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REVIEW

Molecular dynamics of the diatom thylakoid membrane under different light conditions

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Abstract During the last years significant progress was achieved in unraveling molecular characteristics of the thylakoid membrane of different diatoms. With the present review it is intended to summarize the current knowledge about the structural and functional changes within the thylakoid membrane of diatoms acclimated to different light conditions. This aspect is addressed on the level of the organization and regulation of light-harvesting proteins, the dissipation of excessively absorbed light energy by the process of non-photochemical quenching, and the lipid composition of diatom thylakoid membranes. Finally, a working hypothesis of the domain formation of the diatom thylakoid membrane is presented to highlight the most prominent differences of heterokontic thylakoids in comparison to vascular plants and green algae during the acclimation to low and high light conditions.

Keywords Diatom · Light acclimation · Lipid composition · NPQ · Pigment–protein complex · Thylakoid membrane · Xanthophyll cycle

Abbreviations

Chl	Chlorophyll
DDE	Diadinoxanthin de-epoxidase
Ddx	Diadinoxanthin
DEP	Diatoxanthin epoxidase
DGTS	1,2-Diacylglyceryl-3-*O*-4′-(*N*,*N*,*N*-trimethyl)-homoserine
DTT	Dithiothreitol
Dtx	Diatoxanthin
FCP	Fucoxanthin chlorophyll protein
HL	High light
LHC	Light-harvesting complex
LL	Low light
MGDG	Monogalactosyldiacylglycerol
DGDG	Digalactosyldiacylglycerol
NPQ	Non-photochemical quenching of chlorophyll *a* fluorescence
PC	Phosphatidylcholine
PE	Phospatidylethanolamine
PG	Phosphatidylglycerol
PQ	Plastoquinone
PS	Photosystem
qE	High-energy-state quenching
qI	Photoinhibitory quenching
qT	Quenching related to state transitions
SQDG	Sulfoquinovosyldiacylglycerol
VDE	Violaxanthin de-epoxidase
Zx	Zeaxanthin

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Introduction

In the seventies of the twentieth century intensive research was done to document the molecular changes in the structure of the chloroplast during high-light and low-light acclimation. It was shown that in green algae and vascular plants the chloroplasts develop more grana stacks in low

light (Reger and Krauss 1970, Anderson et al. 1973, Lichtenthaler et al. 1981). These grana stacks are enriched in light-harvesting complexes associated with PSII (LHCII). Recently, Haferkamp et al. (2010) have shown that the protein packing within the grana membrane is necessary to improve the light-harvesting function. Therefore, low light-induced grana formation leads to an increase in the functional size of the PSII antenna and to an altered PSII/PSI stoichiometry (Melis 1991). Since the grana membranes contain more LHCII but less PSII reaction centers, the low light chloroplasts have a lower PSII/PSI ratio together with a decreased Chl *a*/*b* ratio. In addition, since low light-acclimated chloroplasts contain more densely packed membranes with a very high chlorophyll concentration, the overall pigment content per chloroplast or per cell is increased. However, due to the package effect of the chlorophylls in the grana membranes, the in vivo absorption coefficient (a^*_{phy}) decreases (Berner et al. 1989). Since a^*_{phy} reflects the absorption efficiency per unit chlorophyll, low light-acclimation leads to the paradoxon that the cells have to invest into pigments whose efficiency for light-harvesting is reduced. Therefore, in low light light-acclimated green algae the photosynthetic efficiency under light limitation is not significantly enhanced compared to high light-acclimated cells. However, the latter show a strongly increased photosynthetic capacity (P_{max}) which is mainly due to the greater capacity of electron transport and carboxylation reactions per absorption unit. Therefore, high light-acclimated cells contain more cytochrome *f*, which is involved in the rate limiting step of the photosynthetic electron transport chain, and more enzymes catalyzing the CO_2 fixation in the Calvin-cycle (Wilhelm 1993).

In the last few years the signal cascade has been elucidated which induces altered gene expression in response to changing light conditions. It was shown that the redox state of the plastoquinone pool (PQ) is a key element in this signal cascade, which regulates the short-term imbalance of the energy distribution between both photosystems via so-called state 1/state 2-transitions (Pfannschmidt et al. 2009). In addition, the redox state of PQ is directly or indirectly involved in the activation of transcription factors encoding for those protein complexes which are altered during high light- low light-acclimation (Pesaresi et al. 2009). However, the documented events during light acclimation hold true only for the green algal lineage but are not applicable to heterokontic algae. In chlorophyll *c*-containing algae the ultrastructure of the chloroplast is characterized by an envelope consisting of four membranes and a so-called "girdle lamella" running in parallel to the envelope inside the chloroplast. The girdle lamella and the remaining thylakoids run in parallel in stacks of three representing the whole photosynthetic membrane system in these algae. In contrast to green algae it was shown that neither in the diatom *Thalassiosira weisflogii* nor in the Eustigmatophyte *Nanochloropsis salina* nor in the dinoflagellate *Prorocentrum* sp. the number of membrane stacks is changing in a HL to LL transition or vice versa and remain always fixed to the number of three. In addition, the ratio of Chl *a*/Chl *c*, which is equivalent to the ratio of Chl *a*/*b* in green algae, showed no or only minor changes. The same holds true for the ratio of Chl *a*/fucoxanthin (see also below). This indicates that, in contrast to green algae, no increase in the antenna size should be observed in heterokontic algae. This assumption was confirmed by Dubinsky et al. (1986) who showed that the absorption cross-section for PSII is not increased under low light conditions in Chl *c*-containing cells. State transitions were found to be absent in diatoms (Owens 1986). In addition, Grouneva et al. (2009) have demonstrated that even in darkness the PQ pool in diatoms is strongly reduced via chlororespiration. Therefore, it is questionable whether the redox state of the PQ pool can act as sensor to modulate the transcription control in diatoms. This assumption is supported by the fact that no redox control of light acclimation via the PQ pool was documented in diatoms. A recent transcriptome study of Nymark et al. (2009) showed that in diatoms the genes encoding for the chlorophyll biosynthesis are down-regulated during the initial phase of high light-acclimation, whereas genes involved in light protection and ROS scavenging are up-regulated. However, the light sensors and the signaling cascade addressing these genes are not yet identified. In summary, in response to high light diatoms reduce only the amount of chlorophyll per cell (e.g., Jakob et al. 2007) by lowering the thylakoid area per chloroplast but maintain the triple structure of the membranes.

