Interaction Between Respiration And Photosynthesis In Phototrophic Prokaryote, Cyanobacterium *Synechocystis* sp. PCC 6803

Department of Integrated Bioscience

Graduate School of Frontier Science

The University of Tokyo

Taku Mohri

2010

Master Thesis

Interaction Between Respiration And Photosynthesis In Phototrophic Prokaryote, Cyanobacterium *Synechocystis* sp. PCC 6803

原核光合成生物シアノバクテリアにおける

呼吸と光合成の相互作用の研究

東京大学大学院新領域創成科学研究科

先端生命科学専攻

毛利　拓

2010

修士論文

Table of contents

ACKOWLEDGMENTS

I would like to express my deepest appreciation to Professor Kintake Sonoike for his constant supervisions, valuable advices meaningful discussions and patient encouragements throughout my study in master course.

I would also like to express my deep appreciation to Professor Yoshikazu Ohya for for his significant discussions and kind encouragements through this study.

I am deeply greateful to Dr. Satoru Nogami, Dr. Hiroshi Ozaki, Dr. Akihiro Saito and Kazumi Shimada for their continuous supervision, fruitful suggestion and technical advice.

I am also grateful to Dr. Aiko Hirata, Dr. Takahiro Negishi, Mizuho Sekiya, Yo Kikuchi and Shinsuke Ohnuki for their helpful communication and discussion.

I wish to thank Keichi Takeda, Tomoko Motohashi, Tomohide Kobayashi, Hayato Ogawa, Hiroki Okada and Miki Maeda for their grate encouragement though my whole life during my master course.

I also wish to thank Youhei Suga, Ryusuke Hiramoto, Ayane Tasai and Seuji Morinaga for their kind communication.

At last, but not at least, I wish to give my best thanks to my family for their financial and moral support.

LIST OF FIGUERS

Figure. 10. Non-photochemical quenching fluorescence measurement in dark analyzed by PAM fluorometer.

Figure. 11. Effects of KCN on state transition.

Figure. 12. Chlorophyll fluorescence of cyanobacteria in strain low light condition by measurement of the PAM fluorometer .

Figure. 13. Chlorophyll fluorescence of cyanobacteria in strain high light condition by measurement of the PAM fluorometer .

Figure. 14. The concentration of chlorophyll *a* and phycocyanin .

Figure. 15. Growth curve of wild type and slr0645 mutant in high light condition (200 μmol s^{-1} m^{-2}).

Figure. 16. Photoheterotrophic growth of WT ctaCI mutant and slr0645 mutant at BG-11 plate containing 5 mM glucose (Glc)

LIST OF TABLES

ABBREVIATION

PSII	photosystem II
PSI	photosystem I
PCC	Pasteur culture collection
F	fluorescence
qN	non-potochemical quenching
qP	photochemical quenching
Fm	the macimum level of fluorescence
Fo	the minimal level of fluorescence
PAM	pulse amplitude modulation
OD	optical density
A	absorbance
DCMU	3-(3,4-dichlorophenyl-)1,1-dimethylurea
KCN	potassium cyanide

SUMMARY

In this study, I found several pieces of evidence that respiration influences photosynthesis. First, I monitored the reduction rate of plastoquinone pool by chlorophyll fluorescence in the presence of KCN. Secondly, I developed a new parameter qN_{dark}, i.e. non-photochemical quenching in the dark, and qN_{KCN}, i.e. non-photochemical quenching in the presence of 1mM KCN in the dark. Finally, I found that low respiration mutant such as *ndhB* mutant had decreased level of chlorophyll *a* and phycocyanin content. These results suggest that change in respiration influences photosynthesis in cyanobacteria.

INTRODUCTION

Photosynthetic organisms possess two major systems for energy metabolism, i.e. photosynthesis and aerobic respiration. Photosynthesis is the process to convert light energy into chemical energy through the electron transport chain and carbon fixation. Respiration is the process to make biochemical energy through glycolytic pathway, TCA cycle and oxidative phosphorylation. The oxidative phosphorylation is the process to produce ATP through proton motive force formed by electron transport chain. In contrast to other heterotrophic organism, substrates for respiration are primarily provided from photosynthesis in the autotrophic organisms. Since light conditions fluctuate rapidly in nature, respiration in photosynthetic organisms is supposed to be influenced by photosynthesis. There must be some mechanisms to regulate photosynthesis and respiration for the survival under the changing environments in photosynthetic organisms.

In higher plants, carbohydrates produced by photosynthesis (photosynthate) are the main substrates for respiration in mitochondria. In general, the maximal respiration rate is only 10% of the maximal photosynthetic rate. Thus, it has been considered that respiration does not play a central role in energy metabolism in photosynthetic organisms. As mentioned above, the condition of photosynthesis inevitably influences the rate of respiration. In plant cells, photosynthetic activity in chloroplasts and oxidative metabolism in mitochondria interact with each other. The

function of photosynthesis in chloroplast is optimized by the complementary nature of mitochondrial respiration in multiple ways: facilitation of export of excess reduced equivalents from chloroplasts, shortening of photosynthetic induction, maintenance of photorespiratory activity. Furthermore, the mitochondrial oxidative electron transport and phosphorylation also protects chloroplasts against photoinhibition (Padmasree et al. 2002).

In the case of cyanobacteria *Synechocystis* sp. PCC 6803, which are prokaryotes, photosynthesis and respiration are not separated in organelles and both systems exist on the same thylakoid membranes (Fig. 1). Furthermore, respiratory electron transport chain shares some components with photosynthetic electron transport, i.e. plastoquinone pool, cytochrome b_6/f complexes and cytochrome c_6. Thus, the changes in the activity of photosynthesis directly influence the redox state of respiratory electron transport chain. The two energy metabolisms in cyanobacteria seem to be tightly coupled and co-regulated with each others. Interaction between photosynthesis and respiration is not limited in metabolic level in cyanobacteria.

Recently, evidence that the regulation of photosynthesis could be influenced by the condition of respiration are provided (Nomura et al. 2007). They reported that a mutant of the *ctaDI*, which is subunit of cytochrome *c* oxidase (terminal oxidase of respiratory electron transport chain) grown under high light condition has reduced amount of photosystem II in *Synechococcus* sp. PCC 7002. Since cytochrome *c* oxidase locates on the downstream of plastoquinone pool in electron transport chain, mutation

on *ctaDI* would lead to the over-reduction of plastoquinone pool, leading to the down-regulation of photosystem II content. Similar phenotype was also observed in the mutants of CtaCI and CtaEI subunits of cytochrome *c* oxidase in the cyanobacterium *Synechocystis* sp. PCC 6803 (Ozaki et al. 2007). These results support the idea that the relationship between photosynthesis and respiration is the mutuall one. Although it has been generally considered that the point of regulatory interaction between photosynthesis and respiration is at the redox state of plastoquinone pool, no direct evidence has been given so far.

The above mentioned cytochrome *c* oxidase mutants of *ctaCI* or *ctaEI* were identified in the screening procedure through the comparison of induction kinetics of chlorophyll fluorescence emitted from intact cells. Furthermore, it have been found that the mutant of *slr0645*, a gene of unknown function, showed very similar chlorophyll fluorescence kinetics to those of CtaCI and CtaEI mutants (Ozaki et al. 2007). Therefore, the *slr0645* might be also involved in respiration.

Cyanobacteria possess functionally distinct multiple NADPH dehydrogenase (NDH-1) complexes that are essential to respiration, cyclic electron flow and CO_2 uptake in upstream of plastoquinone pool. It play a central role in influx electron to plastopuinone pool from respiratory substrate. NDH-1 complexes are the large complexes containing 10-20 subunits (Fig. 3). NdhB is a component of NDH dehydrogenase that plays a major role of respiratory chain at the upstream of plastoquinone pool (Ogawa et al. 2007). NdhF3 is another component of NDH

dehydrogenase that is involved in CO_2 uptake (Ohkawa et al. 2000). Because of the slow growth observed in the *ndhB* mutant (Ohkawa et al. 2000), it has been speculated that influx of electrons to plastopuinon pool is somehow important for cyanobacteria.

The aim of this study is to elucidate how respiration influences the photosynthesis. For this purpose, I investigated five strains, cytochrome *c* oxidase *ctaCI* mutant, the *slr0645* mutant , *ndhB* mutant and *ndhF3* mutant together with the wild type strain (Fig. 2). I compared these strains under low light condition as well as under high light condition to elucidate the regulatory interaction between respiration and photosynthesis. In the process of this study, I developed method to estimate the alteration in respiration by chlorophyll fluorescence.

RESULT

Rate of respiration

Cell of the cyanobacterium, *Synechocystis* sp. PCC6803 was grown under low light (20 μmol m^{-2} s^{-1}) or high light (200 μmol m^{-2} s^{-1}) for 24 hours, and respiration of the intact cells were determined by oxygen electrode. Fig. 6 shows a typical chart of the measurements. The intact cells absorbed oxygen mainly due to respiration (rate 1). The rate of oxygen consumption was enhanced by the addition of 5 mM glucose, a substrate of respiration (rate 2). When cells were illuminated, rapid increase of the oxygen concentration due to photosynthesis was observed (rate 3). After the light was turned off, rapid decrease of the oxygen concentration was observed (rate 4), and this rate is higher than that observed before illumination, possibly due to the accumulation of intracellular photosynthetic product that works as a substrate for respiration. Addition of KCN, an inhibitor of terminal oxidase, suppressed the rate of oxygen consumption (rate 5). The residual decrease of oxygen concentration could be ascribed to the oxygen consumption by the electrode itself. Hereafter, (rate 4 - rate 5) is regarded as rate of respiration, while (rate 3 - rate 4) is regarded as rate of photosynthesis.

When the rate was calculated by per cell basis, there was no significant difference in respiration rate between the cells grown under low light or high light (compare WT of Fig. 7B and 7D). When the rate was calculated based on chlorophyll content, however, the cells grown under low light showed approximately 2.5 times

higher respiration than the cells grown under high light (compare WT of Fig. 7A and 7C). These results suggest that the rate of respiration is constant under different light regimes while chlorophyll (photosynthesis) decreases under high light to avoid excessive light absorption.

I picked up several mutants to characterize the components possibly affects the rate of respiration, i.e CtaCI, a component of cytochrome *c* oxidase (terminal oxidase in respiratory chain), NdhB, an essential component of NDH complex, NdhF3, a component of NDH complex working for CO_2 concentrating mechanism, and Slr0645 that was implied to be working in respiration in a previous study (Ozaki et al. 2007). Respiration rates of *ndhB* mutant were markedly decreased in both high light and low light growth condition. The result suggests that the flux through NDH complex limits the rate of respiration. On the other hand, the *ctaCI* mutant, the *ndhF3* mutant, and the *slr0645* mutant exhibited relatively normal respiratory rate compared to wild type. *ctaCI* and *ndhF3* are the genes present redundantly in the genome, so that other *ctaC* genes or *ndhF* genes might be compensating the role of *ctaCI* and *ndhF3*. As for the slr0645, no concrete role of the gene was reported, and it is hard to conclude anything at this stage.

