Biochimica et Biophysica Acta 1837 (2014) 1463–1471

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbabio

Dark-adapted spinach thylakoid protein heterogeneity offers insights into the photosystem II repair cycle☆

Marjaana Suorsa [a], Marjaana Rantala [a], Ravi Danielsson [c], Sari Järvi [a], Virpi Paakkarinen [a], Wolfgang P. Schröder [d], Stenbjörn Styring [b], Fikret Mamedov [b,*], Eva-Mari Aro [a,**]

[a] Department of Biochemistry, Molecular Plant Biology, University of Turku, FI-20014 Turku, Finland
[b] Molecular Biomimetics, Department of Chemistry, Ångström Laboratory, University of Uppsala, Box 523, SE-75120 Uppsala, Sweden
[c] Department of Biochemistry, Center for Chemistry and Chemical Engineering, Lund University, SE-22100 Lund, Sweden
[d] Umeå Plant Science Center and Department of Chemistry, Linnaeus väg 10, University of Umeå, SE-901 87 Umeå, Sweden

ARTICLE INFO

Article history:
Received 30 September 2013
Received in revised form 18 November 2013
Accepted 22 November 2013
Available online 1 December 2013

Keywords:
PGR5
PGRL1
Photosystem II
PsbS
PsbW
PsbX

ABSTRACT

In higher plants, thylakoid membrane protein complexes show lateral heterogeneity in their distribution: photosystem (PS) II complexes are mostly located in grana stacks, whereas PSI and adenosine triphosphate (ATP) synthase are mostly found in the stroma-exposed thylakoids. However, recent research has revealed strong dynamics in distribution of photosystems and their light harvesting antenna along the thylakoid membrane. Here, the dark-adapted spinach (*Spinacia oleracea* L.) thylakoid network was mechanically fragmented and the composition of distinct PSII-related proteins in various thylakoid subdomains was analyzed in order to get more insights into the composition and localization of various PSII subcomplexes and auxiliary proteins during the PSII repair cycle. Most of the PSII subunits followed rather equal distribution with roughly 70% of the proteins located collectively in the grana thylakoids and grana margins; however, the low molecular mass subunits PsbW and PsbX as well as the PsbS proteins were found to be more exclusively located in grana thylakoids. The auxiliary proteins assisting in repair cycle of PSII were mostly located in stroma-exposed thylakoids, with the exception of THYLAKOID LUMEN PROTEIN OF 18.3 (TLP18.3), which was more evenly distributed between the grana and stroma thylakoids. The TL29 protein was present exclusively in grana thylakoids. Intriguingly, PROTON GRADIENT REGULATION5 (PGR5) was found to be distributed quite evenly between grana and stroma thylakoids, whereas PGR5-LIKE PHOTOSYNTHETIC PHENOTYPE1 (PGRL1) was highly enriched in the stroma thylakoids and practically missing from the grana cores.
This article is part of a Special Issue entitled: Photosynthesis Research for Sustainability: Keys to Produce Clean Energy.

1. Introduction

Photosynthetic light reactions occur in the chloroplast thylakoid membrane, which in higher plants is folded to form stacked grana regions, connected by non-stacked, stroma-exposed thylakoids (Fig. 1A). Light reactions are driven by four multiprotein complexes, photosystem (PS) II, Cytochrome b_6f (Cyt b_6f), PSI and ATP synthase. In higher plants, thylakoid membrane protein complexes show lateral heterogeneity in their distribution: PSII complexes are mostly located in grana stacks, whereas PSI and ATP synthase are mostly found in the stroma-exposed thylakoid membranes, such as grana margins, grana end membranes and stroma thylakoids [1,2]. This lateral heterogeneity is unique to higher plants: the cyanobacterial and diatom thylakoid systems consist of single concentric thylakoids without stacking whereas in green algae, thylakoids can temporarily form stack-like structures, but they are not as well organized as in higher plants [3].

PSII in higher plants consists of more than 30 subunits [4], which are encoded by both the chloroplast and the nuclear genome. The reaction center (RC) complex, capable of charge separation, is made up of five chloroplast-encoded proteins: D1, D2, PsbI and the α and β subunits of the Cyt b_{559} (PsbE and PsbF, respectively). The plastid-encoded chlorophyll (Chl) *a* binding proteins CP43 and CP47 form the inner antenna of PSII, and three nuclear-encoded extrinsic proteins, PsbO, PsbP and PsbQ together with a manganese–calcium cluster form the lumenal-side-located oxygen evolving complex (OEC). In its most active form, dimeric PSII is associated with two to four copies of light harvesting complex II (LHCII), thus forming PSII–LHCII supercomplexes. These supercomplexes have been further shown to form large megacomplexes, in which PSII–LHCII supercomplexes are organized into row-like semi-crystalline arrays in appressed grana membranes [5,6].

☆ This article is part of a Special Issue entitled: Photosynthesis Research for Sustainability: Keys to Produce Clean Energy.

* Corresponding author. Tel.: +46 18 471 6581.
** Corresponding author. Tel.: +358 2 333 5931.
E-mail addresses: fikret.mamedov@kemi.uu.se (F. Mamedov), evaaro@utu.fi (E.-M. Aro).

0005-2728/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.bbabio.2013.11.014

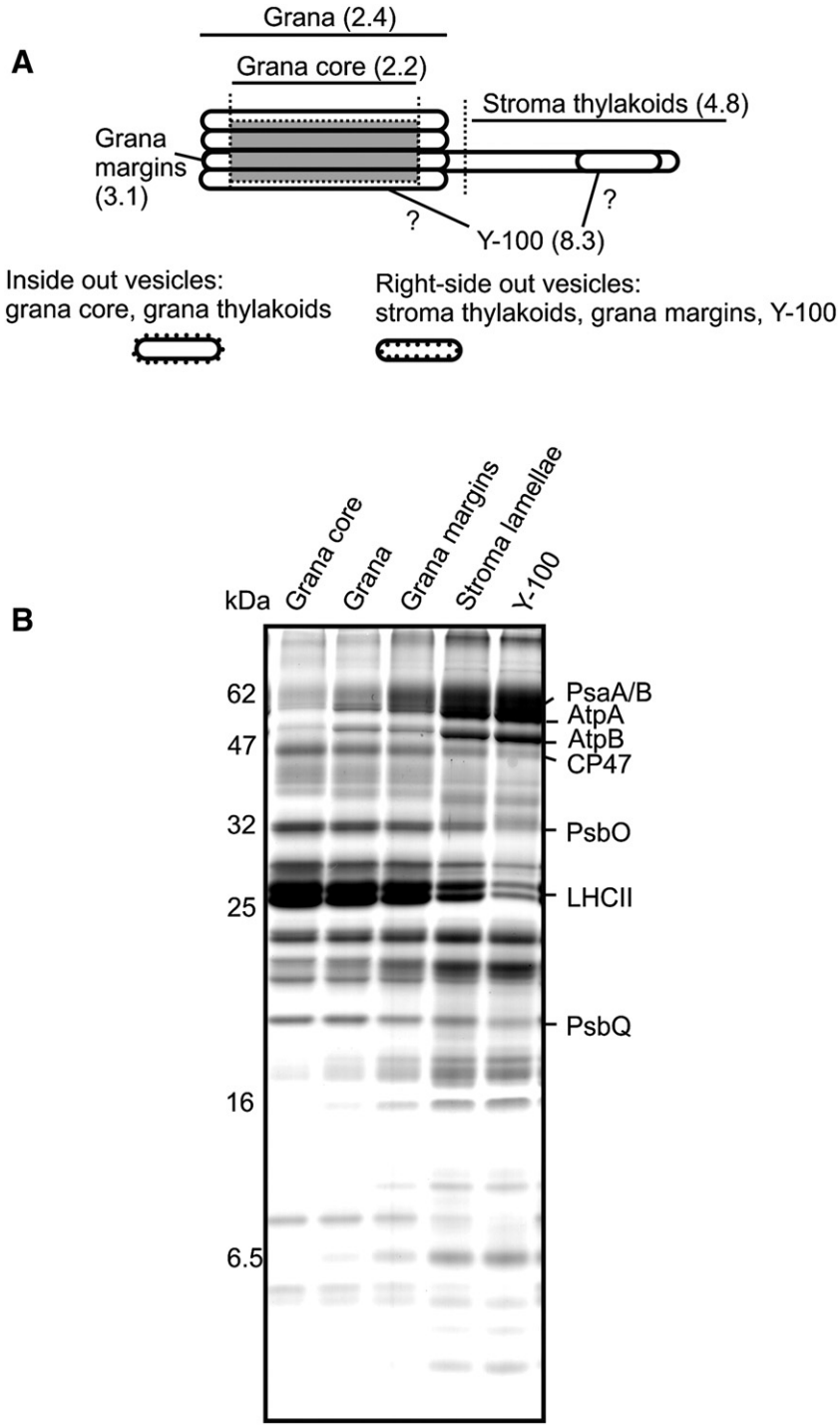

Fig. 1. Spinach thylakoid membrane fractionation and analysis of the individual fractions. A) Schematic diagram of the spinach thylakoid membrane fractions obtained with mechanical fragmentation. The chlorophyll a/b ratio and the topology of each fraction are indicated. The exact origin of the Y-100 fraction is not known. B) Coomassie-stained SDS-PAGE separation of thylakoid membrane proteins from each fraction. Gels were loaded on equal protein basis.

