FIRST INDUCED PLASTID GENOME MUTATIONS IN AN ALGA WITH SECONDARY PLASTIDS: \textit{psbA} MUTATIONS IN THE DIATOM \textit{PHAEODACTYLUM TRICORNUTUM} (BACILLARIOPHYCEAE) REVEAL CONSEQUENCES ON THE REGULATION OF PHOTOSYNTHESIS\(^1\)

\textbf{Arne C. Materna\(^3,5\), Sabine Sturm\(^5\), Peter G. Kroth, and Johann Lavaud\(^2,4\)}

Group of Plant Ecophysiology, Biology Department, Mailbox M611, University of Konstanz, Universitätsstraße 10, 78457 Konstanz, Germany

Diatoms play a crucial role in the biochemistry and ecology of most aquatic ecosystems, especially because of their high photosynthetic productivity. They often have to cope with a fluctuating light climate and a punctuated exposure to excess light, which can be harmful for photosynthesis. To gain insight into the regulation of photosynthesis in diatoms, we generated and studied mutants of the diatom \textit{Phaeodactylum tricornutum} Bohlin carrying functionally altered versions of the plastidic \textit{psbA} gene encoding the D1 protein of the PSII reaction center (PSII RC). All analyzed mutants feature an amino acid substitution in the vicinity of the Q\textsubscript{B}-binding pocket of D1. We characterized the photosynthetic capacity of the mutants in comparison to wildtype cells, focusing on the way they regulate their photochemistry as a function of light intensity. The results show that the mutations resulted in constitutive changes of PSII electron transport rates. The extent of the impairment varies between mutants depending on the proximity of the mutation to the Q\textsubscript{B}-binding pocket and/or to the non-heme iron within the PSII RC. The effects of the mutations described here for \textit{P. tricornutum} are similar to effects in cyanobacteria and green microalgae, emphasizing the conservation of the D1 protein structure among photosynthetic organisms of different evolutionary origins.

\textbf{Key index words:} chlorophyll fluorescence; D1 protein; diatom; electron transport; herbicide; photosystem II; Q\textsubscript{B} pocket

\textbf{Abbreviations:} DCMU, (3-(3, 4-diclorophenyl)-1,1-dimethyleura); LHC, light-harvesting complex; OEC, oxygen evolving complex; PAM, pulse amplitude modulation; PQ, plastoquinone; PSII RC, photosystem II reaction center; Q\textsubscript{A} and Q\textsubscript{B}, quinone A and B; WT, wildtype

Diatoms (Heterokontophyta, Bacillariophyceae) are a major group of microalgae ubiquitous in all marine and freshwater ecosystems. With probably >10,000 species, their biodiversity is among the largest of photosynthetic organisms, just after the higher plants (Mann 1999). Diatoms are assumed to contribute to about 40% of the aquatic primary production (i.e., \approx 20% of the annual global production) and to play a central role in the biochemical cycles of silica (which is part of their cell wall) and nitrogen (Sarthou et al. 2005). Their productivity has contributed largely to the structure of contemporary aquatic ecosystems (Falkowski et al. 2004). In contrast to the supposed primary origin of red algae, green algae, and higher plants, diatoms originate from a secondary endosymbiotic event in which a nonphotosynthetic eukaryote probably engulfed a eukaryotic photosynthetic cell related to red algae and transformed it into a plastid (Keeling 2004). This peculiar evolution has led to complex cellular functions and metabolic regulations recently highlighted by the publication of the genome of two diatom species (Armbrust et al. 2004, Bowler et al. 2008), \textit{Thalassiosira pseudonana} and \textit{Phaeodactylum tricornutum}. The complex cellular functions include aspects of photosynthesis (Wilhelm et al. 2006), photoacclimation (Lavaud 2007), carbon and nitrogen metabolism (Allen et al. 2006, Kroth et al. 2008), and response to nutrient starvation (Allen et al. 2008).

As for most microalgae, the photosynthetic efficiency and productivity of diatoms strongly depend on the underwater light climate (MacIntyre et al. 2000). Planktonic as well as benthic diatoms tend to dominate ecosystems characterized by highly turbulent water bodies (coasts and estuaries) where they have to cope with an underwater light climate with high-frequency irradiance fluctuations coupled with large amplitudes. Depending on the rate of water mixing, diatoms can be exposed to punctual or chronic excess light, possibly generating stressful...
conditions that impair photosynthesis (i.e., photoinactivation/inhibition) (Long et al. 1994, Lavaud 2007). In higher plants and cyanobacteria, the processes of PSI RC photoinactivation/-inhibition are strongly influenced by the redox state of the acceptor side of PSII with quinones (QA and QB) as primary electron acceptor (Vass et al. 1992, Fufezan et al. 2007).