The organization of light-harvesting proteins in diatoms

Diatoms possess fucoxanthin chlorophyll *a*/*c* binding proteins (FCPs) as peripheral antennae of the photosystems. Major progress was made during the last years regarding the investigation of the antenna organization state, pigment binding capacity and protein composition. The antenna of diatoms is organized in oligomeric complexes. In the centric diatom *Cyclotella meneghiniana* a trimeric FCPa-exists besides a hexa- or nonameric FCPb-complex (Büchel 2003; Beer et al. 2006). In contrast, the pennate diatom *P. tricornutum* contains only a single antenna fraction which is basically organized as a trimer (Lepetit et al. 2007; Joshi-Deo et al. 2010), but can be isolated in higher oligomeric states (most probably hexameric) by using very gentle solubilization conditions (Lepetit et al. 2007).

Similarly, higher oligomeric complexes of FCPa and FCPb were found in *C. meneghiniana* by reducing the concentration of detergent during the solubilization (Lepetit et al. 2010). So far, no FCP-PSII supercomplexes comparable to the LHCII-PSII supercomplexes in vascular plants could be isolated. However, CD measurements of intact diatom cells suggest such structures (Szábo et al. 2008), and Nagao et al. (2007, 2010) were able to isolate PSII complexes from *Chaetoceros gracilis* which still contained FCP proteins. However, these proteins were separated from the PSII cores after gel filtration, suggesting a loose binding to the PSII core complex or a co-precipitation (Nagao et al. 2007). Thus, the isolation of functional FCP-PSII supercomplexes in diatoms remains a challenging task for the future.

The FCP complexes contain very high amounts of carotenoids, in particular fucoxanthin (Fx). Although there exist minor differences in the pigment content of FCPs from different diatoms, or in comparison of FCPa and FCPb, the current model for FCP pigmentation can be regarded as a general model for diatoms. Thus, one FCP monomer binds eight molecules of Chl *a*, eight Fx and two Chl *c* (Premvardhan et al. 2010). A minor amount of diadinoxanthin (Ddx) was found to be protein-bound to the FCP, thus adding at least one molecule of Ddx per FCP monomer (Lepetit et al. 2010).

The protein composition of the peripheral FCP now also comes to light. Basically, three different antenna protein families are encoded within the diatom genome. These are (i) the "classical" light-harvesting proteins, called Lhcf; (ii) the Lhcr proteins which are related to the red algal LHCI proteins; and (iii) the ancient Li818 proteins, called Lhcx (Eppard et al. 2000; Green 2007; Koziol et al. 2007). Using immunogold electron microscopy, Westermann and Rhiel (2005) demonstrated that the Lhcr and the Lhcf proteins are present in comparable concentrations in the diatom chloroplast, while the Lhcx proteins are much less abundant. Although this experimental approach can only give an estimation of the relative abundance of the respective antenna proteins, since the different Lhc antibodies might have different binding affinities, a recent analysis based on mass spectrometry basically confirmed these results. In the study of Lepetit et al. (2010), 12 of the 15 Lhcf proteins encoded in the genome of *P. tricornutum* were identified within the FCP (Lepetit et al. 2010). In addition, seven of the 14 Lhcr proteins were detected. However, only Lhcx1 was present in FCPs isolated from cells grown under LL conditions (Lepetit et al. 2010). Furthermore, four FCP-related, but not further annotated proteins, as well as the Lhl1 protein, a member of the newly discovered RedCAP (red lineage of Chl *a*/*b*-binding like proteins; Engelken et al. 2010) were identified. This analysis extended earlier results based on western blots, where only Lhcf and Lhcx proteins (Beer et al. 2006), or only Lhcf and Lhcr proteins (Brakemann et al. 2006) were found within the FCP. To summarize, Lhcf and Lhcr proteins mainly constitute the FCP, but other, more special light-harvesting proteins, are also present. This leads to a huge diversity of FCP complexes, which significantly extends the complexity of the peripheral LHC from vascular plants.

In addition to the peripheral FCP, diatoms contain a specific FCP, which is tightly connected to PSI (Veith and Büchel 2007; Veith et al. 2009; Lepetit et al. 2008, 2010). It contains significantly more protein-bound Ddx and much less Fx than the major FCP. In contrast to the peripheral FCP, this PSI antenna is almost exclusively composed of Lhcr proteins (Lepetit et al. 2010). These proteins are closely related to the PSI antenna of red algae and cryptophytes. In fact, also the Lhcr proteins of red algae bind a very high amount of xanthophyll cycle pigments (Grabowski et al. 2000). Taken together, diatoms contain a peripheral antenna, which delivers energy to both photosystems. In addition, they possess a PSI-specific antenna, whose function is yet a matter of debate (Lepetit et al. 2010).

