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II. Mustila H, Paananen P, Battchikova N, Santana-Sánchez A, Muth-Pawlak D, Hagemann M, Aro EM and Allahverdiyeva Y (2016) The flavodiiron protein Flv3 functions as a homo-oligomer during stress acclimation and is distinct from the Flv1/Flv3 hetero-oligomer specific to the O_2 photoreduction pathway. Plant & Cell Physiology 57: 1468-83.

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Bersanini L, Allahverdiyeva Y, Battchikova N, Heinz S, Lespinasse M, Ruohisto E, Mustila H, Nickelsen J, Vass I and Aro EM (2017) Dissecting the photoprotective mechanism encoded by the *flv4-2* operon: a distinct contribution of Sll0218 in photosystem II stabilization. Plant, Cell & Environment 40:378-389.

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ABBREVIATIONS AND GENETIC NOMENCLATURE

ATP	adenosine triphosphate
BG-11	growth medium for cyanobacteria
BN-PAGE	blue native polyacrylamide gel electrophoresis
bp	base pairs
C_i	inorganic carbon
CBB	Calvin-Benson-Bassham cycle
CCM	carbon concentrating mechanism
cDNA	complementary DNA
CET	cyclic electron transport
Chl *a*	chlorophyll *a*
cis-asRNA	antisense RNA encoded from the complementary strand
Cm^R	chloramphenicol resistance cassette
Cox	cytochrome c oxidase
Cyd	quinol oxidase
Cyt	cytochrome
DCMU	3-(3,4-dichlorophenyl)-1,1-dimethylurea
DMBQ	2,6-dimethyl-p-benzoquinone
DNA	deoxyribonucleic acid
F_0	the minimal level of fluorescence in the dark
F_m	the maximum fluorescence determined in the presence of DCMU under light
F_m'	the maximum level of fluorescence under the light
F_s	the level of steady-state fluorescence under the light
F_v	variable fluorescence, (F_m - F_0)
FAD	flavin adenine dinucleotide
Fed	ferredoxin
FDP, Flv	flavodiiron protein
Fld	flavodoxin
FL	fluctuating light conditions
FMN	flavin mononucleotide
$FNR_{(S/L)}$	ferredoxin:NADP+ oxidoreductase (short/long form)
FTR	ferredoxin:thioredoxin reductase
HC	high CO_2 conditions (3% CO_2)
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
Hik	histidine kinase

HL	high light conditions
Hlip	high-light-inducible protein
LED	light-emitting diode
LET	linear electron transport
LC	low CO_2 conditions, air level CO_2
MIMS	membrane inlet mass spectrometry
mRNA	messenger ribonucleic acid
MOPS	3-Morpholinopropane-1-sulfonic acid
NADH	nicotinamide adenine dinucleotide (reduced)
NADPH	nicotinamide adenine dinucleotide phosphate (reduced)
$NADP^+$	nicotinamide adenine dinucleotide phosphate (oxidized)
ncRNA	noncoding RNA
NDH-1	NAD(P)H dehydrogenase complex type 1
NPQ	non-photochemical quenching
$^1O_2$	singlet oxygen
OCP	orange carotenoid protein
OD_{750}	optical density at 750 nm
OEC	oxygen-evolving complex
$P680/P680^+$	red/ox primary electron donor of PS II
$P700/P700^+$	red/ox primary electron donor of PS I
P	the level of the P700 signal under the light
P_m	the maximum level of oxidizable P700
P_m'	the maximum level of oxidizable P700 under the light
2-PG	2-phosphoglycolate
3-PGA	3-phosphoglycerate
PBS	phycobilisomes
PC	plastocyanin
PCC	Pasteur Culture Collection
PCR	polymerase chain reaction
pH	negative logarithm of the proton concentration
Prx	peroxidases
PS	photosystem
PQH_2/PQ	plastoquinol/plastoquinon
Q_A	the primary electron-accepting plastoquinone of PSII
Q_B	the secondary electron-accepting plastoquinone of PSII
RNA	ribonucleic acid
ROS	reactive oxygen species

RTO	respiratory terminal oxidase
RT-qPCR	real-time quantitative reverse transcription PCR
Rubisco	ribulose bisphosphate carboxylase/oxygenase
SDH	succinate dehydrogenase
SDS	sodium dodecyl sulfate
SH3	Src (sarcoma) homology 3 domain
SOD	superoxide dismutase
sp.	species
SRM	selected reaction monitoring
TES	2-[[1,3-dihydroxy-2-(hydroxymethyl)propan-2-yl]amino]ethanesulfonic acid
TM	thylakoid membrane
WT	wild type
Y(I)	the yield of PS I
Y(II)	the yield of PS II
Y(NA)	the yield of acceptor side limitation of PS I
YFP	yellow fluorescent protein

In bacteria and chloroplasts:

gene (e.g. *sll1521, flv1*)
mutant (Δ*flv1* deletion mutant, mutant deficient in *flv1*)
Antisense RNA (As1_flv4)
protein (flavodiiron protein, Flv1)

In plants and algae:

GENE (*PGR5*)
mutant (*pgr5*)
PROTEIN (PROTON GRADIENT REGULATION 5, PGR5)

ABSTRACT

Cyanobacteria perform oxygenic photosynthesis to fulfill their energy needs in highly dynamic environmental conditions. This requires tight regulation of photosynthetic light reactions for maintenance and optimization of the photosynthetic performance. In this thesis work, I focus on the auxiliary components assisting in the regulation and protection of photosynthetic electron transport in the cyanobacterium *Synechocystis* sp. PCC 6803. Even though light is essential for photosynthesis, excess excitation energy may lead to the formation of reactive oxygen species (ROS) in reaction centers, electron transfer chain and light harvesting antenna systems. In order to prevent the formation of ROS and subsequent damage to the photosynthetic apparatus, cyanobacteria rely on a number of different photoprotective mechanisms. They control the amount of excitation energy reaching the photosynthetic reaction centers and have a capacity to direct excited electrons to several alternative electron transfer routes, many of which utilize O_2 as the terminal electron acceptor. Especially under conditions where the production of photosynthetic electrons exceeds their metabolic need, cyanobacteria use so-called electron valves to safely dissipate the excess electrons.

The flavodiiron proteins Flv1 and Flv3 have a capacity to function as a major alternative photosynthetic electron sink and perform O_2 photoreduction without accumulating ROS. In this work, I show that Flv1 and Flv3 are indispensable during sudden changes in light intensity, as they prevent the over-reduction of the linear electron transport. The Flv1 and Flv3 proteins safeguard photosystem I, and are thus crucial for the survival of cyanobacteria under fluctuating light, a typical light condition in aquatic environments. Both the Flv1 and Flv3 proteins are essential for O_2 photoreduction, and they are probably organized as a hetero-oligomer in order to function. Additionally, homo-oligomers of Flv3 are involved in the acclimation of cells to fluctuating light, and mediate a yet unidentified electron transfer pathway.

The two other flavodiiron proteins in *Synechocystis*, which form a Flv2/Flv4 heterodimer, have been shown to play a role as an electron sink on the acceptor side of photosystem II. These proteins are encoded by the *flv4-2* operon, together with Sll0218, a small membrane protein. I demonstrate that the *flv4-2* operon is transcriptionally controlled by the transcriptionl factor NdhR, and post-transcriptionally by several antisense RNAs. The accumulation of the *flv4-2* operon mRNA and one of the antisense RNAs, As1_flv4, was found to be inversely correlated. The As1_flv4 prevents the premature expression of the *flv4-2* operon-encoded proteins, and the Flv2/4 electron valve is synthetized only if inorganic carbon limitation continues.

Cyanobacteria have several ferredoxins that play a role in photosynthetic electron transfer and environmental stress tolerance. In this thesis work, I show that a bacterial-type ferredoxin 7 (Fed7) is important for the redox regulation of *Synechocystis*. Cells lacking Fed7 cannot properly respond to combined inorganic carbon limitation and high light stress. It is possible that Fed7 mediates the redox signals from the photosynthetic linear electron transport chain and functions indirectly as a redox-dependent regulator.

TIIVISTELMÄ

Syanobakteerit tuottavat kaiken tarvitsemansa energian vettä hajottavan fotosynteesin avulla. Fotosynteesin valoreaktioissa muodostuvat voimakkaat hapettimet ja pelkistimet myös altistavat fotosynteesikoneiston vaurioitumiselle, varsinkin nopeasti vaihtuvissa valo-olosuhteissa. Tästä syystä fotosynteesin valoreaktiot ovat tiukasti säädeltyjä. Väitöskirjatyössäni olen tutkinut *Synechocystis* sp. PCC 6803 -syanobakteerin vaihtoehtoisia elektroninsiirtoreittejä, jotka suojaavat ja säätelevät fotosynteesikoneistoa. Valo on välttämätöntä fotosynteesille, mutta ylimääräinen viritysenergia voi johtaa reaktiivisten happiyhdisteiden muodostumiseen, ja siten vahingoittaa fotosynteesikoneistoa. Vaurioiden välttämiseksi syanobakteereille on kehittynyt useita eri suojamekanismeja liiallisen valoenergian torjumiseksi. Syanobakteerit voivat säädellä reaktiokeskuksiin saapuvan viritysenergian määrää. Fotosynteettisiä elektroneja voidaan lisäksi ohjata useaan vaihtoehtoiseen elektroninsiirtoreittiin, joista monet käyttävät happea viimeisenä elektronin vastaanottajana. Varsinkin olosuhteissa, joissa fotosynteettisten elektronien tuotto ylittää aineenvaihdunnan tarpeet, syanobakteerit käyttävät nk. elektroniventtiilejä elektronien turvalliseen poistamiseen.

Syanobakteereiden spesifiset, rautaa sisältävät flavoproteiinit Flv1 ja Flv3 pystyvät fotosynteettisten valoreaktioiden välityksellä pelkistämään happea vedeksi ilman, että soluun muodostuu reaktiivisia happiyhdisteitä, ja toimivat siten fotosynteesin elektroniventtiileinä. Tässä työssä osoitan, että Flv1 ja Flv3 toimivat korvaamattomana suojamekanismina valon kirkkaudessa tapahtuvien nopeiden vaihteluiden aikana ja estävät lineaarisen elektroninsiirtoketjun ylipelkistymistä. Flv1 ja Flv3 -proteiinit suojaavat valoreaktio I:tä ja ovat siksi ehdottoman tärkeitä syanobakteerien selviytymiselle vaihtelevassa valossa. Nopeat valon vaihtelut ovat tavallisia syanobakteerien elinympäristössä eli pintavedessä. Flv1 ja Flv3 -proteiinit ovat kumpikin välttämättömiä hapen pelkistämisessä, ja toimiakseen ne todennäköisesti muodostavat hetero-oligomeerin. Tämän lisäksi Flv3 voi muodostaa homo-oligomeerin, joka myös auttaa soluja sopeutumaan nopeasti vaihteleviin valo-oloihin, mutta tämän reitin elektroninvastaanottajia ei ole vielä kyetty tunnistamaan.

Synechocystis-synaobakteerilla on lisäksi spesifiset Flv2 ja Flv4 flavoproteiinit, jotka muodostavat heterodimeerin. Flv2/Flv4-heterodimeeri toimii valoreaktio II:n elektroninvastaanottajana. Flv2 ja Flv4 -proteiinit koodautuvat *flv4-2*-operonista yhdessä pienen Sll0218-tylakoidiproteiinin kanssa. Työssäni osoitan, että transkriptiotekijä NdhR säätelee transkriptionaalisesti *flv4-2*-operonin ilmenemistä. Lisäksi operonin säätelyyn osallistuu useampi antisense-RNA-molekyyli, joista As1_flv4-transkriptia kertyy soluun ennen *flv4-2*-operonin lähetti-RNA:ta. As1_flv4 estää solua ilmentämästä *flv4-2* -operonin koodaamia proteiineja ennenaikaisesti, jolloin Flv2/4:n muodostamaa elektroniventtiiliä tuottuu soluun vasta, kun elektroninsiirtoketjun ylipelkistyminen on pysyvää.

Syanobakteereilla on useita erilaisia ferredoksiineja (Fed), jotka välittävät fotosynteesin elektroneja tai osallistuvat stressitekijöiden sietokyvyn kasvattamiseen. Osoitan, että bakteeri-tyypin Fed7 on tärkeä hapetus-pelkistystilojen säätelylle. Solut, joilta puuttuu Fed7, eivät reagoi asianmukaisesti samanaikaiseen epäorgaanisen hiilen vähyyteen ja kirkkaan valon aiheuttamaan stressiin. Fed7 saattaa välittää hapetus-pelkistyssignaaleja fotosynteesin elektroninsiirtoketjulta ja toimia välillisesti säätelymolekyylinä.

1 INTRODUCTION

1.1 Cyanobacteria

Cyanobacteria, also known as blue-green algae, are prokaryotic microorganisms able to perform oxygenic photosynthesis. This variable group of photosynthetic bacteria is found in almost every habitat on Earth, such as oceans, fresh water, soil, deserts and even polar regions (Whitton and Potts 2000). Among cyanobacteria, there are unicellular, colonial and filamentous species and some diazotrophic strains capable of fixing atmospheric nitrogen. In addition, some cyanobacteria form symbiotic relationships with a wide range of eukaryotic hosts, mostly plants and fungi, providing fixed nitrogen or carbon to the host.

The photosynthetic microbial mats and colonies in fossil findings are dated to be 3.5 billion years old (Drews 2011). Around 2.4 billion years ago, O_2 levels in Earth atmosphere started to rise, and the credit of the Great Oxygenation Event has been assigned to ancestors of cyanobacteria (Drews 2011). Predecessors of cyanobacteria, anoxygenic phototrophic microorganisms, also used photosynthesis to obtain energy from sunlight and converted it into chemical energy. However, early cyanobacteria evolved a novel metabolism: light-driven oxidation of water (Cardona et al. 2015). The invention of oxygenic photosynthesis enabled the use of two very abundant raw materials for growth, water and carbon dioxide, and cyanobacteria began to thrive. As a byproduct of water oxidation, cyanobacteria released substantial amounts of O_2, first into the oceans and slowly also to the atmosphere, creating the foundation for more diverse and complex life on Earth. Eventually there was enough O_2 to allow the formation of the ozone layer that shields the Earth from biologically lethal UV radiation. Primitive cyanobacteria are considered to be the ancestors of chloroplasts in modern plants and algae. The primary endosymbiosis event has been estimated to have occurred about 1.5 billion years ago, when an early eukaryotic cell engulfed an ancient cyanobacterium and retained it as a cell organelle (Yoon et al. 2004).

Even today, cyanobacteria are responsible for 30% of the yearly O_2 production on Earth (DeRuyter and Fromme 2008) and half of the production of biologically available nitrogen in the oceans (Stal 2009). Therefore, cyanobacteria are crucial for the net primary production of organic matter and for the global food chains. Cyanobacteria are especially important for the marine ecosystems, as they form a major component of the phytoplankton that thrive in the surface layer of the oceans. Most abundant in the marine environments ranging from 40°N to 40°S latitude are a multitude of unicellular non-diazotrophic *Synechococcus* and *Prochlorococcus* strains

(Partensky et al. 1999). *Prochlorococcus* strains may live up to 200 m depth, where natural light is less than 1 µmol photons m^{-2} s^{-1}, compared to the surface layer where light intensity may reach 1500 µmol photons m^{-2} s^{-1} on a bright day (Partensky et al. 1999). In the Baltic Sea, the filamentous, heterocystous cyanobacteria capable of N_2-fixation are the major components of the phytoplankton (Stal 2009). When conditions are particularly favorable, the population rapidly accumulates and cyanobacteria colonies become visible. This phenomenon is misleadingly referred to as "blooms of blue-green algae", even though prokaryotic cyanobacteria, not eukaryotic algae, are responsible for the green mats on water surface. Vigorous cyanobacterial growth can be observed every summer also in the Baltic Sea, in which eutrophication due to heavy nutrient flow from fields, forestry and waste waters has created conditions that favor cyanobacteria. From a human perspective, the most problematic issue with the cyanobacterial blooms worldwide is that some strains can produce toxins, causing health risks to humans and livestock (Merel et al. 2013).

In recent years, there has been an increasing interest in cyanobacteria due to their great potential for harvesting light energy to produce organic and non-organic biofuels such as hydrogen, ethanol and biodiesel (recently reviewed by Ooms et al. 2016; Nagarajan et al. 2016). Cultivation of the fast growing microalgae (cyanobacteria and single celled eukaryotic algae) in ponds or bioreactors makes it possible to reserve arable land for food production. Furthermore, it is easier to recycle the phosphorus and other nutrients in aquatic culture, or alternatively to cultivate microalgae in the waste water (Whitton et al. 2016). Microalgae could, in addition, be utilized in the production of other high-value chemicals as part of the blue bioeconomy (recently reviewed by Oliver and Atsumi 2014; Cuellar-Bermudez et al. 2015; Chew et al. 2017).

Even though cyanobacteria have great potential as a platform for biorefineries, it is not yet cost effective due to limitations posed by metabolic pathways. Therefore, making use of synthetic biology tools and designing novel metabolic pathways is crucial for creating more efficient cell factories. These modified routes could channel the photosynthetic electrons directly to the pathway(s) leading to a desired high-value end-product. For this purpose, we need to overcome metabolic restrictions and gain more comprehensive understanding of the photosynthetic electron transport routes in cyanobacteria.

1.1.1 Ecological significance and basic physiology

The cyanobacterial cell envelope contains two membranes: an outer membrane and a selectively permeable plasma membrane, giving them a

conventional classification status as gram-negative bacteria (Figure 1A). The peptidoglycan layer connecting the plasma membrane and outer membrane is referred to as the periplasm. The third membrane system of cyanobacteria, the thylakoid membrane, is located in the outer regions of the cytoplasm and consists of several layers. In cyanobacteria, the photosynthetic and respiratory electron transport chains share several components in the thylakoid membrane (reviewed by Mullineaux 2014). Carbon fixation occurs in specialized polyhedral microcompartments, carboxysomes (Shively et al. 1973; Kerfeld and Melnicki 2016). Two evolutionarily distinct forms of Rubisco, the most abundant enzyme for carbon fixation, define *α*-type and *β*-type carboxysomes (Badger et al. 2006). Thus cyanobacteria can be classified into two separate phylogenetic groups: *α*-cyanobacteria, found mostly in marine environments, and *β*-cyanobacteria, found in fresh-water and coastal habitats (Badger and Price 2003).

In addition to carboxysomes, cyanobacteria have other basic cell organelles such as ribosomes, gas vesicles, lipid bodies and storage granules for cyanophycin (a nitrogen-storage polymer), glycogen (polysaccharide) or polyphosphate (a phosphorus and energy storage polymer) (Gonzalez-Esquer et al. 2016). Some strains are able to actively move towards a light (Schuergers et al. 2016) or nutrient source by using pili, which are thin appendages on the cell surface (Bhaya et al. 2000). Cyanobacterial cells may also be able to differentiate from vegetative cells (also known as photosynthetic cells) to specialized cell types. Some of the cells in certain filamentous strains can differentiate into heterocysts in which the fixation of atmospheric N_2 into ammonium occurs (Flores and Herrero 2010). Survival under unfavorable conditions may promote formation of akinetes, dormant spore-like cells, or hormogonia, motile short cell filaments (Singh and Montgomery 2011).

In terms of structure, cyanobacteria have been classified into five morphological subsections (Rippka et al. 1979). Subsections I and II comprise of single-celled cyanobacteria and subsections III–V include filamentous cyanobacteria. These subsections do not, however, follow evolutionary distances: many characteristics of cyanobacteria, such as the ability to form filaments, have evolved multiple times within the Cyanobacteria phylum. Shih et al. (2013) suggested dividing the Cyanobacteria phylum into seven subclades (A-G) based on phylogenetic analysis. Within the cyanobacterium phylum, only heterocystous strains (subsection IV) and ramified strains (subsection V) seem to form a monophyletic group (within subgroup B1). Recently, surveys of genomic datasets from aphotic i.e. dark environments, such as human gut microbiomes and groundwater microbes, revealed the existence of a closely

related sister group to oxygenic cyanobacteria, termed melainabacteria (Di Rienzi et al. 2013). Melainabacteria are non-photosynthetic and produce energy through fermentation in anoxic environments. Interestingly, melainabacteria are more closely related to cyanobacteria than the bacterial phyla capable of anoxygenic phototrophy (including Green and Purple bacteria) (Di Rienzi et al. 2013). As a new approach based on phylogenetic studies, it has been proposed that the Cyanobacteria phylum should actually include three classes: Oxyphotobacteria (oxygenic cyanobacteria), Melainabacteria and an additional, distantly related cyanobacterial lineage ML635J-21 (aphotic and anaerobic) (Soo et al. 2014; Shih et al. 2017).

The Pasteur Culture Collection (PCC) of cyanobacteria at the Institut Pasteur in Paris contains 470 cyanobacterial isolates deposited in their collection that are important for researchers all over the world. A locally important collection is the University of Helsinki Cyanobacteria Collection (UHCC) containing approximately 1200 strains isolated from the Baltic Sea and Finnish lakes. Furthermore, genomic data of cyanobacteria is accumulating, and the cyanobacterial genomic encyclopedia of bacteria and archaea (CyanoGEBA) dataset comprises of 126 sequenced cyanobacterial genomes, including representatives from all five subsections (Shih et al. 2013). This represents only a fraction of all cyanobacterial strains – strains that are new for science are constantly isolated and their genomes sequenced. As information concerning cyanobacterial features and genomes builds up, the classifications can be refined. Whatever the evolutionary relationships are among the strains, the enormous variety among cyanobacteria and their ecological importance are evident.

1.1.2 A model cyanobacterium *Synechocystis* sp. PCC 6803

The unicellular cyanobacterium *Synechocystis* sp. PCC 6803 (hereafter referred to as *Synechocystis*) is widely used as a model organism for cyanobacterial and photosynthesis research. *Synechocystis* was originally isolated from a Californian lake in 1968 (Stanier et al. 1971) and then placed in the PCC (Rippka et al. 1979). It is a unicellular, non-toxic and non-diazotrophic freshwater *β*-cyanobacterium easy to cultivate in laboratory conditions. The genome of *Synechocystis* was the first one to be sequenced among cyanobacteria, and the first draft of the resolved genome was published in 1996 by Kaneko and co-workers. The approximately 3.6 billion base pairs of chromosomal DNA are organized into more than 3000 open reading frames (ORF) (Mitschke et al. 2011), and the circular chromosome exists as multiple copies in the cell. Besides chromosomal DNA, *Synechocystis* cells contain seven plasmids consisting of approximately 400 ORFs. *Synechocystis* is naturally competent and can be readily transformed

by exogenous DNA (Williams 1988). An active homologous recombination mechanism enables the integration of heterologous DNA to the genome of *Synechocystis,* thus allowing targeted mutagenesis (Kufryk et al. 2002). Many laboratories use a glucose-tolerant variant of *Synechocystis* because it can be grown heterotrophically on glucose, which makes it possible to isolate and study viable mutants defective in photosynthesis (Williams 1988; Anderson and McIntosh 1991).

1.2 The photosynthetic machinery of cyanobacteria

Photosynthesis sustains life on Earth, providing both organic matter and the oxygen we breathe. Photosynthesis forms the basis of global food chains, since virtually all life forms use the complex organic compounds derived from photosynthesis as an energy source. In addition, the majority of modern civilization's energy resources originate from fossilized ancient photosynthesizing plants in the form of fossil fuels. Photoautotrophs including cyanobacteria, green algae and plants convert sunlight into chemical energy in the process of oxygenic photosynthesis. Photosynthesis occurs in two stages, 'light' and 'light-independent' reactions, each with multiple steps. In the first stage, light reactions capture the energy of light and use it to generate the energy-storage molecules ATP and NADPH. The light reactions of photoautotrophs occur in the thylakoid membrane. In plants and algae, thylakoid membranes are situated inside specialized organelles called chloroplast. In cyanobacteria, thylakoid membranes are typically arranged in several circular layers within the cell interior (Figure 1A).

The second stage of photosynthesis, light-independent reactions, is fueled by the ATP and NADPH molecules produced in the light reactions. In this stage chemical energy is consumed to capture atmospheric carbon dioxide (CO_2), which is reduced to carbohydrates in the Calvin-Benson-Bassham (CBB) cycle. In plants, CBB cycle reactions take place in the chloroplast stroma, whereas in cyanobacteria they take place in the cell cytoplasm and inside carboxysomes. The glycogen stored during photosynthesis provides raw material for cellular respiration and anabolic pathways that synthetize proteins, lipids and other products.

