Turun yliopisto
University of Turku

REGULATION OF PHOTOSYNTHESIS UNDER DYNAMIC LIGHT CONDITIONS IN *CHLAMYDOMONAS REINHARDTII*: IMPACT ON HYDROGEN PRODUCTION

Martina Jokel

TURUN YLIOPISTON JULKAISUJA - ANNALES UNIVERSITATIS TURKUENSIS
Sarja - ser. AI osa - tom. 563 | Astronomica - Chemica - Physica - Mathematica | Turku 2017

Turun yliopisto
University of Turku

REGULATION OF PHOTOSYNTHESIS UNDER DYNAMIC LIGHT CONDITIONS IN *CHLAMYDOMONAS REINHARDTII*: IMPACT ON HYDROGEN PRODUCTION

Martina Jokel

TURUN YLIOPISTON JULKAISUJA – ANNALES UNIVERSITATIS TURKUENSIS
Sarja - ser. A I osa - tom. 563 | Astronomica - Chemica - Physica - Mathematica | Turku 2017

University of Turku

Faculty of Mathematics and Natural Sciences
Department of Biochemistry
From the Laboratory of Molecular Plant Biology

Supervised by

Dr. Yagut Allahverdiyeva
Laboratory of Molecular Plant Biology,
Department of Biochemistry,
University of Turku, FI-20014 Turku,
Finland

Prof. Eva-Mari Aro
Laboratory of Molecular Plant Biology,
Department of Biochemistry,
University of Turku, FI-20014 Turku,
Finland

Reviewed by

Prof. Dr. Thomas Happe
Laboratory of Photobiotechnology,
Department of Plant Biochemistry,
Ruhr-University Bochum,
Germany

Prof. Yuichiro Takahashi
Department of Biology,
Faculty of Science,
Okayama University,
Japan

Opponent

Dr. Maria L. Ghirardi
Biosciences Center,
National Renewable Energy Laboratory,
Golden,
USA

ISBN 978-951-29-6887-9 (PRINT)
ISBN 978-951-29-6888-6 (PDF)
ISSN 0082-7002 (Print)
ISSN 2343-3175 (Online)
Painosalama Oy - Turku, Finland 2017

LIST OF ORIGINAL PUBLICATIONS

This thesis is composed of the following scientific articles, referred to in the text by their Roman numerals.

I. Jokel M, Kosourov S, Battchikova N, Tsygankov AA, Aro EM and Allahverdiyeva Y. (2015) *Chlamydomonas* Flavodiiron Proteins Facilitate Acclimation to Anoxia during Sulfur Deprivation. *Plant Cell Physiol* 56, 1598-607.

II. Jokel M, Johnson X, Peltier G, Aro EM and Allahverdiyeva Y. (2017) Hunting the main player enabling *C. reinhardtii* growth under fluctuating light: Flavodiiron protein-mediated O_2 photoreduction or the PGR5/PGRL1 operated pathway? (Manuscript).

III. Dang KV, 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, 3036-50.

IV. Volgusheva AA, Jokel M, Allahverdiyeva Y, Kukarskikh GP, Lukashev E, Lambreva, M, Krendeleva T and Antal TK. (2017) Comparative analyses of H_2 photoproduction in magnesium and sulfur starved *Chlamydomonas reinhardtii* cultures. *Physiol Plantarum*. DOI: 10.1111/ppl.12576.

Other publications related to the topic:

- Bersanini L, Battchikova N, Jokel M, ur Rehman A, Vass I, Allahverdiyeva Y and Aro EM. (2014) Flavodiiron protein Flv2/Flv4-related photoprotective mechanism dissipates excitation pressure of PSII in cooperation with phycobilisomes in Cyanobacteria. *Plant Physiol* 164, 805–818.
- Gerotto C, Alboresi A, Meneghesso A, Jokel M, Suorsa M, Aro EM and Morosinotto T. (2016) Flavodiiron proteins act as safety valve for electrons in *Physcomitrella patens*. *PNAS* 113, 12322-12327.

TABLE OF CONTENTS

LIST OF ORIGINAL PUBLICATIONS 3

ABBREVIATIONS 6

ABSTRACT 9

TIIVISTELMÄ 10

1. INTRODUCTION 12
   1.1. Green algae - *Chlamydomonas reinhardtii* as a model organism 12
   1.2. Photosynthetic light reactions 14
      1.2.1. Linear electron transport 14
      1.2.2. Cyclic electron transport 16
   1.3. Alternative electron sinks of photosynthesis 19
      1.3.1. Flavodiiron protein-mediated O_2 photoreduction 20
      1.3.2. Chloroplast O_2 photoreduction processes other than flavodiiron proteins 23
   1.4. Additional mechanisms regulating photosynthetic electron transport 24
      1.4.1. Non-photochemical quenching 24
      1.4.2. Photosynthetic control at Cytochrome b_6f 25
      1.4.3. State transitions 26
   1.5. Hydrogen production in green algae 27
      1.5.1. The role of hydrogenases as an alternative electron sink 29
      1.5.2. Different protocols to induce hydrogen production 30
      1.5.3. Improving hydrogen production via genetic modifications 32

2. AIMS OF THE STUDY 35

3. METHODOLOGY 36
   3.1. Growth conditions and *C. reinhardtii* strains 36
   3.2. Photosynthetic activity measurements 37
      3.2.1. Chl *a* fluorescence and P700 measurements 37
      3.2.2. Electrochromatic shift analysis 37
      3.2.3. Low temperature fluorescence emission spectroscopy 38
      3.2.4. Oxygen exchange/evolution and H_2O_2 analysis 38
   3.3. Transcript analysis 38
      3.3.1. Quantitative Real-time PCR 38
      3.3.2. Next-generation RNA sequencing 39
   3.4. Protein analysis 39

3.5. Hydrogen production 39
3.5.1. Sulfur-deprivation protocol 39
3.5.2. Magnesium-deprivation protocol 40

4. MAIN RESULTS 41
4.1. The interplay of O_2 photoreduction and cyclic electron transport protects photosynthesis 41
4.1.1. Flavodiiron proteins are up-regulated when there is an excess of electrons in the photosynthetic electron transport chain 41
4.1.2. O_2 photoreduction via flavodiiron proteins is crucial for survival under fluctuating light 42
4.1.3. PGRL1-mediated cyclic electron transport is essential during high light transitions 43
4.1.4. The loss of PGRL1 leads to increased mitochondrial respiration and elevated O_2 photoreduction 44
4.1.5. Underlying mechanisms behind the phenotypes of the *pgrl1* and *pgr5* mutants under fluctuating light 45
4.2. Impact of alternative electron transport on hydrogen production 47
4.2.1. Flavodiiron proteins are transiently up-regulated during the shift to sulfur-deprivation 47
4.2.2. Comparison of H_2 production in sulfur- or magnesium-deprivation protocols 47

5. DISCUSSION 49
5.1. The importance of alternative electron transport for photoprotection in dynamic light environments 49
5.2. Flavodiiron proteins work on a faster time-scale than PGR5 or PGRL1 and are indispensible for survival under fluctuating light 50
5.3. PGR5 and PGRL1 proteins have different impacts on photosynthesis 51
5.4. Hydrogen production is influenced by the activity of other alternative electron transport pathways 56

6. CONCLUSIONS AND FUTURE PERSPECTIVES 59

7. ACKNOWLEDGEMENTS 61

8. REFERENCES 62

ORIGINAL PUBLICATIONS 73

ABBREVIATIONS

AA	antimycin A
AET	alternative electron transport
ATP	adenosine triphosphate
CBB	Calvin-Benson-Bassham cycle
CCM	carbon concentrating mechanism
cDNA	complementary DNA
CET	cyclic electron transport
Chl	chlorophyll *a* and *b*
CL	constant light
COX	cytochrome *c* oxidase
Cyt	cytochrome
ΔpH	proton gradient
ΔΨ	electric potential
DCBQ	2,5-Dichloro-1,4-benzoquinone
DNA	deoxyribonucleic acid
ECS	electrochromatic shift
ETR	electron transport rate
F_0	the minimal level of fluorescence in the dark
F_m	the maximum fluorescence in the dark
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
FAD	flavin adenine dinucleotide
Fd (FDX1)	ferredoxin (ferredoxin 1)
FDP, FLV	flavodiiron protein
FeCy	ferricyanide
FL	fluctuating light
FMN	flavin mononucleotide
FNR	ferredoxin:$NADP^+$ oxidoreductase
FQR	ferredoxin plastoquinol reductase

g_{H^+}	proton conductivity
GC MS	gas chromatographic mass spectrometry
GL	growth light
H_2ase	hydrogenase
HC	high CO_2 conditions (3% CO_2)
HL	high light conditions
HSM	high salt minimal medium
LC	low CO_2 conditions, air level CO_2
LET	linear electron transfer
LED	light-emitting diode
LHC	light harvesting complex
LL	low light
MIMS	membrane inlet mass spectrometry
MS/MS	tandem mass spectrometry
NADH	nicotinamide adenine dinucleotide (reduced)
NADPH	nicotinamide adenine dinucleotide phosphate (reduced)
$NADP^+$	nicotinamide adenine dinucleotide phosphate (oxidized)
NDA2	NAD(P)H dehydrogenase
NPQ	non-photochemical quenching
OD_{750}	optical density at 750 nm
P_m	maximum level of oxidizable P700
P_m'	maximum level of oxidizable P700 under the light
P680/$P680^+$	red/ox primary electron donor of Photosystem II
P700/$P700^+$	red/ox primary electron donor of Photosystem I
PAGE	polyacrylamide gel electrophoresis
PAM	pulse amplitude modulation
PAR	photosynthetically active radiation
PC	plastocyanin
PCR	polymerase chain reaction
PET	photosynthetic electron transport
PGR5	proton gradient regulation 5
PGRL1	PGR5-like protein 1

pH	negative logarithm of the proton concentration
pmf	proton motive force
PS	photosystem
PQH_2/PQ	plastoquinol/plastoquinone
Q_A	the primary electron-accepting plastoquinone of PSII
Q_B	the secondary electron-accepting plastoquinone of PSII
qE	energy-dependent NPQ
qI	photoinhibition-dependent NPQ
qT	state transition-dependent NPQ
qRT PCR	quantitative real-time reverse transcription PCR
RNA	ribonucleic acid
RNAi	RNA interference
RNAseq	next-generation RNA sequencing
ROS	reactive oxygen species
RuBisCo	ribulose bisphosphate carboxylase/oxygenase
RuBP	ribulose-1,5-bisphosphate
SDS	sodium dodecyl sulphate
SHAM	salicylhydroxamic acid
SMR	steam methane reforming
TAP (-S/-Mg)	Tris/Acetate/Phosphate medium (without sulfur/magnesium)
wt	wild type
WWC	water-water cycle
Y(I)	effective yield of Photosystem I
Y(II)	effective yield of Photosystem II
Y(NA)	PSI acceptor-side limitation
Y(ND)	PSI donor-side limitation

ABSTRACT

The development of renewable biofuels to ensure sustainable energy supply is one of the biggest global challenges of modern society. Hydrogen has great potential to become the fuel of the future, since it provides energy without CO_2 emission. The green alga *Chlamydomonas reinhardtii* possesses hydrogenase enzymes and is able to photoproduce hydrogen under specific conditons. Hydrogenases, together with flavodiiron proteins (FDPs), PROTON GRADIENT-REGULATION 5 (PGR5), and PGR5-LIKE PHOTOSYNTHETIC PHENOTYPE 1 (PGRL1), represent key players of alternative electron transport (AET) in *C. reinhardtii*. Photosynthetic organisms have evolved AET routes such as these to adjust the photosynthetic apparatus under dynamic environmental conditions, like changing carbon supply or fluctuating light (FL) intensity. The interplay of all of these AET routes was the subject of my doctoral research, which was performed in order to understand AET regulation and to identify possible bottlenecks towards commercially profitable hydrogen production by *C. reinhardtii*.

In the first part of my thesis, I demonstrated that all three proteins; FDPs, PGR5, and PGRL1, contribute to the photoprotection of *C. reinhardtii* under FL. FDPs form a rapid electron sink downstream of PSI and are absolutely crucial for survival under FL conditions. PGR5 operates on a slower time-scale than FDPs and is the next important protein to act under FL stress. A lack of PGR5 inhibits cell growth, even under mild FL conditions. It is possible that PGR5 acts as a redox-dependent regulator of photosynthesis. The importance of PGRL1 on the growth performance is only observed under severe FL conditions, despite the importance of PGRL1-mediated cylic electron transport during high light transients.

In the second section of my thesis, I studied the role of FDPs in H_2-photoproduction during the transition to anaerobiosis. Anoxic culture conditions are necessary to enable the function of oxygen-sensitive hydrogenases. Here, it is shown that FDPs may accelerate the transition of *C. reinhardtii* cells to anaerobiosis during S-deprivation. Furthermore, application of a magnesium (Mg)-deprivation protocol resulted in prolonged H_2 production and improved cell viability. High accumulation levels of FDPs during the H_2 production phase suggested a contribution of these proteins to the maintenance of anoxia when Mg-deprivation is employed to induce H_2 production.

TIIVISTELMÄ

Yksi nyky-yhteiskunnan suurimmista maailmanlaajuisista haasteista on uusiutuvien biopolttoaineiden kehitys kestävän energialähteen turvaamiseksi. Vedyllä on suunnattomat mahdollisuudet tulla tulevaisuuden polttoaineeksi, koska se tarjoaa energiaa ilman hiilidioksidipäästöjä. *Chlamydomonas reinhardtii* viherlevällä on hydrogenaasi entsyymejä, ja tietyissä olosuhteissa se kykenee tuottamaan vetyä valoenergian avulla. Yhdessä FDP-proteiinien (Flavodiiron proteins), PGR5:n (PROTON GRADIENT-REGULATION 5) ja PGRL1:n (PGR5-LIKE PHOTOSYNTHETIC PHENOTYPE 1) kanssa hydrogenaasit ovat vaihtoehtoisten elektroninsiirtoreittien kannalta keskeisiä proteiineja. Yhteyttävät eliöt ovat kehittäneet tämän kaltaisia vaihtoehtoisia elektroninsiirtoreittejä säätääkseen yhteyttämiskoneistoaan muuttuvissa ympäristöolosuhteissa kuten käytettävissä olevan hiilen määrän tai valointensiteetin vaihdellessa. Väitöskirjatutkimukseni aiheena oli kaikkien näiden vaihtoehtoisten elektroninsiirtoreittien välinen vuoropuhelu. Tutkimus suoritettiin vaihtoehtoisten elektroninsiirtoreittien säätelyn ymmärtämiseksi ja *C. reinhardtiin* kaupallisen vedyntuotannon mahdollisten pullonkaulojen tunnistamiseksi.

Väitöskirjani ensimmäisessä osassa osoitin, että kaikki kolme proteiinia, FDPt, PGR5 ja PGRL1, osallistuvat *C. reinhardtiin* valoreaktioiden suojeluun vaihtelevissa valo-olosuhteissa. FDPt muodostavat nopean elektroninielun valoreaktio I:ltä alavirtaan ja ovat elintärkeitä vaihtelevassa valossa selviytymiselle. PGR5 toimii hitaammalla aikaskaalalla kuin FDP:t ja on seuraava tärkeä proteiini, joka toimii vaihtelevan valon stressissä. PGR5:n puuttuminen rajoittaa solujen kasvua jopa vain lievästi vaihtelevassa valossa. On mahdollista, että PGR5 toimii yhteyttämisen hapetus-pelkistys säätelijänä. PGRL1:n merkitys solujen kasvun kannalta tuli ilmeiseksi ainoastaan rajusti vaihtelevissa valo-olosuhteissa, vaikka PGRL1-välitteinen syklinen elektroninsiirto on tärkeä siirryttäessä korkeaan valoon.

Väitöskirjani toisessa osassa tutkin FDP:iden roolia vedyntuotannossa anaerobisiin oloihin siirryttäessä. Hapettomat kasvatusolosuhteet ovat välttämättömiä happiherkkien hydrogenaasien toiminnan mahdollistamiseksi. Tässä työssä näytetään, että FDP:t voivat nopeuttaa *C. reinhardtii* solujen siirtymistä anaerobioosiin rikkipuutoksen aikana. Magnesium-puutoksen käyttö taas johti pidentyneeseen vedyn tuotantoon ja solujen parempaan elinvoimaisuuteen. Kun magnesiumin puutosta sovellettiin vedyntuotannon käynnistämiseen, FDP:iden havaittiin kertyvän korkealle tasolle, mikä viittasi näiden proteiinien osallistuvan hapettomien olosuhteiden ylläpitoon vedyntuotannon aikana.

Es ist schon so: Die Fragen sind es,
aus denen das, was bleibt, entsteht.

- Erich Kästner -

1. INTRODUCTION

1.1. Green algae - *Chlamydomonas reinhardtii* as a model organism

Green algae comprise a large group of photosynthetic eukaryotic organisms including streptophytes, such as Charales and Zygnematales, which are close relatives of the last common ancestor of land plants (Wodniok et al., 2011). Green algae diverged over a billion years ago from stramenopiles (Yoon et al., 2004), thus linking them closely to several other microalgae, including diatoms. The relatively low position of green algae on the evolutionary tree makes them an interesting target for developmental comparisons. Unicellular green algae can occupy both aquatic and terrestrial habitats and can live under photoautotrophic, heterotrophic or mixotrophic conditions when provided with the right carbon source, which in laboratory conditions is commonly acetate. Moreover, green algae possess great variation in cellular architecture and metabolic capacity, making them useful as model organisms for the study of different lifestyles(Harris, 2001).

Green algae own unique features as model systems in research and for commercial purposes (Brennan & Owende, 2013; Scaife et al., 2015). Specifically, the use of green algae for low value, high volume biofuels provides a potential solution to many environmental problems that come with crop-based production (Chisti, 2007). However, to make this economically feasible, a biorefinery approach is required whereby bio-remediation of waste water and/or the production of other economically interesting high value / low volume biomolecules would offset the cost of cell harvest and conversion processes (Sambusiti et al., 2015; Yen et al., 2013). Microalgae are capable of producing valuable compounds like carbohydrates, lipids, proteins, starch, cellulose, polyunsaturated fattyacids, antioxidants, pigments, pharmaceuticals, natural colorants, fertilizer, and after oil extraction, remaining biomass can be utilized as animal feed (Mata et al., 2010; Yen et al., 2013; Trivedi et al., 2015).

The most widely used research model organism among green algae is the unicellular flagellate *Chlamydomonas reinhardtii* (Harris, 2001; Merchant et al., 2007). *C. reinhardtii* is an ~10 µm long, wet soil dwelling green alga with several mitochondria, two flagella for motility and sexual reproduction, and a large cup-shaped chloroplast that contains the photosynthetic apparatus (Dent et al., 2001, Fig. 1). In 1945, Gilbert Smith isolated the first laboratory strain of *C. reinhardtii* in soil samples from Amherst, Massachusetts, USA (Harris, 2001).

