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# High light stress and the one-helix LHC-like proteins of the cryptophyte *Guillardia theta*

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ABSTRACT

Cryptophytes like the cryptomonad *Guillardia theta* are part of the marine phytoplankton and therefore major players in global carbon and biogeochemical cycles. Despite the importance for the cell in being able to cope with large changes in illumination on a daily basis, very little is known about photoprotection mechanisms in cryptophytes. Here, we show that *Guillardia theta* is able to perform non-photochemical quenching, although none of the usual xanthophyll cycle pigments (e.g., zeaxanthin, diadinoxanthin, diatoxanthin) are present at detectable levels. Instead, acclimation to high light intensity seems to involve an increase of alloxanthin. *Guillardia theta* has genes for 2 one-helix "light-harvesting-like" proteins, related to some cyanobacterial genes which are induced in response to high light stress. Both the plastid-encoded gene (*hlipP*) and the nucleomorph-encoded gene (*HlipNm*) are expressed, but transcript levels decrease rather than increase during high light exposure, suggesting that they are not involved in a high light stress response. The HlipNm protein was detected with a specific antibody; expression was constant, independent of the light exposure.


## 1. Introduction

Genes encoding small proteins with one trans-membrane helix related to the first and third trans-membrane helices of the eukaryotic light-harvesting complex (LHC) superfamily proteins are found in all cyanobacteria examined so far [1,2]. They were originally named Hlips, for "high light inducible proteins" [3], but are also referred to as SCPs (for "small CAB-like proteins") in *Synechocystis* sp. PCC 6803, where it has been shown that they are induced not only under high light but also during nutrition or temperature stress [4,5]. Their importance in stabilizing chlorophyll-binding proteins is well documented [6–9], and they might also be important for the tetrapyrrole metabolic pathway [8–10] or act as pigment carriers [7,11]. In support of a role in coping with high light stress, the genome of the high light ecotype of *Prochlorococcus marinus* (strain Med4) encodes at least 24 *hlip* genes [1,12].

Related *hlip* genes (previously annotated *ycf17*) are found in the plastid genomes of red, glaucophyte and cryptophyte algae [13–15], in keeping with the cyanobacterial ancestry of the chloroplast. Plants and green algae do not have plastid *hlip* genes, but they have one or two nuclear genes encoding related one-helix proteins, usually referred to as Ohps [16,17]. The red algae have two *hlip* genes: the plastid gene and a nuclear *hlip* homolog. The latter has acquired an additional N-terminal domain highly enriched in charged residues, preceding the conserved trans-membrane helix (Fig. 1), as well as a predicted transit peptide for chloroplast import (not shown). This suggests that copies of the plastid gene were transferred to the nucleus in both red and green lineages, either before or after the lineages diverged, but the plastid copy was subsequently lost in the green lineage.

In addition to the red algae, there are several major algal groups (those with Chl *a*/*c* LHCs) that acquired their chloroplasts by secondary endosymbiosis from a red algal endosymbiont [18,19]. Many red nuclear genes, including the *hlips*, were transferred to the host nucleus before the endosymbiont nucleus disappeared, and in some cases, the plastid copy was lost. A particularly interesting example is the cryptophyte algae, which are unique in having retained a remnant of the red algal nucleus, called the nucleomorph (Nm), located in the periplastid space between the chloroplast envelope and two additional membranes derived from the red algal plasma membrane and host endomembrane system [19].

In the cryptophyte *Guillardia theta*, only 30 of the 450 nucleomorph genes are predicted to encode proteins that are transported into the plastid. One of these is an *hlip* gene, which we will call *HlipNm* to distinguish it from the plastid gene (*hlipP*). The nucleomorph copy also has the red algal domain enriched in charged amino acids (box in Fig. 1). Since nothing is known about the expression of either gene in

*Abbreviations:* Chl, chlorophyll; HL, high light; Hlip, high light inducible protein; *hlipP*, plastid-encoded *hlip gene*; *HlipNm*, nucleomorph-encoded *hlip gene*; NL, normal light; NPQ, non-photochemical fluorescence quenching; OHP, one-helix protein; SCP, small CAB-like protein

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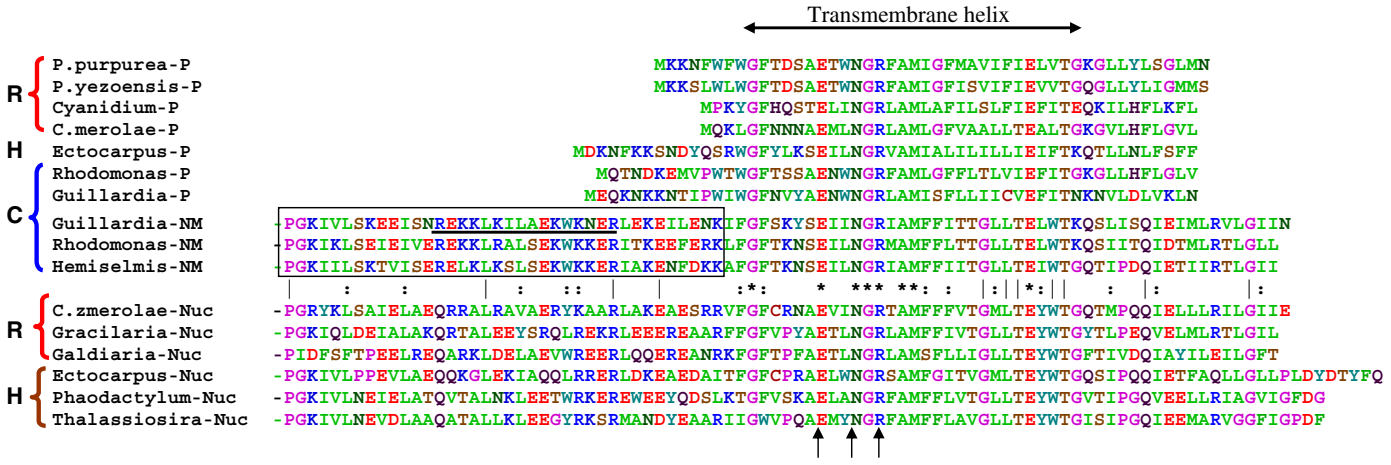


