CMLS, Cell. Mol. Life Sci. 60 (2003) 1591–1606
1420-682X/03/081591-16
DOI 10.1007/s00018-003-3173-x

CMLS Cellular and Molecular Life Sciences

Functional significance of the lipid-protein interface in photosynthetic membranes

T. Páli[a,*], G. Garab[b], L. I. Horváth[a] and Z. Kóta[a]

[a] Institute of Biophysics, Biological Research Centre, Temesvári körút 62, 6726 Szeged (Hungary),
Fax: + 36-62 433133, e-mail: tpali@nucleus.szbk.u-szeged.hu
[b] Institute of Plant Biology, Biological Research Centre, Temesvári körút 62, 6726 Szeged (Hungary)

Abstract. The functional significance of the lipid-protein interface in photosynthetic membranes, mainly in thylakoids, is reviewed with emphasis on membrane structure and dynamics. The lipid-protein interface is identified primarily by the restricted molecular dynamics of its lipids as compared with the dynamics in the bulk lipid phase of the membrane. In a broad sense, lipid-protein interfaces comprise solvation shell lipids that are weakly associated with the hydrophobic surface of transmembrane proteins but also include lipids that are strongly and specifically bound to membrane proteins or protein assemblies. The relation between protein-associated lipids and the overall fluidity of the thylakoid membrane is discussed. Spin label electron paramagnetic resonance spectroscopy has been identified as the technique of choice to characterize the protein solvation shell in its highly dynamic nature; biochemical and direct structural methods have revealed an increasing number of protein-bound lipids. The structural and functional roles of these protein-bound lipids are mustered, but in most cases they remain to be determined. As suggested by recent data, the interaction of the non-bilayer-forming lipid, monogalactosyldyacilglycerol (MGDG), with the main light-harvesting chlorophyll a/b-binding protein complexes of photosystem-II (LHCII), the most abundant lipid and membrane protein components on earth, play multiple structural and functional roles in developing and mature thylakoid membranes. A brief outlook to future directions concludes this review.

Key words. Greening; light-harvesting chlorophyll a/b-binding proteins; lipid-protein interaction; membrane dynamics; nonbilayer lipids; photosynthesis; thermally induced changes; thylakoid membranes.

Introduction

Life on earth depends on photosynthesis, the conversion of light energy to chemical energy. In higher plants, algae and cyanobacteria, photosynthesis takes place in the thylakoid membranes, the internal membranes of chloroplasts and cyanobacteria [1]. In chloroplasts, these membranes – a unique assembly of protein, pigment and lipid molecules – accommodate all light-harvesting and energy-transducing functions. In most higher plants, thylakoid membranes are differentiated into grana and stroma regions, also known as stacked and unstacked regions, respectively. Cylindrical stacks of about 10–20 layers with a diameter of 300–600 nm are interconnected by single thylakoid membranes of several hundred nanometres in length, which are helically wound around the granum (for recent review, see [2]). This fascinating complex organization reflects a compositional and functional differentiation between the two regions: photosystem II (PSII) and its main chlorophyll a/b light-harvesting complex, LHCII, are found predominantly in the stacked membranes; this region is largely deficient in PSI and LHCI, which are enriched in the stroma membranes [3]. Despite this remarkable differentiation and heterogeneity, the thylakoid membrane system is formed from one continuous membrane that is organized into a three-dimensional network. It also encloses one interior aqueous phase, the thylakoid lumen. The continuity of the membrane between the stacked and unstacked regions is warranted by interconnections, via slits on the side of the granum thylakoids. The fusion of two (or more) stroma membranes at their edges ensures continuity for the entire

---

* Corresponding author.

chloroplast. The continuum of the membrane system is important for the diffusion of mobile components between the two regions. The contiguity of inner and outer aqueous phases is essential for the formation of uniform ('delocalized') transmembrane electric field and the pH gradient, which are generated by primary charge separations in the reaction centre complexes and the vectorial electron and proton transport system. The reaction centres are supplied with energy by light-harvesting antenna complexes. In granal chloroplasts, LHCII accounts for about half of the protein and Chlorophyll (Chl) content of the thylakoid membranes, and thus it is the most abundant membrane protein in the biosphere. In addition to their primary, light- harvesting function, these proteins also act as structure proteins. In particular, they have been shown to stabilize the granum ultrastructure and to participate in cation-mediated stacking of membranes [4, 5], and are involved, via electrostatic and osmotic forces, in lateral organization of the complexes [6]. Furthermore, they also participate in multilevel regulatory mechanisms that protect plants against strong light and elevated temperatures [7, 8]. These regulatory processes require sizeable reorganization in the membrane, and thus – in addition to, and together with a structural stability – a substantial degree of structural flexibility [9].

Similar to other energy converting membranes, thylakoid membranes exhibit very high protein:lipid ratios [10]. This is probably required for the large number of cooperative interactions, and – as suggested recently – is most likely adjusted, with a self-regulatory mechanism, by nonbilayer lipids: the high nonbilayer propensity of the thylakoid lipid mixture prevents 'dilution' of membranes [11]. The high protein concentration of thylakoid membranes dictates a large degree of protein-protein contacts. The stoichiometric composition of spinach thylakoid membranes allowed the determination of mean molecular areas occupied by the main membrane constituents [12]. From these data, the lipid phase diffusion space, the fraction of lipids located in the first protein solvation shell (boundary lipids) and the plastoquinone concentration have been derived. The high boundary lipid fraction found in granum thylakoids (60%) suggests that protein complexes are to be found in large assemblies, which are spaced by lipids, in agreement with the macrodomain organization of the granum membranes [9, 13].

The lateral segregation of the two photosystems, 'sorting', has also been shown to be governed by the macrodomain organization of LHCII and LHCII-PSII [6, 9]. The formation of large, ordered arrays is evidently facilitated by the two-dimensional lipid matrix, which prevents unspecific aggregations. As shown in a theoretical study, lipid-mediated interactions, i.e. specific lipid-protein interactions may, in part, also be responsible for the nonuniform distribution of PSI and PSII in the thylakoid membrane [14]. In contrast to PSI- and PSII-associated proteins, glycerolipids appear to be evenly distributed within the plane of the thylakoid membrane, and show no indication of lateral heterogeneity [15].

In photosynthetic membranes, the major lipids are polar glycerolipids, such as glycolipids (galactolipids) and phospholipids [16]. In thylakoid membranes of green plants, monogalactosyldiacylglycerol (MGDG) accounts for ~50% of the total lipid content. The membranes also contain digalactosyldiacylglycerol (DGDG) (~30%), sulfoquinovosyldiacylglycerol (SQDG) (~5–12%) and phosphatidylglycerol (PG) (~5–12%) [10]. Because of its high concentration in the thylakoid membranes, MGDG is the most abundant polar lipid species in nature [17].

In all biomembranes there are lipids that, when isolated, prefer to assume nonbilayer structures such as the reversed hexagonal (H_{II}) phase [18–21]. MGDG, cardiolipin (CL, diphosphatidylglycerol) and phosphatidylethanolamine (PE) are common examples [22]. The structural and functional roles of these lipids are still somewhat enigmatic, and are of great interest [11, 23, 24], also because the energy-transducing functions of thylakoid membranes depend heavily on the bilayer properties. As will be outlined, nonbilayer lipids can play multiple structural-functional roles in thylakoid membranes.

Intrinsic membrane proteins are stabilized primarily by noncovalent, hydrophobic and electrostatic interactions with their neighbouring lipids. These lipids do not only passively host and solvate membrane proteins, but, being structurally exposed to the photosynthetic machinery, they most certainly play defined and specific roles beyond their basic function as a matrix in the bilayer membrane; this will be shown through several examples. Bound lipids have recently been identified in crystal structures of several photosynthetic membrane proteins. Specific lipid-protein interactions have also been determined with the aid of biochemical methods and genetic engineering techniques. These protein-associated lipids evidently influence the overall membrane fluidity. However, we point out that the exact determination of the parameters of the lipid-protein interface and membrane fluidity by different spectroscopic techniques, such as spin label electron paramagnetic resonance (SL EPR) and Fourier transform infrared (FTIR) spectroscopy, requires a cautious approach.

The light-induced biogenesis of the plastid, the maturation of thylakoid membranes during greening, and related structural reorganization, as well as the response of mature thylakoids to different stress factors, such as extreme temperatures or intense light, are active areas in photosynthesis research. The defined array of the pigment-protein-lipid assemblies develop in a coordinated manner. As it will be shown, studying the development of these assemblies is a way to better understand the nature and functional role of lipid-protein interactions.

In this review, we selected recent examples, which show the importance of lipid-protein interactions, but must point out that our understanding concerning the roles played by lipids in these processes are still rudimentary. The synthesis, structure, function and genetic aspects of lipids in photosynthesis are covered in detail elsewhere (see e.g., [1, 10]). Various other aspects and examples of lipid-protein interactions in different membranes and the physical principles of lipid bilayers are treated thoroughly in the literature (see, e.g., [21, 22, 25–28]).

Membrane fluidity and the lipid-protein interface

The most often used, and sometimes misused, characteristic of biological membranes is their fluidity. There is a great deal of inconsistency and confusion in using the term ‘membrane fluidity’ and how and what different techniques report on this parameter. Strictly speaking, fluidity is reciprocal viscosity normalized to unit density. The density of biomembranes varies little as compared with viscosity; hence fluidity is primarily determined by viscosity. Viscosity on a molecular scale cannot be defined and measured as macroscopically but can be tested by molecular probes, of which rotational diffusion is measured at a given temperature and the Stokes-Einstein relation can then be used to calculate the effective viscosity. This can be done in biomembranes with, e.g., a small stable free radical since its rotational correlation time can be directly calculated from EPR spectra [29–31]. This method, however, is unable to explore the vertical mobility gradient, which means increasing fluidity towards the centre of the bilayer. Spin-labelled lipids carry a small doxyl group bearing the EPR active nitroxyl, which probes the rotational mobility of the lipid chain segment at any desired vertical position along the acyl chain [31–33]. Hence, the most detailed studies on membrane fluidity and changes therein have been made with SL EPR technique [34–36]. The simple reason is that the timescale of the technique, which is $\sim 10^{-7}$–10^{-11} s [37, 38], covers the biologically relevant timescales of acyl rotational isomerization and the rotational and translational diffusion of lipid molecules [38]. FTIR is too fast relative to the timescale of molecular motions that contribute to fluidity. Nonetheless, acyl CH_2-stretching vibration measures membrane fluidity indirectly, because the acyl chain disorder increases upon chain melting, which is detected as an up-shift in the maximum of the corresponding vibration band (see, e.g., [39]). Fluorescence techniques, in particular steady-state and time-resolved fluorescence anisotropy, detect rotational diffusion of the probe, which is dependent on the overall membrane fluidity [40], but are also unable to resolve the vertical mobility gradient due to the bulkiness of the fluorescent probe. The rate of lateral diffusion of membrane-embedded particles or probes is an indirect measure of membrane fluidity [41].

