f (cyt b_6/f), a major protein complex of this electron-transfer chain, has, however, been implicated in state transitions through its role in LHCII-kinase activation. State transitions are abolished in cyt b_6/f mutants of green algae and higher plants due to the absence of LHCII reversible phosphorylation (4–8). Gal *et al.* (9) recently reported that the LHCII-kinase was, indeed, associated with cyt b_6/f complexes.

Whereas the PSII and PSI centers are well separated between the stacked and unstacked regions of the thylakoid membranes, cyt b_6/f complexes are found in significant amounts in both membrane domains (10–13). The identity of the long-distance carrier between PSII in the grana regions and PSI in the SL regions has been a matter of debate (14). It has been recently argued that the rapid diffusion of plastoquinones, which transfer electrons between PSII and cyt b_6/f complexes, is limited to small domains containing less than eight PSII centers (15, 16). Therefore linear electron flow should be sustained by plastocyanin diffusing in the luminal space from its binding site on cyt b_6/f complexes in the stacked regions to PSI in the unstacked regions. The fraction of cyt b_6/f complexes located in the unstacked regions next to PSI would then serve cyclic electron flow around PSI.

There is a growing body of evidence that the ATP requirement of the photosynthetic cell controls state transitions (17–19): an increase in the cell demand for ATP leads to state II, whereas making up for this demand leads back to state I. As a working hypothesis, state II can, therefore, be regarded as a state favoring cyclic photophosphorylation around PSI and cyt b_6/f complexes, aimed at ATP production, whereas state I would favor linear photophosphorylation, producing NADPH for carbon fixation. In the present study, we investigated whether state transitions are accompanied by changes in the distribution of cyt b_6/f complexes between grana and SL, which would provide a structural basis for a change in the ratio of linear to cyclic electron flow. Based on immunocytochemical and biochemical analysis of *in vivo* and *in vitro* state transitions in maize and *Chlamydomonas reinhardtii*, we present evidence for an increased localization of cyt b_6/f complexes in the SL regions in state II.

MATERIALS AND METHODS

Maize. Maize seedlings (*Zea mays* L., cv. Dekalb DF 28) were grown in 12-hr day–night cycles for 2–3 weeks. For *in vivo* state

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: cyt b_6/f, cytochrome b_6/f; LHCII, light harvesting complex of photosystem II; PSI, photosystem I; PSII, photosystem II; SL, stroma lamellae.

§To whom reprint requests should be addressed.

transitions, one batch of plants was kept in the dark (state I), while a second batch was exposed to bright light from a tungsten or a halogen metal vapor lamp (state II), as described (20). After 1-hr adaptation, leaves were rapidly harvested and homogenized three times for 5 sec in a homogenizer with replaceable razor blades in ice-cold buffer (350 mM sorbitol/50 mM Tricine, pH 7.4/10 mM $MgCl_2$/1 mM ascorbate). For light-adapted plants, 10 mM NaF was included in the homogenization buffer. The resulting slurry was filtered through three layers of 30-μm nylon mesh, and mesophyll thylakoids were harvested by centrifugation for 10 min at 1500 × *g*.

For *in vitro* state transition, maize thylakoids obtained from dark-adapted leaves (state I, see above) were resuspended in 50 mM Hepes, pH 7.5/10 mM $MgCl_2$, centrifuged at 1000 × *g* for 10 min, resuspended in 100 mM sucrose/50 mM Hepes·NaOH, pH 7.5/10 mM NaCl/10 mM $MgCl_2$, centrifuged again, and resuspended in the same buffer at 200 μg of chlorophyll per ml. Transition to state II was achieved by adding ATP and NaF at 1 mM and 10 mM, respectively, to the suspension before illumination in a flat glass cuvette as in ref. 21. As a control, thylakoids were incubated in the light without ATP and NaF.

The various batches of thylakoids were then either fixed for immunocytochemistry (see below) or used for purification of SL. In the latter case, thylakoids were pelleted by centrifugation, resuspended in 50 mM Hepes, pH 7.5/10 mM $MgCl_2$/1 mM ascorbate/10 mM NaF, and broken three times through a Yeda press at 120 bars (1 bar = 100 kPa). SL were purified by differential centrifugation as in ref. 20.