**Fig. 1.** Hlips in the red lineage. Alignment of Hlip protein sequences, showing conservation of the trans-membrane helix region with predicted Chl-binding residues (arrowheads), and the additional highly charged domain preceding it in the nuclear and nucleomorph (box) proteins. The sequence used for antibody production is underlined. P indicates plastid; NM, nucleomorph; Nuc, nuclear; R, red algal; H, heterokont; C, cryptophyte. In the online version, amino acids are colored according to functional group.

cryptophytes, we asked whether they were both expressed and whether their expression was induced by high light stress as it is in many cyanobacterial and plant homologs. We also assayed the carotenoid content under the same conditions since there is evidence in cyanobacteria that Hlip proteins may bind carotenoids [3] as well as Chl [20].

## 2. Material and methods

### 2.1. Culture conditions and high light treatment

*G. theta* cells (CCMP2712) were obtained from the Provasoli–Guillard National Center for Culture of Marine Phytoplankton. Cultures were grown in Fernbach culture flasks in h/2 media [21] shaken at 18 °C under white fluorescent light (30 μmol photons $m^{-2}$ $s^{-1}$) with light–dark cycle (12 h:12 h) at 18 °C. Cells were harvested in the exponential phase by centrifugation (1500*g* for 10 min). For high light (HL) experiments, a culture grown under normal light (NL; 30 μmol photons $m^{-2}$ $s^{-1}$) was divided into two parts at the end of the dark period (time point 0). One part was transferred to moderate HL (500 μmol photons $m^{-2}$ $s^{-1}$) or very HL (1000 μmol photons $m^{-2}$ $s^{-1}$ or 1500 μmol photons $m^{-2}$ $s^{-1}$); the other was kept under NL. The high light, provided by two attached PHILIPS 100-W halogen lamps, was filtered through a cooling water unit between the lamps and the cell suspension to prevent excess heat exposure. During light treatment, the cells were stirred gently to ensure uniform exposure. The irradiance was measured using a spherical sensor (model QSL-100; Biospherical Instruments Inc., San Diego, CA).

### 2.2. Chlorophyll fluorescence measurements

Variable Chl fluorescence was measured using a PAM 101 fluorometer (Walz, Effeltrich, Germany). Prior to each fluorescence measurement, samples were dark adapted for 30 min and a fresh 2 ml sample was used for each measurement (done in triplicate). A pulse of saturating light (2000 μmol photons $m^{-2}$ $s^{-1}$ for 700 ms) was applied to determine the maximum fluorescence ($F_m$). Once steady state fluorescence was achieved, saturating pulses were applied every 30 s to measure the maximal fluorescence under actinic light ($F_m'$). After the actinic light was turned off, the dark relaxation of fluorescence ($F_{mr}$) was measured by applying a saturating pulse every 60 s. Calculations were performed according to Zhu and Green [22].

### 2.3. Pigment analysis

For pigment analysis, *G. theta* cells grown under NL (30 μmol photons $m^{-2}$ $s^{-1}$) were transferred to continuous light (500 μmol photons $m^{-2}$ $s^{-1}$) for several hours. Another culture was grown under very low light (5 μmol photons $m^{-2}$ $s^{-1}$) for 14 days under diurnal light conditions and then transferred to 30 μmol photons $m^{-2}$ $s^{-1}$. Duplicate aliquots (10 ml) of these different cultures were harvested at each sampling point by filtering onto 25 mm GF/F filters (Whatman, Maidstone, Kent, England), frozen in liquid nitrogen, and stored at −80 °C. Pigment extraction was performed as described in Zapata et al. [23]. The samples were analyzed using a Waters Alliance HPLC which included a 2796 separation module and a Waters Diode Array Detector. The HPLC was calibrated with pigment standards purchased from DHI (Water and Environment, Horsholm, Denmark). Chlorophyll *a*, chlorophyll $c_2$, and alloxanthin were identified and quantified by comparison with the standards. Identification of the other sample pigments was deduced by comparing absorption spectra and retention time with the information available in the literature [23,24].

### 2.4. RNA extraction and qRT–PCR

*G. theta* cells grown under NL on 12:12 light–dark cycle were subjected to high light stress as described above. Time point 0 corresponds to dark adapted cells at the end of the night period. About 200 ml cell suspension was harvested by centrifugation (1500*g* for 10 min) at several time points and cell pellets were frozen in liquid nitrogen and stored at −80 °C. Total RNA was isolated with an RNAqueous™ Kit (Ambion, Austin, TX) followed by DNase treatment for 1 h at 37 °C using a TURBO DNA-free kit (Ambion). Purified total RNA (400 ng) was reverse transcribed into cDNA using Superscript II (Invitrogen, Carlsbad, CA). For quantitative real time RT–PCR (qRT–PCR), gene-specific primers were designed to give products of about 150 bp (Table 1). RNA levels are expressed relative to that of the nuclear actin gene. An iQTM SYBR Green Supermix (Bio-Rad, Hercules, CA) was used for qRT–PCR. The PCR amplification profile was 95 °C for 3 min followed by 40 cycles of 95 °C for 15 s, 61 °C for 30 s, and 72 °C for 30 s (MiniOpticon™; Bio-Rad). Genomic sequences and chromosome locations were obtained from the NCBI database.

