Structure and dynamics of thylakoids in land plants

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Received 1 January 2014; Revised 3 February 2014; Accepted 5 February 2014

Abstract

Thylakoids of land plants have a bipartite structure, consisting of cylindrical grana stacks, made of membranous discs piled one on top of the other, and stroma lamellae which are helically wound around the cylinders. Protein complexes predominantly located in the stroma lamellae and grana end membranes are either bulky [photosystem I (PSI) and the chloroplast ATP synthase (cpATPase)] or are involved in cyclic electron flow [the NAD(P)H dehydrogenase (NDH) and PGRL1–PGR5 heterodimers], whereas photosystem II (PSII) and its light-harvesting complex (LHCII) are found in the appressed membranes of the granum. Stacking of grana is thought to be due to adhesion between Lhcb proteins (LHCCI or CP26) located in opposed thylakoid membranes. The grana margins contain oligomers of CURT1 proteins, which appear to control the size and number of grana discs in a dosage- and phosphorylation-dependent manner. Depending on light conditions, thylakoid membranes undergo dynamic structural changes that involve alterations in granum diameter and height, vertical unstacking of grana, and swelling of the thylakoid lumen. This plasticity is realized predominantly by reorganization of the supramolecular structure of protein complexes within grana stacks and by changes in multiprotein complex composition between appressed and non-appressed membrane domains. Reversible phosphorylation of LHC proteins (LHCPs) and PSII components appears to initiate most of the underlying regulatory mechanisms. An update on the roles of lipids, proteins, and protein complexes, as well as possible trafficking mechanisms, during thylakoid biogenesis and the de-etiolation process complements this review.

Key words: Bifurcation, CURT1, electron microscopy, grana, lateral heterogeneity, margins, phosphorylation, steric hindrance, STN7; STN8, thylakoid, VIPP1.

Introduction

Thylakoids are the internal membranes of chloroplasts and cyanobacteria, and provide the platform for the light reactions of photosynthesis. Chloroplasts of land plants contain grana, characteristic cylindrical stacks of membrane discs with a typical diameter of 400 nm that comprise ~5–20 layers of thylakoid membrane (Mustárdy and Garab, 2003; Mullineaux, 2005). A single granum consists of a central core of appressed membranes, capped by stroma-exposed ‘grana end membranes’ at the top and bottom, and the tightly curved margins that form the periphery of each discoid sac (Albertsson, 2001). Grana stacks are interconnected by stroma-exposed membrane pairs of up to few micrometres in length, the so-called ‘stroma lamellae’. All thylakoid membranes within a chloroplast form a continuous network that encloses a single lumenal space (Shimoni et al., 2005).

Although many biological textbooks include illustrations of the unique and intriguing architecture of thylakoids in land plants, relatively little is known about how it is generated, maintained, and modified. This review summarizes the present state of research on the structure, lateral heterogeneity, dynamics, and biogenesis of thylakoids from land plants and highlights new developments in these fields.
Architecture of thylakoids in land plants

A structural hallmark of thylakoid membranes in plants is their stacking to form the so-called grana thylakoids, which are interconnected by an unstacked but continuous network of stroma lamellae. Grana cylinders are made up of stacks of flat grana membrane discs with a diameter of ~300–600 nm, which are enwrapped in the stroma lamellae. In dark-adapted grana from Arabidopsis thaliana, membrane bilayers are (on average) 4.0 nm thick, lumen thickness is 4.7 nm, and discs are separated by a 3.6 nm gap (Kirkhoff et al., 2011).

The exact three-dimensional architecture of grana is still under debate, and two quite different interpretations of the wealth of electron microscopy data obtained during the past several decades have been proposed: the ‘helical model’ and various ‘fork/bifurcation models’ (reviewed in Daum and Kühlbrandt, 2011; Kirkhoff, 2013a). In the helical model (Paolillo, 1970), thylakoids comprise a fretwork of stroma lamellae, which wind around grana stacks as a right-handed helix, connecting individual grana discs via narrow membrane protrusions (Fig. 1). In its latest form (Mustárdy and Garab, 2003; Mustárdy et al., 2008), the model suggests a bipartite structure consisting of a cylindrical grana body, made of discs piled on top of each other, around which the stroma lamellae are wound as right-handed helices. The grana are connected to each other solely via the stroma lamella helices, which are tilted at an angle of between 10° and 25° with respect to the grana stacks (Mustárdy et al., 2008; Daum et al., 2010; Austin and Staehelin, 2011) and make multiple contacts with successive layers in the grana through slits located in the rims of the stacked discs.

The most significant difference between this helical model and competing alternative models is that the latter postulate that the grana themselves are formed by bifurcations of stroma lamellae. Thus Arvidsson and Sundby (1999) suggested that a grana is composed of piles of repeat units, each containing three grana discs, which are formed by symmetrical invaginations of a thylakoid pair caused by bifurcation of the thylakoid membrane (Fig. 1). With this model it is easier to account for the unstacking and restacking of grana under changing light conditions. More recently, Shimoni et al. (2005) presented another model, in which grana discs are paired units formed by simple bifurcation of stroma thylakoids (Fig. 1). Here, the grana–stroma assembly is formed by bifurcations of the stroma lamellar membranes into multiple parallel discs. The stromal membranes form wide, slightly undulating, lamellar sheets that intersect the grana body roughly perpendicular to the long axis of the grana cylinder. Instead of winding around the grana and fusing to form multiple grana layers at various levels (as in the helical model), each stroma lamellar sheet enters and exits the grana body in approximately the same plane (Shimoni et al., 2005; Brumfeld et al., 2008). Adjacent grana layers are joined not only through the stroma lamellae (as proposed by the helical model), but also via the bifurcations and through direct membrane bridges. The latter are formed by bending of the grana discs, leading to fusion with their neighbours at the edges (Fig. 1). This model has also been used to explain the rearrangements in thylakoids during state transitions (Chuartzman et al., 2008).

The mutual incompatibility of the helical and bifurcation models has led to much debate (Brumfeld et al., 2008; Mustárdy et al., 2008; Austin and Staehelin, 2011; Daum and Kühlbrandt, 2011; Nevo et al., 2012). However, recent tomographic data presented by Daum et al. (2010) and Austin and Staehelin (2011) clearly support the helical model, prompting Daum and Kühlbrandt (2011) to conclude: ‘Taken together, the tomographic data presented by Daum et al. (2010), Austin and Staehelin (2011), and here show that the helix model of thylakoid membrane architecture in chloroplasts is correct. Other models do not describe this architecture correctly and can now be safely discarded.’
Nevertheless, the controversy persists, as Nevo et al. (2012) point out that “…forks or bifurcations were observed and noted in the works of Daum et al. (2010), as well as by Austin and Staehelin (2011; here, termed ‘branches’ or ‘junctonal connections’).”