Primary and secondary electron donors of PSII ($P680^+$ and $TyrZ^+$, respectively) are highly oxidizing and can potentially damage PSII. Furthermore, the presence of oxygen allows the formation of reactive oxygen species (ROS). Despite the existence of efficient detoxification systems and back reactions to scavenge the ROS and PSII radicals, an unavoidable damage to PSII, particularly to the D1 protein, occurs at all light intensities [7,8]. The repair cycle of photodamaged PSII involves detachment of the LHCII complexes from the PSII dimers, monomerization of the PSII complexes and partial disassembly of the complex, including detachment of the CP43 protein and the OEC [9,10]. Furthermore, the damaged D1 protein becomes degraded by the FtsH and Deg proteases, followed by insertion of the newly-synthetized D1 copy to partially disassembled PSII and the concomitant C-terminal processing of the new D1 protein. Subsequent assembly of the OEC complex re-establishes the PSII monomer, which *via* dimerization and re-attachment of the LHCII complexes forms highly active PSII supercomplexes in the grana [9,10]. Several auxiliary proteins have been shown to be essential for ensuring the proper function of the PSII repair cycle [11].

In addition to the abovementioned mechanisms assuring an effective and rapidly ongoing PSII repair cycle at high light intensities,

there are also short-term light acclimation processes occurring concomitantly in the thylakoid membrane. These include the PsbS-dependent dissipation of excess light energy as heat (non-photochemical quenching, NPQ) [12], and "state transitions", which are coordinately regulated by the STN7 kinase and the TAP38/PPH1 phosphatases [13–15]. In addition, diversion of part of the electron flow into cyclic electron transfer (CET) routes, in C3 plants particularly to the antimycin-sensitive ferredoxin (Fd) mediated route, provides thylakoid membranes with functional flexibility upon abrupt changes in light conditions [16]. The PROTON GRADIENT REGULATION5 (PGR5) and PGR5-LIKE PHOTOSYNTHETIC PHENOTYPE1 (PGRL1) proteins have been suggested to mediate the Fd-dependent CET in *Arabidopsis thaliana* (Arabidopsis) [17–19].

Here we attempted to quantify differences in PSII and auxiliary protein content in distinct subdomains of the thylakoid membrane in order to gain more detailed information about PSII repair cycle. Detergent-free mechanical fragmentation (sonication or Yeda press treatment) in combination with aqueous two-phase partition was used to obtain the thylakoid membrane fractions from spinach chloroplasts. The advantage of mechanical fragmentation is that it reduces protein denaturation and disassembly or artificial aggregation of protein complexes due to presence of detergents. Indeed, it has been shown that detergents remove lipids from thylakoids, as the lipid to Chl ratio of the BBY fragments was markedly lowered as compared to mechanically isolated grana thylakoids [20]. The PSII subunits were found mainly in the grana region and in reduced amounts in the stroma thylakoid. However, the auxiliary proteins showed a much more diverse distribution between the thylakoid regions. The PGR5 protein was found to be quite evenly distributed between grana and stroma thylakoids, whereas the PGRL1 was enriched in stroma thylakoids.

2. Materials and methods

2.1. Isolation of thylakoid membrane fractions

Spinach (*Spinacia oleracea* L.) was grown hydroponically under 300 μmol photons m^{-2} s^{-1} in 12 h light period at 20 °C. Mature, two-month-old leaves were dark-adapted for 24 h before thylakoid isolation to reduce the starch content. The dark adaptation was started directly after the 12 h light period. Thylakoid membrane was isolated under weak green light in 4 °C and the samples were kept on ice throughout the isolation. The thylakoid membrane fractions were isolated according to [2] and [21]. In short, thylakoids were broken by sonication, followed by purification of the grana and stroma fractions by an aqueous two-phase system. The so-called Y-100 fraction, on the other hand, was purified from thylakoids with Yeda press and centrifugation [2].

For specific experiments, *A. thaliana* (Arabidopsis) ecotype Colombia was grown under 120 μmol photons m^{-2} s^{-1} in 8 h light period at 23 °C. Thylakoids were isolated from five-week-old rosettes and subfractionated with digitonin as described in [22].

The Chl concentrations were determined according to [23] and protein concentrations according to [24].

2.2. Separation of proteins and Western blotting

SDS-PAGE with 15% of acrylamide, 6 M urea [25] was used for separation of thylakoid membrane proteins. The samples were loaded on equal protein basis. The amount of protein loaded for the Western blotting varied from 4 to 20 μg according to antibody used, as the antibody response had been checked to be in the linear range. After electrophoresis, the proteins were electroblotted to a polyvinylidene difluoride membrane (Millipore) and subsequently blocked with 5% milk (nonfat dry milk, Bio-Rad). Immunoblotting using enhanced chemiluminescence detection was performed according to standard procedures. The PsbS antibody was kindly provided by P. Horton, the CP43 and CP47 antibodies by Dr. R. Barbato, the PsbE antibody by Dr. R.G. Herrmann, the PsbH antibody by Dr. P. Westhoff, the FTSH antibody by Dr. T. Ogava, the SecY antibody by Dr. K. Cline, the Psb27 and LPA1 antibodies by Dr. L. Zhang, the TLP40 antibody by Dr. B. Andersson, the Deg2 antibody by Dr. I Adamska, the PGR5 antibody by Dr. T. Shikanai and the PGRL1 antibody by Dr. D. Leister.

Images from the films were acquired with a CCD camera and quantified with the GeneTools software (Perkin-Elmer). For quantifications, three independent replications were made for each antibody.

3. Results

3.1. Characterization of the thylakoid membrane fractions

The thylakoid membrane was fractionated into the following parts: (*i*) grana core, (*ii*) total grana thylakoids, (*iii*) grana margins, (*iv*) stroma thylakoids and (*v*) Y-100 fraction (Fig. 1A). The clear differences in the Chl a/b ratio *e.g.* between the grana core fraction and the stroma thylakoid (Chl a/b ratios 2.2 and 4.8, respectively) demonstrate that the fractions originate from physically distinct compartments of the thylakoid membrane (Fig. 1A). The Y-100 fraction had remarkably high Chl a/b ratio, 8.3, which supports its likely origin from the "ultimate" stroma thylakoid and/or from the grana end membranes, as has been suggested earlier [26]. It should be stressed that the fractions are vesicles: during isolation, the grana thylakoids re-seal to form inside-out vesicles, whereas the stroma thylakoid fractions re-seal to form right-side-out vesicles (Fig. 1A). Furthermore, the isolated vesicles are not pure preparations of the respective thylakoid membrane domains, but rather should be considered as preparations enriched in fragments from different thylakoid membrane domains [21].

To get preliminary insights into the protein composition and purity of the different fractions, the fractions were analyzed with one-dimensional SDS-PAGE, followed by subsequent staining with Coomassie (Fig. 1B). The diagnostic subunits known to be located in the stroma-exposed thylakoids, PsaA/B and the ATP synthase proteins AtpA and AtpB, were highly enriched in the stroma thylakoid and in the Y-100 fractions, whereas the content of the LHCII proteins was the lowest in stroma fractions and increased gradually when moving towards the grana core fraction (Fig. 1B).

3.2. Distribution of the PSII core proteins in different domains of the thylakoid membrane

The distribution of 13 subunits of PSII in different parts of the thylakoid membrane was semi-quantitatively determined with Western blotting followed by densitometry analyses. Mature leaves were used as a starting material for thylakoid fractionation in order to get insights into the distribution of different phases of the PSII repair cycle along the thylakoid membrane, and conversely to avoid the interference by the *de novo* synthesis of PSII subunits and complexes.

The PSII RC core proteins D1 and D2 as well as the inner light harvesting antenna proteins CP43 and CP47 were highly enriched in grana thylakoids (Table 1, Fig. 2). Altogether 74% of the total D1 and D2 proteins were found from grana thylakoids, grana core and grana margins. Meanwhile 80% of the CP47 protein and 71% of the CP43 protein were detected in the grana domains. The Y-100 fraction hosted roughly 10% of the total D1, D2 and CP43 contents, whereas of the total content of CP47, only 2% was detected in the Y-100 fraction. The distribution of the PSII LMM protein PsbE was very similar to that of the other PSII core proteins, with roughly 30% of the total protein amount being located in Y-100 fraction and stroma thylakoids and around 70% in grana fractions (Table 1).