### 2.5. Protein extraction, SDS–PAGE, and immunoblotting

To harvest the cells, about 500 ml cell suspension was centrifuged at 1500*g* for 10 min. The pellet was washed in buffer 1 (0.4 M sorbitol,

0.05 M $KH_2PO_4$, pH 7) and then resuspended in a small amount of buffer 2 (0.25 M sorbitol, 0.02 M HEPES, pH 7.4, 0.4 mM $Na_2EDTA$). The concentrated pellet was transferred to a 2 ml Eppendorf tube filled halfway with glass beads (0.3 mm) and chilled on ice for 5 min. Cells were broken in the bead-beater in 3 breakage cycles (30 s each cycle) at full speed and cooled on ice in-between the breakage cycles. Protein concentration was determined with a BCA Protein Assay Kit (Pierce, Rockford, IL). SDS–PAGE was performed as described in reference 25 using 17.5% acrylamide and 4 M urea with 30 μg protein loaded per lane. For immunoblotting, proteins were electrotransferred onto nitrocellulose membranes (Bio-Rad) and probed with an antibody raised against the peptide (NH2)-REKKLKILAEKWKNERMC (-CONH2), based on the N-terminal section of *G. theta* HlipNm (underlined in Fig. 1), and produced by AgriSera AB (Sweden). The blots were then incubated in peroxidase-conjugated goat anti-rabbit IgG secondary antibody (Bio-Rad) and the chemiluminescence signal was detected with ECL™ Western blotting detection system (GE Healthcare, Little Chalfont, Buckinghamshire, England).

## 3. Results

### 3.1. Fluorescence quenching in response to transfer from 30 μmol photons $m^{-2}$ $s^{-1}$ to higher light intensities

In order to analyze its ability to perform non-photochemical quenching (NPQ) and the light dependency of this process, *G. theta* cultures grown under normal light (NL; 30 μmol photons $m^{-2}$ $s^{-1}$) were dark adapted for 30 min and fluorescence parameters were measured in a pulse–amplitude modulated (PAM) fluorometer with various levels of actinic illumination. A fresh sample was used for each measurement. The $F_v/F_m$ value was 0.62–0.65, indicating that the cells were in good condition [26]. NPQ increased with actinic light intensity (Fig. 2), reaching a maximum of 1.40 at 764 μmol photons $m^{-2}$ $s^{-1}$. This suggests that light exposure at this level would be expected to induce a high light stress response in NL-grown cells.

### 3.2. Photosynthetic pigments

Cells grown under NL (30 μmol photons $m^{-2}$ $s^{-1}$) were exposed to 500 μmol photons $m^{-2}$ $s^{-1}$ for up to 24 h, starting a few minutes after the start of the light period. An overview of pigments detected by HPLC, simply expressed in terms of peak area, is shown in Fig. 3. The major chlorophylls are Chl *a* and Chl $c_2$, and the major carotenoid is alloxanthin, in agreement with the literature [23,24,27]. Minor carotenoids are monadoxanthin, crocoxanthin, and beta carotene. No diadinoxanthin, diatoxanthin, or zeaxanthin were detected. After 24 h HL, alloxanthin showed a significant increase of 56%, while the minor pigments crocoxanthin and monadoxanthin increased by 47% and 21%, respectively, and Chl *a*, only by 5%.

The quantitative changes in Chl $c_2$ and alloxanthin with respect to Chl *a* were determined after the column was calibrated with standards for these pigments. When cells were transferred from 30 to 500 μmol photons $m^{-2}$ $s^{-1}$, the molar ratio of Chl $c_2$ to Chl *a* did not change significantly over 24 h (Fig. 4A). However, the alloxanthin/Chl *a* ratio changed linearly with increasing time of exposure to HL. The increase after 6 h light stress was only about 10%. This is not the sort of change that is found in algae with the diadinoxanthin–diatoxanthin xanthophyll cycle, where the biggest increase in diatoxanthin occurs in the first few minutes of HL exposure and it increases many-fold over a period of hours (e.g., reference 22; see reference 28 for review).

We also examined the response of cells cultured at very low light levels (5 μmol photons $m^{-2}$ $s^{-1}$) upon transfer to NL levels (30 μmol photons $m^{-2}$ $s^{-1}$). In this case, there was also an increase in the ratio of alloxanthin to Chl *a* over the 24-h period, although the changes were not as great. The cells grown under very low light had lower alloxanthin/Chl *a* ratios, but the ratio increased over the 24 h exposure period, approaching the level of cells grown under NL. It appears that the cells acclimate to a new light intensity by increasing their alloxanthin quotas.

### 3.3. Expression of HlipNm and hlipP over a 24-h period

To investigate the expression of *hlipP* and *HlipNm* during normal growth conditions, *G. theta* cultures grown under NL on a 12:12 light–dark cycle were harvested at different time points and the transcript levels of both genes were determined by quantitative real time RT–PCR (qRT–PCR). As seen in Fig. 5, both *hlipP* and *HlipNm* are expressed throughout the day. Highest expression was observed in cells that had been in darkness for 12 h, i.e., the cells at time points 0 and 24 h. In these dark-adapted cells, the expression of the nucleomorph-located *HlipNm* gene was roughly double that of the plastid-encoded copy in the same experiment. Exposure to normal growth light for 6 h led to decreased expression of both genes. While *HlipNm* transcription decreased 2-fold after exposure to 30 μmol photons $m^{-2}$ $s^{-1}$ for 1 h, the amount of *hlipP* did not change as much.

### 3.4. Effect of HL on HlipNm and hlipP expression

To test whether the two *hlip* genes in *G. theta* are up-regulated by high light, *G. theta* cultures grown under NL on a 12:12 light–dark cycle were exposed to either 500 μmol photons $m^{-2}$ $s^{-1}$ or 1000 μmol photons $m^{-2}$ $s^{-1}$ continuous light and sampled after 0, 1, 2, 3, 4, and 6 h of HL exposure. Transcript levels of both *hlipP* and *HlipNm* determined by qRT–PCR were reduced by HL compared to NL, particularly at the higher light intensity (Fig. 6). After 2 h exposure to 1000 μmol photons $m^{-2}$ $s^{-1}$, the transcript levels of both genes were vanishingly small (Fig. 6B). On a percentage basis, mRNA levels of the Nm gene were more strongly affected than those of the plastid gene: exposure to 1000 μmol photons $m^{-2}$ $s^{-1}$ reduced the *HlipNm* transcription 8-fold within 1 h compared to about 4-fold for *hlipP*. At 500 μmol photons $m^{-2}$ $s^{-1}$, *hlipP*