**Characteristics of lateral heterogeneity**

The various photosynthetic complexes differ not only in abundance (Table 1) but also in their distribution within the photosynthetic membranes. The term ‘lateral heterogeneity’ refers to the observation that stroma lamellae and grana differ in their protein composition (Andersson and Anderson, 1980; Albertsson, 2001; Dekker and Boekema, 2005; Nevo et al., 2012; Kirchhoff, 2013a), with photosystem II (PSII) and light-harvesting complex II (LHCII) being concentrated in the grana—where they can form super- and megacomplexes and semi-crystalline arrays—while photosystem I (PSI) with its light-harvesting complex (LHC1) and the chloroplast ATP synthase (cpATPase) are localized in the unstacked thylakoid regions; that is, the stroma lamellae and grana end membranes (Fig. 2). The allocation of the cytochrome b_{6}f complex (Cyt b_{6}f) between grana and stroma lamellae is less clear, but it is generally assumed that the complex can be found in both appressed and non-appressed regions of thylakoids (reviewed in Dekker and Boekema, 2005; Nevo et al., 2012). The thylakoid complexes involved in cyclic electron flow, the NDH complex and the PGRL1–PGR5 heterodimer, are less abundant than these four major thylakoid heterodimers (see Table 1) and are located in the stroma lamellae (Lennon et al., 2003; Rumeau et al., 2007; Hertle et al., 2013) where they can functionally interact with PSI as electron donor. The bulk of the NDH complex protrudes into the stroma, which precludes its location in grana, but PGRL1 homodimers have been detected in grana (Hertle et al., 2013) (Fig. 2).

Although several of the major thylakoid multiprotein complexes have been detected in margin-enriched fractions of thylakoids, these signals most probably reflect contamination by regions adjacent to grana margins (Dekker and Boekema, 2005). The marked curvature of thylakoid membranes at the grana margins would be expected to exclude larger multiprotein complexes and, indeed, grana margins have been thought to be essentially protein free (reviewed in Dekker and Boekema, 2005). However, following the recent demonstration, by immunogold labelling, that the CURT1 proteins—small polypeptides with two transmembrane regions, a tentative N-terminal amphipathic helix, and a relatively low mol. wt (11.0–15.7 kDa)—are localized to grana margins (Armbruster et al., 2013), this view must be revised. In this instance, envelope-free chloroplasts of wild-type plants and plants containing tagged CURT1A variants were first immunodecorated with specific antibodies and then treated with the appropriate gold-labelled secondary antibodies, which were subsequently visualized by scanning electron microscopy. Because this approach directly localizes proteins without fractionation or extensive fixation steps, it is ideally suited for the detection of proteins located in defined subcompartments within stroma-exposed areas of thylakoids (like margins). Interestingly, the CURT1 proteins appear to control the level of grana stacking, which points to an unsuspected role for grana margins in regulating the extent of thylakoid membranes in the appressed regions present in grana (see below).

**Table 1. Summary of the total and relative abundances of photosynthetic complexes**

In the estimate of the abundance of NDH based on quantification of PsaA (which detects PSI–NDH supercomplexes), the value reported in the literature was halved, because it is likely that each NDH complex is associated with two PSI complexes (Peng et al., 2008). The value for CURT1 was obtained by summing the values reported for CURT1A, B, and C (Armbruster et al., 2013). To calculate the abundance of protein complexes relative to PSI, values obtained from Kirchhoff et al. (2002) are expressed with respect to the value for PSI obtained in that study, whereas values from Hertle et al. (2013) and Armbruster et al. (2013) were related to the value for PSI obtained in Armbruster et al. (2013). For quantitation of the NDH complex based on measurements of NdhI and PsaA, the relative values (with respect to PSI) calculated in the corresponding publications were used.

<table>
<thead>
<tr>
<th>Component</th>
<th>Concentration (mmol/mol of Chl)</th>
<th>Method</th>
<th>% of PSI</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>PSII</td>
<td>2.99±0.22</td>
<td>Absorbance changes</td>
<td>133</td>
<td>Kirchhoff et al. (2002)</td>
</tr>
<tr>
<td>LHCII</td>
<td>33.68±0.76</td>
<td>Coomassie staining</td>
<td>1497</td>
<td>Kirchhoff et al. (2002)</td>
</tr>
<tr>
<td>PSI</td>
<td>2.25±0.17</td>
<td>Absorbance changes</td>
<td>100</td>
<td>Kirchhoff et al. (2002)</td>
</tr>
<tr>
<td></td>
<td>2.16±0.24</td>
<td>PsaD immunoblot</td>
<td>100</td>
<td>Armbruster et al. (2013)</td>
</tr>
<tr>
<td>Cyt b_{6}f</td>
<td>1.29±0.05</td>
<td>Absorbance changes</td>
<td>57</td>
<td>Kirchhoff et al. (2002)</td>
</tr>
<tr>
<td></td>
<td>1.35±0.005</td>
<td>PetD immunoblot</td>
<td>63</td>
<td>Hertle et al. (2013)</td>
</tr>
<tr>
<td>cpATPase</td>
<td>0.95±0.06</td>
<td>Coomassie staining</td>
<td>42</td>
<td>Kirchhoff et al. (2002)</td>
</tr>
<tr>
<td>NDH</td>
<td>0.09±0.005</td>
<td>NdhD immunoblot</td>
<td>4</td>
<td>Hertle et al. (2013)</td>
</tr>
<tr>
<td></td>
<td>0.09±0.005</td>
<td>NdhI immunoblot</td>
<td>1–2</td>
<td>Burrows et al. (1998)</td>
</tr>
<tr>
<td></td>
<td>0.25–2.5</td>
<td>PsaA immunoblot</td>
<td></td>
<td>Peng et al. (2008)</td>
</tr>
<tr>
<td></td>
<td>32</td>
<td>Immunoblot</td>
<td></td>
<td>Hertle et al. (2013)</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>Immunoblot</td>
<td></td>
<td>Hertle et al. (2013)</td>
</tr>
<tr>
<td></td>
<td>19</td>
<td>Immunoblot</td>
<td></td>
<td>Armbruster et al. (2013)</td>
</tr>
</tbody>
</table>
Mechanisms leading to membrane stacking and lateral heterogeneity