The distribution of the OEC complex proteins PsbO, PsbP and PsbQ followed that of the PSII core proteins with around 70% of the total protein pool being located in the grana thylakoids and a little less than 30% in the stroma thylakoids (Table 1, Fig. 2). The PsbS protein, on the other hand, was found to be strikingly less abundant in the Y-100 fraction as

Table 1
Semi-quantitative analyses of the relatively distribution of PSII proteins in different thylakoid membrane fractions of spinach. The values are percentages (mean ± SD, $n = 3$) of total.

	Grana core	Grana	Grana margins	Stroma thylakoids	Y-100
Chl/fraction (%)[a]					
	51	64	13	36	5
PSII core proteins:					
D1	25 ± 1	27 ± 1	22 ± 1	17 ± 1	9 ± 2
D2	25 ± 2	27 ± 8	22 ± 2	19 ± 3	7 ± 2
CP47	31 ± 3	29 ± 4	20 ± 4	18 ± 1	2 ± 1
CP43	23 ± 5	24 ± 5	24 ± 4	19 ± 0	10 ± 4
PsbS	28 ± 1	28 ± 2	26 ± 2	16 ± 2	2 ± 1
OEC proteins:					
PsbO	25 ± 2	23 ± 1	24 ± 2	20 ± 2	8 ± 3
PsbP	26 ± 3	25 ± 4	21 ± 3	20 ± 4	8 ± 1
PsbQ	24 ± 1	25 ± 1	25 ± 1	17 ± 1	9 ± 1
PSII LMM proteins:					
PsbE	24 ± 4	25 ± 2	22 ± 2	19 ± 0	10 ± 4
PsbH	28 ± 5	28 ± 4	20 ± 2	15 ± 4	9 ± 2
PsbR	28 ± 3	26 ± 3	23 ± 2	15 ± 0	8 ± 3
PsbW	30 ± 2	32 ± 4	26 ± 2	11 ± 5	1 ± 1
PsbX	33 ± 3	28 ± 1	23 ± 4	14 ± 0	2 ± 0.6

[a] The Chl yields (as compared to 100% of the Chl yield of total thylakoids) for each fraction was calculated from the countercurrent distribution that provides the yield from each fraction [21,89,90] and from the EPR measurements for the PSII content in the different thylakoid fractions, see [2].

compared to other PSII core proteins, as only approximately 2% of it was found in the Y-100 fraction and more than 80% from the grana fractions (Table 1).

The distribution of PsbH and PsbR followed for the most part that of the PSII RC proteins, the amounts of these proteins being only slightly lower in the stroma thylakoids and Y-100 fraction as compared to the RC core proteins (approximately 25% *versus* 30%, respectively). In contrast, the distribution of the PsbW and PsbX proteins distinctively differed from that of the other PSII LMM proteins. As much as 88% and 84% of PsbW and PsbX (respectively) were found in the grana domains, whereas only 1% of PsbW and 2% of PsbX were present in the Y-100 fraction.

3.3. Distribution of auxiliary proteins assisting PSII biogenesis, assembly and repair cycle along the thylakoid membrane

During the recent years, the knowledge about auxiliary proteins suggested to be involved in assembly and turnover of PSII has increased considerably [11,27]. To gain insights into the function and "operational environment" of several auxiliary proteins suggested to have a role in PSII repair cycle, we analyzed the distribution of those proteins along the thylakoid. The Deg2 and the FtsH1 proteases were enriched in the stroma thylakoid fractions, since roughly 70% of each of them were found from the stroma thylakoids and Y-100 fractions, and only marginal amounts were detected in the grana core (Table 2, Fig. 3A). However, it should be emphasized that the grana margins contained approximately 17% of the total amount of the FtsH1 protease and 11% of Deg2. The distribution of the translocon component protein SecY was very similar with that of the proteases with majority of the protein being located in the stroma thylakoids (Table 2).

The distribution of the LOW PSII ACCUMULATION1 (LPA1) [28] and TLP18.3 (THYLAKOID LUMEN PROTEIN of 18.3) [29] along the thylakoid was found to be rather similar (Table 2). Altogether approximately 50% of these proteins were detected in the stroma thylakoids and Y-100, and around 20% of the total amount was found from both the grana margins and total grana stacks. However, the amount of TLP18.3 in the grana core (around 15% of the total amount) was somewhat higher as compared to that of the LPA1 protein. The distribution of the Psb27 protein

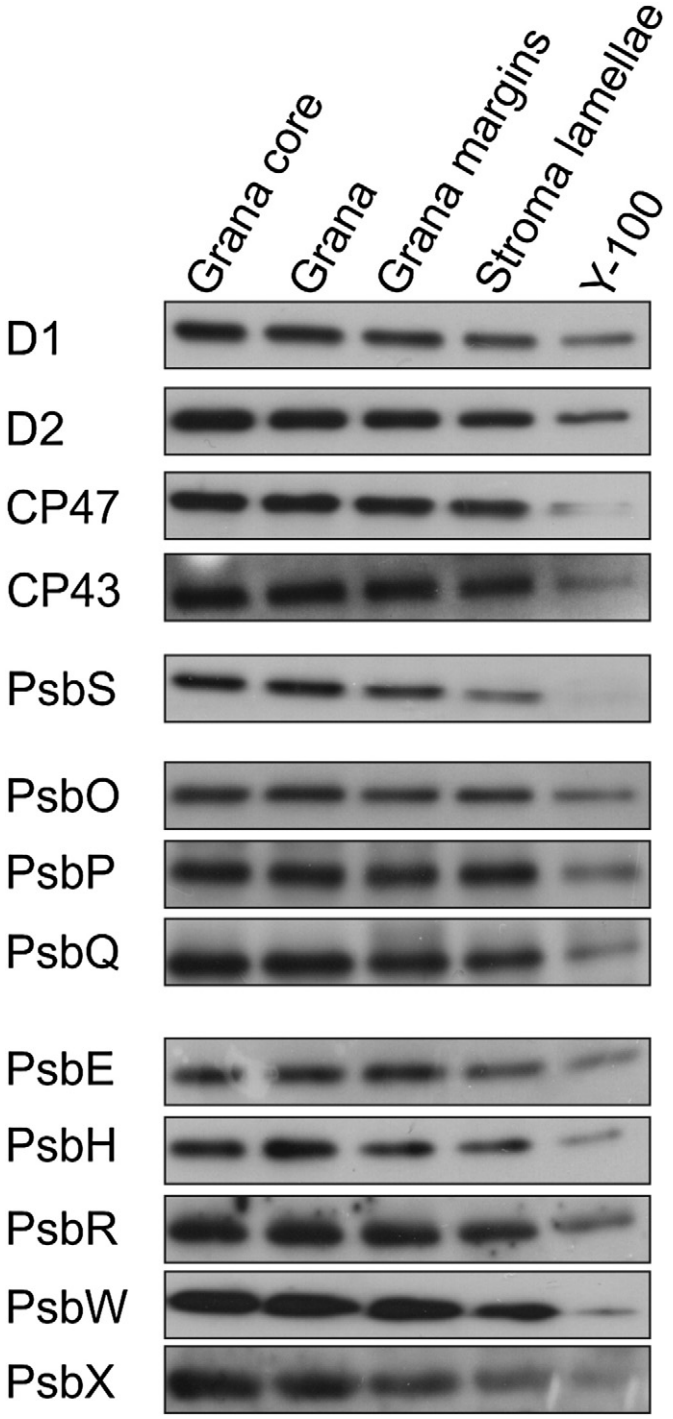

Fig. 2. Distribution of PSII subunits in spinach thylakoid fractions. Immunoblot analyses of the distribution of PSII proteins in mechanically isolated thylakoid membrane fractions from spinach chloroplasts. The gels were loaded on equal protein bases.

[30–32] broadly resembled that of LPA1 and TLP18; however, Psb27 was slightly more abundant in the stroma thylakoid with around 60% of the total protein detected from stroma thylakoids and Y-100 fractions altogether.

The TL29 (thylakoid lumen 29) protein [33] was highly enriched in the grana membranes, as only 7% of the protein was detected in the stroma thylakoid fractions and none in the Y-100 fraction. In contrast, the TLP40 protein (thylakoid lumenal peptidyl–prolyl isomerase of 40 kDa) [34] was found to be present exclusively in the stroma

Table 2
Semi-quantitative analysis of the relatively distribution of chloroplast auxiliary proteins in different thylakoid membrane fractions of spinach. The values are percentages (mean ± SD, $n = 3$) of total.

