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ORIGINAL ARTICLE

The redox state of the plastoquinone (PQ) pool is connected to thylakoid lipid saturation in a marine diatom

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Abstract

The redox state of the plastoquinone (PQ) pool is a known sensor for retrograde signaling. In this paper, we asked, "does the redox state of the PQ pool modulate the saturation state of thylakoid lipids?" Data from fatty acid composition and mRNA transcript abundance analyses suggest a strong connection between these two aspects in a model marine diatom. Fatty acid profiles of *Phaeodactylum tricornutum* exhibited specific changes when the redox state of the PQ pool was modulated by light and two chemical inhibitors [3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) or 2,5-dibromo-3-methyl-6-isopropyl-p-benzoquinone (DBMIB)]. Data from liquid chromatography with tandem mass spectrometry (LC–MS/MS) indicated a ca. 7–20% decrease in the saturation state of all four conserved thylakoid lipids in response to an oxidized PQ pool. The redox signals generated from an oxidized PQ pool in plastids also increased the mRNA transcript abundance of nuclear-encoded C16 fatty acid desaturases (FADs), with peak upregulation on a timescale of 6 to 12 h. The connection between the redox state of the PQ pool and thylakoid lipid saturation suggests a heretofore unrecognized retrograde signaling pathway that couples photosynthetic electron transport and the physical state of thylakoid membrane lipids.

Keywords Diatom · Lipid saturation · PQ pool · Redox state · Retrograde signaling · Thylakoid membrane

Introduction

Light and temperature affect the flux of excitation energy and photosynthesis, but how a photosynthetic cell responds to these environmental signals is not well understood. In eukaryotic algae, more than 90% of the proteins in the plastid are encoded in the nucleus (Abdallah et al. 2000; Allen et al. 2011; Grzebyk et al. 2003; Martin et al. 2002; Rujan and Martin 2001). Indeed, the plastidome possesses only minimal set of genes to support photosynthesis (Oudot-Le Secq et al. 2007; Valentin et al. 1992). Consequently, photosynthetic eukaryotes possess robust retrograde signaling mechanisms that facilitate environmental cues between nuclear gene expression and proteins in the plastid. In this paper, we examine a potential signaling pathway responsible for thylakoid lipid saturation in a model marine diatom.

The oxidation of plastoquinol (PQH_2) at the Q_0 site of cytochrome b_6f complex (cyt b_6f) is the slowest step in linear photosynthetic electron transport (PET) (Haehnel 1977). Hence, the redox state of the plastoquinone (PQ) pool can potentially generate a redox signal to reflect the fluctuation in PET induced by environmental factors such as light intensity, temperature, and to a lesser extent CO_2 concentration (Falkowski and Raven 2013). In response to changes in light intensity, the redox state of the PQ pool regulates the expression of nuclear-encoded genes responsible for light-harvesting pigment protein complexes (Chen et al. 2004; Escoubas et al. 1995; Yang et al. 2001). Similarly, the expression of nuclear-encoded pigment protein complexes is affected by changes in temperature, which has also been associated with the redox

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state of the PQ pool (Maxwell et al. 1994). Furthermore, the redox state of PQ pool transcriptionally regulates the stoichiometry of photosystem II (PSII) and photosystem I (PSI) in cyanobacteria, green algae and terrestrial plants (Karpinski et al. 1997; Li and Sherman 2000; Maxwell et al. 1995; Pfannschmidt et al. 1999). Are there other cellular processes regulated by the redox state of the PQ pool?

The lipid composition in the plastid is characterized by a high abundance of non-phosphate containing glycoglycerolipids including sulfoquinovosyldiacylglycerol (SQDG), monogalactosyldiacylglycerol (MGDG), and digalactosyldiacylglycerol (DGDG), as well as a low abundance of phosphoglycerolipids, such as phosphatidylglycerol (PG), phosphatidylcholine (PC), and phosphatidylinositol (PI) (Block et al. 1983; Douce et al. 1987; Mendiola-Morgenthaler et al. 1985). Among them, SQDG, MGDG, DGDG, and PG make up the four conserved lipids in thylakoid membranes in all oxygenic photoautotrophs. The acyl tails of these lipids are enriched in long-chain unsaturated fatty acids, and their saturation state heavily influences the viscosity of the hydrophobic membrane core (Harwood and Jones 1989; Sarcina et al. 2003). For instance, an increase in thylakoid fatty acid saturation inhibits the rate of linear photosynthetic electron transport (PET) (Horváth et al. 1986; Öquist 1982; Vigh et al. 1985). Decreasing fatty acid saturation is also connected to an increase of diffusion of quinone, a PQ-like lipophilic mobile electron carrier in mitochondrial respiration (Budin et al. 2018). Furthermore, thylakoid membranes are devoid of sterols (Nakamura and Li-Beisson 2016). Without sterols to promote membrane cohesion, these membranes are especially sensitive to very small changes in temperature (Beck et al. 2007; Dufourc 2008a, 2008b). Therefore, a robust and rapid retrograde signaling mechanism is crucial to maintain an optimal biophysical state of thylakoid membranes for photosynthesis. However, little is known about the initial signals that trigger the modulation of the saturation state of thylakoid lipids. This knowledge gap is especially wide in diatoms, a group of unicellular eukaryotic photoautotrophs that often dominates in polar and temperate seas.

In this paper, we present evidence that the redox state of the PQ pool is connected to thylakoid fatty acid saturation. Data from GC–MS analyses revealed that the fatty acid profiles, especially C16 fatty acids, exhibited specific changes in abundance when the redox state of the PQ pool was modulated independent of temperature by light or chemical inhibitors. Further examination with an oxidized PQ pool revealed: (1) decreases in the saturation state of all conserved thylakoid lipids and (2) increases in the mRNA transcript abundance of nuclear-encoded C16 fatty acid desaturase (FADs). These findings strongly suggest the presence of a retrograde signaling pathway.