Regulation of structure and composition of FCPs in response to light acclimation

Based on the results obtained for vascular plants, it could be hypothesized that also diatoms exhibit several mechanisms on the level of the photosynthetic membrane to react to changes in light conditions (e.g., a shift from LL to HL conditions):

(i) changes in the ratio of the number of FCP complexes per photosystem; (ii) a reduction/increase of the amount of total pigment–protein complexes without a change in the FCP/photosystem ratio; (iii) a different pigmentation of the FCPs; (iv) variations in the protein composition of the FCP complexes.

The reduction of the number of antenna complexes per photosystem is a common mechanism during the HL acclimation of vascular plants (Anderson 1986; Kurasova et al. 2002). For *P. tricornutum* and *C. meneghiniana* only a minor decrease of the ratio FCP to photosystems of approximately 10% was observed in HL (Gundermann and Büchel 2008; Lepetit et al. 2010). Based on the Fx to Chl *a* ratio as an indicator for the number of antenna complexes per photosystem, a similarly slight reduction (Anning et al. 2000) or reductions of up to 30% in HL were inferred for other diatoms (Smith and Melis 1988; Van De Poll et al. 2005).

However, several reports show that the total amount of pigment–protein complexes per cell, also including the photosystems, strongly decreased in diatoms exposed to

HL conditions. This can be deduced from the findings that the cellular content of Chl *a* and Fx (Anning et al. 2000; Van de Poll et al. 2005), as well as the thylakoid surface area and the number of thylakoid lamellae, is reduced (Rosen and Lowe 1984; Janssen et al. 2001). The amount of MGDG, a lipid strongly associated with the pigment–protein complexes, is reduced under HL conditions (see below, Lepetit et al. 2010). In addition, RNA transcript levels of most of the genes encoding for FCPs and photosystem core proteins are down-regulated (Nymark et al. 2009). Quantitative 2D-gel electrophoresis revealed that the amount of the proteins belonging to the pigment–protein complexes significantly decreased compared to the ATP synthase and the proteins serving as electron/proton carriers within the thylakoid membrane, such as the $Cytb_6f$ complex (D. Volke, unpublished results). Thus, diatoms seem to co-regulate the number of antennae and photosystem core complexes to fine-tune the amount of absorbed light energy with the biochemical capacity of the cell.

The most significant change in the pigment composition of the peripheral FCP complex upon a transfer from LL to HL conditions is a massive increase in the amount of Ddx cycle pigments (Lepetit et al. 2010), while the content of Fx or Chl *c* per Chl *a* is not affected (Gundermann and Büchel 2008; Gildenhoff et al. 2010; Lepetit et al. 2010). Due to the decrease in the total amount of Chl *a*, the relative increase of Ddx cycle pigments can be extremely high (Lepetit et al. 2010). In contrast, the pigment composition of the PSI-specific FCP is unaffected by changes in the light conditions.

Finally, several modifications in the protein composition of the FCP occur during the acclimation from LL to HL conditions. In different diatoms the analysis of the mRNA transcript level and the amount of proteins revealed that most of the Lhcf proteins are down-regulated in HL (Janssen et al. 2001; Becker and Rhiel 2006; Nymark et al. 2009; Park et al. 2010). In contrast, many of the Lhcx proteins, in particular the FCP-associated Lhcx1 (Beer et al. 2006, Lepetit et al. 2010), are strongly increased (Becker and Rhiel 2006; Beer et al. 2006; Nymark et al. 2009; Bailleul et al. 2010; Park et al. 2010; Zhu and Green 2010). The majority of the lhcr genes is decreased in HL (Nymark et al. 2009), although for some of them the gen and protein expression level was found to be increased (Nymark et al. 2009; Lepetit et al. 2010). Whereas Lhcx proteins contribute to the non-photochemical quenching of Chl *a* fluorescence (NPQ; see below), the Lhcf proteins are clearly functional in light-harvesting. Lhcr proteins can bind high amounts of Ddx cycle pigments (Lepetit et al. 2010). Thus, diatoms modify their antenna protein composition in HL in favor of proteins involved in photoprotection, while light-harvesting proteins are reduced.

Dissipation of excessively absorbed light energy under HL conditions

A shift from LL to HL or vice versa causes an imbalance between the light perception and the utilization capacity of the biochemical machinery of the cell. There are several mechanisms to adapt to changing light conditions by modifications on the level of the thylakoid membrane structure and the embedded photosynthetic pigment–protein complexes (see above) and thylakoid membrane lipids (see below). However, it is conceivable that these modifications may not be an adequate answer to rapidly changing light conditions. In this case, the activation of alternative energy sinks and the dissipation of excessively absorbed light energy by the mechanism of non-photochemical quenching (NPQ) regulate the light utilization in the diatom cell.