C. reinhardtii. Cells of the wild type and of the DUM-1 mutant (isolated by R. Matagne, Université de Liège, ref. 22) were grown in Tris/acetate/phosphate medium (40) under continuous illumination of 1000 lux. Cells were harvested at 4–6 × 10^6 cells per ml and resuspended at 10^7 cells per ml in the same buffer. State I was obtained by a 4000-lux illumination for 20 min in the presence of 10 μM dichlorophenyldimethylurea. State II was obtained by a dark adaptation of wild-type cells for 20 min in the presence of 2 μM antimycin A and 4 mM salicylhydroxamic acid, which block ATP production coupled to mitochondrial electron transport (23). For the DUM-1 mutant, state II was obtained by a dark adaptation with salicylhydroxamic acid only, as this mutant bears a deletion in the mitochondrial gene encoding cytochrome *b* (22). Cells were then fixed in either state I or state II by *p*-benzoquinone treatment as described (24), resuspended in Tris/acetate/phosphate medium (40) supplemented with 10 mM $MgCl_2$ and broken through a Yeda press operating under 100 bars. The resulting broken cells were then pelleted and fixed for immunocytochemistry as described below.

Immunocytochemistry. *C. reinhardtii* cells were fixed in 3% (wt/vol) paraformaldehyde/1% glutaraldehyde in 4 mM potassium phosphate buffer, pH 7.0. Maize thylakoids were fixed in suspension with 1% glutaraldehyde. Both materials were dehydrated in ethanol and embedded in Lowicryl K4M. Thin sections were labeled with specific antibodies and with protein A- or rabbit anti-mouse–gold conjugates as in ref. 25. Labeling was quantitated as in ref. 26. For *C. reinhardtii*, gold granules were counted over well-defined stacked and unstacked membranes, the length of which was measured on a Tektronix graphic tablet, yielding d_u/d_s, the ratio of their labeling densities. With the stacking ratio—i.e., percentage of stacked membranes, measured over wild-type broken cells (σ = 65%)—the proportion of antigen in unstacked membranes (δ) was then deduced by using the formula

$$\delta = \frac{1}{1 + \dfrac{\sigma}{d_u/d_s \times (1 - \sigma)}}.$$

For maize, micrographs were selected where stacked and unstacked membranes were well defined, and δ was obtained directly by counting gold granules over stacked and unstacked membranes. The stacking ratio σ was measured by drawing lines at regular interval perpendicular to the chloroplast axis and counting intersections with stacked and unstacked membranes and used to calculate d_u/d_s.

Antibodies. The antibodies to *C. reinhardtii* cytochrome *f* and p11 (a major LHCII subunit) were prepared by conventional methods, using the polypeptides separated by SDS/PAGE of the purified complexes. Antibodies to cytochrome *b*-559 (27), to PSI (28), and to oligomeric maize LHCII (28) have been described. The antibody to the spinach Rieske protein was from G. Hauska (University of Regensburg, R.F.A.).

Biochemical Analysis. Chlorophyll was determined according to ref. 29. SDS/6 M urea-polyacrylamide gel electrophoresis and Coomassie blue staining were done as described (30). Proteins were transferred to nitrocellulose filters and immunodecorated by using antibodies against PSI, LHCII, or cytochrome *f* and ^{125}I-labeled protein A (Amersham). Autoradiograms of the immunoblots were scanned with a densitometer (Epson EX800 equipped with a laser option). Quantitative estimation of the cytochrome *f* and LHCII contents in whole thylakoids and in the various SL samples were made after correcting for possible variations in the amount of PSI in each sample loaded on the immunoblot: the area computed by scanning either an LHCII band or a cytochrome *f* band was normalized to the scanned area of a PSI band in the same sample (either the PSI-A/PSI-B or the PSI-D/PSI-E/PSI-F bands, each determined on three different exposures) on a similar immunoblot treated with a polyspecific antibody to PSI.