Materials and methods

Culture maintenance, cell count and treatment

Phaeodactylum tricornutum Bohlin (CCAP 1055/1; strain Pt1 8.6) was maintained axenically in batch culture in artificial seawater enriched to F/2 levels at 18 °C (Guillard 1975; Guillard and Ryther 1962). Cells were grown under a 14/10 h light/dark cycle with white-light-emitting diodes at 500 µmol photons m^{-2} s^{-1} and 20 µmol photons m^{-2} s^{-1} for high-light and low-light acclimated cells, respectively. Cells used in all experiments were diluted to maintain log growth phase for at least three consecutive generations (Guillard 1973; Sunda et al. 2005). Cell densities were quantified using a Beckman Coulter Multisizer 3 (Beckman Coulter). To oxidize the PQ pool of the cells, high-light-acclimated cells were either shifted to the low-light condition or incubated with sublethal concentrations of 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU; dissolved in absolute ethanol; Tokyo Chemical Industry) for 24 h. Conversely, low-light acclimated cells were shifted to the high-light condition or incubated with sublethal concentrations of 2,5-dibromo-3-methyl-6-isopropyl-p-benzoquinone (DBMIB; dissolved in dimethyl sulfoxide; Sigma-Aldrich) for equal amount of time to reduce the PQ pool (Durnford et al. 1998). We defined "sublethal concentrations" as doses of DCMU and DBMIB that decreased growth rate but did not lead to complete loss of variable fluorescence (Fig. 3).

Fatty acid composition analysis

For total cellular fatty acid analysis [i.e., fatty acids methyl esters (FAMEs)], cells were harvested by filtration on glass fiber filters (GF/F; Whatman), followed by lipid extraction and transesterification (Bligh and Dyer 1959; Rodríguez-Ruiz et al. 1998). The FAMEs were analyzed using a Shimadzu GCMS- QP2010S Gas Chromatography Mass Spectrometer (GCMS; Shimadzu Scientific Instruments) equipped with a TRACE™ TR- FAME GC Column (60 m × 0.25 mm, film thickness 0.25 µm; Thermo Fisher Scientific), FID detector, and quadrupole mass spectrometer. All detectable FAME species were identified by comparing the mass spectra to the National Institute of Standards and Technology 11 library. The relative abundance of each FAME species was corrected for extraction efficiency (Jenke and Odufu 2012), quantified by referencing to a known amount of heptadecanoic acid internal standard (Sigma-Aldrich), and expressed as molecular percentage of total fatty acid. The double bond index (DBI) was determined

with all detectable fatty acid species as described (Feijão et al. 2017).

We used liquid chromatography with tandem mass spectrometry (LC–MS/MS) to quantify fatty acids by lipid headgroups on the LIPANG platform, Grenoble, France, as described (Jouhet et al. 2017). Briefly, lipid extracts corresponding to 25 nmol of total fatty acids were dissolved in 100 µL of chloroform/methanol [2:1, (v/v); containing 125 pmol of each internal standard]. The internal standards were phosphatidylethanolamine (PE) 18:0–18:0 (Avanti Polar Lipid), diacylglycerol (DAG) 18:0–22:6 (Avanti Polar Lipid), and SQDG 16:0–18:0 extracted from spinach thylakoids (Demé et al. 2014). Lipids were hydrogenated (Buseman et al. 2006). The lipid extract was separated in lipid class, by HPLC (Rainteau et al. 2012) and quantified by MS/MS. The HPLC separation was performed at 40 °C using an Agilent 1200 HPLC system equipped with a 150 mm × 3 mm (length × internal diameter) 5 µm diol column (Macherey–Nagel). The distinct glycerophospholipid classes were eluted successively as a function of the polar head group. The mass spectrometric analysis was done on a 6460 triple quadrupole mass spectrometer (Agilent) equipped with a Jet stream electrospray ion source. This quantification was done by multiple reaction monitoring (MRM) with 50 ms dwell time. Lipid amounts (pmol) were corrected for response differences between internal standards and endogenous lipids and by comparison with a quality control (QC). The QC extract, corresponding to a known *Phaeodactylum* lipid extract, was qualified and quantified by TLC and GC-FID (Jouhet et al. 2017).

Fast repetition rate (FRR) fluorescence measurement of PSII

DCMU oxidizes the PQ pool by irreversibly binding to the Q_B site of PSII, thus, blocking the electron input into the PQ pool and oxidizing it (Fedtke 1982; Trebst 2007). The inhibitory effect of DCMU was assessed by the time constant of Qa reoxidation (τ_{Qa}), using a three-component analysis of the fluorescence relaxation kinetics following a single turnover, saturating flash, using a custom built, miniaturized Fluorescence Induction and Relaxation (mini-FIRe) instrument (Gorbunov and Falkowski 2020; Kolber et al. 1998).

mRNA transcript abundance analysis

For quantitative reverse transcription PCR (RT-qPCR) analysis, total cellular RNA was extracted using TRIzol Reagent (Invitrogen). The extracted RNA was purified by using a RNeasy MinElute Cleanup Kit (Qiagen). RT-qPCR was performed using an Applied Biosystem High-Capacity cDNA Reverse Transcription Kit (Thermo Fisher Scientific) and Power SYBR Green Master Mix (Thermo Fisher Scientific). Primers for the qPCR reaction were designed using Primer Express Software v3.0.0 (Thermo Fisher Scientific) and listed in Table S1. The reactions were assessed using a QuantStudio 3 Real-Time PCR System. The ribosomal protein small subunit 30S (RPS) gene was used as an internal standard (Siaut et al. 2007). The relative mRNA expression ratio (R) of C16 fatty acid desaturases (FADs) was expressed according to Pfaffl (2001).