1.2.1 Photosynthetic linear electron transport (LET)

The thylakoid membrane contains four major types of integral membrane protein complexes that catalyze the light reactions and drive a linear electron transport (LET): photosystem I (PSI), photosystem II (PSII), cytochrome b_6f complex (Cyt b_6f) and ATP synthase (ATPase) (Figure 1B; Hill and Bendall 1960). The photons absorbed by antennae excite the reaction center chlorophylls of the two photosystems, triggering the electron transfer.

Photosystems are multisubunit protein complexes, which bind pigment molecules, chlorophyll *a* (Chl *a*) and carotenoids, capable of absorbing light energy. PSII is organized as a dimer, both units composed of core transmembrane subunits D1 (PsbA) and D2 (PsbD) and intrinsic antenna proteins CP43 (PsbB) and CP47 (PsbC) binding the Chl *a* molecules (See review by Shen 2015). The primary donor of PSII is a specialized Chl *a* pair, P680, bound by the D1 protein. Excitation of P680 leads to primary charge separation, and the electron is transferred to PSII bound pheophytin and subsequently to plastoquinones, Q_A and Q_B. The lost electron of $P680^+$ is replaced by an electron derived from H_2O. The water oxidation of PSII is catalyzed by an oxygen-evolving complex (OEC) with Mn_4O_5Ca cluster in active site. OEC splits two water molecules into four electrons, four protons and an oxygen molecule. The cyanobacterial OEC comprises PsbO protein, like in plant OEC, and additionally the, PsbV, PsbU, CyanoP and CyanoQ proteins, which collectively shield the Mn_4O_5Ca cluster from reductants in the thylakoid lumen (Bricker et al. 2012). In addition, 13 small (<10 kDa) subunits are associated with PSII, although the functions of many of them remain elusive (Heinz et al. 2016). Additionally, a large number of PSII assembly factors are required in PSII formation. The D1 protein of PSII is continuously damaged in the light, and impaired D1 is rapidly degraded and replaced with newly synthetized D1 in a PSII repair cycle (Aro et al. 1993). If the rate of the photodamage exceeds the rate of PSII repair, PSII is said to be photoinhibited.

PSI catalyzes the oxidation of a mobile electron carrier, plastocyanin (PC) or cytochrome c_6 (C_6), on the lumenal side and the reduction of ferredoxin (Fed) on the cytoplasmic side of the thylakoid membrane by absorbed light energy. The PSI complex of cyanobacteria contains 12 subunits and binds 96 Chl *a* molecules and 22 *β*-carotenes, 2 phylloquinones and 3 iron-sulfur clusters (Grotjohann and Fromme 2005). The reaction center proteins of PSI, PsaA and PsaB, harbor the primary electron donor P700 (a dimer of Chl *a* molecules) and a [4Fe-4S] cluster (F_x). Two other [4Fe-4S] clusters (F_A and F_B) are bound by the peripheral PsaC subunit. As in PSII, the P700 becomes oxidized after being excited by a photon and transferring the excited electron to the primary electron acceptor of PSI. On the cytoplasmic side, reduced Feds in turn reduce $NADP^+$ to NADPH via ferredoxin $NADPH^+$-reductase (FNR). In *Synechocystis*, a long isoform of the FNR (FNR_L) is associated with phycobilisome antennae and is responsible for the electron transfer from reduced Fed (van Thor et al. 1999). In cyanobacteria, PSI is predominantly organized as a trimer (Jordan et al. 2001), whereas in plants it is a monomer surrounded by a light-harvesting complex (LHC) I (Jensen et al. 2007). In

some cyanobacterial species, there is also evidence of dimeric and tetrameric forms of PSI (Li et al. 2014).

The catalytic reactions in PSII and in Cyt b_6f generate a proton motive force (*pmf*) across the thylakoid membrane by pumping the protons (H^+) to the lumenal side of the membrane. Consequently, at neutral external pH the cyanobacterial thylakoid lumenal pH is approximately 5.5, and cytosolic pH is 7.2 in the dark, but under light the lumen acidifies and the cytosol becomes more alkaline by 0.5-1 pH units (Belkin et al. 1987; Mangan et al. 2016). Protons flow to the cytoplasmic side of the membrane through ATP synthase (ATPase), which phosphorylates ADP to ATP in the cytoplasm.

Photosystems are surrounded by light-harvesting antennae, which in cyanobacteria are soluble and mostly composed of phycobiliproteins. These antennae, called phycobilisomes (PBS), are attached to the cytoplasmic surface of the thylakoid membrane (Wildman and Bowen 1974). The photons absorbed by the phycobiliproteins are transferred into the antenna core, then into the photosynthetic reaction centers. PBS enable cells to harvest light over a larger surface and a wider region of the light spectrum than a photosystem alone (DeRuyter and Fromme 2008). Phycobiliproteins absorb sunlight mostly from wavelengths within the 470-650 nm range, whereas the absorption maxima for Chl *a* pigment are at the wavelengths of 430–440 and 670 nm (Glazer 1984). The core of a PBS consists of allophycocyanin, which is surrounded by a fan-like array of six cylindrical rods consisting of phycocyanin, phycoerythrin and/or phycoerythrocyanin depending on the organism and environmental condition (Glazer 1984; DeRuyter and Fromme 2008). Phycobiliproteins are assembled with the help of colorless linker polypeptides (Yu and Glazer 1982). Fascinatingly, phycobiliproteins give the characteristic blue-green color of cyanobacteria, which explains the common name ‘blue-green algae’ for these micro-organisms.

Recently, a functional megacomplex composed of a PBS antenna and both photosystems was isolated from *Synechocystis* (Liu et al. 2013). This finding supports a model where PSII is positioned directly under the core of the PBS, while PSI is attached to the PBS core components only on side. Thus, it is postulated that PBS supplies excitation energy to both photosystems, making energy balance regulation between the two photosystems possible. There is also evidence for a PSI specific PBS consisting only of phycocyanin and CpcG3 linker polypeptide (Watanabe et al. 2014).

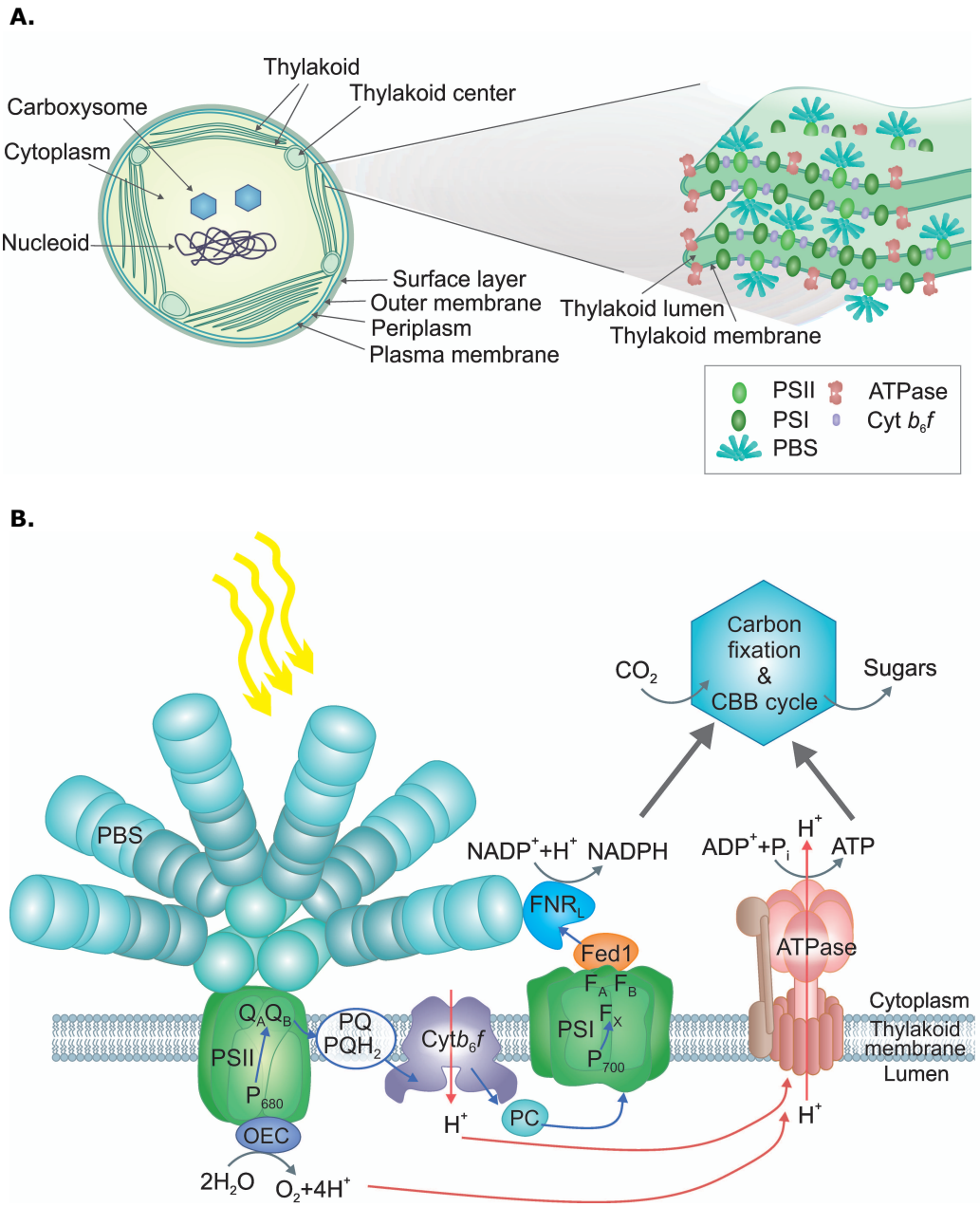

Figure 1. The structure of a typical unicellular cyanobacterium and the organization of the thylakoid membrane (not in scale). **A.** Cyanobacteria are enclosed by an outer membrane and inner plasma membrane. Thylakoid membranes are connected to plasma membranes via thylakoid biogenesis centers, where the biogenesis of thylakoid membranes occurs. **B.** A simplified scheme of the photosynthetic linear electron transport chain in the thylakoid membrane of *Synechocystis*. Blue arrows indicate electron transfer, and red arrows designate proton translocation across the membrane. See the abbreviations from the text.

1.2.2 Ferredoxins (Feds) as important electron distributors

Feds are small and soluble electron carrier proteins containing iron-sulfur clusters. They are important photosynthetic electron distributors to various processes depending on reducing power. In plants, algae and cyanobacteria the primary photosynthetic Fed is a plant-type Fed harboring a [2Fe-2S] cluster, and in cyanobacteria it is designated as Fed1 (Cassier-Chauvat and Chauvat 2014). Fed1 is the primary soluble electron acceptor from PSI, and the photoreduction of ferredoxin occurs upon binding to the cytoplasmic side of PSI. Reduced Fed1 transfers electrons to the FNR, and after a second electron transfer by another Fed1, the FNR catalyzes the reduction of $NADP^+$ to NADPH (Blankenship 2001). The electron transfer between Fed1 and FNR occurs after the redox centers in the complex (the 2Fe–2S cluster of Fed1 and FAD of FNR) are brought into close proximity (Kurisu et al. 2001).

Furthermore, Fed1 is able to transfer electrons to a variety of other soluble partners that are involved in nitrogen and sulfur assimilation, biosynthesis of glutamate and chl, fatty acid metabolism and the thioredoxin system as well as to bidirectional hydrogenase (Poncelet et al. 1998; Gutekunst et al. 2014). The activity of various enzymes is modulated via the thioredoxin system by controlling their redox state. Fed1 donates electrons to thioredoxins via ferredoxin:thioredoxin reductase (FTR), which participates in the regulation of several enzymes involved in carbon assimilation and other metabolic pathways. Although these Fed-dependent enzymes vary in structure, size and prosthetic group composition, they all have the ability to form a functional electron transfer complex with Fed1 (Kurisu et al. 2001).

Synechocystis has nine ferredoxin-like proteins, Fed1–9, which are highly conserved in cyanobacteria (Marteyn et al. 2009). A plant-type ferredoxin, Fed1 (PetF, Ssl0020), is the most abundant in *Synechocystis* (Poncelet et al. 1998). Fed1 is essential for the viability of cyanobacteria in photoautotrophic growth (Van Der Plas et al. 1988). There are three additional plant type Feds with a [2Fe-2S] center in *Synechocystis*, Fed2, Fed3 and Fed4, the first two of which are essential (Cassier-Chauvat and Chauvat 2014). Furthermore, there are two additional [2Fe-2S] center proteins, Fed5 with adrenodoxin-like and Fed6 with bacterial type sequences. Fed4 and Fed5 are encoded by the same operon, along with seven other proteins, and they might function in the assembly of PSII (Wegener et al. 2008). The three remaining Feds contain variable iron sulfur centers: Fed7 [4Fe-4S] centers, Fed8 [3Fe-4S] [4Fe-4S] centers and Fed9 two [4Fe-4S] centers.

The transcription of the *Synechocystis fed1* gene is rapidly induced in light, requiring active photosynthesis and *de novo* transcription (Mazouni et al. 2003). When the cell becomes depleted in iron, Fed1 is replaced by a

flavodoxin (Fld), a non-iron containing electron-transfer protein (Bottin and Lagoutte 1992). Moreover, expression of the *fed1* gene could be turned down by treatment with hydrogen peroxide (H_2O_2) and heavy metals such as cadmium and selenate, which all are toxic and may lead to oxidative stress (Mazouni et al. 2003).

The eight low-abundant Feds in *Synechocystis* play a role in photosynthesis or in tolerance to environmental stresses (Cassier-Chauvat et al. 2017). Several of these Feds respond to metals. Cadmium treatment triggered upregulation of *fed2*, but down-regulation of *fed3*, *fed4*, *fed5* and *fed7* (Mazouni et al. 2003; Houot et al. 2007). Furthermore, selenate (Na_2SeO_4) induced *fed2* expression, and H_2O_2 triggered expression of *fed7* and *fed8* (Mazouni et al. 2003). In accordance with these results, Marteyn et al. (2009) demonstrated that a mutant deficient in *fed7* is more sensitive to both H_2O_2 and selenate than WT *Synechocystis*. According to these authors, Fed7 operates in protection against selenate through a crosstalk pathway where electrons from PSI are transferred via Fed1 to the FTR catalytic chain, which passes the electrons to Fed7 and finally to glutaredoxin (Grx) 2, which then reduces selenate to a non-harmful form. Moreover, the *fed7* transcript has been found to be upregulated during a short term (3-48 h) iron deficiency (Singh and Sherman 2000; Houot et al. 2007), and in response to low inorganic carbon (C_i) availability (Eisenhut et al. 2007). In contrast, a long term iron deficiency (12 days) in a growth environment enriched with 1% CO_2 resulted in significantly reduced Fed7 protein levels (Vuorijoki et al. 2016).

1.2.3 CO_2 fixation and carbon concentrating mechanisms

The majority of the reducing power and ATP produced by light reactions are consumed by carbon fixation. In order to ensure an adequate amount of CO_2 for the carbon fixation, cyanobacteria have developed a carbon concentrating mechanism (CCM) (recently reviewed by Montgomery et al. 2016). The CCM consists of multiple active transport systems for CO_2 and bicarbonate (HCO_3^-), resulting in up to thousand-fold accumulation of CO_2 inside the carboxysome as compared to the extracellular environment (Badger and Price 2003). In cyanobacteria and some proteobacteria, the primary CO_2-fixing enzyme (ribulose-1,5-bisphosphate carboxylase/oxygenase, Rubisco) is encapsulated in carboxysomes, which have an icosahedral, selectively permeable protein shell (Kerfeld et al. 2005; 2016). The importance of Rubisco to all photosynthetic organisms is evident, since it is the most abundant enzyme in the world. Rubisco has a conserved structure and it consists of large and small subunits, RbcL and RbcS, forming a L_8S_8 complex in plants and cyanobacteria (Whitney et al. 2011). The cytoplasmic HCO_3^-

passes through the carboxysome shell and is converted to CO_2 by the carboxysomal carbonic anhydrase enzyme in the vicinity of Rubisco (Kaplan and Reinhold 1999). The first reaction of the CBB cycle catalyzed by Rubisco incorporates CO_2 into ribulose-1,5-bisphosphate (RuBP), forming 3-phosphoglycerate (3-PGA). The three-carbon sugar 3-PGA is exported from the carboxysome and rest of the CBB cycle steps are completed in the cytoplasm.

1.3 Auxiliary electron transport pathways and regulatory proteins

Cyanobacteria are found in various habitats and are exposed to extremely variable environmental conditions. The acclimation of cyanobacteria to rapidly changing quantities of light and varying availability of nutrients is possible due to the remarkable flexibility of the photosynthetic apparatus. In fact, balancing the absorption and conversion of light energy by the photosystems and the energy needs of the metabolic reactions is essential for the optimal functioning of photosynthesis. Auxiliary electron transport pathways that operate alongside the LET route facilitate the regulation of photosynthesis. These alternative routes include cyclic electron flow around PSI and O_2 photoreduction pathways.

1.3.1 Cyclic electron transport (CET) around PSI

Particularly important for the regulation of photosynthesis is the ratio between phosphorylating (ATP) and reducing power (NADPH) supplies. The LET produces ATP at a ratio of 1.33-1.5 per 1 NADPH, depending on the organism and pH (Bendall and Manasse 1995). The ratio of 1.5 ATP/NADPH is close to the requirements of their major consumer, CO_2 fixation. However, ATP is also required for the synthesis of proteins and storage compounds such as glycogen and cyanophycin (Quintana et al. 2011; Saha et al. 2016). Moreover, in cyanobacteria the BCT1 part of the C_i import is ATP-driven. The requirement for extra ATP is predominantly met with the CET around PSI, which generates ATP without net accumulation of reducing equivalents (NADPH). In CET, electrons are recycled from the PSI via PQ to the Cyt b_6f complex, which generates a proton gradient.

Two main pathways for CET have been suggested for plants: the NDH and PGR5 dependent routes (Munekage et al. 2002; 2004).

1.3.2 Cyanobacterial NDH-1 complexes

Most of the photosynthetic organisms possess type I NAD(P)H:Quinone oxidoreductases that resemble mitochondrial Complex I of the respiratory

chain (Berger et al. 1991). The NDH-1 complex transfers electrons from an electron donor to a quinone, generating a *pmf* across the membrane used for ATP synthesis (Battchikova et al. 2011a). In plants, the function of chloroplast NDH is important under different environmental stresses (Wang et al. 2006; Yamori et al. 2011; 2015; Wang et al. 2015). NDH-1 complexes in cyanobacteria are involved in CET around PSI, in CCM and in respiration.

In cyanobacteria, four different types of NDH-1 complexes have been characterized: NDH-1_1- NDH-1_4 (recently reviewed by Peltier et al. 2016). All of these complexes have a common NDH-1M core, consisting of 14 subunits: 8 transmembrane domains residing in the thylakoid membrane (NdhA–C, NdhE, NdhG, NdhL-N) and 6 hydrophilic domains protruding to the cytoplasmic side (NdhH–K, NdhO, NdhS) (Ogawa 1991; Zhang et al. 2005; Birungi et al. 2010; Battchikova et al. 2011b; Zhao et al. 2014). The NdhS subunit, homologous to the SH3 domain in CRR31 of *Arabidopsis*, is postulated to bind reduced Fed (Yamamoto et al. 2011; Battchikova et al. 2011a). Accordingly, recent studies suggest that cyanobacterial NDH-1_1 and NDH-1_2, similar to chloroplast NDH, use reduced Fed1 as an electron donor instead of NADPH (see Peltier et al. 2016). However, taking into account a mild phenotype of *ndhS* mutant cells, the involvement of both electron carriers, NADPH and Fed, in electron donation cannot be completely excluded.

The cyanobacterial NDH-1M core is not functional by itself, but binds a set of hydrophobic NdhD and NdhF subunits, which are encoded by multiple copies of these genes (Ogawa and Mi 2007). In *Synechocystis*, the genome harbors six different ndhD genes (ndhD1–6) and three different ndhF genes (ndhF1, ndhF3-4). The diverse roles of cyanobacterial NDH-1 complexes – in respiration, CET and CO_2 acquisition – are made possible by multiplicity of different subunit compositions. The NDH-1_1 (NDH-1L) complex contains NdhD1 and NdhF1 subunits, and it is important in dark respiration and CET. The NDH-1_2 variant (NDH-1L’) harbors the NdhD2 subunit instead of NdhD1 and it is postulated to be expressed under particular stress conditions (Battchikova et al. 2011a). NDH-1_1 and NDH-1_2 also require NdhP and NdhQ subunits to stabilize the structure (Schwarz et al. 2013; Zhao et al. 2014).

The NDH-1_3 (NDH-1MS) is shown to be involved in low C_i inducible and NDH-1_4 (NDH-1MS') in constitutive CO_2 uptake as part of the CCM (Battchikova et al. 2011a). The NDH-1_3 and NDH-1_4 complexes are also located in the thylakoid membrane (Battchikova et al. 2011a). The NDH-1_3 contains NdhD3 and NdhF3 subunits, and it binds CupA and CupS proteins (Herranen et al. 2004). The fourth complex, NDH-1_4 (NDH-1MS'), contains NdhD4 and NdhF4 subunits, which bind CupB protein in the cytosolic side

(Zhang et al. 2005). The CupA and CupB subunits perform the light dependent hydration of CO_2 to bicarbonate (HCO_3^-) (Shibata et al. 2001; Maeda et al. 2002) while the rest of the complex enables the CO_2-pumping mechanism energized by PSI and performing of CET (Bernát et al. 2011). It has also been proposed that the small soluble FNR isoform, FNR_S, is involved in CET by donating electrons to the NDH-1 complex (Korn 2010). In contrast, the long isoform, FNR_L, has been suggested to function independently from NDH-1 in salt stress-inducible CET (van Thor et al. 2000).

1.3.3 Pgr5-mediated regulation of electron transfer

In plants, PROTON GRADIENT REGULATION 5 (PGR5) is essential for increased acidification of the thylakoid lumen, which is a vital component in induction of thermal dissipation (NPQ) of absorbed energy from PSII antennae (Munekage et al. 2004). First it was suggested that PGR5 functions as a component for an antimycin A-sensitive reaction for a ferredoxin-dependent CET route in plants (Munekage et al. 2002; Nandha et al. 2007) and in cyanobacteria (Yeremenko et al. 2005). However, the mode of action of PRG5 is unknown, since the small protein lacks electron transfer motifs (Munekage et al. 2002). More recently, a crucial role for PGR5 for growth of plants under fluctuating light was demonstrated (Tikkanen et al. 2010). When light intensity is changing rapidly, PGR5 is needed for the photoprotection of PSI. Findings with mutants lacking PGR5 might indicate that PGR5 down-regulates the LET depending on the *pmf*, rather than operating as a component in the CET chain (Suorsa et al. 2012; Tikkanen et al. 2012). It is likely that the PGR5 generates strong proton gradient by hindering the proton translocation from lumen back to stroma either via control of the Cyt b_6f complex (Tikkanen et al. 2012; 2014; Tiwari et al. 2016) or via the ATPase (Kanazawa et al. 2017).

Furthermore, it was proposed that in eukaryotes, PGR5 forms a complex with an integral thylakoid protein PGR5-LIKE PHOTOSYNTHETIC PHENOTYPE1 (PGRL1) (DalCorso et al. 2008). Orthologues of *A. thaliana* PGR5 are found in genomes of plants, algae and cyanobacteria, whereas genes for PGRL1 homologs are found in plants, algae and diatoms, but not in cyanobacteria. The inactivation strain of *pgr5* in *Synechocystis* showed reduced growth in high light but not in low light (Yeremenko et al. 2005). In WT *Synechocystis*, the expression of *pgr5* was found to be induced only transiently in response to a shift from growth light to high light (from 50 to 500 µmol photons m^{-2} s^{-1}) (Sánchez-Riego et al. 2013). Since there is only little experimental data available, the role of the Pgr5-like protein in cyanobacteria remains elusive.

1.4 Multiple terminal O_2 electron sinks of photosynthetic electrons

Photosynthetic organisms rely mostly on three main pathways utilizing O_2 as a terminal electron sink: (i) the Mehler reaction, (ii) the plastid terminal oxidase (PTOX) dependent O_2 reduction pathway and (iii) the photorespiratory pathway. Compared to angiosperms, cyanobacteria have more numerous and efficient systems for O_2 reduction. Besides the above-mentioned pathways they possess: (i) a Mehler-like reaction operating via flavodiiron proteins (FDPs) and (ii) respiratory terminal oxidases (RTOs) that interact and share components with the photosynthetic electron transport chain.