Measurements of the redox state of plastoquinone pool in the dark

Chlorophyll fluorescence is a good way to non-destructively explore the condition of intact cell. I observed that the level of chlorophyll fluorescence in the dark

increased upon addition of KCN (Fig. 8). The level of chlorophyll fluorescence is known to decrease by the oxidation of plastoquinone pool (photochemical quenching), or by the state transition (sate 1 to state 2) caused by the reduction of plastoquinone pool (non-photochemical quenching). The scheme of state transition is described at Fig. 4. Direct effect of KCN would be the reduction of plastoquinone pool through the inhibition of the terminal oxidase, which could be regarded as an "exit" of the electrons from plastoquinone pool. Thus, it is reasonable to assume that the increase of chlorophyll fluorescence is due to the reduction of plastoquinone pool by the electrons from NDH complexes.

In Fig. 8B, the *ctaCI*, the *slr0645*, the *ndhF3* mutants grown under low light showed similar reduction kinetics as wild type. These results suggest that the electron influx from the upstream of plastoquinone pool was not much affected by the mutations. Meanwhile, addition of glucose to wild type cells induced faster reduction, indicating that electron influx from NDH complexes was increased by glucose. On the contrary, the *ndhB* mutant showed no reduction upon addition of KCN. In the case of cells grown under high light, *ctaCI*, slr0645, *ndhF3* mutants and wild type with or without glucose all showed similar reduction kinetics (Fig. 8F). *ndhB* mutant showed no reduction upon addition of KCN also in this case.

Adding DCMU, an inhibitor of electron transfer from Q_A to Q_B in PSII, under illumination induced rapid increase of the fluorescence to maximal level (Fig. 8). DCMU makes the cells in state 2 as well as oxidize Q_A, so that both photochemical

quenching and non-photochemical quenching was diminished to reach Fm level of chlorophyll fluorescence. Both in low light grown cells and high light grown cells, the *ctaCI* mutant and the slr0645 mutant and wild type with or without glucose showed similar rate of fluorescence increase (Fig. 8C, 8F). The *ndhB* mutant showed slower oxidation than wild type in low light grown cells as well as in high light grown cells. The *ndhF* mutant showed slower oxidation than wild type only in high light grown cells. This result suggesting that efflux of PSI got lower by deletion in NdhF which function related to CO_2 uptake. It is noteworthy that the additions of KCN itself largely affect the fluorescence increase upon addition of DCMU under illumination (Fig. 8). Addition of KCN apparently slowed the rate of plastoquinone pool oxidation.

Development of new parameters: qN_{dark} and qN_{KCN}

To monitor the sate transition in the dark, which reflects the redox state of plastoquinone pool, I developed a new parameter qN_{dark} as non-photochemical quenching in dark and qN_{KCN} as non-photochemical quenching in presence of 1mM KCN in dark. The definition of the two parameters is shown in Fig. 10B ($qN_{dark\ or\ KCN}=1-(Fm_{dark\ or\ KCN}-F_o)/(Fm-F_o)$)

. Since these parameters would be expected to represent the redox sate of plastoquinone pool, I thought these parameters could be used to evaluate the rate of respiration in the dark. In the *ctaCI*, *slr0645* and *ndhF3* mutants, values of qN_{dark} were higher than wild type under both low light and high light grown cells (Fig. 10C, 10D). These results

suggesting that their plastoquinone pool were more reduced in the dark in those mutants. In addition, there was slight increase of the parameter when 5mM glucose was added to the high light grown cells. On the other hand, the *ndhB* mutant showed low qN_{dark} in both low light and high light grown cells, suggesting the lack of electron flow from NDH complexes in this mutant.

Values of qN_{KCN} were higher that qN_{dark} in all strains irrespective of the growth light condition. This may reflect the state transition induced by the addition of KCN in each strain. One exception is the *ctaCI* mutant, which showed no significant diffference between qN_{dark} and qN_{KCN} under low light grown cells.

Measurements of chlorophyll fluorescence emission spectra at liquid nitrogen temperature

Chlorophyll fluorescence spectra were determined at 77 K either in state 1 (induced by DCMU and light), state 2 (induced by dark adaptation) or in the presence of KCN in the dark (Fig. 11). State transition from state 1 to state 2 could be monitored by the relative PSI fluorescence around 725 nm in the spectra. In all the mutants, the spectra determined with KCN are quite close to that determined after dark adaptation. In some cases, the relative PSI fluorescence is higher in KCN, possibly due to the plastoquinone reduction induced by KCN.

Photosynthetic characteristics monitored by pulse amplitude modulated (PAM) fluorometer

Photosynthetic parameters of the wild type, the *ctaCI* mutant, the slr0645 mutant, the *ndhB* mutant and *ndhF3* mutant are shown in Fig. 12 and Fig 13. Under the light at 200 μmol m^{-2} s^{-1}, there seem to be not so much difference in all the strains. In ctaCI mutant, some decrease in Fv'/Fm' as well as some increase in qN, NPQ and qP was observed. Steady state measurements under light may not be a good way to monitor the condition of respiration in intact cells.

Growth under normal light, very weak light and continuous high light

Respiration pathway is very important to produce energy even in photosynthetic organisms. The growth of the mutants is not much different from the wild type, except for the *ndhB* mutant (Table. 1). Slow growth of the *ndhB* mutant seems to correspond to the lowest rate of respiration in this mutant.

Since *slr0645* is the gene with no identified function, growth of this mutant was checked for two other conditions. It was reported that some mutants only show the growth defect under continuous high light. I determined the growth of the *slr0645* mutant uder high light (200 μmol m^{-2} s^{-1}) for 3days, but no difference was observed (Fig. 8). I also consider that effect of the defect in respiration pathway might become more

clear under very weak light (i.e. less contribution from photosynthesis). The growth of the *ctaCI* and slr0645 mutants under 5 μmol m^{-2} s^{-1} in the presence of 5 mM glucose is somewhat slower compared with the wild type (Fig. 16). The result indicates that the mutation in the *ctaCI* may affect the growth under light-limiting condition, and Slr0645 may also have some role in respiratory chain.

Measurements of pigment by absorption spectra

Absorption spectra of all strains were measured to characterize photosynthetic pigments. Upon high light acclimation, the concentration of chlorophyll *a* and phycocyanin per cell decreased in half in all strains (Fig. 14). In the *ndhB* and *ndhF3* mutants, concentration of chlorophyll *a* and phycosyanin were lower than those of wild type both in low light grown cells and high light grown cells. No difference from the wild type was observed for *ctaCI* and *slr0645* mutants.

DISCUSSION

Respiration influences photosynthesis

In this study, I found several pieces of evidence that respiration influences photosynthesis. First, I monitored the reduction rate of plastoquinone pool by chlorophyll fluorescence in the presence of KCN (Fig. 8 and Fig. 9). Secondly, I developed a new parameter qN_{dark} as non-photochemical quenching in the dark and qN_{KCN} as non-photochemical quenching in the presence of 1mM KCN in the dark (Fig. 10). Finally, I found that low respiration mutant such as *ndhB* mutant had decreased level of chlorophyll *a* and phycocyanin content (Fig. 14). These results suggest that change in respiration influences photosynthesis in cyanobacteria. In cyanobacteria, respiration and CO_2 uptake are important pathways for carbon assimilation as well as nitrogen assimilation. Intermediary metabolites in these assimilations are also important as starting materials to synthesize chlorophyll and phycobilin (Chew et al. 2007, Brown et al. 1990). It would be reasonable that a mutant, which has deletion in respiration and CO_2 uptake such as *ndhB* mutant, had decreased ability to synthesize chlorophyll and phycobilin (Fig. 14).

Although CtaCI is a component of cytochrome *c* oxidase that plays a major role in respiratory chain at the downstream of plastoquinone pool, there observed no decrease in the rate of respiration in *ctaCI* mutant (Fig. 7). This result suggests that other components might complement the defect in the function of CtaCI. The

cyanobacterium *Synechocystis* sp. PCC 6803 possess alternative respiratory terminal oxidase called CtaII in plasma membrane and cytochrome *bd* oxidase (quinol oxidase) called Cyd that receives electron directory from plastoquinone pool (Fig. 5, Hart et al. 2005). It has been reported that in Mao et al. 1994, the respiratory activity of the strain that lacked cytochrome *c* oxidase is similar to that of wild type strain. The authors discussed that the strain should contain at least one alternative terminal oxidase (Cyd). While branched respiratory chain are common in prokaryotes, so far no direct evidence has been obtained for cyanobacteria. It would be interesting to observe the change in photosynthesis in double the mutant of *ctaCI* and *cyd* in future. Although no decrease in the rate of respiration was observed in the *ctaCI* mutant, it showed decreased contents of PSII under high light condition, resulting in a phenotype of high PSI/PSII ratio (Ozaki et al. 2007). It seems that effect on photosynthesis could be more sensitive way to detect the change in respiration in some cases.

Measurements of plastoquinone pool redox by using values of qN_{dark} and qN_{KCN}

In cyanobacteria, redox state of photosynthetic electron transfer chain is influenced directly by respiration. In the dark, plastoquinone pool is reduced by respiratory electron transfer. In other words, activity of NDH complex should be higher than terminal oxidase. Reduction of plastoquinone pool leads to the induction of state transition, leading to the quenching of chlorophyll fluorescence through the energy transfer from phycobilisome to PSI. The level of state transition could be quantified as a

fluorescence parameter, qN. In the dark, qN shows high value because of the electron influx from NDH complex. When photon flux density becomes higher, plastoquinone pool will be oxidized and qN value would decrease to the level that is determined by the valance between PSI and PSII. Upon further increase in photon flux density, however, qN value will increase again, due to the reduction of plastoquinone pool by the limitation at the cytochrome b_6/f complex. Thus, qN value would be lowest around growth photon flux density.