Here we report on the generation and characterization of four psbA mutants of *P. tricornutum*. All mutants feature distinct amino acid exchanges in the D1 protein of PSI close to or within the QB-binding pocket. The point mutations resulted in a constitutive impairment of the PSI electron transfer in all mutants to different extents. To our knowledge, this is the first report of plastid genome mutants in an alga with secondary plastids.

**MATERIALS AND METHODS**

**Strains and media used for producing the psbA mutants of *P. tricornutum***. *P. tricornutum* (University of Texas Culture Collection, strain 646) was grown at 22°C under continuous illumination at 50 μmol photons m⁻² s⁻¹ in Provasoli’s enriched F/2 seawater medium (Guillard and Ryther 1962) using ‘Tropic Marin’ artificial seawater at a final concentration of 50%, compared to natural seawater. When used, solid media contained 1.2% Bacto Agar (Difco Lab., Becton Dickinson and Co., Sparks, MD, USA).

**Generation of psbA mutants from *P. tricornutum**. Construction of plasmid transformation vectors**: Four transformation vectors were constructed harboring a 795 bp psbA fragment containing the QB-binding pocket. The psbA inserts of each vector carried individual point mutations leading to different substitutions of the amino acid serine encoded by the PsbA (D1) codon 264 (for details and a vector map, see Fig. S1 in the supplementary material). In addition to the nonsynonymous point mutations in codon 264, a second, silent point mutation was introduced into codon 268 (TCT to TCA, nt position 804) without changing the encoded amino acid. The purpose of the second point mutation was to delete a BosI restriction site, thus allowing easy RFLP screening of putative transformants.

**Biologic transformation of *P. tricornutum***. Transformation of *P. tricornutum* was performed using a Bio Rad Biolistic PDS-1000/He Particle Delivery System (Bio-Rad, Hercules, CA, USA) as described previously (Kroth 2007). Gold particles with a diameter of 0.1 μm served as microcarriers for the DNA constructs. Bombarded cells were allowed to recover for 24 h before being suspended in 1 mL of sterile F/2 50% medium. Transformants (250 μL) were selected at 21°C under constant illumination (35 μmol photons m⁻² s⁻¹) on agar plates containing 5 10⁻⁶ M DCMU herbicide (3-(3, 4-dichlorophenyl)-1-dimethylurea) and repeatedly streaked on fresh solid selective medium to obtain full segregation of the mutation.

**Isolation of DNA and sequencing of wildtype and mutant psbA genes**: Total nucleic acids from the wildtype (WT) and mutant cells were isolated via a cetyltrimethylammoniumbromide (CTAB)-based method (Doyle and Doyle 1990). Prior to the mutagenesis of *P. tricornutum*, the WT psbA gene and the surrounding genes were sequenced (NCBI accession no. AV864816) via primer walking (GATC, Konstanz, Germany). For the molecular characterization of mutants, a 795 bp fragment of the psbA gene was amplified as described in Figure S1, and both strands were fully sequenced (GATC, Konstanz, Germany).

**Cell cultivation and preparation for physiological measurements**: *P. tricornutum* WT and mutant cells were grown in 200 mL sterile F/2 50% medium (Guillard and Ryther 1962) at 21°C in airlift columns continuously flushed with sterile air. The cultures were illuminated at a light intensity of 50 μmol photons m⁻² s⁻¹ with white fluorescent tubes (L58W/25, Universal white, OSRAM GmbH, Munich, Germany) with a 16:8 light:dark (L:D) cycle. Cells were harvested during the exponential phase of growth, centrifuged (Allegra 25R; Beckman Coulter GmbH, Krefeld, Germany) at 3,000g for 10 min, and resuspended in their culture medium to a final chl a concentration of 10 μg chl a mL⁻¹. The algae were continuously stirred at 21°C under low continuous light. For oxygen (O₂), chl fluorescence, and thermoluminescence measurements, cells were dark-adapted 20 min prior to measurement.