Pigment composition analysis

Cells were harvested by filtration on GF/F filters (Whatman). The samples were wrapped in foil and flash frozen in liquid nitrogen. Pigment extraction and high-performance liquid chromatography (HPLC) analysis were performed as described (Wright et al. 1991). Briefly, pigment composition was analyzed with an Agilent 1100/1200 series HPLC system equipped with a G1315C Diode Array Detector (scanning wavelengths 275–800 nm) and Zorbax Eclipse Plus C18 column (4.6 × 250 mm; 5 µm). The following HPLC Grade phase eluents were used: Solvent A (80% methanol, 20% aqueous ammonium acetate, pH 7.2); Solvent B (90% acetonitrile, 10% water); and Solvent C (100% ethyl acetate). Peaks were quantified at 440 nm. Pigments were identified using an Agilent software, based on retention time and spectral shape. All peak area quantifications and pigment identifications were manually verified.

Statistical analysis

Normality was assumed in all analyses. Homoscedasticity of data was evaluated with the Levene test. If homoscedastic, an independent Student's *t* test was performed (scipy.stats.ttest_ind, v1.6.2); otherwise, the Mann–Whitney U test was used (scipy.stats.mannwhitneyu, v.1.6.2). One-way analysis of variance (ANOVA) was performed, followed by pairwise Tukey- HSD post-hoc test in Fig. 3 using a Python statistical package, Pingouin version 0.33 (Vallat 2018). All tests were performed at $\alpha = 0.05$.

Results

Total cellular fatty acid profiles

Fatty acid profiles exhibited specific changes depending on the redox state of the PQ pool (Fig. 1). Twelve out of the 14 fatty acid species (ca. 85%) responded identically, suggesting that an oxidized PQ pool modulates fatty acid abundance. When the PQ pool is reduced by high light or DBMIB, 11 out of 14 (ca. 80%) fatty acid species responded in an identical manner. Data suggest that fatty acids respond in opposite directions depending on the redox state of the PQ pool

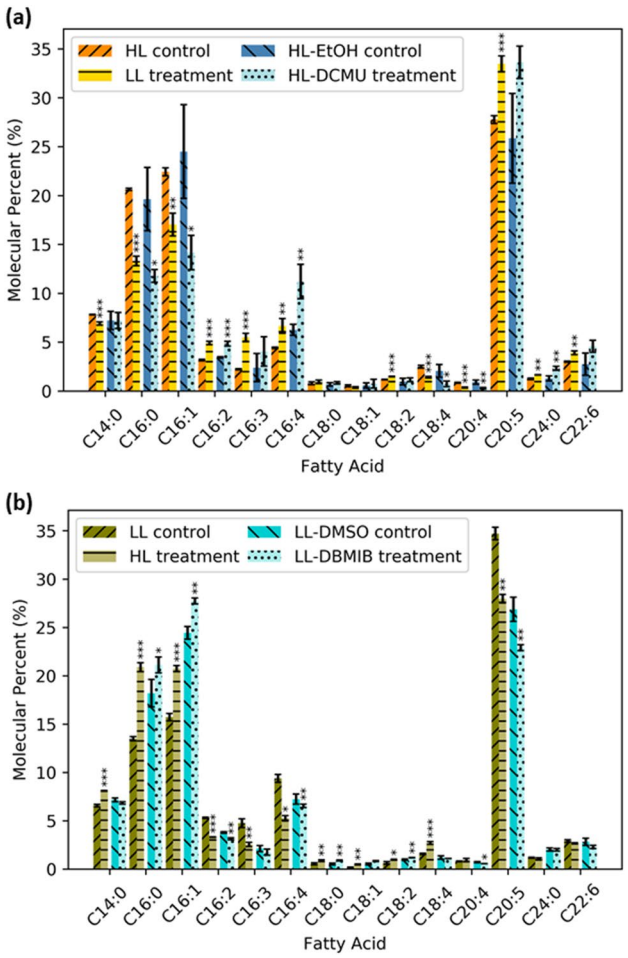

Fig. 1 Cellular fatty acid profiles of *P. tricornutum* measured by gas chromatography-mass spectrometry (GC–MS). **a** Oxidation of the PQ pool with low light (LL) and DCMU treatments for 24 h; **b** Reduction of the PQ pool with high light (HL) and DBMIB treatments for 24 h. Values reported are means ± SD ($n = 3$ biological replicates; except for HL treatment where $n = 2$). "*" represents $p \leq 0.05$, "**" represents $p \leq 0.01$, and "***" represents $p \leq 0.001$ ($\alpha = 0.05$ with independent t test)

(Fig. 2). Specifically, the FAs responsible for the changes were C16:0, C16:1, C16:2, C16:3, C16:4, C20:5, C24:0, and C22:6. Among these "redox sensitive" FAs, C16:0, C16:1, C16:2, and C16:4 exhibited significant and specific changes in response to the redox state of the PQ pool.

The saturation state of cellular fatty acids

We hypothesized that an oxidized PQ pool would lead to a decrease in the fatty acid saturation. Indeed, increased concentrations of DCMU led to increased DBIs (Fig. 3a). Relative to the control, the DBI increased by 20.5%, 22.4%, 22.4%, and 29.2%, as τ_{Qa} increased by 125.1%, 159.0%, 194.0%, and 312.0%. Among the "redox sensitive" fatty acids, the abundance of C16:0 and C16:1 significantly decreased as the concentration of DCMU increased (Fig. 3b). On the other hand, the abundance of less saturated C16:2 and C16:4, and longer C24:0 significantly increased.