RESULTS

To investigate the effect of state transitions on the lateral distribution of cyt b_6/f complexes, we first purified SL from maize thylakoids placed either in state I or in state II. State I thylakoids were obtained from plants incubated in darkness, whereas state II was achieved either *in vivo* by exposing the plants to bright light or *in vitro* by illuminating thylakoids isolated from leaves adapted to state I in the presence of ATP and NaF. Both treatments have been shown to induce phosphorylation of LHCII subunits and their migration to SL (20, 21).

Fig. 1 depicts the results of such an experiment; Fig. 1 *Left* shows the Coomassie blue staining pattern of maize SL polypeptides from thylakoids in state I or in state II after SDS/PAGE. When compared with the polypeptide content of whole thylakoids, all SL fractions displayed a characteristic enrichment in PSI and ATP synthase subunits, whereas they were extensively depleted in the various subunits of the PSII complex and highly deficient in LHCII. To judge the respective efficiencies of the state I-to-state II transitions in this experiment, we first assessed the LHCII content in the various SL by probing immunoblots with a specific antibody. As can be seen (Fig. 1 *Middle*), the SL prepared from thylakoids in state II displayed the expected increase in LHCII content when compared with the SL prepared from thylakoids in state I. This was reflected in their lower chlorophyll a/chlorophyll b ratios (Table 1). The same samples were then analyzed for their content in cytochrome *f*. The immunoblots treated with an antibody against cytochrome *f* (Fig. 1 *Right*) revealed that the SL from state II displayed a higher labeling than the SL from state I. This observation indicates displacement of some cyt b_6/f complexes from stacked to unstacked regions of the membrane upon transition to state II. No movement of cyt b_6/f was seen in *in vitro* control experiments where thylakoids where

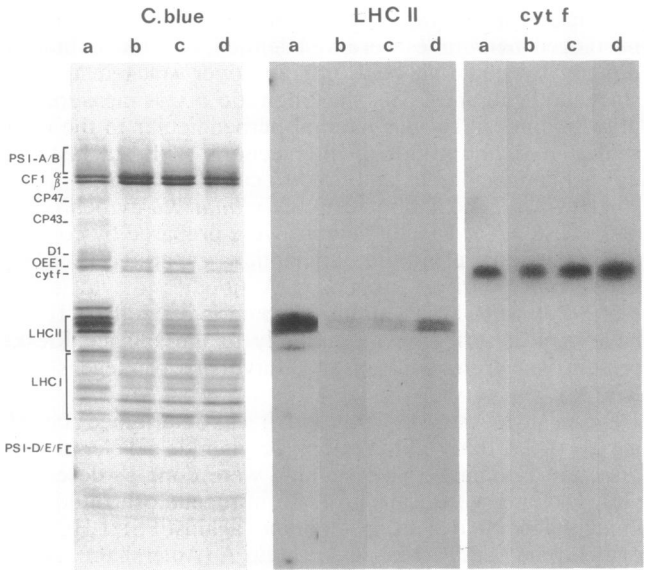

FIG. 1. Characterization of SL fractions. Lanes: a, whole thylakoids; b, SL from state I plants; c, SL from plants adapted to state II *in vivo*; d, SL from plants adapted to state II *in vitro*. (*Left*) Coomassie blue-stained gel. Major bands are identified at left. Note enrichment of the SL fractions in ATPase subunits α and β, in PSI reaction center subunits (PSI-A, -B, -D, -E, -F), and in light harvesting complex of PSI (LHCI) subunits, and their reduced content in PSII subunits (CP47, CP43, D1, OEE1). (*Middle*) Immunoblot treated with the LHCII antibody. (*Right*) Immunoblot treated with the cytochrome *f* antibody.

incubated in the light in the absence of ATP and NaF (data not shown).