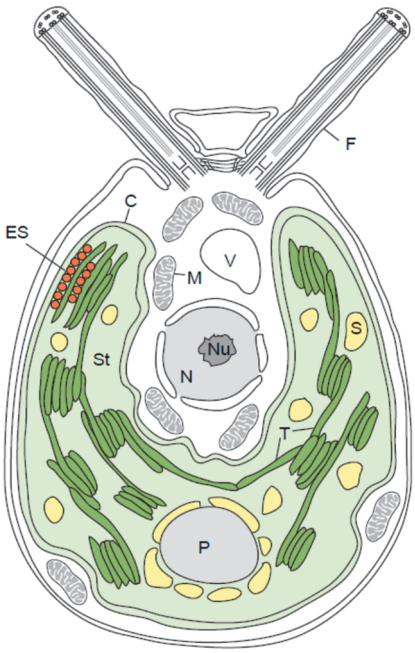

Fig. 1: The morphology of *Chlamydomonas reinhardtii*. The scheme shows the nucleus (N) with the nucleolus (Nu) in the center. It is surrounded by the large cup-shaped chloroplast (C) with the thylakoid membranes (T), stroma (St), starch grains (S) and the pyrenoid (P) inside. The pyrenoid inside the chloroplast houses large amounts of RuBisCo and is the place of CO_2 assimilation into starch. The eye-spot (ES) is located beside the inner envelope membrane of the chloroplast. Two flagella (F) branch from the apical region where is also the contractile vacuole (V) located. In the cytoplasm several mitochondria (M) are situated (adapted from Dent et al., 2001).

C. reinhardtii persists in its haploid stage as one of the two mating types (mt^+ or mt^-) during the vegetative phase (Harris, 2001). The nuclear, mitochondrial and chloroplast genomes are sequenced (Merchant et al., 2007) and the roughly 17 000 genes identified are ~93% correctly annotated, following complementary expression analysis (Blaby et al., 2014). Comparative genomic analyses have revealed that many genes, especially those connected to photosynthesis and plastid metabolism, can be traced back to a common green algae and plant ancestor. However, *C. reinhardtii* also carries many genes related to the animal kingdom that are lost in angiosperms (Merchant et al., 2007).

C. reinhardtii possesses several practical advantages as a model organism. It is easy to cultivate, to make genetic crosses, and there are relatively simple protocols available for genetic manipulation (Graham, 1996). The chloroplast genome can be specifically manipulated by homologous recombination (Day & Goldschmidt-Clermont, 2011). However, the nuclear genome is transformed by random integration and followed by extensive screening of multiple transformants (Debuchy et al., 1989). The generation of a knock-out library, currently consisting of more than 37 000 mutant lines, has thus become an invaluable tool for genetic approaches to photosynthesis research (Li et al., 2016).

1.2. Photosynthetic light reactions

Through oxygenic photosynthesis, cyanobacteria, green algae and higher plants harvest and convert solar energy into chemical energy of carbon compounds via the simultaneous splitting of water into protons, electrons and O_2. Therefore, photosynthesis not only maintains atmospheric O_2 levels but also supplies Earth with energy in form of organic molecules (Blankenship, 2013). In eukaryotic organisms, photosynthesis takes place in chloroplasts, with the synthesis of ATP and NADPH occurring in the light reactions via the major photosynthetic protein complexes located in the thylakoid membrane. The photosynthetic apparatus of photosynthetic organisms is fairly similar, particularly among eukaryotic photosynthetic organisms. However, *C. reinhardtii* has some unique features, which differ from higher plants like *Arabidopsis thaliana.* It also has some features preserved from cyanobacterial ancestors, which have disappeared from *A. thaliana* (Hohmann-Marriott & Blankenship, 2011).

In the stroma, the Calvin-Benson-Bassham (CBB) cycle uses ATP and NADPH to convert inorganic carbon into starch, referred to as the dark reaction (Raines, 2003).

1.2.1. Linear electron transport

Specialized light-harvesting pigment-protein complexes, LHCII and LHCI, harvest sunlight and transfer excitation energy to the reaction centers, Photosystem II (PSII) and Photosystem I (PSI), where the photochemical reactions take place (Blankenship, 2013). In PSII, the energy of the harvested photons is used to excite P680 and to shuttle an electron via pheophytin and a plastoquinone (PQ), Q_A, to another PQ molecule, Q_B (Takahashi et al., 1987; Minagawa & Takahashi, 2004). When Q_B is doubly reduced, it binds two protons from the stroma, is converted to plastoquinol (PQH_2) and is released into the PQ-pool. Simultaneously, water slpitting occurs at the Mn_4CaO_5 cluster on the donor side of PSII (Umena et al., 2011). The protons originating from this reaction are released into the lumen and the electrons reduce oxidized P680.

The PQH_2 molecule is a mobile carrier that moves inside the thylakoid membrane to the Cytochrome (Cyt) b_6f complex where it is oxidized. Cyt b_6f mediates electron transfer between the two photosystems and is a dimeric protein complex. Each monomer consists of eight subunits; the large subunits cytochrome *f*, cytochrome b_6, Rieske iron-sulfur protein and subunit IV, and the small subunits

PetG, PetL, PetM, and PetN (Takahashi et al., 1996; Baniulis et al., 2008). A PQH_2 binds to the Q_0 pocket in Cyt b_6f and transfers one electron onto the Rieske subunit and Cyt f and one electron onto Cyt b_6. Because the next electron carrier, plastocyanin (Pc), can accept only one electron, the so-called Q-cycle in the Cyt b_6f complex regulates this process (Mitchell, 1975). The Q-cycle consists of several consecutive redox reactions and results in one electron being redirected back to the PQ-pool. During the Q-cycle, protons are translocated from the stroma to the lumen. At PSI, photons excite P700, the primary donor of PSI. P700* transfers an electron onto Chl A_0, this is then shuttled via several intermediate acceptors (phylloquinone A_1, the Fe_4-S_4 clusters F_A, F_B, and F_X) onto ferredoxin (Fd) in the stroma (Ben-Shem et al., 2003). Pc in turn, is necessary to reduce $P700^+$. Ultimately, electrons are transferred to nicotinamide adenine dinucleotide phosphate ($NADP^+$) and form NADPH. This reaction is catalyzed by ferredoxin:$NADP^+$ reductase (FNR). This whole electron transport chain from PSII to NADPH is called linear electron transfer (LET) and is depicted in Fig. 2 by black arrows (Haehnel, 1984).

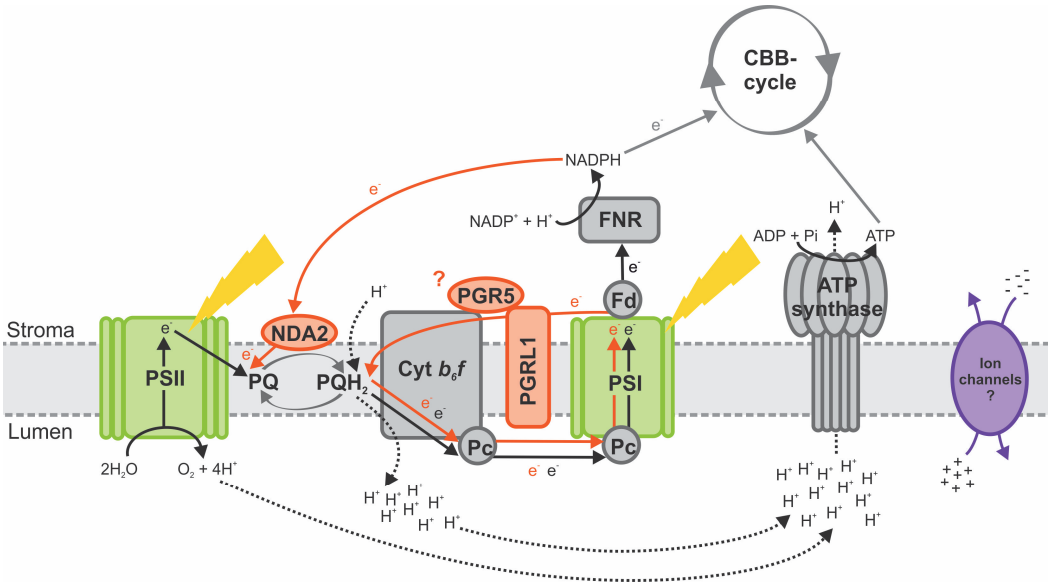

Fig. 2: Simplified scheme of the photosynthetic linear and cyclic electron transfer routes in *C. reinhardtii* thylakoid membranes. LET is marked by black and CET by red arrows. Black dashed arrows illustrate the proton fluxes over the thylakoid membrane. The counter ion fluxes are illustrated in purple. Carbon fixation by the CBB-cycle is marked in grey.

As a result of water splitting at PSII and proton pumping from the stroma via the Q-cycle at Cyt b_6f, the lumen becomes acidified (Sacksteder et al., 2000). This proton gradient, ΔpH, is used to synthesize ATP via the chloroplast F_0F_1-ATP synthase by adding an inorganic phosphate (Pi) to ADP in the stroma (Fig. 2, black dashed arrows). Thus, the final products of the photosynthetic light reactions are NADPH and ATP (Mitchell & Moyle, 1967) that then are used to fuel the carbon fixation by the CBB-cycle (Fig. 2 grey arrows).

Two components comprise the proton motive force (*pmf*): ΔpH and the electric potential at the thylakoid membrane (ΔΨ). They are thermodynamically equal and both contribute to ATP synthesis (Cruz et al., 2001). However, only the ΔpH component induces regulative mechanisms like non-photochemical quenching (NPQ) or photosynthetic control via acidification of the lumen. Recently, it was shown that elevated ΔΨ can also induce alterations in photosynthetic regulation and PSII photodamage (Davis et al., 2016). At low light intensities, the contribution of ΔpH and ΔΨ to *pmf* is equal, but ΔpH increases upon transition to high light and induces photosynthetic regulative mechanisms (Yamamoto et al., 2016). To balance *pmf* it is necessary to regulate the ΔΨ component. Therefore, an efflux of counter cations, like K^+ and Mg^{2+} or influx of anions like Cl^- to the lumen (Fig. 2, purple arrows) is needed to store *pmf* dominantly as ΔpH (Kramer et al., 2003; Shikanai & Yamamoto, 2017).

Most thylakoid ion channels and transporters are characterized in *A. thaliana*, with predicted homologs found in many green algae and cyanobacteria (Spetea et al., 2017). Examples of such channels include: the potassium channel TPK3, which releases K^+ from the thylakoid lumen and thus decreases ΔΨ (Carraretto et al., 2016); the voltage-dependent Cl^- channels VCCN1, VCCN2 (Herdean et al., 2016a) and the CLCe Cl^- channel/transporter (Herdean et al., 2016b), which effects both *pmf* and the H^+ conductivity, g_{H^+}, of the ATP synthase; and the KEA3 K^+/H^+ antiporter, which increases the contribution of ΔΨ to total *pmf* (Armbruster et al., 2014). This KEA3 K^+/H^+ channel is redox-regulated and increases the efficiency of photosynthesis by accelerating the relaxation of NPQ upon a high to low light transition.

1.2.2. Cyclic electron transport

Differences between eukaryotic photosynthetic organisms are evident in the mechanisms that mediate cyclic electron transport (CET) in the thylakoid membrane. Assimilation of one CO_2 in the CBB-cycle requires 3 ATP and 2 NADPH

molecules. The concept of CET around PSI was initially suggested to compensate for the scarcity of ATP produced in LET alone (Allen, 2002).

When four quanta of photons are absorbed by PSII and PSI each, two water molecules are split and four protons and molecular O_2 are released into the thylakoid lumen. The four resulting electrons are transferred onto the intersystem PQ pool. Via LET, these four electrons reduce two $NADP^+$ and translocate eight protons into the thylakoid lumen (Sacksteder et al., 2000). In total 12 protons resulting from LET (Fig. 2, dashed black arrows) can bind to the proton-binding c-subunits that form a ring structure at the CF_0 sub-complex of the ATP synthase. However, there are actually 14 c-subunits, therefore the protons from LET do not completely rotate the CF_0 sub-complex and only produce 2.57 ATP molecules (Vollmar et al., 2009). To achieve the full count of three ATP, one electron needs to be recycled from PSI to Cyt b_6f (Fig. 2, red arrows) where two additional protons are pumped into the lumen. In this way, CET acidifies the chloroplast lumen without creating NADPH (Allen, 2002; Munekage et al., 2004). However, there have been observations of LET yielding three ATP and two NADPH (Steigmiller et al., 2008), questioning the relevance of CET. Nevertheless, ATP and NADPH are also consumed in many other cellular reactions, requiring frequent adjustment between LET and CET. Additionally, CET is needed to increase the ΔpH component of *pmf* to activate NPQ (Shikanai & Yamamoto, 2017).

There are two different CET pathways that have been proposed in *A. thaliana*: the Fd-dependent and the type-I NAD(P)H dehydrogenase (NDH)-dependent CET (Peng et al., 2008). NDH is a huge multisubunit protein complex, which pumps protons into the lumen, similar to complex I in mitochondria. In the past, NAD(P)H has been considered to be the electron donor. However, it was recently demonstrated that Fd in fact donates electrons to NDH, rendering it a ferredoxin-plastoquinone reductase (Munekage et al., 2004; Yamamoto et al., 2011; Peltier et al., 2016). NDH is able to form a supercomplex with PSI via the two antenna proteins LHCA5 and LHCA6 (Peng et al., 2008; Yadav et al., 2017). Higher plant NDH has evolved from a structurally simpler cyanobacterial NDH-1 complex, which can have different subunit composition depending on its metabolic function (Battchikova et al., 2011). Cyanobacterial NDH-1 is also involved in CET and additionally in respiration and the carbon concentrating mechanism (CCM) (Ogawa, 1991). The functions of type-2 NAD(P)H:PQ oxidoreductases (NDH-2) in higher plants and cyanobacteria are not well known, however they do not pump protons and seem to be involved in the α-tocopherol redox cycle and/or vitamin K1 synthesis

in plants (Fatihi et al., 2015) and in even more diverse systems in cyanobacteria (Peltier et al., 2016).

Intriguingly, the NDH-1 complex is missing in *C. reinhardtii* (Maul et al., 2002) and instead NDH-2 (NDA2) reduces PQ and mediates CET without pumping additional protons (Desplats et al., 2009; Jans et al., 2008). NDA2 is thus less effective in establishing ΔpH than the NDH-1 complex. However, its fairly simple structure, comprising only one polypeptide, may be easier to maintain by the cell metabolism.

Two proteins, called PROTON GRADIENT REGULATION 5 (PGR5) and PGR5-LIKE PHOTOSYNTHETIC PHENOTYPE 1 (PGRL1) have been proposed to function in antimycin A (AA)-sensitive CET in *A. thaliana* (DalCorso et al., 2008; Munekage et al., 2002). PGRL1 is an integral thylakoid protein that has two transmembrane domains with both the N- and C-terminus exposed to the stroma (DalCorso et al., 2008). It has been demonstrated that PGRL1 interacts with PGR5 *in vitro* and is able to accept electrons from Fd to reduce PQ. This model suggests that PGRL1 and PGR5 form the elusive ferredoxin-plastoquinone reductase, FQR (Hertle et al., 2013). PGR5 is predicted to be a small extrinsic thylakoid protein without an explicit sequence motif which could predict its function. The regulation of (AA)-sensitive Fd-dependent CET and an interaction of PGR5 with the stromal side of PGRL1 *in vivo* in *A. thaliana* was suggested by DalCorso et al., (2008). Later, it was proposed that the main role of PGR5 is the regulation of LET via photosynthetic control at Cyt b_6f to prevent photodamage of PSI under fluctuating light conditions in *A. thaliana* (Suorsa et al., 2012). In *C. reinhardtii*, PGRL1 has been shown to mediate CET under anoxic and oxic conditions (Terashima et al., 2012; Tolleter et al., 2011), while the involvement of PGR5 in CET has been exclusively demonstrated only under anoxic conditions (Alric, 2014; Johnson et al., 2014). Additionally, in *C. reinhardtii* Fd-mediated CET is not AA-sensitive, probably due to a mutation in the amino acid sequence of PGR5 (Sugimoto et al., 2013).

In *C. reinhardtii*, the molecular machinery for Fd-CET was identified by Iwai et al., (2010) as a supercomplex consisting of PSI–LHCI, LHCII, Cyt b_6f, PETC, PetD, PETO, FNR and PGRL1. In a later study, the Ca^{2+} sensor protein (CAS) and ANAEROBIC RESPONSE 1 (ANR1) were identified as further components of this CET supercomplex, possibly regulating CET efficiency in response to calcium signaling (Terashima et al., 2012). The absence of PGR5 in the functional CET supercomplex suggests that it is not an essential component of CET, but is possibly involved in ensuring efficient CET under certain environmental conditions. Indeed, it has only been possible to isolate the CET supercomplex from anaerobic conditions when the

stromal carriers are over-reduced and the switch from LET to CET is enhanced (Takahashi et al., 2013; Johnson et al., 2014). The formation of a CET supercomplex brings all necessary components for efficient CET in close proximity and thus, possibly eliminates the competition of CET and LET for commonly shared components like PQ, Cyt b_6f, Pc, PSI, Fd and FNR (Johnson, 2011). However, such a CET supercomplex has never been identified in higher plants. Instead, in *A. thaliana* complexes of PSI with NDH or PSI with Cyt b_6f have been identified (Peng et al., 2008; Yadav et al., 2017).

1.3. Alternative electron sinks of photosynthesis

While the recycling of electrons via CET regulates photosynthesis for the metabolic needs of the cell for ATP and NADPH, it does not represent a true sink for excess electrons. In situations where the electron pressure in the PET chain suddenly increases, it is necessary to release these electrons in a safe way to avoid harm to the photosynthetic protein complexes and the production of dangerous reactive oxygen species (ROS) (Asada, 1999). Photosynthetic organisms have developed several alternative electron sinks to fine-tune PET under changing environments. These alternative electron sinks differ between cyanobacteria, green algae and higher plants. Excess ROS production is prevented by O_2 photoreduction processes in the chloroplast. These processes use O_2 as final electron acceptor, reducing it to H_20 (Fig. 3, blue arrows). *C. reinhardtii* mostly relies on O_2 photoreduction via flavodiiron proteins (FDPs), the Water-Water-Cycle (WWC), and the plastoquinole terminal oxidase (PTOX) as alternative electron sinks (Peltier et al., 2010). However, when facing anoxic conditions, *C. reinhardtii* is able to use protons as a final electron acceptor, creating H_2 instead of H_2O (Ghysels et al., 2013; Fig. 3, orange arrows). This H_2ase reaction is described in more detail in section 1.5.1.