	Grana core	Grana	Grana margins	Stroma thylakoids	Y-100	Suggested function[a]
Deg2	1 ± 1	16 ± 6	11 ± 3	33 ± 3	39 ± 3	Degradation of the photodamaged D1 protein [71]
FtsH1	3 ± 5	8 ± 5	17 ± 2	31 ± 4	41 ± 8	Protease, degradation of the photodamaged D1 protein [69,91,92]
Psb27	6 ± 8	18 ± 4	13 ± 4	31 ± 1	32 ± 1	PSII repair cycle [31]. In cyanobacteria: regulation of the reassembly of OEC during PSII repair cycle [32,82], stabilization of the damaged PSII dimers prior to monomerization and repair [81,82]
LPA1	9 ± 5	18 ± 2	21 ± 1	25 ± 3	27 ± 6	Assembly of D1 [28]
TL29	43 ± 10	32 ± 9	18 ± 4	7 ± 4	0	Inactive ascorbate peroxidase? [86]
TLP40	0	2 ± 0	2 ± 1	36 ± 1	60 ± 1	Regulator of the thylakoid phosphatases [75,76], PSII assembly and stabilization [77,78]
TLP18.3	15 ± 2	19 ± 4	17 ± 6	25 ± 4	24 ± 5	Regulation of D1 turnover, PSII dimerization during repair [29]
SecY	4 ± 2	10 ± 0	17±	30 ± 4	39 ± 7	Thylakoid membrane translocon component, co-translational insertion of the novel D1 protein [58]
PGR5	20 ± 6	17 ± 2	20 ± 5	20 ± 3	23 ± 7	Regulation of CET [17,18], regulation of photosynthetic control [87]
PGRL1	2 ± 2	7 ± 2	10 ± 3	32 ± 2	49 ± 4	Regulation of CET [19]

[a] If not otherwise mentioned, the suggested function is based on studies with Arabidopsis and spinach.

thylakoids, as 96% of the total protein amount was detected from the stroma thylakoids and Y-100 fractions (Table 2).

3.4. The presence of the PGR5 and PGRL1 proteins in different domains of the thylakoid membrane

The recent discussion concerning the roles of the PGR5 and PGRL1 [17–19,35,36] and particularly the recently suggested role for PGRL1 as a long-sought ferredoxin plastoquinone reductase (FQR) [37] prompted us to study the distribution of these proteins in spinach thylakoid fractions. The PGR5 protein was found to be very evenly distributed among the different fractions with roughly 20% of the protein present in each fraction (Table 2, Fig. 3A). The PGRL1 protein, on the other hand, was present mainly in stroma thylakoids, as altogether only around 20% of its total pool was detected in grana core, total grana thylakoid fraction and grana margins. To verify this somewhat unexpected result, we investigated the localization of PGR5 and PGRL1 also with Arabidopsis thylakoid fractions obtained with digitonin. The distribution of PGR5 and PGRL1 in Arabidopsis thylakoid fractions highly resembled that obtained with spinach — PGR5 was present evenly between grana and stroma thylakoids, whereas PGRL1 was clearly enriched in stroma thylakoids (Fig. 3B).

4. Discussion

Even though the lateral heterogeneity in the distribution of PSII and PSI along the thylakoid membrane of higher plants has been known for more than 30 years, since the classical publication of Andersson and Anderson [1], the main reason for the segregation of the two photosystems – PSII being located in the grana thylakoids and PSI mostly in the stroma thylakoids – still remains elusive. Tight packing of PSII–LHCII arrays in the grana thylakoids allows efficient energy transfer in PSII–LHCII arrays [5] and the PSII connectivity *via* the shared LHCII pool (the 'lake model') [38]. According to classical view, strict lateral heterogeneity also prevents the "faster" and more efficient photosystem, PSI, from drawing excitation energy from the "slower" PSII [39]. Another explanation for the lateral heterogeneity is CET: in order to maintain the optimal efficiency of the photosynthetic machinery, CET must be physically separated from the more predominant linear electron transfer [40–42]. It has also been suggested that lateral heterogeneity-based tight stacking protects PSII complexes from proteolytic degradation and damage by ROS produced in PSI [43–46].

The overall degree of stacking is dynamic and largely dependent on the light conditions. Firstly, the chloroplasts of shade-grown plants have more stacks than sun-grown plants [43]. The content of the CURVATURE THYLAKOID1 proteins as well as the phosphorylation of thylakoid membrane proteins have been shown to affect the extent of thylakoid stacking [47,48]. Furthermore, it has been recently shown that the thylakoid membranes are highly dynamic *in vivo*. The number of grana per chloroplast as well as the number of stacked thylakoids per granum change markedly even at the time-scale of 10 min [49,50]. Importantly, a single granum undergoes rapid major architectural reorganizations upon changing light conditions: as a consequence of high light exposure, lateral shrinkage and vertical unstacking of the grana stacks as well as swelling of the lumenal space rapidly take place [51–53]. The latter facilitates plastocyanin-mediated electron transfer and ensures fluent release and re-assembly of the OEC during PSII repair cycle and also provides better access to the lumenal auxiliary proteins needed in PSII repair cycle [51].

We addressed the distribution of various PSII proteins in different domains of the thylakoid membrane to gain novel information about the PSII repair cycle and also to study how firmly various PSII subunits are associated with PSII—the latter concerns particularly those subunits specific to higher plants and which thus have not been localized in crystallographic studies (such as PsbR, PsbS, and PsbW). The photodamage of PSII takes place in the grana thylakoids [9], whereas the degradation of the D1 protein and the co-translational insertion of the newly synthetized D1 copy take place in the stroma-exposed thylakoids. Indeed, we found that the stroma thylakoid fraction and the Y-100 fraction hosted altogether around 30% of the studied PSII proteins (Table 1). Our previous study concerning the distribution of PSII complexes in different fractions revealed that stroma thylakoids and the Y-100 fraction are enriched in the PSII RC, PSII core monomers without CP43 and intact PSII core monomer [21], all of which are intermediates of the PSII repair cycle. Therefore, at least 30% of the PSII protein pool can be considered to undergo the repair cycle at certain point in the light period prior to the dark adaptation used in our experiments.

The OEC proteins PsbO, PsbP and PsbQ were found to exhibit highly similar distribution among thylakoid membrane as did the PSII core proteins, with approximately 30% of the proteins being detected from the stroma thylakoids and Y-100 (Table 1, Fig. 1B, Fig. 2). With respect to PSII repair cycle, it has been shown that the presence of CP43 is a prerequisite for the assembly of PsbO to PSII complex [54,55] and that PsbO becomes assembled in the stroma thylakoids whereas PsbP and PsbQ are assembled later, possibly in the grana thylakoids [56]. PsbO is known to be translocated to lumen *via* the Sec pathway [57], and indeed, SecY, responsible also for the co-translational insertion of the novel D1 protein [58], was enriched in the stroma-exposed thylakoids (Table 2). The presence of PsbP and PsbQ also in the stroma thylakoids (Table 1) does not necessarily contradict with previous suggestions concerning their assembly site, as a pool of free, assembly-competent OEC proteins has been shown to be located in the thylakoid lumen [56,59,60]. It is likely that part of the PsbP and PsbQ proteins detected in the stroma thylakoids and Y-100, which both are right-side-out vesicles, represent this soluble lumenal pool.

As compared to other PSII proteins, the nuclear-encoded LMM proteins PsbW and PsbX were more enriched in the grana thylakoids and only marginal amounts of them were detected in the Y-100 fraction (Table 1). The nuclear encoded PsbW has been shown to be associated to the PSII–LHCII supercomplexes [55]; moreover, the PsbW antisense

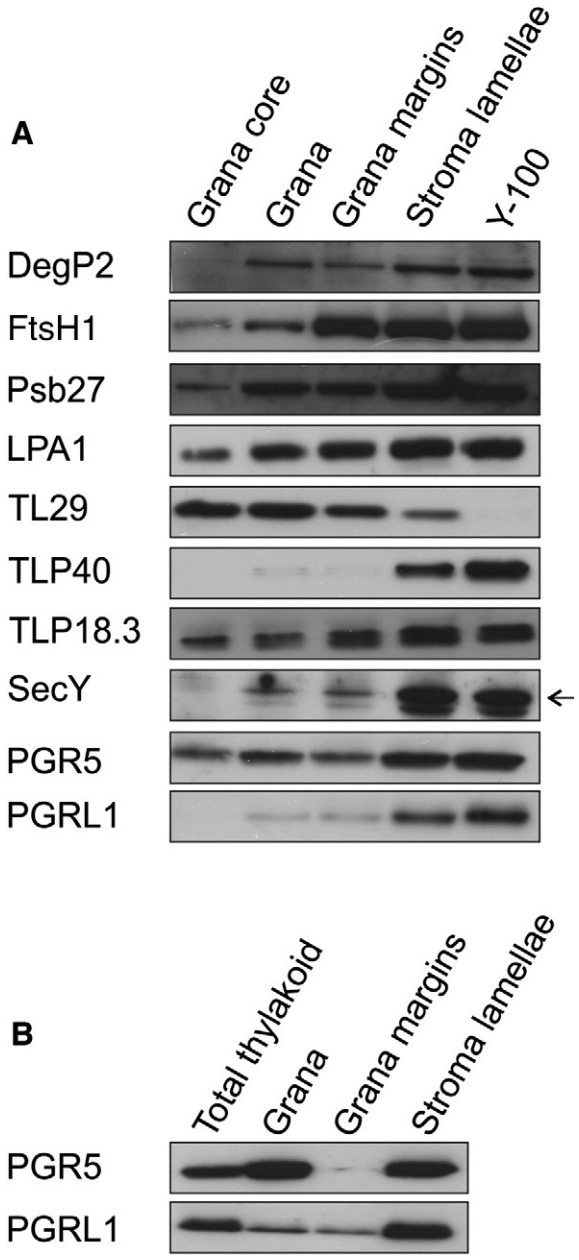

Fig. 3. Distribution of regulatory proteins in thylakoid fractions. A) Immunoblot analyses of the distribution of thylakoid regulatory proteins in mechanically isolated thylakoid membrane fractions from spinach chloroplasts. B) Immunoblot analyses of distribution of PGR5 and PGRL1 between stroma and grana thylakoids of Arabidopsis. Fractions were obtained with digitonin solubilization followed by ultracentrifugations. Thylakoid sample is included as a control. The gels were loaded on equal protein bases.