To monitor respiration, I developed a new parameter qN_{dark} and qN_{KCN} (Fig. 10). I established that the values of qN_{dark} as well as qN_{KCN} reflect the redox change in plastoquinone pool through the the change in state transition. Use of Clark-type oxygen electrode is a general way to determine the rate of respiration. As compared with this measurement, measuring redox condition of plastoquinone pool by using the values of qN_{dark} and qN_{KCN} seems to have more sensitivity to detect the changes in respiration at least in some cases. The difference in the rate of respiration between the *ctaCI* mutant and wild type cells was not possible by using a Clark-type electrode, but was possible by monitoring qN_{dark} and qN_{KCN}. Although NdhF3 is component of NDH dehydrogenase that involved in CO_2 uptake, oxidation of plastoquinone pool cannot be detected by monitoring qN_{dark} and qN_{KCN}. Measurement of qN_{dark} and qN_{KCN} might be not a good way to detect the change in the factors upstream of plastoquinone pool.

slr0645 may be a factor involved in respiration

The slr0645 mutant showed similar phenotype as the *ctaCI* mutant such as qN_{dark} value (Fig. 10), photosynthetic characteristics (Table 1), growth under light-limiting condition (Fig. 16), chlorophyll fluorescence kinetics (Ozaki et al. 2007) and F_{725}/F_{696} (PSI/PSII) ratio (Ozaki et al. 2007). I speculate that Slr0645 has some kind of relation to respiration based on these results. In the cyanbacterium *Synechocystis* sp. strain PCC 6803, there are two terminal oxidases, i.e. cytochrome *c* oxidase and cytochrome *bd* oxidase, at the downstream of plastoquinone pool. Slr0645 itself encodes a hypothetical protein with a von Willebrand factor type A (vWA) domain (http://www.ncbi.nlm.nih.gov) and could not be an oxidase. The vWA domain comprises approximately 200 amino acid residues folded into a classic a/b para-rossmann type of fold. In most cases, some ligand would bind to adhesion site termed as the MIDAS motif that is the characteristic feature of the most of the vWA domain. The binding of ligand is usually mediated by metal ion. The majority of VWAcontaining proteins are extracellular, the most ancient ones, present in all eukaryotes, are all intracellular proteins involved in functions such as transcription, DNA repair, ribosomal and membrane transport, and the proteasome. A common feature seems to be involvement in multiprotein complexes (Whittaker et al. 2002).

Taken together, Slr0645 has some kind of relation to respiration and Slr0645 itself encodes a hypothetical protein with vWA domain. It could be assumed that Slr0645 is transcriptional factor or post-transcriptional regulation factor of terminal

oxidases in respiratory chain.

MATERIALS AND METHODS

Strains and growth conditions

Wild type and mutant cells of *Synechocystis* sp. PCC 6803 were grown in BG-11 medium (Rippka et al. 1979) supplied with 10 mM N-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid (TES)-NaOH (pH 8.0). Cells were grown under continuous illumination at 30°C. Liquid cultures were bubbled with air in 50-ml glass tubes. Solid medium was supplemented with 1.5% (w/v) agar and 0.3% (w/v) sodiumthiosulfate. To support growth under light-limiting condition, 5 mM of glucose was added. Photon flux density at 20 and 200 μmol m^{-2} s^{-1} was regarded as low and high light, respectively. For creating site directed mutants, wild-type strain was transformed by the mutated cosmid (Ozaki et al. 2007), and chloramphenicol-resistant or kanamycin-resistant clones were selected on solid media. Resistant colonies were serially re-streaked at least five times on BG-11 agar plates supplemented with antibiotics to promote the segregation of the mutant genomes. Site-direced mutants were maintained with 20 μg/ml chloramphenicol or kanamycin.

Measurement of respiration rates

Respiration rates were determined using a Clark-type oxygen electrode (Rank Brothers). Cells were harvested and concentrated by centrifugation (3000 rpm, 10 min) at room temperature. The precipitated cells were re-suspended in 7 ml of BG-11. Four

ml were taken from prepared cells and rate of oxygen consumption was measured by oxygen electrode. The rest of cell suspension was used for the measurement of cell density and chlorophyll *a* content. Five min after initiating the respiration measurement, 5 mM glucose were added to the medium. After glucose addition, light were irradiated at 2000 μmol m^{-2} s^{-1} to obtain the rate of photosynthesis. Immediately after 5 min of light exposure, the light was turned off and respiration rate were measured again. During the measurement, temperature of the electrode chamber was maintained at 25°C.

Measurement of the redox state of plastoquinone pool in the dark

Reduction of the plastoquinone pool was measured after 5 min of dark adaptation using pulse-amplitude modulation chlorophyll fluorometer (PAM 101/102/103, Heinz Waltz, Germany). Strains were grown under low or high light condition for 24 hours. During the measurement, fluorescence levels with fully reduced Q_A (Fm_{dark} or Fm_{KCN}) were obtained by applying multiple turnover flashes (XMT103, Heinz Waltz Germany) in the dark. One mM KCN was added to inhibit terminal oxidase in respiration. Then, 10 μM DCMU was added and light (200 μmol m^{-2} s^{-1}) was irradiated to measure maximum value of fluorescence (Fm).

Measurement of fluorescence emission spectra at liquid nitrogen temperature

Low temperature fluorescence emission spectra at liquid nitrogen temperature (77K) were recorded using a custom-made apparatus (Sonoike and Terashima, 1994).

Cell suspension at a chlorophyll concentration of 5 μg/ml in BG-11 medium was injected into brazen sample holders and frozen by immersing them into liquid nitrogen. The cell suspensions were excited by blue light passing through a broad band-pass filter (CS 4-96, Corning Inc., USA). Before the measurement, cells were incubated in darkness for 10 min at room temperature to equilibrate the redox state in photosynthetic electron transfer chain. Under this condition, the intensity ratio of PSI fluorescence at 725 nm and PSII fluorescence at 695 nm gives information on the relative ratio of photosystem contents (photosystem stoichiometry).

Analysis of chlorophyll fluorescence by PAM fluorometer

Chlorophyll fluorescence was measured with a pulse-amplitude modulation chlorophyll fluorometer (PAM 101/102/103, Heinz Waltz, Germany) with a high sensitivity emitter-detector unit (ED-101US, Heinz Waltz, Germany) as described by Sonoike et al. (2001). Cell were dark-adapted for 5 min, and then the measuring light was turned on to obtain the minimal fluorescence level (Fo). The steady state level of fluorescence (Fs) was determined during the exposure of cells to actinic light from a light source (KL 1500, Wiesbaden, Germany) with photon flux density at 200 μmol m^{-2} s^{-1}. The fluorescence level with fully reduced Q_A (Fm') was obtained by applying multiple turnover flashes (XMT103, Heinz Waltz Germany). Far red light was applied just after turning off the actinic light to obtain quenched level of Fo (Fo'). The maximum fluorescence (Fm) was obtained by adding 10 μM DCMU to the sample

under actinic light. Photochemical quenching (qP) and non-photochemical quenching (NPQ) were calculated as (Fm' - Fs)/(Fm' - Fo') and 1 - [(Fm' - Fo')/(Fm - Fo)], respectively. Actual quantum yield of electron transport in the light-acclimated state (ΦII) was calculated as (Fm'-Fs)/Fm'.

FIGURES

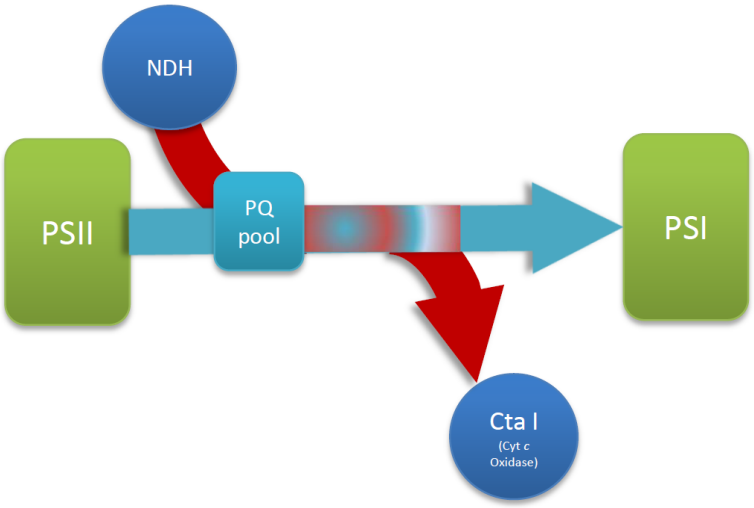


Fig. 1. Model of photosynthesis and respiration electron transport chains in cyanobacteria.
Photosynthesis electron transport chain (blue) and respiration electron transport chain (red) sharing PQ: plast quinone pool, NDH: NADH dehydrogenase, Cta:Cytocrome *c* Oxidase Each arrows represent a direction of the electron flow, PSII: photosystem II, PSI: photosystem I.