The physicochemical forces that control membrane stacking and the lateral segregation of protein complexes are not completely understood. Because of the extremely high density of photosynthetic protein complexes in the thylakoid membranes, membrane appression and lateral segregation of the photosynthetic protein complexes are thought to be intimately linked (Nevo et al., 2012). The unequal distribution of PSI and the cpATPase can be explained in a straightforward way as the bulk of their mass protrudes from the membrane. Therefore, steric hindrance excludes both complexes from grana stacks. Conversely, both LHCII and PSII have relatively flat stromal surfaces that allow them to be accommodated in tightly stacked grana thylakoids, where the distance between neighbouring membranes is only a few nanometres (reviewed in Kirchhoff, 2013). Monomeric PSII complexes that are undergoing repair can be also found in the stroma lamellae (Baena-González and Aro, 2002). NDH and PGR1–PGR5 heterodimers can form complexes with PSI in stroma lamellae (DalCorso et al., 2008; Peng et al., 2008). Note that, in general terms, the protein composition of the end membranes is assumed to be similar to that of stroma lamellae. Tomography and immunogold labelling experiments have confirmed that the cpATPase and Cyt b/f complexes, respectively, are located in end membranes (Daum et al., 2010; Armbruster et al., 2013). Moreover, PGR1 homodimers are enriched in the grana and might represent a pool of physiologically inactive PGR1 molecules that can be recruited under certain conditions (for instance high light) (Hertle et al., 2013). Note that the relative levels of the different multiprotein complexes depicted in the figure do not reproduce the values given in Table 1.

Why and how are thylakoid membranes attracted to each other such that they form grana? Most research on this topic has been guided by the maxim: ‘If LHCII is the major protein component of grana, then grana formation must be mediated by LHCII.’ Indeed, that LHCII polypeptides mediate membrane appression in vitro and thylakoid stacking in vivo was proposed 30 years ago (Day et al., 1984). Subsequently, based on an analysis of the charge distribution on LHCII trimers exposed on the stromal surface, it was suggested that nonspecific interaction of positively charged N-terminal peptides on one membrane with a negatively charged surface patch on trimers protruding from the opposite membrane is likely to play a major role in the cohesion of thylakoid grana (Standfuss et al., 2005). Moreover, constitutive expression of pea Lhcb1 in transgenic tobacco plants indeed leads to increased grana stacking (Labate et al., 2004), and overexpression of certain LhcbM proteins in Chlamydomonas is associated with more tightly appressed membrane regions (Mussgnug et al., 2005), indicating that increased concentrations of LHCII result in

more stacking. This ‘velcro-like’, non-specific interaction of LHCII trimers in apposed thylakoid membranes (Standfuss et al., 2005) would also explain why phosphorylation of LHCII, which effectively neutralizes one positive charge at the N-terminus, weakens this interaction, making it easier for LHCII to relocate towards PSI during state transitions (reviewed in Pesaresi et al., 2011). By analogy with this, it has been suggested that the deficit in PSII phosphorylation in the thylakoid kinase mutant stn8 increases the diameter of grana discs by reducing membrane repulsion due to negatively charged phosphate groups, thus enhancing cohesion between thylakoid membranes of adjacent discs (Fristedt et al., 2009).

However, several observations clearly indicate that the notion that LHCII alone mediates the cohesion of thylakoid membranes in the grana is too simplistic. (i) Arabidopsis mutants that are virtually devoid of LHCII trimers show no defects in grana formation, and their thylakoid architecture is essentially normal (Andersson et al., 2003). In fact, in these LHCII-less plants, the role of LHCII is taken over by a normally minor and monomeric complex, CP26, which is synthesized in large amounts and organized into trimers (Ruban et al., 2003). This at least permits the conclusion that grana stacking does not strictly require LHCII. Instead, it seems likely that CP26 can, in principle, substitute for the putative function of LHCII in grana stacking. (ii) Mutants that lack PSII but still accumulate LHCII, such as hcf136, show markedly enlarged grana that extend almost throughout the chloroplast and display reduced spacing (Meurer et al., 1998), indicating that grana formation is not strictly dependent on the formation of PSII–LHCII aggregates either. However, under variable irradiances, dynamic changes in the stacking of wild-type thylakoid membranes depend on the reversible reorganization of LHCII–PSII supracomplexes and therefore the LHCII/PSII ratio present in grana stacks (Anderson et al., 2012). (iii) More recently, data were presented which suggest that factors located in the grana margins can regulate the dimensions of grana stacks. Thus the level of CURT1 proteins localized to margins appears to control the dimensions of grana stacks without changing the amounts of LHCII and PSII accumulated, such that plants without CURT1 proteins display grana with a significantly increased diameter but far fewer layers of membrane, which form ‘pseudogranal’ without margins (Armbruster et al., 2013) (Fig. 3). This phenotype resembles that of stn8 thylakoids without PSII core phosphorylation (Fristedt et al., 2009) but is much more pronounced, and it is associated with an increase in thylakoid phosphorylation (Armbruster et al., 2013) rather than the decrease that would be expected if PSII core phosphorylation did indeed control the diameter of grana discs as suggested by Fristedt et al. (2009). Therefore, the altered interaction between thylakoid membranes seen in stn8 mutants is unlikely to be due to altered PSII core phosphorylation but rather to altered activity (or oligomerization) of CURT1 proteins, which appear to be reversibly phosphorylatable (Armbruster et al., 2013). But is the total area of appressed thylakoid membrane in a

**Fig. 3.** Effects of CURT1 levels on granum dimensions. Values for granum dimensions of the presented true to scale grana models are derived from Armbruster et al. (2013) and are listed in Table 2. Additional scanning and transmission electron micrographs (SEM/TEM) of the chloroplast thylakoid membrane network of curt1abcd, wild-type, and oeCURT1 plants [left, SEM topview, right, TEM cross-section; adapted from Armbruster U, Labs M, Pribil M, Viola S, Xu W, Scharfenberg M, Hertle AP, Rojahn U, Jensen PE, Rappaport F, Joliot P, Dörmann P, Wanner G, Leister D. 2013. Arabidopsis CURVATURE THYLAKOID1 proteins modify thylakoid architecture by inducing membrane curvature. The Plant Cell 25, 2661–2678, www.plantcell.org] demonstrate the effects of CURT1 levels on grana topology. In curt1abcd thylakoids, margins are absent and pseudogranal are formed.
pseudogranum in the CURT1-less mutant indeed greater than that in a granum—after all, the increase in disc size is accompanied by a decrease in the number of discs found in a single pseudogranum? The measurements made by Armbruster et al. (2013) give an unequivocal answer: the area of thylakoid membrane contained in a pseudogranum is markedly increased in thylakoids of plants without CURT1 proteins compared with wild-type plants (Fig. 3, Table 2). Therefore, in a single pseudogranum of a curt1abcd thylakoid, the area of appressed thylakoid membranes is increased, although the total abundance of PSII and LHCII is not altered. Interestingly, the number of grana per chloroplast is altered both in plants without CURT1 and in CURT1A overexpressors (Table 2). Therefore, not only the total extent of margins (estimated on the basis of the total peripheries of discs present in one chloroplast), but also the number of grana stacks, correlates with the amount of CURT1 proteins (Table 2). It is therefore tempting to conclude that these two features are coupled. Moreover, at the level of the chloroplast, not only curt1abcd but also CURT1A-overexpressing plants display an increase in thylakoid membrane area located in the (pseudo)grana regions when compared with the wild type. This allows one to conclude that, in addition to proteins in the granum core itself, margin-related factors might exert (indirect) effects on the interaction of thylakoid membranes, thereby shaping the architecture of grana.