Alternative energy sinks comprise several mechanisms and biochemical reactions like, e.g., cyclic electron flow in PSII (Prásil et al. 1996) and PSI (e.g., Joliot and Joliot 2006), the Mehler reaction (Asada 1999), CO_2-concentrating mechanisms (Giordano et al. 2005), photorespiration (Badger et al. 2000), and the excretion of carbon (Sukenik et al. 1997). Indeed, in a number of studies the activation of alternative electron sinks in diatoms under exposure to HL illumination was reported (e.g., Flameling and Kromkamp 1998; De Brouwer and Stal 2002; Lavaud et al. 2002b; Feikema et al. 2006; Wagner et al. 2006; Jakob et al. 2007). However, information about mechanistic aspects and components involved in these alternative electron pathways are largely missing for diatom cells.

The thermal energy dissipation by the mechanism of NPQ consists of a high-energy-state (qE), a state transition (qT), and a photoinhibitory (qI) component (e.g., Niyogi 1999). In diatoms, NPQ is dominated by qE, whereas qT is missing (Owens 1986) and qI is strongly reduced (Ting and Owens 1994). Diatoms have in common with vascular plants and green algae that the qE component is modulated by the conversion of XC pigments. In diatoms, this comprises the de-epoxidation of Ddx to Dtx by the enzyme Ddx de-epoxidase (DDE; for a recent review see Goss and Jakob 2010).

NPQ in diatoms is closely correlated with the concentration of Dtx (Lavaud et al. 2002a). On one hand, such a direct correlation of Dtx and NPQ in combination with large pools of XC pigments could promote extraordinarily high values of NPQ under HL illumination in diatoms (Lavaud et al. 2002a; Ruban et al. 2004). On the other hand, for the transition from HL to LL conditions a very efficient conversion of Dtx back to Ddx is indispensible to rapidly switch from a photo-protective to a light-harvesting state (Goss et al. 2006).

Although the linear correlation between the Dtx concentration and the concomittant NPQ values has been shown for a number of different diatom species (Jakob et al. 1999; Lavaud et al. 2004; Goss et al. 2006), the slope of this correlation is subject to variations. This was documented for, e.g., *S. costatum* which exhibits a lower NPQ per Dtx compared to other diatom species and for *T. weissflogii* where the quenching efficiency of Dtx decreases during exposure of the cells to prolonged HL illumination (Lavaud et al. 2004). A comparable result was obtained in a study of Schumann et al. (2007) on *P. tricornutum*, where HL grown cells substantially increased the Ddx cycle pigment pool size but maximum NPQ values remained almost unchanged in comparison to LL-cultivated cells. A recent study of Bailleul et al. (2010) showed that variations in the quenching efficiency of Dtx can be found even in the comparison of different ecotypes of *P. tricornutum*. Thus, several authors hypothesized that the NPQ capacity can be regarded as an adaptive response to ecological conditions (Meyer et al. 2000; Dimier et al. 2007; Lavaud et al. 2007). The NPQ capacity seems to depend on two factors: (i) the binding of Dtx either to the FCPs or the lipid phase of the FCPs and (ii) the expression levels of the stress-related proteins.

(i) Lavaud et al. (2004) were the first to suggest that a lower quenching efficiency of Dtx could be due to the fact that Dtx is not bound to those FCPs which are involved in the NPQ process. Instead, this fraction of Dtx could be present in the lipid phase of the thylakoid membrane. Schumann et al. (2007) provided evidence that under HL conditions the majority of the additionally synthesized Dtx did not contribute to NPQ although it was still associated with the FCPs. Finally, Lepetit et al. (2010) presented experimental evidence that this part of the Dtx pool is located in a lipid shield surrounding the FCPs but is not protein-bound. It could be speculated that these Dtx molecules serve as antioxidants and/or as a reservoir for the synthesis of the light-harvesting pigment Fx after a shift from HL to LL conditions (Lohr and Wilhelm 1999; Lepetit et al. 2010)

(ii) Several studies showed that the up-regulation of proteins, which show homologies with LI818 (like Lhcx proteins, see above), is part of the photoprotective answer of diatoms upon exposure to HL illumination (Oeltjen et al. 2004; Beer et al. 2006; Nymark et al. 2009; Bailleul et al. 2010; Park et al. 2010; Zhu and Green 2010). In addition, Bailleul et al. (2010) provided evidence that the capacity of NPQ is correlated with the expression level of the Lhcx1 protein and that this protein is involved in a constitutive adaptation to environmental conditions

Although the largest part of NPQ is composed of a Dtx-dependent antenna quenching it has to be emphasized that recent results indicate the existence of a reaction center type quenching in diatoms. Eisenstadt et al. (2008) showed in *P. tricornutum* that NPQ can be present in the absence of Dtx. From measurements of Chl *a* fluorescence and thermoluminescence under different experimental conditions it was concluded that excess light treatment causes significant changes in the functional organization of the PSII reaction centre leading to a thermal dissipation of excitation energy. Recently, these results were confirmed by radio isotope discrimination experiments giving evidence that oxygen uptake in the photosynthetic machinery contributes to energy dissipation (Eisenstadt et al. 2010). Due to its independence on Dtx, the reaction centre type of NPQ could be particularly important directly after a rise in the light intensity when the level of Dtx in the cell is still low.