The saturation state of thylakoid lipids

The saturation state of thylakoid fatty acids, i.e., SQDG, MGDG, DGDG, and PG, increased when the PQ pool was oxidized by DCMU (Fig. 4a). The DBIs of non-thylakoid lipids such as PC, DAG, and TAG were also affected, with their DBIs significantly increased by 6.7%, 6.1%, and 27.0%, respectively. The DBIs of PI, PE, and DGTA were unchanged by an oxidized PQ pool. C16 fatty acids and C20:5 contributed to most of the observed changes. C16:2 (45.9%) appeared to be the main contributor of the increase in SQDG (Fig. 4b). The largest significant changes observed in MGDG were C16:1 (− 72.1%), C16:4 (34.3%), and C20:5 (22.2%; Fig. 4c). For DGDG, they were C16:1 (− 35.3%), C16:2 (28.1%), and C20:5 (39.8%; Fig. 4d). Increase in DBI of PG could be mostly attributed to C16:0 (− 26.7%) and C20:5 (32.9%; Fig. 4e). In TAG, C16:0 (− 17.2%) and C20:5 (101.9%) led to the increase in the DBI.

mRNA transcripts of C16 fatty acid desaturases

To examine if there is a connection between an oxidized PQ pool and nuclear-encoded FADs related to C16 fatty acids, we examined the transcription of the potential proteins. In *P. tricornutum*, the FAD PAD (palmitoyl-ACP desaturase; Phatr3_J9316); PTD12 (Phatr3_J48423) on chromosome 17; Putative C16:2 to C16:3 desaturase (Phatr3_EG02619) on chromosome 30; and PTD15 (Phatr3_J41570) on chromosome 31. Is the redox state of the PQ pool connected to the transcription of these genes?

RT-qPCR measurements revealed that within 6 h of PQ pool oxidation, there was a threefold increase in the transcription of PAD and a 1.6-fold increase in the putative C16:2 to C16:3 desaturase (Fig. 5). At 12 h, most FADs were significantly upregulated, i.e., PAD by 2.6-fold; PTD12 by 3.4-fold and putative C16:2 to C16:3 desaturase by 2.3-fold. PTD15 was upregulated by almost tenfold, despite the large standard deviation. It appears that this putative retrograde signaling pathway functions on a time scale of 6 to 12 h. 24 h of incubation did not significantly change the Rs of FADs tested.

Diatom lipid composition

In addition to the saturation state of thylakoid fatty acids, lipid composition can affect thylakoid structure and function by lipid phase behaviors. Thus, we examined if an oxidized PQ pool can modulate thylakoid lipid composition. Among the quartet of conserved thylakoid lipids, we

Fatty acid species	Direction of change relative to control			
	Oxidized PQ pool		Reduced PQ pool	
	LL	*DCMU*	*HL*	*DBMIB*
C14:0	Down***	Unchanged	Up***	Down
C16:0	**Down*** **	**Down***	**Up*** **	**Up***
C16:1	**Down****	**Down***	**Up*** **	**Up****
C16:2	**Up*** **	**Up*** **	**Down*** **	**Down****
C16:3	Up***	Up	Down**	Down
C16:4	**Up****	**Up****	**Down***	**Down****
C18:0	Up	Up	Up**	Up**
C18:1	Down	Up	Up**	Up
C18:2	Up***	Up	Up*	Up**
C18:4	Down***	Down*	Up***	Unchanged
C20:4	Down***	Down**	Unchanged	Down*
C20:5	Up***	Up	Down**	Down**
C24:0	Up**	Up**	Down	Down
C22:6	Up**	Up	Down	Down

Fig. 2 Changes in fatty acid abundance in response to the redox state of the PQ pool. The direction of change reported after 24 h for each treatment are derived from values reported in Fig. 1. Fatty acid species with significant opposite directions of change in oxidized versus reduced PQ pool are in bold. "*" represents $p \leq 0.05$, "**" represents $p \leq 0.01$, and "***" represents $p \leq 0.001$ at $\alpha = 0.05$ with independent t test ($n = 3$ biological replicates; except for HL treatment where $n = 2$ biological replicates)

observed significant changes only in DGDG (-63.0% relative to control; Fig. 6a) that led to an increased in MGDG/DGDG (Fig. 6b). The charge distribution of thylakoid membranes, i.e., neutral/anionic lipids, and composition of negatively charged lipids (SQDG and PG) appeared to be unchanged by the oxidation state of the PQ pool (Fig. 6b). Other lipids exhibiting significant changes include PC (43.4%), DGTA (15.5%), DAG (-49.8%), and TAG (-91.7%; Fig. 6a).

Discussion

The results of our experiments strongly suggest that the redox state of the plastoquinone pools affects the fatty acid saturation in thylakoid membranes of a marine diatom. There was a ca. 85% agreement in changes of fatty acid abundance between LL and DCMU treatments, i.e., an oxidized PQ pool. Reduction of the PQ pool by HL or DBMIB treatments resulted in a ca. 80% agreement in the direction of abundance change (Figs. 1 and 2). DCMU treatments resulted in increased τ_{Qa} (Fig. 3a) and, by inference, a more oxidized PQ pool. Thus, it appears that oxidation of the PQ pool is connected to the increase in the DBI. While the redox state of the PQ pool is biophysically related to the change in variable fluoresce in vivo (e.g., Prášil et al. 2018; Vernotte et al. 1979), chemical analysis of the extracted PQ(H_2) pool by (e.g., HPLC demonstrated in Khorobrykh et al. 2020) might be used, in future experiments. We also attribute most of the increase in total cellular DBI to thylakoid lipids i.e., MGDG, DGDG, and PG (Fig. 4a). C16:0, C16:1, C16:2, and C16:4 in thylakoids appear to be sensitive to the redox state of the PQ pool (Figs. 2, 3, and 4b–e). 20:5 also contributed to the increase of DBI in thylakoid lipids (Fig. 4c–f), albeit its insignificant increase in the total cellular fatty acid analysis (Fig. 3b).