Physiological function(s) of grana

The primary purpose of grana as such is unclear, and suggested functions include the prevention of spillover of excitation energy through physical separation of photosystems, fine-tuning of photosynthesis, facilitation of state transitions, switching between linear and cyclic electron flow, and, in particular, enhancement of light harvesting under low light conditions through the formation of large arrays of PSII–LHCII supercomplexes (Trisil and Wilhelm, 1993; Horton, 1999; Mustárdy and Garab, 2003; Dekker and Boekema, 2005; Mullineaux, 2005; Anderson et al., 2008; Daum and Kühlbrandt, 2011; Nevo et al., 2012). However, grana formation also imposes constraints on photosynthesis, such as the requirement for long-range diffusion of electron carriers between PSII and PSI (Mullineaux, 2008; Kirchhoff et al., 2011) and the relocation of PSI between appressed and non-appressed regions during the PSII repair cycle (Mulo et al., 2008). This complex picture of ‘advantages’ and ‘disadvantages’ of grana formation led Nevo et al. (2012) to conclude that ‘it is quite impossible (and, perhaps, unnecessary) to determine which of the aforementioned potential benefits had been the primary driving force behind grana formation. Along the course of evolution, various functions were gradually gained, concomitant with the acquisition of more refined control mechanisms.’

The availability of the curt1abcd lines with a primary defect in grana architecture—formation of ‘pseudogranum’ (without margin regions) made up of fewer thylakoid discs with enlarged diameters, as well as of grana consisting of more discs with reduced diameters (in CURT1A overexpressors) (Armbruster et al., 2013) (see Fig. 3)—allows one to address the impact of grana on specific photosynthetic functions. Because thylakoids that lack grana stacks represent the primordial type and are ubiquitous in cyanobacteria, it is not surprising that curt1abcd plants are still capable of performing photosynthesis, although they are devoid of canonical grana with margin regions (Armbruster et al., 2013). However, the complex perturbations in the process found in curt1abcd plants suggest that the dramatic increase in the area of thylakoid membrane present in the appressed regions of CURT1-less plants (see Fig. 3) does indeed compromise photosynthesis.

Table 2. Granum dimensions in lines with different CURT1 levels

The term (pseudo)granum refers to the appressed regions in curt1abcd plants which lack margin regions (see Fig. 3). Values for the dimensions of grana from curt1abcd, CURT1A overexpressors (oeCURT1A), and wild-type plants are from Armbruster et al. (2013). The number of discs per (pseudo)granum was calculated according to Kirchhoff et al. (2011). The thylakoid membrane area was calculated according to the equation \( A = \pi x d/4 \) (with \( A \)—circular area and \( d \)—diameter). With respect to the total extent of margins, the total periphery of the discs contained in a granum can be taken as the measure. To calculate the total amount of thylakoid membranes present in a single chloroplast, we measured the number of (pseudo)grana stacks in 6–8 chloroplasts for each genotype, and then calculated membrane areas and peripheries per chloroplast.

<table>
<thead>
<tr>
<th>Granum</th>
<th>curt1abcd</th>
<th>Wild type</th>
<th>CURT1A overexpressor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diameter (µm)</td>
<td>1.656 ± 0.075</td>
<td>0.448 ± 0.016</td>
<td>0.374 ± 0.006</td>
</tr>
<tr>
<td>Height (µm)</td>
<td>0.056 ± 0.002</td>
<td>0.113 ± 0.005</td>
<td>0.173 ± 0.006</td>
</tr>
<tr>
<td>No. of layers in (pseudo)granum</td>
<td>3.4</td>
<td>6.9</td>
<td>10.6</td>
</tr>
<tr>
<td>Membrane area including end membranes (µm²)</td>
<td>14.8</td>
<td>2.2</td>
<td>2.3</td>
</tr>
<tr>
<td>Membrane area without end membranes (µm²)</td>
<td>10.5</td>
<td>1.9</td>
<td>2.1</td>
</tr>
<tr>
<td>Total disc peripheries (µm)</td>
<td>–</td>
<td>9.8</td>
<td>12.5</td>
</tr>
<tr>
<td>Chloroplast</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of (pseudo)grana</td>
<td>13.3 ± 3.3</td>
<td>34.6 ± 8.9</td>
<td>61.1 ± 14.8</td>
</tr>
<tr>
<td>Membrane area including end membranes (µm²)</td>
<td>196.8</td>
<td>75.6</td>
<td>142.5</td>
</tr>
<tr>
<td>Membrane area without end membranes (µm²)</td>
<td>139.5</td>
<td>64.7</td>
<td>129.1</td>
</tr>
<tr>
<td>Total disc peripheries (µm)</td>
<td>–</td>
<td>337.6</td>
<td>761.9</td>
</tr>
</tbody>
</table>
is compatible with the idea that important features of photosynthesis in land plants are indeed dependent on, or at least facilitated by, the lateral heterogeneity provided by grana. One possibility is that, in CURT1-less mutants, the dearth of intermediate regions between appressed regions and stroma lamellae at which processes such as state transitions, PSII repair, and cyclic electron flow normally take place could account for the subtle alterations observed by Armbruster et al. (2013). Moreover, because of the increased diameter of pseudogranules in the curt1abcd mutants, long-range diffusion of electron carriers between PSII and PSI might become limiting, and this could explain the observed drop in linear electron flow.

**The plasticity of thylakoid ultrastructure**

Variations in the spectral composition and intensity of light, and the duration of light exposure, can affect the integrity and efficiency of the photosynthetic complexes in thylakoids. Thus land plants have evolved a variety of adaptations that optimize the excitation of their photosystems and protect them from light-induced damage. These range from developmental adjustments of plant growth [as in the case of shade avoidance reactions (Franklin, 2008)] and positioning of leaves at an appropriate angle with respect to incident solar radiation (Inoue et al., 2008), to intracellular responses leading to the redistribution of chloroplasts within the cytosol. This last process is triggered by blue light and causes chloroplasts to accumulate in a plane either perpendicular or parallel to the direction of incident light, depending on whether light harvesting needs to be increased or decreased (Wada et al., 2003). At the level of chloroplast ultrastructure, the thylakoid membrane turns out to be a highly flexible system, which can respond quickly to changes in ambient light conditions.

