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Localization of cytochrome b_6f complexes implies an incomplete respiratory chain in cytoplasmic membranes of the cyanobacterium *Synechocystis* sp. PCC 6803

Matthias Schultze [a], Björn Forberich [a], Sascha Rexroth [b], Nina Gwendolyn Dyczmons [b], Matthias Roegner [b], Jens Appel [c,*]

[a] Botanisches Institut, Christian-Albrechts-Universität, Am Botanischen Garten 1-9, 24118 Kiel, Germany
[b] Plant Biochemistry, Faculty of Biology and Biotechnology, Ruhr University Bochum, 44780 Bochum, Germany
[c] School of Life Sciences, Arizona State University, 1711 S. Rural Road, Tempe, AZ 85287, USA

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ABSTRACT

The cytochrome b_6f complex is an integral part of the photosynthetic and respiratory electron transfer chain of oxygenic photosynthetic bacteria. The core of this complex is composed of four subunits, cytochrome *b*, cytochrome *f*, subunit IV and the Rieske protein (PetC). In this study deletion mutants of all three *petC* genes of *Synechocystis* sp. PCC 6803 were constructed to investigate their localization, involvement in electron transfer, respiration and photohydrogen evolution. Immunoblots revealed that PetC1, PetC2, and all other core subunits were exclusively localized in the thylakoids, while the third Rieske protein (PetC3) was the only subunit found in the cytoplasmic membrane. Deletion of *petC3* and both of the quinol oxidases failed to elicit a change in respiration rate, when compared to the respective oxidase mutant. This supports a different function of PetC3 other than respiratory electron transfer. We conclude that the cytoplasmic membrane of *Synechocystis* lacks both a cytochrome *c* oxidase and the cytochrome b_6f complex and present a model for the major electron transfer pathways in the two membranes of *Synechocystis*. In this model there is no proton pumping electron transfer complex in the cytoplasmic membrane.
Cyclic electron transfer was impaired in all *petC1* mutants. Nonetheless, hydrogenase activity and photohydrogen evolution of all mutants were similar to wild type cells. A reduced linear electron transfer and an increased quinol oxidase activity seem to counteract an increased hydrogen evolution in this case. This adds further support to the close interplay between the cytochrome *bd* oxidase and the bidirectional hydrogenase.

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1. Introduction

The cytochrome b_6f complex is a multisubunit membrane protein complex [1]. It is the cyanobacterial and plastidial counterpart of the cytochrome bc_1 complexes of prokaryotes and mitochondria. In cyanobacteria it is involved in linear, cyclic photosynthetic and respiratory electron transport. Since the complex catalyzes electron transfer between the plastoquinone pool and either cytochrome c_6 or plastocyanin, it is the major hub and thus, indispensable for all these pathways. The complex is a dimer of two identical monomers composed of four core subunits: cytochrome *b*, cytochrome *f*, subunit IV, the Rieske-type iron sulfur protein PetC and four surrounding small subunits. Crystal structures of cytochrome b_6f complexes are available for the green algae *Chlamydomonas reinhardtii* [2] and the thermophilic cyanobacterium *Mastigocladus laminosus* [3].

When plastoquinol binds to the Q_p (Q_o) site of the complex, one of its electrons is delivered via the Rieske protein to cytochrome *f* and then further on to the soluble electron acceptors cytochrome c_6 or plastocyanin. This is the high-potential chain. The current understanding is that the other electron enters the low-potential chain of the hemes of the cytochrome *b* and is passed to a plastoquinone in the Q_n (Q_i) site of the complex. Simultaneously, two protons are delivered to the p-side of the membrane and, after a second reduction step, a total of two protons are taken up at the n-side. For each plastoquinol oxidized, one electron is recycled back to the plastoquinone pool. Therefore, the complex couples the whole reaction to the transport of two protons per electron. The dimeric structure might promote this so-called Q-cycle since the quinol bound to the Q_n site of one monomer can easily switch to the Q_p site of the adjacent monomer through a cavity formed by the two monomers [4]. For recent reviews on cytochrome bc_1 and b_6f complexes see references [1,5] and [6].

All cyanobacterial genomes contain at least one (*petC1*) gene for the Rieske protein that forms an operon-like structure with *petA*, the gene encoding cytochrome *f*. Whereas the genomes of the marine *Prochlorococcus* and *Synechococcus* species harbor only this gene cluster, some other strains have two to four different *petCs* as in the case of *Nostoc* sp. PCC 7120. Besides PetC1, two additional families,

* Corresponding author. Tel.: +1 480 965 186; fax: +1 480 965 6899.
E-mail address: jens.appel@asu.edu (J. Appel).

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PetC2 and PetC3 are distinguished. However, their distribution across species is erratic and does not correlate with specific metabolic traits such as nitrogen fixation or the presence of terminal respiratory oxidases (see supplementary information).