**Protein extraction and Western blot analysis**: Cells were harvested during the exponential phase of growth as described above and subsequently grind in liquid nitrogen. The homogenized cells were resuspended in preheated (60°C) extraction buffer (125 mM Tris/HCl [pH 6.8], 4% [w/v] SDS, 200 μM PMSF, and 100 mM DTT) and, after heat treatment, extracted with acetone. After wash steps, the dried protein pellet was finally resuspended in extraction buffer. Total protein was separated electrophoretically onto a PVDF membrane (Hybond P, Amersham Biosciences UK Limited, Buckinghamshire, UK) and incubated with an antisera against D1 (Anti-PsbA global antibody, A905 084, Agrisera, Sweden). Detection was performed using the chemiluminescence detection system from Roche Diagnostics (BM Chemiluminescence Blotting Substrate POD; Roche Diagnostics GmbH, Mannheim, Germany).

**Pigment extraction and analysis**: Chl a amount was determined by spectrophotometry using the 90% acetone extraction method. For pigment extraction, cells were deposited on a filter and frozen in liquid nitrogen. Pigments were extracted with a methanol:acetone (70:30, v/v) solution. Pigment analysis was performed via HPLC as previously described (Lavaud et al. 2003). Cell counts were performed using a Thoma hemocytometer (LaborOptik, Friedrichsdorf, Germany).

**Spectroscopy and PSI reaction center (PSI) concentration**. The absorption spectra were obtained at room temperature with a DW-2 Amino (American Instrument Co., Jessup, MD, USA) spectrophotometer, half-bandwith 3 nm, speed 2 nm s⁻¹. OD = 0 at 750 nm, 50% F/2 medium as a reference. P700 quantity relative to chl a was determined as described earlier (Lavaud et al. 2002a) with the DW-2 Amino spectrophotometer in dual beam mode (reference at 730 nm).

**Thermoluminescence**: Thermoluminescence patterns were measured with a self-made thermoluminometer following the procedure previously described (Gilbert et al. 2004). Flashes were single turn-over with duration of 25 μs. Samples were adjusted to 20 μg chl a · mL⁻¹ for measurement.

**Oxygen (O₂) concentration and photosynthetic light-response (P/E) curves**: O₂ concentration was measured with a DWI-Clark electrode (Hansatech Ltd., Norfolk, UK) at 21°C. White light of adjustable intensity (measured with a PAR-sensor, LI-185A; LI-Cor Inc., Lincoln, NE, USA) was provided by a KL-1500 quartz iodine lamp (Schott, Mainz, Germany). Cell culture samples were dark-acclimated for 20 min before measurement. P/E curves were obtained by illuminating a 2 mL sample during 5 min at various light intensities. A new sample was used for each measurement. E∞ is the irradiance for saturation of photosynthetic O₂ emission was estimated from P/E curves.

**Chl fluorescence induction kinetics and DCMU resistance**: Chl a fluorescence induction kinetics were performed with two instruments: a PEA-fluorometer (Walz, Effeltrich, Germany) for short-time kinetics (up to 290 ms), which allowed a classic
OEF analysis (see Fig. S4 in the supplementary materials for details), and a self-made “continuous light” fluorometer (Paréys et al. 2005) for long-time kinetics (up to 100 s). Cells were adjusted to a concentration of 5 µg chl a · mL⁻¹ and 20 µg chl a · L⁻¹ for the PEA- and the self-made fluorometers, respectively.

DCMU resistance was evaluated measuring the inhibition of the PSII activity versus increasing DCMU concentrations (see Fig. S4 for details). The kill curves with DCMU and atrazine were performed by growing the cells on solid medium with increasing concentrations of herbicides; growth conditions were the same as described before.

Chl fluorescence yield. Chl fluorescence yield was monitored with a modified PAM-101 fluorometer (Walz) as described previously (Lavaud et al. 2002a). For each experiment, 2 mL was used. Sodium bicarbonate was added at a concentration of 4 mM to prevent any limitation of the photosynthetic rate by carbon supply. When used, DCMU was incubated with the cell suspension at the beginning of the dark-adaptation period. Fluorescence parameters were defined as described in Figure S4. The parameter used to estimate the fraction of linear electron transport was calculated as follows:

\[ \text{ETR} = \Phi_{\text{PSII}} \times \text{PDF} \times \alpha \times 0.5 \]  (1)