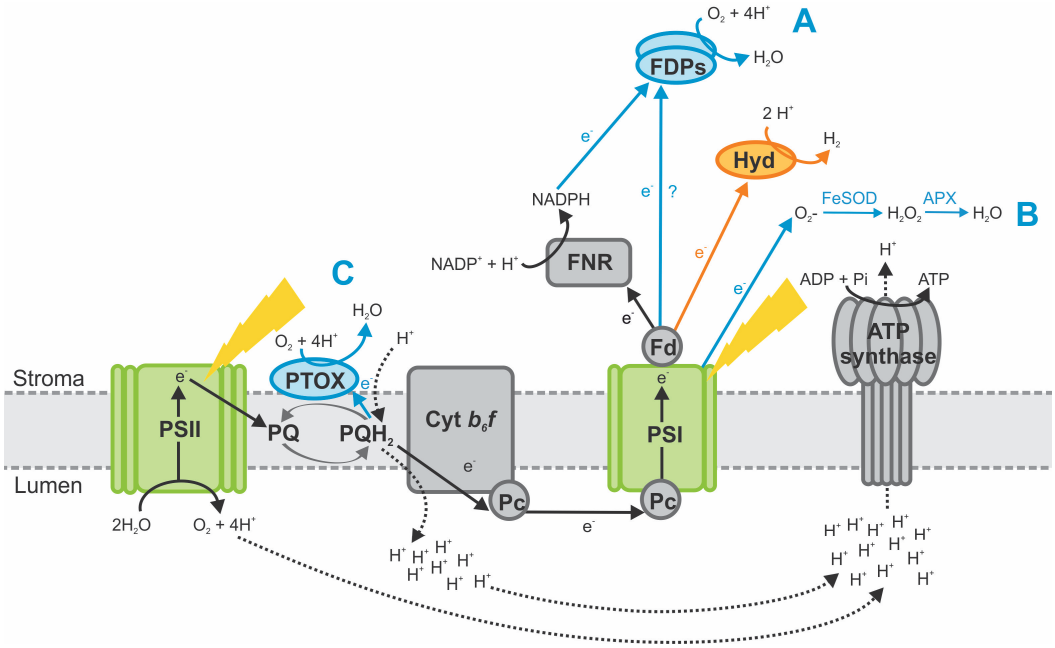

Fig. 3: Alternative electron sinks in *C. reinhardtii* photosynthesis. O_2 photoreduction pathways are marked with blue arrows: FDPs (A), WWC (B) including the superoxide dismutase (FeSOD) and the ascorbate-specific peroxidase (APX), and PTOX (C). Orange arrows illustrate H_2 production used as electron sink under anoxic conditions. Proton fluxes are depicted by black dashed arrows.

1.3.1. Flavodiiron protein-mediated O_2 photoreduction

The most recently elucidated route for O_2 photoreduction in *C. reinhardtii* employs flavodiiron proteins (FDPs) as an electron sink downstream of PSI (Peltier et al., 2010; Allahverdiyeva et al., 2015a). FDPs are a conserved protein family present in prokaryotes (Wasserfallen et al., 1998) and some eukaryotes, including anaerobic protozoa, green algae and lower plants (Zhang et al., 2009; Peltier et al., 2010; Allahverdiyeva et al., 2015b). FDPs are enzymes with nitric oxide (NO) and/or O_2 reductase activity. Thus, they are important in sustaining anoxic conditions in facultative anaerobic organisms and in detoxifying nitrosative stress (Rodrigues et al., 2006; Vicente et al., 2008a; 2008b). It is possible that some FDPs reduce both substrates, O_2 and NO, but usually the substrate selection is exclusive (Seedorf et al., 2007; Vicente et al., 2007).

FDPs have a modular structure with two core domains comprising of metallo-β-lactamase-like (FeFe)-domain at the N-terminus and a C-terminal flavodoxin-like (FMN) domain (Vicente et al., 2002; Fig.4 A).

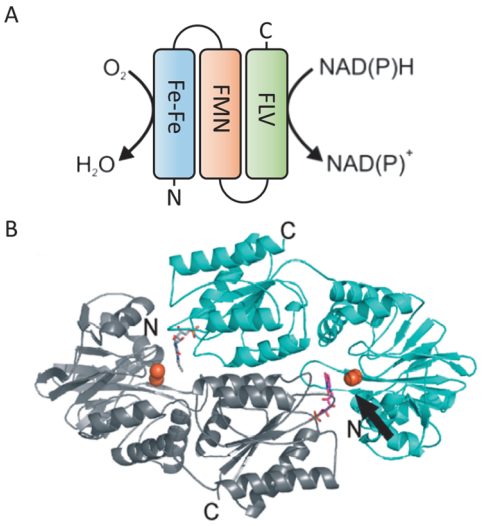

Fig. 4: Function and molecular structure of FDPs. Organization of functional domains in FDPs present in photosynthetic organisms (A). Fe-Fe, *β*-lactamase domain; FMN, flavodoxin domain; and FLV, flavin-reductase domain. The 'head-to-tail' structure of *Synechocystis* Flv2/Flv4-heterodimer (B). The arrow shows the reactive centre with FMN (magenta) of Flv2 (gray) and the diiron (orange) of Flv4 (cyan). Figures modified from Zhang et al., (2012) and Allahverdiyeva et al., (2015b).

These two core domains are part of the most elementary group of FDPs, class A (Vicente et al., 2008a; Allahverdiyeva et al., 2015b). The metallo-*β*-lactamase unit engulfs a non-heme diiron center and forms the active site where NO/O_2 are reduced (Silaghi-Dumitrescu et al., 2003). The flavodoxin-like domain binds FMN as cofactor and functions as the electron donor for the FeFe-domain. However, the FDP monomers are not able to carry out electron transfer by themselves because the FeFe and FMN domains are fairly isolated from each other (Vicente et al., 2008a). Thus, FDPs build a 'head-to-tail' dimer structure that brings the redox centres in close proximity and enables efficient electron transfer (Fig. 4 B). FDPs that have a unique NADPH flavin reductase domain fused to the C-terminus are present exclusively in several photosynthetic organisms and belong to the so called class C FDPs (Wasserfallen et al., 1998; Vicente et al., 2002; Zhang et al., 2009; Allahverdiyeva et al., 2015b).

Class C FDPs comprise the entire electron transport from NADPH to O_2 in a single protein. Genes encoding class C FDP homologs have been conserved in photosynthetic organisms throughout evolution and are found in cyanobacteria, algae, bryophytes, lycophytes and gymnosperms. It is noteworthy that FDPs have not been detected in diatoms, haptophytes or angiosperms (Ilík et al., 2017; Peltier et al., 2010). FDPs present in oxygenic photosynthetic organisms can be phylogenetically grouped into two clusters, A and B, and at least one FDP of each cluster is always present (Zhang et al., 2009). There are several structural differences between FDPs of cluster A or B. FDPs belonging to cluster A are characterized by the absence of canonical ligands in the FeFe center (Gonçalves et al., 2011). This modification possibly hinders electron transfer in homodimers;

therefore the arrangement in heterodimers, one from each cluster, was suggested (Zhang et al., 2012).

Thus far, the majority of studies have focused on the possible role of cyanobacterial FDPs on photosynthesis and cell metabolism. The non-N_2-fixing, unicellular cyanobacterium *Synechocystis* sp. PCC 6803 (hereafter *Synechocystis*) expresses four FDPs. The Flv1 and Flv3 proteins are shown to mediate the light-dependent reduction of O_2 downstream of PSI without the production of ROS (Helman et al., 2003). This pathway of O_2 photoreduction is also called the Mehler-like reaction. The same Flv1 and Flv3 proteins are essential for the protection of photosynthesis, particularly the PSI complex, under fluctuating light conditions (Allahverdiyeva et al., 2013; Allahverdiyeva et al., 2015a). Additionally, Flv1 and Flv3 appear to be capable of forming homodimers that have function aside from light-dependent O_2 reduction (Mustila et al., 2016). The involvement of the other two FDPs, Flv2 and Flv4, in O_2 photoreduction and thus, their electron acceptor is still under debate (Helman et al., 2003; Allahverdiyeva et al., 2015b; Shimakawa et al., 2015). However, these proteins are encoded by the *flv4-sll0218-flv2* operon and form a Flv2/Flv4 heterodimer. The *flv4-flv2* operon functions in the photoprotection of PSII under high light and CO_2 limiting conditions. This is likely by discharging excess electron pressure at the PSII acceptor-side (Zhang et al., 2009; 2012) in a process that is dependent on intact phycobilisomes (Bersanini et al., 2014).

Anabaena sp. strain PCC 7120 (hereafter *Anabaena*), a filamentous heterocystous N_2-fixing cyanobacterium, expresses six FDPs. The heterodimer Flv1A/Flv3A is specific to vegetative cells and it was hypothesized that Flv1A/Flv3A and Flv2/Flv4 function similarly to their homologs in *Synechocystis*. The additional set of Flv1B and Flv3B are exclusively expressed in heterocysts. While the function of Flv1B remains unidentified, it has been demonstrated that Flv3B performs light-induced O_2 photoreduction. Therefore, it maintains a microoxic environment and safeguards nitrogenase in heterocycsts (Ermakova et al., 2013; 2014).

Recent studies have confirmed the essential role of FDPs as a rapid electron sink protecting PSI in the moss *P. patens* under fluctuating light conditions (Gerotto et al., 2016) and at the onset of light in the liverworth *Marchantia* (Shimakawa et al., 2017). Furthermore, the heterologous expression of *P. Patens* FDPs in *A. thaliana* resulted in functional O_2 photoreduction that could, in part, complete the phenotype of the *pgr5* knock-out mutant (Yamamoto et al., 2016).

C. reinhardtii possesses two *flv* genes, *flvA* (Cre12.g531900) and *flvB* (Cre16.g691800). The high homology between the cyanobacterial Flv1 and Flv3 and

the two *C. reinhardtii* FDPs suggests that FLVA and FLVB are also involved in O_2 photoreduction downstream of PSI (Zhang et al., 2009; Peltier et al., 2010; Allahverdiyeva et al., 2015a). Immune-pull-down has shown an interaction of Fd with FLVB, indicating that Fd could also act as an electron donor for FDPs in *C. reinhardtii*, as indicated in Fig. 3 (Peden et al., 2013).

1.3.2. Chloroplast O_2 photoreduction processes other than flavodiiron proteins

Besides FDP-mediated O_2 photoreduction there are three additional mechanisms which use O_2 as a final electron acceptor of PET: O_2 photoreduction by RuBisCo; WWC; and O_2 photoreduction by PTOX.

The active center of RuBisCo can perform a dual function: carboxylation and oxygenation of ribulose-1,5-bisphosphate (RuBP). This oxygenation reaction of RuBisCo is called photorespiration. In plants, photorespiration is considered a physiologically significant process that reduces redox pressure in the stroma when CO_2 assimilation is low (Ort & Baker, 2002; Peltier & Cournac, 2002). However, due to an active CCM, which favors RuBP carboxylation (Moroney & Ynalvez, 2007), it is unlikely that this pathway plays an important role in the cell metabolism in *C. reinhardtii*.

It is also known that O_2 can be directly reduced at the acceptor side of PSI in a process called the Mehler reaction (Mehler, 1951). This process results in the production of superoxide radicals that are then converted to H_2O_2 and O_2 by a superoxide dismutase (FeSOD). An ascorbate-specific peroxidase (APX) reduces the H_2O_2 to H_20 (Fig. 3). This entire process is called the water-water-cycle (WWC) and is able to dissipate excess reducing equivalents from the photosynthetic apparatus under high light or CO_2 limiting conditions (Asada, 1999). As WWC works at the acceptor side of PSI, it contributes to the generation of ΔpH, possibly balancing the ratio of ATP and NADPH in a similar way to CET. The APX reaction also creates a monodehydroascorbate radical (MDA) that is extremely reactive (Miyake & Asada, 1992). This MDA can, in turn, be reduced by an MDA reductase using NADPH as a substrate, or by a glutathione/NADPH system (Asada, 1999). The WWC is not considered a safe way to dissipate excess electrons because of the potential for cell damage by production of ROS (Ort & Baker, 2002). Although, some ROS is required for stress signaling (Baxter et al., 2014).

Another pathway for O_2 photoreduction is the re-routing of electrons from PSII, via the PQ-pool, to a reaction catalyzed by PTOX (Fig. 3), reducing O_2 to H_20 at the expense of CO_2 fixation (Kuntz, 2004). PTOX cannot compete with PSI under

steady-state photosynthetic conditions; however it likely plays an important role under stress conditions (Trouillard et al., 2012) and thus, PTOX is able to act as a safty valve for phototsynthesis. Additionally, PTOX can promote ROS production (Heyno et al., 2009). While higher plants possess only one PTOX gene, *C. reinhardtii* has two, although it has been shown that PTOX2 mostly functions in O_2 photoreduction. Moreover, *C. reinhardtii* PTOX2 is not involved in chloroplast biogenesis or carotenoid biosynthesis like the plant enzyme is (Houille-Vernes et al., 2011). The PTOX reaction directly affects the redox state of the PQ-pool and therefore influences PQ-pool dependent regulative mechanisms, such as state transitions.

1.4. Additional mechanisms regulating photosynthetic electron transport

Fast changes in light availability and other environmental conditions create the need to constantly adjust the electron flow via the PET chain. In addition to the AET pathways and electron sinks described above, there are also other short-term mechanisms regulating photosynthesis and supporting the dissipation of excess electron pressure. These photoprotective mechanisms include non-photochemical quenching, photosynthetic control, and state transitions (Rochaix, 2013).

1.4.1. Non-photochemical quenching

Non-photochemical quenching (NPQ) is the portion of absorbed light energy that exceeds the capacity of the PET chain and therefore has to be dissipated harmlessly as heat. In green algae and plants, NPQ can be separated into three components (Horton et al., 1994; Müller et al., 2001). One component depends on the photoinhibition of PSII (qI) induced by excess light damage; another is credited to state transitions (qT) involving the detachment of phosphorylated LHCII antenna from PSII (Minagawa, 2011), described in more detail below.

However, the main component of NPQ is called energy dependent quenching (qE) and is regulated by the ΔpH over the thylakoid membrane (Niyogi et al., 2000). As already described, ΔpH increases because of a boost in photosynthetic activity and this increased ΔpH in turn down-regulates PET. The qE component involves the reversible conversion of the carotenoid violaxanthin to antheraxanthin and subsequently to zeaxanthin (xanthophyll cycle) under high light conditions (Niyogi et al., 1997). In *A. thaliana*, the xanthophyll cycle makes up a large portion of qE, while its contribution in *C. reinhardtii* is minor (Niyogi et al., 1997; Kawabata & Takeda, 2014; Quaas et al., 2015).

In *A. thaliana,* the protein responsible for qE is a LHC-family protein, PsbS (Li et al., 2002). It is constitutively expressed but only activated by an increase in luminal pH, leading to a rapid and efficient induction of NPQ (Niyogi & Truong, 2013). In *C. reinhardtii*, another stress-related three-helix LHC family protein, LHCSR3, is known to sense ΔpH and quench excited Chl molecules in LHCII (Peers et al., 2009). LHCSR3 expression and activation is triggered by high light and low carbon conditions (Maruyama et al., 2014). Furthermore, protonation of LHCSR3 leads to the activation of the quenching process, linking ΔpH formation with LHCSR3-mediated qE (Bonente et al., 2011). A blue-light photoreceptor and calcium signaling may also promote LHCSR3 expression (Petroutsos et al., 2016). Recently, it was shown that a PSBS protein is present also in *C. reinhardtii*, and is likely involved in the induction of qE (Correa-Galvis et al., 2016). Unlike LHCSR3, the PSBS protein is much less abundant and accumulates transiently during stress conditions (Tibiletti et al., 2016). The contribution of PGRL1-Fd-mediated CET to ΔpH links CET with the induction of NPQ (Kukuczka et al., 2014). Furthermore, a possible cooperation and compensation of LHCSR3-dependent and PGRL1-dependent photoprotection has been proposed (Chaux et al., 2017), demonstrating the importance of the interplay between short-term response mechanisms and AET.

1.4.2. Photosynthetic control at Cytochrome b_6f

Photosynthetic control describes an additional ΔpH-dependent photoprotective mechanism which functions at Cyt b_6f level (Tikhonov, 2014). Although photosynthetic control in the PET chain between PSII and PSI was first proposed long ago (Rumberg & Siggel, 1969), its importance and mechanism have only recently become clearer (Colombo et al., 2016).

It has been suggested that the increase in ΔpH at the thylakoid membrane blocks the reaction between PQH_2 and the Cyt *f* subunit of Cyt b_6f (Nishio & Whitmarsh, 1993). The mechanism of PQH_2 oxidation includes the formation of hydrogen bonds between the hydroxyl group of PQH_2 and the H^+-accepting residues inside the Q_0-center (Crofts et al., 1999). When ΔpH increases, these H^+-accepting residues may be protonated, making them unavailable for PQH_2 oxidation. This photoprotective mechanism is triggered under high light by increased ΔpH, which down-regulates Cyt b_6f activity and limits electron flow, thus protecting PSI under high light stress.

1.4.3. State transitions

In green algae and plants, peripheral light harvesting complexes (LHCs) harvest and transfer light energy to the reaction centers of the photosystems. These LHC antennas consist mostly of chlorophyll (Chl) *a* and *b* and span the thylakoid membrane (Minagawa & Takahashi, 2004; Tokutsu et al., 2004). In *A. thaliana*, the LHCII comprise three types of major antenna proteins, LHCB1-3, organized as heterotrimers. The peripheral LHCII trimers are connected to the PSII core by CP26, CP29 and CP24 monomers. Additional pigments, i.e. neoxanthin, lutein, and carotenoids, are connected with LHCII and work in the dissipation of excess energy. The peripheral antenna of PSI in *A. thaliana* consists of six LHCI subunits, mostly LHCA1-4, with much lower levels of LHCA5 and 6, forming a half-ring on one side of the PSI core (Rochaix, 2011). In *C. reinhardtii*, the monomeric LHCII protein CP24 is missing and CP26 and CP29 are located close to the PSII core complex and connect the major LHCII proteins, LHCBM1-9, to PSII (Drop et al., 2014; Minagawa & Takahashi, 2004). The PSI core is associated with nine LHCI subunits, LHCA1-9, that form a half-ring similar to that in *A. thaliana* (Takahashi et al., 2004; Drop et al., 2011).

The LHCII and LHCI antennae possess different light absorption characteristics. PSII can absorb blue and red light, while PSI can absorb blue and far-red light. These differences result in an imbalance between the excitation of PSII and PSI, especially under rapidly changing light conditions. For optimal photosynthetic electron transport under stress conditions, an adjustment of the relative absorption cross-section is required. Therefore, photosynthetic organisms have developed a mechanism, referred to as a state transition, which balances excitation by connecting LHCs to either PSII or PSI, as required (Murata 1969; Wollman, 2001).