As described above, electron microscopy-based studies have led to a variety of 3-D models for grana (see Fig. 1). However, these models represent snapshots and do not capture the dynamics of the thylakoid system. Exposure of this apparently static membrane structure to different light conditions reveals that it is in fact highly malleable (Fig. 4). Thus, to maximize use of low light levels, the numbers of layers in grana stacks are increased (Anderson, 1986), while high light intensities lead to significant reduction in the diameter, and to partial transversal unstacking, of grana discs (Fristedt et al., 2009; Khatoon et al., 2009; Herbstová et al., 2012). The reduction in grana diameter and modification of the stacked structure under photo-damaging light conditions is thought to facilitate PSII repair in several ways. Damaged PSII complexes, previously embedded within the grana stack, become more accessible to auxiliary proteins, such as FtsH (Nixon et al., 2005), involved in D1 repair, which are otherwise excluded from the grana stacks due to steric hindrance (Kirchhoff, 2013b). Furthermore, lateral shrinkage reduces the time required for damaged D1 to diffuse from the grana core to the margins. In addition, an increase in protein mobility upon exposure to bright light enhances diffusion and turnover of D1 (Herbstová et al., 2012; Kirchhoff, 2014) (Fig. 5A). The partial destacking of grana should further ease migration of LHClI from grana-located PSII to PSI in unstacked thylakoid membranes, a process that is essential for state transitions. Furthermore, swelling of the thylakoid lumen is observed upon dark to light transition, which should facilitate the diffusion of luminal photosynthetic electron carriers such as plastocyanin (Kirchhoff et al., 2011) or of components involved in the degradation of damaged PSII components (such as Deg proteases) (Kirchhoff, 2013b) (Figs 4, 5A). This osmotic expansion in the light is thought to result from an influx of Cl− and Ca2+ ions (Ettinger et al., 1999; Speota and Schoefs, 2010). While some of these structural rearrangements seem to promote molecular photo-protective mechanisms such as the turnover of photodamaged D1 (Herbstová et al., 2012; Kirchhoff, 2013b), other structural changes seem to occur as a consequence of the redistribution of protein components within the thylakoid membrane driven by adaptation mechanisms, including state transitions and other processes, involving reversible protein phosphorylation.

In the following, we turn to the regulatory mechanisms, acting at the molecular and supramolecular levels and operating predominantly in a reversible protein

**Fig. 4.** Schematic depiction of the effects of changes in light conditions on thylakoid membrane organization (adapted from Kirchhoff H, 2013a. Architectural switches in plant thylakoid membranes. Photosynthesis Research 116, 481–487, with kind permission from Springer Science and Business Media). While the switch from dark to light generally induces osmotic swelling of the thylakoid lumen (D→LL/HL), low light specifically promotes an increase in the number of layers per granum (D→L). Structural changes upon exposure to high light levels involve a decrease in grana diameter as well as vertical unstacking of adjacent membrane layers within the granum (D/LL→HL). D, dark; LL, low light; HL, high light.
phosphorylation-dependent manner, which account for the plasticity of the thylakoid ultrastructure.

Alterations in protein complex composition and supramolecular organization

The protein composition of thylakoids (see Fig. 2) can be modulated by alterations in the relative levels of resident complexes, or the stoichiometry of their subunits (Anderson, 1986). For instance, exposure to low light leads to an increase in the ratio of LHCCI to PSII and Cyt b6f (Anderson, 1986; Staehelin, 1986; Kirchhoff et al., 2007). Such changes in protein stoichiometry represent long-term acclimation processes, which require tight coordination of gene expression between the chloroplast and the nucleus. However, the nature of the retrograde signalling pathways that control such responses is still hotly debated (Kleine et al., 2009; Pfannschmidt, 2010). Besides these alterations in protein stoichiometry, differential localization of protein complexes within the thylakoid membrane also exerts a direct influence on its ultrastructure. In particular, the assembly of PSII and LHCCI into super- and megacomplexes (Dekker and Boekema, 2005; Kouřil et al., 2008).
2012) significantly contributes to the formation of grana stacks. Under unfavourable conditions such as low temperature or low light (Garber and Steponkus, 1976; Kirchhoff et al., 2007), these higher order PSII–LHCII complexes can form semi-crystalline arrays that may occasionally span the entire grana disc (Staehelin, 1986; Dekker and Boekema, 2005; Goral et al., 2012). Such PSII macro-structures seem to be susceptible to even minor structural perturbations, as in the case of a lack of CP29 or increased levels of PsbS (Goral et al., 2012). Thus, while the presence of CP29 seems to be essential for formation of PSII–LHCII supercomplexes (Koufil et al., 2012), PsbS appears to suppress the assembly of large PSII arrays (Goral et al., 2012). The latter observation is in line with the idea that non-photochemical quenching (NPQ) involves partial disassembly of PSII–LHCII supercomplexes to allow LHCII aggregation (Betterle et al., 2009; Johnson et al., 2011).

**PSII protein phosphorylation and D1 turnover**

Thylakoid phosphorylation is mainly mediated by the two protein kinases STN7 and STN8 (Bellaﬁore et al., 2005; Bonardi et al., 2005; Vainonen et al., 2005). While STN7 primarily catalyses LHCII phosphorylation (see the following section), STN8 is predominantly responsible for the phosphorylation of PSII core proteins (CP43, D1, D2, and PsbH), especially under high light conditions (Bonardi et al., 2005; Vainonen et al., 2005; Tikkanen et al., 2010). Recent studies on stn7, stn8 and stn7 stn8 double mutants have revealed that phosphorylation of PSII core proteins plays a role in the PSII repair cycle during photoinhibition by facilitating migration of damaged PSII reaction-centre proteins from grana to stroma lamellae, where they undergo degradation (Tikkanen et al., 2008). The PSII repair cycle involves a sequence of steps that includes (de-)phosphorylation of PSII subunits, disassembly and reconstruction of super- and holocomplexes, and degradation/ de novo synthesis of D1 (Aro et al., 1993; Kato and Sakamoto, 2009; Pesaresi et al., 2011). Note, however, that protein phosphorylation by STN7 and STN8 is not essential for the PSII repair cycle (Bonardi et al., 2005), but alters its dynamics, such that disassembly of PSII supercomplexes is impaired in the absence of PSII phosphorylation (Tikkanen et al., 2008). Therefore, it was suggested that STN8-dependent phosphorylation of PSII core proteins modulates thylakoid ultrastructure by enhancing the lateral mobility of thylakoid membrane protein complexes and thus promoting PSII repair (Fristedt et al., 2009; Goral et al., 2010) (Fig. 5A). Indeed, no such increase in mobility is observed in isolated grana membranes, or in stn8 and stn7 stn8 mutants lacking thylakoid phosphorylation (Goral et al., 2010). Fristedt et al. (2009) proposed that the observed increase in the diameter and density of grana stacks in the stn8 and stn7 stn8 mutants reduces lateral diffusion of proteins, including that of photodamaged D1 and of the bulky FtsH complex, which is essential for its degradation (Nixon et al., 2005) and was reported to be spatially separated from PSII in STN8-deﬁcient mutants due to steric hindrance (Fig. 5A). In agreement with this, A. thaliana plants overexpressing STN8 were less sensitive to intense light and exhibited alterations in thylakoid ultrastructure, with grana stacks containing more layers and reduced amounts of PSII supercomplexes (Wunder et al., 2013). An additional architectural adaptation that occurs upon exposure to high light levels is a decrease (of ~20%) in grana diameter. Because no such structural alterations were observed in the stn8 and stn7 stn8 mutants (Herbstová et al., 2012), this decrease can be attributed to the effect of PSII core phosphorylation (Fig. 5A).