where \( \Phi_{\text{PSII}} \) is the PSII quantum yield for photochemistry, PDF is the irradiance, and \( \alpha \) is the PSII antenna size (equivalent to the reciprocal of the steady-state O₂ yield per flash (\( 1/\text{O₂} \)) when measured polarographically with a flash electrode as described by Lavaud et al. (2002b). The flashes were separated by 500 ms allowing the reopening of PSII RCs by reoxidation of QA (Büchel and Wilhelm 1993) was \( 1 - q_P \). Where \( q_P \) is the photochemical quenching of chl fluorescence. The rate of linear electron transport was calculated as follows:

\[ \text{ETS} = \Phi_{\text{PSII}} \times \text{PDF} \times \frac{1}{c} \times 0.5 \]

where \( \Phi_{\text{PSII}} \) is the PSII quantum yield for photochemistry, PDF is the irradiance, and \( c \) is the PSII antenna size (equivalent to \( 1/\text{O₂} \) when measured polarographically).

Oxygen (\( \text{O}_2 \)) yield per flash. The relative \( \text{O}_2 \) yield produced per flash during a sequence of single-turnover saturating flashes (\( \text{O}_2 \) sequence) was measured polarographically at 21°C with a flash electrode as described by Lavaud et al. (2002b). The flashes were separated by 500 ms allowing the reopening of PSII RCs by reoxidation of QA⁻⁻ between each flash. The procedures used to record and calculate the steady-state \( \text{O}_2 \) yield per flash (\( 1/\text{O₂} \), an evaluation of the number of \( \text{O}_2 \) producing PSII RCs relative to chl \( a \)), the reciprocal of the half-saturating flash intensity of flash \( \text{O}_2 \)-evolution saturation curves (\( 1/\text{I}_{1/2} \), an evaluation of the PSII antenna size), and the miss probability per PSII were the ones described in Figure S4.

RESULTS

Generating the P. tricornutum psbA mutants. In an attempt to establish stable plastid transformation in P. tricornutum, we aimed for allele replacement via homologous recombination. To minimize impact on the diatom, we decided to substitute the WT psbA gene with slightly modified versions carrying alternative point mutations in codon 264 (Fig. S2B, green boxes, in the supplementary material). These mutations lead to single amino acid substitutions that were previously reported to induce herbicide resistance and a reduction in the electron transport within the PSII RC (Ohad and Hirshberg 1992, Oettmeier 1999). Sequencing of the target region in several putative transformants revealed a variety of nonsynonymous (and in some cases additional synonymous) point mutations. In all experiments, the observed point mutations occurred apparently random and independently of the respective vector sequence. None of the obtained resistant strains carried the same pair of point mutations that was supposed to be introduced into psbA by the utilized transformation vector (data not shown). Negative control experiments involving exclusive selection without preceding transformation, and biolistic transformation without vector DNA failed to generate resistant colonies. We sequenced 1,000 bp regions surrounding the \( \text{Q}_B \) pocket as well as coding and noncoding areas more distant to the \( \text{psbA} \) locus without finding other mutations than the ones described here. Yet, we cannot exclude the possibility that additional mutations have occurred at unknown loci. However, due to the selection on DCMU, which specifically interacts with the \( \text{Q}_B \) pocket of the D1 protein, additional mutations at other loci are likely to be detrimental and therefore selected against.

Although intriguing, this study is not focusing on the underlying molecular mechanism leading to the elevated mutation rates in the \( \text{psbA} \) gene; it will be the focus of a subsequent work. Instead, we characterized and compared in four selected mutants the physiological effects of different amino acid substitutions in the D1 protein of the PSII RC on the regulation of photosynthesis.

Localization of the mutations in the D1 protein and herbicide resistance of the P. tricornutum psbA mutants. The highly conserved D1 protein is part of the PSII RC in cyanobacteria and all phyla of plastid containing photosynthetic eukaryotes (Fig. S2A). The \( \text{Q}_B \)-binding pocket is located between the DE helix and the transmembrane E helix of the D1 protein (Kern and Renger 2007). The functional relevance of the \( \text{Q}_B \)-binding pocket (Fig. S2B) is highlighted by an amino acid sequence similarity of 97%–98% between pennate and centric diatoms, and a similarity of ~90%–93% between diatoms and members of the red lineage, the green lineage, and even cyanobacteria (Fig. S3 in the supplementary material). Sequencing the \( \text{psbA} \) genes of the four mutant strains revealed point mutations within or near the \( \text{Q}_B \)-binding pocket (Fig. S2B, red squares). The mutant V219I featured an amino acid exchange (Val to Ile at position 219) in transmembrane helix D. In mutant F255I, a Phe was changed to Ile in helix DE close to the \( \text{Q}_B \) pocket. S264A carries a Ser to Ala substitution within the \( \text{Q}_B \) pocket, and in L275W, Leu was changed to Trp in the helix E.