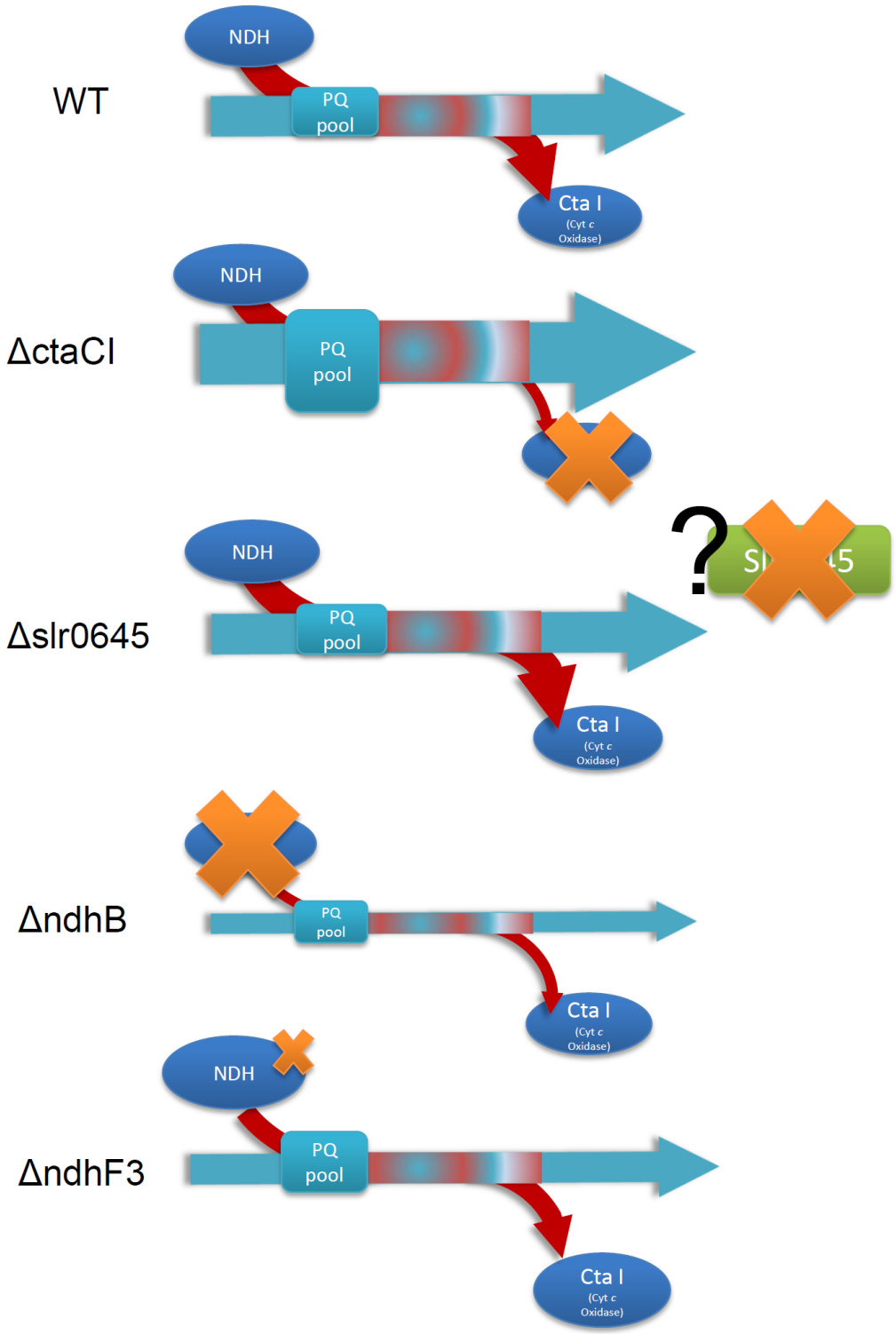


Fig. 2. Model of deletion mutants how influence the photosynthesis and respiration electron transport chains in cyanobacteria.
Arrow reprisents photosynthesis electron transport chain (blue) and respiration electron transport chain (red). The width of arrow reprisenting volume of electron flow. The size of plastoquinone pool reprisents redox state. PQ: plast quinone pool, NDH: NADH dehydrogenase, Cta:Cytocrome *c* Oxidase. Width of arrow represents volume of electrion flow.

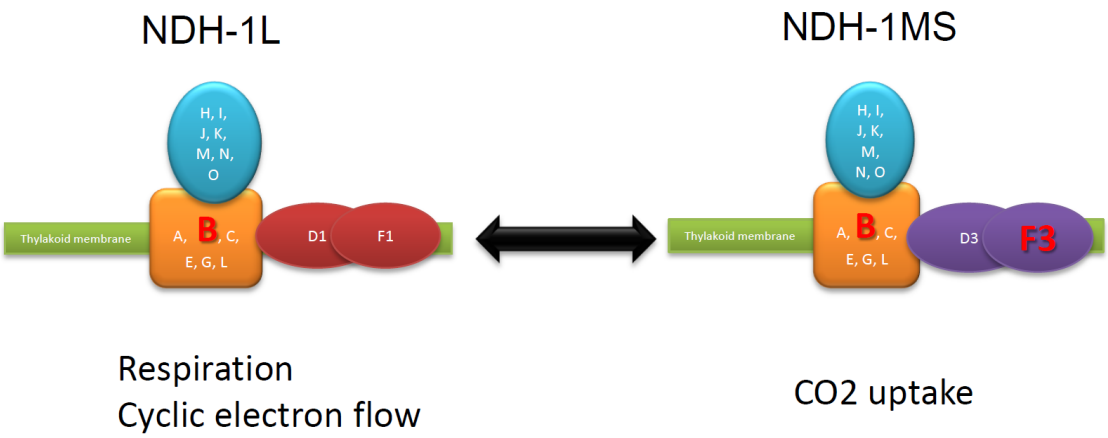


Fig. 3. The scheme of cyanobacterial NADPH dehidorogenase NDH-1 complex. NDH-1L participate in respiration and cyclic electron flow around PSI, while NDH-1MS is involve in CO_2 uptake. The position of NdhB and NdhF3 are indicate in the figure. NdhB plays a cetral role of respiration and CO_2 uptake. While, NdhF is working for CO_2 concetrating mechanism.

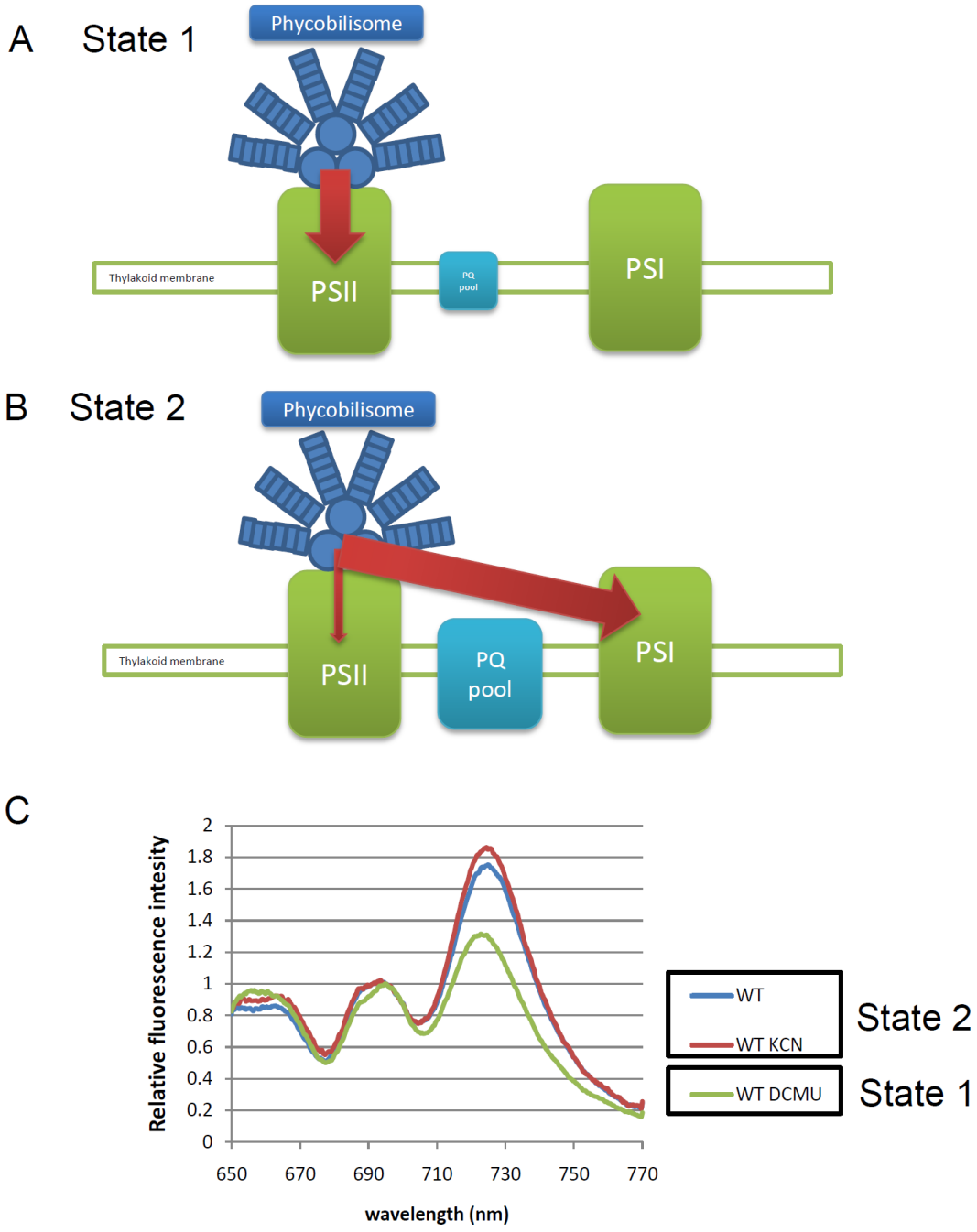


Fig. 4.The scheme of state transition in cyanobacteria.
A and B are regulation of energy distribution between PSII and PSI in cyanobacteria. When plastoquinone pool is oxidized, the energy absorbed by phycobilisome is transferred mainly to PSII (state 1). On the countrary, when plastoquinone pool was reduced , it delibered primarily to PSI (state 2). C is analysis in state transition is estimaed bu chlorophyll foluorescence emission spectra detremine at 77K

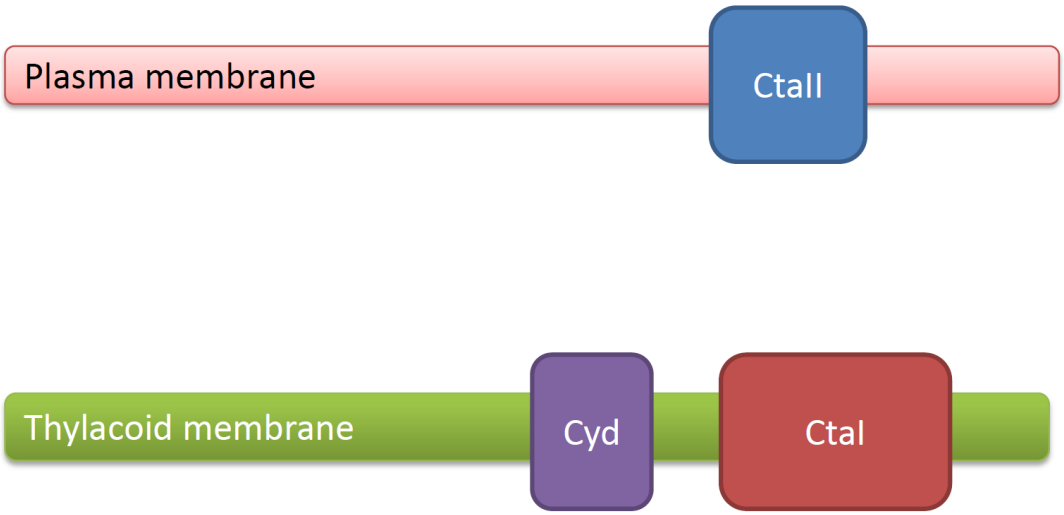


Fig. 5. Localization of terminal oxidases in cyanobacteria.
Cyanobacteria posses three terminal oxidase. Cta II located in plasma membrane. Cta I and Cyd located in thylaciod membrane. Cta I:Cytocrome *c* oxidase, Cta II:Cytocrome *c* oxidase, Cyd: Quinol oxidase.