State transitions rely on the interplay between phosphatases and kinases, regulated by the PQ-pool redox status. Under high light conditions, PSII becomes overexcited and the PQ-pool is reduced. In *C. reinhardtii*, this leads to the binding of PQH2 to the Q_0-centre of Cyt b_6f and in turn activates protein kinases, Stl1 and Stt7 (Bonardi et al., 2005). Respective orthologues in *A. thaliana* are called STN8 and STN7. Subsequently, Stt7 phosphorylates several Lhcb subunits and Stl1 phosphorylates PSII core subunits. Part of LHCII then connects to the PSI–LHCI complex. The formation of this PSI-LHCI-LHCII complex is called state-2 and is reversible, back to state-1. In state-1 the PQ-pool is oxidized, followed by the inactivation of the kinases and then the dephosphorylation of LHCII, which

reconnect to PSII (Minagawa, 2011). The antagonistic phosphatases are not well studied in *C. reinhardtii*. However, in *A. thaliana*, PHOTOSYSTEM II CORE PHOSPHATASE (PBCP) is responsible for the dephosphorylation of PSII core subunits and as such, is the antagonist of STN8 (Samol et al., 2012). The STN7 antagonistic protein phosphatase is PPH1/TAP38 that dephosphorylates several Lhcb subunits (Pribil et al., 2010; Shapiguzov et al., 2010).

As state transitions are regulated by the redox status of the PQ pool, the mechanism can be affected by environmental conditions other than high light. It appears that sulfur-deprivation, ATP deficits and dark anoxia also induce state-2 transitions in *C. reinhardtii* (Wykoff et al., 1998; Cardol et al., 2009; Ghysels et al., 2013). In this context, it is also important to note that both CET and state transitions are redox-regulated, although independently from each other (Takahashi et al., 2013).

Whilst well studied, a consensus on the significance of state-transitions is yet to be reached by the research community. Earlier studies suggested that in green algae about 80% of the LHCII antennae are involved in state transitions, while only about 20% of LHCII participate in this process in plants (Delosme et al., 1996). More recent studies in *C. reinhardtii* indicate that only about 10% of LHCII connect to PSI during state-2. In thylakoids, a major fraction of phosphorylated LHCII detach from PSII but do not attach to PSI, thus forming a self-quenching antennae pool dissipating excess energy (Nagy et al., 2014; Ünlü et al., 2014). Contrary to this, another study has suggested that state transitions redistribute energy between PSI and PSII instead of dissipating it (Nawrocki et al., 2016). Although the role of LHCII and PSII core protein phosphorylation remains unclear in *C. reinhardtii*, research on *A. thaliana* has progressed significantly and has demonstrated a key role for thylakoid protein phosphorylation in light-intensity-dependent interactions and energy distribution between PSII and PSI (Tikkanen et al., 2011; Grieco et al., 2012). Dephosphorylation of thylakoid proteins plays also a significant role in the regulation of photosynthesis (Mekala et al., 2015).

1.5. Hydrogen production in green algae

The largest available energy source on Earth is solar power with ~1300 ZJ of photosynthetically active radiation (PAR) received per year (Oey et al., 2016). This amount of energy is much more than the current global demand of only 0.56 ZJ every year, and is more than all fossil fuel and uranium reserves on earth combined (Stephens et al., 2013). Therefore, the ability to efficient harness solar power for

renewable and sustainable energy sources is highly desirable in resolving problems of global warming and fossil fuel depletion.

The exploitation of biologically produced H_2 is a promising renewable alternative to carbon-based fossil fuels. It has immense advantages in that it is a clean fuel that has the potential to be carbon negative (Dubini & Ghirardi, 2015; Melis & Happe, 2001). The combustion of H_2 generates water as the only product and, when generated by aquatic phototrophs, it does not compete with food production for fertile land, which is a common problem for land-based first- or second-generation biofuels (Ghirardi et al., 2000; Stephens et al., 2013). Furthermore, it has a very high energy density (~120 kJ/g) compared to other hydrocarbon fuels (Gupta, 2014). Currently, commercial H_2 production is based on fossil fuels and is mostly produced via steam methane reforming (SMR), but also coal gasification, industrial oil and naphtha reforming, and fossil fuel driven water electrolysis (Gupta, 2014; Lam & Lee, 2012). These technologies require high energy input, are expensive and emit high levels of CO_2.

Over the past decades, biological H_2 photoproduction by cyanobacteria via the nickel hydrogenase (H_2ase) or the bi-directional H_2ase and by green algae via the [FeFe]-H_2ase has become a focus of research. However, there are several bottlenecks which need to be resolved before H_2 photoproduction becomes economical feasible. One of them is the sensitivity of the [FeFe]-H_2ase to O_2 (Ghirardi et al., 1997; Happe et al., 2002; Ghirardi, 2015). Since O_2 is a byproduct of photosynthesis, H_2 production is only a transient process under natural conditions.

C. reinhardtii is one of the most studied microalga in regards to H_2 production (Eroglu & Melis, 2016; Melis & Happe, 2001; Oey et al., 2016). Furthermore, progress in metabolic engineering and synthetic biology has led to advances in establishing *C. reinhardtii* as a biotechnological platform (Scaife et al., 2015). Most of the strategies employed to reach sustainable H_2 photoproduction in *C. reinhardtii* nowadays follow a common concept. The photosynthetic activity and thus, O_2 evolution, has to be reduced and respiratory processes have to be increased in the cell to establish the anoxic or microoxic conditions that induce H_2 production.

There are three different pathways directing electrons towards the [FeFe]-H_2ase (Fig. 5), of which two are connected with the PET chain. The direct pathway is PSII-dependent (Chochois et al., 2009) and the indirect pathway bypasses PSII, with electrons orginating from starch breakdown arriving at the PQ pool via NDA2 (Jans et al., 2008). However, the starch accumulation is PSII-dependent. The third pathway functions under dark anoxic conditions and is linked to fermentation. It involves

electron transfer via a pyruvate-ferredoxin-oxidoreductase (PFR) from pyruvate to [FeFe]-H_2ase (Hemschemeier & Happe, 2011; Noth et al., 2013). The direct PSII-dependent pathway is the most desirable because it exhibits the most efficient conversion of solar energy into H_2 without intermitten products like starch or pyruvate (Chochois et al., 2009; Eroglu & Melis, 2016; Volgusheva et al., 2013).

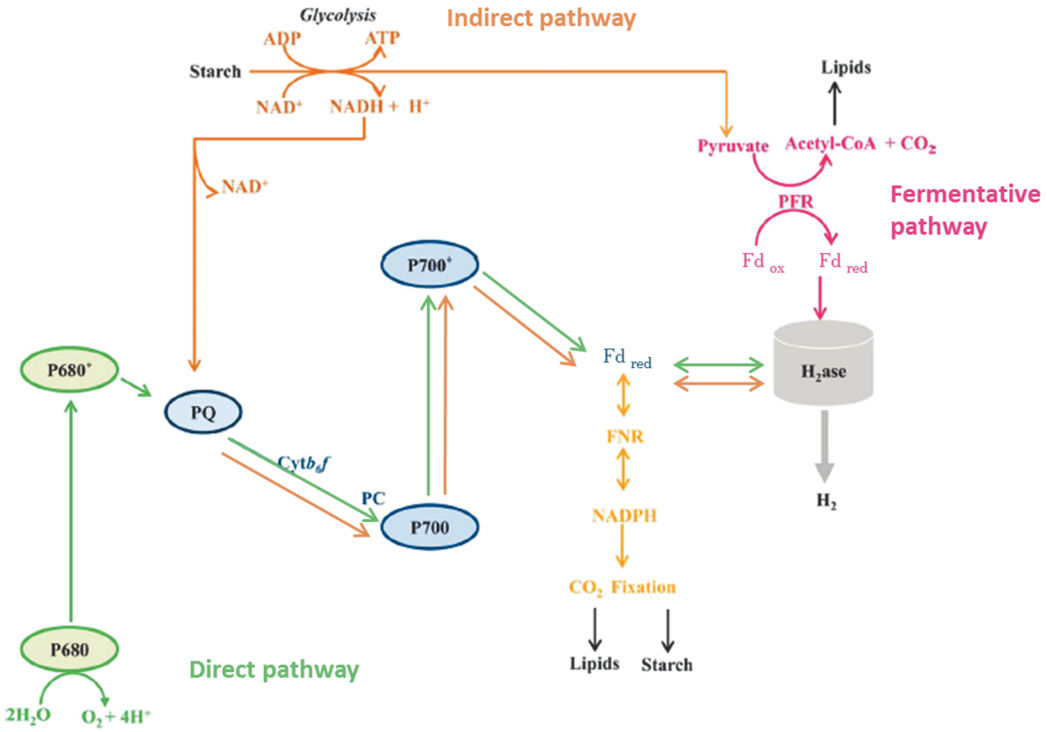

Figure 5: Metabolic pathways fueling H_2 photoproduction. The direct PSII-dependent pathway is depicted in green and the indirect starch-dependent pathway in orange. The third pathway depending on fermentation is shown in pink (adapted from Catalanotti et al., 2013).

1.5.1. The role of hydrogenases as an alternative electron sink

Several microalgae, including *C. reinhardtii*, possess O_2-sensitive [FeFe]-H_2ases that use electrons from Fd to produce H_2 (Fig. 3, orange arrows). These enzymes have been lost in terrestrial plants. It is possible that [FeFe]-H_2ases act as an additional alternative electron sink under anoxic conditions (Melis & Happe, 2001; Melis, 2007; Hemschemeier & Happe, 2011), thus providing a similar dissipation of excess reducing power from the PET chain as PTOX or FDPs under oxic conditions. In cyanobacteria, a bidirectional H_2ase may act as a safety valve during dark to light transitions under anoxic conditions (Appel et al., 2000). This is physiologically

important due to the natural exposure of cyanobacteria and green algae to anoxic or microoxic conditions in their aquatic or wet soil habitats (Clowez et al., 2015), whereas terrestrial plants are constantly surrounded by atmospheric O_2 levels.

The enzymes responsible for H_2 production in *C. reinhardtii* are plastidial soluble [FeFe]-H_2ases located in the stroma (Ghirardi et al., 1997; 2007; Atteia et al., 2013; Stiebritz et al., 2012). The catalytic domain (H-cluster) in *C. reinhardtii* [FeFe]-H_2ases contains only one 4Fe-4S-cluster domain binding one 2Fe-2S cluster, without the addition of a further 4Fe-4S-cluster as found in bacterial H_2ases (Ghirardi et al., 2007; Stripp et al., 2009; Shepard et al., 2014). The [FeFe]-H_2ases mainly accept electrons from FDX1 (Winkler et al., 2010), fueling a double reduction of the proton bound to the distal iron of the 2Fe-2S catalytic center and a subsequent recombination of the resulting hydride anion H^- with another proton bound to the bridging dithiomethylamine ligand. This recombination reaction results in the formation of H_2 (Nicolet et al., 2001). During this reaction the H-cluster becomes accessible to O_2, which inhibits any further H_2ase-activity (Stripp et al., 2009).

C. reinhardtii possesses two genes encoding H_2ases: HydA1, mainly responsible for H_2 production; and HydA2, whose exact function is still under debate (Forestier et al., 2003; Meuser et al., 2012). At least three maturation factors are needed, HydEF and HydG, for the maturation of the H-cluster, that is also O_2-sensitive (King et al., 2006). Whilst early studies indicated that H_2ases are only expressed under anoxic conditions (Happe & Kaminski, 2002), it now appears that O_2 is not the only factor involved in H_2ase expression. It is possible that the redox state of the PQ pool, influenced by starch metabolism or nutrient deprivation, also induces H_2ase expression. Additional factors that may influence H_2ase expression include light-dark photoperiods, the cell cycle (Whitney et al., 2011), and the ΔpH created by CET (Chochois et al., 2009). Whilst H_2ase activity has been well documented under anoxic conditions, a recent study has suggested that H_2ases are also present and able to sustain H_2 production in algal cells under oxic conditions (Liran et al., 2016)

1.5.2. Different protocols to induce hydrogen production

The fermentation driven production of H_2 by green algae under dark anoxic conditions in cooperation with fermentation has been known since the publication of Gaffron and Rubin, (1942). In the same work, light-dependent H_2 production was also demonstrated, following a dark to light shift. Nowadays, a two-stage process involving sulfur-deprivation is the most commonly applied protocol to induce H_2

production in *C. reinhardtii* (Melis et al., 2000). In the first step, *C. reinhardtii* is grown to increase biomass, O_2 is evolved from photosynthesis and reducing power is stored as carbohydrates. During the second step the cells are deprived of sulfur, which initiates specific metabolic changes, leading to H_2 production. Sulfur-deprivation impairs protein synthesis and as a consequence, photodamaged PSII centers are not repaired which severly inhibits electron transport from PSII (Wykoff et al., 1998; Melis et al., 2000; Zhang et al., 2002). Moreover, sulfur-deprivation induces state-2 transition and a simultaneous elevation of CET, which further reduces PSII activity and consequently, O_2 evolution (Wykoff et al., 1998; Melis, 2007). Mitochondrial respiration works at the same level as during sulfur-replete conditions, which allows rapid removal of O_2 from the culture (Melis et al., 2000; Zhang & Melis, 2002). Due to these processes, the culture passes through five stages (Fig. 6): photosynthetically active (I), O_2-consuming (II), anaerobic (III), H_2 producing (IV) and termination (V) (Kosourov et al., 2002; Volgusheva et al., 2013).

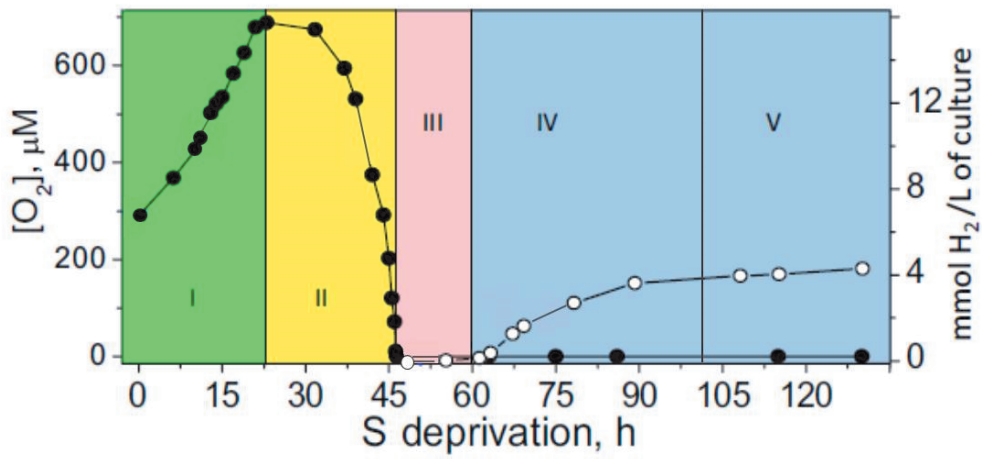

Figure 6: Stages of H_2 photoproduction under sulfur-deprivation; photosynthetic (I), O_2-consumption (II), anaerobic (III), H_2 production (IV) and termination (V). O_2 levels are marked with closed circles and H_2 levels with open circles (adapted from Volgusheva et al., 2013).

Efforts have been made to improve H_2 production yields further by testing deprivation protocols of other nutrients like nitrogen, phosphorus, potassium and magnesium (Philipps et al., 2012; Batyrova et al., 2012; 2015; He et al., 2012; Papazi et al., 2014; Volgusheva et al., 2015). Similar to sulfur-deprivation, the depletion of these nutrients induces a reduced photosynthetic activity, accumulation of starch, the establishment of anoxia and subsequently, H_2 production.

Nitrogen deprivation results in the degradation of numerous proteins of the PET chain and therefore, results in less H_2 production than sulfur-deprivation (Philipps et al., 2012). Phosphorous deprivation is also a less effective stimulus for H_2 production in *C. reinhardtii* (Batyrova et al., 2012). In contrast, magnesium-deprived cells are capable of producing H_2 at similar rates, but for considerably longer periods of time, compared to sulfur-deprived cells (Volgusheva et al., 2015). Magnesium is a component of Chl molecules, therefore its deprivation decreases LHC antenna sizes, which promotes light utilization efficiency in dense cultures. Furthermore, PSII activity remains higher during magnesium-deprivation than during sulfur-deprivation, which may account for the enhanced H_2 production (Volgusheva et al., 2015).

All long-term nutrient deprivation protocols are ultimately terminal for microalgae cultures. Therefore, a periodical two-stage protocol that alternates between aerobic photosynthetic recovery phases and anaerobic H_2 production phases should be applied. This can be realized by transferring cells from the complete growth medium to the nutrient deprivation medium and back (Ghirardi et al., 2009). This is a difficult and expensive task in liquid cultures, which may be improved by the immobilization of cells (Laurinavichene et al., 2006; Kosourov & Seibert, 2009). Moreover, the immobilization of *C. reinhardtii* cells in e.g. Ca^{2+}-alginate films has several advantages in that it substantially increases the conversion efficiency of solar energy to H_2 by accelerated PSII inactivation, altered starch metabolism and thus, enhanced contribution of the indirect pathway to H_2 production (Kosourov & Seibert, 2009). Immobilization possibly also protects the [FeFe]-H_2ase from remaining O_2 in the growth medium.

To date, none of the proposed H_2 production protocols have indicated the potential for an economically feasible process. Thus, more research is required and technical advances are needed to increase current efficiencies.

1.5.3. Improving hydrogen production via genetic modifications

Improvement of H_2 production efficiencies and new insights into production mechanisms have been achieved through genetic engineering approaches. A major target of these approaches has been the generation of an O_2-insensitive H_2ase. However, neither random mutagenesis, nor targeted engineering of the catalytic centre have led to large improvements in O_2-tolerance (Ghirardi et al., 1997; Stiebritz et al., 2012). Another approach is to engineer the heterologous expression of O_2-tolerant H_2ases, such as the clostridial [FeFe]-H_2ase, in *C. reinhardtii* (Noone et

al., 2017). Appealing new candidates are H_2ases able to produce H_2 in the presence of low level O_2 that have recently been identified in two green algae (Hwang et al., 2014).

An alternative approach is to induce anoxic conditions by genetically modifying PSII activity, or by elevating O_2 uptake pathways in the cell. Down-regulation of the O_2 evolution capacity of PSII (Scoma et al., 2012; Torzillo et al., 2009) and the reversible copper-induced regulation of PSII D2 protein synthesis (Surzycki et al., 2007) are strategies demonstrating improved H_2 production. The truncation of LHC antennae (TLA mutants) improved photon conversion efficiencies and altered photoinhibition of PSII, leading to higher H_2 production rates than wt (Kosourov et al., 2011). The reduced LHC antennae make the TLA mutants well-suited to immobilization, as light can penetrate further through the layers of cells. Indeed, immobilized *tla1* mutant cells have been shown to produce H_2 with a maximum specific rate 4–8 times higher than the wt. Similar results were achieved by RNAi knock-down targeting the LHCBM1, 2, 3 and 9 antenna proteins (Grewe et al., 2014; Oey et al., 2013).