In comparison to the WT, the competitive binding of the herbicide DCMU to the \( \text{Q}_B \) pocket was altered to a different degree in all mutants (Fig. S4), among which S264A showed the highest resistance (3,000-fold). The level of resistance was confirmed by growth curves in the presence of increasing concentrations of DCMU (not shown). S264A was also highly resistant against the herbicide atrazine (500-fold).

Photosystem and light-harvesting properties, and growth of the P. tricornutum psbA mutants. At low light
Phaeodactylum tricornutum mutant L275W cells. Cells were grown at 50 \( \mu \text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1} \). The cross-link products of D1 degradation and the cross-link product of \( \sim 83 \text{kDa} \) also resulting from D1 degradation (Ishikawa et al. 1999) were found in a larger amount in L275W but not in the WT.

Fig. 1. Western blot of the D1 protein of the PSII reaction center of Phaeodactylum tricornutum wildtype (WT) and the psbA mutant L275W cells. Cells were grown at 50 \( \mu \text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1} \). Bands representing D1 degradation products of 23 kDa and the cross-link products of \( \sim 83 \text{kDa} \) also resulting from D1 degradation (Ishikawa et al. 1999) were found in a larger amount in L275W but not in the WT.

The concentration of active PSII RCs per chl \( a \) was higher in all the mutants but L275W (Fig. S4). The low concentration for L275W resulted in a decreased photosynthetic efficiency of PSII \( (F_{v}/F_{m}, \text{Fig. S4}) \), which was similar to the WT cells, except for L275W (–19%). When measured at an equivalent irradiance, the effective PSII quantum yield \( (\Phi_{PSII}, \text{Fig. S4}) \) was the same for WT cells and V219I, but lower in the other mutants. These values were in accordance with the steady-state electron transport rate per PSII \( (ET_{0}/CS_{0}, \text{Fig. S4}) \). Addition of DCMU to the WT resulted in a decreased \( \Phi_{PSII} (0.38) \), similar to that of S264A and L275W.

Only L275W showed a reduction in growth rate –26% (\( \mu \), Fig S4) and final maximal biomass (Fig S5 in the supplementary material). Although F255I and S264A reached the same final biomass with the same growth rate as the WT, they showed a 24 h delay (see days 3 and 2, respectively, Fig. S5).

Photosynthetic capacity of the \( P. \ tricornutum \) psbA mutants as a function of the light intensity. The light intensity dependent impairment of the \( Q_{A} - Q_{B} \) electron transfer was evaluated by measuring \( 1 - qP \), a fluorescence parameter that estimates the fraction of reduced \( Q_{A} \) (Büchel and Wilhelm 1993). While \( 1 - qP \) was similar in WT and in V219I, it was the highest in S264A and L275W (Fig. 3A). A difference in the extent of \( Q_{A} \) reduction was also found at rather low light intensities (inset Fig. 3A) as indicated by the ratios of the extent of the I-45 ms peak from the long-time fluorescence induction kinetics of mutant versus WT (see Fig. 2D). In S264A and L275W, \( 1 - qP \) reached saturation earlier (between 250 and 400 \( \mu \text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1} \)) than in WT cells. In F255I, the extent of \( Q_{A} \) reduction was higher than in WT up to a light intensity of 400 \( \mu \text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1} \). The direct consequences of the impaired \( Q_{A} - Q_{B} \) electron transfer were changed amplitudes of the electron transport rate per PSII \( (ETR) \) as well as altered patterns of ETR as a function of light intensity (Fig. 3B). The maximum ETR was decreased in all the mutants but to a different extent, thus confirming the values for \( ET_{0}/CS_{0} \) (Fig. S4). In contrast to WT and the other mutants, ETR was already maximal in S264A and
L275W at a light intensity of 250 μmol photons \( \cdot \) m\(^{-2} \cdot \) s\(^{-1} \); at this light intensity, the extent of Q\(_A\) reduction was close to its maximum (Fig. 3A).

