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Running head

Plastoquinone pool redox regulation in diatoms

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Title

High light acclimation in the secondary plastids containing diatom *Phaeodactylum tricornutum* is triggered by the redox state of the plastoquinone pool

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Abstract

In diatoms the process of energy-dependent chlorophyll fluorescence quenching (qE) has an important role in photoprotection. Three components are essential for qE: 1) the light-dependent generation of a transthylakoidal proton gradient, 2) the de-epoxidation of the xanthophyll diadinoxanthin (Dd) into diatoxanthin (Dt), and 3) specific nuclear-encoded antenna proteins, called LHCX. We used the model diatom *Phaeodactylum tricornutum* to investigate the concerted light acclimation response of the qE key components LHCX, proton gradient and xanthophyll cycle pigments (Dd+Dt), and to identify the intracellular light responsive trigger. At high light exposure, the up-regulation of three of the *LHCX* genes and the *de novo* synthesis of Dd+Dt led to a pronounced rise of qE. By inhibiting either the conversion of Dd to Dt or the translation of *LHCX* genes, the qE amplification was abolished and the diatom cells suffered from stronger photoinhibition. Artificial modification of the redox state of the plastoquinone (PQ) pool via DCMU and DBMIB resulted in a disturbance of the Dd+Dt synthesis in an opposite way. Moreover, we could increase the transcription of two of the four *LHCX* genes under low light conditions by reducing the PQ pool using DBMIB. Altogether, our results underline the central role of the redox state of the PQ pool in the light acclimation of diatoms. Additionally, they emphasize strong evidence for the existence of a plastid-to-nucleus retrograde signaling mechanism in an organism with plastids that derived from secondary endosymbiosis.

List of abbreviations

β -car, β -carotene; Chl *a*, chlorophyll *a*; Chl *c*, chlorophyll *c*; CHX, cycloheximide; Cyt, cytochrome; DBMIB, 2,5-Dibromo-6-isopropyl-3-methyl-1,4-benzoquinone; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; Dd, diadinoxanthin; Dd+Dt, diadinoxanthin+diatoxanthin; Δ pH, transthylakoidal proton gradient; Dt, diatoxanthin; DTT, dithiothreitol; Fx, fucoxanthin; HL, high light; LHC, light harvesting complex; LL, low light; mHL, medium high light; NPQ, non-photochemical fluorescence quenching; $^1\text{O}_2$, singlet oxygen; PQ, plastoquinone; qE, energy-dependent chlorophyll fluorescence quenching; ROS, reactive oxygen species; VDE, violaxanthin de-epoxidase; VDL, violaxanthin de-epoxidase like; VDR, violaxanthin de-epoxidase related

Introduction

Diatoms are eukaryotic microalgae found in any aquatic habitat. They contribute to the global primary production to a similar extent as terrestrial tropical rain forests and grasslands (Geider et al. 2001). As such, they are at the onset of most marine and freshwater food-webs (Armbrust 2009). Because of their ecological importance, the genomes of several diatom species have meanwhile been sequenced and are publically available (see Tirichine and Bowler 2011). Especially the physiological studies on the molecular level of the diatoms *Thalassiosira pseudonana* and *Phaeodactylum tricornutum* revealed a plethora of unique features (Wilhelm et al. 2006; Armbrust 2009; Bowler et al. 2010). Some of these features arise from the chimerical origin of the genome which is due to horizontal gene transfer from bacteria and to the evolution of the diatom chloroplasts via secondary endosymbiosis (Bowler et al. 2010). After each endosymbiotic event, gene transfer from the endosymbiont to the nucleus occurred, permanently modifying the genome composition of the host cell. Still, the diatom chloroplast genome retained a small amount of approximately 130 protein encoding genes (Oudot-Le Secq et al. 2007). As a further consequence of secondary endosymbiosis, diatom plastids are surrounded by an envelope of four membranes reflecting the endosymbiotic origin of these organelles (Green 2011).

Gene transfer from the chloroplast into the nucleus is common to all eukaryotic phototrophs. Because many of the plastid localized proteins are encoded by the nuclear genome and because most of the plastid protein complexes are chimeras of nuclear and plastid encoded proteins, both the plastidic and the nuclear gene expression as well as the respective protein import into the plastids require a fine-tuned regulation (Allen et al. 2011). Thus, anterograde (nucleus to plastid) and retrograde (plastid to nucleus) signaling pathways are widespread processes to coordinate gene expression in green algae and land plants (Kleine et al. 2009). Control of photosynthetic gene expression and enzyme activity is coordinated by a number of

molecular triggers including photosynthetic metabolites, hormones, chlorophyll and carotenoid biosynthesis precursors, photoreceptors, stress-generated reactive oxygen species (ROS), and soluble redox active compounds (Kleine et al. 2009; Pfannschmidt 2010; Foyer et al. 2012). A further important signaling trigger is the redox state of the plastoquinone (PQ) pool of the plastid electron transport chain. Besides its well-known involvement in the irradiance-dependent regulatory process of state-transitions in green microalgae and plants, it has a key role for the regulation of both chloroplast and nuclear gene expression under light changing conditions (Pfannschmidt 2003). In diatoms, the involvement of photoreceptors in photosynthetic regulatory processes has been revealed recently (Depauw et al. 2012), while redox regulation via thioredoxins seem to be of minor importance for the regulation of the Calvin cycle (Kroth et al. 2008), even if thioredoxins may control the activity of carboanhydrases in the pyrenoid (Kikutani et al. 2012). The redox state of the PQ pool is of crucial importance in several photosynthetic regulatory processes such as the PSII electron cycle (Onno Feikema et al. 2006; Lavaud et al. 2007), the non-photochemical fluorescence quenching (NPQ) (Lavaud 2007; Lavaud et al. 2007) and the chlororespiration (Caron et al. 1987; Dijkman and Kroon 2002; Grouneva et al. 2009). The dynamics of the PQ pool redox state as a function of light intensity differ between diatom species and between diatoms and land plants (Ruban et al. 2004; Lavaud 2007; Materna et al. 2009). So far, no evidence for an involvement of the PQ pool in redox signaling in diatoms has been published.

Diatoms possess an outstanding capacity to tolerate light fluctuations regularly punctuated by excess light exposure, which might be one of the reasons for their ecological success (Brunet and Lavaud 2010). The central mechanism for the fast regulation of photochemistry under changing light conditions is the NPQ. It takes places in the light-harvesting complex antennae (LHC) of PSII where excessively absorbed light energy is dissipated as heat (Li et al. 2009; Ruban et al. 2012). In diatoms, the most important part of NPQ is the energy dependent fluorescence quenching, qE, while the photoinhibitory quenching, qI, is low, and the state-

transition quenching, q_T , does not exist at all (Goss and Jakob 2010). q_E requires the irradiance-dependent establishment of a transthylakoidal proton gradient (ΔpH) and the conversion of the xanthophyll diadinoxanthin (Dd) into diatoxanthin (Dt) in the presence of a ΔpH (Goss and Jakob 2010). Together with the reverse reaction in the absence of the ΔpH this process is called ‘xanthophyll cycle’ and is equivalent to the viola-, anthera- and zeaxanthin xanthophyll cycle in land plants (Jahns and Holzwarth 2012). Furthermore the presence of LHCX proteins is essential (Bailleul et al. 2010). In addition to LHCX, diatoms contain other members of the LHC protein superfamily like LHCF which constitute the peripheral light harvesting complex, and LHCR which are tightly linked with PSI and whose function remains still to be elucidated (Lepetit et al. 2012). Located in the plastids, both the enzymes for the interconversion of the xanthophyll cycle pigments diadino- and diatoxanthin (Dd+Dt), the Dd de-epoxidases and the Dt epoxidases (Coesel et al. 2008;Lohr 2011), as well as the LHCX proteins (Bowler et al. 2008;Zhu and Green 2008;Engelken et al. 2012) are encoded in the nucleus. Dd+Dt and LHCX have been shown to be synthesized *de novo* during prolonged high light stress (Olaizola et al. 1994;Bailleul et al. 2010;Zhu and Green 2010). *De novo* synthesis of Dd+Dt does not necessarily lead to higher NPQ (Lavaud et al. 2004;Schumann et al. 2007), but can enhance the antioxidant activity within the thylakoid membranes (Lepetit et al. 2010). Nevertheless, reduced expression of the gene encoding the Dd de-epoxidase clearly leads to both lower Dt synthesis and q_E (Lavaud et al. 2012). Over- or under-expression of LHCX1 promotes higher or lower q_E , respectively, and the up-regulation of LHCX2 and LHCX3 might confer more efficient photoprotection (Bailleul et al. 2010). In the present study we monitored the changes of Dd+Dt and LHCX during prolonged high light exposure and the subsequent low light exposure. Because light stress creates an increased reduction of the electron transport chain, we tested the possible involvement of the redox state of the PQ pool in the q_E acclimation response using different light conditions and inhibitor treatments. By identifying a link between the redox state of the PQ pool and the

regulation of the amount of the qE key players LHCX and Dd+Dt pigments, we deliver a so far missing evidence for plastid-to-nucleus retrograde signaling in diatoms.

Results

Expression of LHCX genes under different light conditions

LHCX gene expression was studied in *P. tricornutum* cells that had been cultured for 33 h in darkness, darkness/ low light (LL, 40 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) or darkness/ medium low light (mHL, 750 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) conditions. Except for the cells kept in the dark, the standard 16/8 h day/ night rhythm was maintained. In prolonged dark conditions, *LHCX4* gene expression showed a significant increase during daytime while gene expression of *LHCX2* and *LHCX3* decreased (Fig. 1A). At 9:00 in the morning, after light had normally been switched on, the transcript level of *LHCX1* reached its maximum, after which it declined to increase again at the same time the next day. This rhythmic diurnal expression suggests the presence of a circadian regulation. LL conditions had a pronounced effect on the expression of *LHCX1*, *LHCX2* and *LHCX3*. The two latter genes were slightly but significantly induced until noon while *LHCX1* was highly up regulated. mHL conditions strongly increased the expression of *LHCX2* and *LHCX3*. During the following night, the respective amounts of mRNA almost reached their initial level indicating a fast regulation of transcript increase and decrease. The extra light intensity at mHL conditions compared to LL also resulted in an increase of *LHCX1* transcription, albeit to a lesser extent. *LHCX4* did not show any differences regarding transcription at both light treatments compared to cells kept in the dark.

In order to evaluate the effect of a high light exposure equivalent to full sunlight in nature, the cells were exposed to 2000 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ (HL) (Long et al. 1994) for 2 h, and their recovery from HL stress was recorded during a subsequent LL exposure of 4 h. In this case the transcription of *LHCX1*, *LHCX2* and *LHCX3* was up-regulated while *LHCX4* appeared to be light insensitive (Fig. 1B). In contrast to cells grown at mHL conditions, *LHCX3* was the most strongly up-regulated gene (more than 100 fold), followed by *LHCX2* and *LHCX1*. After

4 h of recovery at LL, *LHCX3* reached its initial transcript level, indicating an extremely rapid up- and down-regulation. The reduction of transcripts was slower for *LHCX2*, while *LHCX1* transcripts even decreased below its initial level because of the daily regulation pattern.

Changes of pigments and NPQ during mHL and HL treatments

During the mHL exposure (16 h) no alterations were observed regarding the amounts of chlorophyll *c* (Chl *c*) and β -carotene (β -car) per chlorophyll *a* (Chl *a*) (Fig. 2A). The fucoxanthin (Fx) content per Chl *a* increased just after the onset of light and then slightly decreased. Pigments of the violaxanthin cycle, which were observed by Lohr and Wilhelm (1999), could not be recorded under our experimental conditions. The Dd+Dt concentration increased almost fourfold (Fig. 2A) and the Dt amount became higher than Dd. Remarkably, the Dd+Dt pool size kept scaling up during the first 3 h of darkness and did not decrease during the resting night period. After HL treatment (2 h) we detected an equally strong increase of Dd+Dt (Fig. 2B and Fig. 3A). The *de novo* synthesis of Dd+Dt started approximately 30 min after the light onset as already reported before (Lavaud et al. 2004) and rapidly declined within the 4 h of LL recovery. In contrast, during dark recovery the amount of Dd+Dt remained stable (Fig. 3A). Also, we did not observe major differences in the amount of all other pigments indicating a rather independent synthesis and degradation of Dd+Dt from Fx and β -Car. Interestingly, the epoxidation of Dt to Dd occurred rapidly and almost completely during the recovery period, no matter whether LL or darkness was employed (Fig. 3B).

An inverse relationship was observed between NPQ and F_v/F_m during the mHL exposure. The NPQ increased, albeit with a much lower slope during the second half of the light treatment, while F_v/F_m concomitantly decreased (Suppl. Fig. 3). The NPQ did not relax completely during the dark recovery period. A strong increase of NPQ was also observed during the HL

experiments (Fig. 3C). Its maximal value was lower than during the mHL treatment (1.65 to 2.75) which can be explained by the shorter light treatment (2 h vs. 16 h). NPQ completely relaxed during the LL recovery period but not in darkness (Fig. 3C). The addition of NH_4Cl , which usually relaxes NPQ efficiently in *P. tricornutum* (Goss et al. 2006), could not reduce the NPQ persisting in the dark (Fig. 3C).