**Table 1**
Primer sequences used for qRT–PCR.

| Target gene | Amplicon length of cDNA (bp) | Forward (F) and reverse (R) primer sequences |
|---|---|---|
| *hlipP* | 162 | F: 5′-TACCTTGGATTTGGGGCTTT-3′<br>R: 5′-CAAGGTCAAGCACATTTTTATTTG-3′ |
| *HlipNm* | 150 | F: 5′-TTTCTCGATTTGAGATTTCTTCTTT-3′<br>R: 5′-TGCACAATTTTAACGGCATT-3′ |
| *Lhcc4 (nuclear)* | 119 | F: 5′-CCGGAGTCGAGTCTGTCTTC-3′<br>R: 5′-GATGACCATCTCGAGGAAGC-3′ |
| *Actin (nuclear)* | 146 | F: 5′-GTTGGAGTGGGTCAGAAGGA-3′<br>R: 5′-TCGCTGTAGAAGGTGTGGTG-3′ |

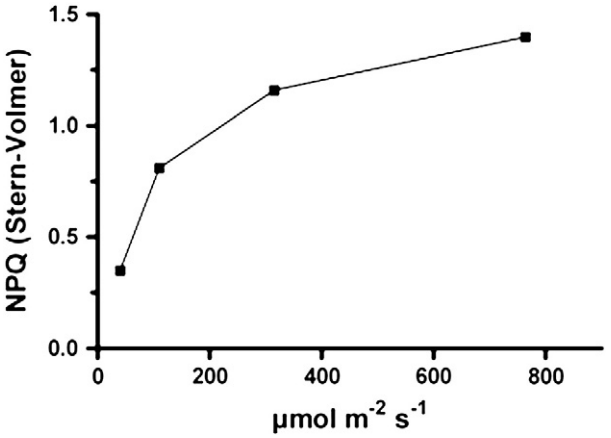


**Fig. 2.** NPQ as a function of light intensity. Cells were grown under NL (30 μmol photons $m^{-2}$ $s^{-1}$), dark adapted for 30 min, and exposed to various light intensities (40, 110, 315, 764 μmol $m^{-2}$ $s^{-1}$) in the PAM fluorometer.

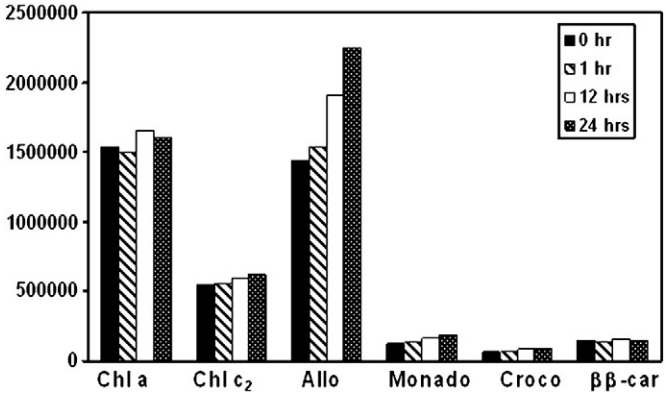


**Fig. 3.** HPLC analysis of pigments from cells grown under NL (30 μmol photons $m^{-2}$ $s^{-1}$) then exposed to HL stress (500 μmol photons $m^{-2}$ $s^{-1}$), expressed as relative peak area in arbitrary units. Chl *a*, Chl $c_2$, alloxanthin, monadoxanthin, crocoxanthin, and β,β-carotene were identified by elution time and absorption spectra. Cells were exposed to high light for 0 h (control, black), 1 h (hatched), 12 h (white), and 24 h (dotted).

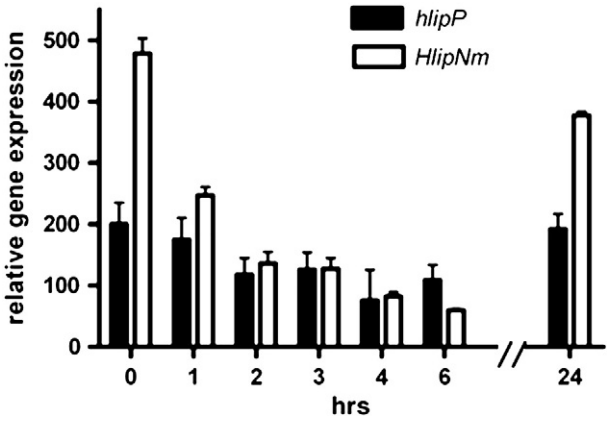


**Fig. 5.** Gene expression of *hlipP* and *HlipNm* under normal growth. Changes in mRNA levels of *hlipP* (black) and *HlipNm* (white) genes in *Guillardia theta* cells grown under NL (30 μmol photons $m^{-2}$ $s^{-1}$) conditions on 12 h day/12 h night cycle. Sampling was started just before the light was turned on, at the end of the night (time points 0 and also 24 h).

expression remained constant at about 25% of control levels from 3 to 6 h, while that of *HlipNm* continued to decrease.

In general, members of the LHC family that are involved in light-harvesting are strongly expressed under low light but reduced under high light to adjust the amount of light energy transferred to the reaction centers for the photosynthetic process [16]. To compare *hlipP* and *HlipNm* expression with one of the "standard" three-helix LHCs, generally assumed to be involved mainly in light-harvesting, a separate qRT–PCR experiment was performed to analyze the expression of *Lhcc4* (GenBank AF268322) using the same cDNA samples (Fig. 7). In dark-adapted cells, directly before the onset of light, *Lhcc4* showed almost its highest level of expression and only increased under control NL conditions during the first hour after the end of the dark phase. In all other samples, transcript levels were lower than at time 0 and decreased with longer exposure. Although HL treatment had an inhibitory effect on the transcription of *Lhcc4*, it was not so strong as that observed for *hlipP* and *HlipNm*.