Mechanistic aspects of Dtx-dependent NPQ in diatoms

In vascular plants the formation of NPQ under HL illumination requires the generation of a trans-thylakoidal proton gradient to activate the de-epoxidase which catalyzes the de-epoxidation of violaxanthin to zeaxanthin (Zx, Hager 1967, 1969). In addition, the decrease of the lumenal pH and the presence of Zx induce structural changes of the LHCs (Horton et al. 1991; Walters et al. 1994). In this process, the PsbS and the LI818 (now termed LHCSR) protein are essential for the sensing of the lumenal pH in vascular plants and green algae, respectively (Li et al. 2000, 2002; Peers et al. 2009). The mechanistic details of the Zx-dependent induction of NPQ have been described by different models which are still under debate. In the direct quenching mechanism (Frank et al. 1994) NPQ is generated by the direct interaction of a Zx and a Chl *a* molecule which results in an energy transfer from Chl *a* to Zx and a thermal dissipation of the energy. According to Holt et al. (2005) Zx, which is bound to the V1 site of LHCII, is suggested to act as the direct quencher of excitation energy. The mechanism proposed by these authors requires the formation of a Zx-Chl *a* radical pair. The indirect quenching mechanism relies on the aggregation of the peripheral LHCII which is allosterically regulated by the presence of Zx (e.g., Horton et al. 1991, 2008). The full induction of NPQ takes place in the presence of Zx which supports the establishment of the actual quenching site within the LHCII which is composed of a Chl *a* homodimer or a Chl *a*/lutein heterodimer (Pascal et al. 2005; Ruban et al. 2007). Recently, Holzwarth et al. (2009) proposed the existence of two different quenching sites in vascular plants. According to their measurements, one quenching

site depends on the presence of Zx and is located in the minor PSII antenna proteins, whereas the second quenching site is independent of Zx and consists of detached and aggregated LHCII complexes.

For diatoms the current knowledge is much less conclusive and does not allow such a detailed description of the mechanism of NPQ. However, there is growing evidence that the formation of NPQ basically relies on comparable mechanisms as in vascular plants and green algae (see also Goss et al. 2006; Goss and Jakob 2010). Ruban et al. (2004) presented a putative sequence of events in the formation of NPQ in diatoms which involves the presence of a proton gradient to activate the de-epoxidation of Ddx to Dtx (Grouneva et al., 2006) and to induce a conformational change of the antenna. In succession, Dtx may be switched into an "activated state" (Lavaud and Kroth 2006) and partitioned into the active NPQ locus of the FCPs. In this way, a fast relaxation of NPQ after the breakdown of the ΔpH may be prevented (Goss et al. 2006). A recent study of Cruz et al. (2011) confirmed that a certain magnitude of the proton gradient is needed for the complete induction of NPQ in diatoms. A reasonable explanation for these results could be found in a conformational change of the antenna induced by the pH decrease of the thylakoid lumen as suggested by Ruban et al. (2004). Indications for such a conformational change in terms of an aggregation of FCPs were presented in Miloslavina et al. (2009). The time-resolved fluorescence data of this study suggest that in *P. tricornutum* and *C. meneghiniana* a part of the FCPs is detached from PSII during exposure to HL illumination and becomes aggregated. In vascular plants such a mechanism was described for LHCII complexes and represents an important part of the total NPQ (Miloslavina et al. 2008).

The hypotheses raised by Ruban et al. (2004) and Lavaud and Kroth (2006) implicate that the binding site of Dtx may play an important role in the regulation of NPQ. Indeed, Gundermann and Büchel (2008) demonstrated that in *C. meneghiniana* Dtx which is bound to the trimeric FCPa leads to a strong fluorescence quenching of the isolated FCP, whereas Dtx bound to the oligomeric FCPb does not induce quenching. This observation of two subpopulations of FCPs which differ with respect to their fluorescence quenching ability correlates with the finding that NPQ in diatoms can be allocated to two different quenching sites (Miloslavina et al. 2009). Quenching site 1 (Q1) can detach from PSII, aggregates to oligomeric complexes (see above) and shows a quenching which is relatively independent of the presence of Dtx. Q1 could be identical with the FCPb complex found in the study of Gundermann and Büchel (2008). The second quenching site (Q2) stays attached to PSII and its quenching is triggered by the formation of Dtx. Thus, it was suggested that Q2 could be identical to the trimeric FCPa.

Vascular plants possess the PsbS protein which belongs to the LHC superfamily and plays an essential role in NPQ (see above). However, no homolog of the PsbS protein was found in the genome of *T. pseudonana* and *P. tricornutum* (Armbrust et al. 2004; Montsant et al. 2005; Bowler et al. 2008). Instead, diatoms possess homologues of the stress-related LHCSR/LI818 proteins known to be involved in the photoprotective answer of green algae (Savard et al. 1996). In *C. meneghiniana fcp6* and *fcp7* were identified as *LI818* homologues (Beer et al. 2006). In *T. pseudonana* and *P. tricornutum* the *Lhcx* family was found to show homologies with the *LI818* gene (Zhu and Green 2010; Nymark et al. 2009). For the fcp6/-7 of *C. meneghiniana* and for different Lhcx proteins of *T. pseudonana* and *P. tricornutum* a correlation between their expression level and the extent of NPQ during a LL to HL transition was demonstrated (Bailleul et al. 2010; Zhu and Green 2010). Thus, Lhcx proteins clearly have a role as molecular effectors of NPQ. However, the diversity of stress-related proteins in diatoms is in contrast to the single PsbS protein in vascular plants and implies different functions of these proteins. In addition, Lhcx proteins do not possess comparable acidic amino acids as they were found for the PsbS/LI818 protein, which makes a pH-sensing function of Lhcx proteins questionable (Bailleul et al. 2010). Instead, Lhcx proteins may play a structural role, e.g., in the binding of de novo-synthesized Dtx as suggested by Zhu and Green (2010). This assumption might be true in particular for Lhcx6 in *T. pseudonana* (Zhu and Green 2010) and fcp6/-7 in *C. meneghiniana* (Beer et al. 2006). On the other hand, a Dtx binding is not likely for *lhcx1* whose expression level is correlated with NPQ, even in the absence of changes in the Dtx content (Bailleul et al. 2010). In this case, Lhcx1 might play a role in NPQ through the induction of conformational changes of FCPs or by influencing the connectivity between antenna and photosystems.