Besides the four conserved thylakoid lipids, the DBIs of DAG, TAG, and PC also significantly increased. DAG serves as a precursor to membrane and storage lipids and is thought to be present in the plastids and endoplasmic reticulum (ER) (Dolch and Maréchal 2015). Although its exact localization in diatoms remains inconclusive with current membrane isolation technique, PC is synthesized in the ER and thought to be the main extraplastidial lipid (Boudière et al. 2014; Dolch and Maréchal 2015; Kroth et al. 2008). The storage lipid, TAG, exists in the form of droplets in *P. tricornutum* (Dolch and Maréchal 2015). The increase of DBI in these

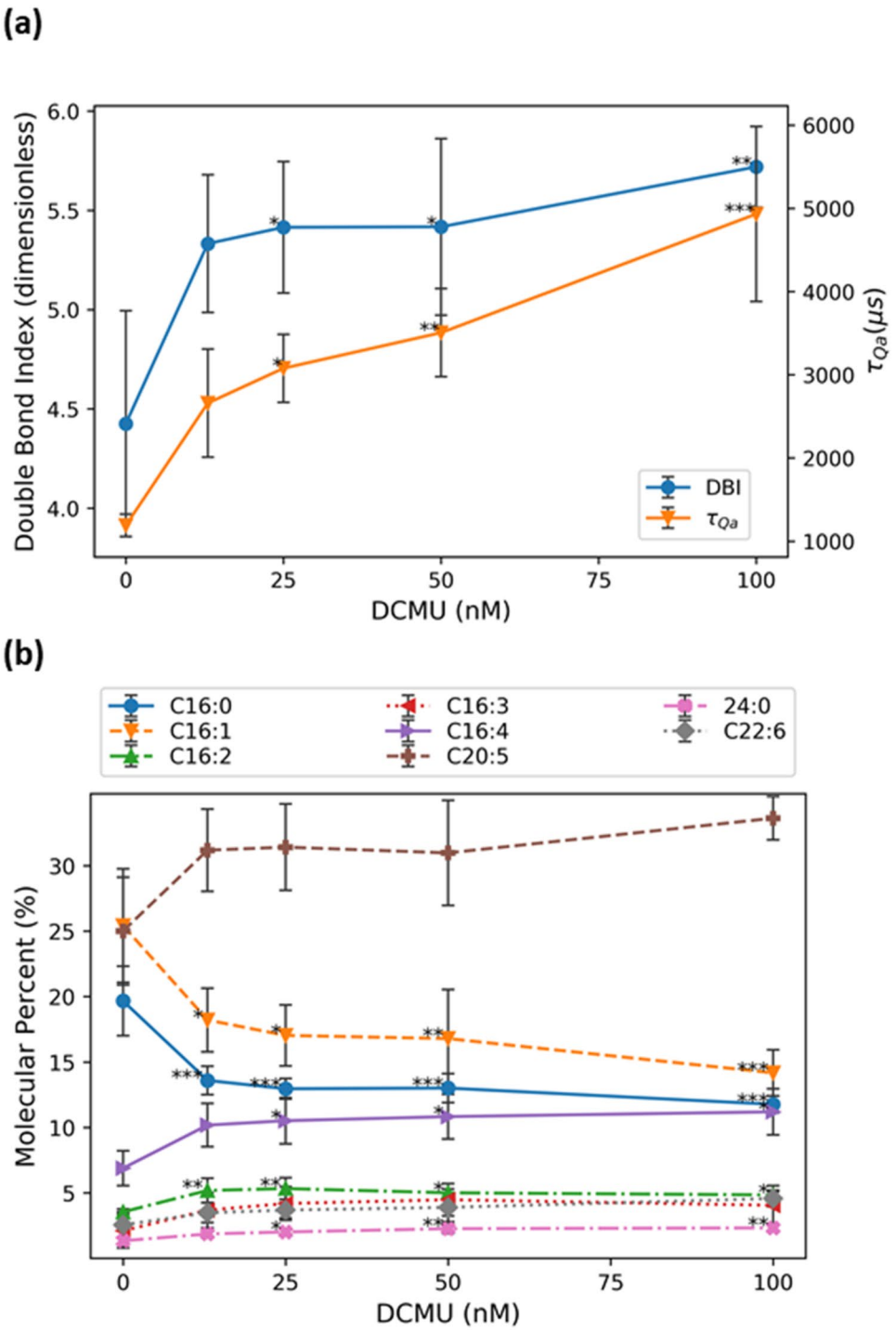

Fig. 3 Fatty acid saturation state and abundance as a function of DCMU concentration. **a** The double bond index (DBI) and time constant of Qa reoxidation (τ_{Qa}), as a function of DCMU concentration. DBI was computed from all detectable fatty acid species. **b** Fatty acid abundance as a function of DCMU. Only the "redox sensitive" fatty acids identified in Fig. 2 are included for brevity. Values reported are means ± SD ($n = 3$ biological replicates). "*" represents $p \leq 0.05$, "**" represents $p \leq 0.01$, and "***" represents $p \leq 0.001$ ($\alpha = 0.05$ with one-way ANOVA and Tukey-HSD post-hoc test)

non-thylakoid lipids and their importance to PET when PQ pool was oxidized is currently unclear. Nonetheless, the increase in DBIs appears to be specific as not all thylakoid lipids were affected indiscriminately. For instance, the DBIs of PI, PE, and DGTA were unchanged (Fig. 4a). Together, these data suggest a potential presence of a specific signaling mechanism in response to an oxidized PQ pool.

Many studies on regulation of FADs by light have been reported on at a transcriptional level using light and dark treatments (Bai et al. 2016; Collados et al. 2006; Hernández et al. 2011; Kis et al. 1998; Nishiuchi et al. 1995). However, light and dark can affect a host of cellular processes. Therefore, it is challenging to pinpoint the sensing signal(s) that regulates FADs. DNA microarray analyses of DCMU- and DBMIB-treated cyanobacteria suggest that FADs are