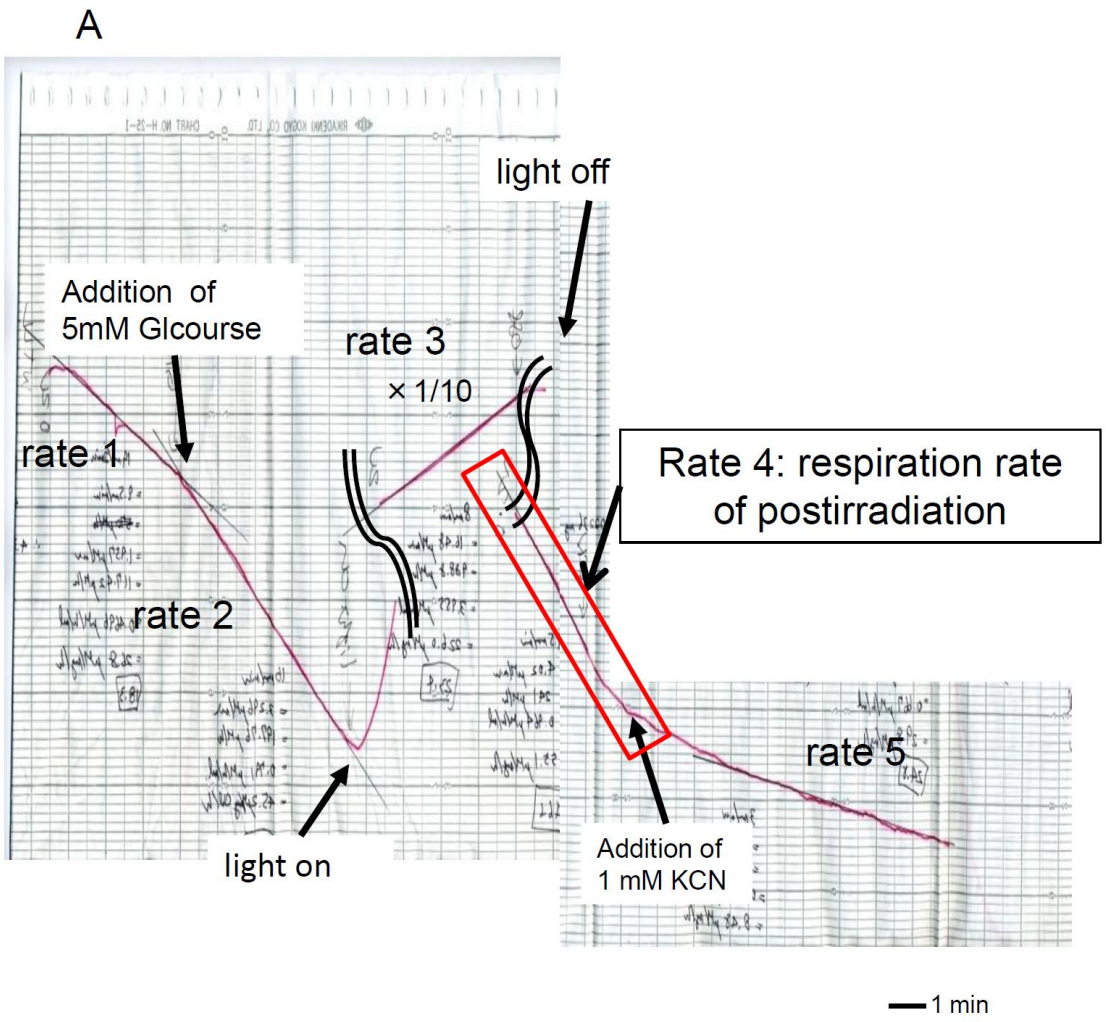


Fig. 6. Chart of mearuring respiration.
Cells which were incubated at 30℃ were concentrated by centrifuge. The condensing cells were prepared at 7 ml. Four ml were taken from prepared cells and measured respiration rate by oxygen electrode. During the measurement 5 mM glucouse were added. After addition of glucose, light were irradiated at 2000 μmol m^{-2} s^{-1}. Then, cells were prepared into dark and measured the respiration rate (red square).

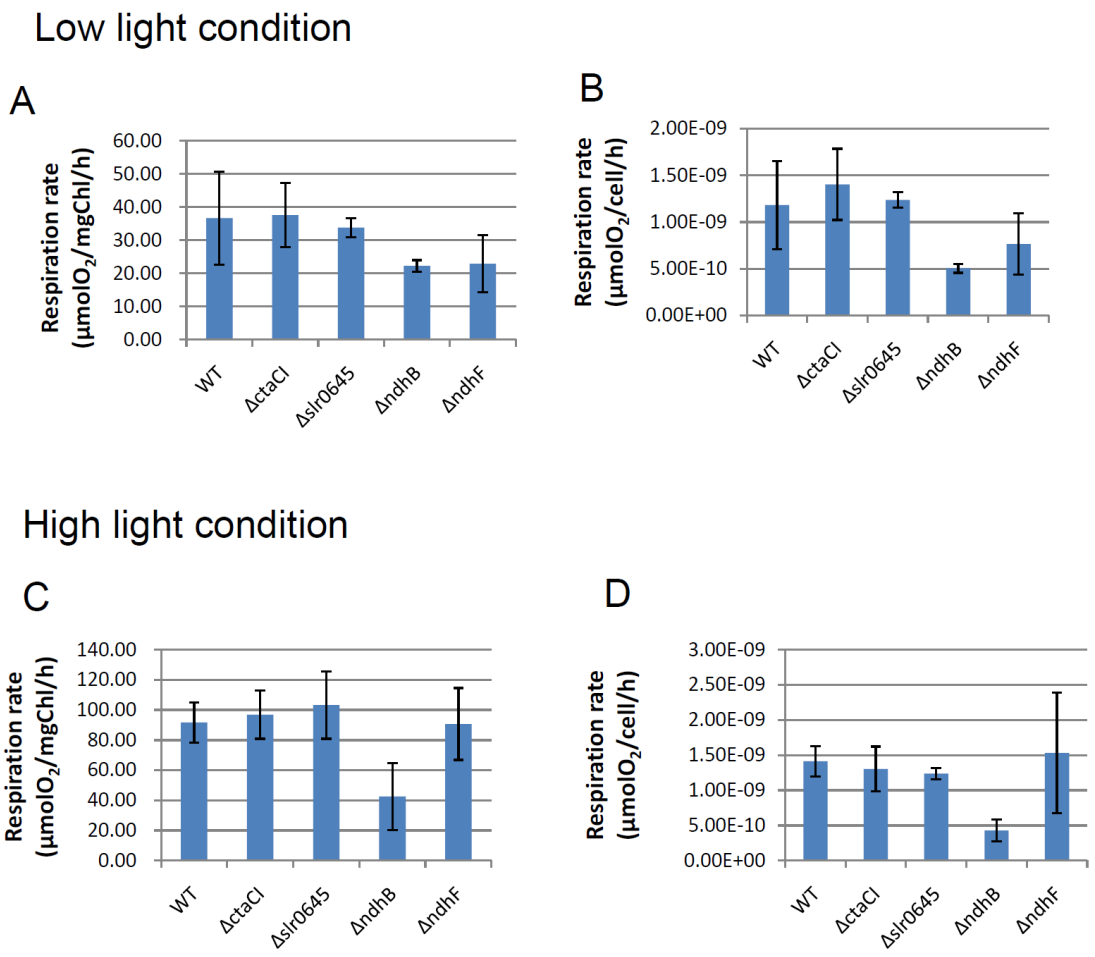


Fig. 7. Measurements of respiration rates.
Respiration rates were measured at low light growth (A , B: 20 µmol m^{-2} s^{-1}) and high light growth (C, D: 200 µmol m^{-2} s^{-1})conditions. A and C are respiration rate that calculated based on chlorophyll content. Whereas, B and D are respiration rate that calculated by cell quantity.

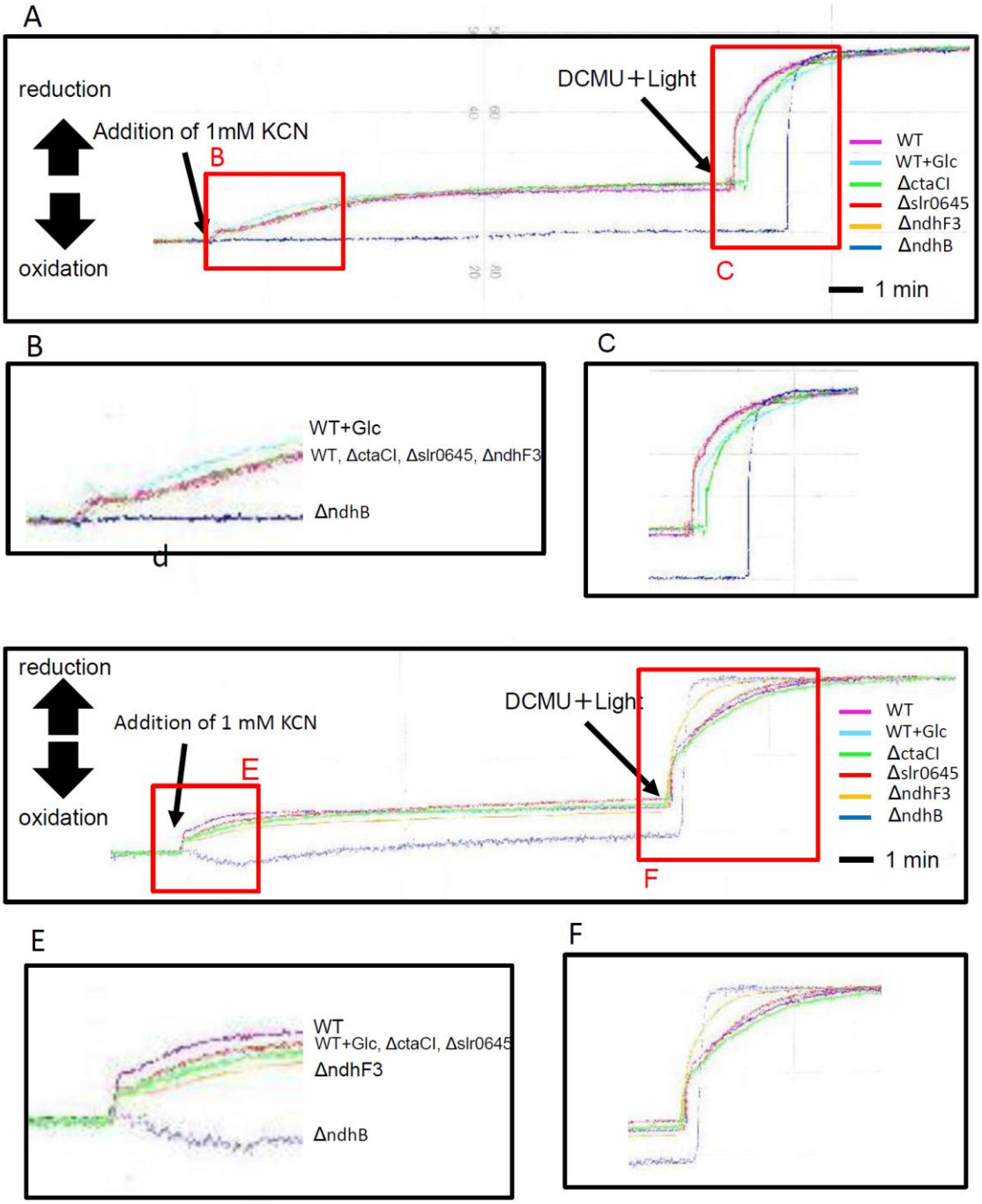


Fig. 8. Measurements of plastoquinone pool redox state by PAM fluorometer

These figures are chart of plastoquinone pool redox state by PAM fluorometer. A, B, C are chart which cells were grown at low light (20 μmol m^{-2} s^{-1}). B and C are close-up figures of A. D, E and F are chart which cells were grown at high light (200 μmol m^{-2} s^{-1}). E and F are close-up figures of D.