The above definition of membrane fluidity based on lipid chain rotational dynamics is the one closest to the (macroscopic) physical definition. In addition, it is this physical characteristic of the biomembrane that is the principal determinant of the mobility of various membrane constituents ranging from small ions or molecular oxygen over plastoquinones to proteins or protein-cofactor assemblies (e.g., [41]). It is this characteristic that appears to be maintained and regulated by the photosynthetic organism within the physiological range under normal conditions. This involves enzymes that tailor lipids in order to ensure optimal mobility for membranous molecules, and hence optimal efficiency of the membrane-associated photosynthetic processes [36, 42].

It was recognized that the overall fluidity of the membrane cannot be simply predicted from the saturated fatty acid contents and phase transition temperatures of the component lipids [43]. Membrane fluidity also depends largely on lipid composition. For instance, following a decrease in the growth temperature of *Dunaliella salina* from 30 to 12 °C, the MGDG/DGDG ratio decreased from 3.5 to 2.1 [44]. Vigh and co-workers compared changes in the lipid compositions of thylakoid and plasma membranes, in a sensitive and in a hardy variety of wheat, induced by frost hardening [45, 46]. In the frost-sensitive variety, the ratios MGDG/DGDG (2.3) and PE/phosphatidylcholine (PC) (0.35) were hardly changed. In contrast, in the resistant variety the same ratios underwent dramatic changes: the MGDG/ DGDG and PE/PC ratios changed from 3.4 to 1.4 and from 0.36 to 0.19, respectively. (In this context, it is important to point out that MGDG and DGDG are readily interconvertible, cf. [44]). Although these changes can bring about significant shifts in phase-transition temperatures, it seems more likely that they are important in allowing the incorporation of more lipids in the membrane, via decreasing the nonbilayer propensity of the membranes [11].

Membrane lipids and integral membrane proteins possess very different rotational and translational mobility, mainly because of the big difference in their mass, giving rise to frictional forces at their colliding molecular interface. This great difference in dynamics between lipids and proteins is certainly required to maintain tight molecular packing, hence a continuous permeability barrier, since fluid lipids can instantaneously match any packing irregularities arising during lateral and rotational movements as well as conformational rearrangements of proteins. The lateral mobility gradient leads to the solvation shell of lipids around integral membrane proteins: lipids suffer motional restriction by interaction with the protein that drives them in the slow motion limit of SL EPR. For instance, the EPR spectra of different spin labels in PSII-enriched membranes showed that the majority of the lipids in these preparations was strongly motionally restricted [47], whereas more than 60% of the total thylakoid lipids were in a fluidlike phase at room temperature [48]. It has been found that the first shell (i.e. solvation shell) lipids have distinctly lower mobility than lipids even just one layer further away from the protein surface [38]. Obviously, the concentration of these less-mobile protein-solvating lipids is a very important structural parameter of lipid-protein interaction in membranes with embedded proteins. Lipids exchange between the interfacial and bulk sites. The on-rate is limited by the rate of lipid lateral diffusion (the diffusion hopping rate is in the order of $\sim 10^7$ s^{-1} in fluid membranes), while strongly bound lipids have slower off-rates or do not exchange at all with bulk lipids [49–51]. These strongly bound lipids, embedded in protein structures or protein assemblies, represent unique and specific functions but are mostly unavailable for techniques that address the solvation shell.

Since photosynthetic organisms are exposed to permanently changing temperature, there are mechanisms to control the membrane fluidity (see, e.g., [52]). When for instance the temperature decreases, cyanobacteria increase the amount of polyunsaturated fatty acids by expressing and activating fatty acid desaturases, provided the photosynthetic machinery is functional [53]. Due to the lateral mobility gradient pointing away from the protein, lipids experience mobile and immobile environments with durations depending on the lipid:protein ratio, lipid specificities and the rate of lateral diffusion of lipids. Therefore, in addition to lipid and acyl chain composition, overall membrane fluidity depends, on the lipid:protein-ratio, as also pointed out above. Recent results suggest that in general, in addition to lipid composition, the role of protein-solvating interfacial lipids needs further attention in low-temperature adaptation (see, e.g., [54]). Interfacial lipids possess slower rotational dynamics than those in the bulk phase [38]; hence, the overall membrane fluidity depends on the size of the lipid-protein interface and consequently on the size of membrane protein assemblies. Their acyl conformational disorder largely depends on molecular details and the three-dimensional structure of the protein-lipid interface and can, in principle, be higher or lower than that of the bulk lipid phase [39, 54, 55]. The fractional increase of interfacial ‘rigidifying’ lipids [38] and accumulation of (poly)unsaturated ‘fluidizing’ lipids (see, e.g., [52, 56]) have an opposite effect on overall membrane fluidity. We shall see below how rigidification by increased lipid-protein interface is compensated by increasing the fatty acid double bond index, e.g. during greening. An extensive study to clearly demonstrate and quantitate the differences in sensitivity of different techniques of ‘seeing’ protein-solvating lipids and membrane fluidity is underway in our laboratory.

Spectroscopic view of the lipid-protein interface

The molecular interactions in the lipid-protein interface are mostly noncovalent. Following the above definition of fluidity, interfacial lipids are less fluid than those in the bulk phase irrespective of their acyl chain conformation. As pointed out above, these lipids are best explored directly by techniques that are sensitive to rotational diffusion on the right timescale, i.e. 10^{-7}–10^{-10} s [38]. Because of the relatively bulky probes, Fluorescence anisotropy data provide limited information on the lipid-protein interface. The protein-associated lipids are not readily resolved in any nuclear magnetic resonance (NMR) experiment as a second component either, because the lipid-protein exchange rates are fast on the timescales typical of NMR experiments [55, 57].

SL (EPR) and FTIR spectroscopies have proven to be particularly informative techniques in studying thylakoid membranes also in relation to the lipid-protein interface [48, 58–61]. These techniques complement each other since EPR detects only the spin label introduced into the membrane and has a time window (10^{-7}–10^{-11} s) that is optimal for lipid chain dynamics [38, 62], while FTIR detects all vibrations simultaneously. FTIR spectroscopy is a noninvasive technique that has proven to be powerful in studying protein secondary structure [63–65] and lipid chain conformational order in model [39, 66, 67] and biological membranes, including thylakoids [39, 54, 60, 68]. The protein-associated lipids are not directly resolved in FTIR of acyl CH_2-stretching vibration because FTIR, as an optical absorption technique, has a very fast timescale ($\sim 10^{-14}$ s) [65]. Therefore, protein-associated and bulk lipids with the same conformational disorder absorb at the same frequency as the bulk lipids irrespective of their rotational mobility.

It appears that the most successful technique in identifying protein-solvating lipids of reduced mobility is again the SL EPR technique [38]. Protein-solvating first-shell lipids can be detected as an immobile component in EPR spectra of spin-labelled lipid analogues. For a detailed description of the structure, dynamics and composition of the lipid-protein interface as seen by this technique, we point the reader to an in-depth review by Marsh and Horváth [38]. Lipid stoichiometry, lipid headgroup selectivity and exchange dynamics at the lipid-protein interface can be determined, in addition to information on the configuration and rotational dynamics of protein-associated lipid chains. Stoichiometry and selectivity are directly related to the intramembranous structure and degree of oligomerization of integral proteins and can be used to study the state of assembly of such proteins in the membrane. In the work by Marsh and Horváth [38], numerous examples of the application of SL EPR in different membranes are shown, but not any in photosynthetic membranes. SL EPR studies on native thylakoid membranes are discussed below. Although the technique provides most detailed data in reconstituted systems, which usefully complement direct structural studies (see, e.g., [69]), we are not aware of such SL EPR studies on proteins isolated from photosynthetic membranes.

In early spin-labelling studies it was found in thylakoid preparations from various plant sources that overall membrane fluidity decreases with chilling-sensitivity, but it was not determined by bulk fluidity alone because lipid-protein interactions also played an important role [70]. In chilling-sensitive thylakoids, spin-labelled MGDG and PG were found to have lower mobility in the bulk lipid phase, and at the same time a higher proportion of lipids were associated with proteins. It was also concluded that MGDG in thylakoid membranes also played a non specific structural role [48].

In our combined FTIR and SL EPR study we found that heavy metals, while exerting a nonspecific rigidifying effect on the thylakoid membrane, also altered membrane proteins and the lipid-protein interface in such a way that heat-induced protein aggregation became more limited [59]. The extent of these effects on lipids and proteins correlated and was dependent on the heavy metals. It was found that those metal ions (Pb, Cu, Zn) that were able to inhibit electron transport also prevented heat-induced protein aggregation and increased the solvation fraction of the lipids, suggesting that the site of action was in part the lipid-protein interface.

Lipid-protein associations in various PSI- and PSII-enriched subthylakoid preparations from higher plants, pea and maize, studied by SL EPR [71], revealed a clear correlation between the immobile EPR fraction of spin-labelled lipids and the protein:lipid ratio of the preparations. In delipidated preparations and timed-labelling experiments, two sets of solvation sites were resolved: a well-defined population of solvation sites exhibited rapid exchange rates with laterally diffusing membrane lipids, whereas lipids in other solvation sites exchanged much more slowly or did not exchange at all. These latter sites constituted ~30% of all the available solvation sites.

FTIR analysis of protein secondary structure, in combination with oxygen electrode polarography, revealed that DGDG exhibited the ability to stimulate oxygen evolution in PSII core complex, which was accompanied by changes in the structures of the proteins [72]. It was also observed that hydrogen-bonding interactions existed between DGDG molecules and PSII proteins [73].

Although both fluidity and acyl chain disorder increases upon lipid chain melting in pure membranes [66], these characteristics are not in general interchangeable in the presence of proteins [54]. However, conformational disorder caused by chain melting and by lipid-protein interactions could be separated in the symmetric stretching vibration band of CH_2 groups if the component frequencies are sufficiently different or have markedly different temperature dependence, provided that the disorder of bulk lipids vanishes while the disorder of protein-associated lipid acyl chains, and other factors influencing acyl chain disorder, do not change upon cooling to low temperatures. These conditions may not hold generally. It was found with deuterium NMR of lipid acyl chains that the mean conformational order of bulk and protein-associated lipids was essentially the same for a range of different membrane proteins [55, 57] (see also the chapter by D. Marsh in this issue). Nevertheless, FTIR could identify protein-associated lipids indirectly. Based on an analysis of the temperature dependence of acyl CH_2 vibrations in thylakoids, it was suggested that protein-solvating lipids influence the overall membrane dynamics [54], and the authors concluded that the characteristic, which was maintained (by fatty acid desaturases), at a constant optimum level in cyanobacteria grown at different temperatures, was the overall lipid (acyl) disorder. Following this hypothesis, it was further argued that a higher protein: lipid ratio increased the amount of overall acyl chain disorder (due to the solvation shell). Therefore, a higher ordering of bulk lipid chains was desirable and vice versa. It remains to be shown in further studies with various techniques whether this astounding phenomenon is a general one or specific to cyanobacteria.

In more recent work on a filamentous cyanobacterium grown at low temperature (25 °C), no protein-associated component was resolved in the lipid CH_2 vibration band. Instead, membrane patches with low dynamics, meaning high acyl chain order, and rich in polar carotenoids were identified by the same authors [74]. It appears therefore that the lipid-protein interface is manifested differently in different cyanobacteria in the fast FTIR timescale, and it is also subject to experimental conditions and the model used for data analysis. Using the same technique, it was found indirectly that the lipid-protein interface was involved in the low temperature adaptation of the cyanobacterium, i.e. via light-induced desaturase gene expression, which provided specific polyunsaturated fatty acids for the lipid-protein interface [75].