**LHCII phosphorylation and state transitions**

State transitions in plants serve to adjust the absorption properties of PSII and PSI at low light intensities so as to optimize utilization of the light available (Allen and Forsberg, 2001). The state 1–2 transition involves the phosphorylation-dependent detachment of LHCII from PSII (in the grana) and its diffusion to PSI (in the stroma lamellae). This reaction is primarily catalysed by the LHCII kinase STN7 (Bellaﬁore et al., 2005; Bonardi et al., 2005), although there is some overlap in substrate specificity between STN7 and the PSII core kinase STN8 (Bonardi et al., 2005). Dephosphorylation of LHCII, which is required to allow LHCII to move back to PSII and thereby initiates the reverse (2–1) transition, is catalysed by the protein phosphatase TAP38/PPH1 (Pribil et al., 2010; Shapiguzov et al., 2010). Moreover, disassembly of the PSII–LHCII supercomplex appears to promote state transitions. Thus, destabilization of PSII supercomplexes, either genetically in the psb27 mutant or by light treatment, accelerates state transitions (Dietzel et al., 2011).

Because LHCII is thought to be the major stabilizer of appressed grana domains (Dekker and Boekema, 2005), the movement of LHCII between grana (PSII) and stroma (PSI) should alter stacking of the grana of higher plants. However, the extent of structural rearrangement that occurs within the thylakoid membrane upon state transitions is disputed. Treatment of thylakoids with dilute salt solutions, which is thought to mimic the state 1–2 transition, leads to extensive structural rearrangements including complete destacking of the appressed membrane regions (Izawa and Good, 1966; Murakami and Packer, 1971). Based on these observations, the movement of LHCII from grana to stroma lamellae was proposed to bring about similar effects (Ryrie, 1983; Arvidsson and Sundby, 1999). However, in vivo studies based on light-induced state transitions revealed only moderate differences in membrane stacking that remained restricted to grana margins and typically did not exceed 10–20% (Kyle et al., 1983; Drepper et al., 1993; Delosme et al., 1996; Rozak et al., 2002; Mustárdy and Garab, 2003; Shimoni et al., 2005). Therefore, assuming that LHCII phosphorylation occurs at the grana margins, it was proposed that LHCII migration between grana and stroma lamellae is associated with changes in the local lipid–protein composition, which in turn cause retraction of the membrane layers and destabilize the grana–stroma interface (Churatzman et al., 2008). Eventually a limited fraction of membrane bridges break and undergo structural rearrangements, such as retraction of neighbouring layers along the granum axis or rotational movements, that cause the layers to be displaced from the granum core.
Biogenesis of thylakoids

The sophisticated thylakoid ultrastructure seen in mature chloroplasts is not observed in the proplastids found in dark-grown tissues, but its formation is rapidly initiated upon exposure to light. The process of thylakoid biogenesis requires the coordinated assembly of lipids, proteins, and chlorophylls, which together account for >98% of the mass of the thylakoid membrane (Murphy, 2008). Strikingly, lipids make up only ~30% of the membrane surface (Kirchhoff et al., 2002). Of these, >50% cannot normally form bilayers under the conditions prevailing in thylakoids (Webb and Green, 1991). Studies on mutant plants have been most helpful in elucidating the interdependence of component assembly during thylakoid biogenesis.

Lipid incorporation

The thylakoid membrane contains five major lipids. The non-bilayer-forming MGDG accounts for 52% of total lipids by weight (Kirchhoff et al., 2002). Due to its small headgroup, MGDG forms inverted hexagonal (HII) structures in solution at physical pH and temperature (Goss and Wilhelm, 2010). Digalactosyldiacylglycerol (DGDG) with 27%, sulfoquinovosyldiacylglycerol (SQDG) with 15%, phosphatidylglycerol (PG) with 3%, and phosphatidylcholine (PC) with 3% account for the rest (Webb and Green, 1991; Kirchhoff et al., 2002). Because the synthesis of all of these lipids is finalized in the chloroplast envelope, a mechanism for their continuous transport to thylakoids must exist (Jouhet et al., 2007; Benning, 2008). Whether this transfer of lipids occurs via (i) a vesicular pathway; (ii) soluble glycerolipid transfer proteins; or (iii) invaginations that directly connect the envelope to thylakoids is not clear (Fig. 6D) (Holthuis and Levine, 2005; Jouhet et al., 2007). A mutant with a defective
MGDG synthase 1 (mgd1) is unable to produce photosynthetically active membranes, but shows invaginations of the inner envelope (Kobayashi et al., 2013). However, the emergence in cold-incubated plants of vesicles (Morré et al., 1991) that resemble the COPII vesicles seen in the cytosol under cold conditions when the energy requirement for fusion with target membranes increases (Saraste et al., 1986; Morré et al., 1989) points to a role for vesicular traffic in the biogenesis and maintenance of thylakoids (Vothknecht and Westhoff, 2001).