To increase O_2 uptake, leghemoglobins that sequester O_2 and are originally found in N_2-fixing rhizobia, have been introduced into *C. reinhardtii* and improved H_2 production compared to wt (Wu et al., 2011). An O_2 consuming *E. coli* enzyme (pyruvate oxidase, PoX) has also been successfully introduced into *C. reinhardtii*, producing double the amount of H_2 than the wt (Xu et al., 2011). In this regard, FDPs could also be considered an interesting target, as they consume O_2 and are endogenous to *C. reinhartii*.

H_2 production may also be limited by electron competition with other pathways like FNR, nitrate reductase, sulphite reductase, glutamate synthase or fatty acid desaturases (Hemschemeier & Happe, 2011). Thus, the fusion of PSI, FDX1 and HydA1 has resulted in efficient electron transfer towards H_2ase and subsequently enhanced H_2 production (Eilenberg et al., 2016; Yacoby et al., 2011). Indirect pathways also compete with H_2ase for electrons, and mutants targeting the down-regulation of RuBisCo (Hemschemeier et al., 2008; Pinto et al., 2013), starch degradation (Chochois et al., 2009), respiration and CET (Kruse et al., 2005; Steinbeck et al., 2015; Tolleter et al., 2011) have all demonstrated increased H_2 production over the wt. The *pgrl1* and *pgr5* mutants hold particular promise in demonstrating the highest yields of H_2 reported thus far (Steinbeck et al., 2015).

The *stm6* mutant, which is locked in state-1 and has an impaired CET, also produces more H_2 than its wt (Kruse et al., 2005), possibly due to an increased

contribution of the direct pathway to H_2 production, via PSII (Volgusheva et al., 2013). To further increase H_2 yields in *stm6*, a hexose symporter from *Chlorella kessleri* was introduced (Doebbe et al., 2007). This mutant, called *stm6glc4*, can uptake externally supplied glucose and use it for heterotrophic growth, supplying more electrons for the H_2ase.

Currently, work is being done to genetically combine several of the above described mutants to push H_2 production yields even further. In addition, new targets for overcoming the bottlenecks of H_2 production are constantly identified.

2. AIMS OF THE STUDY

The green alga *C. reinhardtii* is exposed to variable environmental conditions in its natural habitat and, for that reason, it possesses flexible and efficient acclimation mechanisms to avoid excess electron pressure and the consequent production of harmful ROS. In my thesis, I have focused on the function and regulation of several AET pathways in the protection of photosynthesis under various challenging environmental conditions. In order to gain further insight into the AET network, I used wt and several knock-out mutants of *C. reinhardtii*. A particular emphasis was placed on the evaluation of strategies arising from the findings of my work for the photoproduction of H_2 as a renewable bioenergy source.

My thesis objectives included the following aspects of AET pathways in *C. reinhardtii*:

1. The significance of FDPs as a part of the AET network in protecting the photosynthetic apparatus in *C. reinhardtii*

2. Interplay of the main AET pathways mediated by FDPs, PGRL1 and PGR5 during naturally occurring fluctuations in light intensity

3. Crosstalk between FDP-mediated O_2 photoreduction and H_2 photoproduction

3. METHODOLOGY

3.1. Growth conditions and *C. reinhardtii* strains

Table 1: *C. reinhardtii* strains used in this research. The construction of the mutants is described in detail in the references.

Strain	Description	Paper	Reference
cc 406	Wild-type (wt), cell wall deficient	I	
137c	wt, also CC-124, progenitor of *pgr5* and *pgrl1*	I, II, III, IV	
CC-4533	wt, progenitor of *flv* mutant lines	III	
pgr5	Knock-out of *pgr5*	III	Johnson et al., 2014
***pgr5* c1**	Complementation of *pgr5*	III	Johnson et al., 2014
pgrl1	Knock-out of *pgrl1*	II, III	Tolleter et al., 2011
***flv* 208**	Knock-out of *flvB*, LMJ.RY0402.242208	III	Li et al., 2016
***flv* 308**	Knock-out of *flvB*, LMJ.RY0402.229308	III	Li et al., 2016
***flv* 321**	Knock-out of *flvB*, LMJ.RY0402.052321	III	Li et al., 2016

The *C. reinhardtii* wild-type strains and mutants (Table 1) were maintained in Erlenmeyer flasks under mixotrophic conditions in Tris/Acetate/Phosphate (TAP) medium (Gorman & Levine, 1965). The cultures were kept at ambient air, 25°C, agitation of 90 rpm and under a continuous white fluorescent light (L30W/865 Osram) at an intensity of 50 µmol photons m^{-2} s^{-1}. For preparing experimental cultures the cells were harvested at OD_{750} = ~1.2, diluted to high salt medium (HSM; Sueoka, 1960) to an OD_{750} = ~0.1 and cultivated photoautotrophically at 25°C. Various light intensities from 50 µmol photons m^{-2} s^{-1} (growth light, GL) to 200 µmol photons m^{-2} s^{-1} or 600 µmol photons m^{-2} s^{-1} (high light, HL) were used to grow experimental cultures. In some experiments, the cultures were subject to bubbling with sterile air (low CO_2, LC) or sterile air supplemented with 2% (Paper II) or 3% (paper I) CO_2 (high CO_2, HC). Experiments for paper II were performed in growth chambers (AlgaeTron AG 130-ECO, PSI) equipped with LED white lights at a continuous light intensity of 20 µmol photons m^{-2} s^{-1} (CL 20) or at fluctuating light (FL) where 20 µmol photons m^{-2} s^{-1} background light was interrupted every 5 min by 30 s high light phases of 200 µmol photons m^{-2} s^{-1} (mild FL 20/200) or 600 µmol photons m^{-2} s^{-1} (harsh FL 20/600) for 7 days. Protein and transcript samples were

taken directly from the Erlenmeyer flasks. Prior to biophysical analysis, the cells were transferred into fresh HSM and the Chl *a* and *b* concentration was adjusted to 5 or 10 μg mL^{-1}, depending on the experiment. The Chl concentration was measured spectrophotometrically following pigment extraction with 95% ethanol (Lichtenthaler, 1987).

3.2. Photosynthetic activity measurements

3.2.1. Chl a fluorescence and P700 measurements

A Dual-pulse amplitude modulated (PAM)-100 fluorometer (Walz, Germany) was used for simultaneous measurement of PSII and PSI activity, based on Chl *a* fluorescence and the P700 absorbance changes, respectively (Klughammer & Schreiber, 2008). The P_m value was calibrated and normalized as described in paper II. Saturating pulses (4,000 μmol photons m^{-2} s^{-1} white light, 500 ms) were used to measure maximal fluorescence from dark adapted cells, F_m, and from light adapted cells, F_m'. For mimicking fluctuating light with the DUAL-PAM, the actinic light was set at 5 min 22 μmol photons m^{-2} s^{-1} and 30 s 210 μmol m^{-2} s^{-1} for the high light phase. In this case the cells were not dark-adapted. PSII parameters were calculated as following: the maximum quantum yield of PSII, $F_v/F_m=(F_m-F_0)/F_m$; the effective PSII yield under actinic light, $Y(II)=(F_m'-F_S)/F_m'$; $NPQ=(F_m-F_m')/F_m'$. PSI parameters were calculated as: the effective PSI yield under actinic light, Y(I)=1-Y(ND)-Y(NA); acceptor-side limitation of PSI, $Y(NA)=(P_m-P_m')/P_m$; and donor-side limitation of PSI, Y(ND)=1-P700 red. The analysis of the P700 reduction curves were recorded at 1000 μmol photons m^{-2} s^{-1} actinic white light for 5 s. In this case, the cells were pre-adapted in the dark for 45 min.

3.2.2. Electrochromatic shift analysis

The electrochromatic shift (ECS) absorbance change at 515 nm was measured by using a DUAL-PAM-100 paired with a P515/535 emitter-detector module (Walz, Germany)(Schreiber & Klughammer, 2008). The ECS dark relaxation after 2.5 min of illumination at 210 μmol photons m^{-2} s^{-1} actinic white light was analyzed to estimate the proton motive force (*pmf*). The total amplitude of the rapid decay of the ECS signal represents the total *pmf* and its partitioning in ΔpH and ΔΨ was analyzed according to Kramer and Sacksteder, (1998) and Cruz et al., (2001).

3.2.3. Low temperature fluorescence emission spectroscopy

Low temperature (77 K) fluorescence emission spectra of whole cells were determined using a USB4000-FL-450 (Ocean Optics) spectrofluorometer. The cells for analysis were frozen in liquid nitrogen and excited by 440 nm light obtained via 10 nm width interference filters. The spectra were normalized to PSII emission at 682 nm.

3.2.4. Oxygen exchange/evolution and H_2O_2 analysis

O_2 exchanges were analyzed in the presence of [^{18}O]-enriched O_2 in a thermo-regulated (25°C) reaction chamber connected to a mass spectrometer (model Prima dB; Thermo Electron) via a membrane inlet system (Tolleter et al., 2011). The cell suspensions were treated and the O_2 evolution and uptake rates were determined as described in paper II. For some measurements the mitochondrial respiratory inhibitors myxothiazol (2 mM) and salicylhydroxamic acid (SHAM) (0.4 mM) were added. Extracellular H_2O_2 was detected as described in paper III, using the Amplex Red reagent (Invitrogen). The fluorescence emission at 580 nm (excitation 540 nm) was measured with a SAFAS Xenius fluorescence spectrophotometer.

In paper IV, the O_2 evolution and consumption rates were measured with a Clark-type O_2 electrode (Oxygraph plus system, Hansatech, GB) at 24^{o}C. The net O_2 evolution (photosynthesis rate) was measured in the presence of 10 mM $NaHCO_3$ and under saturating light (2000 μmol photons m^{-2} s^{-1}). Light-saturated O_2 evolution (max. PSII activity) was recorded in the presence of the artificial electron acceptors, 0.5 mM 2,5-dichloro-1,4-benzoquinone (DCBQ) and 0.5 mM ferricyanide (FeCy). Respiration was calculated as the O_2 consumption rate in the dark and chlororespiration was calculated when the mitochondrial respiratory inhibitors myxothiazol (2 μM) and SHAM 0.9 (mM) were added.

3.3. Transcript analysis

3.3.1. Quantitative Real-time PCR

Total RNA was extracted using TRIsure (Bioline) reagent and further purified by phenol/chloroform/isoamylalcohol (25:24:1) extraction. Genomic DNA was removed using the Ambion Turbo DNase kit. The RNA quality and concentration was determined with a NanoDrop ND-1000 spectrophotometer (Thermo Scientific). The cDNA was reverse transcribed with poly(dT)(20) primers and SuperScript III

Reverse Transcriptase (Invitrogen). For the quantitative real-time (qRT) PCR iQ SYBR Green Supermix (Bio-Rad) in 96-well plates was used in a Bio-Rad IQ5 system. The PCR protocol and primers used can be found in paper I. The qbase+ software by Biogazelle was used to calculate relative changes in the gene expression and to normalize them to the appropriate reference genes. The selection process of the reference genes is described in full detail in paper I.

3.3.2. Next-generation RNA sequencing

Total RNA was extracted and treated as described above. The RNA samples were submitted for next-generation RNA sequencing (RNAseq) using a HiSeq 2000 (Illumina) to the Turku Centre for Biotechnology (Turku, Finland). The RNAseq reads were aligned to the genome of *C. reinhardtii* (version 5.1.) downloaded from Phytozome. The alignment using the Tophat algorithm and read-depth quantification was performed using the open source analysis software Chipster (CSC, Finland). Differentially expressed genes were identified by a log2 fold change of 1 with a p-value <0.05.

3.4. Protein analysis

Total and membrane proteins were extracted as described in paper I and separated using 14% SDS-PAGE without urea. Samples were loaded on the gels on equal protein basis, measured by Bradford assay (Bio-Rad, USA) and visualized with Coomassie Brilliant Blue (Bio-Rad, USA). Proteins were then transferred onto a polyvinylidene difluoride membrane (Millipore, USA) and examined with protein-specific antibodies. Antibodies were either purchased from Agrisera (Sweden), provided by collaborators, or newly raised against peptides, as stated in the relevant publication. Protein levels were analyzed using a secondary anti-rabbit antibody linked with horseradish peroxidase (HRP) and visualized with ECL.

3.5. Hydrogen production

3.5.1. Sulfur-deprivation protocol

C. reinhardtii cells cultivated in standard TAP medium and a light intensity of 50 μmol photons m^{-2} s^{-1} were harvested at a Chl concentration of ~25 μg mL^{-1}, washed from sulfur and resuspended in TAP medium without sulfur (TAP-S). The cultures were adjusted to a Chl concentration of 20 μg mL^{-1} and placed into custom-made cylindrical photobioreactors (550 ml) with an inner diameter of 60 mm. The

bioreactors were kept sealed with rubber stoppers and were placed under 75 µmol photons m^{-2} s^{-1} illumination from white fluorescent light (L 30W/865 Osram) at 25°C. Production of H_2 was monitored by gathering the gas in an upside-down graduated cylinder submerged in water. For more detailed continuous monitoring of the O_2 levels, the S-deprivation was performed in a microprocessor-controlled photobioreactor system (Tsygankov et al., 2006). In paper IV, S-deprivation was performed in gas-tight glass vials (total volume of 14 ml, Ø 22 mm).

3.5.2. Magnesium-deprivation protocol

Mg-deprived cultures were obtained via a three-stage process which has been previously described (Volgusheva et al., 2015). In Stage 1, cells were grown under standard conditions in TAP medium (as described above). Stage 2 involved the transfer of cells into TAP-Mg for an aerobic pre-incubation lasting 7 days. For the 3rd stage, cells pre-conditioned to Mg-deprivation were harvested and transferred into fresh TAP-Mg at a concentration of ~4×10^6 cells ml^{-1} (~7 µg Chl mL^{-1}). Aliquots of 11 ml were then placed into gas-tight glass vials (total volume 14 ml, Ø 22 mm) and incubated for 230 h, with constant shaking at 100 rpm, under the temperature and illumination conditions described above.

4. MAIN RESULTS

4.1. The interplay of O_2 photoreduction and cyclic electron transport protects photosynthesis

Genes encoding FDPs were reported in *C. reinhardtii* (Zhang et al., 2009), leading to questions concerning their expression levels and physiological function. Subsequent phylogenetic analysis of *C. reinhardtii* FDP protein structures indicated that FLVA and FLVB could function in a Mehler-like reduction of O_2, in a similar manner to Flv1 and Flv3 in cyanobacteria. I decided to investigate this hypothesis and to explore the cooperation of FDP-mediated O_2 photoreduction with other AET routes like PGRL1-mediated CET and PGR5.

4.1.1. Flavodiiron proteins are up-regulated when there is an excess of electrons in the photosynthetic electron transport chain

To assess the physiological role of FDPs in *C. reinhardtii,* the expression of FDPs on transcript and protein level was studied under different environmental conditions. Prior to analyzing the transcript level of *flvA* and *flvB* with qRT-PCR, I selected the most suitable set of reference genes under the tested conditions required for normalization (Paper I, Fig. 1). The most stable transcript levels under the three studied conditons were found for *cblp* and *ubc8.* Therefore, these were selected as reference genes for transcript analysis.

Cells grown under 50 µmol photons m^{-2} s^{-1} (GL) and 3% CO_2 (HC) were shifted to 150 µmol photons m^{-2} s^{-1} (HL) or air level CO_2 (LC). The cells were also shifted from GLHC conditions to combined HLLC. A sudden shift from GLHC to HL and/or LC creates an accumulation of excess electrons in the PET chain, creating an overreduction of the system. This was confirmed by the reduced effective PSII yield under these conditions, especially during the shift from GLHC to combined HLLC (Paper I, Fig. 2). The shift of *C. reinhardtii* wt cells from HC to LC led to a strong increase in *flvA* and *flvB* transcripts and a moderate increase in the amounts of corresponding proteins (Paper I, Fig. 3). It is noteworthy that the accumulation of the FLVB protein was relatively higher than FLVA. The shift from GL to HL conditions only induced a slight increase in both *flv* transcripts (Paper I, Fig. 4). Under this condition, FLVA accumulation was more strongly induced than FLVB. The shift to combined HLLC conditions resulted in the transient increase of both *flv* transcripts (Paper I, Fig. 5). Interestingly, the protein level of FLVA stayed almost unchanged, whereas FLVB was strongly up-regulated. Overall, it appears that *flv* transcription is

more induced by LC than by HL. This is in line with results from *flv1* and *flv3* in *Anabaena* (Ermakova et al., 2014). However, the amount of protein translation did not always follow transcription levels. Overall, the protein accumulation of FDPs was more stimulated by HL than LC. Under HL conditions, FLVA accumulation was more strongly up-regulated, whilst FLVB was more strongly induced by the shift to combined HLLC.

4.1.2. O_2 photoreduction via flavodiiron proteins is crucial for survival under fluctuating light

Reports of O_2 photoreduction performed by FDPs protecting cyanobacteria and also the moss *P. patens* from FL conditions (Allahverdiyeva et al., 2013; Gerotto et al., 2016) lead to the question whether the same function is possible in *C. reinhardtii*. The availability of *C. reinhardtii flv* knock-out mutants made it possible to study the impact that the lack of FDPs has on photosynthesis. The knock-out mutants missing both FDPs were subjected to photoautotrophic growth conditions and two different light regimes, 5 min of 20 µmol photon m^{-2} s^{-1} interrupted by 30 s of 200 µmol photon m^{-2} s^{-1} (mild FL 20/200) or constant illumination of 20 µmol photon m^{-2} s^{-1} (CL 20). The growth of both *flv* mutants was strongly impaired as compared to the wt under FL 20/200 conditions, while there was no difference under CL 20 illumination (Paper II, Fig. 1). Due to the inability of *flv* mutants to grow under FL 20/200 conditions, all measurements were performed on CL 20 grown cells only.