Assuming that all PSI centers are found exclusively in nonappressed regions of the membrane and that our SL fractions are representative of this domain, we could estimate by densitometric scanning their content in cytochrome *f* and LHCII in states I and II (Table 1). Despite some variability in these estimations, due, in particular, to which PSI immunoreactive band was used for normalization, we consistently observed significant redistribution of cyt b_6/f upon state transitions. The data in Table 1 show that about half of the cyt b_6/f complex located in stacked membranes in state I moved to the unstacked regions upon transition to state II *in vitro*. The ratio of cytochrome *f* content in SL in state II to that in state I was 1.4 ± 0.3 *in vivo* and 2.2 ± 0.4 *in vitro* (mean of eight determinations ± SD). We note that the relative increase in cyt b_6/f content in SL from state II thylakoids was more pronounced *in vitro* than *in vivo*, as was true for LHCII. This result suggests either that full adaptation to state II conditions is more difficult to achieve *in vivo* because of a lower degree of activation of the LHCII kinase or that there was a partial reversion to state I during homogenization of the leaves before NaF inactivated the phosphatase.

Table 1. Maize unstacked regions: Content in cytochrome *f* and in LHCII (percentage of total, as deduced from scanning autoradiograms of immunoblots) and chlorophyll a/b ratios

	State I	State II *in vivo*	State II *in vitro*
Cyt *f*	31–42	37–65	65–80
LHCII	2–3	4.5–8.5	9–11
Chla/b	9.0	7.3	7.1

Percentage of cytochrome *f* (Cyt *f*) and LHCII found in unstacked membranes in each state was computed after normalization to constant PSI. The first figure in each entry relates to normalization with the PSI A/B band; the second relates to normalization with PSI D/E/F. Chla/b, chlorophyll a/b ratios.

Because cytochrome *f* is extracted to a variable extent by the Triton-X100 treatment generally used to prepare grana-derived membranes (31), we have limited our fractionation approach to the study of SL, which can easily be purified by a nondetergent method. As a complementary approach, we used immunocytochemistry to analyze *in situ* the changes in cyt b_6/f distribution during state transitions in maize. In these experiments, we used an antibody raised against the Rieske protein from spinach, one of the four main subunits of the cyt b_6/f complex, which provided a higher labeling on thin sections than the antibody to the *C. reinhardtii* cytochrome *f*, previously used on immunoblots. As shown on Fig. 2 *A–C*, this technique allowed detection of the antigen in both the stacked and unstacked membrane regions. Immunodecoration of the unstacked membrane regions was significantly increased in state II conditions as compared with state I. Quantitation of the labeling (Table 2) indicated that, as seen in the immunoblotting experiments, this enrichment was less pronounced in the *in vivo* than in the *in vitro* transitions. Immunocytochemical analysis with an antibody to LHCII (Fig. 2 *D–F* and Table 2) also showed enrichment of this complex in unstacked membranes in state II as compared with state I. Again, the contrast was lower when the transition was done *in vivo* than *in vitro*. No significant redistribution of the label was seen with antibodies to cytochrome *b*-559 or to CP43, two PSII subunits (Table 2).

A slight destacking was seen in state II, more pronounced when the transition was done *in vitro* (see σ values in Table 2). This destacking is, however, of little amplitude and cannot account for the observed change in label distribution (see *Discussion*). That a specific change had occurred in the affinity of cyt b_6/f for unstacked regions was indicated by examining the relative labeling densities of the two domains. Included in Table 2 are the values of d_u/d_s, the ratio of labeling densities of unstacked vs. stacked regions. An increase in d_u/d_s was seen upon transition to state II for the Rieske protein as well as for LHCII, reflecting a selective