Mutants without the ‘vesicle-inducing protein in plastids’ (VIPP1) lack the aforementioned cold-induced vesicles and are defective in thylakoid biogenesis (Kroll et al., 2001). Because it is found both at the inner envelope and at the thylakoids (Li et al., 1994), a role for VIPP1 in the formation of vesicles that transport lipids and hydrophobic carotenoids to the thylakoids has been proposed (Kroll et al., 2001). Mutants for the cyanobacterial homologue of VIPP1, PspA (bacterial phage shock protein A), also show a thylakoid-defective phenotype (Westphal et al., 2001), and a maintenance function for membrane integrity was suggested for it (Hankamer et al., 2004; Standar et al., 2008). Note that the central α-helical domain, conserved between PspA and Vipp1, is responsible for formation of an oligomeric ring structure (Aseeva et al., 2004), whereas the N-terminal α-helix mediates lipid binding and assembly of a high molecular weight complex (Otters et al., 2013). The dynamics of this complex are controlled by the HSP70B–CDJ2–CGE1 chaperones (Liu et al., 2005; Liu et al., 2007), but HSP90 may promote the disassembly of the multimer and, in its absence, only a few thylakoid membranes are formed (Feng et al., 2014). Other findings hint that VIPP1 might function like its bacterial homologue, namely acting to maintain the chloroplast envelope instead of inducing vesicles, thus having a protective rather than a driving effect on thylakoid biogenesis (Zhang et al., 2012). It has been speculated that thylakoid-associated VIPP1 has a similar function, but conclusive proof is still missing (Vothknecht et al., 2012; Zhang and Sakamoto, 2013). Moreover, VIPP1 was also found to enhance binding of substrates for the cpTat import pathway (Lo and Theg, 2012). Intriguingly, recent results point to a role for VIPP1 in the assembly of thylakoid core complexes (Nordhues et al., 2012). Based on these findings, Rütgers and Schroda (2013) have presented a model in which
VIPP1 fulfills a structural role within thylakoid centres, which are considered as sites from which thylakoid membranes emerge and at which the biogenesis of PSI is at least thought to occur. Furthermore, VIPP1 could create microdomains in the membrane that facilitate the accumulation of specific lipids that, in turn, aid in the function of translocases (Lo and Theg, 2012; Rütgers and Schröda, 2013).

VIPP1 apparently does not play a role in vesicular transport in mature chloroplasts, but several other proteins remain as candidates for such factors. Thus, a bioinformatics approach has identified chloroplast-located homologues of the COPII vesicular pathway between the endoplasmic reticulum and Golgi apparatus (Andersson and Sandelius, 2004). One essential component for assembly of the COPII coat is the GTPase Sar1, whose chloroplast-located homologue cpSar1 also shows GTPase activity in vitro and has been linked to thylakoid biogenesis (Garcia et al., 2010). Although a direct connection with vesicle coat assembly could not be demonstrated, the protein’s presence at the inner envelope and in the stroma is compatible with a function in vesicle initiation. Furthermore, cpSar1 has been detected around cold-induced vesicles (Garcia et al., 2010). While cpSar1 knock-out mutants show developmental arrest before greening, cpSar1 RNAi (RNA interference) lines show an interesting intermediate phenotype with respect to thylakoid biogenesis. In these lines, plastids contain vesicles of various sizes that eventually coalesce and form the typical mature grana stacks (Garcia et al., 2010).

The dynamin family member FZL is also localized at the envelope and thylakoids, and shows GTPase activity, but in FZL knock-out plants disruption of thylakoid ultrastructure is less severe (Gao et al., 2006) than in cpSar1 knock-outs. Although grana stacks are disorganized and vesicles accumulate, FZL is believed to play a more prominent role later in thylakoid development (Gao et al., 2006; Adam et al., 2011).

The THF1 (THylakoid Formation1) protein is also assumed to be involved in vesicular trafficking because in thf1 mutants white/yellow patches appear that completely lack grana stacks or any form of thylakoid membrane but accumulate membrane vesicles (Wang et al., 2004). THF1 is identical to Psb29, which is involved in PSI biogenesis (Keren et al., 2005). This finding is corroborated by the observation that thf1 mutants retain a PSI–LHCII supercomplex in the dark, which implies an important role for THF1/Psb29 in PSI dynamics (Huang et al., 2013). Therefore, it cannot be excluded that THF1/Psb29 might be involved in the fusion of PSI-loaded vesicles emerging from the envelope (Khan et al., 2013), although this seems to vary with the continuous influence of THF1 on leaf development including leaf senescence (Huang et al., 2013).

Given the complexity of COPII vesicular transport, a mechanism dedicated solely to the transport of lipids from the envelope to thylakoids is hard to imagine, especially since the non-bilayer-forming nature of MGDG would complicate such mechanisms. In this context, two observations are of interest: (i) the MGDG:DGDG ratio is three times lower in developing than in mature thylakoids (Andersson et al., 2001) and (ii) the existence of non-bilayer structures in thylakoids and their ability to exchange lipids with the bilayer phase (Krumova et al., 2008). Thus, the integration of high concentrations of MGDG into a lipid bilayer relies on the presence of membrane proteins. If the protein to MGDG ratio is lowered, MGDG cannot be kept within the bilayer, but migrates into non-bilayer structures. However, whether bilayer and non-bilayer phases can co-exist in vesicles too remains speculative. Alternatively, it was suggested that a vesicle pathway might also transport non-lipid components (Westphal et al., 2003; Benning, 2009). This idea is in line with the identification of the plasma membrane as the location of initial photosystem biogenesis in cyanobacteria (Zak et al., 2001), although this apparently does not hold for A. thaliana (Che et al., 2013). In an ongoing bioinformatics analysis, the search for components of the vesicular transport mechanism in plastids has been expanded to associated factors (Khan et al., 2013). In this study, chloroplast-targeted homologues of coat proteins, cargo receptors, tethering factors, and SNAREs were identified. Some 80% of the putative cargo proteins could be linked to functions in thylakoids such as biogenesis, stress responses, and photosynthesis (Khan et al., 2013).

Despite the lack of conclusive experimental proof, the evidence for a vesicular transport system within the chloroplast cannot be easily dismissed (Brandizzi, 2011).

The role of protein complexes in thylakoid biogenesis

The vast majority of the thylakoid surface is occupied by protein complexes, which account for >70% of the total thylakoid membrane area (Kirchhoff et al., 2002). Thus, it seems likely that thylakoid biogenesis is influenced by the insertion of protein complexes into the lipid bilayer matrix. Most of the thylakoid proteins are encoded in the nucleus and synthesized in the cytosol, and must be post-translationally imported into the chloroplast. The pathways mediating this transport, and its evolution and regulation, have been extensively reviewed (Gutensohn et al., 2006; Strittmatter et al., 2010; Shi and Theg, 2013). Recently, it was suggested that lumenal proteins are also essential for thylakoid biogenesis (Shipman-Roston et al., 2010; Järvi et al., 2013). Their proper maturation may be a key step in the assembly of thylakoids, as plants mutant for the processing peptidase PLSP1, which is involved in the maturation of lumenal proteins (such as OE33, OE23, and plastocyanin), have been shown to accumulate large amounts of vesicles in the stroma but fail to develop intact thylakoids in adult plants (Inoue et al., 2005; Shipman and Inoue, 2009; Shipman-Roston et al., 2010). Here, the critical step seems to be the removal of the thylakoid-transfer signal. Without its removal, certain newly imported proteins are not released from the thylakoid membrane (Frielingsdorf and Klösgen, 2007) and are subsequently degraded (Midorikawa and Inoue, 2013).