The existence of different quenching sites within PSII and PSI together with the observation that the xanthophyll cycle pigments are enriched in lipid domains around these sites asks for the specific role of lipids in the thylakoid membrane of diatoms.

Lipid composition of diatom thylakoid membranes in low and high light

In general, the thylakoid membrane of diatoms consists of the same lipid classes as the thylakoids of vascular plants and green algae (for a review see Goss and Wilhelm 2009). The thylakoid membranes of diatoms are, however, enriched in negatively charged lipids compared to the membranes of plants and green algae which are dominated by the neutral galactolipids (Table 1, Vieler et al. 2007a, b,

Table 1 Lipid composition of thylakoid membranes of the diatoms *C. meneghiniana* and *P. tricornutum* grown under low (LL, 10–20 μmol m^{-2} s^{-1} PAR) and high light (HL, 160–180 μmol m^{-2} s^{-1}) intensities

	Cm LL	*Cm HL*	*Pt LL*	*Pt HL*	*Cr*	*So*
MGDG	28.1 ± 7.3	21.1 ± 4.3	37.3 ± 11.7	20.4 ± 4.4	37.9 ± 6.6	44.6 ± 4.6
DGDG	11.9 ± 4.3	13.7 ± 3.0	16.9 ± 3.6	19.1 ± 4.5	20.5 ± 8.3	29.2 ± 3.7
SQDG	32.8 ± 10.7	39.7 ± 3.9	23.9 ± 4.3	34.7 ± 6.3	14.1 ± 8.1	10.6 ± 3.5
PG	11.9 ± 4.7	9.1 ± 2.5	6.7 ± 5.3	10.6 ± 3.6	8.0 ± 2.8	9.2 ± 0.4
PC	15.3 ± 3.5	16.4 ± 2.3	15.2 ± 2.3	14.6 ± 3.8		6.3 ± 1.3
PE	–	–	–	0.6 ± 1.3	4.9 ± 4.0	–
DGTS	–	–	–	–	14.7 ± 3.8	
Ratio neutral/negative lipids	1.23	1.05	2.26	1.19	3.52	4.05

The lipid concentrations are depicted as %lipid of the total thylakoid membrane lipid. This table shows the mean values and standard deviations of 4–8 independent lipid determinations. For a description of lipid extraction, analysis and quantification see Goss et al. (2009)

Cm Cyclotella meneghiniana, *Pt Phaeodactylum tricornutum*, *Cr Chlamydomonas reinhardtii*, *So Spinacia oleracea*

Goss et al. 2009). The most abundant lipid in diatom thylakoids is the anionic lipid SQDG which is found in equal or even higher concentrations than the main lipid of plant thylakoids, the neutral galactolipid MGDG. SQDG and the anionic phospholipids PG amount to more than 40% of the total thylakoid lipid in LL-grown *C. meneghiniana* cells (Goss et al. 2009), whereas in vascular plants the negatively charged lipids represent only 15–20% of the thylakoid membrane lipid (Murata and Siegenthaler 1998; Goss et al. 2009). The high amount of anionic lipids leads to a concomitant decrease of the neutral galactolipids MGDG and DGDG, which represent more than 70% of the thylakoid lipid in vascular plants, but amount to only about 50% in low light grown diatoms. Consequently, in LL-grown *C. meneghiniana* and *P. tricornutum* ratios of neutral to negatively charged lipids of around 1.2 and 2.2 are observed, respectively, whereas this ratio is significantly higher in green algae (3.5) and vascular plants (4) (Table 1, Goss et al. 2009). Illumination of diatom cells with HL intensities leads to a further increase of the concentration of negatively charged lipids. This increase is mostly caused by a higher content of SQDG while the PG concentrations are more stable in low light and high light grown *C. meneghiniana* and *P. tricornutum* (Table 1). The increased SQDG concentration in high light-adapted diatom thylakoids leads to a further reduction of the ratio of neutral to negatively charged lipids, which drops to a value of 1 in *C. meneghiniana* and 1.2 in *P. tricornutum*.

It is of further interest that the diatom thylakoid membranes contain significant concentrations of the phospholipid PC which in vascular plants does not belong to the thylakoid lipid classes, but is described as a contamination with the chloroplast envelope membrane (Joyard et al. 1998; Williams 1998). According to recent results, PC represents a lipid which is tightly bound to the diatom antenna complexes, FCPs (Lepetit et al. 2010).

Interestingly, the FCPs are enriched in MGDG which, besides PC, remains as the only lipid in highly purified antenna complexes from both LL- and HL-adapted *C. meneghiniana* and *P. tricornutum* cultures (Lepetit et al. 2010). It has been proposed that the MGDG, which is associated with the FCPs and forms a shield of lipid molecules around the antenna complexes, serves different functions: (i) it provides as a reservoir for the high concentrations of diadinoxanthin cycle pigments which are synthesized during HL cultivation of diatoms (see above), (ii) it also enables the solubilization of Ddx which is needed for its efficient de-epoxidation by the DDE (Goss et al. 2005, 2007, 2009), (iii) MGDG furthermore forms the so-called inverted hexagonal structures which have been shown to strongly enhance Ddx de-epoxidation (Goss et al. 2005, 2007).