The *Synechocystis* sp. PCC 6803 genome contains all three variants of the PetC. Schneider et al. [7] constructed single mutants of all three genes and the respective double mutants with *petC3*, but could not segregate a *petC1petC2* double mutant. Activity of the cytochrome *bd* oxidase, a quinol oxidase, was upregulated in the *petC1* deletion strain [7], indicating that the electron transfer via the cytochrome b_6f complex was impaired. Therefore, PetC1 seems to be the major Rieske protein of this complex in *Synechocystis* and is only insufficiently replaced by PetC2. PetC1 and PetC3 have been heterologously expressed in *E. coli*, their respective midpoint potentials were +320 and +135 mV [8]. Considering these results, the high degree of similarity between PetC1 and PetC2, and their close phylogenetic clustering, suggest that both are Rieske proteins of the cytochrome b_6f complex oxidizing plastoquinol. Plastoquinone has a midpoint potential in the range of +60 to +100 mV [1], whereas those of the plastoquinol oxidizing Rieske proteins are typically closer to +300 mV [8]. PetC3 either fulfills a different function or, alternatively, is part of a cytochrome b_6f complex oxidizing menaquinols with a redox potential of −60 mV [8].

We constructed all possible single and double mutants of the three genes of the Rieske proteins in *Synechocystis* sp. PCC 6803 (*petC1* sll1316; *petC2* slr1185 and *petC3* sll1182) and tested their segregation under a variety of different conditions. In order to determine the localization of the Rieske proteins (PetC) and the other core subunits of the cytochrome b_6f *complex*, the cytoplasmic and thylakoid membranes of wild type cells and the single mutants were separated. Respiration and electron transfer measurements have been used to support the localization results and the functional role of the different Rieske proteins.

Previous studies showed that inhibition of different electron transfer pathways including cyclic electron transport via photosystem I and respiration (especially via the cytochrome *bd* oxidase), elicits an increased hydrogen production in the light [9,10]. Due to the central position of the cytochrome b_6f complex in these pathways, we sought to determine if its mutation would affect hydrogenase activity and hydrogen evolution.

2. Materials and methods

2.1. Growth conditions

Synechocystis sp. PCC 6803 and mutant strains were grown in BG-11 [11] supplemented with 5 mM TES pH 8. Cultures were grown in 50 ml medium in 100 ml Erlenmeyer flasks on a rotary shaker at 100 rpm, 28 °C and 50 μE m^{-2} s^{-1}. The media were supplemented with 5 μg ml^{-1} gentamycin, 20 μg ml^{-1} chloramphenicol and 25 μg ml^{-1} erythromycin, if appropriate. For physiological measurements, an aliquot was removed and inoculated into 250 ml BG-11 without antibiotics and bubbled with air. At OD_{730} between three and four, cells were harvested and resuspended in fresh BG-11 for measurement. Cultures measured at a lower cell density show the same overall trend as compared to more dense cultures but measuring the latter proved to be much more reproducible.

2.2. Construction of mutants

DNA cloning and PCR amplification were performed using standard procedures [12]. For the construction of deletion mutants and the primer sequences used for amplification of DNA fragments, see supplementary information. Constructs were produced via PCR fusion (adapted from [13]) of 300 bp PCR-products of regions up and downstream of the gene with a resistance cassette as described [14].

2.3. Separation of membranes

Cyanobacterial strains were grown in BG-11 with constant aeration until the culture reached an OD_{730} of 1. Membrane separations at a later growth stage did show similar results but suffered from much higher cross contaminations and were therefore avoided. Cells were harvested and washed two times in 5 mM HEPES, pH 8. Approximately 250 ml of the culture were resuspended in 100 ml lysis buffer (10 mM HEPES pH 8, with 0.6 M sucrose, 50 mM NaCl, 5 mM EDTA and 0.2 μM mercaptoethanol) with 0.25 g lysozyme and incubated at 30 °C for 2 h in the light. After centrifugation, the pellet was washed twice in 20 mM HEPES pH 8 with 0.6 M sucrose and resuspended in 20 ml of the same buffer. 200 U DNase I were added and the suspension passed three times through a French Press at 1200 psi. The cell debris was pelleted and 15 ml of 90% sucrose added to the supernatant. Fifteen milliliters of the sample were centrifuged in a sucrose gradient according to the protocol of Omata and Murata [15]. Fractions were identified by color and harvested as described. The plasma membrane was orange, whereas the thylakoid fraction was green.

2.4. Immunodetection

Purified membranes were applied to Tricine gels according to Schägger and von Jagow [16] and electroblotted on nitrocellulose (Porablot NC amp, Macherey and Nagel, Germany) with transfer buffer (39 mM glycine, 48 mM Tris, 0.0375 M SDS, 20% methanol). As blocking reagent, Tris-buffered saline solution (TBS: 20 mM Tris–HCl, 0.5 mM NaCl) with 5% Blotting Grade Blocker nonfat dry milk (Biorad) was used for one hour. After overnight incubation in TBS with 1% Blotting Grade Blocker and the antibody, the membrane was washed five times with TBS supplemented with 0.05% Tween 20. The detection was carried out using the ECL-system (Amersham, Freiburg, Germany). The antibodies against the PetCs were available [8], the PetB antibody was purchased from Agrisera (Sweden) and the PetA and PetD antibodies have been raised against the corresponding subunits of spinach.