plants were found to be devoid of the PSII–LHCII supercomplexes and also of the semicrystalline macrodomains [61]. However, PsbW has been shown to be present in tobacco Δ*psbE* and Δ*psbF* mutants that lack all PSII core and OEC proteins [54], also in pea it was shown to be present already in etioplasts prior to the assembly of PSII [62]. Thus, the majority of the PsbW protein seems to be present in the thylakoid grana before PSII forms dimers and supra-complexes but it seems not to be under a strict quality control-based regulation that is typical to most other PSII proteins. The PsbX protein is also nuclear encoded and has a molecular mass of 4 kDa, and in contrast to PsbW, it is present also in cyanobacteria [4]. The PsbX antisense plants had less functional PSII complexes, decreased phosphorylation of their PSII proteins and antennae and changes in redox state and plastoquinone pool [63]. Thus contrary to the PsbW protein that at least to some extent seems to be important for the formation of larger complexes and thereby for the turnover of PSII, the PsbX protein seems to be more of functional importance for PSII changing the redox-state of PSII on the acceptor-side [63].

The PsbS protein, essential for the rapid induction of NPQ [64], was found to be present mostly in the appressed regions, as the grana core, grana thylakoids and grana margins together harbor more than 80% of total PsbS pool (Table 1). The distribution of PsbS correlates with the presence of LHCII in different fractions (Fig. 1B). This is in accordance with results from Arabidopsis showing that PsbS is preferentially localized to grana membrane, where it likely regulates the reorganization of the PSII–LHCII supercomplexes, thus allowing the reversible transition between quenching and non-quenching complexes [65–67].

Of the auxiliary proteins known to have a role in PSII repair cycle, the FtsH1 protease was highly enriched in Y-100 (41% of the total pool), stroma thylakoids (32%) and grana margins (17%), and only marginal amounts were found from the grana core (Table 2). In addition to FtsH1 proteases, which are primarily responsible for the degradation of the damaged D1 protein [68], also lumenal-located Deg proteases assist in coordinated degradation of the damaged D1 [69–71]. The Deg2 protein was found to be located preferentially in the stroma-exposed thylakoids (Table 2). The functional form of both the FtsH1 and Deg proteases is a hexamer, which in the case of FtsH1 contains a stromal protuberance of around 6.5 nm [72], whereas the lumenal Deg hexamer has been reported to have a diameter of up to 9 nm [73]. The dense grana stacks cannot fit this kind of bulky molecules. However, the light-induced lateral shrinkage of the grana stacks and the swelling of the lumen width allow both proteases to reach the damaged PSII complexes [51–53]. Also the acidic phosphatase protein TLP18.3 in thylakoid lumen has been shown to be involved in degradation of the photodamaged D1 protein [29,74]. TLP18.3, which has a transmembrane helix and is loosely bound to the thylakoid membrane [29], was found to be rather evenly distributed between the stroma and grana thylakoids (Table 2, Fig. 3A), which is in line with the results previously obtained with Arabidopsis thylakoid membrane fractions isolated with detergent [29]. Indeed, TLP18.3 has a dual role in the PSII repair cycle: in addition to degradation of the D1 protein in stroma thylakoids, it is also assisting in the dimerization of the repaired PSII monomers in grana membranes [29].

TLP40, a lumenal cyclophilin-like protein, contains two functional domains, a PPIase domain and a phosphatase binding module, which was proposed to function as a negative regulator of the thylakoid phosphatases [75,76]. TLP40 was detected practically only in the stroma thylakoid and Y-100 fractions (Table 2). Even though lumenal proteins might have escaped from grana thylakoids during isolation, we believe that this localization holds true, since despite the presence of TLP40 in lumenal proteome [34], most of the protein has been shown to be bound to the membrane at room temperature, and only a small population of the protein was found to be soluble in the lumen [75]. The Arabidopsis ortholog of TLP40, AtCYP38, has been shown to be crucial for the proper folding of D1 and CP47 into PSII, both during biogenesis and repair cycle of PSII [77–79]. These early steps of PSII assembly likely take place in stroma thylakoids. The LPA1 protein is a thylakoid membrane-bound chaperone which has been shown to assure proper translation of novel D1 protein [27,28]. The Arabidopsis *lpa1* mutants showed reduced D1 translation and accumulated unassembled D1 protein. Also LPA1 was found to be present mostly in the Y-100, stroma thylakoids and grana margin fractions (Table 2). However, the stromal location was not as definite as that of TLP40, since grana thylakoids hosted around 30% of LPA1.

The Psb27 protein was enriched in stroma thylakoids and Y-100; however, the combined amount of Psb27 in the grana core and grana fractions was still roughly 20% (Table 2). The cyanobacterial Psb27 protein has been shown to transiently bind to the CP43 protein in PSII monomer intermediate complex during the repair cycle [32,80,81]. This ensures that the re-assembly of the OEC does not occur too early,

which could cause damage. Interestingly, CP43 and Psb27 were found to have interaction with the PSI complexes, which suggests that PSI might have a yet unassigned role in PSII biogenesis and/or repair cycle [81]. Furthermore, the cyanobacterial Psb27 was shown to be transiently bound also to the photodamaged PSII dimer prior to its monomerization and repair, which prevents the release of manganese [82]. The cyanobacterial Psb27 possesses a lipoprotein motif, which is not found from Arabidopsis homologue, so the findings concerning the function of cyanobacterial Psb27 cannot be directly generalized to higher plants. However, the presence of Psb27 protein both in the stroma and grana thylakoids (Table 2) supports the proposed dual role of Psb27 in binding both to the PSII dimer and monomer during the repair cycle. In Arabidopsis, Psb27 has been shown to be required for the efficient repair of photodamaged PSII [31], and another homologue, LOW PSII ACCUMULATION 19 (LPA19) has a role in facilitating the C-terminal processing of the D1 precursor protein [83]. Furthermore, the Arabidopsis *psb27* mutant has been shown to be devoid of the PSII–LHCII supercomplexes, which caused accelerated kinetics of state transitions as compared to WT [84].

The TL29 protein was first found in the thylakoid lumen [85] and was later suggested to be associated with PSII [33]. The TL29 protein was localized almost exclusively to the grana thylakoids (Table 2, Fig. 3A) which corroborates with the earlier studies with Arabidopsis [33]. Despite the fact that the protein has sequence homology to ascorbate peroxidases, it seems not to function as a peroxidase as neither biochemical nor crystal structural analysis at 2.5 Å [86] was able to detect or suggest any peroxidase activity. Thus, the TL29 was suggested to have lost its function as peroxidase and instead was recruited for other functions in the chloroplast, such as assembly and/or chaperon function in PSII, which would be in agreement with the finding in this study.

The PGR5 and PGRL1 proteins are involved in CET [17,18] and have been suggested to form a complex with each other [19]. Furthermore, it was recently suggested that the PGRL1 protein is the actual long-sought ferredoxin-plastoquinone reductase molecule [37]. Intriguingly, PGR5 and PGRL1 showed divergent distribution in different domains of the thylakoid membrane: PGR5 was distributed rather evenly between grana and stroma thylakoids, whereas PGRL1 was highly enriched in stroma thylakoids and Y-100, and was practically missing from the grana cores (Table 2, Fig. 3). This unexpected distribution of the two proteins was further investigated by a digitonin fractionation of Arabidopsis thylakoids, which showed the same distribution (Fig. 3B).