With respect to the de-epoxidation reaction of the Ddx cycle it has to be mentioned that the negatively charged thylakoid lipid SQDG acts as a strong suppressor of the Ddx de-epoxidation reaction (Goss et al. 2009). In lipid systems which resemble the lipid composition of the native thylakoid membrane, low concentrations of SQDG are sufficient to completely block the Ddx de-epoxidation. Since such an inhibition can never be observed in the intact diatom cell, it has been proposed that SQDG has to be confined from the actual places where diadinoxanthin de-epoxidation is taking place (Goss et al. 2009). The observation that highly purified FCPs are enriched in MGDG and do not contain SQDG (Lepetit et al. 2010) supports the view that SQDG forms special domains within the diatom thylakoid membrane which are separated from the antenna complexes. The functional role of these putative SQDG domains is at present unclear. There is, however, evidence that the presence and concentration of negatively charged lipids is essential for the diatom thylakoid membrane. Under sulfate limitation, which strongly decreases the

SQDG content of the membranes, complementary increases of the PG content can be observed, which allows the algae to keep the overall content of negatively charged lipids at a constant level (Sato et al. 2000). With regard to a possible role of SQDG and PG, it is worth mentioning that the negatively charged lipid cardiolipin is highly enriched in membranes designed to generate an electrochemical gradient for ATP synthesis other than the thylakoid membrane (for recent reviews see Schlame et al. 2000; Joshi et al. 2009). A possible function of SQDG and PG in the mechanism of ATP generation is also supported by the observation that SQDG interacts with the plastidic ATP synthase of green algae and vascular plants (Pick et al. 1987).

Future experiments have to show how the diatom thylakoid membrane, which supposedly contains large surface areas with a negative charge, is arranged and how the negative charge is physiologically compensated, e.g., during the course of the light driven electron transport and the generation of the trans-membrane proton gradient.

Domain model of the diatom thylakoid membrane

Figure 1 attempts to incorporate the characteristics of low and high light thylakoids, as outlined in this review, into a working model of the topology of the diatom thylakoid membrane.

Thylakoid membranes from low light-adapted diatoms (Fig. 1a) show the typical arrangement in stacks of three (Gibbs 1962, 1970). The outer lamellae of the stacks are enriched in the negatively charged lipid SQDG and contain a high number of PSI complexes with their specific FCP antennae (Pyszniak and Gibbs 1992). The outer membrane also must contain the ATP synthase of the chloroplast because of the size of its head groups (Böttcher and Gräber 2000). The outer lamellae are not completely free of PSII and the peripheral FCP but contain lower amounts of these pigment–protein complexes than the inner membranes of the stacks. The inner parts of the stacks are enriched in the neutral galactolipid MGDG and contain the majority of the PSII and the peripheral FCP complexes. The FCP complexes in the inner membranes might exist in higher oligomeric states compared with the FCPs in the outer membranes. The Cyt b_6/f complex is equally distributed between the outer and inner lamellae of the stacks (Strzepek and Harrison 2004).

Thylakoid membranes of high light-adapted diatoms (Fig. 1b) show the same regular stacks of three. However, there are important differences in the membrane structure compared with low light membranes. The increased concentration of SQDG leads to a further enrichment of this lipid in the margin regions and SQDG-enriched membrane