The NPQ/Dt relationship reflects the Dt quenching efficiency (Lavaud et al. 2002a; Lavaud et al. 2004), which in the mHL experiments showed a highly linear correlation ($R^2=0.98$) up to a threshold value of 20 mol Dt ($100 \text{ mol Chl } a$)⁻¹ at the 15:00 time point (Suppl. Fig. 4A). Beginning at 18:00, the slope of the relationship strongly decreased. The respective values during the HL treatment yielded a strong linear correlation ($R^2=0.96$) up to a timespan of 60 min and a threshold concentration of 7 mol Dt ($100 \text{ mol Chl } a$)⁻¹, while the slope of the relationship during the second hour of illumination dramatically dropped (Suppl. Fig. 4A and B). Moreover, the values for the steep slopes of the NPQ/Dt relationships in mHL and HL were different (0.125 vs. 0.218) (Suppl. Fig. 4A).

Influence of DTT and CHX on NPQ, Dd+Dt and LHCX synthesis

Because the photoacclimative responses were already fully activated during the two hours of HL exposure, the inhibitor treatments were performed at these conditions. DTT, an inhibitor of the de-epoxidation reaction (Yamamoto and Kamite 1972), had no effect on the increase of the Dd+Dt pool size (Fig. 3A, see 'DTT+'), but blocked the Dd de-epoxidation during the first 30 min of illumination (Fig. 3B). Afterwards, when the Dd+Dt pool size increased, a considerable amount of Dt was produced even in the presence of DTT. To exclude the possibility that the inhibitor effect is reduced after 30 min of HL, DTT was alternatively added every 20 min (Fig. 3B, 'DTT+'). The supplementary DTT dose slowed down the Dt synthesis while not completely inhibiting it. As expected, both DTT treatments (DTT and

DTT+) strongly inhibited the induction of NPQ (Fig. 3C,D) and resulted in a dramatic decrease of the functional fraction of PSII (Fig. 4).

CHX blocks the translation of nuclear encoded genes on cytosolic 80s ribosomes and therefore does not influence gene translation in the organelles (Obrig et al. 1971;Setkov et al. 1992). As a matter of fact, while HL treatment induced the synthesis of nuclear encoded LHCX2 and LHCX3 proteins (Fig. 5) as expected from the transcript level measurements (Fig. 1), the addition of CHX prevented it. In contrast, CHX had no inhibiting effect on the plastid encoded PsbB protein, instead even a slightly increased amount of PsbB was detected as observed before in other diatoms (Wu et al. 2011). We did not notice any differences in the amounts of LHCX1 after HL treatment. This might either be due to an oversaturation of the Western blot because of the already rather high initial LHCX1 amount or to a different regulation on the posttranslational level. Addition of CHX did not alter the *de novo* synthesis of Dd+Dt and the amount of Dt produced during HL exposure (Fig. 3A,B), but it completely prevented the decrease of Dd+Dt during the LL recovery period, similarly to the dark recovery experiments in the absence of the inhibitor. However, after 30 min of HL CHX retarded a further increase of NPQ almost completely (Fig. 3C) and increased the degree of PSII photoinactivation, although not as pronounced as DTT (Fig. 4). As expected, we obtained the highest degree of photoinactivation by adding both CHX and DTT.

Only during the first 30 min of HL and CHX treatment we found a highly linear correlation of Dt versus NPQ ($R^2=1$), thereafter the slope almost dropped close to zero. The threshold concentration for Dt participating in NPQ decreased to a value of around 5 mol Dt (100 mol Chl *a*)⁻¹ (Suppl. Fig. 4B).

Effect of DCMU and DBMIB on the Dd+Dt pool size

In diatoms a chlororespiration process exists which influences the redox state of the PQ pool in the dark (Jakob et al. 1999;Dijkman and Kroon 2002;Wilhelm et al. 2006;Grouneva et al. 2009). As we saw a clear difference in the Dd+Dt pool size during dark or LL recovery, DCMU was utilized to modify the PQ pool redox state. By binding to Q_B , DCMU generates a reduced Q_{A^-} and an oxidized PQ pool (Trebst 2007). Applied during the LL recovery conditions, DCMU prevented the decrease of the Dd+Dt pool (Fig. 3A), comparably to the dark recovery in the absence of the inhibitor. On the other hand, DCMU induced a pronounced de-epoxidation, probably due to the onset of cyclic electron transport around PSI (Fig. 3B). By applying DCMU directly at the beginning of the HL exposure, i.e. by mimicking LL conditions regarding the redox state of the PQ pool, no Dd+Dt pool size increase was observed, but again a strong de-epoxidation occurred (Fig. 3A). In order to verify these results, we used DBMIB which usually shows the opposite effect compared to DCMU regarding the redox state of the PQ pool. DBMIB binds close to the plastoquinol oxidizing Q_o -site of the cytochrome (Cyt) b_6f complex and consequently induces a reduced PQ pool under LL which hence mimics HL conditions (Kurisu et al. 2003;Trebst 2007). Because this effect is known to depend on the concentration of DBMIB, we tested different amounts in order to define a suitable DBMIB concentration necessary to induce a reduced PQ pool following two main criteria: 1) a high F_v/F_m served as a proof that DBMIB does not bind to Q_B which can occur at too high concentrations (Vener et al. 1997), and 2) we checked for a reduced NPQ compared to the control because of the limited proton translocation at the Cyt b_6f complex (Strzepak and Harrison 2004). The suitable DBMIB concentration was defined to be 1 μ M (Suppl. Fig. 5) which is consistent with concentrations used in green microalgae (Steinbrenner and Linden 2003) and in land plants (Tullberg et al. 2000). 1 μ M DBMIB at a light intensity of 100 μ mol photons $m^{-2} s^{-1}$ (LL₁₀₀) was sufficient to support a considerable

electron flow from PSII to PSI while neither inducing any Dd de-epoxidation nor any decrease of F_v/F_m (data not shown). DBMIB + LL₁₀₀ led to a 20 % increase of Dd+Dt in contrast to untreated cells (Fig. 6). A reduced PQ pool could therefore induce an HL response under LL conditions.

Effect of DCMU and DBMIB on the LHCX transcript levels

The described DBMIB + LL₁₀₀ HL mimicking system was then applied for further analysis of *LHCX* transcript levels. While the LL₁₀₀ treatment alone did not induce remarkable transcript changes of any of the *LHCX*, the addition of DBMIB caused a slight rise of the *LHCX1* transcript level (Fig. 7A) and a pronounced increase of *LHCX2* (20 fold after 60 min) (Fig. 7B). In contrast, the amount of *LHCX3* transcripts did not change (Fig. 7C). Hence, for two of the three light induced *LHCX*, gene expression was specifically stimulated by an increased reduction state of the PQ pool. The addition of DCMU during HL treatment did not produce the opposite effect of DBMIB, even when the light intensity was decreased to 500 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ (Suppl. Fig. 6), most probably because the combined effect of HL and DCMU on photosynthesis was higher than the DCMU generated artificial LL effect. Instead, we used DCMU during the DBMIB + LL₁₀₀ treatment in order to stop the electron flow from PSII to the Cyt b₆f, relaxing the reduced PQ pool redox state. This abolished the effect of DBMIB on the transcript levels of *LHCX1* and *LHCX2* (Fig. 7A and B) and demonstrated that DBMIB did not simply induce transcription of these genes due to its presence per se but to its effect on the redox state of the PQ pool. The light insensitive *LHCX4* gene responded differently. Its transcription was slightly induced by the DBMIB + LL₁₀₀ treatment (Fig. 7D) but it was neither abolished by the addition of DCMU nor increased during the HL + DCMU treatment (Suppl. Fig. 6). *LHCX4* apparently reacted specifically on the presence of DBMIB rather than on the changes of the redox state of the PQ pool itself.

Discussion

The role of Dt and LHCX in NPQ

The presented results strengthen previous findings whereupon LHCX1, LHCX2 and LHCX3 but not LHCX4 are involved in the photoacclimative/-protective response of diatoms (Nymark et al. 2009;Bailleul et al. 2010). The Pt4 strain studied here shows a clearer HL response regarding *LHCX1* gene transcription (but not resolved on the protein level) than the Pt1 strain used in the experiments performed by Nymark and co-workers and Bailleul and co-workers. Albeit in both strains LHCX1 is the predominant LHCX protein under LL conditions, Pt1 contains higher levels of LHCX1 mRNA and protein (Bailleul et al. 2010), which might limit its ability to increase their amount under HL.

The increase of gene transcripts is often well paralleled by the respective protein changes (Lee et al. 2011;Dyhrman et al. 2012). After 2 h of HL the huge increase of *LHCX2* and *LHCX3* transcripts was reflected by the respective protein levels. This coincided with a higher Dt concentration, resulting from the de-epoxidation of mostly *de novo* synthesized Dd. As a consequence of the increase of both, Dt and LHCX, a higher NPQ was established. Similar results were also obtained recently in *T. pseudonana* (Zhu and Green 2010). An important question is how these two enhancers of qE may interact. One hint comes from the finding that the amount of Dt alone is only a weak indicator for the quenching capacity (Lavaud et al. 2004;Schumann et al. 2007;Lepetit et al. 2010). If suitable protein binding sites are missing, a larger number of Dt molecules can be dissolved in the thylakoid lipids and then does not participate in NPQ (Lepetit et al. 2010). Indeed, the ratio of NPQ to Dt was considerably different in mHL and HL cultures. Moreover, addition of CHX blocked the increase of NPQ beginning after 30 min of HL treatment. This was also the time when *de novo* synthesis of Dd+Dt started. It therefore can be assumed that, in order to participate in NPQ, the *de novo* synthesized Dt molecules need the equivalent amounts of newly synthesized nuclear encoded

pigment binding proteins. Besides *LHCX1*, *LHCX2* and *LHCX3*, only four *LHCR* genes are stronger expressed at HL compared to LL conditions (Nymark et al. 2009). LHCR proteins constitute the PSI antenna in diatoms (Veith et al. 2009; Lepetit et al. 2010). There are indications that LHCX proteins (except LHCX1/FCP6, see Beer et al. 2006; Lepetit et al. 2010) appear to be rather weakly associated to the thylakoid membrane or to the classical light harvesting complexes (Zhu 2009; Grouneva et al. 2011), similar to their LHCSR/LI818 relatives in *C. reinhardtii* (Richard et al. 2000). As the amount of Dd+Dt bound to LHCR in PSI remains constant at changing light conditions (Lepetit et al. 2010; Juhas and Büchel 2012), we hypothesize that *de novo* synthesized Dd+Dt molecules bind to the newly synthesized LHCX proteins conferring a higher NPQ. Interestingly, PsbS, which is responsible for NPQ in land plants, binds no xanthophylls (Dominici et al. 2002; Bonente et al. 2008), while the LHCX related LHCSR3 in *Chlamydomonas* does (Bonente et al. 2011).

Another peculiarity of our data is the strong difference of NPQ during dark or LL recovery conditions after HL treatment. This difference obviously was not due to photoinhibition, as for both recoveries the light treatment before was identical. It is known that full NPQ recovery in diatoms occurs only at LL conditions, but not in darkness (Goss et al. 2006; Grouneva et al. 2009). So far this effect was related to the Dt epoxidation reaction, which might be limited in darkness due to the chlororespiration driving the buildup of the ΔpH , and due to a shortage of NADPH (Jakob et al. 1999; Goss et al. 2006; Grouneva et al. 2009). In our experiments the epoxidation was not different in LL or darkness. After 4 h of recovery only a minor amount of Dt was present in the cells (1.1 and 0.5 mol Dt (100 mol Chl *a*)⁻¹ in samples from LL and darkness, respectively, Fig. 3A). Moreover, the addition of the uncoupler NH₄Cl did not relax the NPQ persisting in the dark. Different to the experiments by Jakob, Goss and Grouneva and co-workers mentioned above, our cultures were longer exposed to HL. Obviously, some part of the NPQ created here relies on other processes, which are independent of Dt. Dt-independent NPQ already has been demonstrated in diatoms

and was interpreted to be related to a PSII cycle and/or to a rearrangement of the PSII core (Lavaud et al. 2002b; Eisenstadt et al. 2008).

The regulation of xanthophyll cycle pigment synthesis

Our data strongly support the assumption that the redox state of the PQ pool might be the trigger for the HL induced *de novo* synthesis of Dd+Dt. The HL+DCMU treatment, which generates massive amounts of singlet oxygen ($^1\text{O}_2$) (Flors et al. 2006), prevented the increase of Dd+Dt thus excluding a possible triggering role of this ROS, in contrast to its influence on the *LHCX* gene expression (see below). However, as by binding to Q_B DCMU blocks the photochemical linear electron flow towards NADP^+ (Trebst 2007), the amount of NADPH, which is required in the early steps of carotenoid biosynthesis (Lohr et al. 2012), might indirectly limit the *de novo* synthesis of Dd+Dt. In contrast, DBMIB equally reduces linear electron flow by preventing electron flow towards the Cyt b6f complex (Trebst 2007), but induced Dd+Dt *de novo* synthesis. Hence, an NADPH shortage effect as the actual reason for the differences in Dd+Dt *de novo* synthesis seems rather unlikely compared to the triggering by the redox state of the PQ pool.

All known enzymes involved in carotenoid synthesis are nuclear encoded, but the carotenoid synthesis itself takes place in the chloroplast (Coesel et al. 2008; Frommolt et al. 2008; Lohr 2011). The Dd+Dt synthesis was unaffected by the nuclear translation inhibitor CHX, hence its regulation must occur at the post-translational stage and most probably in the chloroplast. Additionally, inhibition of plastid translation does also not reduce Dd+Dt *de novo* synthesis in HL (Olaizola et al. 1994; Wu et al. 2012). While genes encoding Dd synthesizing enzymes are up-regulated in diatoms that are shifted from darkness to light with moderate intensity (Coesel et al. 2008), their expression is not further induced in LL to HL shift experiments similarly to our experimental conditions (Nymark et al. 2009). Thus, the post-translational regulation of

Dd+Dt *de novo* synthesis seems not to require any newly expressed nuclear or plastid encoded factor, which could activate the carotenoid synthesizing enzyme activity. All these features strongly resemble the situation in the green microalga *Haematococcus pluvialis*, where the HL induced *de novo* synthesis of the xanthophyll astaxanthin is triggered by the redox state of the PQ pool via post-translational regulation (Steinbrenner and Linden 2003). In contrast to the Dd+Dt synthesis, the effect of CHX illustrates the need for the expression of nuclear encoded gene(s) during the LL decrease of the Dd+Dt pool size. Unlike in what was proposed by Lohr and Wilhelm (1999), this degradation of Dd+Dt does not result in an increase of the amount of Fx under our experimental conditions but instead Dd+Dt are probably recycled. This fits with recent results pointing out that Fx is not synthesized from Dd but that both xanthophylls share the same precursor pathway (Dambek et al. 2012).