Lipid-protein interactions revealed by genetic engineering and biochemical methods

The physical and (bio)chemical characteristics of membrane lipids depend on the level of unsaturation of their acyl chains [22, 76]. Genetic manipulation of fatty acid desaturase genes and of other lipid-tailoring enzymes is an elegant and powerful tool to study the roles of specific lipids and fatty acids [77–80]. The level of desaturase activity plays an important role in the adaptation and response of photosynthetic organisms to low-temperature stress (see, e.g., [52, 81–84]). It has been recognized that fatty acid desaturation is not only the primary tool for regulation of the overall membrane fluidity but might be important through specific lipid-protein interactions, which result in structural and functional alterations (see, e.g., [78, 85]). The relationship between lipid unsaturation and functioning of the photosynthetic apparatus exposed to elevated temperatures has recently been discussed in [86].

Fatty acid desaturation is one of the crucial steps in cyanobacterial cells in the acclimation processes to low-temperature conditions (see, e.g., [52]). It was found that homeoviscous adaptation [42] via fatty acid desaturation is functional only during photosynthetic activity but not in the dark, suggesting that metabolic or energetic factors of photosynthesis are involved in activating fatty acid desaturase [53]. Unsaturated fatty acids in membrane lipids can also protect the photosynthetic machinery against salt-induced damage [87], and it appears that trienoic fatty acids are required for low-temperature recovery from photoinhibition in *Arabidopsis* [88]. The 32-kDa herbicide-binding D1 subunit of the PSII reaction centre in the thylakoid membrane has a high turnover rate during illumination, which involves photoinhibition, degradation, synthesis and insertion [24]. It was found that increasing the level of lipid desaturation did not influence the inhibition process but accelerated recovery [79, 89].

A further direct functional role of lipids with unsaturated fatty acids was that the lack of unsaturation of fatty acids at precise positions, in cyanobacteria with different desaturase-deficient mutants, blocked the ability of photosynthetic membranes to couple a bioenergetically competent protonmotive force to the ATP formation mechanism at temperatures below 5 °C [90]. These experiments point to direct structural-functional involvement of certain unsaturated lipids in the process, rather than a mechanism based solely on changes in overall membrane fluidity, because the effect was only observed in the absence of the unsaturation of fatty acids in the $\Delta(12)$ and $\Delta(6)$ positions. In relation to light-driven changes in cyanobacteria, it was found that the fatty acids α-18:3 and -18:4 were needed for adaptation of the photosynthetic apparatus to lower temperatures, and fatty acid desaturation was demonstrated to regulate the overall membrane disorder and provide special fatty acids for lipid-protein interactions [75].

In addition to weak forces between membrane proteins and lipids, certain lipid-protein pairs undergo very specific and often strong interactions (table 1). The plant thylakoid ATP synthase was found to be tightly associated with sulfolipids [91]. More recently it was found that SQDG was also indispensable for PSII activity in *Chlamydomonas* by maintaining PSII in its proper state [92]. In higher plants it was found that this sulfolipid, together with PG, contributed to maintaining a negatively charged lipid-water interface, which was thought to be required for proper function of photosynthetic membranes [93]. Preparations of the PSII reaction centre contained high amounts of MGDG [94] which appeared to be associated with certain chlorophyll protein complexes. LHCII had PG strongly linked to the complex, while many galactolipids were lost during the same purification procedure [95].

PG was demonstrated to be essential for the functionality of the PSII reaction centre. Cyanobacterial mutant cells with 90% decrease in PG content suffered a 50% loss of photosynthetic oxygen-evolving activity [96]. In searching for the mechanism, the authors found that the function of the secondary plastoquinone acceptor Q(B) was inhibited by PG deprivation and suggested that PG was indispensable in maintaining the structural integrity of the Q(B) binding site of the PSII reaction centre. PG was found to be essential also for the development of thylakoid membranes in *Arabidopsis* [97, 98]. These findings provide a clear in vivo identification of a specific functional site of PG in the photosynthetic reaction centre [99]. A functional role of this lipid was also demonstrated in vitro by phospholipase A2 treatment (which cuts PG molecules at the *sn*-2 position) of purified PSII core dimers, the formation of which, as revealed by electron crystallography, depends on PG [100]. An array of evidence showed that PG is involved in the dimerization of PSII. An MGDG-deficient *Arabidopsis* mutant showed a 50% lower Chl level and striking defects in chloroplast ultrastructure, strongly suggesting a unique role for MGDG in the structural organization of plastidic membranes [101]. The structural role of MGDG will be addressed in detail in the final section.

Structural view of lipid-protein interactions

Strongly bound lipids in general cannot be detected with SL EPR techniques, because these lipids do not exchange with the exogenous spin label. However, biochemical methods (as discussed above) and direct structural methods identified certain lipids that are tightly associated with or bind to certain thylakoid proteins. These lipids are likely to be delivered via the lipid-protein interface but do not belong to it in the dynamic sense. Although there are already a number of high-resolution structures of membrane proteins available, relatively little is known about the structural details of their interaction with the lipid environment. The situation is rapidly improving with the advance of X-ray and electron crystallography [13, 28, 102–104]. These techniques provide a static picture of lipid-protein interactions at atomic resolution; hence, they are most promising in studying strongly bound lipids that are both less mobile and tend to co-crystallize with proteins (see also the chapter by D. Marsh in this series). The examples identified by X-ray or electron crystallography, summarized in table 2, further demonstrate the

Table 1. Examples of specific lipid-protein interactions and their functional role identified by spectroscopic techniques, genetic engineering and biochemical methods.

Protein (assemblies)	Lipid	Organism	Function	Technique	Reference
Overall	PG, MGDG[a]	plant	structural role, lipid specificity	SL EPR	[48]
Overall	PG	plant	structural: nonexchanging lipids	SL EPR	[71]
Overall	polyunsaturated glycerolipids	CB	adaptation to low temperature	GE, FTIR, activity assays	[75]
overall, LHCII[b]	polyunsaturated MGDG	plant	improved thermal stability, assembly, overall fluidity	GC, SDS-PAGE, SL EPR, FTIR	[124]
LHCII[b]	lipid extracts, MGDG	plant	large-scale assembly with chiral order, structural flexibility, dissipation of absorbed light energy	CD, EM	[142, 147]
PSII	solvation shell lipids	site of heavy plant	metal action	FL, FTIR, SL EPR	[59]
PSII (CC)	DGDG	plant	O_2 evolution, H bonding	FTIR, polarography	[72, 73]
ATP synthase	specific polyunsaturated glycerolipids	CB	required for coupling proton-motive force to ATP synthesis	GE, ATPase activity assays	[90]
D1 subunit of PSII RC	specific polyunsaturated glycerolipids	CB	enhancing insertion, processing and assembly of D1	GE, GLC, O_2 electrode, SDS-PAGE	[79, 89]
LHCII	PG	plant	not addressed	protein and lipid purification	[95]
PSII core proteins	MGDG, PG	plant	not addressed	protein and lipid purification	[94, 95]
PSII RC	PG	CB	integrity of plastoquinon Q(B) binding site	GE, PS activity assays, FL, TL	[96, 99]
PSII	PG	plant	dimerization of PSII	prot. purification, phospholipase A2 treatment, SEC, TLC, EM	[100]
PSII	SQDG	algae	structural, PSII activity	activity assays, polypeptide and lipid analysis	[92]
overall	MGDG	plant	Chl accumulation, chloroplast ultrastructure	GE, EM	[101]

[a] Abbreviations (local to the table): CB, cyanobacterium; CD, circular dichroism; D1, herbicide-binding subunit of photosystem-II reaction centre; EM, electron microscopy; FL, fluorescence; TL, thermoluminescence; GE, genetic engineering; GC, gas chromatography; GLC, gas-liquid chromatography; SEC, size exclusion chromatography; TLC, thin-layer chromatography. [b] Discussed in the final two sections.

functional role of lipids and lipid-protein interactions in photosynthetic membranes.

Recently, X-ray crystallography was used for the purpose of examining the structural details of an interaction between a lipid (CL) and a protein [the photochemical reaction centre (RC) of the photosynthetic bacteria *Rhodobacter sphaeroides*)] [103]. The reaction centre itself is structurally well characterized since it is one of the most-studied integral membrane proteins by this technique. CL, which has four acyl chains and a negative charge at neutral pH, has been shown to be important for optimal activity for a number of membrane proteins, most strikingly for cytochrome c oxidase [105–107]. For the bacterial reaction centre, it was found that binding of CL to RC, close to the transmembrane helix of subunit H, involves both ionic interactions between the protein and the lipid headgroup and van der Waals interactions between the lipid tails and the hydrophobic surface of the protein [103]. Sidechain and backbone groups on the protein as well as bound water molecules contribute to the electrostatic interaction in the headgroup region, whereas the lipid tails sit in hydrophobic grooves in the irregular surface of the protein. The headgroup of CL comes into close contact with all the three subunits of RC. The residues interacting with CL are well conserved among the sequences of the M subunit of bacterial reaction centres. More recent sequence comparison and molecular modelling showed that the size, shape and charge distribution of the site of interaction with CL are well preserved in the reaction centre over a wide range of photosynthetic

Table 2. Specific lipid-protein interactions resolved in the crystal structure of photosynthetic membrane proteins and the ultrastructures of photosynthetic membranes.

Protein	Lipid	Binding, association	Function	Technique	Reference
Bacterial RC[a]	CL	strong	stabilizing subunit assembly (?)	X-ray, 1QOV[(b)]	[103]
Bacterial RC	PE	strong	not addressed	X-ray, 1EYS	[109]
Bacterial RC	CL, PC (PE[c]), GGDG	CL: strong, GGDG, PC (PE): weak	interaction with cofactors causing asymmetry of electron transfer	X-ray, 1M3X	[104]
Bacterial PSI	PG, MGDG	strong (?)	not addressed	X-ray, 1JB0	[111]
Plant LHCII	MGDG PG	strong strong	crystallization trimer formation	EM	[102, 113]
LHCII[d]	MGDG	weak	macroarrays, assembly	EM	[11, 143]

[a] Abbreviations (local to the table): EM, electron microscopy; GGDG, glucosylgalactosyldiacylglycerol.
[b] Protein Data Bank accession codes, if available.
[c] Both PC and PE were detected, but PE in much lower quantity.
[d] Discussed in the final section.

bacteria [108]. This suggests a structural role of CL, which, nevertheless, has yet to be determined.