**DISCUSSION**

Three out of the four psbA mutants showed a phenotype clearly distinct from the WT (see Fig. 4). Obviously, the observed amino acid substitutions hold implications for the phenotype of the mutants. The phenotypic effects described in this study allow various insights into the functionality of mutated residues or domains within the D1 protein.

**A mutation that slightly affects the photosynthetic efficiency: V219I.** In response to the slightly increased reduction state of Q\(_A\) (+10%) and the decreased ETR per PSII (about 5%), in V219I the number of PSII RCs was increased (14% to 22%, depending on the method) to maintain a photosynthetic activity similar to the WT as reflected also by its growth pattern. Hence, the exchange of Val to Ile at the position 219 in the helix D appears to be too distant from the Q\(_B\)-binding pocket to significantly disturb the electron transport within P. tricornutum.

**Effects of mutations within the Q\(_B\)-binding pocket: F255I and S264A.** The residues Phe\(_{255}\) and Ser\(_{264}\) bind the head group of Q\(_B\) (Kern and Renger 2007). The electron transport between Q\(_A\) and Q\(_B\) in F255I was significantly impaired (Fig. 4), slowing down the
reoxidation of QA as illustrated by the increased QA/QB state. It was especially visible with the pattern of thermoluminescence that resembles the one reported in P. tricornutum for WT cells treated with DCMU (abolishment of the B band and increase of the Q band) (Eisenstadt et al. 2008). Backward electron transfer from QB to QA, as illustrated by an enhanced F0 in photochemically inactive PSII RCs (Xiong et al. 1997), might partially explain the increased concentration of QA. As a consequence, the miss probability of the S state cycle was increased, the S1 state was stabilized (Perewoska et al. 1994), and the lifetimes of the redox states S2 and S3 increased (Gleiter et al. 1992), indicating a disturbed OEC operation. To compensate the decreased photochemistry of PSII, in F255I the number of PSII RCs increased (Fig. 4), reflected by a slight increase of chl a per cell as also reported for higher plants (Srivasatava et al. 1994). Nevertheless, the overall amount of pigments per chl a did not change; thus, the antenna size per PSII decreased, leading to a similar increase in Fk. Decreasing the PSII antenna size is known to be a straightforward way to relief from high excitation pressure on PSII due to a slowed down electron flow within the PSII RC because of mutations, herbicides, environmental stress, or other factors. Similarly, Wagner et al. (2006) suggested that in P. tricornutum an increased number of photosynthetic units together with decreased size of these units might allow maximization of photochemistry at different light regimes, which might be the case in mutants F255I and S264A. In spite of all these changes, the potential for photochemistry, qP, was decreased at intermediary irradiances (up to 400 μmol photons · m⁻² · s⁻¹). At high light intensities, the ETR was reduced (Fig. 4), reflecting the decrease of the PSII antenna size and of FPSII.

S264A showed a more drastic reaction compared to F255I regarding the QA–QB reoxidation, the electron back transfer QB to QA, but also the QB/QB² reoxidation (increased QA/QB² state) (Fig. 4). Consequently, the operation of the OEC S-state cycle was strongly disturbed similar to the pattern of the fluorescence induction kinetics, illustrating the consequence of the modified QA–QB redox state on the whole electron transport chain and especially on the redox state of the plastoquinone (PQ) pool (Lazar 2006, Papageorgiou et al. 2007). As F255I, S264A reacted by increasing the PSII number. qP was largely diminished, which usually reflects accumulation of dysfunctional, highly reduced PSII RCs. It led to a decrease in ETR at all light intensities. Both qP and ETR were saturated at a much lower irradiance than in the WT. The exchange of Ser to Ala probably modified the spatial arrangement of the QB pocket (Gleiter et al. 1992, Perewoska et al. 1994), as illustrated by the high DCMU resistance, and greatly impaired not only the redox state of QB but the binding of QB itself (Della Chiesa et al. 1997).