Another interesting observation was that DTT blocked the de-epoxidation effectively only during the first 30 min of HL treatment. Even the addition of supplemental DTT, in order to rule out a reduced efficiency during the treatment, did not restore the full inhibitory effect, although it clearly retarded the synthesis of Dt. So far, no Dt biosynthesis pathway excluding the de-epoxidation from Dd has been identified (Lohr 2011). *P. tricornutum* possesses one violaxanthin de-epoxidase (VDE), two VDE like enzymes (VDL) and one VDE related enzyme (VDR) (Coesel et al. 2008; Bertrand 2010). While the role of VDE is clear, the function of VDL/VDR remains speculative. Studies on *P. tricornutum* mutants containing a reduced amount of VDE indicate that these alternative de-epoxidases are involved in Dd to Dt conversion (Lavaud et al. 2012). It is possible that during DTT inhibition VDR and VDL perform the Dd de-epoxidation reaction, as they might be less sensitive to DTT than VDE. Corresponding to the xanthophyll cycle pigments, their synthesis might be induced in HL, explaining the absence of Dd de-epoxidation in the first 30 min of HL. Indeed, in contrast to the VDE, the transcription of these alternative de-epoxidases is up-regulated during a short term light stress (Nymark et al. 2009).

The regulation of LHCX gene expression

The transcript level of *LHCX1*, *LHCX2* and *LHCX3* responded to light in a dose-dependent manner. As conclusively demonstrated, the transcription could be induced in LL conditions via a reduced PQ pool for *LHCX1* and *LHCX2* but not for *LHCX3*. Blocking the gene expression of *LHCX1* and *LHCX2* with DCMU in HL was not possible. As mentioned above, besides keeping the PQ pool oxidized, DCMU creates high amounts of $^1\text{O}_2$ (Flors et al. 2006), which in plants is the most harmful ROS (Triantaphylidès et al. 2008; Triantaphylidès and Havaux 2009) and which is able to induce nuclear gene expression (op den Camp et al. 2003; Fischer et al. 2004). We assume that *LHCX1* and *LHCX2* are also induced by $^1\text{O}_2$, which superimposes on the effect of the PQ pool redox state. Most likely *LHCX3* is also strongly induced by ROS, as it exhibited a higher relative induction upon addition of DCMU than in HL without DCMU (Suppl. Fig. 6). This suggests a photoprotective role of *LHCX3* at conditions where the light stress is harsh and is in line with our results, where *LHCX3* was the relatively strongest transcribed *LHCX* gene only at $2000 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ light exposure.

LHCX1, *LHCX2* and *LHCX3* are furthermore up-regulated in response to blue light via a cryptochrome mediated pathway (Coesel et al. 2009). Similarly, promoters in Arabidopsis react to various stimuli (Staneloni et al. 2008). Hence, *LHCX* gene expression is apparently fine-tuned by the light quality (cryptochrome photoreceptor) as well as the light intensity either in situations that generate an imbalance between the photosynthetic light reactions and the Calvin cycle (redox state PQ pool) or during prolonged excess light exposure (i.e. ‘light stress’; ROS). Importantly, all of these triggers might participate altogether in the cellular light response, albeit to different extents, as a function of the light conditions.

Which part of the electron transport chain exactly generates the redox signal?

By applying DCMU and DBMIB we were able to mimic a LL or HL phenotype, respectively, similarly like it has been performed in the green alga *Dunaliella tertiolecta* in the pioneer work by Escoubas et al. (1995). Besides green algae, comparable experiments succeeded in cyanobacteria (Li and Sherman 2000) and land plants (Karpinski et al. 1997; Pfannschmidt et al. 1999; Tullberg et al. 2000). While, regarding the effects of DBMIB and DCMU, it is clear that the generated signal is related to the redox state of the PQ pool, its exact localization in the plastidic electron transport chain remains ambiguous (Pfannschmidt 2003). The Q_o-site of the Cyt b₆f complex, which binds plastoquinol, activates state-transitions and probably other redox controlled processes via kinase activation (Zito et al. 1999), but it does not account for all PQ pool redox dependent processes (Baier and Dietz 2005). Because in our experiments DCMU inhibited the DBMIB effect in LL, we proved that the binding of DBMIB to the Cyt b₆f complex alone is not responsible for the redox mediated response. Moreover, in addition to prevent an increase of D_d+D_t in HL due to the oxidized PQ pool, DCMU also inhibited a decrease in LL. In all light conditions DCMU fixed the D_d+D_t pool size to its actual status. As at these distinct conditions different degrees of de-epoxidation are induced (Fig. 3B) which directly depend on the generated ΔpH (Jakob et al. 2001), one can assume either a chlororespiration or a PSI cyclic electron transfer activity. Both processes would change the redox state of the PQ pool. It is therefore tempting to speculate that the PQ pool mediated signal would need not one but two triggers: one is the redox state of the PQ pool itself and the second one might be the redox state of Q_A which works as an on/off switch for the downstream PQ pool redox regulation. Alternatively Q_B could be involved because binding of DCMU here might inactivate this possible regulatory site preventing any regulation by the PQ pool. The rate-limiting step in the photosynthetic electron transport chain is the diffusion and the reoxidation of the PQ pool (Haehnel 1984; Rochaix 2011). In HL conditions Q_A should be

partially oxidized, while the pool itself is reduced, as long as the light reactions are faster than the enzymatic reactions of the Calvin cycle. As a consequence, the synthesis of Dd+Dt is initiated. In LL conditions, Q_A and the PQ pool are oxidized and the Dd+Dt pool size decreases. As long as Q_A is reduced, which in our experiments was induced by adding DCMU or by the sudden transfer of the cells from HL to darkness (as demonstrated by Dijkman and Kroon 2002;Grouneva et al. 2009), there is neither an increase nor a decrease of the Dd+Dt pool size independent of the oxidation state of the PQ pool. Why should diatoms have an additional on/off switch within the PSII core? One explanation might be based on the very unusual electron fluxes in diatoms which are still far from being understood. These include: 1) a vivid exchange of electrons between the electron transport chains of chloroplasts and mitochondria (Prihoda et al. 2012), 2) a cyclic electron transfer in PSII (Lavaud et al. 2002b;Onno Feikema et al. 2006;Lavaud et al. 2007), 3) a chlororespiration process which can modify the redox balance of the quinones and of PQ (Jakob et al. 1999;Dijkman and Kroon 2002;Grouneva et al. 2009;Cruz et al. 2011), and 4) a recombination of Q_A^- and Q_B^- with the oxidized states of the Mn Cluster in the PSII core induced by conformational changes (Eisenstadt et al. 2008). Therefore, an additional switch provided by the acceptor side of PSII (Q_A and Q_B) might help, in combination with the redox state of the PQ pool, to sort the different electron flows and to fine-tune the synthesis of xanthophylls and the transcription of light-responsive genes like *LHCX*, depending on the source of electrons.

Conclusion

The redox state of the PQ pool is used as a widespread physiological mechanism in green algae and land plants for signaling within the plastid as well as for retrograde signaling from the plastid to the nucleus (Pfannschmidt 2003;Baier and Dietz 2005;Fey et al. 2005;Kleine et al. 2009). To our knowledge, no indications for such signaling processes have been reported before in photosynthetic organisms with secondary plastids. In diatoms, a PQ pool redox regulation and retrograde signaling was so far regarded as unlikely (Wilhelm et al. 2006), especially when taking into account their complex evolutionary history (Bowler et al. 2010) and the abundance of several light sensors (Depauw et al. 2012), which might partially replace this function. We now have demonstrated that diatoms also use the redox state of the PQ pool for controlling the light acclimation of the plastid which includes retrograde signaling to the nucleus to regulate photosynthetic gene expression. As the PQ pool oxidation is the slowest step within the electron transport chain, it is the ideal sensor for redox imbalances. This raises the exciting question how the redox signal can cross the four membranes of the plastid envelope, which is even not answered yet in land plants and green algae, although their plastids possess only two membranes (Kleine et al. 2009). Moreover, as diatoms are evolutionary and physiologically closely related to other members of the Heterokontophyta like Phaeophyceae, Chrysophyceae, Xanthophyceae or Pelagophyceae (Adl et al. 2005), it is tempting to assume that such mechanism also exists in these groups and might even be a universal feature of photosynthetic organisms.

Materials and Methods

Cell culturing and light treatments

Phaeodactylum tricornutum (University of Texas Culture Collection, strain 646, so-called 'Pt4') was grown in airlift tubes in a day/night rhythm of 16/8 h with a light intensity of 40 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ (low light, LL) at 20°C. Cells were cultured in Provasoli's enriched f/2 seawater medium (Guillard 1975) using Tropic Marin artificial sea salts (Dr. Biener GmbH, Germany) at a final concentration of 50 % compared to natural seawater. Three days after inoculation the cultures in their exponential phase of growth were concentrated to 10 mg L⁻¹ Chl *a*. They were again acclimated to LL for 1 h before the different experiments were started, and sodium carbonate (4 mM) was added to prevent carbon limitation. Light exposure was provided by four 65 W white light energy saving lamps (Lexman, France) adjusted at distinct distances, in a custom made glass cylinder which was continuously cooled by a water bath at 20°C. Cell dispersal was secured by a magnetic stirrer. A long term experiment started at 24:00 o'clock in darkness. At 8:00 cells were either kept in darkness, exposed to LL or exposed to 750 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ (medium high light, mHL) until 24:00, followed by a further dark cycle of 8 h and a subsequent 1 h darkness or LL/ mHL exposure. In a short term experiment, cells were exposed to 2 h of high light (HL, 2000 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ starting at 12:00), followed by 4 h of recovery using either LL or darkness with a subsequent 1 h HL treatment. In a third experiment cells were exposed for 2 h to 100 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ (LL₁₀₀) only, starting at 12:00.

Sampling

Samples were taken as indicated in the Results section. For HPLC analysis, 400 μL of cell suspension were filtered on a Membrane Isopore Polycarbonate filter 1.2 μm (Millipore, USA) and immediately frozen in liquid nitrogen. Pigments were isolated and analyzed the

same day. For RNA and protein analyses, 4 mL of cell suspension were centrifuged 4 min at 4°C and 4000 g. The supernatant was discarded, and the cells were resolved in 1 mL ice cooled phosphate buffered saline and centrifuged at 14000 g for 1 min. The pellet was frozen in liquid nitrogen and stored at -80°C until further analysis.

Chlorophyll determination

Chl *a* was extracted using successively 10 % Methanol and 90 % Acetone. Its concentration was determined in a spectrophotometer (Hitachi U-5100, Japan) according to Jeffrey and Humphrey (1975).

Inhibitor treatments

Dithiothreitol (DTT, 500 μ M, in H₂O) was added 15 min before HL exposure. In an alternative experiment, DTT in the same concentration was additionally supplied every 20 min during the HL treatment. Cycloheximide (CHX, 2 μ g mL⁻¹, in H₂O) was added 45 min before HL treatment. Ammonium chloride (NH₄Cl, 5 mM, in H₂O) was supplemented after 2 h of LL recovery following 2 h of HL treatment. 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU, 10 μ M, in Ethanol, final concentration of Ethanol 2 ‰) was added either 10 min before the HL treatment or prior to the recovery period. 2,5-Dibromo-6-isopropyl-3-methyl-1,4-benzoquinone (DBMIB, 1 μ M, in Ethanol, final concentration of Ethanol 0.5 ‰) was supplied 10 min before starting the LL₁₀₀ exposure.

Chlorophyll fluorescence measurements

Chlorophyll fluorescence was measured with a Diving-PAM (Walz, Germany), whose optic guide was directly immersed into the algal suspension. Saturating flashes were sent at different time points (see results). As the surrounding light interfered with the measurements, immediately before starting the flash the light was switched off, which was assumed to be fast enough to avoid maximal fluorescence relaxation, in line with previous findings (Havaux et

al. 1991). The maximum photosynthetic efficiency of PSII was determined as $(F_m - F_o)/F_m = F_v/F_m$ and the effective quantum yield of PSII as F_v'/F_m' . Non-photochemical fluorescence quenching (NPQ) was calculated using the Stern-Volmer parameter as $NPQ = F_m/F_m' - 1$. Photoinactivation was determined as $1/F_o - 1/F_m$ according to Park et al. (1995) and He and Chow (2003). It has been shown to well describe the photoinactivation also in diatoms (Wu et al. 2011; Campbell and Tyystjärvi 2012). Here, the value of LL acclimated cells before starting the HL treatment was taken as 100 %. For investigating the optimal concentration of DBMIB, fluorescence kinetics were measured during 5 min of illumination with 2000 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ with a 2500 Schott white light lamp (Germany).

Pigment analysis

Pigment extraction and HPLC analysis followed the protocol established by Jakob et al. (1999). Samples were analyzed with a Hitachi LaChrom Elite HPLC system (Japan) equipped with a 10°C cooled autosampler and a Nucleosil 120-5 C18 column (Maccherey-Nagel, Germany).