The crystal structures of the reaction centre of photosynthetic bacteria and the high-potential iron-sulfur protein from *Thermochromatium tepidum* (a purple bacteria with high temperature stability of its RC) were reported recently [109]. A phosphatidylethanolamine (PE) was identified as being bound by electrostatic interaction and hydrogen bonds in one of the clefts between certain transmembrane helices of the H, L and M subunits of the RC. This binding site is different from that of CL-binding cleft found in the above study [103]. A more recent work on the same protein shed further light on the interaction of the reaction centre proteins with membrane lipids [104]. In addition to CL, glucosylgalactosyldiacylglycerol (GGDG) and PC (and smaller amounts of PE at the same location) were resolved in the crystal structure. These lipids were found to bind to the surface of the protein. They interacted predominantly with hydrophobic amino acids, in particular aromatic residues. In contrast to CL, PC (PE) and GGDG were in close contact with the cofactors. GGDG shields the active bacteriochlorophyll monomer. Electrostatic interactions between the phosphate atom of the PC (PE) and inactive bacteriopheophytin may contribute to electron transfer involving this cofactor. The lipids are asymmetric on the protein surface, which suggests that lipid-cofactor interactions contribute to the difference in the energetics for two branches of cofactors causing the asymmetry in electron transfer [110]. The lipids span a vertical distance of ~30 Å that is consistent with a membrane bilayer. The above study is a remarkable demonstration of the lipid-protein interface, by a high-resolution technique, as the ‘inner shell’ of lipids around membrane proteins that is critical for the function of the reaction centre complexes [104]. Structural aspects of lipid-protein interactions in the purple bacterial RC have been reviewed recently [28].

Four lipids have been resolved in the crystal structure of cyanobacterial photosystem-I (PSI): three phospholipids with a PG headgroup with less well defined acyl chain composition, and one MGDG [111]. The headgroups were hydrogen bonded to central and peripheral subunits of PSI in the stromal side of the membrane plane. The location of at least one phospholipid and the single MGDG, which were close to the core of PSI, and binding of an antenna Chla by another phospholipid suggested that these lipids were important both structurally and functionally.

Crystals of the plant light-harvesting complex have also been examined to study lipid-protein interactions with electron microscopy. The structure of LHCII is known both from electron microscopy and electron diffraction [112–114]. LHCII contains DGDG and more tightly bound PG [95, 102]. Although DGDG can be easily removed by detergents, the complexes can form two-dimensional crystals only in the presence of this lipid, suggesting that DGDG facilitates interactions between the complexes and a role in the structural integrity of the complex. PG is more tightly bound and can only be removed by crude treatments, suggesting that PG is bound specifically to the N-terminal domain of the LHCII apoprotein whereby stabilizing LHCII in the trimeric state. Although DGDG and PG are not required for LHCII formation in vitro, both lipids, together with MGDG, stabilize reconstituted LHCII towards thermal denaturation [115].

The conformation of lipids resolved in the crystal structures of a number of diverse transmembrane proteins are being characterized in detail (unpublished results [116]).

Rearrangements of the lipid-protein interface in developing thylakoid membranes

If a leaf is grown in darkness, proplastids develop into etioplasts, which contain the Chl precursor protochlorophyll(ide) instead of Chl and a remarkably high amount of MGDG [24, 117, 118]. When exposed to light, early events that are related to the conversion of protochlorophyll(ide)s to Chl, the disorganization of the prolamellar bodies and formation of planar thylakoid membranes (primary lamellae) [119–121] are relatively fast processes. These are followed by assembly of the key components of the photosynthetic machinery. The assembly of PSI and PSII supercomplexes, which contain light-harvesting and core complexes, and the organization of PSII and LHCII in extended arrays occur at a slower timescale. It may last for days until mature thylakoids appear [117]. Assembly of LHCII initially occurs via pigmentation of the apoproteins that form monomeric complexes, followed by oligomerization and assembly with the already functional core complex (CC) at later phases of greening [122]. By definition, greening is characterized by chlorophyll accumulation, which is generally used as the parameter to describe the time course of this process [123]. Recently, we studied greening in dark-grown barley seedlings, using biochemical methods, and FTIR and SL EPR spectroscopic techniques [124].

Using a spectral subtraction technique [37, 48, 125] aided with least-squares optimization, the spin label spectra could be decomposed into mobile and immobile components. As discussed above, these were assigned to protein solvation and bulk lipids as in other studies [58, 59, 70, 71]. The big, nonmonotonic change in the immobile component observed during greening, at relatively constant lipid:protein ratio and lipid headgroup composition [117], indicated big changes in the size of the lipid-protein interface which could be quantitated using a simple model [125, 126]. In view of data on gross changes in the secondary structure of membrane proteins, conformation, composition and dynamics of lipid acyl chains, SDS-polyacrylamide gel electrophoresis (PAGE) pattern and thermally induced structural alterations, thylakoid development could be consistently interpreted as a sequence of correlated molecular events grouped into three phases: onset, rearrangement and maturation [124]. The process of reorganization of membrane protein assemblies and the lipid-protein interface was also manifested in these phases. Figure 1 schematically represents changes in the size of protein assemblies and the amount protein-solvating lipids, calculated from the EPR spectra, during greening together with other physical parameters. Bearing in mind that greening is subject to many uncontrolled factors [123], it should be noted that considerable agreement exists with other experiments on the same [122, 127] and other [128] plant species concerning the time course of greening.

Membrane fluidity was lowest in the rearrangement phase, which may have been caused initially by a conversion/disassembly of protein assemblies to smaller units which were subsequently replaced by larger assemblies of newly synthesized proteins, thus resulting in larger and smaller protein-lipid interface, in the rearrangement and maturation phases, respectively, relative to the onset phase. The more than two-fold increase of the linolenic (18:3) acid content appeared to be compensation for the increase in interfacial rigidifying lipids and in protein content. This compensation was probably required to maintain mobility of membrane components. The time course of the accumulation of LHCII (measured by SDS-PAGE), the Chl and the 18:3 fatty acids suggested that the activity of the related fatty acid desaturases [16, 53, 129], Chl synthesis and expression of certain proteins were synchronized. Photosynthetic functions, as revealed by fluorescence induction kinetics, showed good correlation with the above structural rearrangements [130]. It should be noted that the synthesis of various xanthophylls, known to protect and stabilize LHCII assembly [131], followed a time course similar to that of LHCII accumulation [132].

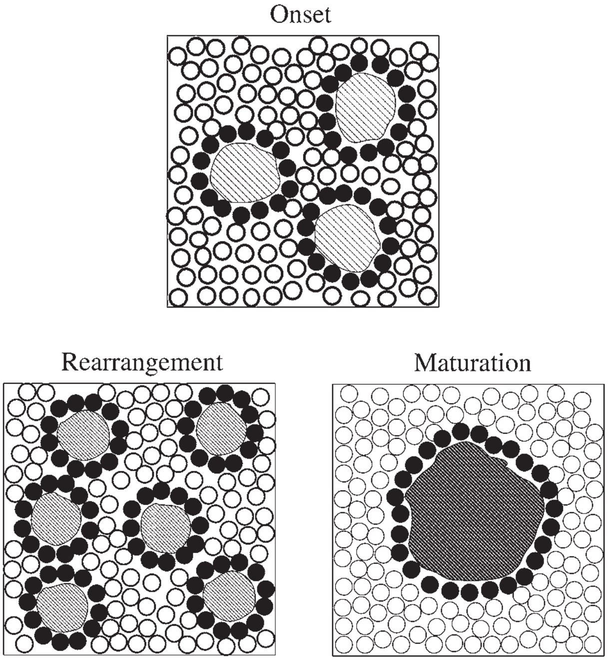

Figure 1. Rearrangement of the lipid-protein interface during onset (*A*), rearrangement (*B*) and maturation (*C*) phases of greening (redrawn according to [124]). The larger objects are protein assemblies, and the lipids are shown with small circles. The lipid-protein interfaces (solvation shell lipids) are shown as black lipids. The size of the lipid-protein interface, the protein:lipid ratio and the size of membrane protein assemblies reflect relative changes that are observed experimentally. The different patterns for the protein assemblies refer to changes in their composition, increasing amount of α-helical and β-sheet forms and improving thermal stability during greening. The patterns for the lipids indicate monotonic increase in linolenic (18:3) acid content.

At any phase of greening, heating of thylakoid preparations caused significant changes in protein secondary structures and the ^{1}H-^{2}H exchange kinetics in deuterated buffer followed by a transition assigned to the exclusion of nonbilayer lipids from the thylakoid at higher temperature [59, 133–135]. Both the thermal stability and amount of thylakoid proteins with well-defined secondary structure increased monotonically during greening as seen by FTIR. A structural coupling between nonbilayer lipids and LHCII assemblies was suggested by the significant reduction of the difference of the midpoint temperatures between these thermal events and the similar greening profile of xanthophyll accumulation [132] and oligomerization of LHCII [122] during the rearrangement and maturation phases [124]. The decrease in the lamellar-to-H_{II} transition temperature was in agreement with an increase in the nonbilayer propensity of MGDG [136] with progressing desaturation during greening. In pea thylakoid membranes, an even closer correlation between heat-induced partial protein unfolding and formation of the H_{II} phase was observed [59]. In addition, the characteristic temperature of these changes agreed with the temperature at which destacking of grana took place in thylakoids [133]. Notably, the lamellar-to-H_{II} transition followed a similar temperature dependence as the loss of oxygen-evolving activity in spinach PSII particles [137], again suggesting an important structural-functional role of these lipids (see also [138]).

The lipid-protein interaction and the fluidity of the thylakoid membrane is determined to a large extent by nonmonotonic changes in protein assemblies and monotonic progression of fatty acid desaturation during greening. The latter affects overall membrane fluidity and increases the nonbilayer propensity of MGDG. The above data strongly imply that as part of a concerted mechanism, the interface between the major protein and lipid components plays a key role in the process of greening such that it allows improving protection against elevated temperature and provides a suitable lipid environment that serves both structural flexibility and stability for lateral organization and the rearrangement of thylakoid proteins.

Interactions between the major lipid and protein components of the thylakoid membrane: the role of nonbilayer lipids

The fact that non-bilayer lipids are precisely regulated implies that they are of considerable functional importance [44–46, 139, 140] (see also the section on membrane fluidity). The nonbilayer H_{II} form is retained when purified thylakoid lipids are mixed, and lamellae can only be observed in galactolipid mixtures if the MGDG content is decreased substantially [136]. The lamellar structure of the thylakoid membranes on the one hand and the nonlamellar structure assumed by thylakoid lipid extracts on the other argue that specific lipid-protein interactions are of paramount importance in these membranes. Indeed, it has been suggested that structural rearrangements seen by electron microscopy [136] are due to dissociation of LHCII and the exclusion and phase-separation of nonbilayer-forming lipids from the thylakoid membrane [141]. This is a further indication that the specific lipid-protein structural balance responsible for keeping nonbilayer lipids in lamellar structures can be lost upon heat treatment, as shown also by our recent results [124, 135]. Indeed, the physical and functional properties of these membranes depend greatly on lipid-protein interactions, and it is generally assumed that photosynthetic light-harvesting proteins suppress the formation the of H_{II} phase [136] in native membranes via lipid-protein interactions (see also [23]) and due to protein aggregation [11]. The temperature of the lamellar-to-H_{II} transition is known to decrease with increasing double-bond content [22, 134, 136], but protein-lipid interactions may have an opposite effect by stabilizing MGDG in the bilayer [11, 23]. It has been shown that lamellar aggregates of LHCII are capable of incorporating substantial amounts of different thylakoid lipids, including MGDG [142]. With the incorporation of lipids, the long-range order of the chromophores, i.e. the chiral organization of macrodomains, was retained, while the ultrastructure of the lipid-protein macroaggregates could be modified significantly. The inverted hexagonal structure was destroyed by LHCII [143], evidently for the high self-aggregation ability of the proteins, which 'absorbed' a high amount of MGDG, more than the thylakoid membranes (calculated on protein basis), and forced them into bilayers [11, 142] (fig. 2). These examples also show the significance of the lipid-protein interactions on the gross ultrastructure of membranes. In particular, MGDG resulted in an onion-like stacked multilayer structure, which differed significantly from the initial lamellar sheets, and was retained in the presence of PG and SQDG – and from the stacked vesicle structure that was obtained upon the addition of DGDG. Further, the effect of MGDG on the structure has also been tested in *Escherichia coli* cells, which became elongated upon overexpressing the enzyme responsible for synthesis of the foreign nonbilayer lipid [144]. The key structural role of these nonbilayer lipids, especially in stabilizing membrane proteins via protein-lipid interaction, has been also suggested and investigated in reconstituted membrane-protein systems with the SL EPR technique (see, e.g., [48, 107, 145]).