Our observation of the enrichment of PGRL1 in stroma thylakoids (Fig. 2) is in line with a recent publication [37], but contradicts with co-localization of PGR5 to the same thylakoid fraction with PGRL1 in Arabidopsis. Indeed, our results with both spinach (Fig. 3A) and Arabidopsis (Fig. 3B) thylakoid fractions revealed differential distribution for the PGRL1 and PGR5 proteins. At present, we cannot explain this discrepancy. Given the highly dynamic rearrangement of the thylakoids according to light conditions [49–53], it should be noted that the observed distribution of the PGR proteins primarily reflects the particular situation under darkness rather than a general feature. However, our recent results from experiments performed with artificially and naturally fluctuating light suggest that the role of the PGR5 protein is far more important than its proposed role of being “only” the component of CET — according to our view, PGR5 is crucial for the regulation of linear electron flow particularly upon sudden high light peaks, and *via* this function in photosynthetic control, it protects PSI from photodamage [87]. The dual role of PGR5, as not only being a CET component but also (and primarily) functioning as a regulator of photosynthetic control, is in line with its localization also in the grana membranes. We suggest that the PGR5 present in grana thylakoids and grana margins functions mainly in photosynthetic control, whereas the pool located in stroma thylakoids might form a complex with PGRL1 and functions as a component of CET. Recently, an additional role was suggested also for PGRL1, as it was shown to form a complex with Plsp1, which is an isoform of the thylakoid processing peptidase that removes the thylakoid-transfer signal peptide of lumen-targeted proteins after translocation [88]. It was suggested that PGRL1 could act as a regulator of thylakoid processing peptidase. Protein translocation likely takes place in stroma thylakoids, and this putative additional role might explain the localization of PGRL1 more exclusively in the stroma thylakoids as compared to its interaction partner PGR5.

To conclude, we have semi-quantitatively determined the localization of 13 PSII subunits and 10 auxiliary proteins along the spinach thylakoid membrane. While most of the PSII subunits showed rather equal distribution with roughly 70% of the proteins located collectively in the grana thylakoids and grana margins, the LMM subunits PsbW and PsbX as well as the PsbS proteins were found to be more exclusively located in grana thylakoids. The auxiliary proteins assisting in repair cycle of PSII were mostly located in stroma-exposed thylakoids. Intriguingly, PGR5 was found to be distributed quite evenly between grana and stroma thylakoids, whereas PGRL1 was highly enriched in the stroma thylakoids and practically missing from the grana cores. As several antibodies against Arabidopsis thylakoid auxiliary proteins did not cross-react with spinach proteins, the next step towards functional characterization of different domains of the thylakoids membrane is to optimize mechanical fractionation method for the Arabidopsis chloroplasts.

Acknowledgements

Our research has been funded by the Academy of Finland (project numbers 138703 to M.S., CoE project 118637 to E.-M.A.), the Swedish Research Council (F.M. and S.S.), the Swedish Energy Agency (F.M. and S.S.) and the Knut and Alice Wallenberg Foundation (F.M. and S.S.).

References

[1] B. Andersson, J.M. Anderson, Lateral heterogeneity in the distribution of chlorophyll–protein complexes of the thylakoid membranes of spinach chloroplasts, Biochim. Biophys. Acta Bioenerg. 593 (1980) 427–440.
[2] R. Danielsson, P.A. Albertsson, F. Mamedov, S. Styring, Quantification of photosystem I and II in different parts of the thylakoid membrane from spinach, Biochim. Biophys. Acta 1608 (2004) 53–61.
[3] I. Grouneva, P.J. Gollan, S. Kangasjarvi, M. Suorsa, M. Tikkanen, E.M. Aro, Phylogenetic viewpoints on regulation of light harvesting and electron transport in eukaryotic photosynthetic organisms, Planta 237 (2012) 399–412.
[4] L.X. Shi, M. Hall, C. Funk, W.P. Schroder, Photosystem II, a growing complex: updates on newly discovered components and low molecular mass proteins, Biochim. Biophys. Acta Bioenerg. 1817 (2012) 13–25.
[5] J. Dekker, E. Boekema, Supramolecular organization of thylakoid membrane proteins in green plants, Biochim. Biophys. Acta Bioenerg. 1706 (2005) 12–39.
[6] T.K. Goral, M.P. Johnson, C.D. Duffy, A.P. Brain, A.V. Ruban, C.W. Mullineaux, Light-harvesting antenna composition controls the macrostructure and dynamics of thylakoid membranes in *Arabidopsis*, Plant J. 69 (2012) 289–301.
[7] E. Tyystjärvi, E.M. Aro, The rate constant of photoinhibition, measured in lincomycin-treated leaves, is directly proportional to light intensity, Proc. Natl. Acad. Sci. U. S. A. 93 (1996) 2213–2218.
[8] E.M. Aro, I. Virgin, B. Andersson, Photoinhibition of Photosystem II. Inactivation, protein damage and turnover, Biochim. Biophys. Acta Bioenerg. 1143 (1993) 113–134.
[9] E.M. Aro, M. Suorsa, A. Rokka, Y. Allahverdiyeva, V. Paakkarinen, A. Saleem, N. Battchikova, E. Rintamaki, Dynamics of photosystem II: a proteomic approach to thylakoid protein complexes, J. Exp. Bot. 56 (2005) 347–356.
[10] E. Baena-Gonzalez, E.M. Aro, Biogenesis, assembly and turnover of photosystem II units, Philos. Trans. R. Soc. Lond. B Biol. Sci. 357 (2002) 1451–1459(discussion 1459-60).
[11] P. Mulo, S. Sirpio, M. Suorsa, E.M. Aro, Auxiliary proteins involved in the assembly and sustenance of photosystem II, Photosynth. Res. 98 (2008) 489–501.
[12] P. Horton, A.V. Ruban, R.G. Walters, Regulation of light harvesting in green plants, Annu. Rev. Plant Physiol. Plant Mol. Biol. 47 (1996) 655–684.
[13] M. Pribil, P. Pesaresi, A. Hertle, R. Barbato, D. Leister, Role of plastid protein phosphatase TAP38 in LHCII dephosphorylation and thylakoid electron flow, PLoS Biol. 8 (2010) e1000288.
[14] A. Shapiguzov, B. Ingelsson, I. Samol, C. Andres, F. Kessler, J.D. Rochaix, A.V. Vener, M. Goldschmidt-Clermont, The PPH1 phosphatase is specifically involved in LHCII dephosphorylation and state transitions in *Arabidopsis*, Proc. Natl. Acad. Sci. U. S. A. 107 (2010) 4782–4787.
[15] M. Tikkanen, M. Grieco, E.M. Aro, Novel insights into plant light-harvesting complex II phosphorylation and ‘state transitions’, Trends Plant Sci. 16 (2011) 126–131.
[16] T. Shikanai, Cyclic electron transport around photosystem I: genetic approaches, Annu. Rev. Plant Biol. 58 (2007) 199–217.