Mehler reaction: In 1951, Alan Mehler observed that in illuminated spinach chloroplasts, O_2 is reduced to H_2O_2 (Mehler 1951). Later on, the reaction was specified to occur when over-reduced PSI transfers electrons from Fe-S centers or Feds directly to O_2, and the primary product was identified to be superoxide anion radical (O_2^-) (Asada 1999). In plant thylakoids, the O_2^- is rapidly reduced to H_2O_2 by superoxide dismutase (SOD) and further to H_2O by ascorbate-specific peroxidase (APX). This reaction is known as the water-water cycle (Asada 1999). The magnitude of the Mehler reaction is controversial. The data accumulated during last several years related to the function of FDP originated Mehler-like reaction requires revisiting the classical Mehler -reaction and distinguish between these two processes (see 1.4.2). However, it is clear that the rate of the Mehler reaction changes depending on environmental conditions and organism.

PTOX is a diiron quinol oxidase that accepts electrons from reduced PQ and transfers them to O_2, producing H_2O. Thus, PTOX regulates the redox-state of the intersystem chain. Depending on substrate concentration it can also produce superoxide radicals, putatively participating in signaling pathways (Krieger-Liszkay and Feilke 2016). The PTOX gene is present in only 15 sequenced cyanobacterial strains, and it is missing from *Synechocystis* (McDonald et al. 2011).

Photorespiration: Because of the chemical similarity of CO_2 and O_2, Rubisco may also catalyze an oxygenation reaction, producing 2-phosphoglycolate (2-PG), which is toxic to the cell. The role of the carboxysome in concentrating the substrate CO_2 in the vicinity of Rubisco is crucial, but does not completely eliminate the oxygenation reaction. Thus, the detoxifying photorespiratory 2-PG metabolism is essential in cyanobacteria (Eisenhut et al. 2006; 2008), and the direct evidence of the existence of photorespiratory O_2 uptake was shown in *Synechocystis* under C_i-deprived conditions (Allahverdiyeva et al. 2011).

1.4.1 Flavodiiron proteins (FDPs)

FDPs, previously called as A-type flavoproteins, form a large family of enzymes that share sequence similarity. The FDPs are dioxygen (O_2) and/or nitric oxide (NO) reductases widely spread among anaerobic as well as some aerobic Bacteria (such as cyanobacteria) and Archaea, and also found in eukaryotes, particularly anaerobic protozoa and evolutionary more ancient photosynthetic organisms like green algae, mosses, lycophytes and gymnosperms. FDPs are modular proteins consisting minimally of two conserved core modules: the flavodoxin domain harbors the flavin mononucleotide (FMN) binding site and the two irons are in the active site of the metallo-*β*-lactamase-like domain in the N-terminus (Vicente et al. 2002). The coenzyme FMN is a derivative of riboflavin (vitamin B2) and serves as an electron carrier. The active non-heme diiron site is responsible for the reduction of NO to N_2O or O_2 to H_2O (Vicente et al. 2008a).

As depicted in Figure 2, FDPs can be classified into five different structural classes (A–E) based on the additional C-terminal modules fused to the two core modules (Vicente et al. 2008b; Romão et al. 2016). The class A FDPs are the simplest and most widespread FDPs among prokaryotes, consisting merely of the two core modules. The class B FDPs have an additional C-terminal rubredoxin domain with a Fe-S center, and are found in enterobacteria such as *Escherichia coli*. In bacterial FDPs, the electrons are transferred from NADPH produced in glycolysis to the active site of FDP, either through an external rubredoxin enzyme or through the fused rubredoxin-like domain of FDPs in Class A and B, respectively.

In cyanobacterial (photosynthetic) class C FDPs, an NAD(P)H:flavin oxidoreductase (Flv) domain is fused at the C-terminus of FDPs allowing the condensation of a multi-component pathway into a single protein (Vicente et al. 2002; Vicente et al. 2008b). Thus, cyanobacterial FDPs may be able to receive electrons directly from NAD(P)H and transfer them to the catalytic diiron center without the participation of rubredoxin. In addition, Class C FDPs can be found in eukaryotic photosynthetic species, and their biological role has been evaluated in *Chlamydomonas reinhardtii*, a single celled soil-dwelling green alga, in a moss *Physcomitrella patens* and a liverworth *Marchantia* (Dang et al. 2014; Jokel et al. 2015; Gerotto et al. 2016; Shimakawa et al. 2017). Recent data shows the existence of homologous genes for Fv1 and Flv3 in gymnosperms as well, but diatoms, haptophytes or angiosperms do not possess FDPs (Peltier et al. 2010; Ilík et al. 2017). In *C. reinhardtii*, FlvA and FlvB were shown to help in acclimation to anoxia during sulfur deprivation, perhaps by actively assisting in decreasing the O_2 level inside the chloroplast (Jokel et al. 2015). Most recently it was demonstrated that FLVA and FLVB of *P. patens* and *Marchanta*, similar to cyanobacterial

Flv1 and Flv3, function as an electron sink downstream of PSI after a rapid change in light intensity and act as a safety valve for electrons (Gerotto et al. 2016; Shimakawa et al. 2017).

Considering the importance of FDPs in a wide range of photosynthetic oxygenic organisms, it is rather surprising that FDPs are not present in angiosperms (Zhang et al. 2009; Allahverdiyeva et al. 2015; Ilík et al. 2017). Recently, Yamamoto and co-workers (2016) showed that FDPs from *P. patens* are capable of accepting electrons from PSI when expressed in *A. thaliana*. It is highly possible that angiosperms try to avoid wasting electrons to O_2 and instead use more sophisticated regulatory pathways, efficient NPQ systems and reactive oxygen species (ROS) scavenging enzymes that have compensated for the loss of FDPs during the evolution of higher plants.

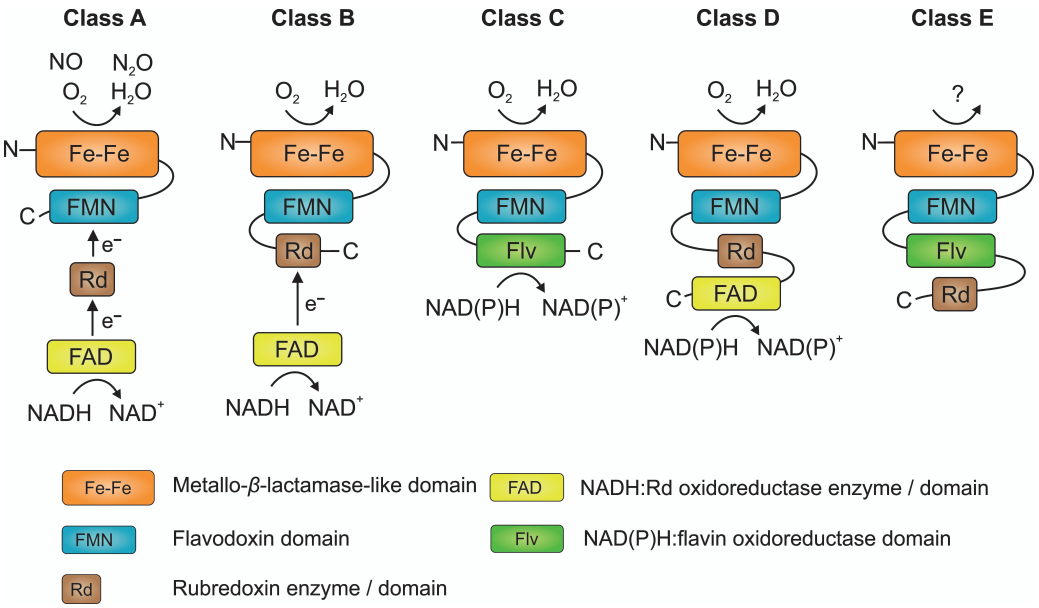

Figure 2. Structural modules of the class A-E FDPs. Figure modified from Romão et al. (2016).

Moreover, based on gene sequences, some protozoa and clostridiales are predicted to have Class D and E enzymes with both an extra rubredoxin and either a NAD(P)H:rubredoxin oxidoreductase-like (FAD) domains or a flavin reductase (Flv) domain, respectively (Romão et al. 2016). The functional unit of FDPs in anaerobic prokaryotes and eukaryotic protozoa has been determined to form a homodimer arranged in a head-to-tail orientation, in which the diiron site from one monomer and the FMN cofactor of the other monomer are close to each other, ensuring an efficient electron transfer

across the two centers (Figure 3; Vicente et al. 2008b; Zhang et al. 2009; 2012). In some anaerobes, the FDPs have been isolated and crystallized as a homotetramer (dimer of dimers) (Seedorf et al. 2007). Prokaryotic FDPs are expected to be cytoplasmic soluble enzymes (Zhang et al. 2012; Romão et al. 2016).

The current model by Frederick and co-workers (2015) predicts that under oxic conditions, FDPs contain an oxidized FMN_{ox}–$Fe^{III}Fe^{III}$ active site. Upon accepting four electrons, the active site is reduced to $FMNH_2$–$Fe^{II}Fe^{II}$. The O_2 turnover proceeds by formation of a peroxo intermediate linking the two ferric ions. After reducing the peroxo-diferric by two electrons facilitated by protonation, two H_2O molecules are generated as a result.

1.4.2 Regulation and function of FDPs in cyanobacteria

In cyanobacteria, the FDPs seem to be important for protecting the photosystems against oxidative stress, suggesting an early evolutionary appearance. One indication of their importance is that almost all sequenced cyanobacterial strains contain several copies (2–6) of genes encoding FDPs (*flv*), forming a small family encoding different FDP paralogs (Zhang et al. 2009; Allahverdiyeva et al. 2015). The genome of the *Synechocystis* contains four genes encoding FDPs: *sll1521* (Flv1), *sll0550* (Flv3) and a tricistronic operon for *sll0219* (Flv2), *sll0218* and *sll0217* (Flv4) (Zhang et al. 2009). The Flv1-Flv3 pair is present in all class C FDP containing phototrophs, whereas the Flv2-Flv4 pair, usually organized as an operon, is present only in some *β*-cyanobacteria (Allahverdiyeva et al. 2015). The structure of the *flv4-ORF-flv2* operon is highly conserved, unlike for *flv1* and *flv3* organization. The majority of *α*-cyanobacteria and some *β*-cyanobacteria encode an operon of *flv3-flv1* genes. Yet, many *β*-cyanobacteria, such as *Synechocystis*, have *flv1* and *flv3* spread out in the genome.

Furthermore, FDPs from oxygenic photosynthetic organisms group into two phylogenetically separate clusters: cluster A including, Flv1 and Flv2, and cluster B, including Flv3 and Flv4 (Zhang et al. 2009). The cyanobacterial FDPs in cluster B have conserved residues accurately corresponding to the canonical ligands known to participate in iron coordination, while cluster A FDPs have considerable dissimilarities in these residues forming the active site (Zhang et al. 2009; Gonçalves et al. 2011; 2014). Since FDPs occur as pairs in the cyanobacterial genomes, it has been proposed that they arrange in heterodimers, so that one protein from each cluster is used to form an active protein complex (Figure 3; Zhang et al. 2009; 2012).

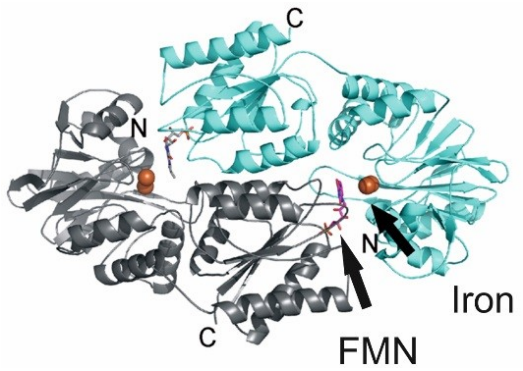

Figure 3. Homology model of the *Synechocystis* Flv2/Flv4 heterodimer without Flv domain, constructed based on *Moorella thermoacetica* FprA. The Flv2 monomer is shown as gray and the Flv4 monomer as cyan. Orange spheres represent the diiron site. Figure modified from Zhang et al. (2012).

In 2002, Vicente et al. demonstrated that *Synechocystis* Flv3, heterologously produced in *E. coli*, is able to catalyze NAD(P)H-mediated O_2 reduction to H_2O *in vitro*. Moreover, Helman and co-workers (2003) could prove that both Flv1 and Flv3 of *Synechocystis* are essential for photoreduction of O_2 *in vivo*. Upon illumination, dark adapted *Synechocystis* WT cells showed rapid O_2 consumption, unlike Δ*flv1* or Δ*flv3* inactivation mutants (Helman et al. 2003; Allahverdiyeva et al. 2013). The reaction mediated by Flv1 and Flv3 was designated as Mehler-like reaction to distinguish it from the non-enzymatic ROS producing Mehler-reaction (Allahverdiyeva et al. 2013).

Induction of the *flv* genes under certain conditions may provide hints that can be used to predict their physiological role. Transcriptomics data of *Synechocystis* has shown that the transcript levels of *flv3* and the *flv4-2* operon is enhanced under C_i limitation (Wang et al. 2004; Eisenhut et al. 2007; Mitschke et al. 2011) and repressed upon iron limitation (Hernández-Prieto et al. 2012). The response of Flv3, Flv4 and Flv2 to C_i limitation has been verified by measuring protein levels (Zhang et al. 2009). Conversely, Flv1 and Flv3 were suppressed in terms of protein level only during short term iron deprivation, and Flv1 was in fact upregulated after 12 days under iron depletion while Flv3 remained below the abundance threshold (Vuorijoki et al. 2016). The transcripts of *flv3* and *flv4-2* also respond to irradiance, exhibiting a low expression level under darkness (Kucho et al. 2005; Ermakova et al. 2013), and the *flv4-2* operon is enhanced upon shifting to a high light regime (Singh et al. 2008; Mitschke et al. 2011). Moreover, the Flv3, and to some extent, also the Flv1 proteins, showed reduced protein amounts under light-activated heterotrophic conditions, indicating that their

function is correlated with autotrophic growth under light (Kurian et al. 2006).

Interestingly, the transcript of *Synechocystis flv1* gene does not respond to light or carbon regime. Instead, a slight enhancement in *flv1* transcript amount was seen under nitrosative stress (S-nitrosogluthatione treatment), under oxidative stress (methyl viologen and H_2O_2 treatment) and heat stress (shift from 25°C to 38°C) conditions (Kobayashi et al. 2004; Houot et al. 2007; Rowland et al. 2010). In these conditions, the response of *flv3* transcription was similar.

Even though class C cyanobacterial FDPs contain an NAD(P)H:flavin oxidoreductase (Flv) domain, Flv1 and Flv3 in *Synechocystis* (Hanke et al. 2011), and FLBA and FLVB in *C. reinhardtii* (Peden et al. 2013) were found to interact with Fed1 and FDX1, respectively. Hanke and co-workers (2011) suggested that the interaction between Flvs and Fed1 could occur directly or through FNR as a participant of a metabolic channeling complex, which ensures an efficient supply of NADPH. The latter hypothesis is supported by data, where *Synechocystis* Flv3 heterologously expressed in *E. coli* was shown to exhibit an NAD(P)H dependent O_2 reduction to H_2O (Vicente et al. 2002). A recent finding from Cassier-Chauvat and co-workers (2014) provides still another possibility. By applying the bacterial adenylate cyclase two-hybrid (BACTH) system, they found that Fed9 physically interacts with the Flv3. Given that the predicted redox potential of both Fed1 and Fed9 is high, -420mV, it is conceivable that they both are able to transfer electrons to Flv3 (Cassier-Chauvat and Chauvat 2014).

Filamentous heterocystous cyanobacteria have additional pairs of *flv1-flv3* in heterocyst cells, designated as *flv1a-flv3a* for vegetative cells and *flv1b-flv3b* for heterocysts. In *Anabaena* sp. PCC 7120, the Flv1B and Flv3B have been shown to be heterocyst-specific with mutant strains expressing Flv-YFP fusion proteins, and the transcript levels of *flv1b* and *flv3b* increase under N_2-fixing conditions (Ermakova et al. 2013). Furthermore, it was demonstrated that Flv3B-mediated O_2 uptake ensures a microoxic environment in the heterocysts for the function of the O_2 sensitive nitrogenase enzyme under the light (Ermakova et al. 2014).

1.4.3 The role of *flv4-2* operon-encoded proteins

The other FDP pair in *Synechocystis*, Flv2 and Flv4, is encoded from the *flv4-sll0218-flv2* operon, together with a small Sll0218 protein. The Δ*sll0218-flv2* and Δ*flv4* mutants were found to be more susceptible to high light induced photoinhibition of PSII than the WT or Δ*flv1* and Δ*flv3* mutant strains (Zhang et al. 2009). Accordingly, Zhang et al. suggested a crucial function of the Flv2

and Flv4 proteins in photoprotection of PSII. Flv2 and Flv4 were shown to form a heterodimer in the cytoplasm, yet they showed strong association with the thylakoid membrane in the presence of divalent cations (Zhang et al. 2012). In contrast, Sll0218 is predicted to contain four transmembrane helices, and thus it is most likely integrated in the thylakoid membrane.

Further studies suggested that the Flv2/Flv4 heterodimer releases excess electrons from the Q_B pocket on the acceptor side of PSII, triggered by an increased redox potential of Q_B (Zhang et al. 2012; Bersanini et al. 2014; Chukhutsina et al. 2015a). The *flv4-flv2* operon helps to maintain the PQ pool in an oxidized state under C_i-limited and high light conditions. The accumulation of Flv4/2 and Sll0218 is restricted in a mutant lacking PBS, whereas their amount highly increased in mutants lacking OCP and hence the NPQ-mediated photoprotective mechanism (Bersanini et al. 2014). Therefore, the *flv4-flv2* operon could provide a complementary photoprotection mechanism to OCP and other carotenoids against singlet oxygen mediated damage.

Bersanini and co-workers (2017) studied in detail the separate functions of Sll0218 and Flv2/4 heterodimer with a mutant lacking only Sll0218, while expressing the Flv2 and Flv4 proteins. It was shown that Sll0218 stabilizes the early assembly and repair intermediates of PSII. In addition, the YFP-tagged Sll0218 protein was localized at specific sites of the thylakoid membrane, correlating to the sites where the early biogenesis steps of PSII occur (Bersanini et al. 2017). In the absence of Sll0218, the relative content of PSII dimers decreases, which leads to increased detachment of the PBS antenna from the reaction centers and decreased light harvesting in a mutant lacking the *flv4-2* operon-encoded proteins (Chukhutsina et al. 2015a; Bersanini et al. 2017).

1.4.4 Respiratory terminal oxidases (RTOs)

The breakdown of the organic compounds generated through photosynthesis occurs via the process of aerobic respiration, which also requires the O_2 produced by photosynthesis. Cyanobacteria accumulate glycogen as a main polysaccharide storage molecule during the day and then use it as a carbon source during the night (Saha et al. 2016). In heterotrophic energy generation, cyanobacteria catabolize glucose via the oxidative pentose phosphate (OPP) pathway, the glycolytic pathway, the Entner-Doudoroff pathway and a cyanobacteria specific tricarboxylic acid (TCA) cycle to produce ATP, NADPH and carbon skeletons, which are used as anabolic precursors for nucleotides, amino acids and fatty acids (Zhang and Bryant 2011; Chen et al. 2016). Respiration is essentially a reverse version of the photosynthesis process; sugars are converted to CO_2 and H_2O, and energy is

released in the form of ATP. In cyanobacteria, RTOs function as a terminal electron sink for the respiratory pathways reducing O_2 to H_2O. *Synechocystis* has three distinct RTOs: an alternative respiratory terminal oxidase (ARTO), a cytochrome bd-quinol oxidase complex (Cyd) and an aa_3-type cytochrome *c* oxidase complex (Cox) (Pils et al. 1997).

Cyanobacteria have two sites for aerobic respiration: the plasma membrane and the thylakoid membrane, where the respiratory electron transport shares some components with the LET. ARTO and putatively the Cyd are localized in the plasma membrane (Howitt and Vermaas 1998; Pils and Schmetterer 2001). In the plasma membrane, a succinate dehydrogenase (SDH) and one or more of three different type 2 NAD(P)H dehydrogenases (NDH-2) serve as electron donors (Howitt et al. 1999). Cox and Cyd have been localized to the thylakoid membrane, where the NDH-1, SDH and putatively NDH-2 are transferring respiratory electrons to the PQ pool (Howitt and Vermaas 1998; Berry et al. 2002). The SDH catalyzes the oxidation of succinate to fumarate coupled to the reduction of PQ to PQH_2 as one step of the TCA cycle. Respiratory PQH_2 oxidation can then occur via either thylakoid-bound Cyd or Cyt b_6f, and further via soluble redox carriers to either PSI or to Cox (Howitt and Vermaas 1998; Ermakova et al. 2016).

All sequenced cyanobacterial strains contain at least one set of genes encoding Cox, indicating it to be the major terminal oxidase (Lea-Smith et al. 2013). In *Synechocystis*, genes encoding the subunits of Cox and *ndbC* encoding one NDH-2 exhibit rhythms with peak expression around the time that day turns into night (Kucho et al. 2005; Layana and Diambra 2011). This might help the cells adjust their physiology to the approaching nighttime circumstances. It was also shown that in *Synechocystis,* Cox is important for light-activated heterotrophic growth (Pils et al. 1997) and for dark respiration (Howitt and Vermaas 1998; Ermakova et al. 2016). Interestingly, the respiratory electron transport chain is not essential for the survival of *Synechocystis* in phototropic conditions in unstressed cells. Conversely, the thylakoid-localized Cox and Cyd are required when responding to sudden light stress (Lea-Smith et al. 2013; Ermakova et al. 2016). Furthermore, Ermakova et al. (2016) demonstrated that Cyd is the principal RTO functioning under light at PQ level and capable of light induced O_2 uptake. Putatively, Cyd contributes to the thylakoid membrane potential by using protons on the cytoplasmic side to generate H_2O by oxidizing PQH_2.

1.5 Other photoprotective mechanisms

Light is a vital component for the growth and survival of cyanobacteria and other photosynthetic organisms. Simultaneously, in the highly oxidizing

environment of the PSII containing cells, excess light can be harmful because ROS are generated in the reaction centers and in the antennae. In PSII, singlet oxygen ($^1O_2$) is produced via photosensitized Chl (Reviewed by Telfer 2014), and PSI is mostly responsible for the generation of superoxide anion radicals (O_2^-) (Latifi et al. 2009). The reduction of O_2^- produces hydrogen peroxide (H_2O_2), which can be further reduced to extremely reactive hydroxyl radical ($OH\cdot$) via the Fenton reaction. The ROS can act as signal molecules and activate physiological responses (Schmitt et al. 2014). However, when produced in abundance, the ROS can irreversibly damage lipids, proteins, pigments and DNA (Latifi et al. 2009). In addition to light, other environmental stress factors like nutrition limitation, salinity or temperature variations may trigger oxidative stress.

Like all photosynthetic organisms, cyanobacteria possess several ROS detoxifying scavenging enzymes. *Synechocystis* has one SOD catalyzing the disproportionation of O_2^- to H_2O_2. Dismutation of H_2O_2 to O_2 and H_2O is catalyzed by catalases and peroxidases. Besides one catalase, *Synechocystis* has five genes encoding different peroxidases (Prx), at least one from each of the four subgroups: one 1-Cys Prx, one 2-Cys Prx, two atypical 2-Cys Prxs (PrxQ) and one type II Prx (Kobayashi et al. 2004; Stork et al. 2005). However, the ROS scavenging systems of cyanobacteria are not as efficient as in algae and higher plants. Cyanobacteria lack ascorbate peroxidases common in chloroplastic organisms (Passardi et al. 2007), but their function may be partially replaced by e.g. the thioredoxin-using peroxidases (Matthijs et al. 2012). Nevertheless, cyanobacteria are considerably more sensitive to external H_2O_2 than eukaryotic phytoplankton (Matthijs et al. 2012). It is conceivable that cyanobacteria rather try to avoid the production of H_2O_2 (e.g. having FPDs) than scavenge it.

In addition, UV-irradiation can, directly or indirectly, through the production of ROS, cause damage to DNA and proteins. To protect themselves, cyanobacteria are able to produce UV-absorbing compounds like scytonemin and mycosporine-like amino acids (MAAs) that function both as a UV-screen and an antioxidant (Wada et al. 2013). Furthermore, several carotenoids like *β*-carotene, zeaxanthin and echinenone protect against photooxidative damage by acting as sunscreen pigments and antioxidants (Latifi et al. 2009). Certain carotenoids are specialized in absorbing and quenching energy from the reaction centers of the photosystem.