Effects of a mutation close to the nonheme iron-binding site: L275W. Leu275 is close to one of the histidines binding the nonheme iron atom (His272 in helix E, grey bar in Fig. S2), as well as at nearly equal distance between QA and QB (Kern and Renger 2007).
The effect of the L275W mutation on the photosynthetic ability per PSII was similar to the point mutation S264A (Fig. 4) but showed a highly disturbed OEC operation along with increased QA reduction. QA reduction was already elevated (1.2- to 1.3-fold compared to WT) at light intensities that were even below the intensity used for growing the cells. Ultimately, L275W showed a decreased growth rate under low light as well as the inability to reach the same final maximal biomass. The main difference compared to the other mutants was the reduced amount of active PSII (Fig. 4), demonstrated by a disturbed D1 repair cycle. Mutations close to or within the Qb pocket have been reported to modify the D1 turnover either by accelerating its damage and/or by inhibiting its proteolysis and/or synthesis (Della Chiesa et al. 1997, Nishiyama et al. 2006). Thus, it is very likely that in L275W there is a mixed population of active and inactive PSII, even at low light intensities (Mohanty et al. 2007), which is supported by the high $F_0$ level and the lowest $E_F/E_m$. In contrast to the other mutants, L275W responded to the point mutation by modifying the architecture of the photosynthetic apparatus as illustrated by the increase of the PSI:PSII stochiometry (Fig. 4). This attempt to maintain a reasonable photosynthetic activity might lead to an increased capacity for PSI cyclic electron flow as in higher plants with deficient linear electron transport (Kotakis et al. 2006).

In a series of papers (reviewed in van Rensen et al. 1999), Govindjee et al. showed that the exchange of the residue Leu275 significantly perturbs the QA−Fe−Qb2 structure, the protonation of Qb2− (Xiong et al. 1997), and subsequently the PQ redox state. It is thus likely that the phenotype of L275W is due to the close vicinity of the point mutation to both the Qb pocket and the nonheme iron atom binding sites, which functionally affects the properties of both the QA and Qb pockets (Vermaas et al. 1994).
Effects of similar mutations in cyanobacteria and green algae. The effects of the mutations described here for the diatom *P. tricornutum* are similar to effects of the same mutations reported in other photosynthetic organisms. For example, it has also been concluded that in the green alga *Chlamydomonas reinhardtii* (Erickson et al. 1989), the V219I amino acid substitution does not significantly disturb the electron transport within the PSI RC. Also, the effects of the F255I, S264A, and L275W mutations have been described in cyanobacteria (*Synechocystis* and *Synechococcus*) and *C. reinhardtii* (Erickson et al. 1989, Etienne et al. 1990, Gleiter et al. 1992, Kless et al. 1994, Perewoska et al. 1994). Remarkably, the S264A-induced DCMU resistance was much higher in *P. tricornutum* than in all previously studied organisms (Gleiter et al. 1992).

**CONCLUSION**

Our results illustrate that not only the substitution loci but also the nature of the exchanged amino acids are essential in modifying the spatial arrangement and properties of the D1 protein (Kless and Vermaas 1994). Ultimately, such structural changes, especially in the Qb-binding pocket, are defining the electron transport rate within the PSI RC (Lardans et al. 1998, Oettmeier 1999). The fact that photosynthesis is impaired at different levels in the *P. tricornutum* psbA mutants described here (see Fig. 4) provides a unique opportunity to further study the regulation of photosynthesis in diatoms. This work was supported by the European network MarGenes (QLRT-2001-01226 to P. G. K.), the University of Konstanz (University of Konstanz, ‘Anreisystem zur Frauenförderung’ to S. S. and J. L.), and the DFG (grant LA2368-2-1 to J. L.). We thank I. Adamska (University of Leipzig) for access to some of the instruments used here and for helpful discussions; D. Ballert for technical assistance; V. Reiser and P. Huesgen (University of Konstanz), C. Bowler (ENS Paris), and C. Wilhelm (University of Leipzig) for help with some of the experiments. This work is part of the PhD project of A. C. M. and of the diploma work of S. S.


---

**Supplementary Material**

The following supplementary material is available for this article:

**Figure S1.** Construction of the pGEM-T D1 transformation vectors.

**Figure S2.** Multiple sequence alignments and phylogenetic reconstruction.

**Figure S3.** Pair-wise comparison chart of D1 (PsbA) amino acid similarities and distances.

**Figure S4.** Pigment composition (in mol·100 mol⁻¹ chl a) and photosynthetic properties of the wildtype (WT) and the psbA mutants of *Phaeodactylum tricornutum*.

**Figure S5.** Growth curves of the wildtype (WT) and the four psbA mutants (V219I/F255L/S264A/L275W) of *Phaeodactylum tricornutum* cells.

This material is available as part of the online article.

Please note: Wiley-Blackwell are not responsible for the content or functionality of any supplementary materials supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.