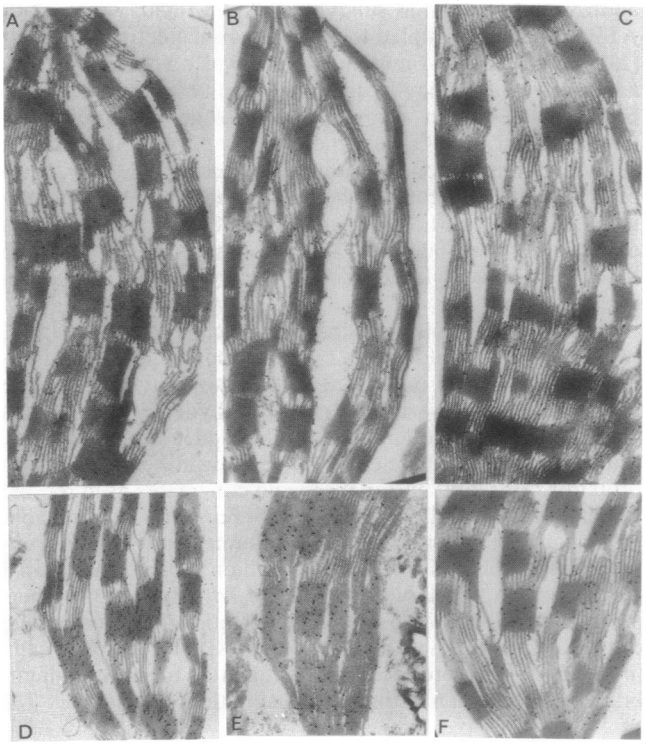

FIG. 2. Immunogold labeling of maize thylakoids. (*A–C*) Rieske antibody. (*D–F*) LHCII antibody. (*A* and *D*) State I. (*B* and *E*) State II *in vivo*. (C and F) State II *in vitro*. (×15,100.)

Table 2. Quantitation of immunogold labeling experiments on maize thylakoids

Antigen	State I			State II *in vivo*			State II *in vitro*		
	δ	σ	d_u/d_s	δ	σ	d_u/d_s	δ	σ	d_u/d_s
Rieske	46.5 ± 5.3	66.0	1.69	50.2 ± 7.0	63.8	1.79	67.1 ± 4.8	59.3	2.97
LHCII	15.3 ± 2.2	67.2	0.37	27.5 ± 3.9	66.9	0.77	38.2 ± 3.4	62.0	1.07
Cyt *b*-559	19.1 ± 1.4	ND	ND	13.1 ± 1.2	ND	ND	24.0 ± 1.3	ND	ND
CP43	20.8 ± 1.7	ND	ND	ND	ND	ND	24.0 ± 3.7	ND	ND

δ is the percentage of label found in unstacked regions (± SE at 0.01 confidence), σ is the stacking ratio measured over the same set of micrographs, and d_u/d_s is the ratio of labeling densities over unstacked vs. stacked regions, as deduced from δ and σ. Cyt, cytochrome; ND, not done.

enrichment of both antigens in unstacked regions. In both cases, this increase was larger when the transition was done *in vitro* than *in vivo*.

We then extended our immunocytochemical analysis to the unicellular green algae *C. reinhardtii*, which displays particularly extensive state transitions *in vivo* (32). In this case the antibody to cytochrome *f*, which was raised against the *C. reinhardtii* antigen, displayed the best labeling efficiency. State I was obtained by preillumination of the cells in the presence of dichlorophenyldimethylurea, whereas state II was obtained by blocking ATP production in the dark, as reported (32). *C. reinhardtii* thylakoids do not form grana stacks but are arranged instead in a complex network, where thylakoids come irregularly in contact with each other (see ref. 12). For this reason, analysis of the label distribution required measurement of labeling densities of selected areas where stacked and unstacked membranes could be clearly identified; this measurement gives d_u/d_s directly. The proportion of label in unstacked areas was then calculated, assuming a constant stacking ratio of 65%. Because only statistical analysis of many micrographs can reveal changes in label distribution, the results are presented directly in their tabulated form. As shown in Table 3, LHCII and cytochrome *f* were present in a larger proportion in the unstacked membrane regions in state II as compared with state I, both in the wild type and the DUM-1 mutant. In contrast, the fraction of the PSII core subunit P6 (which corresponds to CP43 from higher plants) detected in unstacked membranes did not differ significantly between state I and state II.