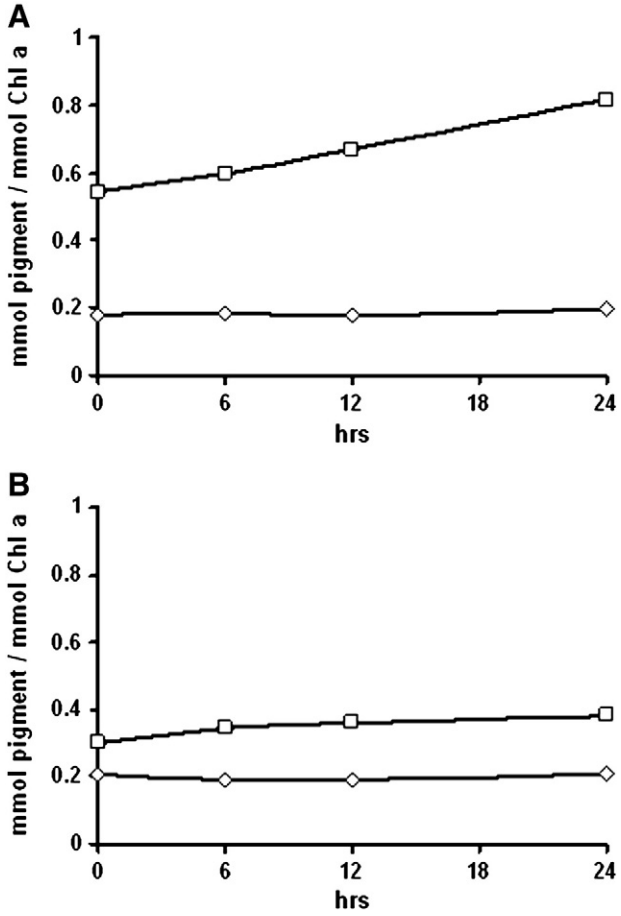


**Fig. 4.** Quantitative changes in alloxanthin and Chl $c_2$ after transfer to a higher light intensity for up to 24 h. A) NL grown cells were transferred to HL (500 μmol photons $m^{-2}$ $s^{-1}$). B) Cells grown at very low light (5 μmol photons $m^{-2}$ $s^{-1}$) transferred to NL (30 μmol photons $m^{-2}$ $s^{-1}$). Ratio of Chl $c_2$/Chl *a* (diamonds), alloxanthin/Chl *a* (squares).

In eukaryotes, translational and post-translational regulation often results in different protein expression compared to gene expression. To analyze the expression of HlipNm at the protein level, peptide-directed antibodies recognizing the N-terminus of this protein were produced by AgriSera (Sweden). It was not possible to produce specific antibodies for the plastid-encoded protein because most of the sequence is hydrophobic and there is too much sequence conservation. *G. theta* cultures grown at 30 μmol photons $m^{-2}$ $s^{-1}$ in a day/night cycle with 12 h light were exposed to either 500 μmol photons $m^{-2}$ $s^{-1}$ or 1000 μmol photons $m^{-2}$ $s^{-1}$ continuous light and sampled after various times of exposure. Proteins were separated by SDS–PAGE and immunostained using the anti-HlipNm antibody (Fig. 8). The differences between samples are not significant, showing that neither the NL treatment nor the normal diurnal cycle affected levels of this protein.

## 4. Discussion

Marine phytoplankton account for a significant fraction of the global net primary biomass production [29]. Cryptomonads like *G. theta* are part of this very phylogenetically diverse group. To optimize aquatic photosynthesis, light capture has to be carefully balanced with the photoprotective capacity to avoid over-excitation of the photosystems. The fastest response to high light stress is provided by non-photochemical quenching (NPQ), consisting of two major components: energy quenching (qE), operating within seconds to minutes, and inhibitory quenching (qI) which is slower and relaxes within 1–2 h in the dark [30]. In vascular plants, green and brown algae, qE activation requires the conversion of violaxanthin to zeaxanthin by the enzyme violaxanthin de-epoxidase (VDE) in the so-called xanthophyll cycle [28,31]. However, the main xanthophyll cycle in most of the algae with Chl *c* involves the conversion of diadinoxanthin to diatoxanthin [28,32]. We verified the existence of NPQ in *G. theta* but could not detect any of the xanthophyll cycle carotenoids.

In agreement with the literature, we found that the major carotenoid under all conditions was alloxanthin, with minor amounts of crocoxanthin and monadoxanthin [27,33]. The ratio of alloxanthin

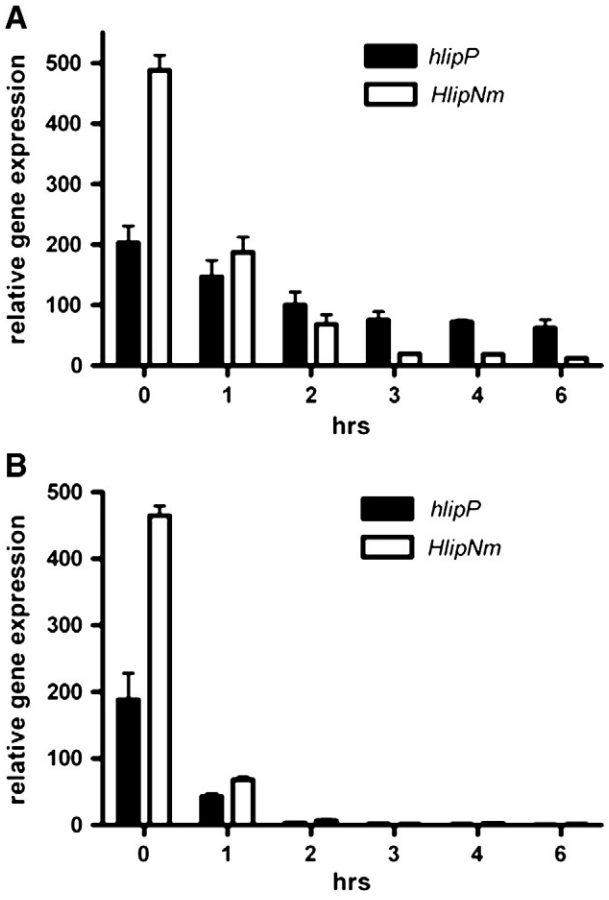


**Fig. 6.** Gene expression of *hlipP* and *HlipNm* under high light stress. Changes in mRNA levels of *hlipP* (black) and *HlipNm* (white) genes in *Guillardia theta* cells grown under NL (30 μmol photons $m^{-2}$ $s^{-1}$) conditions on 12 h day/12 h night cycle after exposure to 500 μmol photons $m^{-2}$ $s^{-1}$ (A) and 1000 μmol photons $m^{-2}$ $s^{-1}$ (B). Sampling was started (time point 0) at the end of the night, just before the light was turned on.