Analysis of the *flv* mutants with pulse-amplitude fluorometer (PAM) mimicking FL 20/200 conditions revealed that the effective PSII yield, Y(II), and the yield of PSI, Y(I), were drastically reduced at the onset of the HL phase, as compared to the wt progenitor (Paper II, Fig. 4). The lack of FDPs in the mutants created a strong acceptor-side limitation at PSI, Y(NA), that in turn led to a lack of donor-side limitation during the HL phase. Analysis of the P700 oxidation kinetics in wt cells during a shift from darkness to HL illumination resulted in a wave phenomenon - the fast oxidation of P700, then its reduction and re-oxidation within 1 s at the onset of light (Paper II, Fig. 5). In previous studies, this fast P700 “re-oxidation rise” was described as a FDP-mediated electron sink acting after PSI (Allahverdiyeva et al., 2013; Ilík et al., 2017). Accordingly, the anaerobic incubation of wt cells resulted in the loss of this P700 “re-oxidation rise” and this trend was also missing from the P700 oxidation kinetics of the *flv* mutants. Next, the proton motive force (*pmf*) and its partitioning into a proton gradient (ΔpH) and an electric potential (ΔΨ) were analyzed by measuring the electrochromic shift (ECS) at the thylakoid membrane

(Joliot and Joliot, 1989; Kramer and Sacksteder, 1998; Cruz et al., 2001). The *flv* mutants displayed a smaller *pmf* than the wt, which was caused by a decrease in the ΔpH component, while the ΔΨ component was comparable to that of the wt (Paper II, Fig. 6). The reduced ΔpH in the *flv* mutants demonstrates that FDP-mediated O_2 photoreduction contributes to the establishment of ΔpH, similar to the water-water cycle. Accordingly, the buildup of NPQ is delayed in the *flv* mutants (Paper II, Suppl. Fig. 4). However, contribution of FDPs to ΔpH must be a transient effect. This is also supported by the fact that the *flv* mutants were able to accumulate LHCSR3 protein and establish NPQ similar to the wt (Paper II, Fig. 7). The *flv* mutant cells appeared to compensate for the loss of FDPs via up-regulation of RbcL and O_2-consuming processes, as indicated by the elevated protein level of FeSOD and COXIIb. It appears that CET is not one of the compensatory processes, because accumulation of NDA2 did not change and PGRL1 even decreased slightly in the *flv* mutants compared to the wt. Interestingly, the accumulation of PGR5 increased, indicating that PGR5 may have an additional, yet unknown function besides CET.

4.1.3. PGRL1-mediated cyclic electron transport is essential during high light transitions

Another AET pathway in *C. reinhardtii* photosynthesis is CET mediated by PGRL1, and the availability of a *pgrl1* knock-out mutant (Tolleter et al., 2011) made it possible to study this pathway and its cooperation with other AET routes. In paper III, similar environmental conditions were applied as in paper I, although in steady-state mode: low light (LL, 50 µmol photons m^{-2} s^{-1}); high light (HL, 200 µmol photons m^{-2} s^{-1}); high CO_2 (HC, 2% CO_2 in air); and low CO_2 (LC, air). Under HCLL, HCHL and LCLL conditions, the electron transport rate (ETR) and NPQ of *pgrl1* and the wt showed no significant differences (Paper III, Fig. 1). In contrast, under LCHL conditions, *pgrl1* had a reduced ETR and stronger NPQ. To investigate which component was responsible for this elevated NPQ, state transitions (qT) were analyzed by low temperature (77K) chlorophyll fluorescence emission and LHCII phosphorylation (Paper III, Fig. 2). Under LCHL conditions, *pgrl1* showed a strongly increased transition to state-2 and a stronger phosphorylation of the LHCII proteins CP29, CP26 and LHCP11 than the wt (Paper III, Fig. 2). Thus, the high NPQ in *pgrl1* under LCHL conditions is likely due to increased state-2 transition. Nevertheless, the fast induction of NPQ was reduced within 1 min in *pgrl1*.

The growth of *pgrl1* in 1 L photobioreactors, operated as turbidostats, was not affected under the steady-state conditions described (Paper III, Fig. 3). However,

the observation that *pgrl1* showed impaired growth under LCHL conditions on solid media (Paper III, Fig. 3), led to the question of whether *pgrl1* is sensitive to changes in light intensity during the plating of cells from liquid culture onto solid medium. Indeed, the growth of *pgrl1* in photobioreactors was severely impaired during the first 4 h after the shift to HL (800 µmol photons m^{-2} s^{-1}). Under FL (5 min 50 µmol photon m^{-2} s^{-1} and 1 min 800 µmol photon m^{-2} s^{-1}) and LC conditions, *pgrl1* also showed impaired growth as compared to wt. These results together suggest that PGRL1-Fd-mediated CET is important during transitions to HL when CO_2 levels limit photosynthesis, but is not strictly necessary during steady-state growth.

4.1.4. The loss of PGRL1 leads to increased mitochondrial respiration and elevated O_2 photoreduction

Under LC and HL conditions, which usually invoke increased ATP demand for metabolic processes, the wt up-regulated PGRL1 protein amounts (Paper III, Fig. 7 and Suppl. Fig. 3). Thus, it could be expected that growth of the *pgrl1* mutant would be impaired in these circumstances. However, as already described, the *C. reinhardtii pgrl1* mutant and wt demonstrated approximately equal growth under LC and HL conditions. This indicates that there are other compensatory mechanisms contributing to the ATP production in *pgrl1*. One possible compensatory mechanism is an elevated cooperation with mitochondrial respiration. Thus, the effect of respiratory inhibitors on photosynthetic activity was analyzed (Paper III, Figure 4). Indeed, when salicylhydroxamic acid (SHAM) (inhibitor of the alternative oxidase) and myxothiazol (inhibitor of the mitochondrial cytochrome bc_1 complex) were added to the cells, *pgrl1* exhibited a reduced PSII yield, accompanied with more reduced PSII electron acceptors (1-qP), than the wt progenitor. These results suggest that the loss of PGRL1 makes the *C. reinhardtii* photosynthetic activity more dependent on the cooperation with mitochondrial respiration.

The light-dependent O_2 exchange in wt and *pgrl1* were analyzed by Membrane Inlet Mass Spectrometer (MIMS) with [^{18}O] labelled O_2 in order to dissect the nature of the O_2 reducing processes involved in the compensatory mechanisms. The net O_2 production did not notably differ between wt and *pgrl1*, except under the LCHL condition, where less activity was shown in *pgrl1*. The light-dependent O_2 uptake was slightly higher in *pgrl1* than in wt under LCLL conditions (Paper III, Fig. 5). To estimate the contribution of mitochondrial respiration and O_2 photoreduction to the light-dependent O_2 uptake, the same inhibitors of mitochondrial respiration were used as for the photosynthetic activity analysis described above. Under LCHL

conditions, the light-dependent O_2 uptake in *pgrl1* was clearly less affected by the inhibitors than in the wt, suggesting that O_2 photoreduction pathways other than mitochondrial respiration are also active. The LCHL condition also increased extracellular H_2O_2 levels compared to the other conditions. This indicated increased activity of the true Mehler reaction and was observed for both wt and *pgrl1* cultures, although in *pgrl1* the increase was more prominent (Paper III, Fig. 6). Immunoblot analysis showed that both FDPs are strongly up-regulated in *pgrl1* during all conditions except for LCHL. Under LCHL conditions, FLVB was moderately up-regulated and FLVA protein levels were unchanged as compared to the wt (Paper III, Fig. 6). A shift from HC to LC at 500 µmol photon m^{-2} s^{-1} (HL) revealed large increases in both FDP levels over the first 6 h, but levels had lowered again by 24 h. However, FLVB accumulation remained at higher levels than FLVA (Paper III, Fig. 7). Collectively, these results show that O_2 photoreduction processes, the true Mehler reaction and FDP-mediated O_2 photoreduction are activated in the *pgrl1* mutant. It is likely that FDPs function under conditions that create a moderate electron pressure on the PET chain, while the true Mehler reaction becomes the main compensatory O_2 photoreduction mechanism under LCHL, when the electron pressure on the PET chain is higher.

4.1.5. Underlying mechanisms behind the phenotypes of the pgrl1 and pgr5 mutants under fluctuating light

To evaluate the function of PGRL1 and PGR5 in protecting photosynthesis during fluctuating light, the knock-out mutants of *pgr5* and *pgrl1* were subjected to the same mild FL 20/200 illumination regime as described in section 4.1.2. The *pgr5* mutant showed severely impaired growth under mild FL 20/200 conditions, whereas the growth of *pgrl1* was not affected. It was only under harsh FL 20/600 conditions that inhibiton of the growth of *pgrl1* cells was observed (Paper II, Fig. 1). Under mild FL 20/200 conditions, the wt and *pgrl1* mutant exhibited very similar effective PSII and PSI yields (Paper II, Fig. 2 and Fig. 3). The *pgrl1* mutant grown in mild FL 20/200 showed only a slightly lower PSI yield than the wt under the HL phase. Additionally, the PSI donor-side limitation and PSI acceptor-side limitation were only mildly affected under mild FL 20/200 conditions. Immunoblot analysis showed an increased accumulation of FDPs in the *pgrl1* mutant (Paper II, Fig. 7). Remarkably, this increased accumulation of FDPs in *pgrl1* even caused a decrease in PSI acceptor-side limitation during the HL phase when the cells were grown in CL 20 conditions (Paper II, Fig. 3). Immunoblot analysis also revealed a low

accumulation of Cyt *f* in *pgrl1* under FL 20/200 illumination, suggesting limited electron flow to PSI. This, together with increased FDP accumulation, allows *pgrl1* to protect PSI and to cope with mild FL stress.

When *pgr5* was grown under FL 20/200 conditions, the effective yields of PSII and PSI were reduced as compared to the wt (Paper II, Fig. 2). PSI acceptor-side limitation was greatly increased and the donor-side of PSI was not limited. This led to greatly increased electron pressure on PSI in the *pgr5* mutant under FL 20/200 conditions. The increased electron pressure on PSI results in a decrease of PSI over time, as was evidenced by reduced levels of PsaA protein under FL 20/200 (Paper II, Fig. 7). Next generation RNA sequencing (RNAseq) showed the down-regulation of several photosynthesis related transcripts including *psaH, lhcbm6*, *lhcbm7* and *lhcbm9* in *pgr5* (Paper II, Suppl. Tab.1). Furthermore, the *curt1* transcript level is reduced in the *pgr5* mutant, indicating the possibility of a disturbed thylakoid architecture. Immunoblot analysis revealed that the problem in *pgr5* is likely associated with the missing donor-side limitation, given that proteins working as electron acceptors downstream of PSI (FDX1, RbcL and FDPs) were up-regulated (Paper II, Fig. 7). The *pgr5* and *pgrl1* mutants exhibited an increased level of proteins involved in O_2 consuming processes (COXIIb, FeSOD and FDPs) under CL 20 conditions and, to a lesser extent, under FL 20/200 conditions (Paper II, Fig. 7). These results were similar to the observations made for *pgrl1* under steady-state conditions in paper III and thus could indicate similar compensation mechanisms involving mitochondrial respiration and O_2 photoreduction for *pgr5*.

Interestingly, the P700 redox kinetics revealed that, similar to the *flv* mutants, the fast "re-oxidation rise" was almost completely lacking in the *pgr5* mutant (Paper II, Fig. 5). This finding suggests that although FDPs are present, they are not capable to cope with the high electron flux towards P700 in *pgr5*. Low temperature (77K) fluorescence emission analysis showed an increased transition to state-2 in *pgr5* under FL 20/200 conditions, that was not observed for wt or *pgrl1* cells. Thus, *pgr5* stromal electron carriers are likely over-reduced and may be influencing FDP-activity.

Analysis of the ECS signal in *pgr5* revealed that the total *pmf* was diminished due to the reduction of both ΔpH and, particularly, ΔΨ components (Paper II, Fig. 6). Therefore, NPQ and LHCSR3 accumulation were also drastically reduced (Paper II, Suppl. Fig. 4 and Fig. 7). Surprisingly, the *pgrl1* mutant exhibited a slightly higher *pmf* than the wt. However, ΔpH was reduced under FL 20/200 conditions due to the lack of PGRL1-mediated CET and thus, the accumulation of NPQ and LHCSR3 were

also reduced. The increased *pmf* in *pgrl1* was a result of an increased ΔΨ component. This result indicates that ion channels in the thylakoid membrane may be differently regulated in *pgrl1* than in wt or the *pgr5* mutant.

4.2. Impact of alternative electron transport on hydrogen production

Hydrogen (H_2) production represents an additional AET pathway because it can work as an electron sink for photosynthesis under specific conditions, e.g. during the shift from dark anoxia to light (Appel et al., 2000; Happe et al., 2002). It has been shown that the knock-out of *pgrl1* and *pgr5* enhances H_2 photoproduction during sulfur (S)-deprivation (Steinbeck et al., 2015). Therefore, the impact of other pathways in the AET network on H_2 production is an area of increased interest.

4.2.1. Flavodiiron proteins are transiently up-regulated during the shift to sulfur-deprivation

In order to understand the impact of FDP-mediated O_2 photoreduction on H_2 production, gene transcription and protein expression of FDPs were studied during cell acclimation to S-deprivation. S-deprived *C. reinhardtii* cultures go through five successive stages during the acclimation process: active photosynthesis, increased O_2 consumption, anaerobic lag phase, H_2 production and termination (see Introduction, Fig. 6). The samples for transcript and protein analysis were collected at representative time points for each acclimation stage (Paper I, Fig. 6). Both *flvA* and *flvB* transcripts were greatly up-regulated after only 2 h of S-deprivation (Paper I, Fig. 6). The *flvA* transcript amount decreased only after 40 h, whilst *flvB* had decreased by 24 h. Immunoblot analysis demonstrated a strong up-regulation of FLVA over the first 24 h of the shift to S-deprivation followed by a return to initial levels. In contrast, FLVB levels were elevated during the first 24 h, but then declined to become undetectable by the end of the experiment. These results indicate that FDPs are important during the early hours of S-deprivation and possibly accelerate the acclimation to anoxic conditions.

4.2.2. Comparison of H_2 production in sulfur- or magnesium-deprivation protocols

Sulfur (S)-deprivation is one of the regularly used protocols to establish anoxic conditions needed for H_2 photoproduction in *C. reinhardtii* (Melis et al., 2000). However, in a recent study a magnesium (Mg)-deprivation protocol has been tested and the Mg-deprived *C. reinhardtii* cells were able to produce more H_2 than known from S-deprived

cells (Volgusheva et al., 2015). Therefore, the reasons for this enhanced H_2 production were studied by comparing side by side the two different protocols.

The H_2 production capacities of *C. reinhardtii* cells during both S- and Mg-deprivation exhibited the typical five acclimation stages described above. The Mg-deprived cells produced H_2 at a similar rate to the S-deprived cells and were able to maintain the production for a longer time (Paper IV, Fig. 1). Excluding the pre Mg-deprivation stage, the O_2 evolution phase was shorter in the Mg-deprived cells and the overall O_2 yield was also lower. Accordingly, anoxia was established earlier in the Mg-deprived cells. The effective PSII yield showed a sharp drop when O_2 was entirely consumed from the media, possibly because of a redox-dependent inactivation of PSII. Instead, in Mg-deprived cells, *ΔF/Fm'* decreased much more slowly than in S-deprived cells. The starch content was also lower in Mg-deprived cells, and the starch break-down began earlier (Paper IV, Fig. 1). Low temperature (77K) fluorescence analysis showed that Mg-deprivation induced a transition to state-2. This indicated that CET was possibly increased, as both phenomenona are induced by the redox status of the PQ-pool (Paper IV, Fig. 6).

The apparent increase in PSII activity in Mg-deprived cells, as compared to S-deprived cells, led to a more thorough study of O_2 exchange. The net O_2 evolution in light, or photosynthesis rate, and maximal PSII activity were measured using a Clark-type O_2 electrode. The photosynthesis rate and maximal PSII activity exhibited a rapid drop after 24 h in S-deprivation (Paper IV, Fig. 3). In contrast to this, Mg-deprivation resulted in a higher photosynthesis rate and PSII activity, which only dropped after 120 h. These results correlate with the higher levels of PsbA, Cyt *f* and RbcL found in Mg-deprived cells and their rapid decline in S-deprived cells (Paper IV, Fig. 5).

Respiration increased during the first 48 h in S-deprivation and then slowly decreased, whilst respiration in Mg-deprived cells remained constant and only decreased after 120 h. The contribution of chlororespiration to dark O_2 consumption was estimated by adding the mitochondrial respiratory inhibitors myxothiazol and SHAM to the cells (Paper IV, Fig. 4). It is probable that the remaining O_2 uptake activity mainly represents the activity of PTOX (Antal et al., 2009), which was enhanced during Mg-deprivation. Immunoblot analysis suggested that other O_2-consuming processes were also up-regulated during Mg-deprivation, as FeSOD levels were elevated under both conditions and FDPs remained at higher levels than during S-deprivation. These results indicate that a combination of different O_2 reduction processes create a micro-oxic environment inside the chloroplast to protect the O_2-sensitive hydrogenase under Mg-deprivation.

5. DISCUSSION

5.1. The importance of alternative electron transport for photoprotection in dynamic light environments

Many laboratory studies concerning photosynthesis are carried out under steady state environmental conditions, or changes are tracked following a change in only one environmetal factor. However, in nature, photosynthetic organisms are repeatedly exposed to changing environmental conditions, such as light intensity, temperature or nutrient availability. In particular, aquatic photosynthetic microorganisms experience frequent fluctuations in light due to turbulence, the lens effect of waves and the shading caused by clouds or leaves (Allahverdiyeva et al., 2015a). Resulting sudden shifts to high light create an electron pressure in the PET chain that leads to harmful side products like reactive oxygen species (ROS) and as a result, oxidative damage of the photosynthetic apparatus. Therefore, oxygenic photosynthetic organisms developed elaborate and efficient mechanisms to fine-tune the photosynthetic apparatus and cope with changing light conditions.

In cyanobacteria, Flv1 and Flv3 (the homologs of *C. reinhardtii* FDPs) protect PSI from excess electron pressure under FL conditions by forming a heterooligomer and acting as an electron sink downstream of PSI (Allahverdiyeva et al., 2013). It is noteworthy that cyanobacteria do not possess a Pgrl1 homolog and only have a Pgr5-like protein. The Pgr5-like protein is believed to be involved in CET (Yeremenko et al., 2005), but it is not involved in the protection of *Synechocystis* during fluctuations in light intensity (Allahverdiyeva et al., 2013). In contrast, for higher plants that do not possess homologous genes of FDPs, the PGR5 protein is crucial for survival under FL conditions. This is the case for *A. thaliana* (Suorsa et al., 2012) and rice (Yamori et al., 2016). Higher plants also possess PGRL1 which is likely involved in CET and this pathway could also be beneficial during fluctuating light. To my knowledge, the impact of PGRL1 on the photosynthesis of higher plants under FL conditions has not yet been studied. In the moss *P. patens* and the liverwort *Marchantia*, which are lower in the evolutionary tree, the important photoprotective function of FDPs under fluctuating light conditions has been previously shown (Gerotto et al., 2016; Shimakawa et al., 2017). These organisms, like C*. reinhardtii*, possess all of the proteins already discussed as important under FL: FDPs, PGR5 and PGRL1. However, a comparative study determining which of these proteins is the main player in the photoprotection of PSI, has yet to be published. Therefore, my doctoral research focussed on the function and interplay of these AET pathways, to

identify which pathway is most important and if these pathways can complement each other.