DISCUSSION

In a previous immunocytochemical study, we had shown that cyt b_6/f complexes were located in both the unstacked and stacked membrane regions of higher plants and green algae thylakoids (12). The present study demonstrates that the distribution of cyt b_6/f complexes along the membranes is, nevertheless, variable and depends on the state I/II to which thylakoids have been adapted before such an analysis. The proportion of cyt b_6/f complexes found in the SL is significantly higher in state II conditions than in state I conditions. Our experiments with maize thylakoids consistently showed the same extensive lateral displacement of cyt b_6/f complexes upon state I-to-state II transition *in vitro*, leading to ≈70% of the complexes in the SL region. In contrast, similar transitions *in vivo* yielded more variable results, ranging from no significant change in lateral distribution of cyt b_6/f complexes to a near doubling in the proportion found in unstacked membrane regions. In all cases, we observed similar variations in the extent of LHCII displacement between the two membrane domains. Therefore, the lower efficiency of the transition done with maize *in vivo* that we observe in the present study is more likely to pertain to our experimental conditions than to a genuine limitation of the regulation mechanism operating *in vivo*. Interestingly, the population of cyt b_6/f complexes in the unstacked regions of the thylakoid membranes adapted *in vivo* to a given state, was higher in maize than in *C. reinhardtii*. No such difference was noted in our previous study (12) comparing spinach and *C. reinhardtii*, where no special care was taken to maintain the thylakoids in a predetermined state. The figures obtained in that study for *C. reinhardtii* were close to those we find here in state II, whereas the spinach data were close to that of state I in maize. Indeed, the conditions used in ref. 12 can be expected to place higher plant thylakoids in state I, due to oxidizing conditions during homogenization, whereas *C. reinhardtii* cells fall in anerobic conditions and adapt irreversibly to state II, when rapidly broken after being pelleted by centrifugation (24).

State transitions have long been considered a mechanism by which photosynthetic organisms regulate the distribution of light excitation between the two photosystems. The present study shows that both the light-harvesting antenna and the electron-transport chain undergo reorganizations during state transitions. Our quantitative analysis of the immunocytochemical and immunoblotting experiments suffers from the intrinsic limitations of each technique, so that the estimates for the proportion of LHCII and cytochrome *f* in each domain should be taken as indicative only. Nevertheless, our study clearly shows that the proportion of cyt b_6/f involved in this reorganization (30–40%) is, in fact, larger than that of LHCII (10–20%). The change in LHCII distribution is only more dramatic because the state I SL contain extremely little, if any, LHCII (a correct assessment of LHCII concentration is difficult to achieve in this situation by either of the two methods used in this study).

The present study adds support to a dual organization of electron transport based on the existence of two populations of cyt b_6/f complexes, one in the grana regions allowing linear electron flow from PSII to PSI through plastocyanin diffusion

Table 3. Quantitation of immunogold labeling experiments on *C. reinhardtii*

	State I				State II			
	Wild type		DUM-1		Wild type		DUM-1	
	∂	d_u/d_s	∂	d_u/d_s	∂	d_u/d_s	∂	d_u/d_s
Antibody to Cyt *f*	18.1 ± 3.0	0.41	14.3 ± 5.3	0.31	39.6 ± 3.0	1.22	39.3 ± 9.2	1.23
Antibody to LHCII	11.0 ± 3.5	0.23	16.6 ± 6.3	0.37	35.7 ± 7.5	1.03	28.2 ± 8.6	0.73
Antibody to CP43	6.1 ± 2.6	0.12	17.4 ± 6.2	0.39	9.7 ± 2.2	0.20	11.0 ± 4.9	0.23

d_u/d_s is the ratio of the labeling densities over unstacked versus stacked regions. ∂ is the percentage of label found in unstacked regions (± SE at 0.01 confidence), as deduced from d_u/d_s, assuming a constant stacking ratio of 65%. Cyt *f*, cytochrome *f*.

in the lumen and the other population in the unstacked membrane regions serving cyclic electron flow. Because state II can be induced by intracellular ATP depletion (17) and is accompanied by an increase in cyt b_6/f concentration next to PSI centers in the SL regions, state transitions have the features of a regulation mechanism aimed at controlling ATP production via the balance between linear and cyclic electron flow.

In addition, the movement of cyt b_6/f to unstacked regions in state II may play a critical role in the mechanism of LHCII–kinase activation: because an association of the kinase with the cyt b_6/f complex has been recently reported (9), the lateral distribution of the kinase may vary between state I and state II, which may, in turn, exert a control on its activation state and/or on its access to its substrate, LHCII.