1.5.1 Non-photochemical quenching (NPQ)

In plants, the low lumenal pH under strong illumination induces a photoprotective mechanism increasing the energy dissipation as heat via non-photochemical quenching (NPQ) of Chl fluorescence. Thereby, NPQ

decreases the amount of energy arriving at the PSII reaction center and prevents the generation of ROS. In contrast to plants, cyanobacterial NPQ is not induced by lumenal acidification, but light activation of a soluble orange carotenoid protein (OCP) (Wilson et al. 2006). OCP is a small soluble protein containing a single covalently bound carotenoid. Absorption of blue-green light or strong white light induces conformational changes in the structure, converting the orange form of OCP to the active red form able to bind to the PBS core and dissipate energy as heat (Wilson et al. 2008). Another protein, fluorescence recovery protein (FRP), accelerates the conversion of active OCP back to ground state and facilitates the detachment of OCP from the PBS (Boulay et al. 2008; Kirilovsky and Kerfeld 2016). In addition, OCP efficiently scavenges singlet oxygen, which is formed in the PSII reaction centers during the light reactions (Sedoud et al. 2014).

Recent studies indicate that high-light-inducible proteins (Hlips) are also capable of performing NPQ mediated by one Chl *a* and *β*-carotene molecule bound to the Hlips (Knoppová et al. 2014; Niedzwiedzki et al. 2016). Cyanobacteria have a family of Hlips that are homologous to the LHC superfamily of plants and algae. *Synechocystis* contains four genes encoding Hlips, while a high-light adapted marine cyanobacterium, *Prochlorococcus* MED4, contains more than 20 *hli* genes (Komenda and Sobotka 2016). It was further suggested that in *Synechocystis* Hlips assist during the final steps of the Chl *a* biosynthesis and the early stages of PSII assembly by protecting the assembly intermediates from photodamage via thermal dissipation (Knoppová et al. 2014; Komenda and Sobotka 2016; Niedzwiedzki et al. 2016).

1.5.2 State transitions

In natural environments, photosynthetic organisms need to acclimate rapidly to varying quality and quantity of light to optimize their photosynthetic capacity. To accomplish this, the functional organization of their light harvesting antennae can be changed according to the light conditions. The redox state of the PQ pool and of the Cyt b_6f complex regulate the energy distribution between the photosystems, which is known as state transitions (Bonaventura and Myers 1969; Murata 1969). In state I, light harvesting antennae (LHCII) of higher plants or PBS of cyanobacteria are energetically coupled to PSII, and the excitation energy captured by the antenna system is mostly directed to PSII (Campbell et al. 1998). In state II, the situation is opposite, and most of the excitation energy is directed to PSI. In plants and algae, the reduction of the PQ pool upon illumination induces the activation of a specific kinase and the phosphorylation of LHCII, resulting in a re-distribution of excitation energy from PSII to PSI (state II). Cyanobacteria,

however, are in state II in darkness due to a reduced PQ pool as a result of respiratory activity. In the light, cyanobacterial cells shift towards state I and PBS serve primarily PSII.

Careful analysis of different thylakoid domains has shown that in *A. thaliana*, both photosystems are energetically connected to LHCII antenna: the LHCII phosphorylation enhances the interaction and thus efficient energy transfer to PSI, yet the phosphorylation is not a prerequisite for the energetic connectivity (Tikkanen and Aro 2014; Grieco et al. 2015). In addition to the reversible phosphorylation of the LHCII proteins, phosphorylation of the PSII core proteins is crucial for balancing the excitation energy between the photosystems (Mekala et al. 2015). The phosphorylation of the core and antenna is regulated independently, yet both phosphorylations are dependent on the light intensity and duration of the prevailing light condition.

Several models involving movements of PBS or photosystems for cyanobacterial state transitions have been suggested (reviewed by Kirilovsky 2015). Recent studies, however, demonstrate that the principal mechanism for the energy redistribution between the photosystems is reversible PBS decoupling from PSI upon illumination (Chukhutsina et al. 2015b). Moreover, continuous high light leads to excitonic uncoupling and detachment of PBS from both photosystems in *Synechocystis* (Tamary et al. 2012). Antenna decoupling may be important under sustained stress conditions, as a final photoprotective response aimed at down-regulating the PSII activity when the other photoprotective responses are not adequate against photo-oxidative damage.

1.6 Regulation by modification of photosynthetic components

Cyanobacteria have rather restricted motility, and thus acclimation to the varying nutritional, temperature and light conditions surrounding them is crucial. Upon changes in light intensity, cyanobacteria can thermally dissipate excess absorption energy relatively fast and rearrange their PBS antennae. These photoprotective mechanisms ensure the balance of excitation energy reaching the photosynthetic apparatus and with the cell's capacity to utilize the light energy. In plants, fine tuning the protein activity via thylakoid protein phosphorylation and other post-translational modifications is crucial for short-term regulation of the photosynthetic light reactions (Reviewed by Tikkanen and Aro 2012). Phosphorylation of photosynthetic components was recently observed in *Synechocystis*, suggesting that phosphorylation may be important for the regulation of photosynthetic performance in cyanobacteria as well (Spät et al. 2015; Angeleri et al. 2016).

1.6.1 Changes in the protein content and stoichiometry

Ultimately, cyanobacteria need to sense and respond to environmental cues in order to regulate photosynthesis and their metabolism. Different signals are integrated and transduced into changes in gene expression and protein activity via complex regulatory networks. In photosynthetic organisms, changes in the redox state of the components in the photosynthetic LET chain are particularly important for transcriptional regulation. The two-component regulatory systems such as the histidine kinase and response regulator pair Hik33–RpaB and the ferredoxin/thioredoxin system play central roles as regulators of the transcription of photosynthetic genes (Wilde and Hihara 2016; Kadowaki et al. 2016). In the simplest case, the signal transduction chains lead to the binding of transcription factors to the promoters of their target genes, where they activate or prevent transcription.

Under long-term exposure to high light or upon other environmental changes, the stoichiometry of photosystems and the protein content of the cell are altered. Cyanobacteria have 2 to 8 PSI complexes per PSII complex (Kirilovsky 2015), and PSI trimers bind about 90% of the total cellular chlorophyll (Schneider et al. 2001). Under high light, both PSII and PSI content is decreased, but the down-regulation of PSI is more prominent, leading to a change in photosystem stoichiometry (Murakami et al. 1997; Sonoike et al. 2001). Reduction in the content of photosystems and Chl *a* is accompanied with a reduction in the number and/or size of PBS (Raps et al. 1985; de Lorimier et al. 1992; Miskiewicz et al. 2002).

1.6.2 Post-transcriptional regulation

In addition to transcriptional regulation, gene expression is also controlled through post-transcriptional control. Recent transcriptome analyses predicted 3500 putative noncoding RNAs (ncRNA) for *Synechocystis* (Mitschke et al. 2011). Short ncRNAs include *cis*-encoded antisense RNA (*cis*-asRNA) from the opposite complementary strand of an annotated gene. More than half of the predicted ncRNAs are in the intergenic region, some of them functioning as small regulatory RNAs and having a partial antisense complementarity to their target RNAs (*trans*-acting ncRNA). NcRNAs are typically 50–200 nt in length, while the average length of asRNAs is longer, up to 700 nt in *Synechocystis* (Georg et al. 2009). These findings suggest the existence of a significant number of asRNAs that may have a role in the transcriptional and post-transcriptional regulation of mRNA degradation or stability as well as translation activation or repression.

In *Synechocystis*, two members of the *psbA* gene family encoding the D1 protein, *psbA2* and *psbA3*, were shown to be regulated by asRNAs. The asRNA

PsbA2R binds to the 5'-untranslated region of *psbA2* mRNA and stabilizes it by blocking the access of RNase E endoribonuclease to its cleavage sites, and thus prevents degradation of the mRNA (Sakurai et al. 2012). An opposite outcome has been shown for asRNA IsrR for *isiA* protein: IsrR regulates the accumulation of *isiA* by making the IsR:*isiA* duplex more prone to degradation (Dühring et al. 2006). IsiA forms an antenna ring around PSI under prolonged iron limitation and oxidative stress, but via post-transcriptional control with asRNA, expensive premature accumulation of IsiA can be prevented.

2 AIMS OF THE STUDY

Oxygenic photosynthesis supports all life, and its basic principles are long-established. The basis of linear photosynthetic electron transport was drafted as the so called Z-scheme already in 1960. Since then, the tandem function of the two light harvesting photosystems together with Cyt b_6f and soluble electron carriers have been clarified, and the crystal structures of all major photosynthetic protein complexes have been obtained. Nevertheless, the complex network of auxiliary components assisting in the regulation of the photosynthetic electron transport under rapidly changing environmental conditions is poorly understood. During the course of evolution, a multitude of different photoprotective and regulatory mechanisms have developed in cyanobacteria, algae and plants. The early evolutionary steps of oxygenic photosynthesis and oxidative stress prevention mechanisms are represented in cyanobacteria. The aim of my thesis research was to clarify the function and regulation of different components in the alternative electron transport routes involved in the photoprotection of cyanobacteria.

The specific aims were as follows:

1) Elucidation of the molecular mechanisms of the acclimation of *Synechocystis* cells to FL, and the role of FDPs under rapidly changing light intensities that mimic the natural aquatic environment

2) Dissection of the photoprotective function of the Flv1 and Flv3 homo-oligomers

3) Identification of the regulatory mechanisms controlling the expression of the *flv4-2* operon

4) Evaluation of a role of the Fed7, one of the low abundant Feds in *Synechocystis*

3 MATERIAL AND METHODS

3.1 Cyanobacterial strains and growth conditions

***Synechocystis* sp. PCC 6803**					
Strain	**Deleted genes**	**Phenotype**	**Reintroduced or modified genes**	**Paper**	**Reference**
WT				I-IV	
Δ*flv1*	*sll1521*::Cm^R	FL-sensitive		I, II	Helman et al. 2003
Δ*flv3*	*sll0550*::Sp^R	FL-sensitive		I, II	Helman et al. 2003
Δ*flv1*/Δ*flv3*	*sll1521*::Cm^R	FL-sensitive		I, II	Allahverdiyeva et al. 2011
	sll0550::Sp^R				
Δ*pgr5*	*ssr2016*::Km^R			I	Paper I
Δ*flv1::flv1*	*sll1521*::Cm^R		*sll1521*::$Sp^{R(1)}$	I, II	Paper I
Δ*flv1*/oe*flv3*	*sll1521*::Cm^R	FL-sensitive	*sll0550*::$Sp^{R(1)}$	II	Paper II
Δ*flv3*/oe*flv1*	*sll0550*::Sp^R	FL-sensitive	*sll1521*::$Km^{R(1)}$	II	Paper II
Δ*psbA2*	*slr1311*::Sp^R			II	Zhang et al. 2012
As1_flv4[+]			P-*petJ* -As1_flv4$^{(2)}$	III	Paper III
MpILA-as1_flv4			P-as1_flv4-*luxAB*	III	Paper III
MpILA-*flv4-2*			P-*flv4-2*-luxAB	III	Paper III
Δ*ndhR*	*sll1594*::Sp^R	LC-sensitive		III	Wang et al. 2004
Δ*sll0822*	*sll0822*::km^R	Slow growth		III	Ishii and Hihara 2008
Δ*fed7*	*sll0662*::km^R			IV	Paper IV
***Anabaena* sp. PCC 7120**					
WT				I	
Δ*flv1*A	*all3891*::Nm^R	FL-sensitive		I	Paper I
Δ*flv3*A	*all3895*::Nm^R	FL-sensitive		I	Paper I

Table 1. Cyanobacterial strains used in this work. A detailed description of the mutants can be found in the provided references. Integration site in the genome (1) *psbA2* and (2) *spkA*. P=promoter.

3.1.1 Culture Conditions

A non-motile glucose tolerant wild-type (WT) strain of *Synechocystis* sp. PCC 6803 and mutant strains (Table 1) were grown at 30°C in the BG-11 medium (Rippka et al. 1979) supplemented with 20 mM HEPES-NaOH (pH 7.5) or 10 mM TES-KOH (pH 8.2). Two different light sources were used in the experiments: fluorescent lamps and LED light. Cells were illuminated continuously with white fluorescent light at an intensity of about 50 µmol photons m^{-2} s^{-1} (Growth Light: GL) (Papers I-IV). Lower and higher light intensities were used for certain experiments in Paper I (20 and 500 µmol photons m^{-2} s^{-1}) and in Paper IV (200 µmol photons m^{-2} s^{-1}). AlgaeTron AG 130-ECO (PSI) growth chambers equipped with LED white light were used for culturing cells under FL and, for the controls, growing them under constant GL (Papers I-II). Two different light regimes for FL were used in Papers I and II: FL 20/500 (20 µmol photons m^{-2} s^{-1} for 5 min and 500 µmol photons m^{-2} s^{-1} for 30 s) and FL 50/500 (50 µmol photons m^{-2} s^{-1} for 5 min and 500 µmol photons m^{-2} s^{-1} for 30 s).

Liquid cultures were grown in Erlenmeyer flasks shaking at 150 r.p.m. to ensure continuous gas exchange and kept at high CO_2 (air enriched with 3% CO_2; HC) or at low CO_2 (ambient air with 0.039% CO_2; LC), depending on the experiment. Cells were maintained with antibiotics, but experimental cultures were not supplemented with antibiotics. Absence of contamination with heterotrophic bacteria was routinely checked by spreading liquid culture on LB and R2A agar plates.

For physiological experiments, cells were harvested at the logarithmic growth phase (OD_{750} between 0.6 and 1.1). Prior to biophysical measurements, the cells were pelleted and resuspended in fresh BG-11 medium, and adjusted to achieve equal Chl concentration (10-15 µg ml^{-1}). Chl concentration was determined spectrophotometrically in 90% methanol and calculated by using an absorption coefficient at 665 nm of 78.74 l g^{-1} cm^{-1} (Meeks and Castenholz 1971).

3.1.2 Construction of mutant strains

In order to construct an inactivation strain in *Synechocystis*, the ORF of the gene targeted for mutagenesis and its surroundings (a flanking region of about 300 bp on both sides) were amplified from *Synechocystis* WT chromosomal DNA by polymerase chain reaction (PCR) with specific primers. The amplified gene fragment was ligated into a cloning vector, and an antibiotic resistance gene was inserted into or used to replace the coding region of targeted gene in the same orientation. A kanamycin resistance (Km^R) cassette was used to interrupt *fed7* and *pgr5* genes to gain Δ*fed7* and

Δ*pgr5* mutants, respectively. A neomycin resistance cassette (Nm^R) was used to gain Δ*flv1A* and Δ*flv3A* mutants in *Anabaena*. A more detailed description of the cloning plasmids and specific primers is provided in Papers I and IV.

The Flv1 and Flv3 overexpression and complementation mutants, Δ*flv1*/oe*flv3, Δflv3*/oe*flv1* and *Δflv1::flv1*, were generated by introducing the *flv1* or *flv3* gene to a single mutant background (Papers I and II). The constructs were obtained by inserting the *flv1* or *flv3* gene into the *NdeI-NheI* sites of a modified pPSBA2KS plasmid (obtained from Dr. Marion Eisenhut), which contains the up- and downstream regions of the *psbA2* gene. A spectinomycin resistance (Sp^R) or Km^R cassette was used for selection. Homologous recombination of the construct to the *Synechocystis* genome results in the insertion of the *flv* gene under the control of the *psbA2* promoter. The As1_flv4[+] overexpression strain was generated by fusing the *as1_flv4* fragment with the *petJ* promoter and the Km^R cassette and integrated into a neutral site in the genome, in the *spkA* gene (Paper III). The MpILA-as1_flv4 and MpILA-*flv4-2* promoter probe strains were constructed by fusing the 300 and 700 nt promoter sequences of the genes encoding the asRNA As1_flv4 and the *flv4-2* operon, respectively, with the *luxAB* genes encoding a LuxAB luminescence reporter (Paper III).

The resulting inactivation and overexpression constructs were used to transform *Synechocystis* WT or mutant cells by applying 1 μg of the plasmid to concentrated cells and further incubating them overnight at 30°C in gentle agitation. The transformed cells were spread on solid BG-11 plates with 1/5 of the full antibiotic concentration. After around two weeks, the transformants were picked up for a fresh BG-11 plate, and antibiotic concentration was gradually increased until they were fully segregated with all copies of the chromosome mutated. Complete segregation of the strains was confirmed by PCR from cultures grown without antibiotics. *Anabaena* was transformed by triparental mating (Elhai and Wolk 1988).

3.2 Measurements of photosynthesis and respiration

3.2.1 O_2 exchange by a Clark-type electrode

Photosynthetic oxygen evolution was measured *in vivo* with a Clark-type oxygen electrode and Oxygraph control unit (Hansatech) at 30°C under saturating white light of 1300 μmol photons m^{-2} s^{-1}. The whole-chain photosynthetic electron transfer rates were measured in the presence of 10 mM $NaHCO_3$ and the PSII electron transfer rates were measured in the presence of 2 mM 2,6-dimethyl-p-benzoquinone (DMBQ).

3.2.2 Gas-exchange analyses with membrane inlet mass spectrometry (MIMS)

Real-time gas-exchange analyses with MIMS was performed to measure O_2 and CO_2 fluxes. The method makes it possible to distinguish light induced O_2 uptake from photosynthetic O_2 evolution by applying the $^{18}O_2$ isotope in gaseous phase, which is consumed by the oxygen uptake reactions, while the O_2 is evolved by the PSII by consuming the $^{16}O_2$ isotope of water (Beckmann et al. 2009). Online measurements were monitored using a mass spectrometer (Thermo Scientific) model Prima-B in Paper I and model Prima PRO in Paper II. The membrane inlet system is comprised of a modified oxygen electrode chamber that is attached to the vacuum line of a mass spectrometer through a thin gas-permeable membrane. Temperature was maintained at 30°C in the chamber, and cell suspensions were continuously mixed with a magnetic stirrer. Actinic white light intensity of 20 or 500 µmol photons m^{-2} s^{-1} was provided when required.

3.2.3 PSII and PSI functional parameters

A pulse amplitude modulated fluorometer, Dual-PAM-100 (Walz), was used to monitor Chl *a* fluorescence and P700 oxido-reduction in intact cells. F_0, the minimum fluorescence, was determined using dark-adapted cells. Red (620 nm) actinic light was used to induce photosynthesis, and saturating pulses (5000 µmol photons m^{-2} s^{-1}, 300 ms) were applied to close all PSII centers transiently in order to measure Fm' and Pm'. The Fm was measured under light in the presence of 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU), an inhibitor of electron transport between the Q_A and Q_B sites in PSII. The maximal photochemical efficiency of PSII (Fv/Fm) was calculated as $(Fm-F_0)/Fm$. To determine the Pm value, a far-red light for 10 s and a subsequent saturating pulse were applied to dark adapted cells. The acceptor side limitation of PSI, Y(NA), was calculated as Y(NA) = (Pm-Pm')/Pm.

The single flash induced Chl fluorescence rise and the following decay were measured with a fluorometer FL 3500 (PSI Instruments). The cells were dark adapted for 5 min before the application of a single 10 µs saturating flash in the absence or presence of 10 µM DCMU.

Fluorescence emission spectra of intact cells frozen in liquid nitrogen **at 77 K** were measured using a USB4000-FL-450 (Ocean Optics) spectrofluorometer. Fluorescence emission spectra were recorded by excitation with 580 nm or 440 nm light, and normalized to the PSI emission peak at 725 nm.

3.3 Transcript analyses

3.3.1 Isolation of total RNA

Total RNA was isolated from exponentially growing cultures by the hot-phenol method according to Tyystjärvi et al. (2001). After the removal of any residual of genomic DNA, RNA concentration and purity were measured with a NanoDrop spectrophotometer (Thermo Scientific). RNA integrity was verified by agarose gel electrophoresis.

3.3.2 Real-time quantitative PCR (RT-qPCR)

First strand cDNA was synthesized from purified RNA using the SuperScript III Reverse Transcriptase kit (Invitrogen) and used as a template for the RT-qPCR. For comparison, reference genes with a constitutive transcription were used: *rnpB* encoding the RNA subunit of RNase P was used in Paper IV and additionally *rimM* and *cysK* in Paper II. All primers were designed to generate a similar amplicon length (about 150 bp). RT-qPCR was performed with a Bio-Rad IQ5 detection system using iQ SYBR Green Supermix (BioRad) containing a fluorescent dye to detect the accumulation of amplicons. Samples lacking reverse transcriptase or template were used as negative controls. Melting curve analysis was performed after 40 cycles of PCR for each run to verify the specificity of the expected PCR product. The annealing temperatures were optimized, and the efficiencies of each reaction were estimated using a LinRegPCR program.

3.3.3 Transcriptional profiling

DNA Microarray analysis was performed at the Finnish DNA Microarray and Sequencing Centre (Turku, Finland). Total RNA was hybridized to the Agilent 8x15K custom cyanobacterium *Synechocystis* sp. PCC 6803 array. The array covers 2-4 specific and non-overlapping probes for each 3262 ORF. Microarrays were analyzed by pairwise comparisons between groups using the LinearModels for Microarray Data (Limma) package from Bioconductor software. A gene was considered up-regulated if $\log_2$ of the fold change was ≥ 1 and down-regulated if fold change was ≤ -1. Genes with statistically significant ($p < 0.05$) values were considered as differentially expressed. Gene annotation was performed with definitions listed in the CyanoBase. (http://genome.annotation.jp/cyanobase/Synechocystis).

3.3.4 Northern blot analysis

For mRNA analysis, total RNA samples were mixed with denaturation solution, denatured for 10 min at 70°C and the RNA was separated according to size in 1.3% agarose gels containing 7% formaldehyde in MOPS. For small

RNA studies, RNA samples were denatured for 10 min at 70°C in RNA loading dye (Fermentas) and separated in 10% polyacrylamide-urea gels. The separated RNA was transferred to Hybond-N nylon membranes by capillary blotting (mRNA) or by electroblotting for 1 h (small RNAs). Membranes were cross-linked with UV-light, and even loading and blotting were verified using methylene blue staining. The specific RNA fragments were detected with [α-^{32}P]CTP-labeled DNA probes or [α-^{32}P]UTP-incorporated transcripts. The DNA probes were prepared with the Prime-a-Gene® Labeling System (Promega), and the single-stranded radiolabeled RNA probes were synthesized by *in vitro* transcription with the MAXIscript kit (Invitrogen). Overnight hybridization with a specific probe was performed in hybridization buffer at 62°C. Remaining probe was removed the next day by washing the membrane. Signals were detected with autoradiography on x-ray films.

3.4 Protein analyses

3.4.1 Protein isolation, SDS-PAGE, BN-PAGE and immunodetection

Total cell extracts and the soluble and membrane fractions of *Synechocystis* cells were isolated as described by Zhang et al. (2009). **Sodium dodecyl sulfate-Polyacrylamide gel electrophoresis (SDS-PAGE)** was applied to analyze denatured proteins. Protein samples were solubilized in Laemmli sample buffer (Laemmli 1970) and separated using 10-12% (w/v) SDS-PAGE containing 6 M urea. Protein complexes in their native form were analyzed by **blue-native electrophoresis (BN-PAGE)**. The BN gel was prepared as described by Zhang et al. (2012). The membrane samples were washed and then solubilized with 2% dodecyl-β-D-maltoside (DM). The soluble samples were diluted in 4xBN buffer. Prior to loading, both membrane and soluble samples were supplemented with a 1/10 volume of sample buffer and loaded on an equal protein basis in a 4-10% or 5-15% acrylamide gradient gel. For **two-dimensional (2D)** separation, the strips from the first dimension BN-PAGE were excised and the proteins were denatured in Laemmli sample buffer. The strip was then placed onto a 12% SDS-PAGE gel containing 6 M urea to separate the protein subunits of the complexes. The proteins were electrotransferred to a polyvinylidene fluoride membrane (Immobilon-P; Millipore) and immunodetected with protein-specific primary antibodies. Horseradish peroxidase (HRP) conjugated secondary antibody (anti-rabbit IgG from donkey) was used for recognizing the primary antibodies.

3.4.2 Quantitative SRM-based proteomics assay

Protein extracts from *Synechocystis* were reduced, alkylated and digested with trypsin according to the protocol described in Vuorijoki et al. (2016).