In our earlier studies, loosely stacked lamellar macroaggregates of LHCII were shown to be capable of undergoing light-induced reversible structural changes and changes in photophysical pathways which resembled those in thylakoid membranes exposed to excess light [146]. This structural flexibility of LHCII was found to depend critically on the lipid content of the preparations [147] and was absent in delipidated LHCII preparations, e.g. in LHCII microcrystals. In intact thylakoid membranes pigment molecules with long-range chiral order

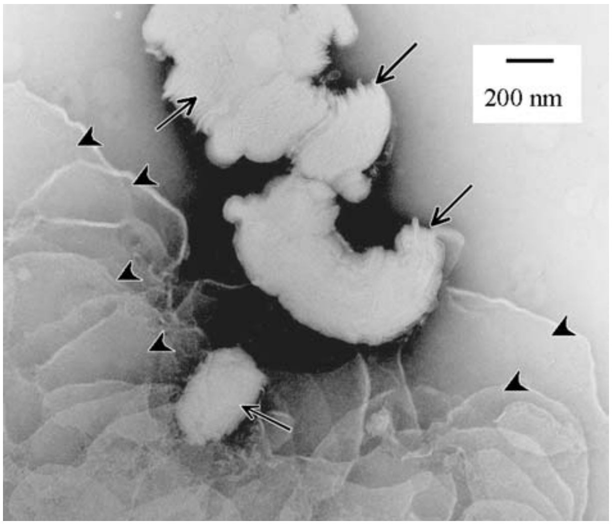

Figure 2. Electron micrograph of negatively stained MGDG-LHCII aqueous dispersions obtained by adding saturating amounts of MGDG to freshly prepared lamellar aggregates of LHCII (courtesy of Drs I. Simidjiev, S. Stoylova and A Holzenburg; reproduced from [11] with permission of Elsevier Science Ltd.). The lamellar and inverse hexagonal H_{II} phase (arrowheads and arrows, respectively) of MGDG can be identified, as well as the interaction of LHCII in the lamellar phase with MGDG in the H_{II} phase (lower left arrow). The interaction leads to the destruction of the H_{II} phase, shown by small-angle X-ray scattering [143], and the 'uptake' of the lipids in the lamellar phase.

[147] displayed high stability below 40 °C, but these macrodomains were gradually disassembled between 50 and 60 °C [148]. Addition of thylakoid lipids to lamellar aggregates of LHCII significantly enhanced the ability of the complexes to undergo light-induced structural changes. It has also been shown that these structural changes are given rise by fast local thermal transients, a thermooptic effect [149]. The lipid-protein interface around LHCII complexes probably plays a significant role in determining the structural flexibility of the macroaggregates. Involvement of specific lipid-protein interactions cannot be ruled out, since both the macroorganization and the trimeric structure depend on lipids (see above). However, it is equally possible that the main role of lipids is to provide a structurally flexible matrix which transduces the local heat effect to the protein, where it can cause longer-lasting effects. Lipid vesicles have been shown to be very sensitive to small variations in temperature [150].

Detailed understanding of the functional and structural role of nonbilayer lipids and the mechanism of their action on membrane proteins and vice versa is beginning to emerge. In addition to their roles in membrane fusion and the formation of junctions, via transient nonbilayer structures, and specific interactions with certain proteins (reviewed in [21]), these lipids – for their conical shapes – are responsible for membrane curvatures [145, 151]. In a recent theoretical model, derived from flash photolysis data on bovine rhodopsin reconstituted in membranes containing nonbilayer lipids, it was suggested that matching the spontaneous curvature of the lipid bilayer to the mean curvature of the lipid-water interface adjacent to the protein balances lipid-protein solvation energy [152]. As discussed above, nonbilayer lipids also facilitate packing of membrane proteins [23, 24, 107, 126, 145], as also shown for LHCII [143]. At the same time aggregation of proteins spatially inhibits formation of nonbilayer structures under normal conditions [11, 107], but allows adaptation and rearrangement of the protein-protein and lipid-protein interface during development or under stress conditions, including protection against elevated temperature and intense light [124, 148]. This fine interplay and regulation of the lipid:protein ratio [11] ensures both the structural flexibility and stability hypothesized long ago [48, 70]. The structural balance between LHCII and MGDG can be lost upon, e. g., elevated temperature when the proteins suffer structural changes [59, 124, 133, 135]. This thermal unfolding is only partial, since LHCII unfolds at a much higher temperature [153]. It probably occurs in the relatively loose loop regions between the transmembrane α-helices. This suggests that MGDG-LHCII interaction in the region of the membrane-water interface is of great significance.

Conclusion and outlook

A number of examples of specific, weak and strong, lipid-protein associations in photosynthetic membranes have been shown. Such an inventory cannot be complete, but it clearly demonstrates that the lipid-protein interface not only seals proteins in the membrane and motionally couples them with the dynamic bulk lipid environment required for the function of the complex photosynthetic machinery, but is a very important site of specific modulation of protein function. Since the heterogeneity of lipid and fatty acid composition of photosynthetic membranes is high, with potentially numerous interacting lipid-protein pairs that are still unknown, as compared with assigned functions, there is clearly room for research in this field, and we expect that new functions of particular lipid-protein interactions will be explored.

The results on greening demonstrate the promises of combining techniques (FTIR and SL EPR in particular) and further substantiate the applicability of the protein-solvating lipid concept in studying photosynthetic membranes [59, 124]. The role of fatty acid desaturation and in particular the molecular details of the mechanism of its action in adaptation and other processes is likely to remain in the focus of research. For instance, What is the molecular basis of selective dependence on the precise site of the desaturation of fatty acids of some processes [90]? In addition, the fact that the nonbilayer-forming propensity of MGDG depends on the level of unsaturation [56] has received little attention so far in in vivo studies. Also, the hypothesis that nonbilayer lipids are essential in determining the dynamic properties of thylakoid membranes such as dynamic release and uptake of lipids upon, e. g., heating or changes in the physicochemical environment of the membrane [11] require detailed investigations.

A useful approach to explore the molecular details of the interaction of nonbilayer lipids with membrane proteins, and the role of the degree of acyl chain unsaturation, might be studies in reconstituted systems. In these, structurally more relevant data on the lipid-protein interface can be obtained [38]. In addition, lipid-protein interactions can also be tested in genetically engineered and site-specifically spin- or fluorescent-labelled proteins combined with molecular modelling (see, e. g., [154–156]). In view of recent advances in two- and three-dimensional crystallization of membrane proteins [143, 157–162], new high-resolution structures of thylakoid membrane proteins, also from photosynthetic bacteria, and lipid-reconstituted molecular assemblies are expected to appear that will most certainly explore important details of the lipid-protein interface in photosynthetic membranes.

Acknowledgments. Work from the author's laboratories was supported by the Volkswagen-Stiftung and the Hungarian National Science Fund (grants T029458, T043425, T30324, T42696, T34188). We thank Drs Derek Marsh and Zoltán Gombos for reading and commenting on the manuscript.

1 Siegenthaler P. A. and Murata N. (1998) Lipids in Photosynthesis: Structure, Function and Genetics, Kluwer Academic Publishers, Dordrecht
2 Mustárdy L. and Garab G. (2003) Granum revisited. A three-dimensional model – where things fall into place. Trends Plant Sci. **8:** 117–122
3 Andersson B. and Anderson J. M. (1980) Lateral heterogeneity in the distribution of chlorophyll-protein complexes of the thylakoid membranes of spinach chloroplasts. Biochim. Biophys. Acta **593:** 427–440
4 Arntzen C. J. (1978) Dynamic structural features of chloroplast lamellae. Curr. Topics Bioenerget. **8:** 111–160
5 Barber J. (1982) Influence of surface charges on thylakoid structure and function. Annu. Rev. Plant Physiol. **33:** 261–295
6 Garab G., Kieleczawa J., Sutherland J. C., Bustamante C. and Hind G. (1991) Organization of pigment-protein complexes into macrodomains in the thylakoid membranes of wild-type and chlorophyll b-less mutant of barley as revealed by circular dichroism. Photochem. Photobiol. **54:** 273–281
7 Anderson J. M. and Andersson B. (1988) The dynamic photosynthetic membrane and regulation of solar energy conversion. Trends Biochem. Sci. **13:** 351–355
8 Horton P., Ruban A. V. and Walters R. G. (1996) Regulation of light harvesting in green plants. Annu. Rev. Plant Physiol. Plant Mol. Biol. **47:** 655–684
9 Garab G. and Mustárdy L. (1999) Role of LHCII-containing macrodomains in the structure, function and dynamics of grana. Austral. J. Plant Physiol. **26:** 649–658
10 Douce R. and Joyard J. (1996) Biosynthesis of thylakoid membrane lipids. In: Advances in Photosynthesis/Oxygenic Photosynthesis: The Light Reactions. pp. 69–101, Ort D. R. and Yocum C. F. (eds), Kluwer Academic Publishers, Dordrecht
11 Garab G., Lohner K., Laggner P. and Farkas T. (2000) Self-regulation of the lipid content of membranes by non-bilayer lipids: a hypothesis. Trends Plant Sci. **5:** 489–494
12 Kirchhoff H., Mukherjee U. and Galla H. (2002) Molecular architecture of the thylakoid membrane: Lipid diffusion space for plastoquinone. Biochemistry **41:** 4872–4882
13 Boekema E., van Breemen J., van Roon H. and Dekker J. (2000) Arrangement of photosystem II supercomplexes in crystalline macrodomains within the thylakoid membrane of green plant chloroplasts. J. Mol. Biol. **301:** 1123–1133
14 Rojdestvenski I., Ivanov A., Cottam M., Borodich A., Huner N. and Oquist G. (2002) Segregation of photosystems in thylakoid membranes as a critical phenomenon. Biophys. J. **82:** 1719–1730
15 Duchene S. and Siegenthaler P. (2000) Do glycerolipids display lateral heterogeneity in the thylakoid membrane? Lipids **35:** 739–744
16 Murata N. and Siegenthaler P. A. (1998) Lipids in Photosynthesis: An Overview. In: Advances in Photosynthesis: Lipids in Photosynthesis: Structure, Function and Genetics, vol. **6**, pp. 1–20, Siegenthaler P. A. and Murata N. (eds), Kluwer Academic Publishers, Dordrecht
17 Gounaris K. and Barber J. (1983) Monogalactosyldiacylglycerol – the most abundant polar lipid in nature. Trends Biochem. Sci. **8:** 378–381
18 Seddon J. M., Cevc G., Harlos K., Kaye R. D. and Marsh D. (1986) Polymorphism and phase diagrams of saturated phosphatidyl ethanolamines. In: Surfactants in Solution, pp. 783–791, Mittal K. L. and Bothorel P. (eds), Plenum Press, New York
19 Selstam E., Brentel I. and Lindblom G. (1990) Phase structure of lipids of the photosynthetic membrane. In: Plant Lipid Biochemistry, Structure and Utilization, pp. 39–46, Quinn P. J. and Harwood J. L. (eds), Portland Press Limited, London
20 Seddon J. M. and Templer R. H. (1995) Polymorphism of lipid-water system. In: Structure and Dynamics of Membranes, pp. 97–161, Lipowsky R. and Sackmann E. (eds), Elsevier, Amsterdam
21 Epand R. M. (1998) Lipid polymorphism and protein-lipid interactions. Biochim. Biophys. Acta **1376:** 353–368
22 Marsh D. (1990) CRC Handbook of Lipid Bilayers, CRC Press, Boca Raton, FL
23 de Kruijff B. (1997) Lipids beyond the bilayer. Nature **386:** 129–131
24 Bruce B. D. (1998) The role of lipids in plastid protein transport. Plant Mol. Biol. **38:** 223–246
25 Cevc G. and Marsh D. (1987) Phospholipid Bilayers. Physical Principles and Models, vol. 5, Wiley-Interscience, New York
26 Lipowsky R. and Sackmann E. (1995) Structure and Dynamics of Membranes: From Cells to Vesicles, vol. 1A, Elsevier, Amsterdam
27 White S. and Wimley W. (1999) Membrane protein folding and stability: Physical principles. Annu. Rev. Biophys. Biomol. Struct. **28:** 319–365
28 Jones M. R., Fyfe P. K., Roszak A. W., Isaacs N. W. and Cogdell R. J. (2002) Protein-lipid interactions in the purple bacterial reaction centre. Biochim. Biophys. Acta Biomembranes **1565:** 206–214
29 Kivelson D. (1960) Theory of ESR line-widths of free radicals. J. Chem. Phys. **33:** 1094–1107
30 Seelig J. (1976) Anisotropic motion in liquid crystalline structures. In: Spin Labeling. Theory and applications, vol. 1, pp. 373–409, Berliner L. J. (ed), Academic Press, New York
31 McConnell H. M. (1976) Molecular motion in biological membranes. In: Spin Labeling. Theory and Applications, vol. 1, pp. 525–560, Berliner L. J. (ed), Academic Press, New York