[17] Y. Munekage, M. Hojo, J. Meurer, T. Endo, M. Tasaka, T. Shikanai, PGR5 is involved in cyclic electron flow around photosystem I and is essential for photoprotection in *Arabidopsis*, Cell 110 (2002) 361–371.
[18] Y. Munekage, M. Hashimoto, C. Miyake, K. Tomizawa, T. Endo, M. Tasaka, T. Shikanai, Cyclic electron flow around photosystem I is essential for photosynthesis, Nature 429 (2004) 579–582.
[19] G. DalCorso, P. Pesaresi, S. Masiero, E. Aseeva, D. Schunemann, G. Finazzi, P. Joliot, R. Barbato, D. Leister, A complex containing PGRL1 and PGR5 is involved in the switch between linear and cyclic electron flow in *Arabidopsis*, Cell 132 (2008) 273–285.
[20] S. Haferkamp, W. Haase, A.A. Pascal, H. van Amerongen, H. Kirchhoff, Efficient light harvesting by photosystem II requires an optimized protein packing density in Grana thylakoids, J. Biol. Chem. 285 (2010) 17020–17028.
[21] R. Danielsson, M. Suorsa, V. Paakkarinen, P.A. Albertsson, S. Styring, E.M. Aro, F. Mamedov, Dimeric and monomeric organization of photosystem II. Distribution of five distinct complexes in the different domains of the thylakoid membrane, J. Biol. Chem. 281 (2006) 14241–14249.
[22] E. Baena-Gonzalez, R. Barbato, E. Aro, Role of phosphorylation in the repair cycle and oligomeric structure of photosystem II, Planta 208 (1999) 196–204.
[23] R.J. Porra, W.A. Thompson, P.E. Kriedemann, Determination of accurate extinction coefficients and simultaneous-equations for assaying chlorophyll-a and chlorophyll-b extracted with 4 different solvents—verification of the concentration of chlorophyll standards by atomic-absorption spectroscopy, Biochim. Biophys. Acta Bioenerg. 975 (1989) 384–394.
[24] O.H. Lowry, N.J. Rosebrough, A.L. Farr, R.J. Randall, Protein measurement with the Folin phenol reagent, J. Biol. Chem. 193 (1951) 265–275.
[25] U.K. Laemmli, Cleavage of structural proteins during the assembly of the head of bacteriophage T4, Nature 227 (1970) 680–685.
[26] T. Henrysson, C. Sundby, Characterization of photosystem-II in stroma thylakoid membranes, Photosynth. Res. 25 (1990) 107–117.
[27] W. Chi, J. Ma, L. Zhang, Regulatory factors for the assembly of thylakoid membrane protein complexes, Philos. Trans. R. Soc. Lond. B Biol. Sci. 367 (2012) 3420–3429.
[28] L. Peng, J. Ma, W. Chi, J. Guo, S. Zhu, Q. Lu, C. Lu, L. Zhang, Low PSII ACCUMULATION1 is involved in efficient assembly of photosystem II in *Arabidopsis thaliana*, Plant Cell 18 (2006) 955–969.
[29] S. Sirpio, Y. Allahverdiyeva, M. Suorsa, V. Paakkarinen, J. Vainonen, N. Battchikova, E.M. Aro, TLP18.3, a novel thylakoid lumen protein regulating photosystem II repair cycle, Biochem. J. 406 (2007) 415–425.
[30] J.B. Peltier, O. Emanuelsson, D.E. Kalume, J. Ytterberg, G. Friso, A. Rudella, D.A. Liberles, L. Soderberg, P. Roepstorff, G. von Heijne, K.J. van Wijk, Central functions of the lumenal and peripheral thylakoid proteome of *Arabidopsis* determined by experimentation and genome-wide prediction, Plant Cell 14 (2002) 211–236.
[31] H. Chen, D. Zhang, J. Guo, H. Wu, M. Jin, Q. Lu, C. Lu, L. Zhang, A Psb27 homologue in *Arabidopsis thaliana* is required for efficient repair of photodamaged photosystem II, Plant Mol. Biol. 61 (2006) 567–575.
[32] M.M. Nowaczyk, R. Hebeler, E. Schlodder, H.E. Meyer, B. Warscheid, M. Rögner, Psb27, a cyanobacterial lipoprotein, is involved in the repair cycle of photosystem II, Plant Cell 18 (2006) 3121–3131.
[33] I. Granlund, P. Storm, M. Schubert, J.G. Garcia-Cerdan, C. Funk, W.P. Schröder, The TL29 protein is lumen located, associated with PSII and not an ascorbate peroxidase, Plant Cell Physiol. 50 (2009) 1898–1910.
[34] H. Fulgosi, A.V. Vener, L. Altschmied, R.G. Herrmann, B. Andersson, A novel multi-functional chloroplast protein: identification of a 40 kDa immunophilin-like protein located in the thylakoid lumen, EMBO J. 17 (1998) 1577–1587.
[35] B. Nandha, G. Finazzi, P. Joliot, S. Hald, G.N. Johnson, The role of PGR5 in the redox poising of photosynthetic electron transport, Biochim. Biophys. Acta Bioenerg. 1767 (2007) 1252–1259.
[36] D. Leister, T. Shikanai, Complexities and protein complexes in the antimycin A-sensitive pathway of cyclic electron flow in plants, Front. Plant. Sci. 4 (2013) 161.
[37] A.P. Hertle, T. Blunder, T. Wunder, P. Pesaresi, M. Pribil, U. Armbruster, D. Leister, PGRL1 is the elusive ferredoxin-plastoquinone reductase in photosynthetic cyclic electron flow, Mol. Cell 49 (2013) 511–523.
[38] D.M. Kramer, G. Johnson, O. Kiirats, G.E. Edwards, New fluorescence parameters for the determination of q(a) redox state and excitation energy fluxes, Photosynth. Res. 79 (2004) 209–218.
[39] H.W. Trissl, C. Wilhelm, Why do thylakoid membranes from higher plants form grana stacks? Trends Biochem. Sci. 18 (1993) 415–419.
[40] P. Albertsson, A quantitative model of the domain structure of the photosynthetic membrane, Trends Plant Sci. 6 (2001) 349–358.
[41] P. Joliot, D. Beal, A. Joliot, Cyclic electron flow under saturating excitation of dark-adapted *Arabidopsis* leaves, Biochim. Biophys. Acta Bioenerg. 1656 (2004) 166–176.
[42] G.N. Johnson, Cyclic electron transport in C3 plants: fact or artefact? J. Exp. Bot. 56 (2005) 407–416.
[43] J.M. Anderson, E.M. Aro, Grana stacking and protection of photosystem-II in thylakoid membranes of higher-plant leaves under sustained high irradiance—an hypothesis, Photosynth. Res. 41 (1994) 315–326.
[44] S.E. Tjus, H.V. Scheller, B. Andersson, B.L. Moller, Active oxygen produced during selective excitation of photosystem I is damaging not only to photosystem I, but also to photosystem II, Plant Physiol. 125 (2001) 2007–2015.
[45] M. Khatoon, K. Inagawa, P. Pospisil, A. Yamashita, M. Yoshioka, B. Lundin, J. Horie, N. Morita, A. Jajoo, Y. Yamamoto, Y. Yamamoto, Quality control of photosystem II: thylakoid unstacking is necessary to avoid further damage to the D1 protein and to facilitate D1 degradation under light stress in spinach thylakoids, J. Biol. Chem. 284 (2009) 25343–25352.
[46] A. Krieger-Liszkay, P.B. Kos, E. Hideg, Superoxide anion radicals generated by methylviologen in photosystem I damage photosystem II, Physiol. Plant. 142 (2011) 17–25.
[47] R. Fristedt, A. Willig, P. Granath, M. Crevecoeur, J.D. Rochaix, A.V. Vener, Phosphorylation of photosystem II controls functional macroscopic folding of photosynthetic membranes in *Arabidopsis*, Plant Cell 21 (2009) 3950–3964.
[48] U. Armbruster, M. Labs, M. Pribil, S. Viola, W. Xu, M. Scharfenberg, A.P. Hertle, U. Rojahn, P.E. Jensen, F. Rappaport, P. Joliot, P. Dormann, G. Wanner, D. Leister, *Arabidopsis* CURVATURE THYLAKOID1 proteins modify thylakoid architecture by inducing membrane curvature, Plant Cell 25 (2013) 2661–2678.
[49] J.M. Anderson, P. Horton, E.H. Kim, W.S. Chow, Towards elucidation of dynamic structural changes of plant thylakoid architecture, Philos. Trans. R. Soc. Lond. B Biol. Sci. 367 (2012) 3515–3524.
[50] P. Rozak, R. Seiser, W. Wacholtz, R. Wise, Rapid, reversible alterations in spinach thylakoid appression upon changes in light intensity, Plant Cell Environ. 25 (2002) 421–429.
[51] H. Kirchhoff, C. Hall, M. Wood, M. Herbstova, O. Tsabari, R. Nevo, D. Charuvi, E. Shimoni, Z. Reich, Dynamic control of protein diffusion within the granal thylakoid lumen, Proc. Natl. Acad. Sci. U. S. A. 108 (2011) 20248–20253.
[52] M. Herbstova, S. Tietz, C. Kinzel, M.V. Turkina, H. Kirchhoff, Architectural switch in plant photosynthetic membranes induced by light stress, Proc. Natl. Acad. Sci. U. S. A. 109 (2012) 20130–20135.
[53] H. Kirchhoff, Architectural switches in plant thylakoid membranes, Photosynth. Res. 116 (2013) 481–487.
[54] M. Suorsa, R.E. Regel, V. Paakkarinen, N. Battchikova, R.G. Herrmann, E.M. Aro, Protein assembly of photosystem II and accumulation of subcomplexes in the absence of low molecular mass subunits PsbL and PsbJ, Eur. J. Biochem. 271 (2004) 96–107.
[55] A. Rokka, M. Suorsa, A. Saleem, N. Battchikova, E.M. Aro, Synthesis and assembly of thylakoid protein complexes: multiple assembly steps of photosystem II, Biochem. J. 388 (2005) 159–168.
[56] A. Hashimoto, W.F. Ettinger, Y. Yamamoto, S.M. Theg, Assembly of newly imported oxygen-evolving complex subunits in isolated chloroplasts: sites of assembly and mechanism of binding, Plant Cell 9 (1997) 441–452.
[57] M. Schubert, U.A. Petersson, B.J. Haas, C. Funk, W.P. Schroder, T. Kieselbach, Proteome map of the chloroplast lumen of *Arabidopsis thaliana*, J. Biol. Chem. 277 (2002) 8354–8365.
[58] L. Zhang, V. Paakkarinen, M. Suorsa, E.M. Aro, A SecY homologue is involved in chloroplast-encoded D1 protein biogenesis, J. Biol. Chem. 276 (2001) 37809–37814.
[59] A. Hashimoto, Y. Yamamoto, S.M. Theg, Unassembled subunits of the photosynthetic oxygen-evolving complex present in the thylakoid lumen are long-lived and assembly-competent, FEBS Lett. 391 (1996) 29–34.
[60] W.F. Ettinger, S.M. Theg, Physiologically active chloroplasts contain pools of unassembled extrinsic proteins of the photosynthetic oxygen-evolving enzyme complex in the thylakoid lumen, J. Cell Biol. 115 (1991) 321–328.
[61] J.G. Garcia-Cerdan, L. Kovacs, T. Toth, S. Kereiche, E. Aseeva, E.J. Boekema, F. Mamedov, C. Funk, W.P. Schroder, The PsbW protein stabilizes the supramolecular organization of photosystem II in higher plants, Plant J. 65 (2011) 368–381.
[62] E. Kanervo, M. Singh, M. Suorsa, V. Paakkarinen, E. Aro, N. Battchikova, E.M. Aro, Expression of protein complexes and individual proteins upon transition of etioplasts to chloroplasts in pea (*Pisum sativum*), Plant Cell Physiol. 49 (2008) 396–410.
[63] J.G. Garcia-Cerdan, D. Sveshnikov, D. Dewez, S. Jansson, C. Funk, W.P. Schröder, Antisense inhibition of the PsbX protein affects PSII integrity in the higher plant *Arabidopsis thaliana*, Plant Cell Physiol. 50 (2009) 191–202.
[64] X.P. Li, O. Bjorkman, C. Shih, A.R. Grossman, M. Rosenquist, S. Jansson, K.K. Niyogi, A pigment-binding protein essential for regulation of photosynthetic light harvesting, Nature 403 (2000) 391–395.
[65] S. Kereiche, A.Z. Kiss, R. Kouril, E.J. Boekema, P. Horton, The PsbS protein controls the macro-organisation of photosystem II complexes in the grana membranes of higher plant chloroplasts, FEBS Lett. 584 (2010) 759–764.
[66] A.Z. Kiss, A.V. Ruban, P. Horton, The PsbS protein controls the organization of the photosystem II antenna in higher plant thylakoid membranes, J. Biol. Chem. 283 (2008) 3972–3978.
[67] P. Horton, Optimization of light harvesting and photoprotection: molecular mechanisms and physiological consequences, Philos. Trans. R. Soc. Lond. B Biol. Sci. 367 (2012) 3455–3465.
[68] R. Wagner, H. Aigner, C. Funk, FtsH proteases located in the plant chloroplast, Physiol. Plant. 145 (2012) 203–214.
[69] Y. Kato, X. Sun, L. Zhang, W. Sakamoto, Cooperative D1 degradation in the photosystem II repair mediated by chloroplastic proteases in *Arabidopsis*, Plant Physiol. 159 (2012) 1428–1439.
[70] H. Schuhmann, I. Adamska, Deg proteases and their role in protein quality control and processing in different subcellular compartments of the plant cell, Physiol. Plant. 145 (2012) 224–234.
[71] K. Haussuhl, B. Andersson, I. Adamska, A chloroplast DegP2 protease performs the primary cleavage of the photodamaged D1 protein in plant photosystem II, EMBO J. 20 (2001) 713–722.
[72] R. Suno, H. Niwa, D. Tsuchiya, X. Zhang, M. Yoshida, K. Morikawa, Structure of the whole cytosolic region of ATP-dependent protease FtsH, Mol. Cell 22 (2006) 575–585.
[73] J. Kley, B. Schmidt, B. Boyanov, P.C. Stolt-Bergner, R. Kirk, M. Ehrmann, R.R. Knopf, L. Naveh, Z. Adam, T. Clausen, Structural adaptation of the plant protease Deg1 to repair photosystem II during light exposure, Nat. Struct. Mol. Biol. 18 (2011) 728–731.
[74] H.Y. Wu, M.S. Liu, T.P. Lin, Y.S. Cheng, Structural and functional assays of AtTLP18.3 identify its novel acid phosphatase activity in thylakoid lumen, Plant Physiol. 157 (2011) 1015–1025.