The digested peptide mixture was injected into the liquid chromatograph-mass spectrometer (LC-MS) in biological triplicate. The peptides were analyzed on a triple quadrupole mass spectrometer (TSQ Vantage, Thermo Scientific) equipped with a nanoelectrospray source according to Vuorijoki et al. 2016. The data was analyzed in Skyline software and statistics work was performed in the MSstats package integrated in Skyline. For the selected reaction monitoring (SRM) assay, up to five proteotypic peptides unique for each target protein were selected.

4 RESULTS

4.1 Flv1 and Flv3 proteins are crucial for cyanobacterial survival under natural light environment

The Flv1 and Flv3 proteins are a major electron sink during dark to light transitions, and in harsh C_i-deprived conditions up to 40-60% of electrons extracted from water can be redirected to O_2 by Flv1/3 (Helman et al. 2005; Allahverdiyeva et al. 2011). Thus, Flv1 and Flv3 have high capacity to harmlessly dissipate excess electrons via light-induced O_2 consumption without producing ROS. The ability of photoprotective pathways to rapidly react to changes in light intensity is especially important in aquatic environments, where the focus of sunlight can rapidly change due to surface waves and create a lens effect, resulting in a bright spot. Water movement and clouds may cause sudden changes of light intensity that reach cyanobacterial cells, ranging from very dim to very bright light. In Paper I, I studied the possible role of the Flv1/3 mediated O_2 photoreduction under fluctuating light intensities when the growth light is repeatedly interrupted by high-light pulses.

4.1.1 Flv1 and Flv3 protect PSI under fluctuating light

Even though a significant amount of electrons can be directed to Flv1 and Flv3 pathways, the mutants defected in either or both of the proteins, namely the Δ*flv1*, Δ*flv3* and Δ*flv1*/Δ*flv3* mutant strains, did not show any distinctive growth phenotype under any constant light regimes varying 20-500 µmol photons m^{-2} s^{-1}. However, under harsh fluctuating light (FL 20/500 regime) where low background illumination was interrupted every five minutes by repetitive high-light pulses for 30 seconds, the growth of the deletion mutants was completely arrested (Paper I, Fig. 1A-B). Under the less severe FL 50/500 regime (50 µmol photons m^{-2} s^{-1} for 5 min and 500 µmol photons m^{-2} s^{-1} for 30 s), the mutant cells were able to grow, but drastically more slowly than WT cells (Paper I, Fig. 1A, C). Growth phenotype was also investigated in filamentous *Anabaena* strains lacking Flv1A and Flv3A, the closest homologs of *Synechocystis* Flv1 and Flv3 proteins. Growth of the *Anabaena* Δ*flv1*A and Δ*flv3*A strains was likewise strongly inhibited by FL, indicating a conserved role for the Flv1 and Flv3 proteins in cyanobacteria (Paper I, Supplemental Fig. S1D).

The WT and mutant cells were treated with FL for up to 3 days and their gas exchange was monitored in real-time with MIMS so that the measurement light was also fluctuated to resemble the growth conditions. During high-light pulses, a strong O_2 uptake was observed in WT cells, indicating the function

of Flv1 and Flv3 proteins (Paper I, Fig. 2A). The Δ*flv1*/Δ*flv3* mutant showed low-light-stimulated O_2 uptake comparable to WT rate (Paper I, Fig. 2C). However, no high light induced O_2 uptake was recorded in the Δ*flv1*/Δ*flv3* mutant. The low-light-induced O_2 uptake in the Δ*flv1*/Δ*flv3* mutant was abolished upon addition of KCN, an inhibitor of Cox and Cyd, indicating the function of RTOs in the mutant (Paper I, Fig. 3). Compared to the WT, the Δ*flv1*/Δ*flv3* mutant showed only one-third of photosynthetic O_2 evolution and net photosynthesis rates during the low-light phases. Moreover, in contrast to WT, high-light pulses did not induce strong CO_2 uptake in Δ*flv1*/Δ*flv3*, indicating severe limitation in the delivery of light reaction products, ATP and NADPH, for the carbon assimilation pathway (Paper I, Fig. 2B, D).

The properties of electron transport and the photosynthetic apparatus were studied in more detail by analyzing the functional status of photosystems using Dual-PAM fluorometry. The Chl fluorescence originating from PSII and the P700 absorbance change demonstrating the status of the reaction center Chl of PSI were monitored simultaneously *in vivo*. The Δ*flv1*/Δ*flv3* mutant showed lower effective quantum yield of PSII and PSI, Y(II) and Y(I), respectively, compared with WT (Paper I, Fig. 5A-B and supplemental Fig. S3A). The Pm parameter, representing the maximal change of the P700 signal from the fully reduced to the fully oxidized state, was only 0.06 in the mutant cells, compared with 0.33 in the WT.

Lower Pm signal for the Δ*flv1*/Δ*flv3* mutant indicates a severe problem in PSI. To identify the target of damage in the reaction center more accurately, the donor and acceptor sides of PSI were investigated. The electron transfer from PC, used to reduce photo-oxidized $P700^+$, represents the donor (lumenal) side of PSI, and the reactions from oxidized $P700^+$ to ferredoxin are on the acceptor (cytosolic) side of PSI. Application of a strong saturating pulse during the high-light phases of FL was able to fully oxidize P700 in the WT, but not in the Δ*flv1*/Δ*flv3* mutant (Fig.5A-C). This demonstrated a strong acceptor-side limitation in the Δ*flv1*/Δ*flv3* mutant due to a lack of electron sinks downstream of PSI. An examination of constant light grown cells revealed that during the low- to high-light transition, P700 became strongly oxidized in the WT cells, reaching a steady-state oxidation level instantly (Paper I, supplemental Fig. S4). In contrast, the Δ*flv1*/Δ*flv3* mutant cells showed completely different $P700^+$ kinetics. First, rapid and transient peak oxidation of P700 was recorded, yet the cells reached a steady-state oxidation level only after approximately 15 s from the onset of the high-light phase (Paper I, Fig. 6C). Such a major delay in P700 oxidation in mutants confirmed the function of Flv1 and Flv3 as powerful electron sinks during the low- to high-light transitions of FL.

Furthermore, the photosystem modifications were reflected at the level of photosynthetic proteins detected by immunoblotting (Paper I, Fig. 7). PsaB, the core protein of the PSI reaction center, showed approximately half of the WT level in the Δ*flv1*/Δ*flv3* mutant after 4 days of growth under FL, when expressed on the protein basis. The amount of D1, the PSII reaction center protein, was diminished in the Δ*flv1*/Δ*flv3* mutant only after 7 days under FL. In addition, RbcL, the large subunit of Rubisco, showed a reduced level in the Δ*flv1*/Δ*flv3* mutant. The single mutants, Δ*flv1* and Δ*flv3*, showed changes in protein levels under FL similar to the Δ*flv1*/Δ*flv3* double mutant, and the complementation strain Δ*flv1*::*flv1* behaved as WT in terms of growth rate, biophysical measurements and protein content. Moreover, immunodetection of carbonyl groups introduced into the proteins was used as an indicator of oxidative modifications caused by ROS. The Δ*flv1*/Δ*flv3* cells showed increased amounts of carbonylated proteins compared to the WT upon shift from constant to FL, indicating more ROS production in the mutant cells resulting from the over-reduction of LET (Paper I, supplemental Fig. S6).

4.1.2 Lessons learned from transcript analysis of WT, Δ*flv1* and Δ*flv1*/Δ*flv3*

The effects of fluctuating light (FL 20/500) on gene expression in the WT strain as well as the Δ*flv1* and Δ*flv1*/Δ*flv3* mutants of *Synechocystis* were examined by using full genome DNA microarrays. In the WT, 48 hour exposure to FL 20/500 induced alterations in the expression of 108 genes compared to constant GL conditions. Particularly significant down-regulation of transcripts involved in carbon-concentrating mechanisms (CCMs) was observed. These include the genes in operon *ndhF3/ndhD3/cupA/cupS* encoding subunits for NDH-1$_3$ involved in CO_2 acquisition, *cmpA-D* operon encoding BCT1 and *sbtA/sbtB* operon encoding high affinity HCO_3^- transporters. In addition, transcript levels of the *flv4-2* operon and *flv3* were diminished. In contrast, several genes associated with high-light stress, *hliA-C* encoding high-light-inducible proteins (Hlips), the *hspA* chaperone, the *htrA* protease and the *sll1862-3* operon were induced in WT cells exposed to FL. Instead, the reductants and ATP from light reactions were likely directed to nitrogen assimilation, supported by the enhancement of genes encoding ATP-dependent nitrate/nitrite transporters (*nrtC-D*) and ferredoxin dependent nitrate reductase (*narB*). Unexpectedly, the ROS scavenging pathways were not affected. The differences in the transcript levels of numerous genes in the Δ*flv1*/Δ*flv3* double mutant treated with constant GL and FL for 48 hours are described in Paper II and in Figure 4.

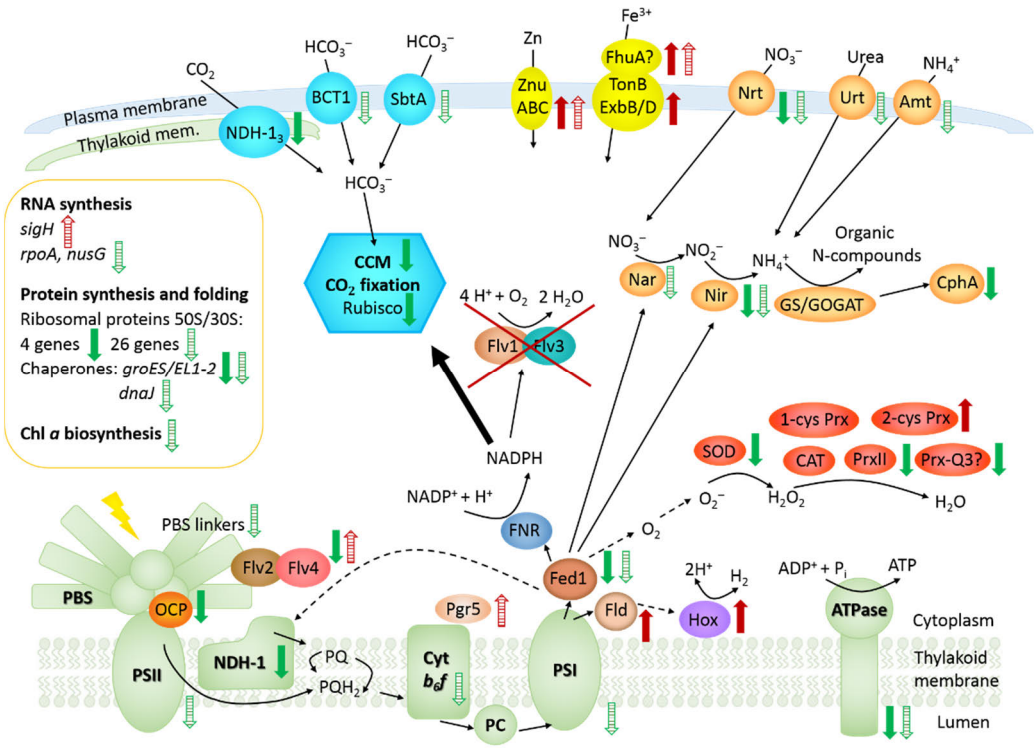

Figure 4. Effect of mutation of the *flv1* and *flv3* genes on gene expression after 48-hour treatment under constant GL (solid arrow) or under FL conditions (dashed arrow). Down-regulated (green arrows) and up-regulated (red arrows) genes related to pathways in photosynthesis, cell metabolism as well as gene and protein synthesis are shown.

Along with the Δ*flv1*/Δ*flv3* double mutant, differences in gene expression in the Δ*flv1* single mutant were also compared to the WT. Interestingly, many alterations were specific only to the Δ*flv1*/Δ*flv3* mutant, and not detected in Δ*flv1*. Among these, the *fed1* gene was significantly down-regulated only upon disruption of *flv3*, both under constant GL and FL. The transcript level of *fld*, the *slr0458* gene encoding a 2-PG phosphatase and several genes functioning in the homeostasis of metals were up-regulated solely in the Δ*flv1*/Δ*flv3* mutant but not in the single mutant under GL. These results suggest that certain transcripts are primarily affected due to the lack of *flv3*.

4.1.3 Neither overexpressed Flv1 nor Flv3 homo-oligomer alone is capable of O_2 photoreduction

In Paper I, I showed that single Δ*flv1* and Δ*flv3* mutants as well as the Δ*flv1*/Δ*flv3* double mutant strain have a similar growth phenotype and all mutants lack light induced O_2 uptake. However, in terms of protein levels

Flv1 and Flv3 have co-regulation, since in Δ*flv1* the level of Flv3 is lower than in WT, and *vice versa*, in Δ*flv3* the level of Flv1 is lower than in WT (Paper II, Fig. 3A). This raised the question of whether Flv1 and Flv3 could function also as homodimers, yet with restricted function due the low protein levels in the single mutants.

In order to distinguish whether crosswise downregulation of the Flv proteins in the single mutants is the cause for the lack of O_2 uptake, I constructed mutants overproducing only either Flv3 or Flv1. The Flv proteins were expressed under the strong *psbA2* promoter of *Synechocystis*. The obtained strain ∆*flv1*/oe*flv3* lack Flv1 and presumably express Flv3 homo-oligomers, and correspondingly, the ∆*flv3*/oe*flv1* strain lacks Flv3 but presumably express Flv1 homo-oligomers. In the ∆*flv1*/oe*flv3* strain, Flv3 was expressed 16-fold compared to WT and the homo-oligomer formation was confirmed with immunodetection upon separation with BN-PAGE (Paper II, Fig. 3A, 4). However, only a 0.68-fold expression of the Flv1 protein was reached in ∆*flv3*/oe*flv1* in comparison to the WT, indicating that the expression of Flv1 is dependent on the expression of Flv3 (Paper II, Fig. 3C). In a control strain ∆*flv1*::*flv1*, in which native Flv3 is present along with the Flv1 complementing the disrupted native Flv1, strong expression of Flv1 was achieved. It is noteworthy that the transcript level of *flv1* was similar both in ∆*flv3*/oe*flv1* and ∆*flv1*::*flv1* (Paper II, Fig. 3B).

As seen in Paper I, neither ∆*flv1* nor ∆*flv3* was able to grow under the FL 20/500 light regime. In contrast, the overexpression strains containing only the Flv1 or the Flv3 homo-oligomer were able to sustain growth under FL conditions (Paper II, Fig. 5). The growth difference in FL was linked to the PsaB protein content, which was strongly decreased in the ∆*flv1*/∆*flv3* mutant, while in the overexpression strains the PsaB amount was similar to WT (Paper II, Fig. 6B).

The next question was whether the improvement in the growth and PSI content of the overexpression strains was due to light-induced O_2 uptake owing to the Flv1 or Flv3 homo-oligomer. The gas exchange of the strains was monitored with MIMS using cells grown under the FL 20/500 regime (Paper II, Fig. 7). Both the overexpression strains and the deletion mutants demonstrated a lack of efficient O_2 photoreduction under the FL conditions. Intriguingly, unlike deletion strains, both overexpression strains demonstrated only about 11–13% lower net O_2 evolution rates and about a 30% decrease in CO_2 uptake compared with the WT, which explains the improved growth. Hence it appears that homo-oligomers of Flv1 or Flv3 are

not able function in O_2 photoreduction *in vivo*, but are involved in another electron transport route.

4.2 The *flv4-2* operon is controlled by several transcriptional and post-transcriptional regulators

Cyanobacteria show strong induction of specific genes in response to a shift from high to low C_i availability. The *flv4-2* operon is among the most upregulated transcripts in *Synechocystis,* and the crucial functions of the respective Flv2, Flv4 and Sll0218 proteins have been demonstrated under these conditions. In Paper III, I provide evidence concerning the transcriptional and post-transcriptional regulation of the *flv4-2* operon.

Four candidate ncRNAs were predicted to be associated with the *flv4-2* operon by using tiling microarray-based screening and differential RNA sequencing analysis of the *Synechocystis* genome (Georg et al. 2009; Mitschke et al. 2011). These comprise two asRNAs to *flv4*, As1_flv4 and As2_flv4, one asRNA to *flv2* named As_flv2, and one putative ncRNA designated as Ncr0080 (Paper III, Fig. 1A). As1_flv4 and As2_flv4 originate from the reverse complementary strand of *flv4* and As_flv2 from the antisense strand to the *flv2* gene. Ncr0080 originates from the intergenic space between *flv4* and the neighboring *ctpB* gene. In Paper III, the existence and lengths of these ncRNAs were verified by Northern blot analysis (Paper III, Fig. 1B) and the role of As1_flv4 was further studied.

4.2.1 Accumulation of the *flv4-2* operon mRNA and As1_flv4 transcripts is inversely correlated

Upon shift from HC to LC conditions, both *flv4-2* mRNA and respective proteins first appeared after 3 h (Paper III, Fig. 2). The *flv4-2* mRNA was most abundant after 24 h, whereas on the protein level, Flv2 and Flv4 reached the peak value after 12 h and Sll0218 only after 24 h. The transcript of As1_flv4 accumulated in a reverse manner. The transcript was present under HC conditions and reached the highest accumulation transiently 1-3 hours after the shift from HC to LC, after which it declined. The accumulation kinetics of As2_flv4 RNA was similar but relative abundance was lower. The As_flv2 transcript levels increased after 3 h upon the LC shift reaching a plateau, whereas the expression of Ncr0080 was transiently down-regulated for the first 6 h after the LC shift and recovered to the original HC level after 12 h.

The expression pattern suggested regulation of the *flv4-2* operon by As1_flv4, putatively by pairing with the target mRNA. Thus, the inversely

accumulated As1_flv4 was further studied by overexpressing the As1_flv4 transcript under an inducible *petJ* promoter. The increased amount of As1_flv4 RNA was apparently able to delay and reduce the transcript levels from the *flv4-2* operon (Paper III, Fig. 3). A promoter activity analysis with a bioluminescent luciferase reporter confirmed that the as1_flv4 promoter was transiently induced upon the LC shift, whereas the activity of the *flv4-2* promoter increased over time (Paper III, Fig. 4). Interestingly, both promoters were already active under HC.

4.2.2 Transcriptional regulators NdhR and Sll0822 control the *flv4-2* operon via different mechanisms

Because both the *flv4-2* operon promoter and the As1_flv4 RNA promoter were found to be C_i-controlled, two known C_i-dependent candidates for transcriptional regulators, NdhR and the AbrB-like protein Sll0822, were chosen for further analysis. A deletion mutant in *ndhR* accumulated reduced amounts of the *flv4-2* operon transcript, whereas expression of the As1_flv4 was not altered in Δ*ndhR* (Paper III, Fig. 5). Thus, it is conceivable that NdhR controls the promoter activity of the *flv4-2* operon.

In the Δ*sll0822* mutant, the expression of As1_flv4 was heavily enhanced under HC and shortly after the shift to LC conditions (Paper III, Fig. 6). The accumulation of *flv4-2* operon mRNA remained lower as compared with WT cells, supposedly due to post-transcriptional regulation by the enhanced As1_flv4 expression. These results indicate that Sll0822 is a negative transcriptional regulator for the promoter activity of As1_flv4.

4.3 Fed7 facilitates *Synechocystis* cells to manage photooxidative stress

Since *Synechocystis* has nine different ferredoxins and there is no consensus on the interaction partner(s) of FDPs, it raises the question of whether some of the low-abundance Feds could serve as electron donors for FDPs instead of or along with Fed1. Based on transcriptomics data, the most prominent candidate was Fed7. The *flv3* and *flv4-2* transcripts are enhanced upon low Ci availability, similar to *fed7* (Eisenhut et al. 2007). Moreover, *fed7* was the only *fed* gene found to be differentially regulated in the Δ*flv3* mutant (Hackenberg et al. 2009). In order to investigate whether Fed7 and Flv3 could possibly be part of the same electron transport pathway, I constructed a mutant deficient in Fed7 (Δ*fed7)*. The results did not indicate a direct connection, yet *fed7* seems to have an important role in a regulatory pathway used to manage photooxidative stress, as reported in Paper IV.

4.3.1 Loss of Fed7 negatively affects photosynthesis under photooxidative stress conditions

First, it was confirmed with RT-qPCR that the *fed7* transcript is enhanced in WT upon shift from HC to LC regime under GL (50 µmol photons m^{-2} s^{-1}), and even more upon shift to high light (HL, 200 µmol photons m^{-2} s^{-1}), indicating a function under photooxidative stress promoting conditions (Paper IV, Fig. 1). The tricistronic operon *ssl1263-sll0661-fed7* structure is highly conserved throughout cyanobacteria (Paper IV, Fig. 2A, supplemental Fig. A.6). Yet, Fed7 is not essential for the survival of the cells, since a fully segregated Δ*fed7* mutant was obtained and the growth of Δ*fed7* was not different from the WT under any conditions tested (HC+GL, HC+HL, LC+GL and LC+HL) (Paper IV, Fig. 2, Supplemental Fig. A.2).

However, Δ*fed7* showed alterations in pigment composition and photosynthetic performance. Chl *a* content was elevated in Δ*fed7* compared to the WT under LC conditions (Paper IV, Fig. 3B, Supplemental Fig. A.3). Moreover, while the WT downregulated the Chl *a* content under HL treatment, in the mutant cells this reduction was less distinct (Paper IV, Fig. 3C). Accordingly, the PsaB level of Δ*fed7* remained the same under HL as under GL, whereas in the WT PsaB was reduced (Paper IV, Fig. 6, 7). In addition, elevated Pm values pointed to increased PSI amount in the Δ*fed7* mutant in comparison to the WT (0.47 and 0.39, respectively).

Even though D1 amounts in Δ*fed7* were only slightly lower than in the WT cells upon HL treatment, Δ*fed7* showed lower Fv/Fm ratios compared to the WT, indicating a reduced maximum quantum yield of PSII (0.20 and 0.28, respectively) (Paper IV, Fig. 6). PSII-dependent net photosynthesis measured by O_2 evolution rate in the presence of DMBQ as an artificial electron acceptor was also significantly declined in the mutant (Paper IV, Fig. 4C-D). Moreover, in HL grown Δ*fed7* cells, the PSI effective yield, Y(PSI), decreased and PSI donor side limitation Y(ND) increased at lower light intensities than in the WT (Paper IV, Fig. 4F). It is conceivable that the PSI photochemistry is restricted in Δ*fed7* due to the low capacity of PS II (Paper IV, Fig. 4E).

Along with reduced PSII activity, Δ*fed7* contained reduced PBS, Flv2/4 and Sll0218 amounts (Paper IV, Fig. 4, 7; Table 7). The amount of Flv3 was not altered. The loss of Flv2/4 protection was compensated for by increased carotenoid levels in the Δ*fed7*, except that OCP was not altered (Paper IV, Fig. 3, 7). The Δ*fed7* mutant characteristics also included impaired induction of the CCM components under LC conditions.

4.3.2 Two transcriptional regulators controlling *psaAB* are differentially expressed in the Δ*fed7* mutant

Interestingly, according to DNA microarray analysis, the expression of four transcription factors was differentially regulated in Δ*fed7* (Paper IV, Table 2-3). The transcript levels of a gene encoding a regulator of PBS association (RpaB) were significantly reduced. It has been shown that RpaB regulates HL stress responsive genes and PSI genes (Kappell and van Waasbergen 2007; Seino et al. 2009). In contrast, *slr0846*, which encodes an Rrf2-type transcriptional regulator, was up-regulated in the Δ*fed7*. Slr0846 acts as transcriptional activator for both the *psaA* and *psaB* genes (Midorikawa et al. 2009). The expression of the *psaAB* operon was unaltered in Δ*fed7*. RpaB and Slr0846 are both required for correct light dependent expression of *psaAB*, perhaps in concert with other, individually activated transcriptional regulators. Moreover, *sll0649*, which encodes for a putative orphan response regulator, was down-regulated and *hik5,* which encodes a two-component histidine kinase, were up-regulated in the Δ*fed7*. Sll0646 has been shown to be involved in cadmium tolerance and metal homeostasis (Chen et al. 2014).

5 DISCUSSION

5.1 Electron transport pathways in the thylakoid membrane of cyanobacteria are highly complex

In order to grow, produce biomass and compete with other species, cyanobacteria require a sufficient amount of light to efficiently run their photosynthetic light reactions. On the other hand, the absorption of excessive light energy may lead to over-reduction of photosystems and oxidative damage to the photosynthetic machinery. To balance the absorbed light energy with the consumption capacity, several alternative electron transport routes and a multitude of photoprotective mechanisms have evolved. The regulation of photosynthetic processes is especially important under changing light conditions.