32 Marsh D. and Watts A. (1982) Diffusible spin labels used to study lipid-protein interactions with rhodopsin and bacteriorhodopsin. Methods Enzymol. **88:** 762–772

33 Moser M., Marsh D., Meier P., Wassmer K.-H. and Kothe G. (1989) Chain configuration and flexibility gradient in phospholipid membranes. Comparison between spin-label electron spin resonance and deuteron nuclear magnetic resonance, and identification of new conformations. Biophys. J. **55:** 111–123

34 Griffith O. H. and Jost P. C. (1976) Lipid spin labels in biological membranes. In: Spin Labeling. Theory and Applications, vol. 1, pp. 453–523, Berliner L. J. (ed), Academic Press, New York

35 Marsh D., Watts A. and Knowles P. F. (1977) Cooperativity of the phase transition in single and multibilayer lipid vesicles. Biochim. Biophys. Acta **465:** 500–514

36 Quartacci M., Pinzino C., Sgherri C., Dalla Vecchia F. and Navari-Izzo F. (2000) Growth in excess copper induces changes in the lipid composition and fluidity of PSII-enriched membranes in wheat. Physiologia Plantarum **108:** 87–93

37 Marsh D. (1989) Experimental methods in spin-label spectral analysis. In: Spin Labeling. Theory and Applications, vol. 8, pp. 255–303, Berliner L. J. and Reuben J. (eds), Plenum, New York

38 Marsh D. and Horváth L. I. (1998) Structure, dynamics and composition of the lipid-protein interface. Perspectives from spin-labelling. Biochim. Biophys. Acta **1376:** 267–296

39 Kóta Z., Debreczeny M. and Szalontai B. (1999) Separable contributions of ordered and disordered lipid fatty acyl chain segments to *v*CH2 bands in model and biological membranes: a Fourier transform infrared spectroscopic study. Biospectroscopy **5:** 169–178

40 Páli T., Nemet B. and Pesti M. (1996) Phase transition of membrane lipids. In: Manual on membrane lipids, pp. 80–111, Prasad R. (ed.), Springer, Berlin

41 Sarcina M., Tobin M. and Mullineaux C. (2001) Diffusion of phycobilisomes on the thylakoid membranes of the cyanobacterium *Synechococcus* 7942 – effects of phycobilisome size, temperature and membrane lipid composition. J. Biol. Chem. **276:** 46830–46834

42 Cossins A. R. (1994) Temperature Adaptation of Biological Membranes, Portland, London

43 Orr G. R. and Raison J. K. (1987) Compositional and thermal properties of thylakoid polar lipids of nerium-oleander l in relation to chilling sensitivity. Plant Physiol. **84:** 88–92

44 Harwood J. L. (1998) Involvement of chloroplast lipids in the reaction of plants submitted to stress. In: Advances in Photosynthesis: Lipids in Photosynthesis: Structure, Function and Genetics, vol. 6, pp. 287–302, Siegenthaler P. A. and Murata N. (eds), Kluwer, Dordrecht

45 Vigh L., Horváth I., Van Hasselt P. R. and Kuiper P. J. C. (1985) Effect of frost hardening on lipid and fatty-acid composition of chloroplast thylakoid membranes in 2 wheat-varieties of contrasting hardiness. Plant Physiol.**79:** 756–759

46 Vigh L., Horváth I., Woltjes J., Farkas T., Van Hasselt P. R. and Kuiper P. J. C. (1987) Combined electron-spin-resonance, X-ray-diffraction studies on phospholipid-vesicles obtained from cold-hardened wheats. 1. An attempt to correlate electron-spin-resonance spectral characteristics with frost-resistance. Planta **170:** 14–19

47 Li G., Horváth L. I., Knowles P. F., Murphy D. J. and Marsh D. (1989) Spin label saturation transfer ESR studies of protein-lipid interactions in photosystem II-enriched membranes. Biochim. Biophys. Acta **987:** 187–192

48 Li G., Knowles P. F., Murphy D. J., Nishida I. and Marsh D. (1989) Spin-label ESR studies of lipid-protein interactions in thylakoid membranes. Biochemistry **28:** 7446–7452

49 Horváth L. I., Brophy P. J. and Marsh D. (1988) Exchange rates at the lipid-protein interface of myelin proteolipid protein studied by spin-label electron spin resonance. Biochemistry **27:** 46–52

50 Horváth L. I., Brophy P. J. and Marsh D. (1993) Exchange rates at the lipid-protein interface of the myelin proteolipid protein determined by saturation transfer electron spin resonance and continuous wave saturation studies. Biophys. J. **64:** 622–631

51 Ryba N., Horváth L. I., Watts A. and Marsh D. (1987) Exchange at the lipid-protein interface. Spin label ESR studies of rhodopsin-dimyristoyl phosphatidylcholine recombinants. Biochemistry **26:** 3234–3240

52 Kiseleva L. L., Horváth I., Vígh L. and Los D. A. (1999) Temperature-induced specific lipid desaturation in the thermophilic cyanobacterium *Synechococcus vulcanus*. FEMS Microbiol. Lett. **175:** 179–183

53 Kis M., Zsiros O., Farkas T., Wada H., Nagy F. and Gombos Z. (1998) Light-induced expression of fatty acid desaturase genes. Proc. Natl. Acad. Sci. USA **95:** 4209–4214

54 Szalontai B., Nishiyama Y., Gombos Z. and Murata N. (2000) Membrane dynamics as seen by Fourier transform infrared spectroscopy in a cyanobacterium, *Synechocystis* PCC 6803. The effect of lipid unsaturation and the protein-to-lipid ratio. Biochim. Biophys. Acta Biomembranes **1509:** 409–419

55 Meier P., Sachse J.-H., Brophy P. J., Marsh D. and Kothe G. (1987) Integral membrane proteins significantly decrease the molecular motion in lipid bilayers: a deuteron NMR relaxation study of membranes containing myelin proteolipid apoprotein. Proc. Natl. Acad. Sci. USA **84:** 3704–3708

56 Coolbear K., Berde C. and Keough K. (1983) Gel to liquid-crystalline phase-transitions of aqueous dispersions of polyunsaturated mixed-acid phosphatidylcholines. Biochemistry **22:** 1466–1473

57 Bloom M. and Smith I. C. P. (1985) Manifestations of lipid-protein interactions in deuterium NMR. In: Progress in Protein-Lipid Interactions, vol. 1, pp. 61–88, Watts A. and De Pont J. J. H. H. M. (eds), Elsevier, Amsterdam

58 Li G., Knowles P. F., Murphy D. J. and Marsh D. (1990) Lipid-protein interactions in stacked and destacked thylakoid membranes and the influence of phosphorylation and illumination. Spin label ESR studies. Biochim. Biophys. Acta **1024:** 278–284

59 Szalontai B., Horváth L. I., Debreczeny M., Droppa M. and Horváth G. (1999) Molecular rearrangements of thylakoids after heavy metal poisoning, as seen by Fourier transform infrared (FTIR) and electron spin resonance (ESR) spectroscopy. Photosynth. Res. **61:** 241–252

60 Szalontai B., Nishiyama Y., Gombos Z. and Murata N. (1999) Membrane dynamics studied by FTIR spectroscopy in thylakoid and cytoplasmic membranes of *Synechocystis* PCC6803. Lipids and the effect of protein to lipid ratios. In: Spectroscopy of Biological Molecules: New Directions, pp. 385–386, Greve J., Puppels G. J. and Otto C. (eds), Kluwer, Dordrecht

61 Kóta Z., Szalontai B., Droppa M., Horváth G. and Páli T. (1999) Fourier transform infrared and electron paramagnetic resonance spectroscopic studies of thylakoid membranes. J. Mol. Struct. **481:** 395–400

62 McConnell H. M. (1976) Molecular motion in biological membranes. In: Spin Labelling – Theory and Applications, pp. 525–560, Berliner L. J. (ed.), Academic Press, New York

63 Arrondo J. L. R., Muga A., Castresana J. and Goni F. M. (1993) Quantitative studies of the structure of proteins in solution by Fourier transform infrared spectroscopy. Prog. Biophys. Mol. Biol. **59:** 23–56

64 Jackson M. and Mantsch H. (1995) The use and misuse of FTIR spectroscopy in the determination of protein structure. Crit. Rev. Biochem. Mol. Biol. **30:** 95–120

65 Mantsch H. H. and Chapman D. (1996) Infrared Spectroscopy of Biomolecules, Wiley-Liss, New York

66 Casal H. L. and Mantsch H. H. (1984) Polymorphic phase behaviour of phospholipid membranes studied by infrared spectroscopy. Biochim. Biophys. Acta **779:** 381–401