2.5. Chlorophyll fluorescence measurements

Chlorophyll fluorescence was measured with a PAM (puls amplitude modulated) fluorimeter (Walz, Germany) equipped with the ED-101US/M unit for liquid samples. The measuring light was pulsed at 1.6 kHz at an intensity of 7, which corresponds to approximately 0.15 μE m^{-2} s^{-1}. Actinic light of differing intensities was provided by a KL-1500 (Schott, Germany) for five minutes. At the end of this phase, a single saturating light flash of 8000 μE m^{-2} s^{-1} of 1 s duration of a KL-1500 (Schott, Germany) was given to measure F_m. In a phase of 30 s before the next light intensity, the PQ pool was oxidized by applying far red light of 5 W m^{-2}. Prior to measurement, the cell suspension was set to 2.5 μg chlorophyll ml^{-1}. Photochemical and non-photochemical quenching were determined according to Campbell et al. [17]. Photochemical quenching was calculated according to $q_P = (F_m' - F')/(F_m' - F_0')$. F_m' being the maximum fluorescence during the saturating light flash, F' the steady state fluorescence at the actinic light intensity and F_0' the fluorescence during far red illumination directly after the actinic light period. Non-photochemical quenching was derived from $q_N = 1 - (F_m' - F_0')/(F_{m(DCMU)} - F_0)$. F_0 being the fluorescence in darkness prior to applying any actinic light. $F_{m(DCMU)}$ was the maximum fluorescence measured with a fresh sample in the presence of 10 μM DCMU and 50 μE m^{-2} s^{-1}. Under these conditions the maximal possible fluorescence can be obtained from cyanobacteria [17]. The light intensities used during growth (50 μE m^{-2} s^{-1}) and during the

experiment (0 to 400 μE m^{-2} s^{-1}) are well below the high light intensities (1000 to 1500 μE m^{-2} s^{-1}) required to elicit the fluorescence quenching related to the orange carotenoid protein (OCP) [18]. In addition, cultures were grown under iron replete conditions, so except photoinhibition and state transition no other fluorescence quenching mechanisms were expected to contribute considerably.

2.6. P700 measurements

P700 re-reduction was measured with a DUAL-PAM (Walz, Germany). In this case part of the culture was harvested by centrifugation and resuspended in fresh BG-11 to a cell density of 50 μg chlorophyll ml^{-1}. 500 μl of this suspension with 10 μM DCMU was used in a glass cuvette with an inner diameter of 2 mm. This cuvette was placed between the two measuring heads of the DUAL-PAM and a white light pulse of 10,000 μmol m^{-2} s^{-1} was applied for 100 ms. Saturation of the light pulse was proven by using a number of different light intensities. The decay of the signal at the end of the light phase was fitted assuming first order kinetics.

2.7. Hydrogen and respiration measurements

Hydrogenase activity and photohydrogen evolution were measured with a Clark-type electrode set to a potential of −600 mV, as previously described [10,19]. Respiratory rate was measured in a cell suspension of 20 μg chlorophyll ml^{-1} in the presence of 10 mM glucose. Cells were preincubated for 5 min in the presence of glucose in the oxygen electrode cuvette (DW 2 Liquid Clark electrode, Hansatech Inst., Norfolk, UK) prior to starting the dark measurement.

3. Results

3.1. Construction of mutants

We used PCR fusion [14] to make deletion constructs of all three *petC* genes. Cell lines were exposed to a variety of growth conditions, including photoautotrophic, mixotrophic, photoheterotrophic and heterotrophic (LAHG, light activated heterotrophic growth). Cells switch to purely heterotrophic growth under LAHG conditions, permitting the segregation of photosystem mutants [20]. All single mutants and the *petC1petC3* and *petC2petC3* double mutants could be segregated as previously described [7]. If PetC1 and PetC2 are essential for photosynthetic electron transport but dispensable for respiration we would have expected segregation of the double mutant under these conditions. However, we saw no segregation in spite of streaking cells more than 10 times. In all instances cells remained merodiploids (data not shown). These results indicate that either PetC1 or PetC2 are required for cytochrome b_6f complex functionality.

3.2. Localization of the Rieske proteins

To localize the different Rieske proteins, we separated plasma and thylakoid membranes from wild type cells and single mutants, according to Omata and Murata [15]. Total plasma and thylakoid membranes were loaded on a Tricine gel and immunoblotted (Fig. 1). As a quality control of the membrane preparations we used two marker proteins, the nitrate transporter protein, NrtA, exclusively localized to the plasma membranes, and CP47, the core antenna protein of photosystem II, found only in thylakoids [21]. Our plasma membrane preparation was free of thylakoids, however, we consistently observed a minor contamination by plasma membrane in the thylakoid fraction (Fig. 1A).