The driving force for the movement of cyt b_6/f complexes between the stacked and unstacked regions is at present unknown. As can be seen in Table 2, transition to state II in maize is accompanied by a slight destacking, even though we used a high Mg^{2+} concentration in our *in vitro* experiments (10 mM, to which we should substract the 1 mM needed to complex the ATP added). This change is of similar amplitude to that seen in pea at 5 mM Mg^{2+} (33) and may be understood in the frame of the electrostatic theory of stacking (34) as reflecting the increased amount of proteins that are excluded from stacked regions, even at maximum electrostatic screening. Such a destacking can by itself increase the proportion of cyt b_6/f in unstacked regions and, therefore, its proximity with PSI, with no need for a change in the relative affinity of cyt b_6/f for unstacked regions. However, the change we observed cannot be accounted for solely by this mechanism, as evidenced by the increase in d_u/d_s. A specific mechanism is at work, by which the affinity of cyt b_6/f for unstacked regions is increased.

Because the redistribution of cyt b_6/f complexes is induced *in vitro* in similar conditions as that of LHCII (light plus ATP), phosphorylation may also be involved. However, no reversible phosphorylation has been reported thus far for any subunit of the cyt b_6/f complex. Alternatively, movement may result from the association of cyt b_6/f with the mobile pool of LHCII. We have already suggested (12) that such an association may exist in *C. reinhardtii*, based on the observation that cyt b_6/f mutants display a reduction in the size of a subpopulation of the particles located on the protoplasmic faces (PFs), which are known to contain LHCII.

Another type of mechanism should, however, be considered, based on the existence of supercomplexes associating the cytochrome *bc*-type complexes with cytochrome oxidase in bacteria (35, 36) and with the photochemical reaction centers in photosynthetic bacteria (37). It has recently been found that experimental conditions close to state II produce an increase in the flash-induced oxidation rate of cytochrome *f* by PSI (38). Some of us have observed an increase in the proportion of cyt b_6/f complexes comigrating with PSI complexes after sucrose-gradient centrifugation of solubilized thylakoid membranes from *C. reinhardtii* after the cells had been placed in state II as compared with state I (39). Therefore, we suggest that state II conditions could favor supercomplex formation between cyt b_6/f and PSI. If cyt b_6/f complexes can diffuse freely between stacked and unstacked membrane regions, then enhanced trapping of cyt b_6/f complexes by PSI in supercomplexes in state II would increase the proportion of that complex found in SL.

We are indebted to M. Recouvreur for expert technical assistance in electron microscopy. We thank P. Joliot and R. Delosme for stimulating discussions, Y. Pierre for help with antibody preparation, and D. Béal for setting up the scanning device. This work was supported by the Centre National de la Recherche Scientifique (UM3 and URA D1187) and by a Ministère de la Recherche et de la Technologie grant to J.O. and F.-A.W. (89C0665).