Generally, it is difficult to determine unambiguously the importance of integral membrane proteins for thylakoid biogenesis, since their absence results in significant perturbation of photosynthetic activity. In the following, defects in the assembly of the major thylakoid protein complexes will be reviewed in the context of their effects on thylakoid biogenesis.
Mutants without PSI are incapable of photoautotrophic growth. First identified in a series of high chlorophyll fluorescence (hcf) mutants (Meurer et al., 1996), hcf101 is depleted of PSI and showed an impaired thylakoid ultrastructure completely devoid of stroma lamellae (Stöckel and Oelmüller, 2004). HCF101 was found to be involved in the provision of Fe–S clusters required for PSI assembly (Lezhneva et al., 2004; Schwenkert et al., 2010). Interestingly, other mutants specifically lacking PSI form fragmentary stroma lamellae but still express near wild-type levels of the light-harvesting complexes. These include strains defective for the PSI assembly factor PPD1 (PsbP-domain protein1) (Liu et al., 2012), PSI-F, a subunit of PSI (Haldrup et al., 2000), and Pale yellow green7 (Pyg7) (Stöckel et al., 2006), as well as hcf101, hcf113, and hcf140 (Amann et al., 2004). Therefore, it can be concluded that the presence of PSI is essential for thylakoid biogenesis, more specifically the formation of the stroma lamellae.

The aforementioned PSII assembly mutant hcf136 forms enlarged and denser grana stacks, while light-harvesting complexes assemble normally (Meurer et al., 1998). In Low PSII Accumulation1 (LPA1) lines, which retain 20% of the wild-type PSII amount, grana stacks are shorter and thinner, but the overall effect on thylakoid ultrastructure is less severe (Peng et al., 2006). In the absence of AtCtpA, a protein required for maturation of the PSII reaction centre protein D1, no functional PSII complexes, and few grana stacks, could be assembled (Che et al., 2013). Conversely, overexpression of maize plastidial transglutaminase in tobacco increased the numbers of PSII centres in the appressed grana, leading to larger grana stacks and reduced stroma lamellae (Ioannidis et al., 2009). The lack of ATAB2 (Arabidopsis homologue of Chlamydomonas Tab2), which is presumably involved in the biogenesis of both photosystems, results in an intermediate thylakoid phenotype (Dauvillée et al., 2003; Barneche et al., 2006). In atab2 mutants, PSI complexes are absent, PSII is decreased 5-fold, while the Cyt b/f and ATPase complexes are expressed normally, leading to a significant decrease in stroma lamellae and a general decrease in thylakoid membrane content (Barneche et al., 2006).

Light-harvesting complex proteins (LHCPs)

Post-translational insertion of the LHCPs into thylakoids is mediated by the signal recognition particle (cpSRP) pathway (Schünemann, 2004). In the case of LHCPs, this works in close cooperation with FtsY and Albino3 (ALB3) (Tu et al., 1999; Moore et al., 2000; Woolhead et al., 2001), with ALB3 being responsible for cpSRP-dependent LHCP integration into the thylakoid membrane (Bals et al., 2010; Falk et al., 2010). The importance of ALB3, and hence of the LHCPs, for the biogenesis of thylakoid membranes is striking. In the alb3 mutant, a significant loss in thylakoid membrane and grana stacking is observed (Sundberg et al., 1997). As mentioned above, the impact on grana formation of a specific lack of LHCII trimers or alterations in their subunit composition is less severe.

The assembly of the LHCIIIs also relies on the incorporation of chlorophyll b (Horn et al., 2007). In a mutant devoid of chlorophyll b (chl-3), the concentration of LHCII was decreased, with no LHCII trimers detectable (Kim et al., 2009). This led to smaller chloroplasts and a 30% decrease in numbers of grana per chloroplast area. A cross of chl-3 and lhcb5 showed a further decrease in LHCII monomers and resulted in a loss of >60% grana area (Kim et al., 2009). This decrease in grana stacking due to reduced LHCII levels was attributed to a decline in van der Waals attraction, lower electrostatic interaction between opposite charges across the partitioning gap, and impaired formation of PSII–LHCII aggregates, which together appear to exert stronger negative effects on grana formation than the positive effects caused by the weaker electrostatic repulsion due to the lack of LHCII (Chow et al., 1991, 2005; Kim et al., 2009).

Others

The level and stability of the CF1,CFo ATP synthase is strongly reduced in the alb4 mutant (Benz et al., 2009), decreasing the degree of appression in grana stacks (Gerdes et al., 2006). Mutants affected in the assembly of the Cyt b/f complex (Lennartz et al., 2001; Dreyfuss et al., 2003; Maiwald et al., 2003; Xiao et al., 2012) have not yet been characterized with respect to thylakoid ultrastructure. Nevertheless, ultrastructural data are available for a mutant with markedly reduced levels of Cyt b/f (Manara et al., 2014). However, these lines show normal thylakoid formation, indicating that the Cyt b,f complex does not play a significant role in the establishment of the thylakoid ultrastructure (Manara et al., 2014).

Interdependency of protein and lipid supply

The composition of thylakoid membranes varies little between photosynthetically active organisms (Siegenthaler, 1998; Vigh et al., 2005). Lipid membranes serve as habitats for the proteins involved, playing important roles in their stability and functionality (Mizusawa and Wada, 2012; Boudière et al., 2014). Liposomes consisting of DGDG and MGDG are able to stabilize LHCII trimers, while the absence of DGDG slightly destabilizes the complex (Yang et al., 2006). Interestingly, increasing MGDG content in LHCII–PSII liposomes increases the antenna cross-section and boosts photosynthetic activity (Zhou et al., 2009). SQDG may be similarly involved in stabilizing PSI (Sugimoto et al., 2010).

Conversely, proteins can modulate the phase behaviour of MGDG. Thus, by increasing the amounts of LHClI, the inverted hexagonal phase can be progressively transformed into ordered lamellar structures (Simidjiev et al., 2000). It has been hypothesized that the amount of thylakoid-incorporated non-bilayer-forming lipid is controlled by the current state of the membrane (Garab et al., 2000), such that changes in protein content and distribution cause excess MGDG to be forced into a non-bilayer phase or be recruited from there (Garab et al., 2000). The existence of such a non-bilayer phase and its exchange with the membrane was shown by Krumova et al. (2008) (see above). Only the tight packing of proteins into the membrane is compatible with the high
concentration of MGDG, and vice versa. Consequently, the protein-rich appressed grana stacks were found to have a higher MGDG:DGDG ratio than stroma lamellae (Gounaris et al., 1983, 1986), in agreement with the idea that non-bilayer-forming lipids mediate stacking (Lec, 2000).

Acknowledgements

We thank Paul Hardy for critical reading of the manuscript. Carolina Galgenmüller is thanked for supporting statistical evaluations. This work has been supported by the DFG-grant LE 1265/21-1.

References