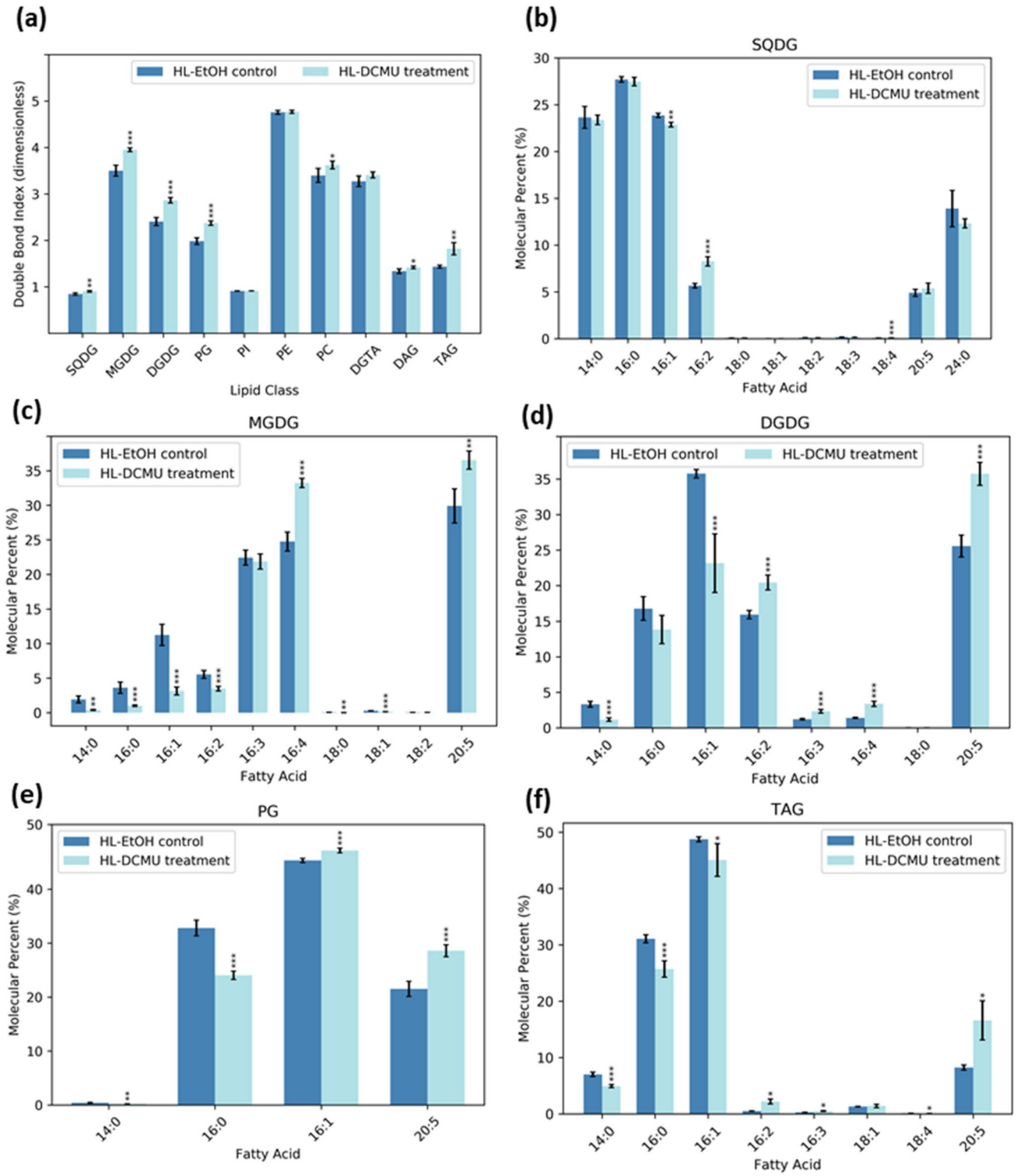

Fig. 4 The double bond index (DBI) of *P. tricornutum* measured by liquid chromatography with tandem mass spectrometry (LC–MS/MS). **a** DBI of all detectable lipid species in diatoms treated with ethanol (EtOH; carrier control) and 25 nM DCMU for 24 h; DBI of **b** SQDG **c** MGDG **d** DGDG **e** PG **f** TAG fatty acid tails. Values reported are means ± SD ($n = 4$ biological replicates). "*" represents $p \leq 0.05$, "**" represents $p \leq 0.01$, and "***" represents $p \leq 0.001$ ($\alpha = 0.05$ with independent t test for homoscedastic data and Mann–Whitney U test for non-homoscedastic data). Abbreviations: SQDG, sulfoquinovosyldiacylglycerol; MGDG, monogalactosyldiacylglycerol; DGDG, digalactosyldiacylglycerol; PG, phosphatidylglycerol; PI, phosphatidylinositol; PE, phosphatidylethanolamine; PC, phosphatidylcholine; DGTA, diacylglyceryl hydroxymethyltrimethyl-β-alanine; DAG, diacylglycerol; TAG, triacylglycerol