5.2. Flavodiiron proteins work on a faster time-scale than PGR5 or PGRL1 and are indispensible for survival under fluctuating light

FDPs are up-regulated during the acclimation process to stress conditions that lead to the reduction of the PET chain, i.e. HL and/or LC (Paper I Fig. 3, 4 and 5). Furthermore, the accumulation levels of FLVA and FLVB do not necessarily correlate with each other, indicating that besides functioning as heterooligomers, these proteins possibly also form homooligomers as demonstrated for *Synechocystis* Flv3 and Flv1 (Mustila et al., 2016). Indeed, alignment of the flavodoxin domain demonstrated specific changes in the aminoacid sequence of the flavodoxin domain in cluster B (Fig. 7). This suggests that homooligomers of FLVA or FLVB would exhibit different features, rather than heterooligomers, and thus may be involved in different processes than Mehler-like O_2 photoreduction. Apparently, the protein accumulation of FLVB also affects either the stability or the translation of FLVA, because the *flvB* knock-out mutant lines are not able to accumulate FLVA in the absence of the heterodimer with FLVB (Paper II Suppl. Fig. 2).

Compared to the *pgr5* and *pgrl1* mutants, the *flv* mutants showed the most severe phenotype even under mild FL 20/200 conditions (Paper II Fig. 1). During the HL phase, the PET chain is completely blocked, demonstrated by a fully limited PSI acceptor-side in the *flv* mutants (Paper II Fig. 4). These results and the fast P700 oxido-reduction kinetics (Paper II Fig. 5) led to the conclusion that FDPs work as a fast electron sink, within 1 second of the onset of HL, similar to previous studies in cyanobacteria, the moss *P. patens* and the liverwort *Merchantia* (Allahverdiyeva et al., 2013; Gerotto et al., 2016; Shimakawa et al., 2017). Furthermore, the important contribution of this rapid electron sink to the fast induction of photoprotective mechanisms, i.e. NPQ and photosynthetic control of Cyt b_6f, via the establishment of ΔpH was demonstrated (Paper II Fig. 7 and Suppl. Fig. 4).

From these data, I concluded that the soluble nature of FDPs makes a fast interaction with available reduced donors, NADPH or FDX, possible. Thus, FDP-mediated O_2 photoreduction could provide a strong sink for the majority of electrons arriving from PSI over a short time period, at the onset of HL. On the other hand, the redox-dependent activation of PGRL1-mediated CET via thioredoxin and the reduction of PGRL1 likely need several seconds to activate (Hertle et al., 2013). Due

to this time difference between FDP-, PGR5- and PGRL1-mediated AET, a lack of FDPs cannot be rescued by PGR5 or PGRL1-mediated CET.

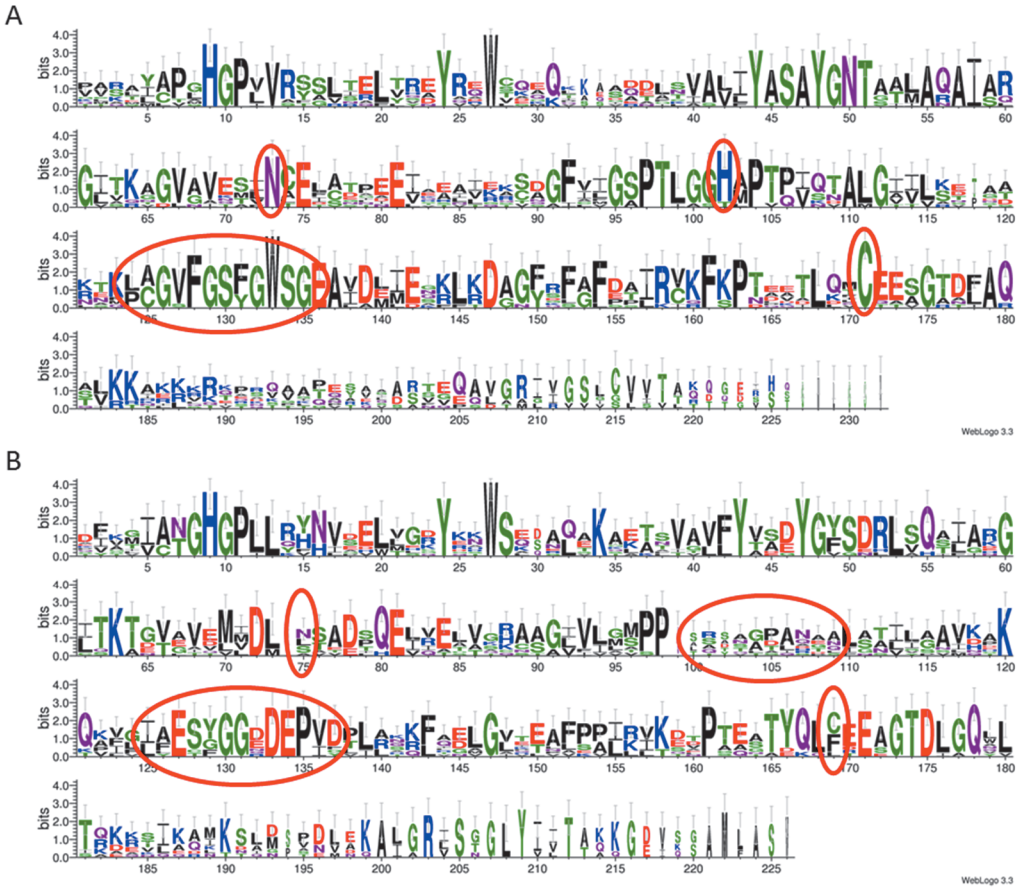

Fig. 7: Sequence logo of the flavodoxin domain of cluster A (A) and B (B). The alignments were performed with the FDP aminoacid sequences from *Synechocystis* sp. PCC 6803, *Anabaena* sp. PCC 7120, *Chlamydomonas reinhardtii*, *Micromonas pusilla CCMP1545, Ostreococcus lucimarinus, Physcomitrella patens and Selaginella moellendorffii*. Red circles indicate conserved aminoacids that are missing in the cluster B flavodoxin domain and the hydrophobic area from position 126 to 135, which is also missing in flavodoxin B.

5.3. PGR5 and PGRL1 proteins have different impacts on photosynthesis

The ability of *C. reinhardtii* cells to grow under different cultivation modes – mixotrophic, heterotrophic and photoautotrophic, and the common tradition to favour heterotrophic growth, makes the interpretation of photosynthesis research, particularly that focused on CET, challenging. CET rates have been estimated from cells grown in the presence of acetate (Petroutsos et al. 2009; Alric 2014; Johnson et

al. 2014) and from short (2 h) shifts in TAP medium to minimal medium (Terashima et al., 2012; Tolleter et al., 2011). Complications arise because in mixotrophic grown *C. reinhardtii* cells, up to 50% of the carbon is actually acquired from acetate and not from photosynthetic driven CO_2 fixation (Heifetz et al., 2000). Furthermore, the effective PSII yield and O_2 evolution are reduced in mixotrophic grown cells, while respiration remained unchanged (Endo and Asada, 1996). The presence of acetate in the medium also reduces the expression of several photosynthesis related genes in *C. reinhardtii* (Kindle, 1987; Sheen, 1994). To avoid masking photosynthetic responses to FL conditions by acetate metabolism, I undertook experiments in completely photoautotrophic conditions, growing cells for 7 days in minimal medium.

My data show the importance of PGRL1 during the transition to HL, especially under LC conditions (Paper III Fig. 3). These data also correlate with the impaired growth of the *pgrl1* mutant under harsh FL 20/600 conditions (Paper II Fig. 1). The following modifications in the photosynthetic apparatus of *pgrl1* are able to rescue the loss of PGRL1-mediated CET under steady state conditions and under mild FL 20/200: The up-regulation of FDPs (Paper II, Fig. 7); with an increased mitochondrial respiration (Paper III, Fig. 4 and 5); and protection of PSI by the down-regulation of Cyt *f* under FL 20/200 conditions (Paper II, Fig. 7). It is possible that the loss of PGRL1 destabilizes the Cyt b_6f complex and creates this down-regulation of Cyt *f*.

While the role of PGRL1 in CET is relatively clear, that of PGR5 is not so straightforward. The *pgr5* mutant shows a more severe phenotype even under mild FL 20/200 conditions. This cannot be simply ascribed to impaired CET, because the *pgrl1* mutant can still cope under these conditions (Paper II Fig. 1 and 2). It is noteworthy that Cyt *f* accumulation is not altered in the *pgr5* mutant and therefore electrons reach PSI unhindered (Paper II Fig. 2 and 7). The induction of NPQ is reduced in *pgr5* and *pgrl1*, although more severly in *pgr5*. This correlates with the LHCSR3 protein accumulation in these mutants (Paper II Fig. 7 and Suppl. Fig. 4). In contrast to wt and *pgrl1,* the 77K low temperature spectroscopy of FL 20/200 grown cells showed that the *pgr5* mutant is mostly in state-2 (Paper II Suppl. Fig. 5). This indicates a more reduced redox status of stromal components in the absence of PGR5 and thus, hints towards a possible role for PGR5 as a redox- regulator of photosynthetic electron transport.

This different redox status of the *pgr5* mutant compared to wt or *pgrl1* under FL 20/200 illumination could also explain the differences in the electric potential

component (ΔΨ) of the *pmf* (Paper II Fig. 6). While the *pgrl1* mutant demonstrates increased ΔΨ, the *pgr5* mutant exhibits a decreased ΔΨ component. This could indicate a modification of ion fluxes over the thylakoid membrane (Finazzi et al., 2015; Höhner et al., 2016). The redox-regulated thylakoid K^+/H^+ antiporter KEA3 has been shown to increase ΔΨ while decreasing ΔpH, and thus accelerate NPQ relaxation during a transition from HL to LL in *A. thaliana* (Armbruster et al., 2014). A homolog of KEA3 is present in *C. reinhardtii* and could thus be a candidate for the difference observed in ΔΨ between *pgr5* and *pgrl1*.

It is interesting that FDP-mediated O_2 photoreduction cannot rescue the loss of PGR5. The fast P700 oxido-reduction kinetics of the *pgr5* mutant closely resembles that of the *flv* mutants (Paper II Fig. 5). Thus, even though FDPs accumulate in *pgr5*, their activity as an electron sink downstream of PSI seems to be insufficient to contribute to the oxidation of P700. It is possible that despite high FDP accumulation (Paper II Fig. 7) and an increased light-induced O_2 uptake (Steinbeck et al., 2015), the elevated influx of electrons to PSI cannot be quenched in the *pgr5* mutant. However, this also does not exclude a possible redox-dependent down-regulation of FDP-activity via one or more of the conserved cysteins present in FDPs in the *pgr5* mutant, which exhibites an altered redox status (Paper II Suppl. Fig. 5).

The results of my own doctoral research and other recently published work on the function of PGRL1 and PGR5 show distinct differences in the regulation of the knockout mutants. I observed differences in growth performance under FL conditions, the induction and partitioning of *pmf*, and the redox state of the cells between the two mutants. In other works, the CET rate has been reported as drastically reduced in the *pgrl1* mutant, whilst altered only under anoxia in *pgr5* (Tolleter et al., 2011; Alric, 2014; Johnson et al., 2014). Moreover, the *pgr5* mutant has been demonstrated to produce H_2 much more efficiently than the *pgrl1* mutant (Steinbeck et al., 2015). In the same work, the *pgr5* mutant also showed stronger PSI photoinhibition than *pgrl1*, similar to the results presented in this thesis. The apparent differences between the two mutants indicate that PGRL1 and PGR5 do not have exclusive function together in the same pathway. In *A. thaliana*, the PGR5 protein plays a crucial role in the protection of PSI under FL conditions (Suorsa et al., 2012; Tiwari et al., 2016) and thus, the data for the *pgr5* mutant presented here suggests a similar role for *C. reinhardtii.*

The function of PGRL1 in *C. reinhardtii* is closely linked to CET under oxic and anoxic conditions (Iwai et al., 2010; Petroutsos et al., 2009; Terashima et al., 2012;

Tolleter et al., 2011) and it was proposed to be the elusive FQR, functioning in a redox-dependent manner in *A. thaliana* (Hertle et al., 2013). In that study, the function of the six conserved cysteins in PGRL1 was elucidated *in vitro*. While the first two cysteins are important for the dimerization of PGRL1, these cysteins, together with two more, bind the iron in the active centre. The last two cysteins appear to be necessary for the structural stability of the protein. It was also suggested that the last four cysteins are involved in the interaction of PGRL1 with PGR5 (Hertle et al., 2013; Dang, 2015). In PGR5, only one cystein is conserved, thus the interaction via disulfide bonds is probably not very strong (Dang, 2015). It is likely that PGR5 is not directly involved in CET via PGRL1, but is actually more of a redox sensor, manipulating the efficiency of electron transport via PGRL1. In this regard, it is also possible to explain the fairly extensive consequences that the lack of PGR5 has on many different processes in *C. reinhardtii*, as described in this doctoral thesis. As a redox sensor, PGR5 may also interacts with other targets and influence several metabolic pathways, such as: thylakoid ion channel activity; ATP synthase H^+ conductivity; and the functionality of FDPs. The stimulating effect that the lack of PGR5 has on ATP synthase H^+ conductivity has been reported in *A. thaliana* (Avenson et al., 2005; Wang et al., 2015). Interestingly, a recent study in *A. thaliana* showed the redox-dependent regulation of the ATP synthase H^+ conductivity by the chloroplast NADPH thioredoxin reductase C (Carrillo et al., 2016). It is possible that the redox sensing function of PGR5 is connected to the thioredoxin regulative system in the chloroplast.

From the new data presented in this thesis, together with current literature on the topic, a model of the mechanisms regulating photosynthesis during the exposure to fluctuating light is proposed (Fig. 8).

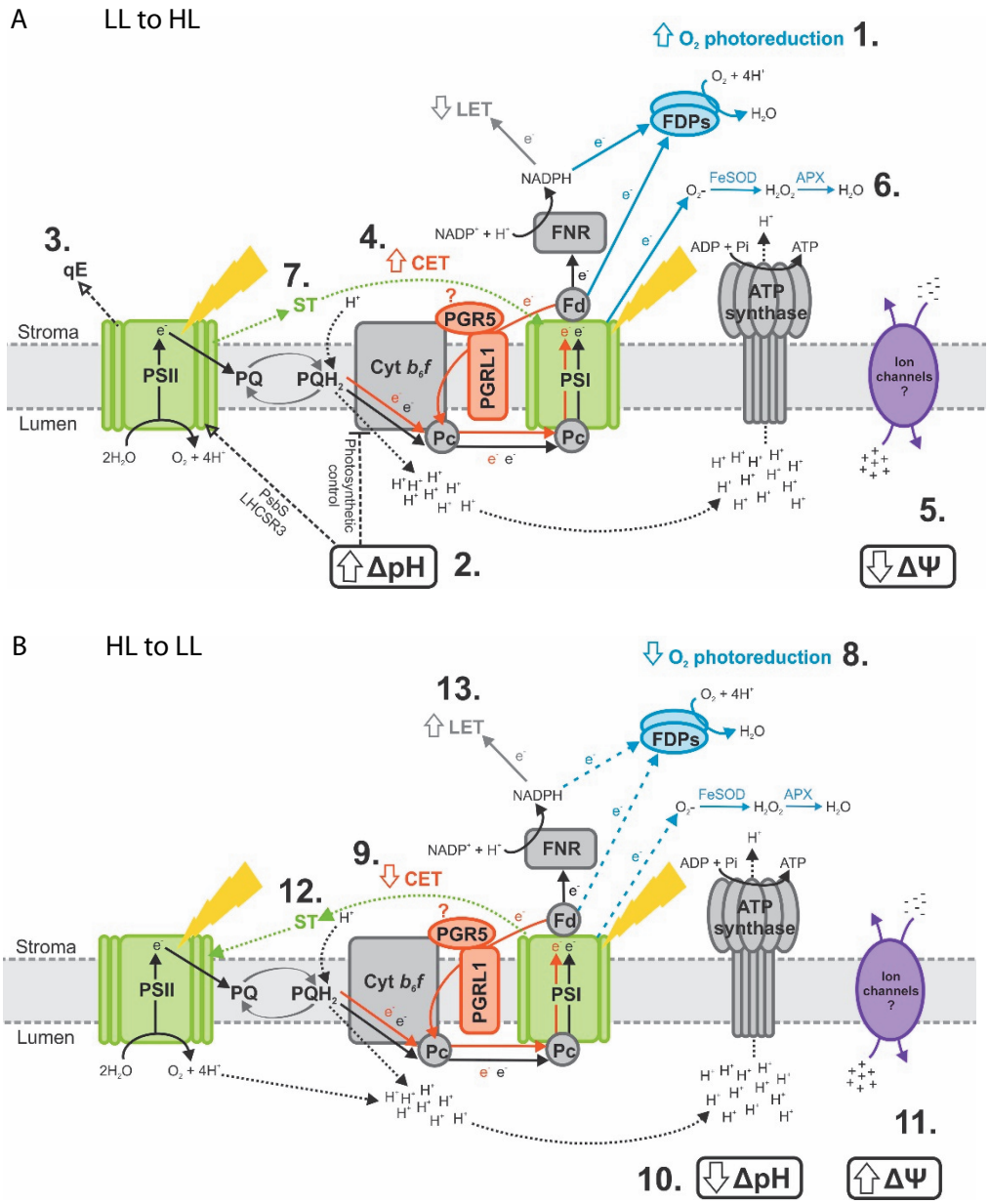

Fig. 8: Schematic representation of photosynthetic and alternative electron transport pathways demonstrating events occurring during a shift from LL to HL (A) and from HL to LL (B) in *C. reinhardtii*. LET is indicated by black and grey arrows; CET by red arrows. Blue arrows indicate O_2 photoreduction, mediated by FDPs or the true Mehler-reaction. Proton translocation is indicated by black dashed arrows and ion movement over the thylakoid membrane by purple arrows. Green dashed arrows represent state transitions. At the onset of HL (A), FDP-mediated O_2 photoreduction functions within seconds and forms a strong electron sink downstream of PSI (1). Thus, FDPs support the entire PET and contribute to proton pumping into the thylakoid lumen (2). Subsequently, increased ΔpH induces other photoprotective mechanisms including photosynthetic control at Cyt *b*$_6$*f* level (Colombo et al.,

2016) and the activation of LHCSR3 and PsbS (Correa-Galvis et al., 2016; Peers et al., 2009), which in turn induce NPQ (3). After several seconds, PGRL1-mediated CET accelerates, possibly influenced by the redox-sensor PGR5. Elevated CET contributes further to the establishment of ΔpH (4). At this point, PGRL1-mediated CET probably replaces FDP-mediated O_2 photoreduction as the major AET route. To balance *pmf* and regulate the ΔΨ component, counter ions are translocated across the thylakoid membrane (Finazzi et al., 2015; Höhner et al., 2016) by ion channels, which either release cations into the stroma or transport anions into the lumen (5). After a prolonged period of HL, possibly several minutes, the excess electron pressure leads to ROS production at PSI which activates true Mehler reaction and subsequently the WWC (6), ultimately making FDPs redundant as an electron sink. Also after several minutes, the reduced PQ-pool, responsible for activating specific kinases, leads to the transition of LHCII from PSII to PSI (Minagawa, 2011) into state-2 (7). On returning from HL to LL illumination (B), these processes are reversed. Limited electron availability causes a decrease in FDP-mediated O_2 photoreduction (8) and PGRL1-mediated CET (9). The transition to LL also requires rapid dissipation of NPQ and photosynthetic control at Cyt b_6f, this happens via the dissipation of ΔpH (10). Proton translocation performed solely via ATP synthase is probably not fast enough, thus the KEA3 channel may also releases protons from the thylakoid lumen (Armbruster et al., 2014) and ion channels operate so that ΔΨ is increased (11). On a slower time-scale, probably in the range of minutes, state transitions are reversed (12), the PET chain returns to its balanced state and LET becomes the major PET pathway (13). For simplicity reasons, NDA2-mediated CET is not depicted in this figure.