to Chl *a* increased significantly upon high light exposure, although the increase was gradual, reaching 56% only after 24 h exposure. There were also increases in the relative amounts of crocoxanthin and monadoxanthin. Differences in alloxanthin/Chl *a* between cultures acclimated to low (23 μmol photons $m^{-2}$ $s^{-1}$) versus high (554 μmol photons $m^{-2}$ $s^{-1}$) light have been reported for several other cryptophyte species [34]. This suggests that our cultures were in the process of acclimating to a higher growth light intensity during the course of the experiment and that an increase in alloxanthin (and possibly the other carotenoids) is a normal part of this process.

An important component of NPQ in vascular plants is the light-harvesting like (Lil) protein PsbS [35], which induces a conformational change in the light-harvesting complex of Photosystem II [36,37]. In green algae, a different Lil protein, LhcSR (formerly called LI818), has been shown to be involved in NPQ [38]. LhcSR orthologs (also called Lhcx) are found in various chromalveolates (reviewed in reference 39). In the diatom *Thalassiosira pseudonana*, the expression of one of them (Lhcx6) appears to be correlated with qI, the slow-relaxing phase of NPQ [22]. However, no homologs of either of these proteins could be detected in the draft nuclear genome of *G. theta* (B.R. Green, J. A. D. Neilson, D.G. Durnford, unpublished).

Based on their sequence homology, the Hlips belong to the large group of Lil proteins, which encompasses all the members of the LHC superfamily that are not involved in light-harvesting [39]. They are also related to the cyanobacterial Hlips/SCPs which can be induced by high light as well as other stresses [4,5]. We therefore asked if the Hlip proteins could be playing a role in NPQ and/or high light stress response in *G. theta*.

Our data show conclusively that both *HlipNm* and *hlipP* are expressed at the RNA level under normal growth conditions. In the case of HlipNm, immunodetection with a specific antibody showed that it was expressed at the protein level regardless of light intensity. Therefore, the plastid as well as the nucleomorph-encoded copy are functional genes. However, the effect of high light stress on *hlipP* and *HlipNm* transcript accumulation is very different from the other Lil proteins, including PsbS and LhcSR [22,38]. Instead of being up-regulated, their expression pattern is down-regulated in a manner more similar to that of genes involved in light-harvesting, although the down-regulation is more extreme at very high light intensities. To our knowledge, this is the first time that the effect of a stress on expression of a nucleomorph gene has been studied.

Clearly, these hlips are not high light-induced, and they are not the only members of this family who do not live up to their name [4]. Unfortunately, the alternatives are no better: "one-helix protein" is too vague and "small CAB-like" [3] is misleading because they are no more related to the Chl *a/b* proteins of the green lineage than to any of the other members of the LHC superfamily. Neither do they fit well into any particular "Lil" category (Fig. 1 in [39]). This is not the first example

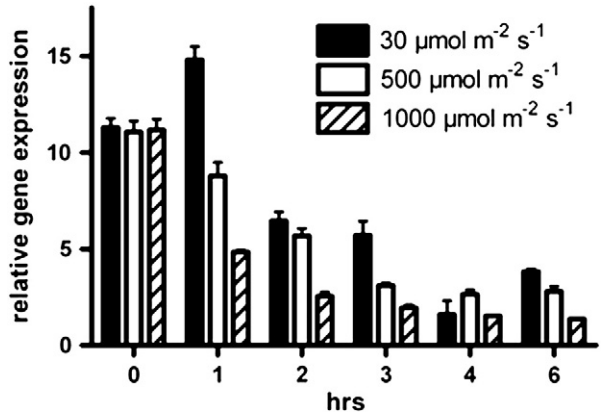


**Fig. 7.** Gene expression of *Lhcc4* under high light stress. Changes in mRNA levels of *Lhcc4* in *Guillardia theta* cells grown on 12 h day/12 h night cycle after growth at NL (30 μmol photons $m^{-2}$ $s^{-1}$) (black), exposure to 500 μmol photons $m^{-2}$ $s^{-1}$ (white) and 1000 μmol photons $m^{-2}$ $s^{-1}$ (striped). Harvest was started (time point 0) at the end of the night, just before the light was turned on.

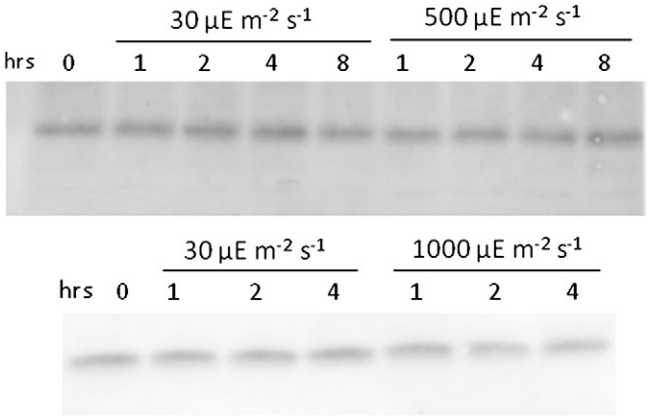


**Fig. 8.** Protein expression of HlipNm under high light stress. Immunodecoration of HlipNm with molecular mass of 13 kDa using a peptide-directed antibody recognizing the N-terminus of the protein (underlined in Fig. 1). Total cell extract was isolated from *Guillardia theta* cells grown on 12 h day/12 h night cycle under NL (30 μmol photons $m^{-2}$ $s^{-1}$) or after exposure to 500 μmol photons $m^{-2}$ $s^{-1}$ (upper panel) or 1000 μmol photons $m^{-2}$ $s^{-1}$ (lower panel). Harvest was started (time point 0, lane 1) at the end of the night, just before the light was turned on. Thirty micrograms of protein was loaded per lane.

of a gene family that has outgrown its original name, and a resolution of this problem will have to be left for a future publication.