The b_6f complex proteins were detected by antibodies against the different PetCs [8] and against the cytochrome *b* subunit, PetB. All PetC antibodies, except that directed against PetC2, were specific and

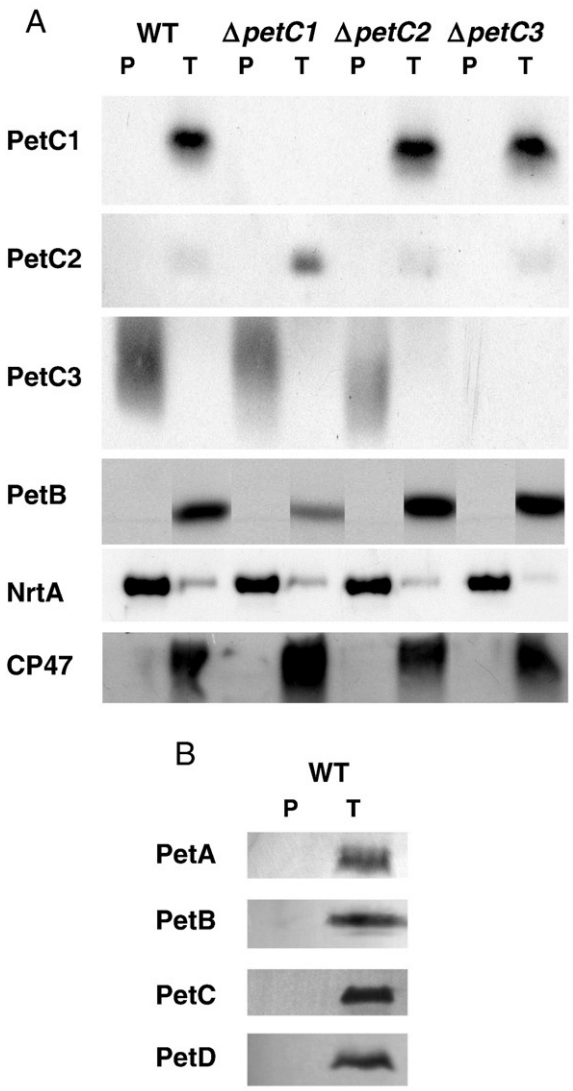

Fig. 1. The Rieske proteins PetC1 and PetC2 and the core subunits of the cytochrome b_6f complex are exclusively localized in the thylakoids, while PetC3 is only found in plasma membrane fractions of *Synechocystis* sp. PCC 6803. (A) Membranes of mutant and wild type cells were separated on a sucrose gradient, applied on Tricine gels, electroblotted on nitrocellulose, and probed with antibodies against PetC1, PetC2, PetC3, PetB, NrtA, and CP47. NrtA is used as a marker for plasma membranes (P) and CP47 as a marker for the thylakoids (T). For a better comparison, the arrangement of the blot of the PetB antibody was changed electronically. (B) Separated membranes of wild type were probed with antibodies against PetA, cytochrome *f*; PetB, cytochrome *b*; PetC, Rieske protein, and PetD, subunit IV. The isolated complex was used as a control. All blots are representative results of at least two independent experiments.

showed no reaction with the respective deletion mutants. Since the PetC2 antibody detects protein of the same molecular weight in the Δ*petC2*, it has some cross reactivity to PetC1. In summary, the immunoblots show that PetC1, PetC2 and PetB are confined to the thylakoids, whereas PetC3 is exclusively found in the plasma membrane.

Our data expands and confirms recent studies on the tat-dependent targeting of PetC-GFP fusions. Confocal laser scanning microscopy showed that PetC1 and PetC2 are only in the thylakoids, whereas PetC3 seemed to be confined to the cytoplasmic membrane [22].

PetB is downregulated in the Δ*petC1* (Fig. 1A). These findings indicate that, in this mutant, the b_6f complex is downregulated.

We also probed the fractions obtained from the wild type with antibodies directed against all four core subunits (PetA, cytochrome *f*; PetB, cytochrome *b*; PetC, Rieske protein and PetD, subunit IV) of the

cytochrome b_6f complex (Fig. 1B). None of these core subunits could be detected in the plasma membrane fraction. It is therefore highly unlikely that PetC3 is a Rieske protein of the cytochrome b_6f complex.

3.3. Respiration of the Rieske protein mutants

To determine respiratory activity, oxygen uptake in the dark was measured in the wild type and the *petC* mutants (Fig. 2). All single and double mutants showed similar respiration rates compared to the wild type cells. Mutants of *petC2* (Δ*petC2*, Δ*petC2*Δ*petC3*) had slightly reduced respiration, while *petC1* deletion mutants (Δ*petC1*, Δ*petC1*Δ*petC3*) demonstrated a slightly increased respiration.

Since PetC3 was the only Rieske protein found in plasma membranes, we hypothesized that a respiratory chain in these membranes would be non-functional if *petC3* was deleted in conjunction with any of the possible quinol oxidase genes. Therefore, we introduced the *petC3* deletion in all the mutants of the cytochrome *bd* oxidase (*cyd*) and the alternative respiratory terminal oxidase (*ctaII*), constructed previously [23]. All these *petC3* mutants had the same respiratory rate as their original oxidase mutants. These data imply that PetC3 is not part of a cytochrome b_6f complex.

3.4. Electron transport

Chlorophyll fluorescence measurements are widely used to characterize photosynthetic electron flow. Two quenching parameters, photochemical (q_P) and non-photochemical quenching (q_N), are indicative of cyanobacterial electron transfer [17]. The higher the photochemical quenching of chlorophyll fluorescence of photosystem II, the more rapidly are excited electrons transferred from PSII to the PQ pool. Non-photochemical quenching is the sum of three different processes: excitation energy redistribution in the cells (state transition), energy dissipation, and photoinhibition. At higher q_N, less available light energy is channeled into PSII. Cyanobacteria, especially, regulate distribution of their light harvesting complexes, the phycobilisomes, and their excitation energy transfer to the two photosystems by state transition [24,25]. The cytochrome b_6f complex is central to this process because it senses the redox state of the PQ pool and elicits a signal that results in redistribution of the excitation energy transfer [26].