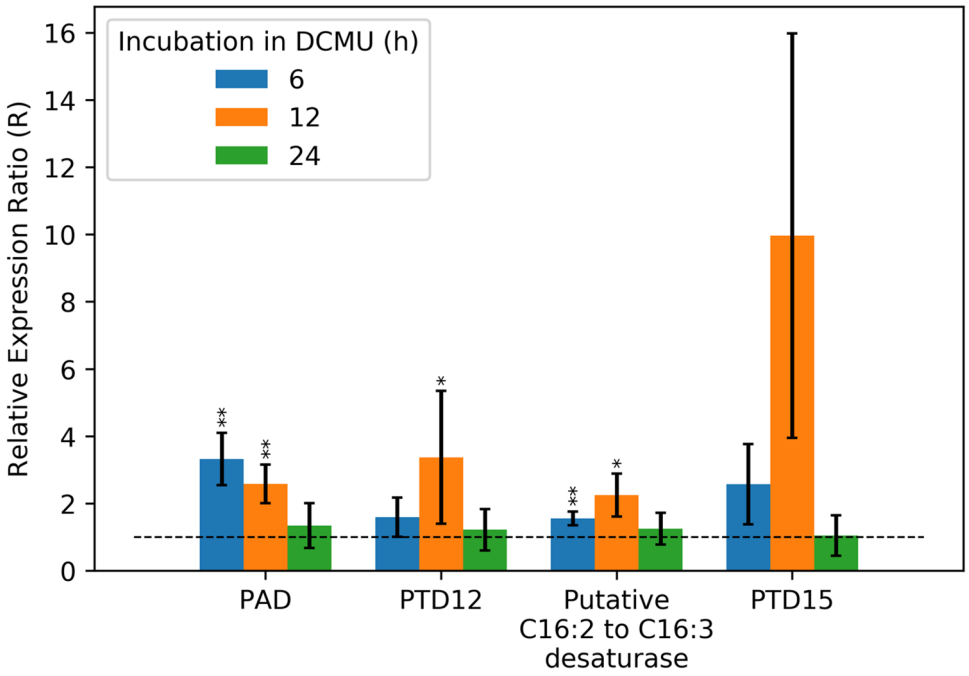

Fig. 5 Relative mRNA expression ratio (R) of C16 fatty acid desaturases (FADs) after incubation in 25 nM of DCMU for 6, 12 and 24 h. R of a FAD is expressed in comparison to ribosomal protein small subunit 30S (RPS) gene (Siaut et al. 2007), in DCMU treatments versus respective carrier controls (ethanol) according to Pfaffl (2001). We referred to Dolch and Maréchal (2015) and Smith et al. (2021) for the function of each FAD. Dashed line at R of 1 indicates no change in expression level. Values reported are means ± SD ($n = 3$ biological replicates, except for PTD15 where $n = 4$ biological replicates). "*" represents $p \leq 0.05$ and "**" represents $p \leq 0.01$ ($\alpha = 0.05$ with independent t test between target gene and RPS). Gene name (function) and Ensembl Protists ID: PAD (desaturates C16:0 to C16:1), Phatr3_J9316; PTD12 (desaturates C16:1 to C16:2), Phatr3_J48423; Putative C16:2 to C16:3 desaturase, Phatr3_EG02619; PTD15 (desaturates C16:3 to C16:4), Phatr3_J41570

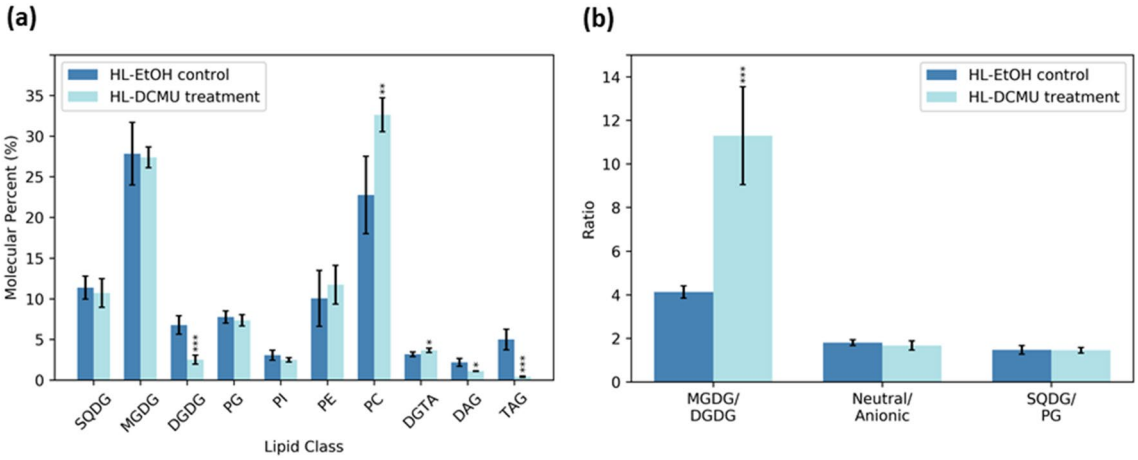

Fig. 6 Lipid composition of *P. tricornutum* measured by liquid chromatography with tandem mass spectrometry (LC–MS/MS). **a** Sum of molecular abundance by lipid category. Diatom cells were treated with ethanol (EtOH; carrier control) and 25 nM DCMU for 24 h. **b** The ratios of lipid classes. Neutral: MGDG and DGDG; Anionic: SQDG and PG. Values reported are means ± SD ($n = 4$ biological replicates). "*" represents $p \leq 0.05$, "**" represents $p \leq 0.01$, and "***" represents $p \leq 0.001$ ($\alpha = 0.05$ with independent t test with homoscedastic data; Mann–Whitney U test for non-homoscedastic data). Abbreviations: SQDG, sulfoquinovosyldiacylglycerol; MGDG, monogalactosyldiacylglycerol; DGDG, digalactosyldiacylglycerol; PG, phosphatidylglycerol; PI, phosphatidylinositol; PE, phosphatidylethanolamine; PC, phosphatidylcholine; DGTA, diacylglyceryl hydroxymethyltrimethyl-β-alanine; DAG, diacylglycerol; TAG, triacylglycerol

redox-responsive (Hihara et al. 2003). However, the changes in expression were similarly repressed and this might be attributed to the physiological artifacts from the higher concentration of inhibitors used (an order of magnitude higher than the present study). A recent study in diatoms pointed out that lower light intensity led to decrease in fatty acid saturation, and vice versa (Conceição et al. 2020). However, the signal(s) that led to this observation remain elusive and unexplored.

Diatoms are secondary endosymbionts that possess four layers of plastid envelope membranes (Bowler et al. 2008, 2010). Retrograde signaling was once considered to be unlikely in diatoms because a signal from plastids must travel through these membranes to the nucleus (Wilhelm et al. 2006). Here, our data suggest that the change in fatty acid profile is connected to the redox state of the PQ pool transcriptionally (Fig. 5). An oxidized PQ pool led to an initial increase in R of PAD by 3.3-fold at 6 h, which desaturates C16:0 to C16:1 (Smith et al. 2021). This increase in C16:1 may serve as the substrate for downstream fatty acid desaturation, which is reflected by the peak upregulation of most C16 FADs at 12 h. Once the new lipid homeostasis achieved at 24 h, all FADs exhibited unchanged expression relative to the internal standard gene. Together with the corresponding GC–MS and LC–MS/MS data, this expression pattern, in which FADs were upregulated to attain a new state of lipid homeostasis and downregulated once the homeostasis was attained, suggests that the redox state of the PQ pool is an initial signal in a retrograde signal that regulates the transcription of specific, nuclear-encoded FADs.