It is worth pointing out that the red algae (the donors of the cryptophyte plastid) also do not have any sort of xanthophyll cycle, although they do demonstrate non-photochemical quenching of fluorescence [40–42]. Red algae harvest light using complex phycobiliprotein structures (phycobilisomes), as well as membrane-intrinsic members of the LHC family, and it has been suggested that physical decoupling of the phycoerythrin component of the phycobilisome could be responsible for the observed energy dissipation [42,43]. Cryptophytes like *G. theta* also use phycobilins for light-harvesting, but they are organized in a much simpler structure and are located in the thylakoid lumen rather than on the stromal surface [44]. No work has yet been done to determine whether they are involved in NPQ nor do we know to what proteins the extra alloxanthin molecules synthesized during high light growth are bound. Considering the great variety of ways in which photosynthetic organisms deal with changing environments, it would not be surprising to find that cryptophytes have invented novel solutions to the problem of energy dissipation that are not shared with other lineages.

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## References

[1] D. Bhaya, A. Dufresne, D. Vaulot, A. Grossman, Analysis of the hli gene family in marine and freshwater cyanobacteria, FEMS Microbiol. Lett. 215 (2002) 209–219.
[2] S. Bailey, A. Grossman, Photoprotection in cyanobacteria: regulation of light harvesting, Photochem. Photobiol. 84 (2008) 1410–1420.
[3] N.A. Dolganov, D. Bhaya, A.R. Grossman, Cyanobacterial protein with similarity to the chlorophyll a/b binding proteins of higher plants: evolution and regulation, Proc. Natl. Acad. Sci. USA 92 (1995) 636–640.
[4] C. Funk, W. Vermaas, A cyanobacterial gene family coding for single-helix proteins resembling part of the light-harvesting proteins from higher plants, Biochemistry 38 (1999) 9397–9404.
[5] Q. He, N. Dolganov, O. Bjorkman, A.R. Grossman, The high light-inducible polypeptides in *Synechocystis* PCC6803. Expression and function in high light, J. Biol. Chem. 276 (2001) 306–314.
[6] Q. Wang, S. Jantaro, B. Lu, W. Majeed, M. Bailey, Q. He, The high light-inducible polypeptides stabilize trimeric photosystem I complex under high light conditions in *Synechocystis* PCC 6803, Plant Physiol 147 (2008) 1239–1250.
[7] D. Vavilin, D. Yao, W. Vermaas, Small Cab-like proteins retard degradation of photosystem II-associated chlorophyll in *Synechocystis* sp. PCC 6803: kinetic analysis of pigment labeling with 15N and 13C, J. Biol. Chem. 282 (2007) 37660–37668.
[8] H. Xu, D. Vavilin, C. Funk, W. Vermaas, Small Cab-like proteins regulating tetrapyrrole biosynthesis in the cyanobacterium *Synechocystis* sp. PCC 6803, Plant Mol. Biol. 49 (2002) 149–160.
[9] H. Xu, D. Vavilin, C. Funk, W. Vermaas, Multiple deletions of small Cab-like proteins in the cyanobacterium *Synechocystis* sp. PCC 6803: consequences for pigment biosynthesis and accumulation, J. Biol. Chem. 279 (2004) 27971–27979.
[10] R. Sobotka, S. McLean, M. Zuberova, C.N. Hunter, M. Tichy, The C-terminal extension of ferrochelatase is critical for enzyme activity and for functioning of the tetrapyrrole pathway in *Synechocystis* strain PCC 6803, J. Bacteriol. 190 (2008) 2086–2095.
[11] D. Yao, T. Kieselbach, J. Komenda, K. Promnares, M.A. Prieto, M. Tichy, W. Vermaas, C. Funk, Localization of the small CAB-like proteins in photosystem II, J. Biol. Chem. 282 (2007) 267–276.
[12] F. Partensky, L. Garczarek, *Prochlorococcus*: advantages and limits of minimalism, Ann. Rev. Mar. Sci. 2 (2010) 305–331.
[13] S.E. Douglas, S.L. Penny, The plastid genome of the cryptophyte alga. *Guillardia theta*: complete sequence and conserved synteny groups confirm its common ancestry with red algae, J. Mol. Evol. 48 (1999) 236–244.
[14] M. Reith, J. Munholland, Complete nucleotide sequence of the *Porphyra purpurea* chloroplast genome, Plant Mol. Biol. Rep. 13 (1995) 333–335.
[15] V.L. Stirewalt, C.B. Michalowski, W. Löffelhardt, H.J. Bohnert, D.A. Bryant, Nucleotide sequence of the cyanelle genome from *Cyanophora paradoxa*, Plant Mol. Biol. Rep. 13 (1995) 327–332.
[16] S. Jansson, A guide to the Lhc genes and their relatives in *Arabidopsis*, Trends Plant Sci. 4 (1999) 236–240.
[17] S. Jansson, A protein family saga: from photoprotection to light-harvesting (and back?), in: B. Demmig-Adams (Ed.), Photoprotection, Photoinhibition, Gene Regulation and Environment, Springer, The Netherlands, 2005, pp. 145–153.
[18] S.P. Gibbs, The chloroplasts of some algal groups may have evolved from endosymbiotic eukaryotic algae, Ann. N.Y. Acad. Sci. 361 (1981) 193–208.
[19] C.E. Moore, J.M. Archibald, Nucleomorph genomes, Annu. Rev. Genet. 43 (2009) 251–264.
[20] P. Storm, M.A. Hernandez-Prieto, L.L. Eggink, J.K. Hoober, C. Funk, The small CAB-like proteins of *Synechocystis* sp. PCC 6803 bind chlorophyll. In vitro pigment reconstitution studies on one-helix light-harvesting-like proteins, Photosynth. Res. 98 (2008) 479–488.