RNA extraction and LHCX transcript level analysis by Real-time PCR

RNA was extracted using Trizol (Invitrogen, USA) and the RNeasy Mini kit (Qiagen, Germany). Contaminations of DNA were removed with TurboDNase (Ambion, USA). An equal amount of RNA was used for all samples to be transcribed into cDNA using the QuantiTect reverse transcription kit (Qiagen, Germany). Resulting cDNA was diluted 2-fold in Nuclease-free water. For Real-time PCR analysis, 1 μL of cDNA was used in a 20 μL qPCR assay containing also the primer pairs and the GoTaq Mastermix (Promega, USA). A geNorm v.3.5 analysis (Vandesompele et al. 2002) was performed according to the authors instructions, showing that *18S* and *RPS* were both well suited as endogenous reference genes under conditions used for growing of the cells (Suppl. Fig. 1). Disadvantageous for *18S* as

reference gene were the lower Ct-values compared to *LHCX* genes due to its high abundance (around 14 compared to 20-30 for the *LHCX* genes), leading to a decreased sensitivity for weaker expressed genes. However, when DBMIB and DCMU were supplied, *18S* expression was clearly more stable than *RPS* expression (Suppl. Fig. 2). Therefore, the gene encoding *18S* was chosen as the endogenous reference gene. Primer sequences can be found in Suppl. Table 1. Real-Time PCR was performed on a Stratagene Mx3005P system (Agilent, USA) using a two-step protocol. Samples were measured in triplicates. At the end of each run melting curve analysis was applied to confirm the specific amplification of the template and to exclude false positive fluorescence due to side reactions. C_T-values were obtained by utilizing PCR Miner 3.0 (Zhao and Fernald 2005). Relative transcript levels were calculated according to Pfaffl et al. (2002) and using the REST© software tool.

LHCX protein analysis

Protein extraction, Western blot and ECL detection followed the protocol described in Coesel et al. (2009) but using a 14% Lithiumdodecylsulfate PAGE for protein separation. Anti-LHCSR from *Chlamydomonas reinhardtii* (kind gift of Dr. Graham Peers, UC Berkely, USA), which was already shown to detect the LHCX proteins in *P. tricornutum* (Bailleul et al. 2010), was applied in a 1:5000 dilution overnight. Anti-PsbC (CP47, Agrisera, Sweden) was used as a loading control. Chemiluminescence signals were recorded with the CCD camera LAS4000 (Fujifilm Global, Japan).

Supplemental Data

Supplemental Fig. 1: geNorm analysis of putative housekeeping genes

Supplemental Fig. 2: C_T -Values of *18S* and *RPS* for the different light and inhibitor treatments

Supplemental Fig. 3: Effective PSII quantum yield and NPQ during the 33 h experiment

Supplemental Fig. 4: Diatoxanthin versus NPQ relationship in mHL, HL, and HL plus cycloheximide treated cells

Supplemental Fig. 5: Effect of DBMIB and DCMU on effective PSII quantum yield and NPQ

Supplemental Fig. 6: Relative gene expression levels of *LHCX1*, *LHCX2*, *LHCX3* and *LHCX4* under HL illumination in the presence or absence of DCMU

Supplemental Table 1: Primer sequences used for the gene expression analysis

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Literature Cited

- Adl SM, Simpson AGB, Farmer MA, Andersen RA, Anderson OR, Barta JR, Browser SS, Brugerolle G, Fensome RA, Fredericq S, James TY, Karpov S, Kugrens P, Krug J, Lane CE, Lewis LA, Lodge J, Lynn DH, Mann DG, McCourt RM, Mendoza L, Moestrup O, Mozley-Standridge SE, Nerad TA, Shearer CA, Smirnov AV, Spiegel FW, Taylor MFJR** (2005) The new higher level classification of eukaryotes with emphasis on the taxonomy of protists. *J Eukaryot Microbiol* **52**: 399-451
- Allen JF, de Paula WBM, Puthiyaveetil S, Nield J** (2011) A structural phylogenetic map for chloroplast photosynthesis. *Trends Plant Sci* **16**: 645-655
- Armbrust EV** (2009) The life of diatoms in the world's oceans. *Nature* **459**: 185-192
- Baier M, Dietz KJ** (2005) Chloroplasts as source and target of cellular redox regulation: a discussion on chloroplast redox signals in the context of plant physiology. *J Exp Bot* **56**: 1449-1462
- Bailleul B, Rogato A, de Martino A, Coesel S, Cardol P, Bowler C, Falciatore A, Finazzi G** (2010) An atypical member of the light-harvesting complex stress-related protein family modulates diatom responses to light. *Proc Natl Acad Sci USA* **107**: 18214-18219
- Beer A, Gundermann K, Beckmann J, Büchel C** (2006) Subunit composition and pigmentation of fucoxanthin-chlorophyll proteins in diatoms: Evidence for a subunit involved in diadinoxanthin and diatoxanthin binding. *Biochemistry* **45**: 13046-13053
- Bertrand M** (2010) Carotenoid biosynthesis in diatoms. *Photosynth Res* **106**: 89-102
- Bonente G, Howes BD, Caffarri S, Smulevich G, Bassi R** (2008) Interactions between the photosystem II subunit PsbS and xanthophylls studied in vivo and in vitro. *J Biol Chem* **283**: 8434-8445
- Bonente G, Ballottari M, Truong TB, Morosinotto T, Ahn TK, Fleming GR, Niyogi KK, Bassi R** (2011) Analysis of LhcSR3, a Protein Essential for Feedback De-Excitation in the Green Alga *Chlamydomonas reinhardtii*. *PLoS Biol* **9**: e1000577
- Bowler C, Allen AE, Badger JH, Grimwood J, Jabbari K, Kuo A, Maheswari U, Martens C, Maumus F, Otilar RP, Rayko E, Salamov A, Vandepoele K, Beszteri B, Gruber A, Heijde M, Katinka M, Mock T, Valentin K, Verret F, Berges JA, Brownlee C, Cadoret JP, Chiovitti A, Choi CJ, Coesel S, De Martino A, Dettler JC, Durkin C, Falciatore A, Fournet J, Haruta M, Huysman MJJ, Jenkins BD, Jiroutova K, Jorgensen RE, Joubert Y, Kaplan A, Kroger N, Kroth PG, La Roche J, Lindquist E, Lommer M, Martin-Jezequel V, Lopez PJ, Lucas S, Mangogna M, McGinnis K, Medlin LK, Montsant A, Oudot-Le Secq MP, Napoli C, Obornik M, Parker MS, Petit JL, Porcel BM, Poulsen N, Robison M, Rychlewski L, Rynearson TA, Schmutz J, Shapiro H, Siaut M, Stanley M, Sussman MR, Taylor AR, Vardi A, von Dassow P, Vyverman W, Willis A, Wyrwicz LS, Rokhsar DS, Weissenbach J, Armbrust EV, Green BR, Van De**

- Peer Y, Grigoriev IV** (2008) The *Phaeodactylum* genome reveals the evolutionary history of diatom genomes. *Nature* **456**: 239-244
- Bowler C, Vardi A, Allen AE** (2010) Oceanographic and Biogeochemical Insights from Diatom Genomes. *Annu Rev Marine Sci* **2**: 333-365
- Brunet C, Lavaud J** (2010) Can the xanthophyll cycle help extract the essence of the microalgal functional response to a variable light environment? *Journal of Plankton Research* **32**: 1609-1617
- Campbell DA, Tyystjärvi E** (2012) Parameterization of photosystem II photoinactivation and repair. *BBA-Bioenergetics* **1817**: 258-265
- Caron L, Berkaloff C, Duval JC, Jupin H** (1987) Chlorophyll Fluorescence Transients from the Diatom *Phaeodactylum tricornutum* - Relative Rates of Cyclic Phosphorylation and Chlororespiration. *Photosynth Res* **11**: 131-139
- Coesel S, Mangogna M, Ishikawa T, Heijde M, Rogato A, Finazzi G, Todo T, Bowler C, Falciatore A** (2009) Diatom PtCPF1 is a new cryptochrome/photolyase family member with DNA repair and transcription regulation activity. *EMBO Rep* **10**: 655-661
- Coesel S, Obornik M, Varela J, Falciatore A, Bowler C** (2008) Evolutionary origins and functions of the carotenoid biosynthetic pathway in marine diatoms. *PLoS One* **3**: e2896
- Cruz S, Goss R, Wilhelm C, Leegood R, Horton P, Jakob T** (2011) Impact of chlororespiration on non-photochemical quenching of chlorophyll fluorescence and on the regulation of the diadinoxanthin cycle in the diatom *Thalassiosira pseudonana*. *J Exp Bot* **62**: 509-519
- Dambek M, Eilers U, Breitenbach J, Steiger S, Büchel C, Sandmann G** (2012) Biosynthesis of fucoxanthin and diadinoxanthin and function of initial pathway genes in *Phaeodactylum tricornutum*. *J Exp Bot* **63**: 5607-5612
- Depauw FA, Rogato A, Ribera d'Alcala M, Falciatore A** (2012) Exploring the molecular basis of responses to light in marine diatoms. *J Exp Bot* **63**: 1575-1591
- Dijkman NA, Kroon BMA** (2002) Indications for chlororespiration in relation to light regime in the marine diatom *Thalassiosira weissflogii*. *J Photochem Photobiol B: Biol* **66**: 179-187
- Dominici P, Caffarri S, Armenante F, Ceoldo S, Crimi M, Bassi R** (2002) Biochemical Properties of the PsbS Subunit of Photosystem II Either Purified from Chloroplast or Recombinant. *J Biol Chem* **277**: 22750-22758
- Dyhrman ST, Jenkins BD, Rynearson TA, Saito MA, Mercier ML, Alexander H, Whitney LP, Drzewianowski A, Bulygin VV, Bertrand EM, Wu Z, Benitez-Nelson C, Heithoff A** (2012) The Transcriptome and Proteome of the Diatom *Thalassiosira pseudonana* Reveal a Diverse Phosphorus Stress Response. *PLoS One* **7**: e33768

- Eisenstadt D, Ohad I, Keren N, Kaplan A** (2008) Changes in the photosynthetic reaction centre II in the diatom *Phaeodactylum tricornutum* result in non-photochemical fluorescence quenching. *Environmental Microbiology* **10**: 1997-2007
- Engelken J, Funk C, Adamska I** (2012) The Extended Light-Harvesting Complex (LHC) Protein Superfamily: Classification and Evolutionary Dynamics. in R Burnap, W Vermaas, eds, *Functional Genomics and Evolution of Photosynthetic Systems*, Volume 33, Springer 265-284
- Escoubas JM, Lomas M, LaRoche J, Falkowski PG** (1995) Light intensity regulation of cab gene transcription is signaled by the redox state of the plastoquinone pool. *Proc Natl Acad Sci USA* **92**: 10237-10241
- Fey V, Wagner R, Bräutigam K, Pfannschmidt T** (2005) Photosynthetic redox control of nuclear gene expression. *J Exp Bot* **56**: 1491-1498
- Fischer BB, Krieger-Liszkay A, Eggen RIL** (2004) Photosensitizers Neutral Red (Type I) and Rose Bengal (Type II) Cause Light-Dependent Toxicity in *Chlamydomonas reinhardtii* and Induce the Gpxh Gene via Increased Singlet Oxygen Formation. *Environ Sci Technol* **38**: 6307-6313
- Flors C, Fryer MJ, Waring J, Reeder B, Bechtold U, Mullineaux PM, Nonell S, Wilson MT, Baker NR** (2006) Imaging the production of singlet oxygen in vivo using a new fluorescent sensor, Singlet Oxygen Sensor Green. *J Exp Bot* **57**: 1725-1734
- Foyer CH, Neukermans J, Queval G, Noctor G, Harbinson J** (2012) Photosynthetic control of electron transport and the regulation of gene expression. *J Exp Bot* **63**: 1637-1661
- Frommolt R, Werner S, Paulsen H, Goss R, Wilhelm C, Zauner S, Maier UG, Grossman AR, Bhattacharya D, Lohr M** (2008) Ancient recruitment by chromists of green algal genes encoding enzymes for carotenoid biosynthesis. *Mol Biol Evol* **25**: 2653-2667
- Geider RJ, Delucia EH, Falkowski PG, Finzi AC, Grime JP, Grace J, Kana TM, La Roche J, Long SP, Osborne BA, Platt T, Prentice IC, Raven JA, Schlesinger WH, Smetacek V, Stuart V, Sathyendranath S, Thomas RB, Vogelmann TC, Williams P, Woodward FI** (2001) Primary productivity of planet earth: biological determinants and physical constraints in terrestrial and aquatic habitats. *Global Change Biol* **7**: 849-882
- Goss R, Jakob T** (2010) Regulation and function of xanthophyll cycle-dependent photoprotection in algae. *Photosynth Res* **106**: 103-122
- Goss R, Pinto EA, Wilhelm C, Richter M** (2006) The importance of a highly active and delta pH regulated diatoxanthin epoxidase for the regulation of the PSII antenna function in diadinoxanthin cycle containing algae. *J Plant Physiol* **163**: 1008-1021
- Green BR** (2011) Chloroplast genomes of photosynthetic eukaryotes. *Plant J* **66**: 34-44
- Grouneva I, Jakob T, Wilhelm C, Goss R** (2009) The regulation of xanthophyll cycle activity and of non-photochemical fluorescence quenching by two alternative electron