The connection between the redox state of the PQ pool and saturation state of thylakoid fatty acids may have important physiological implications to the organism. Many crucial processes in photosynthesis, such as PET and PSII repair, rely heavily on diffusion (Goral et al. 2010; Kirchhoff 2014; Kirchhoff et al. 2000). In PET, PQ and its reduced form, PQH_2, are the only lipophilic mobile electron carriers that diffuse in the acyl tails of thylakoid lipids. Hence, decreased thylakoid fatty acid saturation induced by low light or DCMU (Figs. 1a, 2, 3a, and 4a) is likely to decrease the viscosity of the microenvironment between PSII and cyt b_6f. This may promote the rate of diffusion of PQ and PQH_2 to encourage higher electron input to PET. Conversely, saturated thylakoid membranes may retard the rates of PQ and PQH_2 diffusion to restrict electron input to PET in cells exposed to high light or DBMIB. We also explored the possibility if the redox state of PQ pool can act as a thermal sensor to modulate the saturation level of thylakoid fatty acids. According to the photostasis theory, low temperature and high light both similarly exert high excitation pressure at PSII (Ensminger et al. 2006; Huner et al. 1998, 2002; Wilson et al. 2006). Theoretically, DBMIB and low-temperature treatments (inversely, DCMU and high temperature treatments) should result in similar fatty acid profiles. However, we did not observe these similarities in our cultures (data not shown). Thus, it is possible that additional sensors (or checks) are needed to trigger thermal modulation of thylakoid fatty acids. To the extent these experimental results apply, the redox state of the PQ pool may serve as one of the signaling mechanisms to modulate FADs.

An oxidized PQ pool increases the abundance of light-harvesting complexes and the associated Chl *a* (Escoubas et al. 1995; Maxwell et al. 1995). In accordance with literature, we observed ca. 70% increase in Chl *a* in DCMU-treated cells (Fig. S1). Instead of light-harvesting complex II in terrestrial plants, diatoms utilize fucoxanthin chlorophyll a/c binding proteins (FCP) as the major peripheral light-harvesting antennae for the photosystems. There are eight molecules of Chl *a*, eight fucoxanthin (Fx), and two Chl *c* bound to each monomeric FCP (Premvardhan et al. 2010). Thus, a lack of corresponding increase in Fx and Chl *c* suggests that oxidation of the PQ pool alone did not significantly increase the abundance of FCPs but mainly the reaction centers. According to the percolation diffusion theory, high protein density encloses diffusion domain and prevents long-range diffusion of smaller hydrophobic molecules (Almeida and Vaz 1995; Kirchhoff 2014; Saxton 1989). Thus, an increase in the abundance of reaction centers, with unchanged thylakoid lipid abundance, may increase thylakoid protein density and impede lateral diffusion of lipophilic molecules. Therefore, decreasing the saturation level of thylakoid fatty acids may counter this increase in membrane viscosity, for maintenance of lateral diffusion in the hydrophobic membrane core.

An oxidized PQ pool also led to changes in cellular lipid composition. We observed a ca. 60% decrease in DGDG (Fig. 6a) and corresponding 1.74-fold increase in MGDG/DGDG (Fig. 6b). MGDG (conical) does not promote bilayer forming whereas DGDG (cylindrical and forms hydrogen bonds with polar heads of lipids in adjacent bilayers) does (Demé et al. 2014; Williams 1998). MGDG tends to form hexagonal II (HII) phase i.e., inverted tubules where lipid head groups form the center of the tubule and fatty acids are pointing outward to the aqueous phase (Jouhet 2013; Seddon 1990; Shipley et al. 1973). Decreasing saturation in fatty acids also favors the formation of the HII phase. Together, it appears that thylakoid membranes may favor the HII phase but not the lamellar phase (bilayer forming and crucial for the maintenance of ultrastructure) when the PQ pool is oxidized. Data from ^{31}P-NMR experiments on spinach thylakoids suggest that non-bilayer phases may play an important role in structural dynamics of thylakoid membranes (Garab et al. 2017). The HII phase is important for the activities of some enzymes, including de-epoxidation of violaxanthin (Latowski et al. 2004). However, the cells examined exhibited decreased abundance of Ddx and unchanged Dtx (Fig. S1). They did not

appear to activate non-photochemical quenching. Thus, this complex structure–function relationship of thylakoid lipids remains enigmatic. In phosphorus limiting condition, deficiency in PC could be replaced by DGTA (Abida et al. 2015). However, our cells were grown in nutrient replete condition, and thus, the link between the redox state of PQ pool to these lipids is unclear. Future study should focus on the roles of these lipids in photosynthesis, especially in PET. Although it may seem straightforward, changes in fatty acid saturation have profound structural implications to biophysical properties of thylakoid membranes. In an *Arabidopsis* mutant deficient in *fad5*, semicrystalline PSII arrays were observed (Tietz et al. 2015). Instead of impeding the diffusion of small lipophilic molecules, the authors reported an enhancement of mobility of PQ and xanthophylls due to altered supramolecular protein organization. In diatoms, cryo-ET studies revealed semicrystalline arrays of PSII core subunits (Levitan et al. 2019). It will be interesting to study the effects of the PQ redox state on the supramolecular organization of PSII and PET.

This work provides a reference for future studies on elucidating the molecular intermediates connecting the plastid signal to nuclear gene expression in this retrograde signaling pathway.

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Author contribution All authors contributed to the study conception and design. Material preparation, data collection, and analysis were performed by KYC and JJ. The first draft of the manuscript was written by KYC, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Data availability The data that support the findings of this study are available from the corresponding author upon reasonable request.

Code availability Not applicable.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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