Fluorescence measurements at various light intensities revealed that Δ*petC1* and Δ*petC1*Δ*petC3* have a slightly lower q_P and a higher q_N than the wild type cells (Fig. 3A). All other single or double mutants behaved similarly to wild type. These results suggest that the PQ pool is more rapidly reduced, under all conditions, if *petC1* is deleted, causing a shift of excitation energy from PSII to PSI resulting in a higher q_N. The higher q_N is directly visible in the original fluorescence traces (Fig. 3B), indicating a more pronounced shift of excitation energy from growth light (50 μE m^{-2} s^{-1}) to the DCMU inhibited state in the *petC1* mutant as compared to the wild type cells (Fig. 3B).

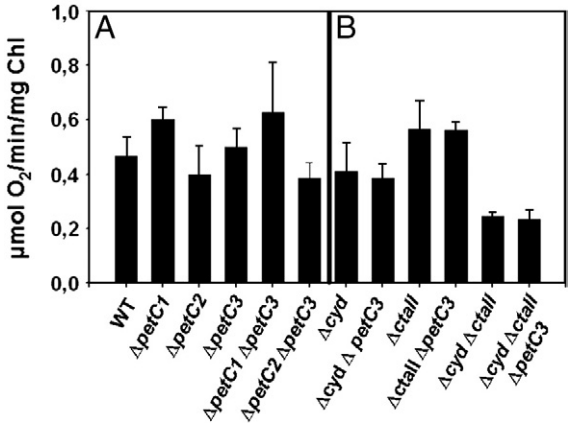

Fig. 2. Respiration of deletion mutants of *petC3* in conjunction with different terminal oxidases is not changed in comparison to the respective oxidase mutants. Dark respiration in the presence of 10 mM glucose of the different Rieske protein mutants (A) and mutants of *petC3* in conjunction with putative quinol oxidase genes (*cyd* and *ctaII*) (B) was measured with a Clark electrode. The given values are the average of duplicate measurements of three independent cultures. The standard deviation is indicated.