67 Mendelsohn R., Davies M. A., Brauner J. W., Schuster H. F. and Dluhy R. A. (1989) Quantitative determination of conformational disorder in the acyl chains of phospholipid bilayers by infrared spectroscopy. Biochemistry **28:** 8934–9

68 Moore D. J. and Mendelsohn R. (1994) Adaptation to altered growth temperatures in *Acholeplasma laidlawii B*: Fourier transform infrared studies of acyl chain conformational order in live cells. Biochemistry **33:** 4080–4085

69 Páli T., Finbow M. E., Holzenburg A., Findlay J. B. C. and Marsh D. (1995) Lipid-protein interactions and assembly of the 16-kDa channel polypeptide from *Nephrops norvegicus*. Studies with spin-label electron spin resonance spectroscopy and electron microscopy. Biochemistry **34:** 9211–9218

70 Li G., Knowles P. F., Murphy D. J. and Marsh D. (1990) Lipid-protein interactions in thylakoid membranes of chilling-resistant and -sensitive plants studied by spin label electron spin resonance spectroscopy. J. Biol. Chem. **265:** 16867–16872

71 Ivancich A., Horváth L. I., Droppa M., Horváth G. and Farkas T. (1994) Spin label ESR study of lipid solvation of supramolecular photosynthetic protein complexes in thylakoids. Biochim. Biophys. Acta Biomembranes **1196:** 51–56

72 Yang Z., Wang Z., Li L. and Kuang T. (2002) Oxygen-evolving activity in photosystem II core complex of photosynthetic membrane in the presence of native lipid. Chinese J. Chem. **20:** 576–582

73 Gabashvili I., Menikh A., Segui J. and Fragata M. (1998) Protein structure of photosystem II studied by FTIR spectroscopy – effect of digalactosyldiacylglycerol on the tyrosine side chain residues. J. Mol. Struct. **444:** 123–133

74 Várkonyi Z., Masamoto K., Debreczeny M., Zsiros O., Ughy B., Gombos Z. et al. (2002) Low-temperature-induced accumulation of xanthophylls and its structural consequences in the photosynthetic membranes of the cyanobacterium *Cylindrospermopsis raciborskii*: an FTIR spectroscopic study. Proc. Natl. Acad. Sci. USA **99:** 2410–2415

75 Zsiros O., Kis M., Mustárdy S., Farkas T., Várkonyi Z., Gombos Z. et al. (2002) Light-driven structural changes in thylakoid and cytoplasmic membranes of a cyanobacterium, *Synechocystis* PCC 6803. J. Plant Physiol. **159:** 403–414

76 Murata N. and Wada H. (1995) Acyl-lipid desaturases and their importance in te tolerance and acclimatization to cold of cyanobacteria. Biochem. J. **308:** 1–8

77 Gombos Z., Wada H. and Murata N. (1992) Unsaturation of fatty acids in membrane lipids enhances tolerance of the cyanobacterium *Synechocystis* PCC6803 to low-temperature photoinhibition. Proc. Natl. Acad. Sci. USA **89:** 9959–9962

78 Tasaka Y., Gombos Z., Nishiyama Y., Mohanty P., Ohba T., Ohki K. et al. (1996) Targeted mutagenesis of acyl-lipid desaturases in *Synechocystis*: evidence for the important roles of polyunsaturated membrane lipids in growth, respiration and photosynthesis. EMBO J. **15:** 6416–6425

79 Gombos Z., Kanervo E., Tsvetkova N., Sakamoto T., Aro E. and Murata N. (1997) Genetic enhancement of the ability to tolerate photoinhibition by introduction of unsaturated bonds into membrane glycerolipids. Plant Physiol. **115:** 551–559

80 Dormann P., Balbo I. and Benning C. (1999) *Arabidopsis* galactolipid biosynthesis and lipid trafficking mediated by DGD1. Science **284:** 2181–2184

81 Wada H., Gombos Z. and Murata N. (1990) Enhancement of chilling tolerance of a cyanobacterium by genetic manipulation of fatty acid desaturation. Nature **347:** 200–203

82 Zsiros O., Várkonyi Z., Kovács A., Farkas T., Gombos Z. and Garab G. (2000) Induction of polyunsaturated fatty-acid synthesis enhances tolerance of a cyanobacterium, *Cylindrospermopsis raciborskii*, to low-temperature photoinhibition. Indian J. Biochem. Biophys. **37:** 470–476

83 Várkonyi Z., Zsiros O., Farkas T., Garab G. and Gombos Z. (2000) The tolerance of cyanobacterium *Cylindrospermopsis raciborskii* to low-temperature photo-inhibition affected by the induction of polyunsaturated fatty acid synthesis. Biochem. Soc. Trans. **28:** 892–894

84 Routaboul J., Fischer S. and Browse J. (2000) Trienoic fatty acids are required to maintain chloroplast function at low temperatures. Plant Physiol. **124:** 1697–1705

85 Ariizumi T., Kishitani S., Inatsugi R., Nishida I., Murata N. and Toriyama K. (2002) An increase in unsaturation of fatty acids in phosphatidylglycerol from leaves improves the rates of photosynthesis and growth at low temperatures in transgenic rice seedlings. Plant Cell Physiol. **43:** 751–758

86 Morgan-Kiss R., Ivanov A., Williams J., Khan M. and Huner N. (2002) Differential thermal effects on the energy distribution between photosystem II and photosystem I in thylakoid membranes of a psychrophilic and a mesophilic alga. Biochim. Biophys. Acta Biomembranes **1561:** 251–265

87 Allakhverdiev S., Kinoshita M., Inaba M., Suzuki I. and Murata N. (2001) Unsaturated fatty acids in membrane lipids protect the photosynthetic machinery against salt-induced damage in *Synechococcus*. Plant Physiol. **125:** 1842–1853

88 Vijayan P. and Browse J. (2002) Photoinhibition in mutants of *Arabidopsis* deficient in thylakoid unsaturation. Plant Physiol. **129:** 876–885

89 Gombos Z., Wada H. and Murata N. (1994) The recovery of photosynthesis from low-temperature photoinhibition is accelerated by the unsaturation of membrane lipids: a mechanism of chilling tolerance. Proc. Natl. Acad. Sci. USA **91:** 8787–8791

90 Dilley R., Nishiyama Y., Gombos Z. and Murata N. (2001) Bioenergetic responses of *Synechocystis* 6803 fatty acid desaturase mutants at low temperatures. J. Bioenerget. Biomembranes **33:** 135–141

91 Pick U., Gounaris K. and Barber J. (1987) Dynamics of photosystem-II and its light harvesting system in response to light changes in the halotolerant alga *Dunaliella salina*. Plant Physiol. **85:** 194–198

92 Minoda A., Sato N., Nozaki H., Okada K., Takahashi H., Sonoike K. et al. (2002) Role of sulfoquinovosyl diacylglycerol for the maintenance of photosystem II in *Chlamydomonas reinhardtii*. Eur. J. Biochem. **269:** 2353–2358

93 Yu B., Xu C. and Benning C. (2002) *Arabidopsis* disrupted in SQD2 encoding sulfolipid synthase is impaired in phosphate-limited growth. Proc. Natl. Acad. Sci. USA **99:** 5732–5737

94 Murata N., Higashi S. and Fujimura Y. (1990) Glycerolipids in various preparations of photosystem-II from spinach-chloroplasts. Biochim. Biophys. Acta **1019:** 261–268

95 Tremolieres A., Dainese P. and Bassi R. (1994) Heterogenous lipid distribution among chlorophyll-binding proteins of photosystem-II in maize mesophyll chloroplasts. Eur. J. Biochem. **221:** 721–730

96 Hagio M., Gombos Z., Várkonyi Z., Masamoto K., Sato N., Tsuzuki M. et al. (2000) Direct evidence for requirement of phosphatidylglycerol in photosystem II of photosynthesis. Plant Physiol. **124:** 795–804

97 Hagio M., Sakurai I., Sato S., Kato T., Tabata S. and Wada H. (2002) Phosphatidylglycerol is essential for the development of thylakoid membranes in *Arabidopsis thaliana*. Plant Cell Physiol. **43:** 1456–1464

98 Xu C., Hartel H., Wada H., Hagio M., Yu B., Eakin C. et al. (2002) The pgp1 mutant locus of *Arabidopsis* encodes a phosphatidylglycerolphosphate synthase with impaired activity. Plant Physiol. **129:** 594–604

99 Gombos Z., Várkonyi Z., Hagio M., Iwaki M., Kovács L., Masamoto K. et al. (2002) Phosphatidylglycerol requirement for the function of electron acceptor plastoquinone QB in the photosystem II reaction center. Biochem. **41:** 3796–3802

100 Kruse O., Hankamer B., Konczak C., Gerle C., Morris E., Radunz A. et al. (2000) Phosphatidylglycerol is involved in the dimerization of photosystem II. J. Biol. Chem. **275:** 6509–6514

101 Jarvis P., Dormann P., Peto C., Lutes J., Benning C. and Chory J. (2000) Galactolipid deficiency and abnormal chloroplast development in the *Arabidopsis* MGD synthase 1 mutant. Proc. Natl. Acad. Sci. USA **97:** 8175–8179

102 Nussberger S., Dörr K., Wang D. N. and Kühlbrandt W. (1993) Lipid-protein interactions in crystals of plant light-harvesting complex. J. Mol. Biol. **234:** 347–356

103 McAuley K., Fyfe P., Ridge J., Isaacs N., Cogdell R. and Jones M. (1999) Structural details of an interaction between cardiolipin and an integral membrane protein. Proc. Natl. Acad. Sci. USA **96:** 14706–14711

104 Camara-Artigas A., Brune D. and Allen J. P. (2002) Interactions between lipids and bacterial reaction centers determined by protein crystallography. Proc. Natl. Acad. Sci. USA **99:** 11055–11060

105 Powell G. L., Knowles P. F. and Marsh D. (1985) Association of spin-labelled cardiolipin with dimyristoylphosphatidylcholine-substituted bovine heart cytochrome c oxidase. A generalized specificity increase rather than highly specific binding sites. Biochim. Biophys. Acta **816:** 191–194

106 Powell G. L., Knowles P. F. and Marsh D. (1987) Spin label studies on the specificity of interaction of cardiolipin with beef heart cytochrome oxidase. Biochemistry **26:** 8138–8145

107 Powell G. L., Knowles P. F. and Marsh D. (1990) Incorporation of cytochrome oxidase into cardiolipin bilayers and induction of non-lamellar phases. Biochemistry **29:** 5127–5132

108 Wakeham M., Sessions R., Jones M. and Fyfe P. (2001) Is there a conserved interaction between cardiolipin and the type II bacterial reaction center? Biophys. J. **80:** 1395– 1405

109 Nogi T., Fathir I., Kobayashi M., Nozawa T. and Miki K. (2000) Crystal structures of photosynthetic reaction center and high-potential iron-sulfur protein from *Thermochromatium tepidum*: Thermostability and electron transfer. Proc. Natl. Acad. Sci USA **97:** 13561–13566

110 Heller B. A., Holten D. and Kirmaier C. (1995) Control of electron-transfer between the L-side and M-side of photosynthetic reaction centers. Science **269:** 940–945