5.1.1 The hetero-oligomers of Flv1 and Flv3 operate transiently during rapid changes in light intensity

Cyanobacterial Flv1 and Flv3 proteins form an important alternative electron transport route by directing electrons to O_2, thus functioning as a safety valve for the electrons. Flv1 and Flv3 allow the cells to avoid acceptor side limitation of PSI by reoxidizing $NADPH^+$ and consequently preventing the over-reduction of the LET chain (Paper I, II). The scarcity of electron acceptors downstream of PSI may lead to photoinhibition of PSI. Unlike for PSII, there is no efficient repair machinery for PSI, and consequently, the recovery of photodamaged PSI is very slow (Sonoike et al. 1995; Munekage et al. 2002; Kudoh and Sonoike 2002; Tiwari et al. 2016). Accordingly, Flv1 and Flv3 play a crucial role in protecting PSI by dissipating excess electrons in a harmless way (Figure 5). Indeed, a severe reduction in PSI proteins was characteristic for the FL-treated *Synechocystis* cells deficient in Flv1 and Flv3 (Paper I, II). The impaired performance was reflected in low net photosynthesis in the Δ*flv1*/Δ*flv3*, leading to decreased CO_2 fixation and slow growth (Paper I, II).

Compared to other photoprotective mechanisms in cyanobacteria, the Flv1 and Flv3 mediated pathway functions in a very fast time scale and with high capacity, which makes it crucial when encountering rapid changes in light intensity. It is noteworthy that the O_2 photoreduction capacity of membrane bound RTOs, Cyd and Cox might be limited due to a crowded thylakoid membrane, a problem that soluble Flv1 and Flv3 do not encounter (Ermakova et al. 2016). Along with CET, an important role for Flv1 and Flv3 is to regulate the NADPH/ATP balance by consuming the NADPH and hence providing cyanobacterial cells with extra ATP.

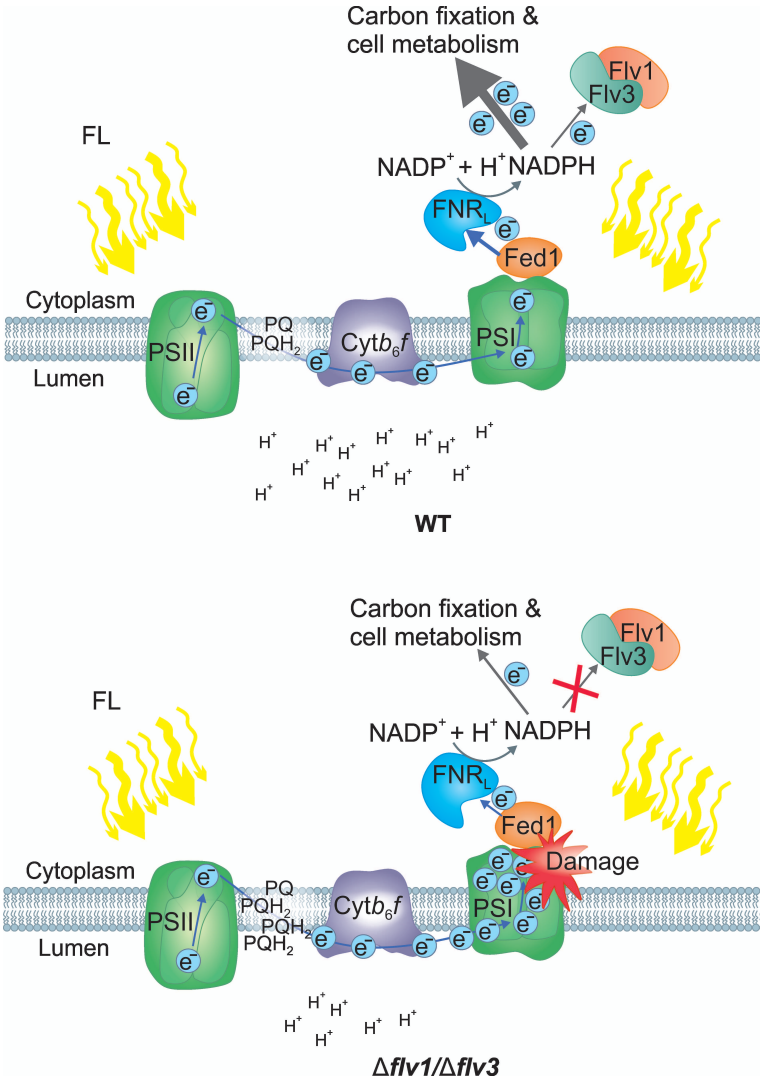

Figure 5. The role of Flv1 and Flv3 in photoprotection of PSI under fluctuating light. Flv1 and Flv3 are required as an electron sink beyond PSI. Upon the sudden shift from low to high light, the Δ*flv1*/Δ*flv3* mutant fails to consume the electrons on the acceptors side of PSI, leading to over-reduction of PSI electron transport chain and blockage of the linear electron flow. Therefore, the PSI is the primary target of damage, and supposedly the iron-sulfur centers of PSI are destroyed. Impairment of photosynthetic light reactions in the Δ*flv1*/Δ*flv3* mutant is accompanied by lower carbon fixation as well as reduced nitrogen and sulfur assimilation capacities.

It seems that during evolution, the function of FDPs has been replaced by other mechanisms in flowering plants. It is conceivable that plants try to avoid wasting electrons, having therefore developed other mechanisms to protect PSI. In *A. thaliana*, PGR5 has been shown to be important under FL (Tikkanen et al. 2010; Suorsa et al. 2012). Intriguingly, Δ*pgr5* mutant in

Synechocystis did not show any specific phenotype under FL, instead growing just as well as the WT (Paper I, supplemental Fig. S1). Furthermore, the more efficient H_2O_2 scavenging systems in plants may have compensated for the loss of FDPs (Matthijs et al. 2012). In plants, ROS play important role in signaling, not only in the chloroplast but also in nuclear gene expression and inducing defense mechanisms (Nott et al. 2006). Especially H_2O_2 has a relatively long lifetime and may initiate signaling cascades and activate CET (Karpinski et al. 2003; Strand et al. 2015). Thus, it can be hypothesized that the avoidance of producing of H_2O_2 achieved with the FDPs might not be as beneficial to plants as it is for cyanobacteria.

5.1.2 *In vitro* studies did not reveal the whole story: the functional organization of Flv1 and Flv3 *in vivo*

In Paper I, I showed that the single Δ*flv1* and Δ*flv3* mutants as well as the Δ*flv1*/Δ*flv3* double mutant have a similar growth phenotype in FL and all mutants lack light induced O_2 uptake. Based on this data and earlier studies, it has been assumed that Flv1 and Flv3 form a heterodimer in order to function. In fact, FDPs always function as dimers or tetramers since the head-to-tail organization is essential for the electron transfer to take place (Vicente et al. 2008a). Contradictory to the probable heterodimer formation is the fact that the transcriptional regulation of *flv1* and *flv3* is very different. The genes of *flv1* and *flv3* are separately located in the genome of *Synechocystis*, and the transcript level of *flv1* is not induced by low C_i or light like *flv3*, instead *flv1* expression is relatively constant (Allahverdiyeva et al. 2015). The different regulation of the subunits of one protein complex is rather unexpected. On the protein level, Flv1 and Flv3 have co-regulation to some extent (Paper II). It seems that the Flv1 protein is expressed only in the presence of Flv3, and the control might be mediated by a post-transcriptional control mechanism. Indeed, *cis*-encoded asRNA in the opposite strand of the *flv1* gene has been predicted (Mitschke et al. 2011). I was unfortunately not able to detect the asRNA with Northern blot in order to examine the possible mode of action of the asRNA in regulation of *flv1* (data not published). Furthermore, Hackenberg and co-workers (2009) showed separate functions for Flv1 and Flv3 under certain conditions.

Taking into account these facts of discrepancy in Flv1 and Flv3 regulation, there were several ways to clarify the organization of these proteins. (i) It is possible that Flv1 and Flv3 predominantly form heterodimers (or hetero-oligomers). Even though the transcriptional control of *flv1* and *flv3* varies, the post-transcriptional and/or post-translational regulations may ensure an adequate level of both subunits to form a functional Flv1/Flv3 heterodimer when required. Perhaps Flv3 is more prone to damage and has faster

turnover rate, and thus also has a more active transcription rate than *flv1*. (ii) Flv1 and Flv3 may also function as homo-oligomers, but their function is masked by the low level in the single deletion mutants. (iii) Flv1 and Flv3 may form both homo- and hetero-dimers depending on the environmental trigger. Interestingly, Ermakova et al. (2014) were able to show that in *Anabaena* the heterocyst-specific Flv3B is capable of functioning independently from Flv1B, since only the Δ*flv3B* mutant, but not Δ*flv1B*, was deficient in O_2 photoreduction. (iv) One possible scheme is that Flv1 acts as an auxiliary protein, helping in the organization of the Flv3 homodimer or homotetramer. (v) Could Flv1 and/or Flv3 have interactions with Flv2 and Flv4? These are the questions I aimed to answer in Paper II.

Biochemical analysis with BN-PAGE confirmed the presence of the Flv3 dimer in strains deficient in Flv1, indicating a homodimer formation of Flv3 *in vivo* (Paper II, Allahverdiyeva et al. 2011). Previously, the O_2 reduction function of Flv3, independent from Flv1, was shown *in vitro* with recombinant Flv3 (Vicente et al. 2002). In addition, tetramer formation of Flv3 *in vivo* cannot be ruled out, since Flv3 expressed in *E. coli* was also able to form tetramers (Paper II).

The excess and exclusive formation of the Flv3 homodimer in the Δ*flv1*/oe*flv3* and moderate expression of the Flv1 homodimer in the Δ*flv3*/oe*flv1* could significantly improve, but not fully complement, the growth phenotype of both strains under FL (Paper II). Neither strain was capable of performing light-induced O_2 uptake, and therefore the improved growth phenotype was not due to a gain of function of the Mehler-like reaction. Consequently, both Flv1 and Flv3 are necessary for the O_2 photoreduction *in vivo*. However, the recovery of CO_2 uptake and net photosynthesis in the overexpression strains strongly suggest that Flv1 and Flv3 homo-oligomers mediate another electron transport pathway. Although this is highly speculative, Flv1 or Flv3 may also be able to use NO as electron acceptor. Moreover, *flv1* gene transcription was induced upon short term (1 h), and both *flv1* and *flv3* after long (12 h) term nitrosative stress (Gonçalves et al. 2011). Yet, considering the low expression of *flv1*, it is more likely that in the WT cells the abundant Flv3 is responsible for forming the homo-oligomer in addition to the Flv1/3 heterodimer. To conclude, Flv3 homo-oligomer formation was shown in a mutant lacking the opportunity to form hetero-oligomers. Thus, it remains to be shown whether the Flv3 mediated route is operational in the WT and what the ratio is between hetero- and homo-oligomers. In addition, the putative coorganization of Flv1, Flv3 and Flv2/4 remains to be elucidated.

5.1.3 Auxiliary electron transport pathways may partially compensate for each other

The global transcript profiles of the WT and mutant strains (Papers II and IV) demonstrated well the complexity of different auxiliary electron transport routes in *Synechocystis* and the ways they can partially compensate for each other (Figure 6). The compensatory abilities are evident based on the fact that none of the mutations in the genes participating in the auxiliary electron transport routes is lethal. Nevertheless, all of the electron transport pathways are important under certain conditions to ensure the optimal acclimation of the cyanobacterial cells. In addition, the state transitions and changes in photosystem stoichiometry participate in fine-tuning the regulatory mechanisms. The co-operation of all of the regulatory and protective mechanisms is not yet fully understood.

The global analysis of gene transcripts revealed that when one important electron sink, the Flv1/Flv3 is disrupted, *Synechocystis* cells down-regulate the components of the whole LET chain from PSII to Fed1 under FL (Paper II). Rather unexpectedly, OCP and Flv2/Flv4 mediated photoprotective pathways were also diminished in Δ*flv1*/Δ*flv3* under constant GL, and Flv2/Flv4 also under FL. It might simply be a consequence of diminished PSII and PBS contents or it could indicate cooperation between Flv1/Flv3 and Flv2/4 pathways. As the LET was diminished, another major consumer of the energy from the light reactions, the CO_2 fixation pathway, was downregulated. Similarly, the WT showed down-regulation of LET and CCM components under FL in comparison to constant GL. In addition, the WT showed up-regulation of nitrogen assimilation pathways, which was putatively used by WT cells as an alternative electron sink downstream of PSI. In contrast, Δ*flv1*/Δ*flv3* grown under the FL was unable to enhance nitrogen assimilation pathways, perhaps due to the downregulation of Fed1 and ATPase. Under constant GL, Δ*flv1*/Δ*flv3* promoted a shortcut from PSI to Fld, presumably directing the PSI electrons to an alternative electron transport pathway involving Fld and bidirectional hydrogenase (Gutekunst et al. 2014). It has also been suggested that the bidirectional hydrogenase may function as electron valve (Appel et al. 2000).

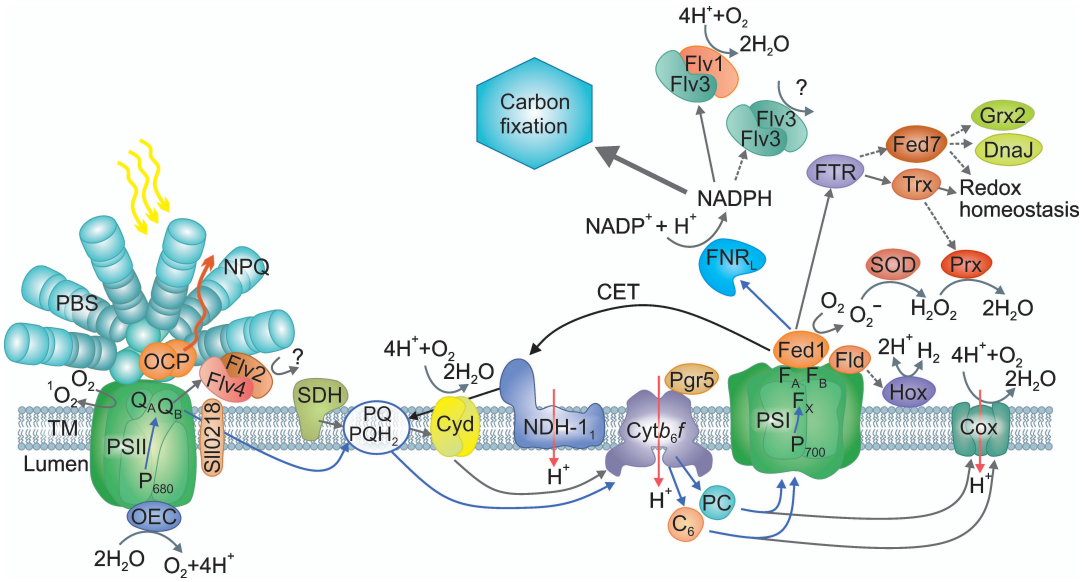

Figure 6. The complexity of the alternative electron transport routes and regulatory pathways in the cyanobacterial thylakoid membranes (TM). Blue arrows indicate linear electron transport, black arrows cyclic electron transport (CET) and red arrows proton translocation across the membrane. Grey solid and dotted arrows depict verified and unverified electron transport, respectively. Flv1/Flv3 and the terminal oxidases, Cyd and Cox, mediate O_2 photoreduction. The acceptors of the Flv3/Flv3 homo-oligomer and the Flv2/Flv4 heterodimer are unknown. NDH-1_1 and Cytb_6f mediate CET. OCP is required for the NPQ from PSII, and Sll0218 for the stabilization of the PSII biogenesis. Flavodoxin (Fld) substitutes ferredoxin (Fed1) under iron deprivation and certain stress conditions. Bidirectional hydrogenase (Hox) may also function as electron valve under certain conditions. Thioredoxin (Trx) and Fed7 mediated pathways may contribute to the redox control of the cells and regulation of photosynthesis. Superoxide dismutase (SOD) and peroxidases (Prx) function as ROS scavengers.

5.2 Changes in environmental cues control the expression of electron transport components by a number of different mechanisms

Under changing conditions, it is vital to combine various environmental and metabolic cues to initiate the correct acclimation processes. A multitude of signaling cascades provide feedback from the redox-status of the LET components and control the acclimation to different conditions. Transcriptional regulators mediate the signals to gene expression, which can further be controlled post-transcriptionally through regulatory ncRNAs. The fine-tuning of protein synthesis is important in order for cells to respond only to long term environmental changes instead of wasting energy on unnecessary acclimation processes upon transient changes. In Papers III and IV, I provide novel insights to some of these regulatory pathways.

The Flv2/Flv4 mediated electron valve is important for the photoprotection of PSII under low C_i conditions, and becomes crucial under combined C_i limitation and HL conditions (Zhang et al. 2012; Bersanini et al. 2014). In Paper III it is shown that *flv4-2* operon is under the control of NdhR transcription factor. NdhR belongs to the LysR family of transcriptional regulators, and it is a global regulator of carbon assimilation in cyanobacteria. Recently, the binding regions for *Anabaena* PacR, the closest homolog of *Synechocystis* NdhR, were identified upstream of the *flv1A* and *flv4* genes (Picossi et al. 2015). In *Synechocystis*, NdhR binding sites in the promoter regions of the *flv4-2* operon could not be identified (Paper III), thus it is probable that, unlike in *Anabaena*, the regulation by NdhR in *Synechocystis* is indirect.

In addition to control via transcriptional regulator(s), the *flv4-2* operon is under the control of several small ncRNAs. The delicately regulated asRNA As1_flv4 establishes a transient threshold for *flv4-2* expression in the initial phase of acclimation to reduced C_i levels (Paper III). The promoter activity studies revealed that both the asRNA and *flv4-2* mRNA are transcribed under HC conditions. Presumably, the asRNA As1_flv4 pairs with the *flv4-2* mRNA, and the formed double-stranded RNA is then rapidly degraded. As long as the asRNA As1_flv4 transcript is more abundant, the target *flv4-2* mRNA is being titrated out. Only when the C_i deprived condition is prolonged, the *flv4-2* mRNA becomes available for ribosome binding and protein synthesis. Therefore, As1_flv4 prevents the premature synthesis of the proteins from the *flv4-2* operon by delaying the response to environmental cues. Only when the Flv2, Flv4 and Sll0218 mediated protection for PSII is required, they are adequately synthesized. Post-transcription fine-tuning of the protein expression is vital to avoid unnecessary and costly protein synthesis. In the case of iron binding FDPs, the avoidance of nonessential synthesis of these proteins is crucial, since aquatic environments are frequently limited in biologically available iron.

It is conceivable that the *flv4-2* operon could, in addition, be redox-regulated by the PQ pool. Under combined LC and HL conditions, the PQ pool becomes highly-reduced, which might induce a signaling cascade for the redox-controlled components. As shown in Paper IV, one of the players in the redox regulation network is Fed7. In the absence of Fed7, the cyanobacterial cells cannot properly respond to these photooxidative stress promoting conditions. The Δ*fed7* mutant fails to enhance the CCM and Flv2/4 components in LC conditions. In addition, the proper downregulation of PSI and Chl *a* biosynthesis under HL is impaired. The Δ*fed7* mutants are hence more susceptible to the generation of ROS. Therefore, it is likely that Fed7 functions in coping with photooxidative stress via a regulatory pathway. These signal

pathways might be connected with the RpaB and Slr0846 transcriptional regulators in order to ensure optimal photosynthetic performance.

It is also possible that Fed7 constitutes a redox-responsive regulatory element in photoprotection. Fed7 is the closest homolog to a distinct domain in chloroplast DnaJ-like (CDJ) proteins in *C. reinhardtii* (Paper IV, supplemental Fig. A.6). It has been suggested that the CDJ3–5 proteins function as redox switches for interaction with HSP70B, a major chloroplast chaperone assisting in the repair of photodamaged D1 (Dorn et al. 2010). Furthermore, interaction with Fed7 and DnaJ-like protein has been identified (Cassier-Chauvat and Chauvat 2014). This supports our hypothesis that in photosynthetic eukaryotes, the Fed7-domain is fused with the DnaJ-domain forming a single redox-responsive protein. Furthermore, Cassier-Chauvat and Chauvat (2014) suggested that upon oxidative stress, the [4Fe4S] cluster of Fed7 is converted into a [3Fe4S] center by liberating one of the active site cysteines, C56. Thereby the C56 cysteine could form a redox responsive disulfide bridge with the C100 cysteine and Fed7 would functionally resemble a thioredoxin A (TrxA) -like protein.

6 CONCLUSIONS AND FUTURE PERSPECTIVES

The results presented in Papers I-IV unquestionably indicate the importance of FDPs and other auxiliary electron transport components in cyanobacteria in the maintenance of the optimal performance of oxygenic photosynthesis in varying environmental conditions. To conclude:

1. In my thesis work, a biological significance for the seemingly wasteful photosynthetic electron transfer to molecular oxygen mediated by the FDPs Flv1 and Flv3 is presented for the first time. (Paper I)

a) Under fluctuating light, a characteristic light condition in aquatic habitats, an Flv1 and Flv3 mediated Mehler-like reaction enables the growth of cyanobacteria (*Synechocystis* and *Anabaena*) by safeguarding the photosynthetic apparatus, particularly PSI.

b) Under constant light conditions (including high light intensities) Flv1 and Flv3 are dispensable, thus suggesting that the Flv1/Flv3-dependent Mehler-like reaction occurs only transiently during darkness-to-high-light and low-light-to-high-light shifts. Under steady state, the Mehler-like reaction is not significant, and it is compensated with other protective mechanisms.

2. Flv1 and Flv3 can also form homo-oligomers, whose function is required in the acclimation of cells to stress conditions, e.g. FL. Based on my results it became evident that the terminal acceptor of Flv1 and Flv3 homo-oligomers is not O_2. Considering the small quantity of Flv1, it is probable that mostly Flv3 homo-oligomers are functional in WT cells. The components of this electron transport pathway remain to be identified. In addition, the cooperation of all four FDPs in *Synechocystis* requires further characterization. (Paper II)

3. A small regulatory RNA, As1_flv4, is involved in the C_i-dependent regulation of the Flv2/4 proteins by establishing a safety threshold to prevent an unfavorable expression of the *flv4-2* operon. Only when conditions promoting oxidative stress continue, such as upon C_i limitation and high light, the Flv2/4 electron valve is synthetized. (Paper III)

4. Through a putative interaction with FTR and DnaJ, Fed7 mediates the redox signals from the photosynthetic LET chain and functions indirectly as a redox-dependent regulator. (Paper IV)

The alternative electron transport routes and especially FDPs have become an increasingly popular topic in photosynthesis research. Besides the interest

for the basic research on photosynthesis, cyanobacteria are appealing platforms for biotechnological applications. While the efficiency of photosynthetic charge separation is 100%, generally only about 1-2% of absorbed solar energy is stored in the biomass (Ghirardi et al. 2009). It is tempting to consider what the implications could be if this superb conversion efficiency could, to some extent, be directed to solar fuel or high value compound production instead of being stored as biomass. Therefore, it is essential to identify the destination of the electrons derived from primary charge separation. Flv1 and Flv3 form an important photosynthetic electron sink, but like everything in nature they have a specific purpose: their function is transient and is essential for the survival of the cells under natural light conditions. However, during evolution FDPs were replaced by less wasteful photoprotection mechanisms in plants, perhaps simultaneously with the transition of life from aquatic habitats to the land.