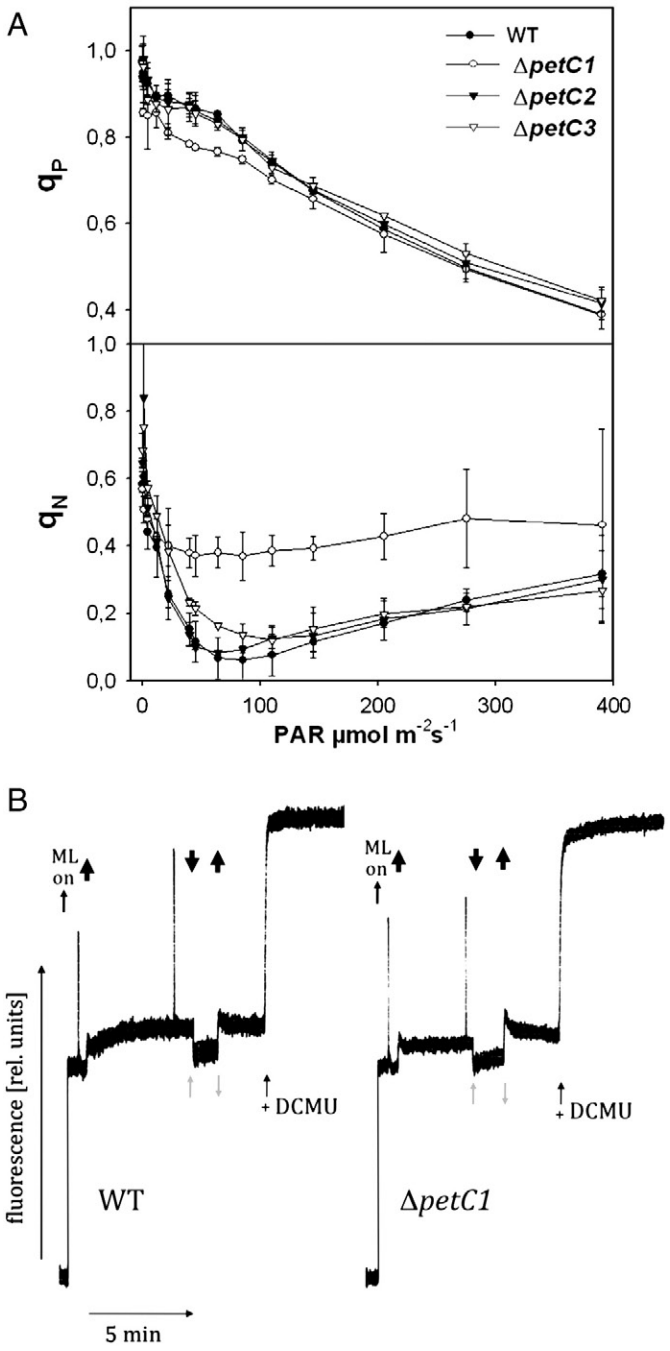

Fig. 3. The electron transfer of the Δ*petC1* is impaired. (A) Fluorescence measurements of photochemical (q_P) and non-photochemical quenching (q_N) were performed at the PAM-fluorimeter at different light intensities. Only the measurements of wild type cells and the single mutants are given. Results of the Δ*petC1*Δ*petC3* and Δ*petC2*Δ*petC3* were identical to the respective single mutants and omitted for clarity. The given values are the average of duplicate measurements of three independent cultures. The standard deviation is indicated and the actinic light intensity is given as photosynthetic active radiation (PAR). In (B) original fluorescence traces of wild type cells and Δ*petC1* are shown for comparison. The measuring light (ML) was switched on at the first arrow. Actinic light was set to 50 μE m^{-2} s^{-1} and switched on and off at the respective bold arrows. The grey arrows indicate illumination with far red light and at the end of the measurement 10 μM DCMU was added.

Table 1
Half-time of P700 re-reduction of wild type and the different *petC* deletion mutants.

Strain	Half-life [ms]
Wild type	131 ± 10
Δ*petC1*	197 ± 4
Δ*petC2*	128 ± 5
Δ*petC3*	96 ± 12
Δ*petC1*Δ*petC3*	188 ± 3
Δ*petC2*Δ*petC3*	118 ± 11

Measurements were made at least in triplicates. Representative results with standard deviations are given.

In addition, cyclic electron transfer was measured by oxidizing photosystem I in white light in the presence of DCMU (Table 1). These measurements indicate that the deletion of *petC1* results in an impaired cyclic transfer as seen by the increased half-time of P700 re-reduction in the Δ*petC1* and the Δ*petC1*Δ*petC3*. In contrast to this the deletion of *petC3* caused a decreased half-time in the Δ*petC3* and Δ*petC2*Δ*petC3*.

3.5. Hydrogenase and hydrogen evolution

Hydrogenase activity was found to be the same for all different single and double mutants of the *petC* genes (data not shown). In addition, photoinduced hydrogen evolution under anaerobic conditions as described by Gutthann et al. [10] was the same for all mutants and wild type cells. All strains produced approximately 4.8 nmol H_2/mg Chl at a rate of 12.5 nmol H_2/min/mg Chl in agreement with previous studies [10]. Therefore, an impaired cytochrome b_6f complex does not affect electron transfer to the hydrogenase.

4. Discussion

In cyanobacteria, the cytochrome b_6f complex is the only complex that catalyzes transfer of electrons between the two photosystems. The complex is involved in both linear and cyclic photosynthetic electron transport as well as in respiration. A newly discovered molybdopterin-dependent membrane oxidoreductase is hypothesized to perform a similar function in the anoxygenic photosynthetic bacterium *Chloroflexus aurantiacus* [27]. It is, however, absent in cyanobacteria.

We were unable to segregate a double mutant of the *petC1* and *petC2* genes, even when grown under strictly heterotrophic growth conditions. The corresponding proteins colocalize with all core subunits of the b_6f complex in the thylakoids (Fig. 1). Furthermore, deletion of *petC1* resulted in impaired electron transfer between the photosystems (Fig. 3) and inhibited cyclic electron transfer (Table 1). An involvement of PetC2 in respiration is especially suggested by the oxygen uptake measurements depicted in Fig. 2. Whereas the deletion of *petC1* causes an upregulation of O_2 uptake, the deletion of *petC2* results in a slight downregulation. Similar effects are not visible in the Δ*petC3*, thus suggesting that PetC2 is involved in respiratory electron transfer. Therefore, these data support that PetC1 and PetC2 are Rieske proteins of the cytochrome b_6f complex and essential for photosynthesis and respiration. In contrast, PetC3 was localized exclusively to the plasma membrane and did not colocalize with PetB or any of the other core subunits (Fig. 1). Its deletion has no effect on respiration, even in mutants of all putative quinol oxidases (Fig. 2). These findings imply that PetC3 is not a Rieske protein of the cytochrome b_6f complex.

PetC3, because of its low midpoint potential, would be well-suited to oxidize a menaquinol like phylloquinone. In cyanobacteria, the latter is believed to be synthesized only to provide the quinones present in the photosystem I reaction centers. Mutants of phylloquinone biosynthesis genes can be segregated. The PSI reaction centers of the respective strains substitute plastoquinone for phylloquinone [28]. Currently, there is no evidence for an additional function of phylloquinone other than as an electron transfer cofactor in PSI [28,29]. In contrast, our attempts to delete *ubiA* (slr0926), the putative prenyl transferase of the plastoquinone biosynthesis pathway, failed even under heterotrophic conditions. These results suggest that plastoquinone is absolutely essential and probably the only diffusible quinone in the electron transfer pathways of *Synechocystis*. The low midpoint potential of PetC3 and the absence of phylloquinone in the membranes provide an additional hint that it is not part of a cytochrome b_6f complex.