- flows in the diatoms *Phaeodactylum tricornutum* and *Cyclotella meneghiniana*. BBA-Bioenergetics **1787**: 929-938
- Grouneva I, Rokka A, Aro EM** (2011) The Thylakoid Membrane Proteome of Two Marine Diatoms Outlines Both Diatom-Specific and Species-Specific Features of the Photosynthetic Machinery. J Proteome Res **10**: 5338-5353
- Guillard RRL** (1975) Culture of phytoplankton for feeding marine invertebrates. In WL Smith, MH Chaney, eds, Culture of marine invertebrate animals, Plenum Book Publ Corp, New York, 29-60
- Haehnel W** (1984) Photosynthetic Electron Transport in Higher Plants. Annu Rev Plant Physiol **35**: 659-693
- Havaux M, Strasser RJ, Greppin H** (1991) A theoretical and experimental analysis of the qP and qN coefficients of chlorophyll fluorescence quenching and their relation to photochemical and nonphotochemical events. Photosynth Res **27**: 41-55
- He J, Chow WS** (2003) The rate coefficient of repair of photosystem II after photoinactivation. Physiol Plant **118**: 297-304
- Jahns P, Holzwarth AR** (2012) The role of the xanthophyll cycle and of lutein in photoprotection of photosystem II. BBA-Bioenergetics **1817**: 182-193
- Jakob T, Goss R, Wilhelm C** (1999) Activation of diadinoxanthin de-epoxidase due to a chlororespiratory proton gradient in the dark in the diatom *Phaeodactylum tricornutum*. Plant Biology **1**: 76-82
- Jakob T, Goss R, Wilhelm C** (2001) Unusual pH-dependence of diadinoxanthin de-epoxidase activation causes chlororespiratory induced accumulation of diatoxanthin in the diatom *Phaeodactylum tricornutum*. J Plant Physiol **158**: 383-390
- Juhas M, Büchel C** (2012) Properties of photosystem I antenna protein complexes of the diatom *Cyclotella meneghiniana*. J Exp Bot **63**: 3673-3681
- Karpinski S, Escobar C, Karpinska B, Creissen G, Mullineaux PM** (1997) Photosynthetic electron transport regulates the expression of cytosolic ascorbate peroxidase genes in Arabidopsis during excess light stress. Plant Cell **9**: 627-640
- Kikutani S, Tanaka R, Yamazaki Y, Hara S, Hisabori T, Kroth PG, Matsuda Y** (2012) Redox regulation of carbonic anhydrases via thioredoxin in chloroplast of the marine diatom *Phaeodactylum tricornutum*. J Biol Chem **287**: 20689-20700
- Kleine T, Voigt C, Leister D** (2009) Plastid signalling to the nucleus: messengers still lost in the mists? Trends Genet **25**: 185-192
- Kroth PG, Chiovitti A, Gruber A, Martin-Jezequel V, Mock T, Parker MS, Stanley MS, Kaplan A, Caron L, Weber T, Maheswari U, Armbrust EV, Bowler C** (2008) A model for carbohydrate metabolism in the diatom *Phaeodactylum tricornutum* deduced from comparative whole genome analysis. PLoS One **3**: e1426
- Kurusu G, Zhang H, Smith JL, Cramer WA** (2003) Structure of the Cytochrome b6f Complex of Oxygenic Photosynthesis: Tuning the Cavity. Science **302**: 1009-1014

- Lavaud J** (2007) Fast regulation of photosynthesis in diatoms: mechanisms, evolution and ecophysiology. *Func Plant Sci Biotech* **1**: 267-287
- Lavaud J, Rousseau B, Etienne AL** (2004) General features of photoprotection by energy dissipation in planktonic diatoms (Bacillariophyceae). *J Phycol* **40**: 130-137
- Lavaud J, Rousseau B, Van Gorkom HJ, Etienne AL** (2002a) Influence of the diadinoxanthin pool size on photoprotection in the marine planktonic diatom *Phaeodactylum tricornutum*. *Plant Physiol* **129**: 1398-1406
- Lavaud J, Strzepak RF, Kroth PG** (2007) Photoprotection capacity differs among diatoms: Possible consequences on the spatial distribution of diatoms related to fluctuations in the underwater light climate. *Limnol Oceanogr* **52**: 1188-1194
- Lavaud J, Van Gorkom HJ, Etienne AL** (2002b) Photosystem II electron transfer cycle and chlororespiration in planktonic diatoms. *Photosynth Res* **74**: 51-59
- Lavaud J, Materna AC, Sturm S, Vugrinec S, Kroth PG** (2012) Silencing of the Violaxanthin De-Epoxidase Gene in the Diatom *Phaeodactylum tricornutum* Reduces Diatoxanthin Synthesis and Non-Photochemical Quenching. *PLoS One* **7**: e36806
- Lee MV, Topper SE, Hubler SL, Hose J, Wenger CD, Coon JJ, Gasch AP** (2011) A dynamic model of proteome changes reveals new roles for transcript alteration in yeast. *Mol Syst Biol* **7**: 514
- Lepetit B, Goss R, Jakob T, Wilhelm C** (2012) Molecular dynamics of the diatom thylakoid membrane under different light conditions. *Photosynth Res* **111**: 245-257
- Lepetit B, Volke D, Gilbert M, Wilhelm C, Goss R** (2010) Evidence for the existence of one antenna-associated, lipid-dissolved, and two protein-bound pools of diadinoxanthin cycle pigments in diatoms. *Plant Physiol* **154**: 1905-1920
- Li H, Sherman LA** (2000) A Redox-Responsive Regulator of Photosynthesis Gene Expression in the Cyanobacterium *Synechocystis* sp. Strain PCC 6803. *J Bacteriol* **182**: 4268-4277
- Li Z, Wakao S, Fischer BB, Niyogi KK** (2009) Sensing and responding to excess light. *Ann Rev Plant Biol* **60**: 239-260
- Lohr M, Wilhelm C** (1999) Algae displaying the diadinoxanthin cycle also possess the violaxanthin cycle. *Proc Natl Acad Sci USA* **96**: 8784-8789
- Lohr M** (2011) Carotenoid metabolism in phytoplankton. In S Roy, CA Llewellyn, E Skarstadt Egeland, G Johnsen, eds, *Phytoplankton Pigments: Characterization, Chemotaxonomy and Applications in Oceanography*, Cambridge University Press, New York, 113-161
- Lohr M, Schwender J, Polle JEW** (2012) Isoprenoid biosynthesis in eukaryotic phototrophs: A spotlight on algae. *Plant Science* **185–186**: 9-22
- Long SP, Humphries S, Falkowski PG** (1994) Photoinhibition of Photosynthesis in Nature. *Ann Rev Plant Physiol Plant Mol Biol* **45**: 633-662

- Materna A, Sturm S, Kroth PG, Lavaud J** (2009) First induced plastid genome mutations in an alga with secondary plastids: *psbA* mutations in the diatom *Phaeodactylum tricorutum* (Bacillariophyceae) reveal consequences on the regulation of photosynthesis. *J Phycol* **45**: 838-846
- Nymark M, Valle KC, Brembu T, Hancke K, Winge P, Andresen K, Johnsen G, Bones AM** (2009) An integrated analysis of molecular acclimation to high light in the marine diatom *Phaeodactylum tricorutum*. *PLoS One* **4**: e7743
- Obrig TG, Culp WJ, McKeehan WL, Hardesty B** (1971) The Mechanism by which Cycloheximide and Related Glutarimide Antibiotics Inhibit Peptide Synthesis on Reticulocyte Ribosomes. *J Biol Chem* **246**: 174-181
- Olaizola M, LaRoche J, Kolber Z, Falkowski PG** (1994) Non-photochemical fluorescence quenching and the diadinoxanthin cycle in a marine diatom. *Photosynth Res* **41**: 357-370
- Onno Feikema W, Marosvolgyi MA, Lavaud J, van Gorkom HJ** (2006) Cyclic electron transfer in photosystem II in the marine diatom *Phaeodactylum tricorutum*. *BBA-Bioenergetics* **1757**: 829-834
- op den Camp RGL, Przybyla D, Ochsenbein C, Laloi C, Kim C, Danon A, Wagner D, Hideg E, Göbel C, Feussner I, Nater M, Apel K** (2003) Rapid Induction of Distinct Stress Responses after the Release of Singlet Oxygen in Arabidopsis. *Plant Cell* **15**: 2320-2332
- Oudot-Le Secq M-P, Grimwood J, Shapiro H, Armbrust E, Bowler C, Green B** (2007) Chloroplast genomes of the diatoms *Phaeodactylum tricorutum* and *Thalassiosira pseudonana*: comparison with other plastid genomes of the red lineage. *Mol Gen Genomics* **277**: 427-439
- Park YI, Chow WS, Anderson JM** (1995) Light inactivation of functional photosystem II in leaves of peas grown in moderate light depends on photon exposure. *Planta* **196**: 401-411
- Pfaffl MW, Horgan GW, Dempfle L** (2002) Relative expression software tool (REST) for group-wise comparison and statistical analysis of relative expression results in real-time PCR. *Nucleic Acids Res* **30**: e36
- Pfannschmidt T** (2003) Chloroplast redox signals: how photosynthesis controls its own genes. *Trends Plant Sci* **8**: 33-41
- Pfannschmidt T** (2010) Plastidial retrograde signalling - a true plastid factor or just metabolite signatures? *Trends Plant Sci* **15**: 427-435
- Pfannschmidt T, Nilsson A, Allen JF** (1999) Photosynthetic control of chloroplast gene expression. *Nature* **397**: 625-628
- Prihoda J, Tanaka A, de Paula WBM, Allen JF, Tirichine L, Bowler C** (2012) Chloroplast-mitochondria cross-talk in diatoms. *J Exp Bot* **63**: 1543-1557
- Rochaix JD** (2011) Regulation of photosynthetic electron transport. *BBA-Bioenergetics* **1807**: 375-383

- Ruban AV, Lavaud J, Rousseau B, Guglielmi G, Horton P, Etienne AL** (2004) The super-excess energy dissipation in diatom algae: comparative analysis with higher plants. *Photosynth Res* **82**: 165-175
- Ruban AV, Johnson MP, Duffy CDP** (2012) The photoprotective molecular switch in the photosystem II antenna. *BBA-Bioenergetics* **1817**: 167-181
- Schumann A, Goss R, Jakob T, Wilhelm C** (2007) Investigation of the quenching efficiency of diatoxanthin in cells of *Phaeodactylum tricornutum* (Bacillariophyceae) with different pool sizes of xanthophyll cycle pigments. *Phycologia* **46**: 113-117
- Setkov NA, Kazakov VN, Rosenwald IB, Makarova GF, Epifanova OI** (1992) Protein synthesis inhibitors, like growth factors, may render resting 3T3 cells competent for DNA synthesis: a radioautographic and cell fusion study. *Cell Proliferation* **25**: 181-191
- Staneloni RJ, Rodriguez-Batiller MJ, Casal JJ** (2008) Abscisic Acid, High-Light, and Oxidative Stress Down-Regulate a Photosynthetic Gene via a Promoter Motif Not Involved in Phytochrome-Mediated Transcriptional Regulation. *Mol Plant* **1**: 75-83
- Steinbrenner J, Linden H** (2003) Light induction of carotenoid biosynthesis genes in the green alga *Haematococcus pluvialis*: Regulation by photosynthetic redox control. *Plant Mol Biol* **52**: 343-356
- Strzpek RF, Harrison PJ** (2004) Photosynthetic architecture differs in coastal and oceanic diatoms. *Nature* **431**: 689-692
- Tirichine L, Bowler C** (2011) Decoding algal genomes: tracing back the history of photosynthetic life on Earth. *Plant J* **66**: 45-57
- Trebst A** (2007) Inhibitors in the functional dissection of the photosynthetic electron transport system. *Photosynth Res* **92**: 217-224
- Triantaphylidès C, Havaux M** (2009) Singlet oxygen in plants: production, detoxification and signaling. *Trends Plant Sci* **14**: 219-228
- Triantaphylidès C, Krischke M, Hoeberichts FA, Ksas B, Gresser G, Havaux M, Van Breusegem F, Mueller MJ** (2008) Singlet oxygen is the major reactive oxygen species involved in photooxidative damage to plants. *Plant Physiol* **148**: 960-968
- Tullberg A, Alexciev K, Pfannschmidt T, Allen JF** (2000) Photosynthetic electron flow regulates transcription of the *psaB* gene in pea (*Pisum sativum* L.) chloroplasts through the redox state of the plastoquinone pool. *Plant Cell Physiol* **41**: 1045-1054
- Vandesompele J, De Preter K, Pattyn F, Poppe B, Van Roy N, De Paepe A, Speleman F** (2002) Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. *Genome Biol* **3**: 0034.1
- Veith T, Brauns J, Weisheit W, Mittag M, Büchel C** (2009) Identification of a specific fucoxanthin-chlorophyll protein in the light harvesting complex of photosystem I in the diatom *Cyclotella meneghiniana*. *BBA-Bioenergetics* **1787**: 905-912