1 mM KCN was added to inhibit terminal respiration oxidase.10 μM DCMU was added and light (200 μmol m^{-2} s^{-1}) was irradiated to measure maximum value of fluorescence.

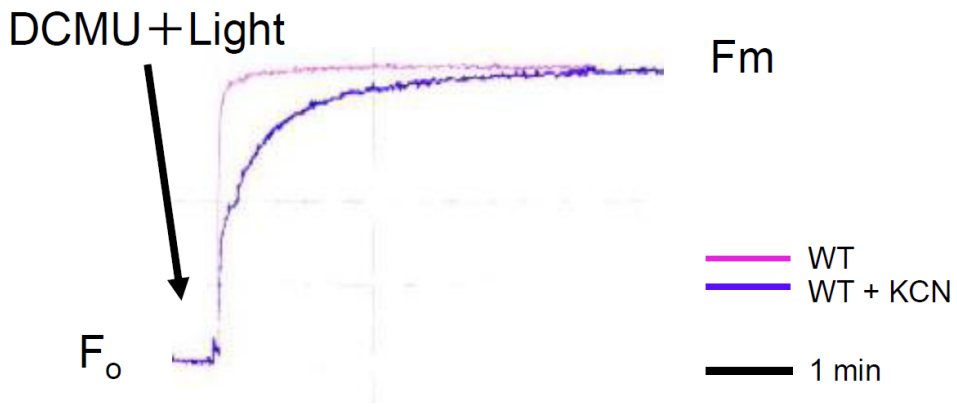


Fig. 9. Measurements of dustribution of state transition by PAM fluorometer.
Cells were grown at low light (20 μmol m^{-2} s^{-1}). F_o is initial fluorescence level. 1 mM KCN was added to inhibit terminal respiration oxidase berfore measurment (blue line). 10 μM DCMU was added and light (200 μmol m^{-2} s^{-1}) was irradiated to measure maximum fluorescence (Fm).

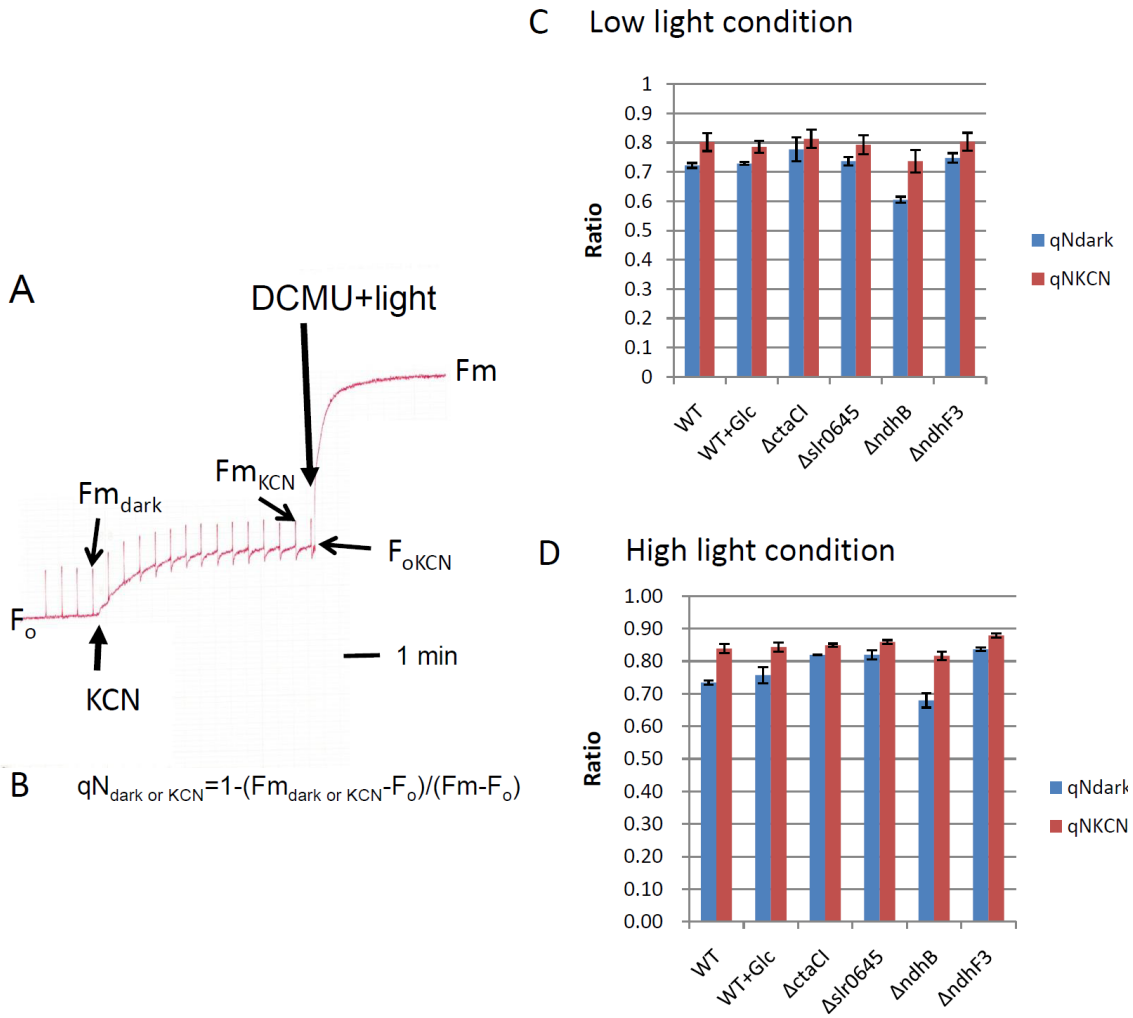


Fig. 10. Non-photochemical quenching fluorescence measurement in dark analyzed by PAM fluorometer.

(A), chart of photochemical quenching fluorescence. (B), equation to determine qN_{dark} and qN_{KCN}. (C), qN_{dark} and qN_{KCN} of low light grown cell. (D), qN_{dark} and qN_{KCN} high light grown cell. Bars indicate the standard deviation of three independent experiments. Cells were grown at low light (20 µmol $m^{-2}s^{-1}$) and high light (200 µmol $m^{-2}s^{-1}$) for 24h.

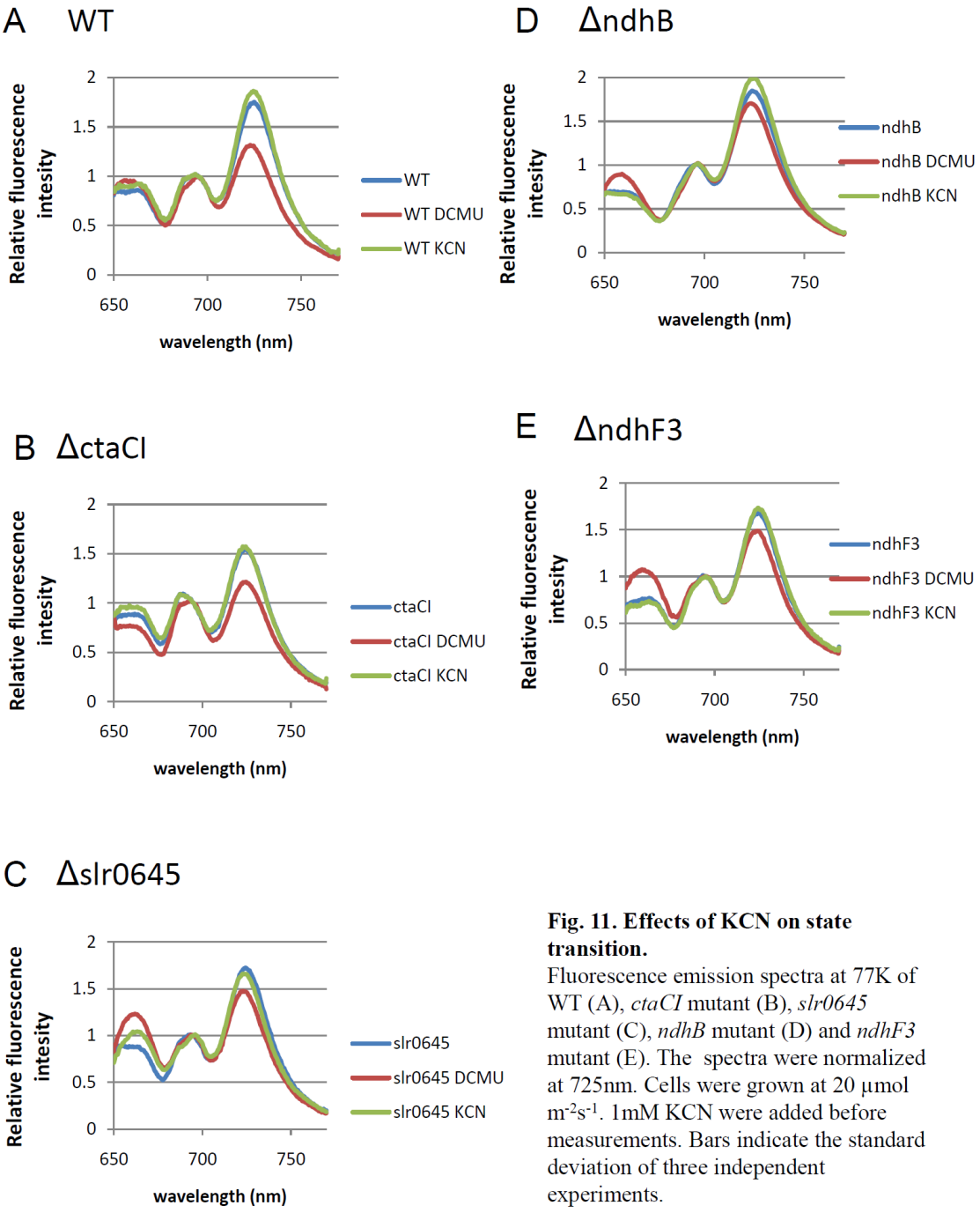
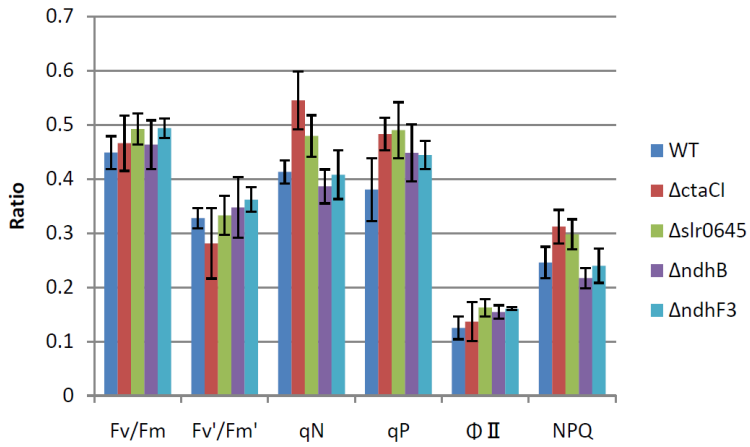


Fig. 11. Effects of KCN on state transition.
Fluorescence emission spectra at 77K of WT (A), *ctaCI* mutant (B), *slr0645* mutant (C), *ndhB* mutant (D) and *ndhF3* mutant (E). The spectra were normalized at 725nm. Cells were grown at 20 µmol $m^{-2}s^{-1}$. 1mM KCN were added before measurements. Bars indicate the standard deviation of three independent experiments.