Our results show that there are two different cytochrome b_6f complexes in the thylakoids, containing either PetC1 or PetC2. The complex in the Δ*petC1* mutant is downregulated, as seen from the immunoblot with the antibody against PetB (Fig. 1A). Decreased expression is supported by the slightly lower q_P and significantly higher q_N of the mutant (Fig. 3) and the impaired cyclic electron transport (Table 1). Consequently, linear, as well as cyclic electron transport, are inhibited. In this respect it might be of interest that CP47 was upregulated in the Δ*petC1*. Although highly speculative, this could be taken as evidence for an increased turnover of photosystem II (PSII), a larger number of assembly intermediates of PSII [30], or maybe even a higher quenching capacity analogous to the role of IsiA under iron deficient conditions [31].

As the inhibition of cyclic electron transfer results in a higher photohydrogen evolution [9], we expected the Δ*petC1* to produce higher amounts of hydrogen. Since this was not found, this effect must be counterbalanced by its lower overall linear electron transfer providing fewer reducing equivalents to drive hydrogen production and/or its upregulated quinol oxidase activity [7](Fig. S1, Supplementary information), which supports our previous finding that this oxidase is able to withdraw electrons from the plastoquinone pool even under low oxygen concentrations [10].

Recent microarray studies show that *petC2* is expressed under normal conditions and upregulated under microaerobic conditions [32]. This suggests a division of labor between the two b_6f Rieske proteins, one working under normal and the second preferentially expressed under low oxygen concentrations.

The four core subunits of the b_6f complex were not detected in conjunction with PetC3, implying a role in a yet-undescribed electron transfer complex. The cyanobacterial PetC3 cluster in a well-separated group in the phylogenetic tree of Rieske proteins of the cytochrome b_6f and bc_1 complexes [33]. Although membrane associated the PetC3 are clearly not part of these complexes. However, it is remarkable that all the *petC3* mutants show a decreased half-time of P700 re-reduction. It might indicate that PetC3 is involved in alternative pathways withdrawing reducing equivalents otherwise available for the reduction of photosystem I. Further investigations are needed to clarify the role of PetC3.

Previous investigations suggest the absence of the NDH-1 complex in the plasma membrane [34,35]. Up to now a single study suggests the presence of the cytochrome c oxidase in the plasma membrane of *Synechocystis* [36], however the purity of the investigated membranes was not confirmed and the used antibody probably cross reacts with the subunits of the alternative heme-copper oxidase, ctaII [37]. The latter was detected in the plasma membrane by a proteomics investigation, but not the cytochrome c oxidase [38]. Therefore, we infer the existence of an incomplete respiratory chain in these membranes as outlined in our model on the photosynthetic and respiratory electron transport in thylakoid and cytoplasmic membranes, which is shown in Fig. 4. It is tempting to propose that there is only a NAD(P)H oxidizing module in the cytoplasmic membrane, such as NDH-2 and/or the succinate dehydrogenase [39–41] delivering electrons to the plastoquinone pool and a quinol oxidase reoxidizing this pool. Apart from the ctaII, the cytochrome *bd* oxidase, cyd, would be another candidate for this quinol oxidase, since it has been shown to withdraw electrons from the thylakoid PQ pool [42] and has also been suggested to be localized in the plasma membrane as well [43].