1. Bonaventura, C. & Myers, J. (1969) *Biochim. Biophys. Acta* **189**, 366–383.
2. Murata, N. (1969) *Biochim. Biophys. Acta* **172**, 242–251.
3. Barber, J. (1986) *Photosynth. Res.* **10**, 243–253.
4. Gal, A., Shahak, Y., Schuster, G. & Ohad, I. (1987) *FEBS Lett.* **221**, 205–210.
5. Lemaire, C., Girard-Bascou, J. & Wollman, F.-A. (1987) *Prog. Photosynth. Res.* **4**, 655–658.
6. Wollman, F.-A. & Lemaire, C. (1988) *Biochim. Biophys. Acta* **933**, 85–94.
7. Bennett, J., Shaw, E. K. & Michel, H. (1988) *Eur. J. Biochem.* **171**, 95–100.
8. Coughlan, S. J. (1988) *Biochim. Biophys. Acta* **933**, 413–422.
9. Gal, A., Hauska, G., Herrmann, R. & Ohad, I. (1990) *J. Biol. Chem.* **265**, 19742–19749.
10. Cox, R. P. & Andersson, G. (1981) *Biochem. Biophys. Res. Commun.* **103**, 1336–1342.
11. Allred, D. R. & Staehelin, L. A. (1985) *Plant Physiol.* **78**, 199–202.
12. Olive, J., Vallon, O., Wollman, F.-A., Recouvreur, M. & Bennoun, P. (1986) *Biochim. Biophys. Acta* **851**, 239–248.
13. Goodchild, D. J., Anderson, J. M. & Andersson, B. (1985) *Cell Biol. Int. Rep.* **9**, 715–721.
14. Whitmarsh, J. (1986) *Encycl. Plant Physiol.* **19**, 508–525.
15. Joliot, P., Lavergne, L. & Beal, D. (1990) in *Current Research in Photosynthesis*, ed. Balcheffsky, M. (Kluwer, Boston), Vol. 2, pp. 879–882.
16. Lavergne, J. & Joliot, P. (1991) *Trends Biochem. Sci.* **16**, 129–134.
17. Bulté, L., Gans, P., Rebéillé, F. & Wollman, F.-A. (1990) *Biochim. Biophys. Acta* **1020**, 72–80.
18. Horton, P., Lee, P. & Fernyhough, P. (1990) *Biochim. Biophys. Acta* **1017**, 160–166.
19. Turpin, D. & Bruce, D. (1990) *FEBS Lett.* **263**, 90–103.
20. Bassi, R., Giacometti, G. & Simpson, D. (1988) *Biochim. Biophys. Acta* **935**, 152–165.
21. Bassi, R., Rigoni, F., Barbato, R. & Giacometti, G. (1988) *Biochim. Biophys. Acta* **936**, 29–38.
22. Matagne, R. F., Michel-Wolwertz, M. R., Munaut, C., Duyckaerts, C. & Sluse, F. (1989) *J. Cell Biol.* **108**, 1221–1226.
23. Rebéillé, F. & Gans, P. (1988) *Plant Physiol.* **88**, 973–975.
24. Bulté, L. & Wollman, F.-A. (1990) *Biochim. Biophys. Acta* **1016**, 253–258.
25. Vallon, O., Wollman, F.-A. & Olive, J. (1985) *FEBS Lett.* **183**, 245–250.
26. Vallon, O., Wollman, F.-A. & Olive, J. (1986) *Photobiochem. Photobiophys.* **12**, 203–220.
27. Vallon, O., Tae, G. S., Cramer, W., Simpson, D., Høyer-Hansen, G. & Bogorad, L. (1989) *Biochim. Biophys. Acta* **975**, 132–141.
28. Di Paolo, M. L., Dal Belin Perufo, A. & Bassi, R. (1990) *Planta* **181**, 275–286.
29. Porra, R. J., Thompson, W. A. & Kriedermann, P. E. (1989) *Biochim. Biophys. Acta* **975**, 384–394.
30. Bassi, R., Høyer-Hansen, G., Barbato, R. & Giacometti, G. M. (1987) *J. Biol. Chem.* **262**, 13333–13341.
31. Dunahay, T. G., Staehelin, L. A., Seibert, M., Ogilvie, P. D. & Berg, S. P. (1984) *Biochim. Biophys. Acta* **764**, 179–193.
32. Wollman, F.-A. & Delepelaire, P. (1984) *J. Cell Biol.* **98**, 1–7.
33. Telfer, A., Hodges, M., Millner, P. A. & Barber, J. (1984) *Biochim. Biophys. Acta* **766**, 554–562.
34. Barber, J. (1980) *FEBS Lett.* **118**, 1–10.
35. Berry, E. A. & Trumpower, B. L. (1985) *J. Biol. Chem.* **260**, 2458–2467.
36. Sone, N., Sekimachi, M. & Kutohe, E. (1987) *J. Biol. Chem.* **262**, 15386–15391.
37. Joliot, P., Verméglio, A. & Joliot, A. (1989) *Biochim. Biophys. Acta* **975**, 336–345.
38. Delosme, R. (1991) *Photosynth. Res.*, in press.
39. Wollman, F.-A. & Bulté, L. (1990) in *Photoconversion Processes for Energy and Chemicals*, eds. Hall, D. O. & Grassi, G. (Elsevier, London), pp. 198–207.
40. Gorman, D. S. & Levine, R. P. (1965) *Proc. Natl. Acad. Sci. USA* **79**, 4352–4356.