- Vener AV, van Kan PJM, Rich PR, Ohad I, Andersson B** (1997) Plastoquinol at the quinol oxidation site of reduced cytochrome b_f mediates signal transduction between light and protein phosphorylation: Thylakoid protein kinase deactivation by a single-turnover flash. *Proc Natl Acad Sci USA* **94**: 1585-1590
- Wilhelm C, Büchel C, Fisahn J, Goss R, Jakob T, LaRoche J, Lavaud J, Lohr M, Riebesell U, Stehfest K, Valentin K, Kroth PG** (2006) The regulation of carbon and nutrient assimilation in diatoms is significantly different from green algae. *Protist* **157**: 91-124
- Wu H, Cockshutt AM, McCarthy A, Campbell DA** (2011) Distinctive photosystem II photoinactivation and protein dynamics in marine diatoms. *Plant Physiol* **156**: 2184-2195
- Wu H, Roy S, Alami M, Green B, Campbell DA** (2012) Photosystem II Photoinactivation, Repair and Protection in Marine Centric Diatoms. *Plant Physiol* **160**: 464-476
- Yamamoto HY, Kamite L** (1972) The effects of dithiothreitol on violaxanthin de-epoxidation and absorbance changes in the 500-nm region. *BBA-Bioenergetics* **267**: 538-543
- Zhao S, Fernald RD** (2005) Comprehensive algorithm for quantitative real-time polymerase chain reaction. *J Comput Biol* **12**: 1047-1064
- Zhu SH** (2009) Characterization of LI818-like genes under various stress conditions in the marine diatom *Thalassiosira pseudonana*. PhD thesis. University of British Columbia, Vancouver
- Zhu SH, Green BR** (2008) Light harvesting and photoprotection in diatoms: Identification and expression of LI818-like proteins. In JF Allen, E Gantt, JH Golbeck, B Osmond, eds, *Energy from the Sun*, Springer, 261-264
- Zhu SH, Green BR** (2010) Photoprotection in the diatom *Thalassiosira pseudonana*: Role of LI818-like proteins in response to high light stress. *BBA-Bioenergetics* **1797**: 1449-1457
- Zito F, Finazzi G, Delosme R, Nitschke W, Picot D, Wollman FA** (1999) The Q_o site of cytochrome b₆f complexes controls the activation of the LHCII kinase. *EMBO J* **18**: 2961-2969

Figure legends

Fig. 1: *LHCX* transcription levels at different light regimes. Expression ratios are given in \log_2 transformed values as indicated by the color bar and represent relative amounts referring to *18S* gene expression. All levels were normalized to the respective initial value and represent the mean of four independent measurements. Asterisks represent significant changes with a p-value <0.05 (Pair Wise Fixed Reallocation Randomization Test© performed by REST©). A-C: 33 h experiment with A) darkness all the time, B) $40 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ illumination (LL) from 8:00 until 24:00, C) $750 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ illumination (mHL) from 8:00 until 24:00; D,E) 6 h experiment with D) LL illumination all the time, and E) $2000 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ illumination (HL) for the first 2 h followed by 4 h of LL recovery.

Fig. 2: Pigment evolution during the mHL (A) and HL (B) experiment. In A), the recovery period was performed at darkness, while in B) recovery was monitored at LL conditions. After 4 h of recovery from HL, cells were again exposed to 1 h of HL treatment. Values are the mean of three (A) and five (B) independent experiments ($\pm\text{SD}$).

Fig. 3: Diadinoxanthin+diatoxanthin (Dd+Dt) (A) and diatoxanthin (Dt) (B) content and NPQ (C,D) during 2 h of HL treatment, 4 h of LL recovery and subsequently 1 h of HL treatment. Control: cultures without inhibitors; dark recovery: after 2 h of HL exposure 4 h of darkness followed instead of 4 h of LL; CHX: cultures were incubated with cycloheximide (CHX, $2 \mu\text{g mL}^{-1}$) 45 min before starting the experiment; DTT: cultures were incubated with DTT ($500 \mu\text{M}$) 15 min before starting the experiment; DTT+: DTT ($500 \mu\text{M}$) was added additionally every 20 min during the HL treatment and 20 min before the second HL treatment; LL rec+DCMU: DCMU ($10 \mu\text{M}$) was added before starting the LL recovery period; DCMU+dark rec: DCMU ($10 \mu\text{M}$) was added 10 min before light exposure; recovery was performed in darkness; dark rec+ NH_4Cl : recovery was performed in darkness; after 2 h of darkness, NH_4Cl (5 mM) was added (indicated with an arrow). Samples represent the mean of two to five independent experiments ($\pm\text{SD}$), except for the NPQ values of DCMU treated samples during the recovery time, where only one experiment was performed.

Fig. 4: Functional fraction of PSII (in %) during 2 h of HL treatment as an indicator of PSII photoinactivation calculated by $1/F_o-1/F_m$. The ratio $1/F_o-1/F_m$ obtained before the start of the light treatment was taken as 100%. For explanation of the legend, see Fig. 3. DTT+CHX: DTT (500 μM) and cycloheximide (CHX, 2 $\mu\text{g mL}^{-1}$) were added 15 and 45 min before light exposure, respectively. $\pm\text{SD}$

Fig. 5: Western blot of LL, HL and HL+cycloheximide (CHX) treated cultures. Indicated are the samples before (0) and after (120) 2 h of LL or HL treatment, respectively. A polyclonal LHCSR antibody of *C. reinhardtii* was employed, which detects all of the LHCX proteins. Arrowheads indicate LHCX2 and LHCX3, which can be distinguished from LHCX1 by their larger size. As a loading control, an antibody against the plastid encoded PsbB (CP47) was applied.

Fig. 6: Evolution of the diadinoxanthin+diatoxanthin (Dd+Dt) pool size during illumination with 100 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ (LL_{100}). In these conditions, the diatoxanthin amount was less than 2 % of the total diadinoxanthin+diatoxanthin pool size in both experimental conditions. In case of the inhibitor treatment, 1 μM DBMIB was added 10 min before the start of the experiment. Depicted are the mean values of four independent measurements ($\pm\text{SD}$).

Fig. 7: Relative gene expression of *LHCX1* (A), *LHCX2* (B), *LHCX3* (C), and *LHCX4* (D) during illumination with 100 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ (LL_{100}). Relative *LHCX* transcription levels referring to *18S* gene expression were normalized to the initial amount. White: no chemical treatment; dark grey: 1 μM DBMIB was applied 10 min before the light treatment; light grey: in addition to DBMIB 10 μM DCMU was applied 10 min before the light treatment. Analyses were performed at the indicated time points and values represent the mean of three independent samples ($\pm\text{SD}$). Asterisks represent significant changes (p-value <0.05, Pair Wise Fixed Reallocation Randomization Test© performed by REST©) of the DBMIB treated samples compared to the respective control samples and, when available, also compared to the respective DBMIB+DCMU treated samples. *LHCX4* was significantly up-regulated in the presence of DBMIB compared to the control conditions at each time point. As this

significance was abolished by comparing DBMIB treated with DBMIB+DCMU treated samples, these are false positive results and therefore significance is also not indicated for time points 45 and 120 min.

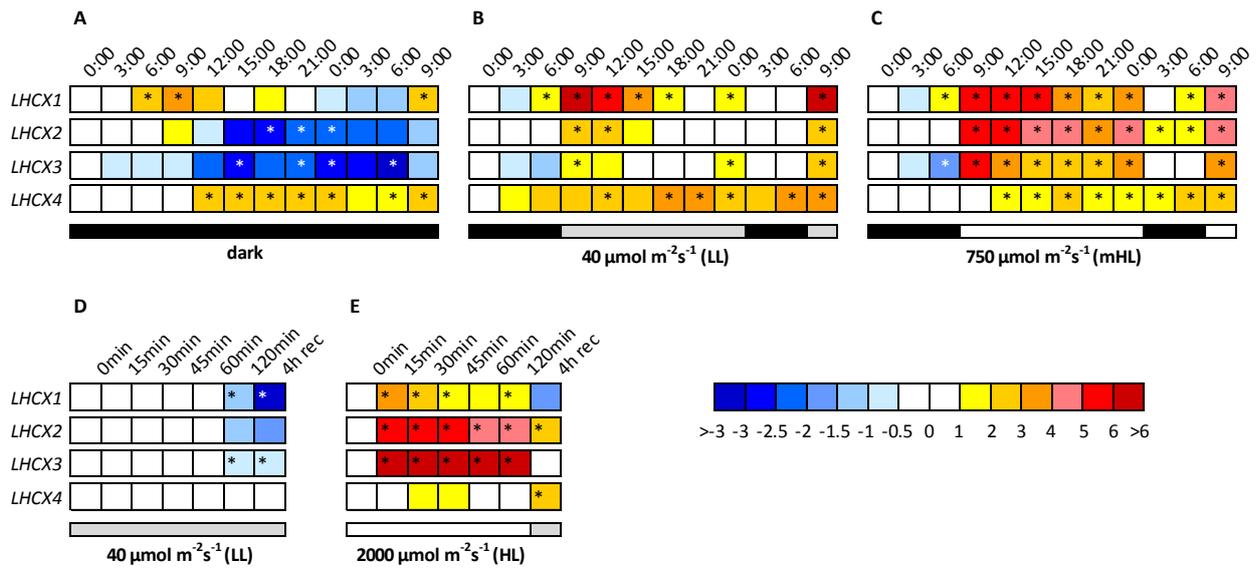


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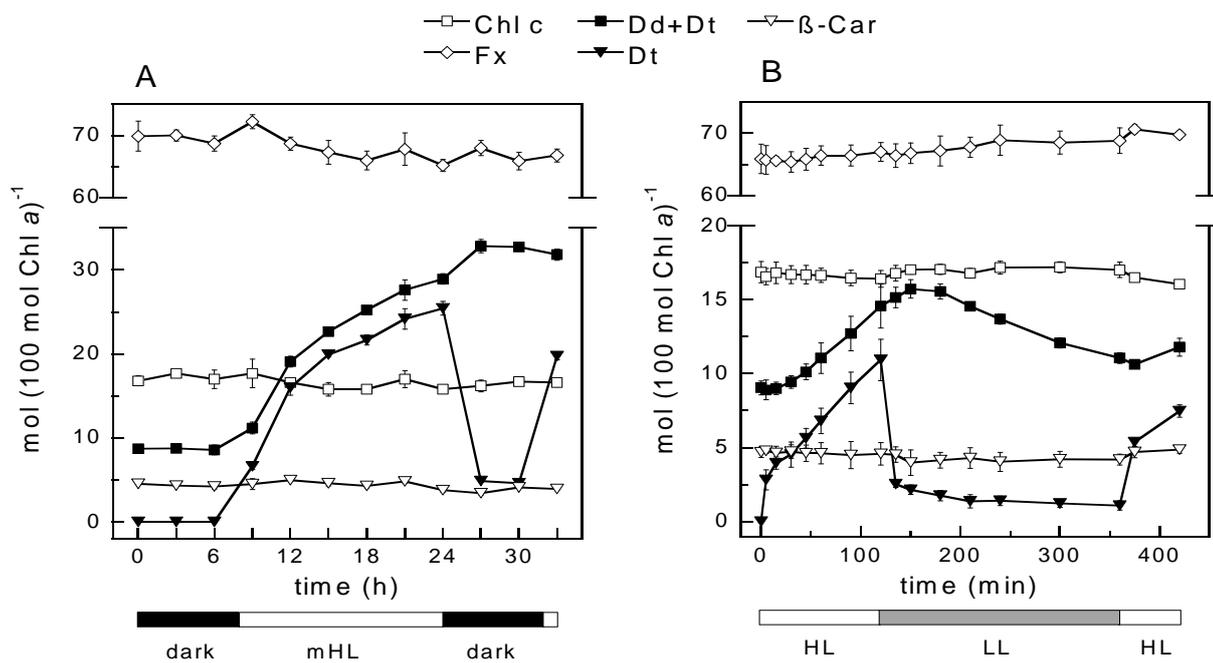


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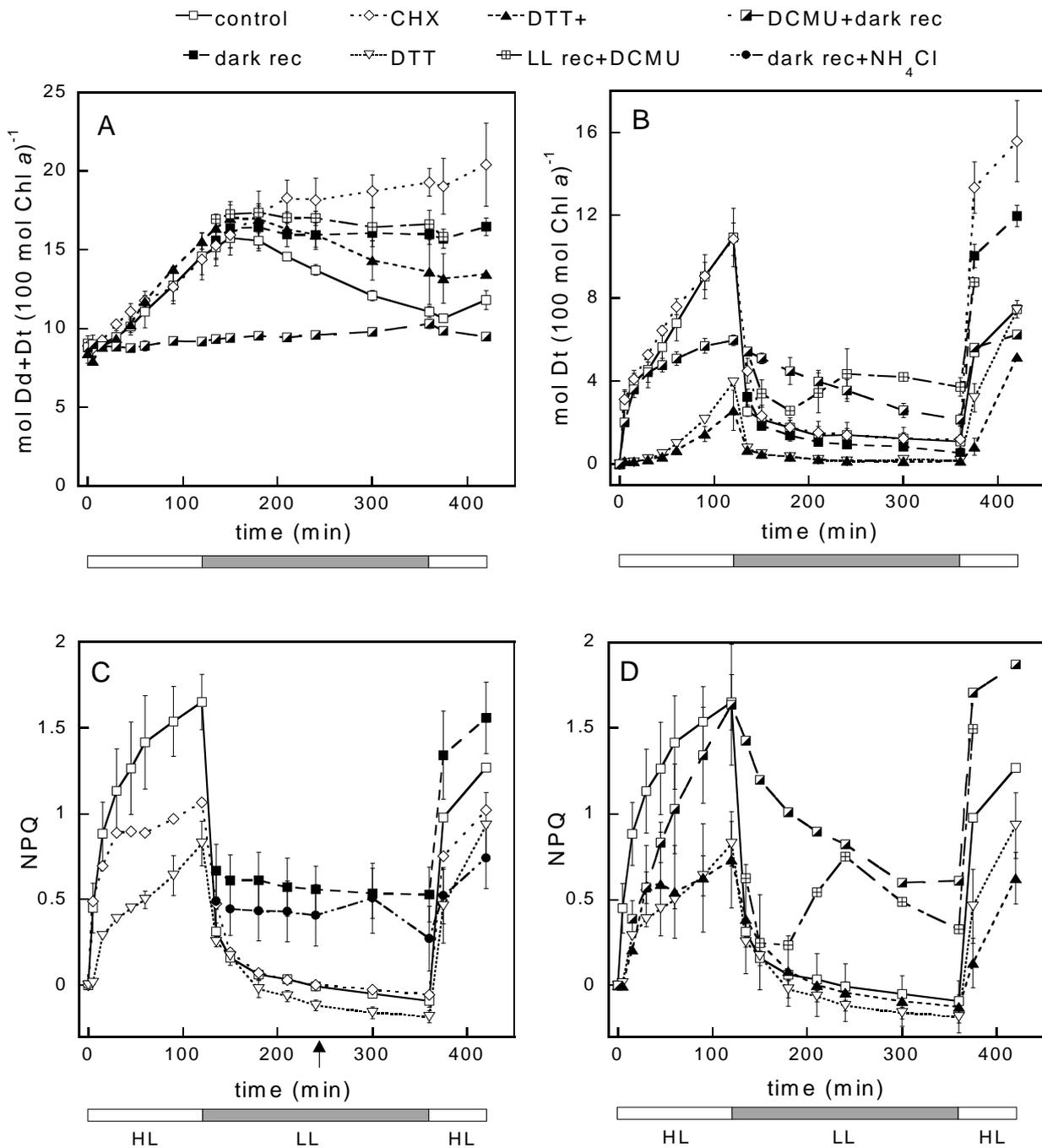