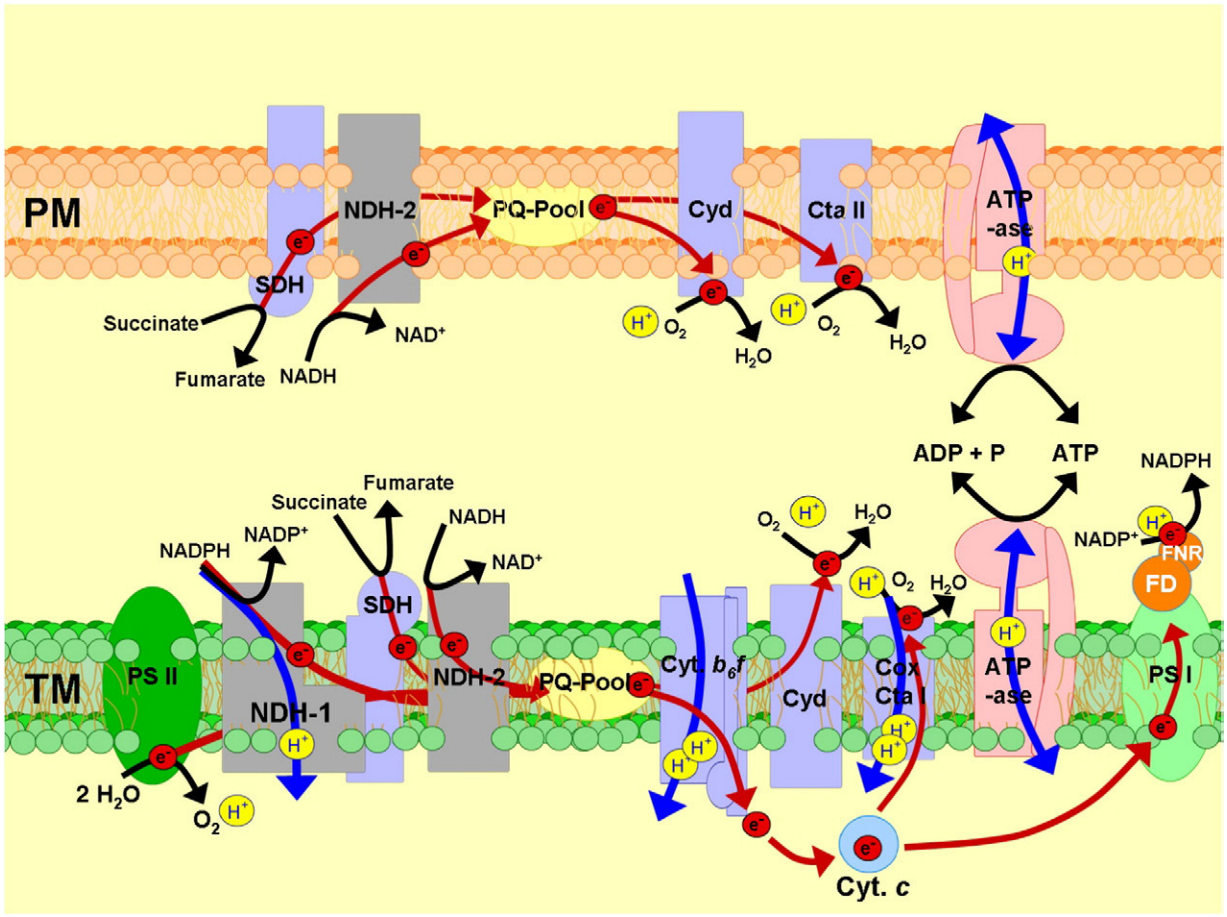

Fig. 4. Suggested distribution of electron transfer complexes in the membranes of *Synechocystis* sp. PCC 6803. Our results support the existence of a rudimentary respiratory chain with a type 2 NADH dehydrogenase (NDH-2) and a quinol oxidase in the plasma membrane (cyd). The cta II, also called alternative terminal oxidase, is another putative quinol oxidase. The available data suggest that thylakoids contain all the respiratory complexes, including NDH-2 [37]. The proton gradient at the plasma membrane is either formed by the oxidation of plastoquinol by the quinol oxidase at the periplasmic side of the membrane, with the concomitant release of two protons to the outside, or by the ATPase driven in the reverse or both. The cytochrome b_6f complex exists exclusively in the thylakoid membrane (with Rieske-copies PetC1 and/or PetC2) and is lacking in the cytoplasmic membrane (only PetC3 being present with unknown function). If the cta II is exclusively in the plasma membrane is currently unknown as well as the localization of the succinate dehydrogenase (SDH). Previous investigations did not find a NDH-1 complex in the plasma membrane [34,35]. The cytochrome *bd* oxidase (cyd) was found in the thylakoids [40] and was suggested to be in the plasma membrane as well [41]. Proteomics data suggest the absence of the cytochrome *c* oxidase but the presence of the ctaII in the plasma membrane [37]. The complexes are not drawn to scale. The abbreviations used are: PM, plasma membrane; TM, thylakoid membrane; NDH-2, type-2 NADH dehydrogenase; SDH, succinate dehydrogenase; PQ, plastoquinone; cyd, cytochrome bd oxidase (quinol oxidase); cta II, alternative respiratory terminal oxidase; PS II and PSI, photosystem II and I; NDH-1, NADH dehydrogenase (complex I); Cyt. b_6f, cytochrome b_6f complex; cox(ctaI), cytochrome *c* oxidase; Fd, ferredoxin and FNR, ferredoxin NADPH reductase.

In summary, our findings suggest that the plasma membrane of *Synechocystis* sp. PCC 6803 harbors respiratory complexes that do not function as proton pumps (Fig. 4). Nevertheless, a proton gradient would form when plastoquinone is reduced at the cytoplasmic side of the membrane and oxidized by a quinol oxidase expelling its protons to the periplasm as described for the cytochrome *bd* oxidase of *Escherichia coli* [44]. Alternatively, particularly in the light, energization of thylakoids causes high ATP levels that, in turn, could drive the plasma membrane ATPase in reverse to pump protons out of the cell. This corresponds to previous studies which indicate that both oxidation of NAD(P)H and ATP hydrolysis result in a proton gradient across the plasma membrane [45]. It could be speculated that this organization of electron transfer complexes could facilitate the oxidation of a surplus of NAD(P)H at the plasma membrane. Since a smaller fraction of the electrochemical potential of the reduction of oxygen by NAD(P)H would be used for the generation of a proton gradient, this system could be used for energy dissipation.

Definition of the role of this plasma membrane-located system contributes to our understanding of its functions and adaptive advantages. In contrast to *Synechocystis* sp. PCC 6803, *Synechococcus* sp. PCC 6301 expresses a high level of cytochrome *c* oxidase in the plasma membrane [36]. Predictably, it also has a high level of the cytochrome b_6f complex in the same membrane. Other strains with three different Rieske protein genes, like *Synechococcus* sp. PCC 7002, do not even tolerate the deletion of its *petC1* gene [46]. Therefore, cyanobacteria maintain a surprising variety of cellular organizations, that are probably indicative of adaptations to different ecological niches.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbabio.2009.06.010.

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