A



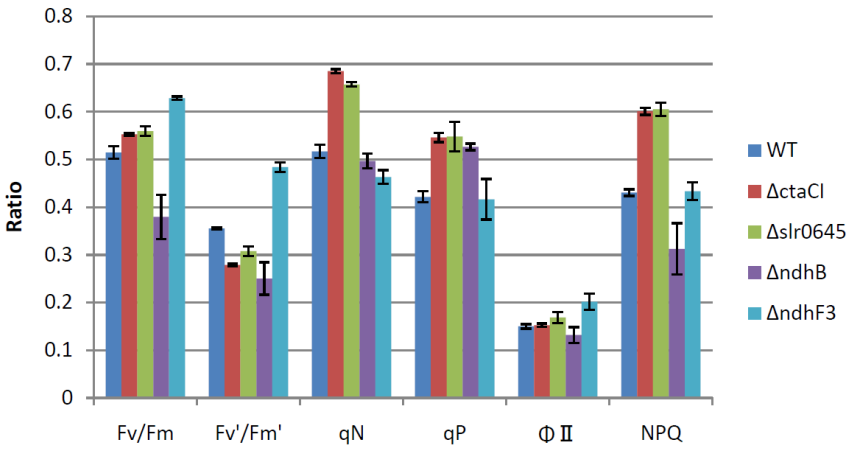
B

	Fv/Fm	Fv'/Fm'	qN	qP	ΦⅡ	NPQ
Low light grown cells						
WT	0.45 ± 0.03	0.33 ± 0.02	0.41 ± 0.02	0.38 ± 0.06	0.12 ± 0.02	0.25 ± 0.03
ΔctaCI	0.47 ± 0.05	0.28 ± 0.06	0.55 ± 0.05	0.48 ± 0.03	0.14 ± 0.04	0.31 ± 0.03
Δslr0645	0.49 ± 0.03	0.33 ± 0.04	0.48 ± 0.04	0.49 ± 0.05	0.16 ± 0.02	0.30 ± 0.03
ΔndhB	0.46 ± 0.04	0.35 ± 0.06	0.39 ± 0.03	0.45 ± 0.05	0.15 ± 0.01	0.22 ± 0.02
ΔndhF3	0.49 ± 0.02	0.36 ± 0.02	0.41 ± 0.05	0.44 ± 0.03	0.16 ± 0.00	0.24 ± 0.03

Fig. 12. Chlorophyll fluorescence of cyanobacteria in strain low light condition by measurement of the PAM fluorometer .

Actinic light intensity were measured at 200 μmol m^{-2} s^{-1}. Values represent the average ±S.D. of three independent measurements. Fv/Fm: maximum quantum yield of PS II in dark, Fv'/Fm': effective quantum yield of photosystem II, NPQ and qN: parameters reflecting any process other than photochemistry that lowers the yield of fluorescence, qP: an indicator of the redox state of plastoquinone pool, ΦII: quantum yield of electron transfer through PS II.

A



B

	Fv/Fm	Fv'/Fm'	qN	qP	ΦⅡ	NPQ
High light grown cells						
WT	0.51 ± 0.01	0.36 ± 0.00	0.52 ± 0.01	0.42 ± 0.01	0.15 ± 0.00	0.43 ± 0.01
ΔctaCI	0.55 ± 0.01	0.28 ± 0.01	0.69 ± 0.00	0.55 ± 0.03	0.15 ± 0.01	0.60 ± 0.01
Δslr0645	0.56 ± 0.00	0.31 ± 0.00	0.66 ± 0.01	0.55 ± 0.00	0.17 ± 0.00	0.61 ± 0.02
ΔndhB	0.38 ± 0.05	0.25 ± 0.03	0.50 ± 0.02	0.53 ± 0.01	0.13 ± 0.02	0.31 ± 0.05
ΔndhF3	0.63 ± 0.00	0.48 ± 0.01	0.46 ± 0.01	0.42 ± 0.04	0.20 ± 0.02	0.43 ± 0.02

Fig. 13. Chlorophyll fluorescence of cyanobacteria in strain high light condition by measurement of the PAM fluorometer .

Actinic light intensity were measured at 200 μmol m^{-2} s^{-1}. Values represent the average ±S.D. of three independent measurements. Fv/Fm: maximum quantum yield of PS II in dark, Fv'/Fm': effective quantum yield of photosystem II, NPQ and qN: parameters reflecting any process other than photochemistry that lowers the yield of fluorescence, qP: an indicator of the redox state of plastoquinone pool, ΦII: quantum yield of electron transfer through PS II.

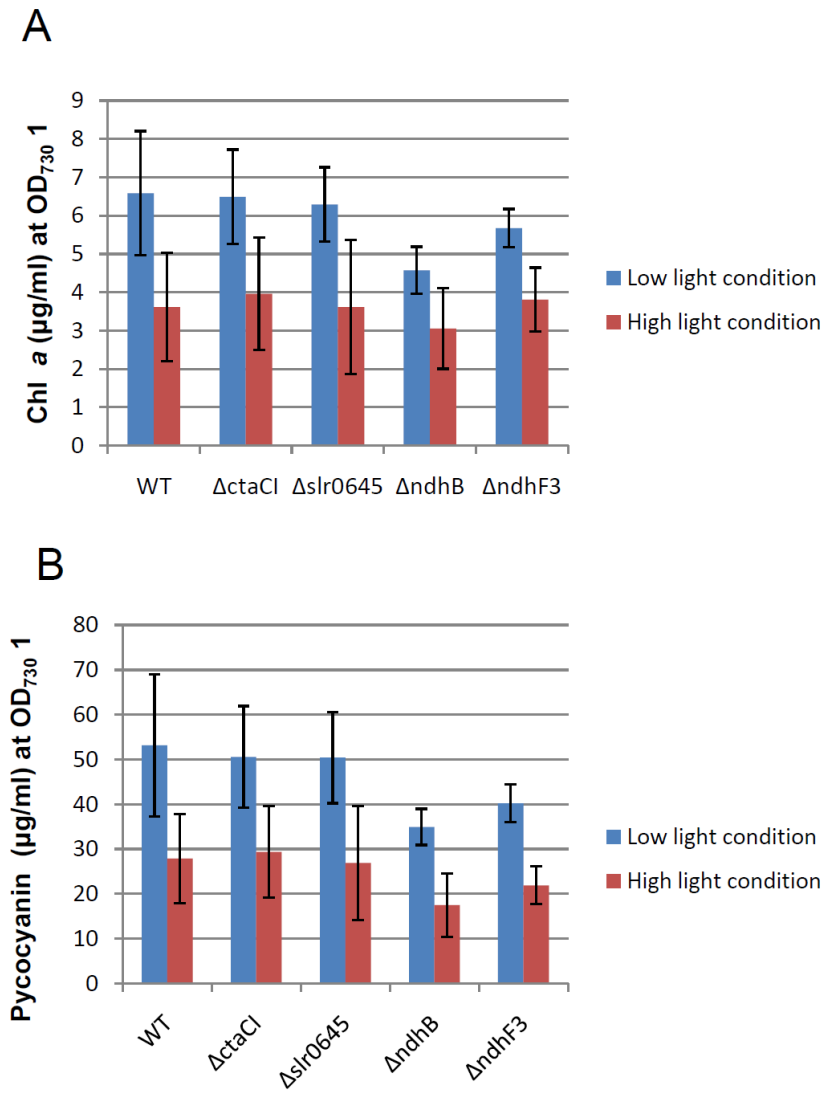


Fig. 14. The concentration of chlorophyll *a* (A) and phycocyanin (B) .
Strains were grown at low light (blue column: 20 µmol $m^{-2}s^{-1}$) and high light (red column: 200 µmol $m^{-2}s^{-1}$) condition for 24 hours. These concentrations determined at absorption and normalized at OD_{730}. Bar indicate the mean ± SD of three independent experiments.

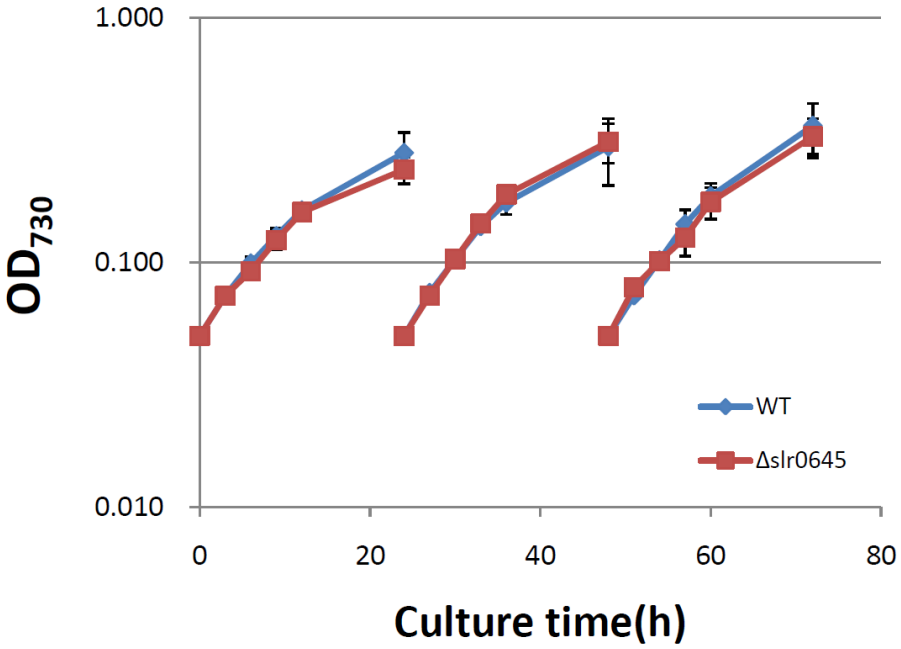


Fig. 15. Growth curve of wild type and slr0645 mutant in high light condition (200 µmol s^{-1} m^{-2}).
Cell densities were measured at OD_{730}. Samples were grown for 72 hours in the liquid cultuer of BG-11. Every start points were began at OD_{730} 0.05. Values in the parenthesis are standard deviation (SD) of three independent experiments.