111 Jordan P., Fromme P., Witt H. T., Klukas O., Saenger W. and Krauss N. (2001) Three-dimensional structure of the cyanobacterial photosystem I at 2.5 Angstrom resolution. Nature **411:** 909–917

112 Kühlbrandt W. and Wang D. N. (1991) Three-dimensional structure of plant light-harvesting complex determined by electron crystallography. Nature **350:** 130–134

113 Kühlbrandt W., Wang D. G. and Fujiyoshi Y. (1994) Atomic model of plant light-harvesting complex by electron-crystallography. Nature **367:** 614–621

114 Kühlbrandt W. (1994) Structure and function of the plant light-harvesting-complex, LHCII. Curr. Opin. Struct. Biol. **4:** 519–528

115 Reinsberg D., Booth P., Jegerschold C., Khoo B. and Paulsen H. (2000) Folding, assembly, and stability of the major light-harvesting complex of higher plants, LHCII, in the presence of native lipids. Biochemistry **39:** 14305–14313

116 Reference removed in proof

117 Selstam E. (1998) Development of thylakoid membranes with respect to lipids. In: Lipids in Photosynthesis: Structure, Function and Genetics, pp. 209–224, Siegenthaler P. A. and Murata N. (eds), Kluwer, Dordrecht

118 Sarafis V. (1998) Chloroplasts – a structural approach. J. Plant Physiol. **152:** 248–264

119 Virgin H. I., Kahn A. and von Wettstein D. (1963) The physiology of chlorophyll formation in relation to structural changes in chloroplasts. Photochem. Photobiol. **2:** 83–91

120 Thompson W. F. and White M. J. (1991) Chloroplast development and gene expression. Ann. Rev. Plant Physiol. Plant Mol. Biol. **42:** 423–466

121 von Wettstein D., Gough S. and Kannangara C. G. (1995) Chlorophyll biosynthesis. Plant Cell **7:** 1039–1057

122 Dreyfuss B. W. and Thornber J. P. (1994) Assembly of the light-harvesting complexes (LHCs) of photosystem II. Monomeric LHCIIb complexes are intermediates in the formation of oligomeric LHCIIb complexes. Plant. Physiol. **106:** 829–839

123 Droppa M., Masojidek J. and Horváth G. (1990) Changes of the polypeptide composition in thylakoid membranes during differentiation. Z. Naturforsch. **45c:** 253–257

124 Kóta Z., Horváth L., Droppa M., Horváth G., Farkas T. and Páli T. (2002) Protein assembly and heat stability in developing thylakoid membranes during greening. Proc. Natl. Acad. Sci. USA **99:** 12149–12154

125 Marsh D. (1997) Stoichiometry of lipid-protein interaction and integral membrane protein structure. Eur. Biophys. J. **26:** 203–208

126 Murphy D. J. (1986) The molecular organisation of the photosynthetic membranes of higher plants. Biochim. Biophys. Acta **864:** 33–94

127 Mysliwa-Kurdziel B., Barthelemy X., Strzalka K. and Franck F. (1997) The early stages of photosystem II assembly monitored by measurements of fluorescence lifetime, fluorescence induction and isoelectric focusing of chlorophyll-proteins in barley etiochloroplasts. Plant Cell Physiol. **38:** 1187–1196

128 Guseinova I. M., Suleimanov S. Y., Zulfugarov I. S. and Aliev J. A. (2000) Assembly of the light-harvesting complexes during plastid development. J. Fluorescence **10:** 255–259

129 Wada H. and Murata N. (1989) *Synechocystis* PCC6803 mutants defective in desaturation of fatty-acids. Plant Cell Physiol. **30:** 971–978

130 Droppa M., Kóta Z., Páli T., Szalontai B., Horváth L. I. and Horváth G. (1999) Structural-functional organization of thylakoids in developing chloroplasts. In: The Chloroplasts: From Molecular Biology to Biotechnology, pp. 55–60, Argyroudi-Akoyunoglou J. H. and Senger H. (eds), Kluwer, Dordrecht

131 Siefermann-Harms D. (1987) The light-harvesting and protective functions of carotenoids in photosynthetic membranes. Physiol. Plant. **69:** 561–568

132 Krol M., Ivanov A. G., Janson S., Kloppstech K. and Huner N. P. A. (1999) Greening under high light in cold temperature affects the level of xanthophyll-cycle pigments, early light-inducible proteins and light-harvesting polypeptides in wild-type barley and the Chlorina f2 mutant. Plant Physiol. **120:** 193–203

133 Gounaris K., Brain A. R. R., Quinn P. J. and Williams W. P. (1984) Structural reorganisation of chloroplast thylakoid membranes in response to heat-stress. Biochim. Biophys. Acta **766:** 198–208

134 Thomas P. G., Dominy P. J., Vigh L., Mansourian A. R., Quinn P. J. and Williams W. P. (1986) Increased thermal stability of pigment-protein complexes of pea thylakoids following catalytic hydrogenation of membrane lipids. Biochim. Biophys. Acta **849:** 131–140

135 Kóta Z., Szalontai B., Droppa M., Horváth G. and Páli T. (2002) The formation of an inverted hexagonal phase from thylakoid membranes upon heating. Cell. Mol. Biol. Lett. **7:** 126–128

136 Williams W. P. (1998) The physical properties of thylakoid membrane lipids and their relation to photosynthesis. In: Lipids in Photosynthesis: Structure, Function and Genetics, pp. 103–118, Siegenthaler P. A. and Murata N. (eds), Kluwer, Dordrecht

137 Nash D., Miyao M. and Murata N. (1985) Heat inactivation of oxygen evolution in photosystem II particles and its acceleration by chloride depletion and exogenous manganese. Biochim. Biophys. Acta **807:** 127–133

138 Lee A. G. (2000) Membrane lipids: It’s only a phase. Curr. Biol. **10:** R37–R380

139 Wieslander A., Christiansson A., Rilfors L. and Lindblom G. (1980) Lipid bilayer stability in membranes – regulation of

lipid-composition in *Acholeplasma – laidlawii* as governed by molecular shape. Biochemistry **19:** 3650–3655

140 Goldfine H., Johnston N. C., Mattai J. and Shipley G. G. (1987) Regulation of bilayer stability in clostridium-butyricum – studies on the polymorphic phase-behavior of the ether lipids. Biochem. **26:** 2814–2822

141 Takeuchi T. S. and Thornber J. P. (1994) Heat-induced alterations in thylakoid membrane protein composition in barley. Austral. J. Plant Physiol. **21:** 759–770

142 Simidjiev I., Barzda V., Mustárdy L. and Garab G. (1998) Role of thylakoid lipids in the structural flexibility of lamellar aggregates of the isolated light-harvesting chlorophyll a/b complex of photosystem II. Biochemistry **37:** 4169–4173

143 Simidjiev I., Stoylova S., Amenitsch H., Jávorfi T., Mustárdy L., Laggner P. et al. (2000) Self-assembly of large, ordered lamellae from non-bilayer lipids and integral membrane proteins in vitro. Proc. Natl. Acad. Sci. USA **97:** 1473–1476

144 Gad M., Awai K., Shimojima M., Yamaryo Y., Shimada H., Masuda T. et al. (2001) Accumulation of plant galactolipid affects cell morphology of *Escherichia coli*. Biochem. Biophys. Res. Commun. **286:** 114–118

145 Marsh D. (1996) Components of the lateral pressure in lipid bilayers deduced from HII phase dimensions. Biochim. Biophys. Acta **1279:** 119–123

146 Barzda V., Istokovics A., Simidjiev I. and Garab G. (1996) Structural flexibility of chiral macroaggregates of light-harvesting chlorophyll a/b pigment-protein complexes. Light-induced reversible structural changes associated with energy dissipation. Biochem. **35:** 8981–8985

147 Simidjiev I., Barzda V., Mustárdy L. and Garab G. (1997) Isolation of lamellar aggregates of the light-harvesting chlorophyll a/b protein complex of photosystem II with long-range chiral order and structural flexibility. Anal. Biochem. **250:** 169–175

148 Cseh Z., Rajagopal S., Tsonev T., Busheva M., Papp E. and Garab G. (2000) Thermooptic effect in chloroplast thylakoid membranes. Thermal and light stability of pigment arrays with different levels of structural complexity. Biochemistry **39:** 15250–15257

149 Garab G., Cseh Z., Kovács L., Rajagopal S., Várkonyi Z., Wentworth M. et al. (2002) Light-induced trimer to monomer transition in the main light-harvesting antenna complex of plants: thermo-optic mechanism. Biochemistry **41:** 15121–15129

150 Lipowsky R. (1995) The morphology of lipid membrane. Curr. Opin. Struct. Biol. **5:** 531–540

151 Gruner S. M. (1985) Instrinsic curvature hypothesis for biomembrane lipid composition: a role for nonbilayer lipids. Proc. Natl. Acad. Sci. USA **82:** 3665–3669

152 Botelho A., Gibson N., Thurmond R., Wang Y. and Brown M. (2002) Conformational energetics of rhodopsin modulated by nonlamellar-forming lipids. Biochemistry **41:** 6354–6368

153 Nolan W. G., Hopkins H. P. J. and Kalini S. A. M. (1992) Differential scanning calorimetric investigation of pea chloroplast thylakoids and thylakoid fractions. Arch. Biochem. Biophys. **297:** 19–27

154 Stopar D., Jansen K. A. J., Páli T., Marsh D. and Hemminga M. A. (1997) Membrane location of spin-labeled M13 major coat protein mutants determined by paramagnetic relaxation agents. Biochemistry **36:** 8261–8268

155 Páli T. and Marsh D. (2002) Structural studies on membrane proteins using non-linear spin label EPR spectroscopy. Cell. Mol. Biol. Lett. **7:** 87–91

156 Bashtovyy D., Hemminga H. M., Marsh D. and Páli T. (2001) Constrained modelling of spin-labelled major coat protein mutants from M13 bacteriophage in a phospholipid bilayer. Protein Sci. **10:** 979–987

157 Kühlbrandt W. (1995) Structure of the plant light-harvesting complex determined by electron crystallography of 2D crystals. Protein Eng. **8:** 42–42 Suppl. S

158 Chiu M., Nollert P., Loewen M., Belrhali H., Pebay-Peyroula E., Rosenbusch J. et al. (2000) Crystallization in cubo: general applicability to membrane proteins. Acta Crystallogr. Biol. Crystallogr. **56:** 781–784

159 Caffrey M. (2000) A lipid's eye view of membrane protein crystallization in mesophases. Curr. Opin. Struct. Biol. **10:** 486–497

160 Rosenbusch J. (2001) Stability of membrane proteins: relevance for the selection of appropriate methods for high-resolution structure determinations. J. Struct. Biol. **136:** 144–157

161 Faham S. and Bowie J. (2002) Bicelle crystallization. A new method for crystallizing membrane proteins yields a monomeric bacteriorhodopsin structure. J. Mol. Biol. **316:** 1–6

162 Ruban A. V., Wentworth M., Yakushevska A. E., Andersson J., Lee P. J., Keegstra W. et al. (2003) Plants lacking the main light-harvesting complex retain photosystem II macro-organization. Nature **421:** 648–652