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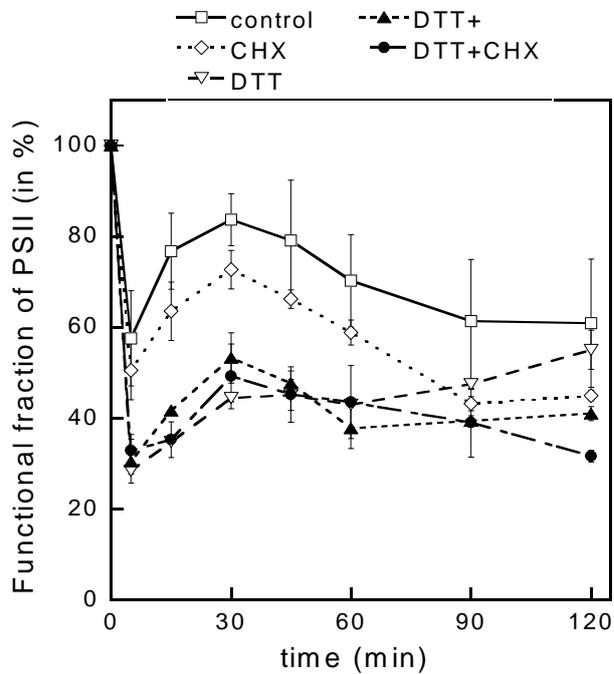


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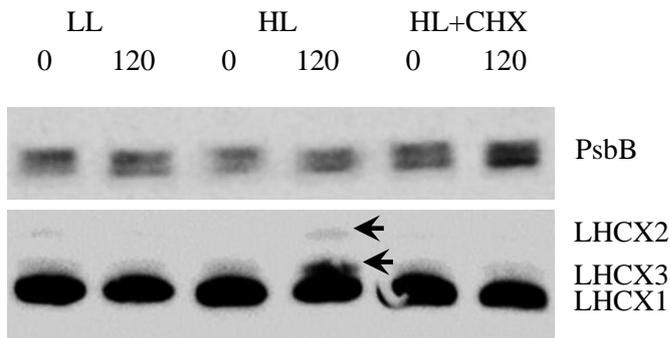


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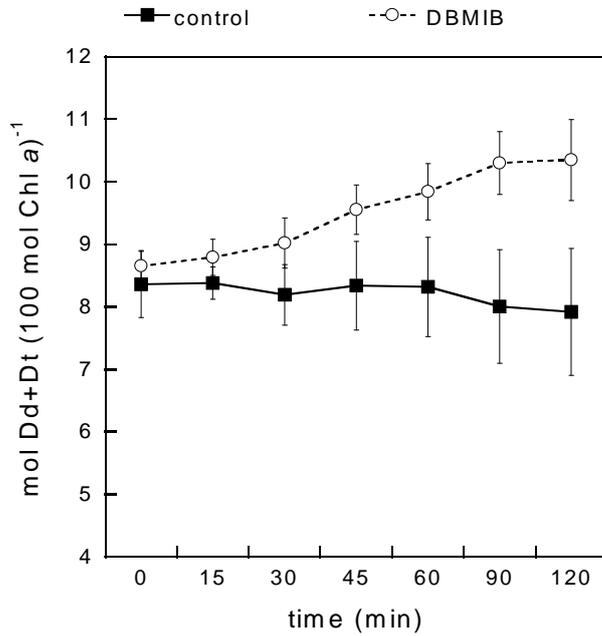


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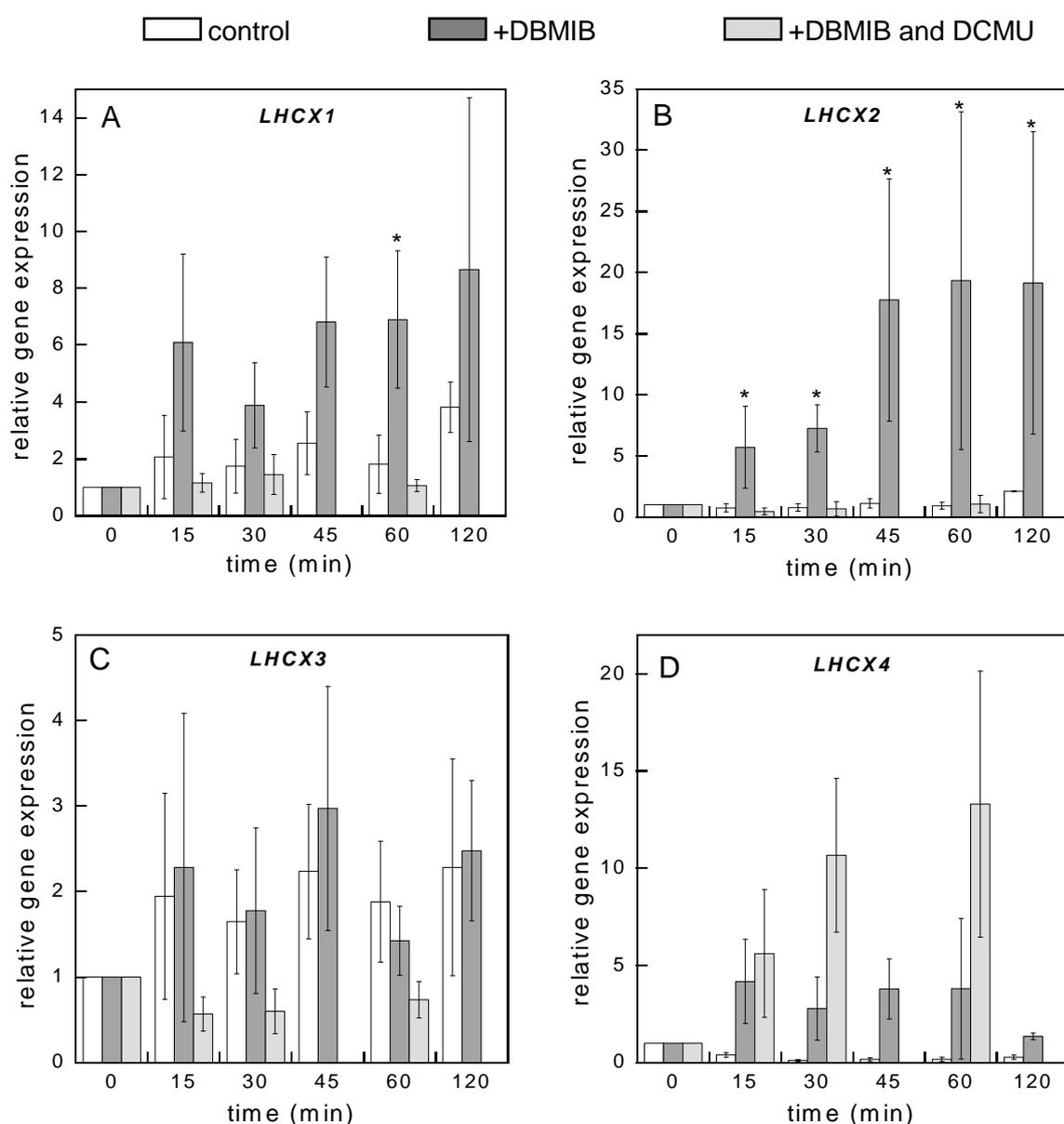
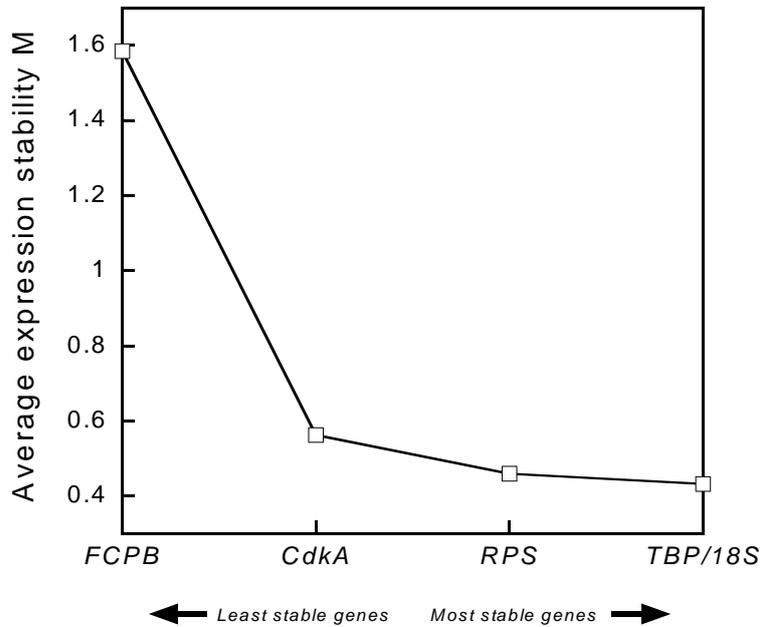


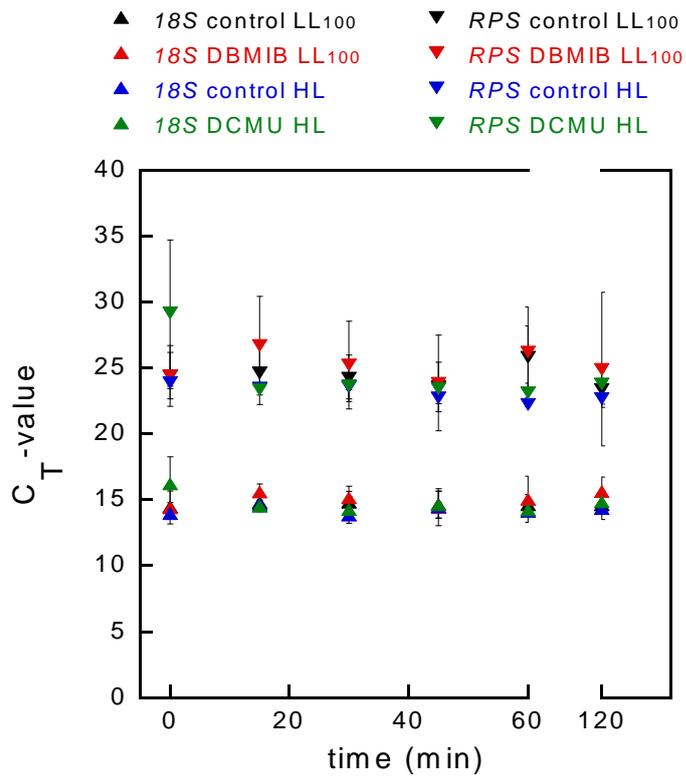
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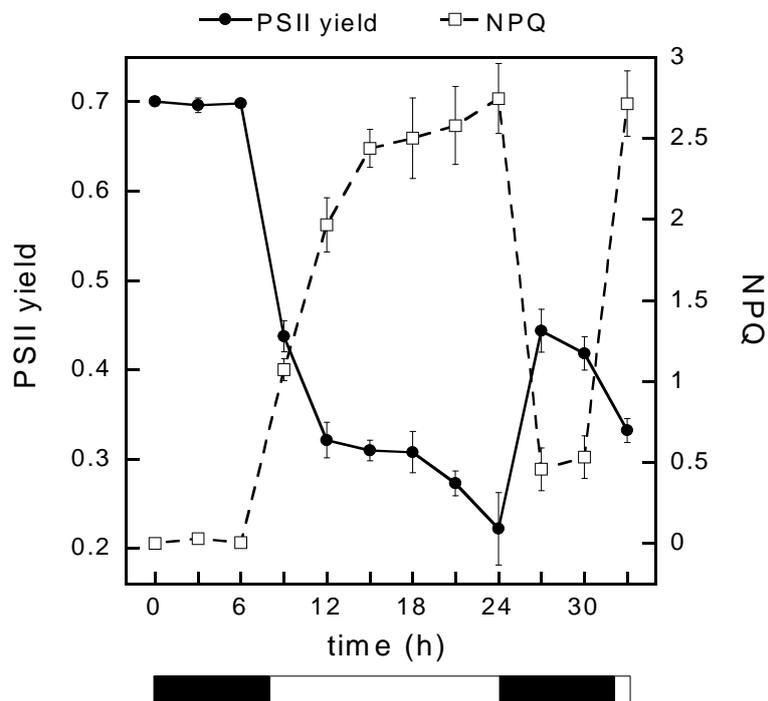
Supplemental Data