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REFERENCE

Allahverdiyeva Y, Ermakova M, Eisenhut M, Zhang P, Richaud P, Hagemann M, Cournac L, Aro E (2011) Interplay between flavodiiron proteins and photorespiration in *Synechocystis* sp. PCC 6803. J Biol Chem 286: 24007-24014

Allahverdiyeva Y, Mustila H, Ermakova M, Bersanini L, Richaud P, Ajlani G, Battchikova N, Cournac L, Aro E (2013) Flavodiiron proteins Flv1 and Flv3 enable cyanobacterial growth and photosynthesis under fluctuating light. Proc Natl Acad Sci U S A 110: 4111-4116

Allahverdiyeva Y, Suorsa M, Tikkanen M, Aro E (2015) Photoprotection of photosystems in fluctuating light intensities. J Exp Bot 66: 2427-2436

Anderson SL, McIntosh L (1991) Light-activated heterotrophic growth of the cyanobacterium *Synechocystis* sp. strain PCC 6803: a blue-light-requiring process. J Bacteriol 173: 2761-2767

Angeleri M, Muth-Pawlak D, Aro E, Battchikova N (2016) Study of O-phosphorylation sites in proteins involved in photosynthesis-related processes in *Synechocystis* sp. strain PCC 6803: application of the SRM approach. J Proteome Res 15: 4638-4652

Appel J, Phunpruch S, Steinmüller K, Schulz R (2000) The bidirectional hydrogenase of *Synechocystis* sp. PCC 6803 works as an electron valve during photosynthesis. Arch Microbiol 173: 333-338

Aro EM, Virgin I, Andersson B (1993) Photoinhibition of Photosystem II. Inactivation, protein damage and turnover. Biochim Biophys Acta 1143: 113-134

Asada K (1999) The water-water cycle in chloroplasts: scavenging of active oxygens and dissipation of excess photons. Annu Rev Plant Physiol Plant Mol Biol 50: 601-639

Badger MR, Price GD (2003) CO_2 concentrating mechanisms in cyanobacteria: molecular components, their diversity and evolution. J Exp Bot 54: 609-622

Badger MR, Price GD, Long BM, Woodger FJ (2006) The environmental plasticity and ecological genomics of the cyanobacterial CO_2 concentrating mechanism. J Exp Bot 57: 249-265

Battchikova N, Eisenhut M, Aro E (2011a) Cyanobacterial NDH-1 complexes: novel insights and remaining puzzles. Biochim Biophys Acta 1807: 935-944

Battchikova N, Wei L, Du L, Bersanini L, Aro E, Ma W (2011b) Identification of novel Ssl0352 protein (NdhS), essential for efficient operation of cyclic electron transport around photosystem I, in NADPH:plastoquinone oxidoreductase (NDH-1) complexes of *Synechocystis* sp. PCC 6803. J Biol Chem 286: 36992-37001

Beckmann K, Messinger J, Badger MR, Wydrzynski T, Hillier W (2009) On-line mass spectrometry: membrane inlet sampling. Photosynth Res 102: 511-522

Belkin S, Mehlhorn RJ, Packer L (1987) Proton gradients in intact cyanobacteria. Plant Physiol 84: 25-30

Bendall DS, Manasse RS (1995) Cyclic photophosphorylation and electron transport. Biochim Biophys Acta 1229: 23-38

Berger S, Ellersiek U, Steinmüller K (1991) Cyanobacteria contain a mitochondrial complex I-homologous NADH-dehydrogenase. FEBS Lett 286: 129-132

Bernát G, Appel J, Ogawa T, Rögner M (2011) Distinct roles of multiple NDH-1 complexes in the cyanobacterial electron transport network as revealed by kinetic analysis of $P700^+$ reduction in various *ndh*-deficient mutants of *Synechocystis* sp. strain PCC6803. J Bacteriol 193: 292-295

Berry S, Schneider D, Vermaas WFJ, Rögner 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

Bersanini L, Allahverdiyeva Y, Battchikova N, Heinz S, Lespinasse M, Ruohisto E, Mustila H, Nickelsen J, Vass I, Aro E (2017) Dissecting the photoprotective mechanism encoded by the *flv4-2* operon: a dstinct contribution of Sll0218 in photosystem II stabilization. Plant Cell Environ 40: 378-389

Bersanini L, Battchikova N, Jokel M, Rehman A, Vass I, Allahverdiyeva Y, Aro E (2014) Flavodiiron protein Flv2/Flv4-related photoprotective mechanism dissipates excitation pressure of PSII in

cooperation with phycobilisomes in Cyanobacteria. Plant Physiol 164: 805-818

Bhaya D, Bianco NR, Bryant D, Grossman A (2000) Type IV pilus biogenesis and motility in the cyanobacterium *Synechocystis* sp. PCC6803. Mol Microbiol 37: 941-951

Birungi M, Folea M, Battchikova N, Xu M, Mi H, Ogawa T, Aro E, Boekema EJ (2010) Possibilities of subunit localization with fluorescent protein tags and electron microscopy examplified by a cyanobacterial NDH-1 study. Biochim Biophys Acta 1797: 1681-1686

Blankenship RE (2001) It takes two to tango. Nat Struct Biol 8: 94-95

Bonaventura C, Myers J (1969) Fluorescence and oxygen evolution from *Chlorella pyrenoidosa*. Biochim Biophys Acta 189: 366-383

Bottin H, Lagoutte B (1992) Ferredoxin and flavodoxin from the cyanobacterium *Synechocystis* sp PCC 6803. Biochim Biophys Acta 1101: 48-56

Boulay C, Abasova L, Six C, Vass I, Kirilovsky D (2008) Occurrence and function of the orange carotenoid protein in photoprotective mechanisms in various cyanobacteria. Biochim Biophys Acta 1777: 1344-1354

Bricker TM, Roose JL, Fagerlund RD, Frankel LK, Eaton-Rye JJ (2012) The extrinsic proteins of Photosystem II. Biochim Biophys Acta 1817: 121-142

Campbell D, Hurry V, Clarke AK, Gustafsson P, Oquist G (1998) Chlorophyll fluorescence analysis of cyanobacterial photosynthesis and acclimation. Microbiol Mol Biol Rev 62: 667-683

Cardona T, Murray JW, Rutherford AW (2015) Origin and evolution of water oxidation before the last common ancestor of the cyanobacteria. Mol Biol Evol 32: 1310-1328

Cassier-Chauvat C, Chauvat F (2014) Responses to oxidative and heavy metal stresses in cyanobacteria: recent advances. Int J Mol Sci 16: 871-886

Cassier-Chauvat C, Dive V, Chauvat F (2017) Cyanobacteria: photosynthetic factories combining biodiversity, radiation resistance, and genetics to facilitate drug discovery. Appl Microbiol Biotechnol 101: 1359-1364

Chen L, Zhu Y, Song Z, Wang J, Zhang W (2014) An orphan response regulator Sll0649 involved in cadmium tolerance and metal homeostasis in photosynthetic *Synechocystis* sp. PCC 6803. J Proteomics 103: 87-102

Chen X, Schreiber K, Appel J, Makowka A, Fähnrich B, Roettger M, Hajirezaei MR, Sönnichsen FD, Schönheit P, Martin WF, Gutekunst K (2016) The Entner-Doudoroff pathway is an overlooked glycolytic route in cyanobacteria and plants. Proc Natl Acad Sci U S A 113: 5441-5446

Chew KW, Yap JY, Show PL, Suan NH, Juan JC, Ling TC, Lee D, Chang J (2017) Microalgae biorefinery: High value products perspectives. Bioresour Technol 229: 53-62

Chukhutsina V, Bersanini L, Aro E, van Amerongen H (2015a) Cyanobacterial *flv4-2* operon-encoded proteins optimize light harvesting and charge separation in photosystem II. Mol Plant 8: 747-761

Chukhutsina V, Bersanini L, Aro E, van Amerongen H (2015b) Cyanobacterial light-harvesting phycobilisomes uncouple from photosystem I during dark-to-light transitions. Sci Rep 5: 14193

Cuellar-Bermudez SP, Garcia-Perez JS, Rittmann BE, Parra-Saldivar R (2015) Photosynthetic bioenergy utilizing CO_2: an approach on flue gases utilization for third generation biofuels. Journal of Cleaner Production 98: 53-65

Dang K, Plet J, Tolleter D, Jokel M, Cuiné S, Carrier P, Auroy P, Richaud P, Johnson X, Alric J, Allahverdiyeva Y, Peltier G (2014) Combined increases in mitochondrial cooperation and oxygen photoreduction compensate for deficiency in cyclic electron flow in *Chlamydomonas reinhardtii*. Plant Cell 26: 3036-3050

de Lorimier RM, Smith RL, Stevens SE (1992) Regulation of phycobilisome structure and gene expression by light intensity. Plant Physiol 98: 1003-1010

DeRuyter Y, Fromme P (2008) Molecular structure of the photosynthetic apparatus. In Herrero A, Flores E (eds), The cyanobacteria: Molecular biology, genomics and evolution. Caister Academic Press, Norfolk, UK, pp 217-268

Di Rienzi SC, Sharon I, Wrighton KC, Koren O, Hug LA, Thomas BC, Goodrich JK, Bell JT, Spector TD, Banfield JF, Ley

RE (2013) The human gut and groundwater harbor non-photosynthetic bacteria belonging to a new candidate phylum sibling to Cyanobacteria. Elife 2: e01102

Dorn KV, Willmund F, Schwarz C, Henselmann C, Pohl T, Hess B, Veyel D, Usadel B, Friedrich T, Nickelsen J, Schroda M (2010) Chloroplast DnaJ-like proteins 3 and 4 (CDJ3/4) from *Chlamydomonas reinhardtii* contain redox-active Fe-S clusters and interact with stromal HSP70B. Biochem J 427: 205-215

Drews G (2011) The evolution of cyanobacteria and photosynthesis. In Peschek GA, Obinger C, Renger G (eds), Bioenergetic processes of cyanobacteria - From evolutionary singularity to ecological diversity. Springer, The Netherlands, pp 265-284

Dühring U, Axmann IM, Hess WR, Wilde A (2006) An internal antisense RNA regulates expression of the photosynthesis gene *isiA*. Proc Natl Acad Sci U S A 103: 7054-7058

Eisenhut M, Aguirre von Wobeser E, Jonas L, Schubert H, Ibelings BW, Bauwe H, Matthijs HCP, Hagemann M (2007) Long-term response toward inorganic carbon limitation in wild type and glycolate turnover mutants of the cyanobacterium *Synechocystis* sp. strain PCC 6803. Plant Physiol 144: 1946-1959

Eisenhut M, Kahlon S, Hasse D, Ewald R, Lieman-Hurwitz J, Ogawa T, Ruth W, Bauwe H, Kaplan A, Hagemann M (2006) The plant-like C2 glycolate cycle and the bacterial-like glycerate pathway cooperate in phosphoglycolate metabolism in cyanobacteria. Plant Physiol 142: 333-342

Eisenhut M, Ruth W, Haimovich M, Bauwe H, Kaplan A, Hagemann M (2008) The photorespiratory glycolate metabolism is essential for cyanobacteria and might have been conveyed endosymbiontically to plants. Proc Natl Acad Sci U S A 105: 17199-17204

Elhai J, Wolk CP (1988) Conjugal transfer of DNA to cyanobacteria. Methods in Enzymology 167: 747-754

Ermakova M, Battchikova N, Allahverdiyeva Y, Aro E (2013) Novel heterocyst-specific flavodiiron proteins in *Anabaena* sp. PCC 7120. FEBS Lett 587: 82-87

Ermakova M, Battchikova N, Richaud P, Leino H, Kosourov S, Isojärvi J, Peltier G, Flores E, Cournac L, Allahverdiyeva Y, Aro E (2014) Heterocyst-specific flavodiiron protein Flv3B enables oxic diazotrophic growth of the filamentous cyanobacterium *Anabaena* sp. PCC 7120. Proc Natl Acad Sci U S A 111: 11205-11210

Ermakova M, Huokko T, Richaud P, Bersanini L, Howe CJ, Lea-Smith DJ, Peltier G, Allahverdiyeva Y (2016) Distinguishing the roles of thylakoid respiratory terminal oxidases in the cyanobacterium *Synechocystis* sp. PCC 6803. Plant Physiol 171: 1307-1319

Flores E, Herrero A (2010) Compartmentalized function through cell differentiation in filamentous cyanobacteria. Nat Rev Microbiol 8: 39-50

Frederick RE, Caranto JD, Masitas CA, Gebhardt LL, MacGowan CE, Limberger RJ, Kurtz DM (2015) Dioxygen and nitric oxide scavenging by *Treponema denticola* flavodiiron protein: a mechanistic paradigm for catalysis. J Biol Inorg Chem 20: 603-613

Georg J, Voss B, Scholz I, Mitschke J, Wilde A, Hess WR (2009) Evidence for a major role of antisense RNAs in cyanobacterial gene regulation. Mol Syst Biol 5: 305

Gerotto C, Alboresi A, Meneghesso A, Jokel M, Suorsa M, Aro E, Morosinotto T (2016) Flavodiiron proteins act as safety valve for electrons in *Physcomitrella patens*. Proc Natl Acad Sci U S A 113: 12322-12327

Ghirardi ML, Dubini A, Yu J, Maness P (2009) Photobiological hydrogen-producing systems. Chem Soc Rev 38: 52-61

Glazer AN (1984) Phycobilisome a macromolecular complex optimized for light energy transfer. Biochim Biophys Acta 768: 29-51

Gonçalves VL, Saraiva LM, Teixeira M (2011) Gene expression study of the flavodi-iron proteins from the cyanobacterium *Synechocystis* sp. PCC6803. Biochem Soc Trans 39: 216-218

Gonçalves VL, Vicente JB, Pinto L, Romão CV, Frazão C, Sarti P, Giuffrè A, Teixeira M (2014) Flavodiiron oxygen reductase from *Entamoeba histolytica*: modulation of substrate preference by

tyrosine 271 and lysine 53. J Biol Chem 289: 28260-28270

Gonzalez-Esquer CR, Smarda J, Rippka R, Axen SD, Guglielmi G, Gugger M, Kerfeld CA (2016) Cyanobacterial ultrastructure in light of genomic sequence data. Photosyn Res 129: 147-157

Grieco M, Suorsa M, Jajoo A, Tikkanen M, Aro E (2015) Light-harvesting II antenna trimers connect energetically the entire photosynthetic machinery - including both photosystems II and I. Biochim Biophys Acta 1847: 607-619

Grotjohann I, Fromme P (2005) Structure of cyanobacterial photosystem I. Photosyn Res 85: 51-72

Gutekunst K, Chen X, Schreiber K, Kaspar U, Makam S, Appel J (2014) The bidirectional NiFe-hydrogenase in *Synechocystis* sp. PCC 6803 is reduced by flavodoxin and ferredoxin and is essential under mixotrophic, nitrate-limiting conditions. J Biol Chem 289: 1930-1937

Hackenberg C, Engelhardt A, Matthijs HCP, Wittink F, Bauwe H, Kaplan A, Hagemann M (2009) Photorespiratory 2-phosphoglycolate metabolism and photoreduction of O_2 cooperate in high-light acclimation of *Synechocystis* sp. strain PCC 6803. Planta 230: 625-637

Hanke GT, Satomi Y, Shinmura K, Takao T, Hase T (2011) A screen for potential ferredoxin electron transfer partners uncovers new, redox dependent interactions. Biochim Biophys Acta 1814: 366-374

Heinz S, Liauw P, Nickelsen J, Nowaczyk M (2016) Analysis of photosystem II biogenesis in cyanobacteria. Biochim Biophys Acta 1857: 274-287

Helman Y, Barkan E, Eisenstadt D, Luz B, Kaplan A (2005) Fractionation of the three stable oxygen isotopes by oxygen-producing and oxygen-consuming reactions in photosynthetic organisms. Plant Physiol 138: 2292-2298

Helman Y, Tchernov D, Reinhold L, Shibata M, Ogawa T, Schwarz R, Ohad I, Kaplan A (2003) Genes encoding A-type flavoproteins are essential for photoreduction of O_2 in cyanobacteria. Curr Biol 13: 230-235

Herranen M, Battchikova N, Zhang P, Graf A, Sirpiö S, Paakkarinen V, Aro E (2004) Towards functional proteomics of membrane protein complexes in *Synechocystis* sp. PCC 6803. Plant Physiol 134: 470-481

Hill R, Bendall F (1960) Function of the two cytochrome components in chloroplasts: a working hypothesis. Nature 186: 136-137

Houot L, Floutier M, Marteyn B, Michaut M, Picciocchi A, Legrain P, Aude J, Cassier-Chauvat C, Chauvat F (2007) Cadmium triggers an integrated reprogramming of the metabolism of *Synechocystis* PCC6803, under the control of the Slr1738 regulator. BMC Genomics 8: 350

Howitt CA, Udall PK, Vermaas WF (1999) Type 2 NADH dehydrogenases in the cyanobacterium *Synechocystis* sp. strain PCC 6803 are involved in regulation rather than respiration. J Bacteriol 181: 3994-4003

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

Ilík P, Pavlovič A, Kouřil R, Alboresi A, Morosinotto T, Allahverdiyeva Y, Aro E, Yamamoto H, Shikanai T (2017) Alternative electron transport mediated by flavodiiron proteins is operational in organisms from cyanobacteria up to gymnosperms. New Phytol

Ishii A, Hihara Y (2008) An AbrB-like transcriptional regulator, Sll0822, is essential for the activation of nitrogen-regulated genes in *Synechocystis* sp. PCC 6803. Plant Physiol 148: 660-670

Jensen PE, Bassi R, Boekema EJ, Dekker JP, Jansson S, Leister D, Robinson C, Scheller HV (2007) Structure, function and regulation of plant photosystem I. Biochim Biophys Acta 1767: 335-352

Jokel M, Kosourov S, Battchikova N, Tsygankov AA, Aro EM, Allahverdiyeva Y (2015) *Chlamydomonas* flavodiiron proteins facilitate acclimation to anoxia during sulfur deprivation. Plant Cell Physiol 56: 1598-1607

Jordan P, Fromme P, Witt HT, Klukas O, Saenger W, Krauss N (2001) Three-dimensional structure of cyanobacterial photosystem I at 2.5 A resolution. Nature 411: 909-917

Kadowaki T, Nagayama R, Georg J, Nishiyama Y, Wilde A, Hess WR, Hihara Y (2016) A feed-forward loop consisting of the response regulator RpaB and the small RNA PsrR1 controls light acclimation of

photosystem I gene expression in the cyanobacterium *Synechocystis* sp. PCC 6803. Plant Cell Physiol 57: 813-823

Kanazawa A, Ostendorf E, Kohzuma K, Hoh D, Strand DD, Sato-Cruz M, Savage L, Cruz JA, Fisher N, Froehlich JE, Kramer DM (2017) Chloroplast ATP synthase modulation of the thylakoid proton motive force: implications for photosystem I and photosystem II photoprotection. Front Plant Sci 8: 719

Kaneko T, Sato S, Kotani H, Tanaka A, Asamizu E, Nakamura Y, Miyajima N, Hirosawa M, Sugiura M, Sasamoto S, Kimura T, Hosouchi T, Matsuno A, Muraki A, Nakazaki N, Naruo K, Okumura S, Shimpo S, Takeuchi C, Wada T, Watanabe A, Yamada M, Yasuda M, Tabata S (1996) Sequence analysis of the genome of the unicellular cyanobacterium *Synechocystis* sp. strain PCC6803. II. Sequence determination of the entire genome and assignment of potential protein-coding regions. DNA Res 3: 109-136

Kaplan A, Reinhold L (1999) CO_2 concentrating mechanisms in photosynthetic microorganisms. Annu Rev Plant Physiol Plant Mol Biol 50: 539-570

Kappell AD, van Waasbergen LG (2007) The response regulator RpaB binds the high light regulatory 1 sequence upstream of the high-light-inducible *hliB* gene from the cyanobacterium *Synechocystis* PCC 6803. Arch Microbiol 187: 337-342

Karpinski S, Gabrys H, Mateo A, Karpinska B, Mullineaux PM (2003) Light perception in plant disease defence signalling. Curr Opin Plant Biol 6: 390-396

Kerfeld CA, Melnicki MR (2016) Assembly, function and evolution of cyanobacterial carboxysomes. Curr Opin Plant Biol 31: 66-75

Kerfeld CA, Sawaya MR, Tanaka S, Nguyen CV, Phillips M, Beeby M, Yeates TO (2005) Protein structures forming the shell of primitive bacterial organelles. Science 309: 936-938

Kirilovsky D (2015) Modulating energy arriving at photochemical reaction centers: orange carotenoid protein-related photoprotection and state transitions. Photosyn Res 126: 3-17

Kirilovsky D, Kerfeld CA (2016) Cyanobacterial photoprotection by the orange carotenoid protein. Nat Plants 2: 16180

Knoppová J, Sobotka R, Tichy M, Yu J, Konik P, Halada P, Nixon PJ, Komenda J (2014) Discovery of a chlorophyll binding protein complex involved in the early steps of photosystem II assembly in *Synechocystis*. Plant Cell 26: 1200-1212

Kobayashi M, Ishizuka T, Katayama M, Kanehisa M, Bhattacharyya-Pakrasi M, Pakrasi HB, Ikeuchi M (2004) Response to oxidative stress involves a novel peroxiredoxin gene in the unicellular cyanobacterium *Synechocystis* sp. PCC 6803. Plant Cell Physiol 45: 290-299

Komenda J, Sobotka R (2016) Cyanobacterial high-light-inducible proteins — Protectors of chlorophyll-protein synthesis and assembly. Biochim Biophys Acta 1857: 288-295

Korn A (2010) Respective roles of the ferredoxin:NADP-oxidoreductase isoforms in the cyanobacterium *Synechocystis* sp. PCC 6803. PhD thesis. Biological Physics, Université Paris Sud.

Krieger-Liszkay A, Feilke K (2016) The dual role of the plastid terminal oxidase PTOX: between a protective and a pro-oxidant function. Front Plant Sci 6:1147

Kucho K, Okamoto K, Tsuchiya Y, Nomura S, Nango M, Kanehisa M, Ishiura M (2005) Global analysis of circadian expression in the cyanobacterium *Synechocystis* sp. strain PCC 6803. J Bacteriol 187: 2190-2199

Kudoh H, Sonoike K (2002) Irreversible damage to photosystem I by chilling in the light: cause of the degradation of chlorophyll after returning to normal growth temperature. Planta 215: 541-548

Kufryk GI, Sachet M, Schmetterer G, Vermaas WFJ (2002) Transformation of the cyanobacterium *Synechocystis* sp. PCC 6803 as a tool for genetic mapping: optimization of efficiency. FEMS Microbiol Lett 206: 215-219

Kurian D, Jansèn T, Mäenpää P (2006) Proteomic analysis of heterotrophy in *Synechocystis* sp. PCC 6803. Proteomics 6: 1483-1494

Kurisu G, Kusunoki M, Katoh E, Yamazaki T, Teshima K, Onda Y, Kimata-Ariga Y, Hase T (2001) Structure of the electron transfer complex between ferredoxin and ferredoxin-$NADP^+$ reductase. Nat Struct Biol 8: 117-121

Latifi A, Ruiz M, Zhang C (2009) Oxidative stress in cyanobacteria. FEMS Microbiol Rev 33: 258-278

Layana C, Diambra L (2011) Time-course analysis of cyanobacterium transcriptome: detecting oscillatory genes. PLoS ONE 6: e26291

Lea-Smith DJ, Ross N, Zori M, Bendall DS, Dennis JS, Scott SA, Smith AG, Howe CJ (2013) Thylakoid terminal oxidases are essential for the cyanobacterium *Synechocystis* sp. PCC 6803 to survive rapidly changing light intensities. Plant Physiol 162: 484-495

Li M, Semchonok DA, Boekema EJ, Bruce BD (2014) Characterization and evolution of tetrameric photosystem I from the thermophilic cyanobacterium *Chroococcidiopsis* sp TS-821. Plant Cell 26: 1230-1245

Liu H, Zhang H, Niedzwiedzki DM, Prado M, He G, Gross ML, Blankenship RE (2013) Phycobilisomes supply excitations to both photosystems in a megacomplex in cyanobacteria. Science 342: 1104-1107

Maeda S, Badger MR, Price GD (2002) Novel gene products associated with NdhD3/D4-containing NDH-1 complexes are involved in photosynthetic CO_2 hydration in the cyanobacterium, *Synechococcus* sp. PCC7942. Mol Microbiol 43: 425-435

Mangan NM, Flamholz A, Hood RD, Milo R, Savage DF (2016) pH determines the energetic efficiency of the cyanobacterial CO_2 concentrating mechanism. Proc Natl Acad Sci U S A 113: 5354

Marteyn B, Domain F, Legrain P, Chauvat F, Cassier-Chauvat C (2009) The thioredoxin reductase-glutaredoxins-ferredoxin crossroad pathway for selenate tolerance in *Synechocystis* PCC6803. Mol Microbiol 71: 520-532

Matthijs HCP, Visser PM, Reeze B, Meeuse J, Slot PC, Wijn G, Talens R, Huisman J (2012) Selective suppression of harmful cyanobacteria in an entire lake with hydrogen peroxide. Water Res 46: 1460-1472

Mazouni K, Domain F, Chauvat F, Cassier-Chauvat C (2003) Expression and regulation of the crucial plant-like ferredoxin of cyanobacteria. Mol Microbiol 49: 1019-1029

McDonald AE, Ivanov AG, Bode R, Maxwell DP, Rodermel SR, Hüner NPA (2011) Flexibility in photosynthetic electron transport: the physiological role of plastoquinol terminal oxidase (PTOX). Biochim Biophys Acta 1807: 954-967