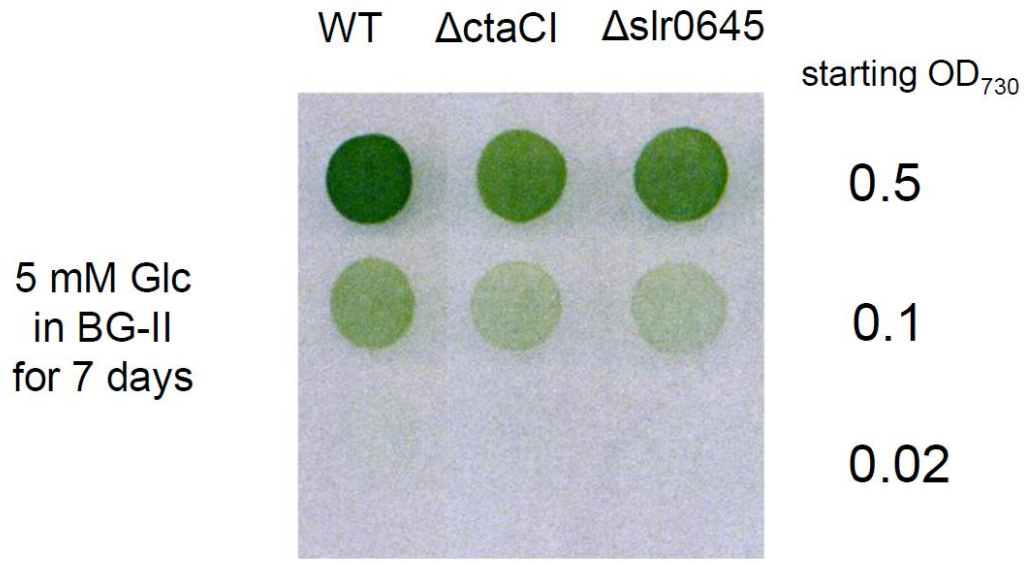


Fig. 16. Photoheterotrophic growth of WT ctaCI mutant and slr0645 mutant at BG-11 plate containing 5 mM glucose (Glc). Light intensity was 5 μmol s^{-1} m^{-2}. each sample were adjust at OD_{730} 0.5, 0.1, 0.02 and 10 μl were load at 5mM Glc BG-11 plate. Samples were grown for 7 days.

TABLES

A

	0h	24h
Low light grown cells (OD_{730})		
WT	0.05	0.18± 0.04
ΔctaCI	0.05	0.18± 0.04
Δslr0645	0.05	0.17± 0.03
ΔndhB	0.05	0.11± 0.03
ΔndhF3	0.05	0.23± 0.04

B

	0h	24h
High light grown cells(OD_{730})		
WT	0.05	0.27± 0.07
ΔctaCI	0.05	0.30± 0.06
Δslr0645	0.05	0.34± 0.07
ΔndhB	0.05	0.15± 0.05
ΔndhF3	0.05	0.33± 0.09

Table. 1 . Growth of wild type and mutants in low light (A, B: 20 µmol s^{-1} m^{-2}) and high light condition (C, D: 200 µmol s^{-1} m^{-2}).
Cell densities were measured at OD_{730}. Samples were grown for 24 hours. Start points were began at OD_{730} 0.05. Values in the parenthesis are standard deviation (SD) of three independent experiments.

REFERENCES

Badger MR, Price GD (2003) CO2 concentrating mechanisms in cyanobacteria: molecular components, their diversity and evolution. *Journal of experimental botany* **54:** 609-622

Berry S, Schneider D, Vermaas WF, Rogner M (2002) Electron transport routes in whole cells of Synechocystis sp. strain PCC 6803: the role of the cytochrome bd-type oxidase. *Biochemistry* **41:** 3422-3429

Brown SB, Houghton JD, Vernon DI (1990) Biosynthesis of phycobilins. Formation of the chromophore of phytochrome, phycocyanin and phycoerythrin. *Journal of photochemistry and photobiology* **5:** 3-23

Chew AG, Bryant DA (2007) Chlorophyll biosynthesis in bacteria: the origins of structural and functional diversity. *Annual review of microbiology* **61:** 113-129

Endo T, Ishida S, Ishikawa N, Sato F (2008) Chloroplastic NAD(P)H dehydrogenase complex and cyclic electron transport around photosystem I. *Molecules and cells* **25:** 158-162

Fuhrmann E, Gathmann S, Rupprecht E, Golecki J, Schneider D (2009) Thylakoid membrane reduction affects the photosystem stoichiometry in the cyanobacterium Synechocystis sp. PCC 6803. *Plant physiology* **149:** 735-744

Fujimori T, Hihara Y, Sonoike K (2005) PsaK2 subunit in photosystem I is involved in state transition under high light condition in the cyanobacterium Synechocystis sp. PCC 6803. *The Journal of biological chemistry* **280:** 22191-22197

Gutthann F, Egert M, Marques A, Appel J (2007) Inhibition of respiration and nitrate assimilation enhances photohydrogen evolution under low oxygen concentrations in Synechocystis sp. PCC 6803. *Biochimica et biophysica acta* **1767:** 161-169

Hart SE, Schlarb-Ridley BG, Bendall DS, Howe CJ (2005) Terminal oxidases of cyanobacteria. *Biochemical Society transactions* **33:** 832-835

Howitt CA, Vermaas WF (1998) Quinol and cytochrome oxidases in the cyanobacterium Synechocystis sp. PCC 6803. *Biochemistry* **37:** 17944-17951

Huang C, Yuan X, Zhao J, Bryant DA (2003) Kinetic analyses of state transitions of the cyanobacterium Synechococcus sp. PCC 7002 and its mutant strains impaired in electron transport. *Biochimica et biophysica acta* **1607:** 121-130

Lee S, Ryu JY, Kim SY, Jeon JH, Song JY, Cho HT, Choi SB, Choi D, de Marsac NT, Park YI (2007) Transcriptional regulation of the respiratory genes in the cyanobacterium Synechocystis sp.

PCC 6803 during the early response to glucose feeding. *Plant physiology* **145:** 1018-1030

Ma W, Deng Y, Ogawa T, Mi H (2006) Active NDH-1 complexes from the cyanobacterium Synechocystis sp. strain PCC 6803. *Plant & cell physiology* **47:** 1432-1436

Mao HB, Li GF, Ruan X, Wu QY, Gong YD, Zhang XF, Zhao NM (2002) The redox state of plastoquinone pool regulates state transitions via cytochrome b6f complex in Synechocystis sp. PCC 6803. *FEBS letters* **519:** 82-86

Muro-Pastor MI, Reyes JC, Florencio FJ (2005) Ammonium assimilation in cyanobacteria. *Photosynthesis research* **83:** 135-150

Noguchi K, Yoshida K (2008) Interaction between photosynthesis and respiration in illuminated leaves. *Mitochondrion* **8:** 87-99

Nomura CT, Persson S, Shen G, Inoue-Sakamoto K, Bryant DA (2006) Characterization of two cytochrome oxidase operons in the marine cyanobacterium Synechococcus sp. PCC 7002: inactivation of ctaDI affects the PS I:PS II ratio. *Photosynthesis research* **87:** 215-228

Ogawa T, Mi H (2007) Cyanobacterial NADPH dehydrogenase complexes. *Photosynthesis research* **93:** 69-77

Ohkawa H, Price GD, Badger MR, Ogawa T (2000) Mutation of ndh genes leads to inhibition of CO(2) uptake rather than HCO(3)(-) uptake in Synechocystis sp. strain PCC 6803. *Journal of bacteriology* **182:** 2591-2596

Ozaki H, Sonoike K (2009) Quantitative analysis of the relationship between induction kinetics of chlorophyll fluorescence and function of genes in the cyanobacterium Synechocystis sp. PCC 6803. *Photosynthesis research* **101:** 47-58

Padmasree K, Padmavathi L, Raghavendra AS (2002) Essentiality of mitochondrial oxidative metabolism for photosynthesis: optimization of carbon assimilation and protection against photoinhibition. *Critical reviews in biochemistry and molecular biology* **37:** 71-119

Pils D, Schmetterer G (2001) Characterization of three bioenergetically active respiratory terminal oxidases in the cyanobacterium Synechocystis sp. strain PCC 6803. *FEMS Microbiol Lett* **203:** 217-222

Pils D, Wilken C, Valladares A, Flores E, Schmetterer G (2004) Respiratory terminal oxidases in the facultative chemoheterotrophic and dinitrogen fixing cyanobacterium Anabaena variabilis strain ATCC 29413: characterization of the cox2 locus. *Biochimica et biophysica acta* **1659:** 32-45

Schluchter WM, Zhao J, Bryant DA (1993) Isolation and characterization of the ndhF gene of Synechococcus sp. strain PCC 7002 and initial characterization of an interposon mutant. *Journal of bacteriology* **175:** 3343-3352

Schultze M, Forberich B, Rexroth S, Dyczmons NG, Roegner M, Appel J (2009) Localization of cytochrome b6f complexes implies an incomplete respiratory chain in cytoplasmic membranes of the cyanobacterium Synechocystis sp. PCC 6803. *Biochimica et biophysica acta* **1787:** 1479-1485

Sirpio S, Allahverdiyeva Y, Holmstrom M, Khrouchtchova A, Haldrup A, Battchikova N, Aro EM (2009) Novel nuclear-encoded subunits of the chloroplast NAD(P)H dehydrogenase complex. *The Journal of biological chemistry* **284:** 905-912

Whittaker CA, Hynes RO (2002) Distribution and evolution of von Willebrand/integrin A domains: widely dispersed domains with roles in cell adhesion and elsewhere. *Molecular biology of the cell* **13:** 3369-3387

Zhang P, Battchikova N, Jansen T, Appel J, Ogawa T, Aro EM (2004) Expression and functional roles of the two distinct NDH-1 complexes and the carbon acquisition complex NdhD3/NdhF3/CupA/Sll1735 in Synechocystis sp PCC 6803. *The Plant cell* **16:** 3326-3340

Zhang P, Battchikova N, Paakkarinen V, Katoh H, Iwai M, Ikeuchi M, Pakrasi HB, Ogawa T, Aro

EM (2005) Isolation, subunit composition and interaction of the NDH-1 complexes from Thermosynechococcus elongatus BP-1. *The Biochemical journal* **390:** 513-520