[75] A. Rokka, E.M. Aro, R.G. Herrmann, B. Andersson, A.V. Vener, Dephosphorylation of photosystem II reaction center proteins in plant photosynthetic membranes as an immediate response to abrupt elevation of temperature, Plant Physiol. 123 (2000) 1525–1536.
[76] A.V. Vener, A. Rokka, H. Fulgosi, B. Andersson, R.G. Herrmann, A cyclophilin-regulated PP2A-like protein phosphatase in thylakoid membranes of plant chloroplasts, Biochemistry 39 (2000) 2130.
[77] S. Sirpiö, A. Khrouchtchova, Y. Allahverdiyeva, M. Hansson, R. Fristedt, A.V. Vener, H.V. Scheller, P.E. Jensen, A. Haldrup, E.M. Aro, AtCYP38 ensures early biogenesis, correct assembly and sustenance of photosystem II, Plant J. 55 (2008) 639–651.
[78] A. Fu, Z. He, H.S. Cho, A. Lima, B.B. Buchanan, S. Luan, A chloroplast cyclophilin functions in the assembly and maintenance of photosystem II in *Arabidopsis thaliana*, Proc. Natl. Acad. Sci. U. S. A. 104 (2007) 15947–15952.
[79] D. Vasudevan, A. Fu, S. Luan, K. Swaminathan, Crystal structure of *Arabidopsis* cyclophilin38 reveals a previously uncharacterized immunophilin fold and a possible autoinhibitory mechanism, Plant Cell 24 (2012) 2666–2674.
[80] H. Liu, R.Y. Huang, J. Chen, M.L. Gross, H.B. Pakrasi, Psb27, a transiently associated protein, binds to the chlorophyll binding protein CP43 in photosystem II assembly intermediates, Proc. Natl. Acad. Sci. U. S. A. 108 (2011) 18536–18541.
[81] J. Komenda, J. Knoppova, J. Kopecna, R. Sobotka, P. Halada, J. Yu, J. Nickelsen, M. Boehm, P.J. Nixon, The Psb27 assembly factor binds to the CP43 complex of photosystem II in the cyanobacterium *Synechocystis* sp. PCC 6803, Plant Physiol. 158 (2012) 476–486.
[82] N. Grasse, F. Mamedov, K. Becker, S. Styring, M. Rögner, M.M. Nowaczyk, Role of novel dimeric photosystem II (PSII)–Psb27 protein complex in PSII repair, J. Biol. Chem. 286 (2011) 29548–29555.
[83] L. Wei, J. Guo, M. Ouyang, X. Sun, J. Ma, W. Chi, C. Lu, L. Zhang, LPA19, a Psb27 homolog in *Arabidopsis thaliana*, facilitates D1 protein precursor processing during PSII biogenesis, J. Biol. Chem. 285 (2010) 21391–21398.
[84] L. Dietzel, K. Brautigam, S. Steiner, K. Schuffler, B. Lepetit, B. Grimm, M.A. Schöttler, T. Pfannschmidt, Photosystem II supercomplex remodeling serves as an entry mechanism for state transitions in *Arabidopsis*, Plant Cell 23 (2011) 2964–2977.
[85] T. Kieselbach, Å. Hagman, B. Andersson, W.P. Schroder, The thylakoid lumen of chloroplasts. Isolation and characterization, J. Biol. Chem. 273 (1998) 6710–6716.
[86] E. Lundberg, P. Storm, W.P. Schroder, C. Funk, Crystal structure of the TL29 protein from *Arabidopsis thaliana*: an APX homolog without peroxidase activity, J. Struct. Biol. 176 (2011) 24–31.
[87] M. Suorsa, S. Järvi, M. Grieco, M. Nurmi, M. Pietrzykowska, M. Rantala, S. Kangasjärvi, V. Paakkarinen, M. Tikkanen, S. Jansson, E.M. Aro, PROTONGRADIENTREGULATION5 is essential for proper acclimation of *Arabidopsis* photosystem I to naturally and artificially fluctuating light conditions, Plant Cell 24 (2012) 2934–2948.
[88] J.K. Endow, K. Inoue, Stable complex formation of thylakoidal processing peptidase and PGRL1, FEBS Lett. 587 (2013) 2226–2231.
[89] L. Wollenberger, H. Stefansson, S. Yu, P. Albertsson, Isolation and characterization of vesicles originating from the chloroplast grana margins, Biochim. Biophys. Acta Bioenerg. 1184 (1994) 93–102.
[90] P. Albertsson, The structure and function of the chloroplast photosynthetic membrane—a model for the domain organization, Photosynth. Res. 46 (1995) 141–149.
[91] S. Bailey, E. Thompson, P.J. Nixon, P. Horton, C.W. Mullineaux, C. Robinson, N.H. Mann, A critical role for the Var2 FtsH homologue of *Arabidopsis thaliana* in the photosystem II repair cycle in vivo, J. Biol. Chem. 277 (2002) 2006–2011.
[92] Y. Kato, E. Miura, K. Ido, K. Ifuku, W. Sakamoto, The variegated mutants lacking chloroplastic FtsHs are defective in D1 degradation and accumulate reactive oxygen species, Plant Physiol. 151 (2009) 1790–1801.