Meeks JC, Castenholz RW (1971) Growth and photosynthesis in an extreme thermophile, *Synechococcus lividus* (Cyanophyta). Arch Mikrobiol 78: 25-41

Mehler AH (1951) Studies on reactions of illuminated chloroplasts. I. Mechanism of the reduction of oxygen and other Hill reagents. Arch Biochem Biophys 33: 65-77

Mekala NR, Suorsa M, Rantala M, Aro E, Tikkanen M (2015) Plants actively avoid state transitions upon changes in light intensity: role of light-harvesting complex II protein dephosphorylation in high light. Plant Physiol 168: 721-734

Merel S, Walker D, Chicana R, Snyder S, Baurès E, Thomas O (2013) State of knowledge and concerns on cyanobacterial blooms and cyanotoxins. Environ Int 59: 303-327

Midorikawa T, Matsumoto K, Narikawa R, Ikeuchi M (2009) An Rrf2-type transcriptional regulator is required for expression of *psaAB* genes in the cyanobacterium *Synechocystis* sp. PCC 6803. Plant Physiol 151: 882-892

Miskiewicz E, Ivanov AG, Huner NPA (2002) Stoichiometry of the photosynthetic apparatus and phycobilisome structure of the cyanobacterium *Plectonema boryanum* UTEX 485 are regulated by both light and temperature. Plant Physiol 130: 1414-1425

Mitschke J, Georg J, Scholz I, Sharma CM, Dienst D, Bantscheff J, Voss B, Steglich C, Wilde A, Vogel J, Hess WR (2011) An experimentally anchored map of transcriptional start sites in the model cyanobacterium *Synechocystis* sp. PCC6803. Proc Natl Acad Sci U S A 108: 2124-2129

Montgomery BL, Lechno-Yossef S, Kerfeld CA (2016) Interrelated modules in cyanobacterial photosynthesis: the carbon-concentrating mechanism, photorespiration, and light perception. J Exp Bot 67: 2931-2940

Mullineaux CW (2014) Co-existence of photosynthetic and respiratory activities in cyanobacterial thylakoid membranes. Biochim Biophys Acta 1837: 503-511

Munekage Y, Hashimoto M, Miyake C, Tomizawa K, Endo T, Tasaka M, Shikanai T (2004) Cyclic electron flow

around photosystem I is essential for photosynthesis. Nature 429: 579-582

Munekage Y, Hojo M, Meurer J, Endo T, Tasaka M, Shikanai T (2002) PGR5 is involved in cyclic electron flow around photosystem I and is essential for photoprotection in *Arabidopsis*. Cell 110: 361-371

Murakami A, Kim SJ, Fujita Y (1997) Changes in photosystem stoichiometry in response to environmental conditions for cell growth observed with the cyanophyte *Synechocystis* PCC 6714. Plant Cell Physiol 38: 392-397

Murata N (1969) Control of excitation transfer in photosynthesis. I. Light-induced change of chlorophyll a fluorescence in *Porphyridium cruentum*. Biochim Biophys Acta 172: 242-251

Nagarajan D, Lee D, Kondo A, Chang J (2016) Recent insights into biohydrogen production by microalgae - From biophotolysis to dark fermentation. Bioresour Technol

Nandha B, Finazzi G, Joliot P, Hald S, Johnson GN (2007) The role of PGR5 in the redox poising of photosynthetic electron transport. Biochim Biophys Acta 1767: 1252-1259

Niedzwiedzki DM, Tronina T, Liu H, Staleva H, Komenda J, Sobotka R, Blankenship RE, Polívka T (2016) Carotenoid-induced non-photochemical quenching in the cyanobacterial chlorophyll synthase-HliC/D complex. Biochim Biophys Acta 1857: 1430-1439

Nott A, Jung H, Koussevitzky S, Chory J (2006) Plastid-to-nucleus retrograde signaling. Annu Rev Plant Biol 57: 739-759

Ogawa T (1991) A gene homologous to the subunit-2 gene of NADH dehydrogenase is essential to inorganic carbon transport of *Synechocystis* PCC6803. Proc Natl Acad Sci U S A 88: 4275-4279

Ogawa T, Mi H (2007) Cyanobacterial NADPH dehydrogenase complexes. Photosyn Res 93: 69-77

Oliver JWK, Atsumi S (2014) Metabolic design for cyanobacterial chemical synthesis. Photosyn Res 120: 249-261

Ooms MD, Dinh CT, Sargent EH, Sinton D (2016) Photon management for augmented photosynthesis. Nat Commun 7: 12699

Partensky F, Hess WR, Vaulot D (1999) *Prochlorococcus*, a marine photosynthetic prokaryote of global significance. Microbiol Mol Biol Rev 63: 106-127

Passardi F, Bakalovic N, Teixeira FK, Margis-Pinheiro M, Penel C, Dunand C (2007) Prokaryotic origins of the non-animal peroxidase superfamily and organelle-mediated transmission to eukaryotes. Genomics 89: 567-579

Peden EA, Boehm M, Mulder DW, Davis R, Old WM, King PW, Ghirardi ML, Dubini A (2013) Identification of global ferredoxin interaction networks in *Chlamydomonas reinhardtii*. J Biol Chem 288: 35192-35209

Peltier G, Aro E, Shikanai T (2016) NDH-1 and NDH-2 plastoquinone reductases in oxygenic photosynthesis. Annu Rev Plant Biol 67: 55-80

Peltier G, Tolleter D, Billon E, Cournac L (2010) Auxiliary electron transport pathways in chloroplasts of microalgae. Photosyn Res 106: 19-31

Picossi S, Flores E, Herrero A (2015) The LysR-type transcription factor PacR is a global regulator of photosynthetic carbon assimilation in *Anabaena*. Environ Microbiol 17: 3341-3351

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, Gregor W, Schmetterer G (1997) Evidence for in vivo activity of three distinct respiratory terminal oxidases in the cyanobacterium *Synechocystis* sp. strain PCC6803. FEMS Microbiology Letters 152: 83-88

Poncelet M, Cassier-Chauvat C, Leschelle X, Bottin H, Chauvat F (1998) Targeted deletion and mutational analysis of the essential (2Fe-2S) plant-like ferredoxin in *Synechocystis* PCC6803 by plasmid shuffling. Mol Microbiol 28: 813-821

Quintana N, Van der Kooy F, Van de Rhee, Miranda D, Voshol GP, Verpoorte R (2011) Renewable energy from Cyanobacteria: energy production optimization by metabolic pathway engineering. Appl Microbiol Biotechnol 91: 471-490

Raps S, Kycia JH, Ledbetter MC, Siegelman HW (1985) Light intensity adaptation and phycobilisome composition

of *Microcystis aeruginosa*. Plant Physiol 79: 983-987

Rippka R, Deruelles J, Waterbury JB, Herdman M, Stanier RY (1979) Generic assignments, strain histories and properties of pure cultures of cyanobacteria. Microbiology 111: 1-61

Romão CV, Vicente JB, Borges PT, Victor BL, Lamosa P, Silva E, Pereira L, Bandeiras TM, Soares CM, Carrondo MA, Turner D, Teixeira M, Frazão C (2016) Structure of *Escherichia coli* flavodiiron nitric oxide reductase. J Mol Biol 428: 4686-4707

Rowland JG, Pang X, Suzuki I, Murata N, Simon WJ, Slabas AR (2010) Identification of components associated with thermal acclimation of photosystem II in *Synechocystis* sp. PCC6803. PLoS ONE 5: e10511

Saha R, Liu D, Hoynes-O'Connor A, Liberton M, Yu J, Bhattacharyya-Pakrasi M, Balassy A, Zhang F, Moon TS, Maranas CD, Pakrasi HB (2016) Diurnal regulation of cellular processes in the cyanobacterium *Synechocystis* sp. strain PCC 6803: insights from transcriptomic, fluxomic, and physiological analyses. MBio 7:

Sakurai I, Stazic D, Eisenhut M, Vuorio E, Steglich C, Hess WR, Aro E (2012) Positive regulation of *psbA* gene expression by cis-encoded antisense RNAs in *Synechocystis* sp. PCC 6803. Plant Physiol 160: 1000-1010

Sánchez-Riego AM, López-Maury L, Florencio FJ (2013) Glutaredoxins are essential for stress adaptation in the cyanobacterium *Synechocystis* sp. PCC 6803. Front Plant Sci 4: 428

Schmitt F, Renger G, Friedrich T, Kreslavski VD, Zharmukhamedov SK, Los DA, Kuznetsov VV, Allakhverdiev SI (2014) Reactive oxygen species: re-evaluation of generation, monitoring and role in stress-signaling in phototrophic organisms. Biochim Biophys Acta 1837: 835-848

Schneider D, Berry S, Rich P, Seidler A, Rögner M (2001) A regulatory role of the PetM subunit in a cyanobacterial cytochrome b_6f complex. J Biol Chem 276: 16780-16785

Schuergers N, Lenn T, Kampmann R, Meissner MV, Esteves T, Temerinac-Ott M, Korvink JG, Lowe AR, Mullineaux CW, Wilde A (2016) Cyanobacteria use micro-optics to sense light direction. Elife 5:

Schwarz D, Schubert H, Georg J, Hess WR, Hagemann M (2013) The gene *sml0013* of *Synechocystis* species strain PCC 6803 encodes for a novel subunit of the NAD(P)H oxidoreductase or complex I that is ubiquitously distributed among Cyanobacteria. Plant Physiol 163: 1191-1202

Sedoud A, López-Igual R, Ur Rehman A, Wilson A, Perreau F, Boulay C, Vass I, Krieger-Liszkay A, Kirilovsky D (2014) The Cyanobacterial Photoactive Orange Carotenoid Protein Is an Excellent Singlet Oxygen Quencher. Plant Cell 26: 1781-1791

Seedorf H, Hagemeier CH, Shima S, Thauer RK, Warkentin E, Ermler U (2007) Structure of coenzyme F420H2 oxidase (FprA), a di-iron flavoprotein from methanogenic Archaea catalyzing the reduction of O_2 to H_2O. FEBS J 274: 1588-1599

Seino Y, Takahashi T, Hihara Y (2009) The response regulator RpaB binds to the upstream element of photosystem I genes to work for positive regulation under low-light conditions in *Synechocystis* sp. Strain PCC 6803. J Bacteriol 191: 1581-1586

Shen J (2015) The structure of photosystem II and the mechanism of water oxidation in photosynthesis. Annu Rev Plant Biol 66: 23-48

Shibata M, Ohkawa H, Kaneko T, Fukuzawa H, Tabata S, Kaplan A, Ogawa T (2001) Distinct constitutive and low-CO_2-induced CO_2 uptake systems in cyanobacteria: genes involved and their phylogenetic relationship with homologous genes in other organisms. Proc Natl Acad Sci U S A 98: 11789-11794

Shih PM, Hemp J, Ward LM, Matzke NJ, Fischer WW (2017) Crown group Oxyphotobacteria postdate the rise of oxygen. Geobiology 15: 19-29

Shih PM, Wu D, Latifi A, Axen SD, Fewer DP, Talla E, Calteau A, Cai F, Tandeau de Marsac N, Rippka R, Herdman M, Sivonen K, Coursin T, Laurent T, Goodwin L, Nolan M, Davenport KW, Han CS, Rubin EM, Eisen JA, Woyke T, Gugger M, Kerfeld CA (2013) Improving the coverage of the cyanobacterial phylum using diversity-driven genome sequencing. Proc Natl Acad Sci U S A 110: 1053-1058

Shimakawa G, Ishizaki K, Tsukamoto S, Tanaka M, Sejima T, Miyake C (2017) The liverwort, *Marchantia*, drives alternative electron flow using a flavodiiron protein to protect PSI. Plant Physiol 173: 1636-1647

Shively JM, Ball F, Brown DH, Saunders RE (1973) Functional organelles in prokaryotes: polyhedral inclusions (carboxysomes) of *Thiobacillus neapolitanus*. Science 182: 584-586

Singh AK, Sherman LA (2000) Identification of iron-responsive, differential gene expression in the cyanobacterium *Synechocystis* sp. strain PCC 6803 with a customized amplification library. J Bacteriol 182: 3536-3543

Singh AK, Elvitigala T, Bhattacharyya-Pakrasi M, Aurora R, Ghosh B, Pakrasi HB (2008) Integration of carbon and nitrogen metabolism with energy production is crucial to light acclimation in the cyanobacterium *Synechocystis*. Plant Physiol 148: 467-478

Singh SP, Montgomery BL (2011) Determining cell shape: adaptive regulation of cyanobacterial cellular differentiation and morphology. Trends Microbiol 19: 278-285

Sonoike K, Hihara Y, Ikeuchi M (2001) Physiological significance of the regulation of photosystem stoichiometry upon high light acclimation of *Synechocystis* sp. PCC 6803. Plant Cell Physiol 42: 379-384

Sonoike K, Terashima I, Iwaki M, Itoh S (1995) Destruction of photosystem I iron-sulfur centers in leaves of *Cucumis sativus* L. by weak illumination at chilling temperatures. FEBS Lett 362: 235-238

Soo RM, Skennerton CT, Sekiguchi Y, Imelfort M, Paech SJ, Dennis PG, Steen JA, Parks DH, Tyson GW, Hugenholtz P (2014) An expanded genomic representation of the phylum cyanobacteria. Genome Biol Evol 6: 1031-1045

Spät P, Maček B, Forchhammer K (2015) Phosphoproteome of the cyanobacterium *Synechocystis* sp. PCC 6803 and its dynamics during nitrogen starvation. Front Microbiol 6: 248

Stal LJ (2009) Is the distribution of nitrogen-fixing cyanobacteria in the oceans related to temperature? Environ Microbiol 11: 1632-1645

Stanier RY, Kunisawa R, Mandel M, Cohen-Bazire G (1971) Purification and properties of unicellular blue-green algae (order *Chroococcales*). Bacteriol Rev 35: 171-205

Stork T, Michel K, Pistorius EK, Dietz K (2005) Bioinformatic analysis of the genomes of the cyanobacteria *Synechocystis* sp. PCC 6803 and *Synechococcus* elongatus PCC 7942 for the presence of peroxiredoxins and their transcript regulation under stress. J Exp Bot 56: 3193-3206

Strand DD, Livingston AK, Satoh-Cruz M, Froehlich JE, Maurino VG, Kramer DM (2015) Activation of cyclic electron flow by hydrogen peroxide in vivo. Proc Natl Acad Sci U S A 112: 5539-5544

Suorsa M, Järvi S, Grieco M, Nurmi M, Pietrzykowska M, Rantala M, Kangasjärvi S, Paakkarinen V, Tikkanen M, Jansson S, Aro E (2012) PROTON GRADIENT REGULATION5 is essential for proper acclimation of *Arabidopsis* photosystem I to naturally and artificially fluctuating light conditions. Plant Cell 24: 2934-2948

Tamary E, Kiss V, Nevo R, Adam Z, Bernát G, Rexroth S, Rögner M, Reich Z (2012) Structural and functional alterations of cyanobacterial phycobilisomes induced by high-light stress. Biochim Biophys Acta 1817: 319-327

Telfer A (2014) Singlet oxygen production by PSII under light stress: mechanism, detection and the protective role of β-carotene. Plant Cell Physiol 55: 1216-1223

Tikkanen M, Aro E (2014) Integrative regulatory network of plant thylakoid energy transduction. Trends Plant Sci 19: 10-17

Tikkanen M, Aro E (2012) Thylakoid protein phosphorylation in dynamic regulation of photosystem II in higher plants. Biochim Biophys Acta 1817: 232-238

Tikkanen M, Grieco M, Kangasjärvi S, Aro E (2010) Thylakoid protein phosphorylation in higher plant chloroplasts optimizes electron transfer under fluctuating light. Plant Physiol 152: 723-735

Tikkanen M, Grieco M, Nurmi M, Rantala M, Suorsa M, Aro E (2012) Regulation of the photosynthetic apparatus under fluctuating growth light. Philos Trans R Soc Lond B Biol Sci 367: 3486-3493

Tikkanen M, Mekala NR, Aro E (2014) Photosystem II photoinhibition-repair cycle protects Photosystem I from irreversible damage. Biochim Biophys Acta 1837: 210-215

Tiwari A, Mamedov F, Grieco M, Suorsa M, Jajoo A, Styring S, Tikkanen M, Aro E (2016) Photodamage of iron-sulphur clusters in photosystem I induces non-photochemical energy dissipation. Nat Plants 2: 16035

Tyystjärvi T, Herranen M, Aro EM (2001) Regulation of translation elongation in cyanobacteria: membrane targeting of the ribosome nascent-chain complexes controls the synthesis of D1 protein. Mol Microbiol 40: 476-484

Van Der Plas J, De Groot R, Woortman M, Cremers F, Borrias M, Van Arkel G, Weisbeek P (1988) Genes encoding ferredoxins from *Anabaena* sp. PCC 7937 and *Synechococcus* sp. PCC 7942: structure and regulation. Photosyn Res 18: 179-204

van Thor JJ, Gruters OW, Matthijs HC, Hellingwerf KJ (1999) Localization and function of ferredoxin:NADP(+) reductase bound to the phycobilisomes of *Synechocystis*. EMBO J 18: 4128-4136

van Thor JJ, Jeanjean R, Havaux M, Sjollema KA, Joset F, Hellingwerf KJ, Matthijs HC (2000) Salt shock-inducible photosystem I cyclic electron transfer in *Synechocystis* PCC6803 relies on binding of ferredoxin:NADP(+) reductase to the thylakoid membranes via its CpcD phycobilisome-linker homologous N-terminal domain. Biochim Biophys Acta 1457: 129-144

Vicente JB, Carrondo MA, Teixeira M, Frazão C (2008a) Structural studies on flavodiiron proteins. Meth Enzymol 437: 3-19

Vicente JB, Gomes CM, Wasserfallen A, Teixeira M (2002) Module fusion in an A-type flavoprotein from the cyanobacterium *Synechocystis* condenses a multiple-component pathway in a single polypeptide chain. Biochem Biophys Res Commun 294: 82-87

Vicente JB, Justino MC, Gonçalves VL, Saraiva LM, Teixeira M (2008b) Biochemical, spectroscopic, and thermodynamic properties of flavodiiron proteins. Meth Enzymol 437: 21-45

Vuorijoki L, Isojärvi J, Kallio P, Kouvonen P, Aro E, Corthals GL, Jones PR, Muth-Pawlak D (2016) Development of a quantitative SRM-based proteomics method to study iron metabolism of *Synechocystis* sp. PCC 6803. J Proteome Res 15: 266-279

Wada N, Sakamoto T, Matsugo S (2013) Multiple roles of photosynthetic and sunscreen pigments in cyanobacteria focusing on the oxidative stress. Metabolites 3: 463-483

Wang C, Yamamoto H, Shikanai T (2015) Role of cyclic electron transport around photosystem I in regulating proton motive force. Biochim Biophys Acta 1847: 931-938

Wang H, Postier BL, Burnap RL (2004) Alterations in global patterns of gene expression in *Synechocystis* sp. PCC 6803 in response to inorganic carbon limitation and the inactivation of *ndhR*, a LysR family regulator. J Biol Chem 279: 5739-5751

Wang P, Ye J, Shen Y, Mi H (2006) The role of chloroplast NAD(P)H dehydrogenase in protection of tobacco plant against heat stress. Sci China, C, Life Sci 49: 311-321

Watanabe M, Semchonok DA, Webber-Birungi MT, Ehira S, Kondo K, Narikawa R, Ohmori M, Boekema EJ, Ikeuchi M (2014) Attachment of phycobilisomes in an antenna-photosystem I supercomplex of cyanobacteria. Proc Natl Acad Sci U S A 111: 2512-2517

Wegener KM, Welsh EA, Thornton LE, Keren N, Jacobs JM, Hixson KK, Monroe ME, Camp DG, Smith RD, Pakrasi HB (2008) High sensitivity proteomics assisted discovery of a novel operon involved in the assembly of photosystem II, a membrane protein complex. J Biol Chem 283: 27829-27837

Whitney SM, Houtz RL, Alonso H (2011) Advancing our understanding and capacity to engineer nature's CO_2-sequestering enzyme, Rubisco. Plant Physiol 155: 27-35

Whitton BA, Potts M (2000) Introduction to the cyanobacteria. In Whitton BA, Potts M (eds), The ecology of cyanobacteria - Their diversity in time and space. Kluwer Academic Publishers, The Netherlands, pp 1-11

Whitton R, Le Mével A, Pidou M, Ometto F, Villa R, Jefferson B (2016) Influence of microalgal N and P composition on wastewater nutrient remediation. Water Res 91: 371-378

Wilde A, Hihara Y (2016) Transcriptional and posttranscriptional regulation of cyanobacterial photosynthesis. Biochim Biophys Acta 1857: 296-308

Wildman RB, Bowen CC (1974) Phycobilisomes in blue-green algae. J Bacteriol 117: 866-881

Williams JGK (1988) Construction of specific mutations in photosystem II photosynthetic reaction center by genetic engineering methods in *Synechocystis* 6803. Methods in enzymology 167: 766-778

Wilson A, Ajlani G, Verbavatz J, Vass I, Kerfeld CA, Kirilovsky D (2006) A soluble carotenoid protein involved in phycobilisome-related energy dissipation in cyanobacteria. THE PLANT CELL 18: 992-1007

Wilson A, Punginelli C, Gall A, Bonetti C, Alexandre M, Routaboul J, Kerfeld CA, van Grondelle R, Robert B, Kennis JTM, Kirilovsky D (2008) A photoactive carotenoid protein acting as light intensity sensor. Proc Natl Acad Sci U S A 105: 12075-12080

Yamamoto H, Peng L, Fukao Y, Shikanai T (2011) An Src homology 3 domain-like fold protein forms a ferredoxin binding site for the chloroplast NADH dehydrogenase-like complex in *Arabidopsis*. Plant Cell 23: 1480-1493

Yamamoto H, Takahashi S, Badger MR, Shikanai T (2016) Artificial remodelling of alternative electron flow by flavodiiron proteins in *Arabidopsis*. Nat Plants 2: 16012

Yamori W, Sakata N, Suzuki Y, Shikanai T, Makino A (2011) Cyclic electron flow around photosystem I via chloroplast NAD(P)H dehydrogenase (NDH) complex performs a significant physiological role during photosynthesis and plant growth at low temperature in rice. Plant J 68: 966-976

Yamori W, Shikanai T, Makino A (2015) Photosystem I cyclic electron flow via chloroplast NADH dehydrogenase-like complex performs a physiological role for photosynthesis at low light. Sci Rep 5: 13908

Yeremenko N, Jeanjean R, Prommeenate P, Krasikov V, Nixon PJ, Vermaas WFJ, Havaux M, Matthijs HCP (2005) Open reading frame *ssr2016* is required for antimycin A-sensitive photosystem I-driven cyclic electron flow in the cyanobacterium *Synechocystis* sp. PCC 6803. Plant Cell Physiol 46: 1433-1436

Yoon HS, Hackett JD, Ciniglia C, Pinto G, Bhattacharya D (2004) A molecular timeline for the origin of photosynthetic eukaryotes. Mol Biol Evol 21: 809-818

Yu MH, Glazer AN (1982) Cyanobacterial phycobilisomes. Role of the linker polypeptides in the assembly of phycocyanin. J Biol Chem 257: 3429-3433

Zhang P, Allahverdiyeva Y, Eisenhut M, Aro E (2009) Flavodiiron proteins in oxygenic photosynthetic organisms: photoprotection of photosystem II by Flv2 and Flv4 in *Synechocystis* sp. PCC 6803. PLoS ONE 4: e5331

Zhang P, Battchikova N, Paakkarinen V, Katoh H, Iwai M, Ikeuchi M, Pakrasi HB, Ogawa T, Aro E (2005) Isolation, subunit composition and interaction of the NDH-1 complexes from *Thermosynechococcus elongatus* BP-1. Biochem J 390: 513-520

Zhang P, Eisenhut M, Brandt A, Carmel D, Silén HM, Vass I, Allahverdiyeva Y, Salminen TA, Aro E (2012) Operon *flv4-flv2* provides cyanobacterial photosystem II with flexibility of electron transfer. Plant Cell 24: 1952-1971

Zhang S, Bryant DA (2011) The tricarboxylic acid cycle in cyanobacteria. Science 334: 1551-1553

Zhao J, Gao F, Zhang J, Ogawa T, Ma W (2014) NdhO, a subunit of NADPH dehydrogenase, destabilizes medium size complex of the enzyme in *Synechocystis* sp. strain PCC 6803. J Biol Chem 289: 26669-26676

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