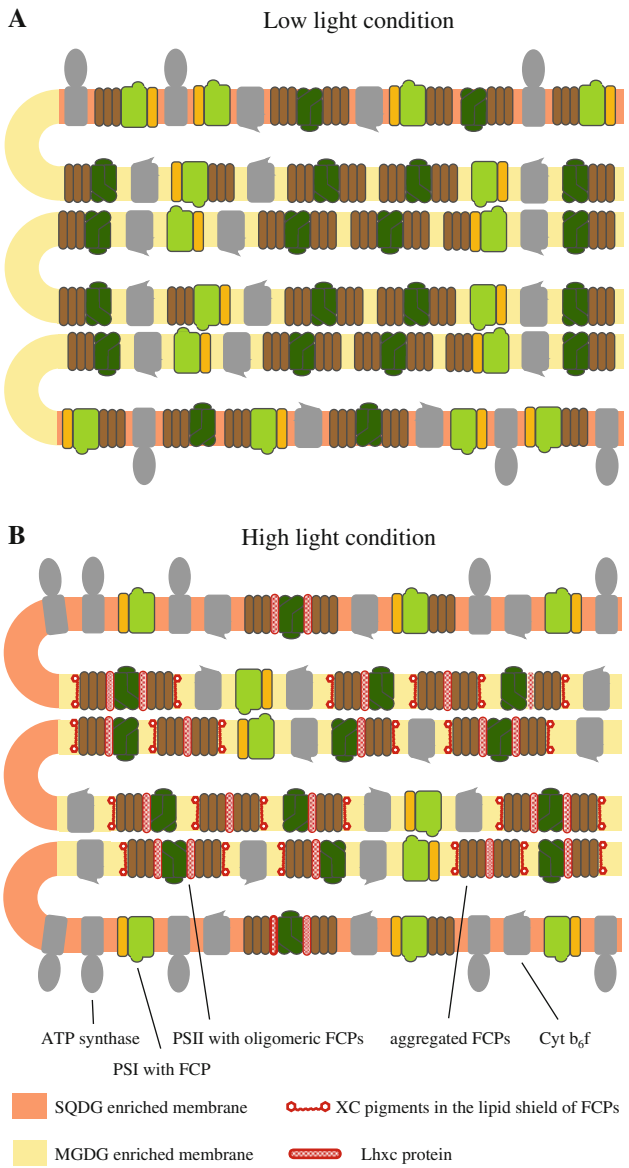

Fig. 1 A working hypothesis depicting the the functional characteristics of the diatom thylakoid membrane in acclimation to low-light (**a**) and high-light (**b**) conditions. Based on the current knowledge, this model represents a simplification of the structures in the native membrane to highlight the prominent changes in the structure of thylakoid membrane in adaptation to different light conditions. It is, however, not intended to reflect the exact stoichiometries of the components of the thylakoid membrane in diatoms

parts may even protrude into the inner lamellae of the stacks. High light illumination also induces the synthesis of Lhcx proteins which are preferentially associated with PSII due to their photoprotective function. The strong increase of the diadinoxanthin cycle pigment concentration after exposure to high light intensities leads to the incorporation of high amounts of xanthophyll cycle pigments into the MGDG molecules which are closely associated with the FCP complexes in the inner membranes. Furthermore, it is

possible that a part of the peripheral FCP complexes detaches from the PSII core complexes and forms higher order aggregates. High light illumination of diatom cells also leads to a strongly enhanced electron transport and photophosphorylation which is realized by an increased content of the Cyt b_6/f and ATP synthase complex.

The working model depicted in Fig. 1 is based on the assumption that the diatom thylakoid membrane is divided into structural domains. These domains are not as strictly separated as the grana and stroma domains of the vascular plant thylakoid membrane, yet they provide the opportunity for a patch-work like separation and regulation of different functional tasks of the thylakoid as shown for the chlorophyll *c*-containing xanthophyte *Pleurochloris meiringensis* by Büchel and Wilhelm (1992). The enrichment of SQDG in the outer lamellae and in the margin regions ensures that SQDG does not inhibit the Ddx de-epoxidation which is preferentially taking place at the FCP complexes associated with PSII in the inner membranes. The high concentration of SQDG in the outer parts of the stacks is also in line with the localization of the ATP synthase, taking into account that SQDG represents the thylakoid lipid that is preferentially associated with this enzyme complex (Pick et al. 1985). A restriction of SQDG to the outer lamellae of the membrane stacks seems to be likely when the negative charge of the lipid is considered. High concentrations of SQDG in opposing membranes of the inner part of the stacks would lead to strong repulsive forces which are not in line with the rather tight packing of these membrane parts (Pyszniak and Gibbs 1992). The separation of the two photosystems to a certain degree, i.e., an enrichment of PSI in the outer lamellae and a higher concentration of PSII in the inner part of the stacks, may prevent a strong spill-over from PSII to PSI. The MGDG-enriched inner parts of the thylakoid stacks, together with the embedded PSII and the oligomeric FCP, could represent attraction centres for the xanthophyll cycle enzyme Ddx de-epoxidase. De-epoxidation of Ddx to Dtx within the close vicinity of the FCP/PSII is in line with two different photoprotective mechanisms. Lipid-dissolved Dtx acts as a potent antioxidant and prevents damage by reactive oxygen species. Protein-bound Dtx quenches fluorescence non-photochemically and harmlessly dissipates the excessive excitation energy. Protection of PSII may also be realized in the inner membrane parts by the oligomeric FCP complexes which after their detachment from the core complex and a specific aggregation form strong quenching centres. Note, however, that protection against ROS and an over-excitation is also provided for PSI in the outer lamellae by the FCP complex which is tightly associated with the PSI core and which is enriched in the Ddx cycle pigments.

According to our opinion, the working model presented in Fig. 1 is well-suited to integrate the present day knowledge on the functional characteristics of the diatom thylakoid membrane. The model may also provide information why differences exist between these processes in the diatom membrane and the thylakoids from vascular plants and green algae. However, it has to be noted that, the model is a first approach to define functional macrodomains and represents a simplification of the situation in the native membrane. The distribution of the two photosystems, for example, may not be as heterogeneous as depicted in Fig. 1. There is only experimental evidence for a slight enrichment of PSI in the outer parts of the stacks which together with a higher PSII to PSI ratio (values between 1.3 and 4 have been published for different diatom species, e.g., Strzepek and Harrison 2004; Smith and Melis 1988) points to a certain enrichment of PSI in the outer and PSII in the inner membranes, respectively. It is also not clear, if there is a different FCP composition in the outer and inner lamellae. Earlier studies, which reported on a more equal distribution of the FCP complexes (Pyszniak and Gibbs 1992), have to be re-evaluated taking into account our increased knowledge about individual antenna proteins and different oligomeric states of the complexes. In addition, it has to be mentioned that further micro-heterogeneities might exist with respect to the lipid distribution. The Cyt b_6/f complex, for example, is strongly inhibited in the presence of SQDG (Yan et al. 2000), which means that Cyt b_6/f complexes located in the outer lamellae of the stacks have to be surrounded by a shield of MGDG molecules to ensure their efficient operation.

Therefore, further experimental evidence is needed to either support or contradict our model of the diatom thylakoid membrane as presented in Fig. 1.

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