[21] R.R.L. Guillard, Culture of Phytoplankton for Feeding Marine Invertebrates, Plenum Press, New York, USA, 1975.
[22] S.H. Zhu, B.R. Green, Photoprotection in the diatom *Thalassiosira pseudonana*: role of LI818-like proteins in response to high light stress, Biochim. Biophys. Acta 1797 (2010) 1449–1457.
[23] M. Zapata, F. Rodriguez, J.L. Garrido, Separation of chlorophylls and carotenoids from marine phytoplankton: a new HPLC method using a reversed phase C-8 column and pyridine-containing mobile phases, Mar. Ecol. Prog. Ser. 195 (2000) 29–45.
[24] L. Van Heukelem, C.S. Thomas, Computer-assisted high-performance liquid chromatography method development with applications to the isolation and analysis of phytoplankton pigments, J. Chromatogr. A 910 (2001) 31–49.
[25] C. Funk, W.P. Schroder, A. Napiwotzki, S.E. Tjus, G. Renger, B. Andersson, The PSII-S protein of higher plants: a new type of pigment-binding protein, Biochemistry 34 (1995) 11133–11141.
[26] P. Juneau, P.J. Harrison, Comparison by PAM fluorometry of photosynthetic activity of nine marine phytoplankton grown under identical conditions, Photochem. Photobiol. 81 (2005) 649–653.
[27] F.C. Pennington, F.T. Haxo, G. Borch, S. Liaaen-Jensen, Carotenoids of Cryptophyceae, Biochem. System. Ecol. 13 (1985) 215–219.
[28] R. Goss, T. Jakob, Regulation and function of xanthophyll cycle-dependent photoprotection in algae, Photosynth. Res. 106 (2010) 103–122.
[29] C.B. Field, M.J. Behrenfeld, J.T. Randerson, P. Falkowski, Primary production of the biosphere: integrating terrestrial and oceanic components, Science 281 (1998) 237–240.
[30] K.K. Niyogi, Photoprotection revisited: genetic and molecular approaches, Annu. Rev. Plant Physiol. Plant Mol. Biol. 50 (1999) 333–359.
[31] R. Bassi, S. Caffarri, Lhc proteins and the regulation of photosynthetic light harvesting function by xanthophylls, Photosynth. Res. 64 (2000) 243–256.
[32] M. Lohr, C. Wilhelm, Xanthophyll synthesis in diatoms: quantification of putative intermediates and comparison of pigment conversion kinetics with rate constants derived from a model, Planta 212 (2001) 382–391.
[33] S.W. Wright, S.W. Jeffrey, R.F.C. Mantoura, C.A. Llewellyn, T. Bjørnland, D. Repeta, N. Welschmeyer, Improved HPLC method for the analysis of chlorophylls and carotenoids from marine phytoplankton, Mar. Ecol. Prog. Ser. 77 (1991) 183–196.
[34] L. Schlüter, T.L. Lauridsen, G. Krogh, T. Jørgensen, Identification and quantification of phytoplankton groups in lakes using new pigment ratios—a comparison between pigment analysis by HPLC and microscopy, Freshwater Biology 51 (2006) 1474–1485.
[35] C. Funk, The PsbS protein: a Cab-protein with a function of its own, in: E.-M. Aro, B. Andersson (Eds.), Advances in Photosynthesis: Regulation of Photosynthesis, vol. 11, Kluwer Academic Publisher, The Netherlands, 2001, pp. 453-467.
[36] A.Z. Kiss, A.V. Ruban, P. Horton, The PsbS protein controls the organization of the photosystem II antenna in higher plant thylakoid membranes, J. Biol. Chem. 283 (2008) 3972–3978.
[37] X.P. Li, O. Bjorkman, C. Shih, A.R. Grossman, M. Rosenquist, S. Jansson, K.K. Niyogi, A pigment-binding protein essential for regulation of photosynthetic light harvesting, Nature 403 (2000) 391–395.
[38] G. Peers, T.B. Truong, E. Ostendorf, A. Busch, D. Elrad, A.R. Grossman, M. Hippler, K. K. Niyogi, An ancient light-harvesting protein is critical for the regulation of algal photosynthesis, Nature 462 (2009) 518–521.
[39] J.A.D. Neilson, D.G. Durnford, Evolutionary distribution of light-harvesting complex-like proteins in photosynthetic eukaryotes, Genome 53 (2010) 68–78.
[40] E. Gantt, B. Grabowski, F.X. Cunningham Jr., Antenna systems of red algae: phycobilisomes with photosystem II and chlorophyll complexes in photosystem I, in: B.R. Green, W.W. Parson (Eds.), Light-Harvesting Antennas in Photosynthesis, Kluwer Academic, The Netherlands, 2003, pp. 307–322.
[41] E. Delphin, J.-C. Duval, A.-L. Etienne, D. Kirilovsky, State transitions or ΔpH-dependent quenching of photosystem II fluorescence in red algae, Biochemistry 35 (1996) 9435–9445.
[42] L.-N. Liu, A.T. Elmalk, T.J. Aartsma, J.C. Thomas, G.E.M. Lamers, B.-C. Zhou, Y.Z. Zhang, Light induced energetic decoupling as a mechanism for phycobilisome-related energy dissipation in red algae: a single molecule study, PLoS One 3 (2008) e314.
[43] L.-N. Liu, T.J. Aartsma, J.C. Thomas, B.C. Zhou, Y.Z. Zhang, FRAP analysis on red alga reveals the fluorescence recovery is ascribed to intrinsic photoprocesses of phycobilisomes than large-scale diffusion, PLoS One 4 (2009) e5295.
[44] K.E. Wilk, S.J. Harrop, L. Jankova, D. Edler, G. Keenan, F. Sharples, R.G. Hiller, P.M.G. Curmi, Evolution of a light-harvesting protein by addition of new subunits and rearrangement of conserved elements: crystal structure of a cryptophyte phycoerythrin at 1.63 Å resolution, Proc. Natl. Acad. Sci. USA 96 (1999) 8901–8906.