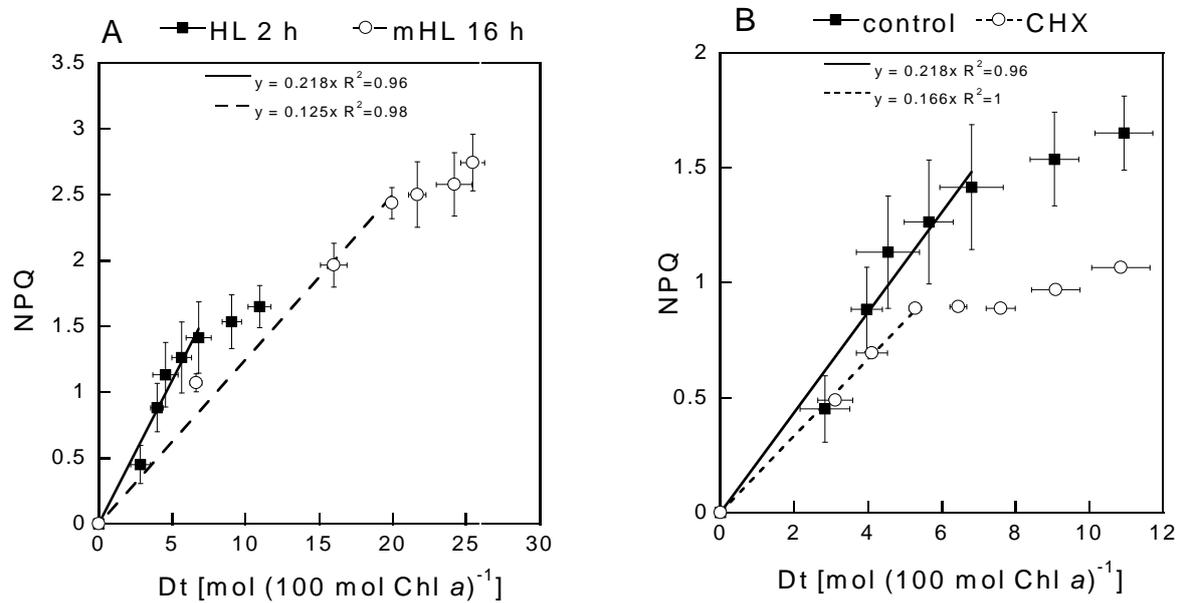
Supplemental Fig. 1: geNorm (v.3.5) analysis of putative housekeeping genes (cyclin dependent kinase A *CdkA* (20262, protein ID in the JGI database), ribosomal protein S1 *RPS* (44451), TATA-box binding protein *TBP* (10199) and 18S ribosomal ribonucleic acid *18S* (56377)), and *FCPB* (25172), which is known to have a strong daily regulation pattern (Siaut et al. 2007). Lower M-values indicate more stable expression. A total of 12 different samples were analyzed. *18S* and *RPS* are exhibiting comparable high expression stabilities and therefore are suitable candidates as endogenous reference genes at LL conditions.



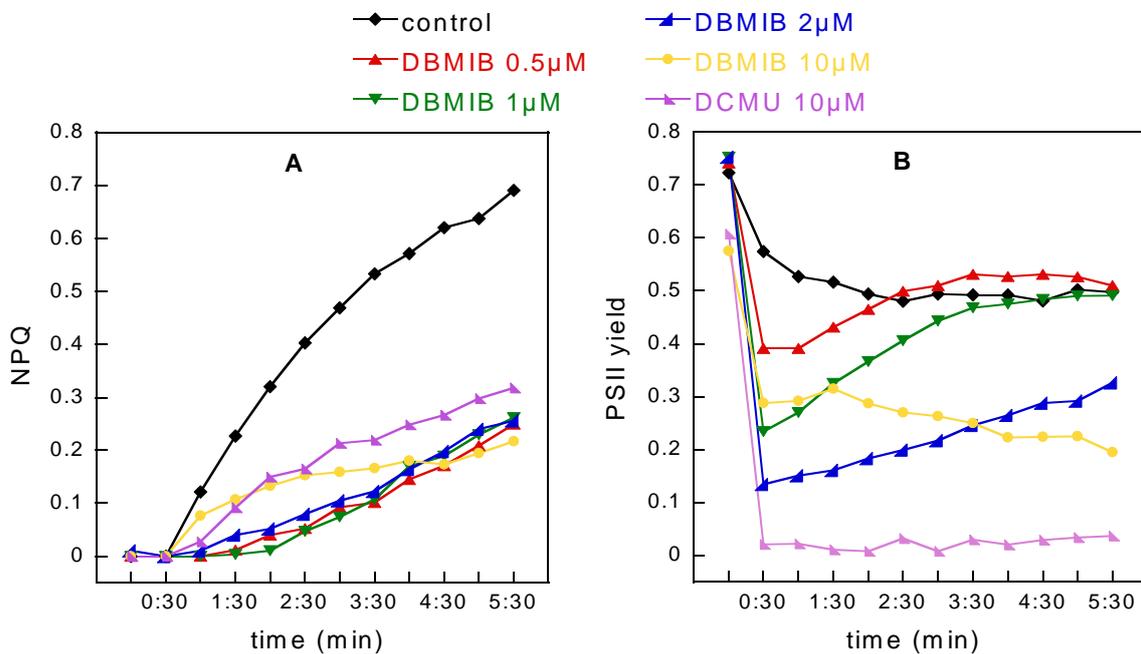
Supplemental Fig. 2: C_T-Values obtained by Real-time PCR analysis of *18S* and *RPS* for the different light and inhibitor treatments. LL₁₀₀, 100 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$; HL, 2000 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$. Cells were incubated with DBMIB (1 μM) or DCMU (10 μM) 10 min before light exposure. Note that the variations of the C_T-values as well as their standard deviations are weaker for *18S* than for *RPS*. Data represent the mean of two (HL) or three (LL₁₀₀) independent samples ($\pm\text{SD}$).



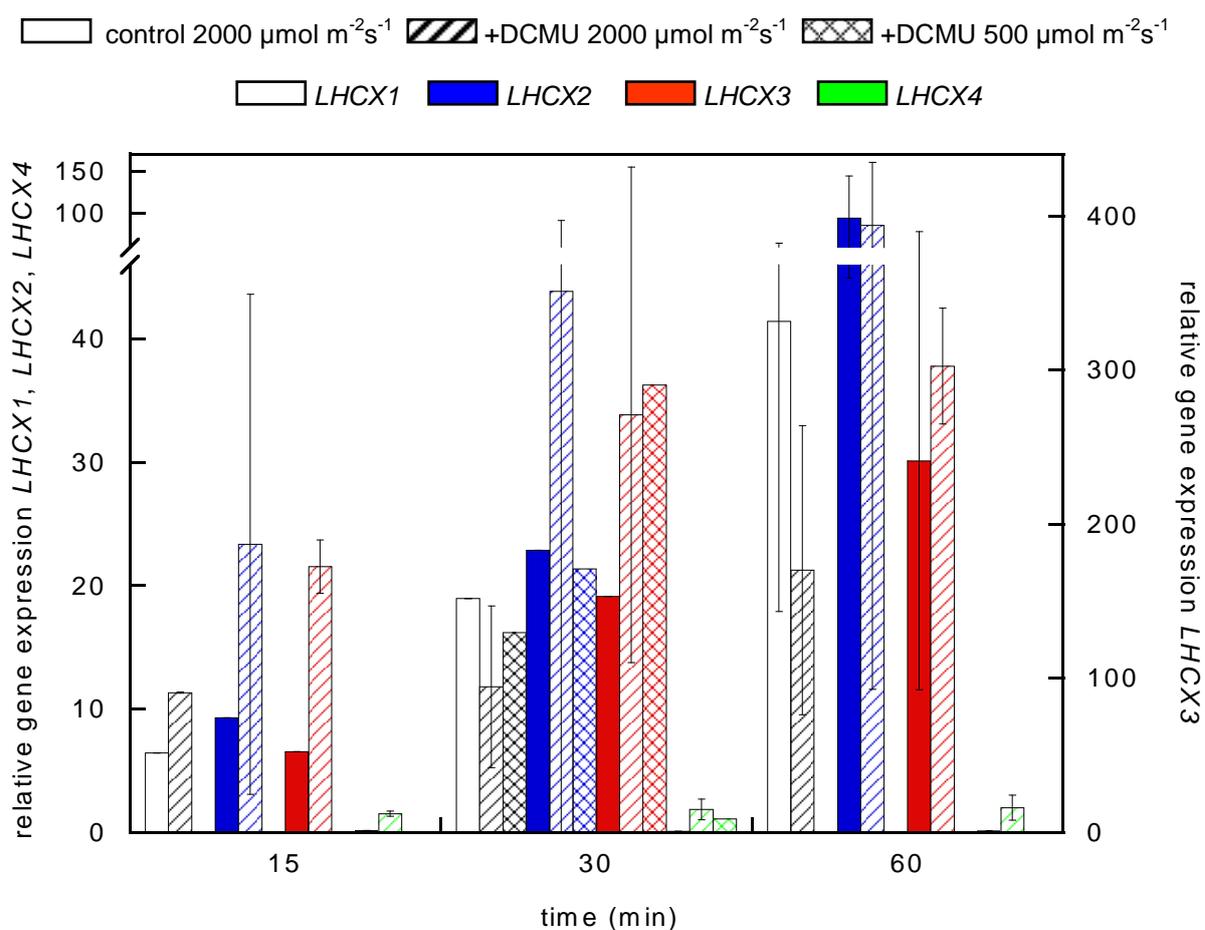
Supplemental Fig. 3: Effective PSII quantum yield and NPQ monitored during the 33 h experiment. The timespan of darkness and illumination with mHL are indicated with the black and white bar, respectively. Values represent the mean of three independent experiments (\pm SD).



Supplemental Fig. 4: Diatoxanthin (Dt) versus NPQ relationship of HL and mHL treated cells (A) and of HL (control) and HL plus cycloheximide (CHX) treated cells (B). For details, see legend of Fig. 3. To obtain an excellent linear correlation, the threshold concentration of HL treated cells was 7 mol Dt (100 mol Chl a)⁻¹ after 60 min of HL exposure, 5 mol Dt (100 mol Chl a)⁻¹ after 30 min of HL for the HL+CHX treated cells, and 20 mol Dt (100 mol Chl a)⁻¹ at 15:00 o'clock after 7 h of mHL exposure for the mHL treated cells. \pm SD



Supplemental Fig. 5: Effect of different concentrations of DBMIB and DCMU on NPQ (A) and the effective PSII quantum yield (B) during 5 min of illumination with HL. DCMU diminished the NPQ capacity and decreased the effective PSII quantum yield close to 0. NPQ was similarly low for concentrations of 0.5, 1, 2 and 10 μM DBMIB. The effective PSII quantum yield after 5 min of HL illumination was identical for the control and the two lowest DBMIB concentrations, while it was lower for 2 and 10 μM DBMIB. Note that in cells treated with 10 μM DBMIB the drop in the effective PSII quantum yield at the beginning of the HL treatment is lower than in cells treated with 1 or 2 μM DBMIB. Here one needs to take into account that at this concentration DBMIB quenches fluorescence (note the lower maximal PSII yield at the beginning of the measurement) and that the subsequent measurements are therefore unavoidably distorted. As 0.5 and 1 μM DBMIB had a similar effect on NPQ and the effective PSII quantum yield, we chose the higher DBMIB concentration for conducting the experiments, in order to prevent a possible weakening of the DBMIB effect during the 2 h of light treatment .



Supplemental Fig. 6: Relative gene transcript levels of *LHCX1*, *LHCX2*, *LHCX3* and *LHCX4* during exposure at 2000 or 500 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$. Relative *LHCX* transcription levels referring to *18S* gene expression were normalized to the initial amount at time point 0, which was accordingly 1 (not indicated). As the relative gene expression of *LHCX3* was much higher than the relative gene expression of all other three *LHCX* genes, an extra y-scale on the right was introduced. When indicated (+DCMU), DCMU (10 μM) was applied to the samples 10 min before starting the light exposure. Regarding the DCMU 500 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ conditions, only time points 0 and 30 min of only one sample were analyzed. Values of the DCMU 2000 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ dataset represent the mean of two independent samples ($\pm\text{SD}$). Regarding control conditions, only time points 0 and 60 min of two independent samples were analyzed, as a more detailed analysis was already performed under these conditions presented in Fig. 1.

Gene	Forward primer sequence	Reverse primer sequence
<i>LHCX1</i>	5'-AAGGTTTCGTCGTTCTCTTCG-3'	5'-CGGAAGCACCGATAGCAATAGT-3'
<i>LHCX2</i>	5'-CGCCATTACTCACCTCAACCAG-3'	5'-CAACCCAACCAATTTGAGCG-3'
<i>LHCX3</i>	5'-TCTTGAACGAGGACTACACCCC-3'	5'-TCCGTTCTGGAGTTCCTTGGT-3'
<i>LHCX4</i>	5'-ATGGCCGCGTTGCTATGTT-3'	5'-TATAGCAGGCCCCGAAACTTG-3'
<i>18S</i>	5'-TGCCCTTTGTACACACCGC-3'	5'-AAGTTCTCGCAACCAACACCA-3'

Supplemental Table 1: Primer sequences used for the gene expression analysis.

Literature Cited

Siaut M, Heijde M, Mangogna M, Montsant A, Coesel S, Allen A, Manfredonia A, Falciatore A, Bowler C (2007) Molecular toolbox for studying diatom biology in *Phaeodactylum tricornutum*. *Gene* **406**: 23-35