5.4. Hydrogen production is influenced by the activity of other alternative electron transport pathways

Several microalgae, including *C. reinhardtii*, are able to use solar energy and photoproduce H_2 metabolically. However, direct conversion of light to H_2 is very low, because the H_2ase is O_2-sensitive (Ghirardi et al., 1997; 2007; Stripp et al., 2009). Consequently, H_2 production yields are not high enough for commercialization of this process. Thus, different H_2 production protocols and genetic modifications of *C. reinhardtii* have been tested for their H_2 production capability.

An increased understanding of photosynthesis in microalgae has led to the discovery of new targets for enhancing H_2 production via genetic manipulation. The knockout mutant of PGRL1, lacking CET, is able to produce about 5-times more H_2 than the wt (Steinbeck et al., 2015; Tolleter et al., 2011). The *pgr5* mutant is, even more impressive, able to produce 8-times more H_2 compared to the wt, making it the best biological H_2 producer up-to-date. This enhanced H_2 production in *pgrl1* and *pgr5* has been explained by more remaining PSII activity and increased O_2 consumption (Steinbeck et al., 2015). The data obtained during my doctoral research corroborates the increased O_2 consumption in *pgrl1* and suggests that this is due to a combined effect of enhanced mitochondrial respiration and O_2-photoreduction via

FDPs (Paper III Fig. 5 and 6). However, whether FDP-mediated O_2-photoreduction contributes to the enhanced O_2 consumption in *pgr5* is still under question, because there is the possibility that FDPs are not fully active in *pgr5* under certain conditions (Paper II Fig. 5). It is possible that the redox status of the *pgr5* mutant favors the formation of FDP homooligomers which could function in a different AET route to the Mehler-like reaction as proposed for Flv1 and Flv3 homooligomers in *Synechocystis* (Mustila et al., 2016).

The data presented in this doctoral thesis also suggest that FDP-mediated O_2 photoreduction is an important factor accelerating the acclimation of algal cells to anaerobiosis induced by S-deprivation (Paper I Fig. 6). To sustain the metabolism of the cell under anoxia, the activity of the H_2ase and of fermentative pathways is needed (Hemschemeier and Happe, 2011; Catalanotti et al., 2013). Several enzymes of these pathways are sensitive to O_2 (Atteia et al., 2013). Therefore, a rapid shift to complete anoxia in the cell and subsequent efficiency of fermentation and H_2ase are important for the survival of *C. reinhardtii*, which regularly faces microoxic or anoxic conditions in its natural aquatic habitat. Because of their possible role in the acclimation process to anoxia, FDPs are an interesting target for genetic modification towards improved H_2 production yields.

Different production protocols, used to induce anaerobiosis via the depletion of nutrients like nitrogen, phosphorus, potassium and magnesium, have been the focus of much of the recent research on *C. reinhardtii* H_2 production (Philipps et al., 2012; Batyrova et al., 2012; 2015; He et al., 2012; Papazi et al., 2014; Volgusheva et al., 2015). Indeed, Mg-deprivation has resulted in the production of about twice as much H_2 as S-deprivation, this being due to a prolonged H_2 production phase (Paper IV Fig. 1). The contribution of FDP-mediated O_2 photoreduction to the removal of remaining O_2 inside the chloroplast is indicated by a greater accumulation of FDPs during H_2 production in Mg-deprived cells than in S-deprived cells (Paper IV Fig. 5). Recently, it was shown that H_2 production is also possible under micro-oxic conditions (Liran et al., 2016). Under these conditions, FDPs may be particularly useful in the removal of O_2 from the chloroplast to ensure the activity of the H_2ase. In Mg-deprived cells, mitochondrial respiration and PTOX activity are also increased (Paper IV Fig. 3 and 4). The enhancement of these O_2 consumptive processes is necessary because the activity of PSII remains higher in Mg-deprived cells than in S-deprived ones (Paper IV Fig. 1, 2 and 3). Although the energy for H_2 production is mostly supplied by the indirect pathway via starch breakdown, it is highly likely that the enhanced PSII activity in Mg-deprived cells has positive effects on the

metabolism and viability of the cells and is the reason for the prolonged H_2 production phase.

Biological H_2 production by microalgae holds both challenge and promise for the future. Further advances in molecular engineering, such as the deployment of the CRISPR/Cas9 method, novel H_2 production protocols and bioreactor design are needed to feasibly employ solar fuel systems in the context of a sustainable energy economy. A combination of several of the discussed mutations with the right production protocol will be required to ensure efficient long-term production of clean H_2 fuel, which has the potential to be an important step toward the reduction of global green house gas emissions.

6. CONCLUSIONS AND FUTURE PERSPECTIVES

The data presented in this doctoral thesis give valuable new insight into the regulation of photosynthesis via AET in the green algae *C. reinhardtii*. My results suggest that the regulation of photosynthesis via AET under dynamic light environments is a more complex process than is currently understood. Through my doctoral research, I have demonstrated that in *C. reinhardtii*;

- FDPs are indispensable for survival under FL and function as a strong and transient electron sink of the PET chain downstream of PSI
- Following FDPs, PGR5 is the next important player to act under FL conditions. In the absence of PGR5, FDPs are not sufficient to fully rescue cells from FL stress
- PGRL1 is dispensible under mild FL conditions, but becomes more important during periodic shifts from low background light to very strong HL transients and thus, contributes to growth under harsh FL conditions
- The successive function of all three AET pathways; FDPs, PGR5, and PGRL1-mediated CET, together with the cooperation of mitochondrial respiration are necessary to protect photosynthesis under changing environmental conditions
- FDPs function as O_2 quenchers during S-deprivation and contribute to the maintenance of anoxia inside the chloroplast during the acclimation phase
- Mg-deprivation results in prolonged H_2 production due to the positive effect of increased PSII activity and O_2 consumption via FDPs and other pathways on the metabolism of the cell

Additional research is needed to clarify the exact role of PGR5 as a possible redox-regulator of PET and/or its function in establishing photosynthetic control. In particular, more extensive studies on the different possible targets of PGR5 are needed to understand how PGR5 regulates photosynthesis. Additionally, *in vivo* experiments with cystein substitution mutants of PGR5 and PGRL1 remain to be performed. My doctoral research indicates a close link between PGR5, PGRL1 and the regulation of ion fluxes over the thylakoid membrane. Further studies are

needed to identify thylakoid ion channels in *C. reinhardtii* and evaluate their impact on PET.

The protection of photosynthesis via the FDP-mediated O_2 photoreduction pathway is evolutionarily conserved in cyanobacteria, green algae, bryophytes, lycophytes and gymnosperms. Therefore, the question arises as to why this pathway is lost in angiosperms, and what mechanism compensates for this loss. More work is needed to properly address this question, although efficient CET was proposed as a possible candidate mechanism in *A. thaliana* (Yamamoto et al., 2016).

The discovery of the function of FDPs in AET and the fact that the H_2ases are also part of this network, led to the identification of FDPs as a new target for improving H_2 photoproduction in microalgae. Whether the knockout of FDPs and thus, the removal of an electron competitor to the H_2ase, is beneficial for H_2 photoproduction is yet to be established. However, the approach demonstrates the value in better understanding photosynthesis and AET toward the employment of microalgae for efficient bioenergy production.

7. ACKNOWLEDGEMENTS

The Laboratory of Molecular Plant Biology of University of Turku is an excellent place to conduct innovative research and learn the ins and outs of being a good scientist. For financial support, the Academy of Finland, Turku University Foundation, Kone Foundation, and the ISB and DPMLS doctoral programs are gratefully acknowledged.

I am very thankful to Academy Prof. Eva Mari Aro for her everlasting support and trust set in me. I am deeply grateful to my supervisor Dr. Yagut Allahverdiyeva-Rinne for giving me the freedom to be self-responsible and to grow independent scientific thinking, but still guiding my doctoral research and directing me towards the right path. My fellow Chlamy-person Dr. Sergey Kosourov receives my warmest thanks for his careful, thorough and cheerful help in working with and understanding Chlamy.

Furthermore, I want to thank everybody who contributed to the scientific research presented here; with special thanks to Dr. Natalia Battchikova, Dr. Kieu-Van Dang, Dr. Gilles Peltier, and Dr. Alena Volgusheva. For her language proof-reading of this thesis Dr. Fiona Lynch and for translating the abstract to Finnish Minna Koskela are thanked. I am grateful to Prof. Dr. Thomas Happe and Prof. Yuichiro Takahashi for critically reviewing the thesis and providing the last finishing touches. I want to thank my opponent Dr. Maria L. Ghirardi in advance for coming all the way to Finland and discuss my research with me. Prof. Eevi Rintamäki is acknowledged for the guidance through the formalities and bureaucracy that come with finishing a PhD thesis. For technical support and keeping the order in the lab Eveliina, Kurt, Maija, Mika, Tapio, and Vipu are warmly thanked.

Especially I want to thank my past and present fellow PhD students, also called friends ❤: Julia, Luca, Martina, Masha, Ninni, and Veronica. For making the process of writing the thesis quite enjoyable I want to thank my thesis-support group: Gayathri, Henna, and Linda ❤. Anniina is warmly thanked for her friendship, help in the lab, and for organizing the Karonkka party.

I am deeply grateful to all my outside friends; Anna, Annette, Irene, Eveliina, Sabrina, and Steffi for all the good things in life; like the best holidays, nice relaxing chats on summery balconies or cozy winter knitting sessions ❤.

Ich möchte mich herzlichst bei meinen Eltern bedanken, die immer für mich da sind und mich in all meinen Vorhaben unterstützen.

Extra special thanks go to Jarno, who is my home ❤.

8. REFERENCES

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

Allahverdiyeva, Y., Isojärvi, J., Zhang, P., & Aro, E.-M. (2015b). Cyanobacterial Oxygenic Photosynthesis is Protected by Flavodiiron Proteins. *Life*, 5, 716–743.

Allahverdiyeva, Y., Mustila, H., Ermakova, M., Bersanini, L., Richaud, P., Ajlani, G., Battchikova, N., Cournac, L., & Aro, E.-M. (2013). Flavodiiron proteins Flv1 and Flv3 enable cyanobacterial growth and photosynthesis under fluctuating light. *PNAS*, 110, 4111–6.

Allen, J. F. (2002). Photosynthesis of ATP—Electrons, Proton Pumps, Rotors, and Poise. *Cell*, 110, 273–276.

Alric, J. (2014). Redox and ATP control of photosynthetic cyclic electron flow in Chlamydomonas reinhardtii: (II) Involvement of the PGR5–PGRL1 pathway under anaerobic conditions. *BBA - Bioenergetics*, 1837, 825–834.

Antal, T. K., Volgusheva, A. A., Kukarskih, G. P., Krendeleva, T. E., & Rubin, A. B. (2009). Relationships between H2 photoproduction and different electron transport pathways in sulfur-deprived Chlamydomonas reinhardtii. *Int. J. Hydrogen Energy*, 34, 9087–9094.

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.

Armbruster, U., Carrillo, L. R., Venema, K., Pavlovic, L., Schmidtmann, E., Kornfeld, A., Jahns, P., Berry, J. A., Kramer, D. M., & Jonikas, M. C. (2014). Ion antiport accelerates photosynthetic acclimation in fluctuating light environments. *Nat. Commun*, 222, 5439.

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.

Atteia, A., van Lis, R., Tielens, A. G. M., & Martin, W. F. (2013). Anaerobic energy metabolism in unicellular photosynthetic eukaryotes. *BBA - Bioenergetics*, 1827, 210–223.

Avenson, T. J., Cruz, J. A., Kanazawa, A., & Kramer, D. M. (2005). Regulating the proton budget of higher plant photosynthesis. *PNAS*, 102, 9709–13.

Baniulis, D., Yamashita, E., Zhang, H., Hasan, S. S., & Cramer, W. A. (2008). Structure-Function of the Cytochrome b6f Complex. *Photochem Photobiol,* 84, 1349–1358.

Battchikova, N., Eisenhut, M., & Aro, E.-M. (2011). Cyanobacterial NDH-1 complexes: Novel insights and remaining puzzles. *BBA - Bioenergetics,* 1807, 935–944.

Batyrova, K., Gavrisheva, A., Ivanova, E., Liu, J., & Tsygankov, A. (2015). Sustainable Hydrogen Photoproduction by Phosphorus-Deprived Marine Green Microalgae Chlorella sp. *Int J Molecular Sciences*, 16, 2705–2716.

Batyrova, K. A., Tsygankov, A. A., & Kosourov, S. N. (2012). Sustained hydrogen photoproduction by phosphorus-deprived Chlamydomonas reinhardtii cultures. *Int. J. Hydrogen Energy*, 37, 8834–8839.

Baxter, A., Mittler, R., & Suzuki, N. (2014). ROS as key players in plant stress signalling. *J Exp Bot*, 65, 1229–1240.

Ben-Shem, A., Frolow, F., & Nelson, N. (2003). Crystal structure of plant photosystem I. *Nature*, 426, 630–635.

Bersanini, L., Battchikova, N., Jokel, M., Rehman, A., Vass, I., Allahverdiyeva, Y., & Aro, E.-M. (2014). Flavodiiron protein Flv2/Flv4-related photoprotective mechanism dissipates excitation pressure of PSII in cooperation with phycobilisomes in Cyanobacteria. *Plant Physiol*, 164, 805–18.

Blaby, I. K., Blaby-Haas, C. E., Tourasse, N., Hom, E. F. Y., Lopez, D., Aksoy, M., … Prochnik, S. (2014). The Chlamydomonas genome project: a decade on. *Trends Plant Sci*, 19, 672–680.

Blankenship, R. E. (2013). Molecular Mechanisms of Photosynthesis. John Wiley & Sons.

Bonardi, V., Pesaresi, P., Becker, T., Schleiff, E., Wagner, R., Pfannschmidt, T., … Leister, D. (2005). Photosystem II core phosphorylation and photosynthetic acclimation require two different protein kinases. *Nature*, 437, 1179–1182.

Bonente, G., Ballottari, M., Truong, T. B., Morosinotto, T., Ahn, T. K., Fleming, G. R., … Bassi, R. (2011). Analysis of LhcSR3, a Protein Essential for Feedback De-Excitation in the Green Alga Chlamydomonas reinhardtii. *PLoS Biology*, 9, e1000577.

Brennan, L., & Owende, P. (2013). Biofuels from Microalgae: Towards Meeting Advanced Fuel Standards. In Advanced Biofuels and Bioproducts (pp. 553–599). New York, NY: Springer New York.

Cardol, P., Alric, J., Girard-Bascou, J., Franck, F., Wollman, F.-A., & Finazzi, G. (2009). Impaired respiration discloses the physiological significance of state transitions in Chlamydomonas. *PNAS*, 106, 15979–84.

Carraretto, L., Teardo, E., Checchetto, V., Finazzi, G., Uozumi, N., & Szabo, I. (2016). Ion Channels in Plant Bioenergetic Organelles, Chloroplasts and Mitochondria: From Molecular Identification to Function. *Mol Plant*, 9, 371–395.

Carrillo, L. R., Froehlich, J. E., Cruz, J. A., Savage, L. J., & Kramer, D. M. (2016). Multi-level regulation of the chloroplast ATP synthase: the chloroplast NADPH thioredoxin reductase C (NTRC) is required for redox modulation specifically under low irradiance. *Plant J*, 87, 654–663.

Catalanotti, C., Yang, W., Posewitz, M. C., & Grossman, A. R. (2013). Fermentation metabolism and its evolution in algae. *Frontiers in Plant Science*, 4, 37-53.

Chaux, F., Johnson, X., Auroy, P., Beyly-Adriano, A., Te, I., Cuiné, S., … al., et. (2017). PGRL1 and LHCSR3 Compensate for Each Other in Controlling Photosynthesis and Avoiding Photosystem I Photoinhibition during High Light Acclimation of Chlamydomonas Cells. *Molecular Plant*, 10, 216–218.

Chisti, Y. (2007). Biodiesel from microalgae. *Biotechnology Advances*, 25, 294–306.

Chochois, V., Dauvillée, D., Beyly, A., Tolleter, D., Cuiné, S., Timpano, H., … Peltier, G. (2009). Hydrogen production in Chlamydomonas: photosystem II-dependent and -independent pathways differ in their requirement for starch metabolism. *Plant Physiology*, 151, 631–40.

Clowez, S., Godaux, D., Cardol, P., Wollman, F.-A., & Rappaport, F. (2015). The involvement of hydrogen-producing and ATP-dependent NADPH-consuming pathways in setting the redox poise in the chloroplast of Chlamydomonas reinhardtii in anoxia. *The Journal of Biological Chemistry*, 290, 8666–76.

Colombo, M., Suorsa, M., Rossi, F., Ferrari, R., Tadini, L., Barbato, R., & Pesaresi, P. (2016). Photosynthesis Control: An underrated short-term regulatory mechanism essential for plant viability. *Plant Signaling & Behavior,* 11, e1165382.

Correa-Galvis, V., Redekop, P., Guan, K., Griess, A., Truong, T. B., Wakao, S., … Jahns, P. (2016). Photosystem II Subunit PsbS Is Involved in the Induction of LHCSR Protein-dependent Energy Dissipation in Chlamydomonas reinhardtii. *The Journal of Biological Chemistry,* 291, 17478–87.

Crofts, A. R., Barquera, B., Gennis, R. B., Kuras, R., Guergova-Kuras, M., & Berry, E. A. (1999). Mechanism of Ubiquinol Oxidation by the bc1 Complex: Different Domains of the Quinol Binding Pocket and Their Role in the Mechanism and Binding of Inhibitors. *Biochemistry*, 38, 15807–15826.

Cruz, J. A., Sacksteder, C. A., Kanazawa, A., & Kramer, D. M. (2001). Contribution of Electric Field (Δψ) to Steady-State Transthylakoid Proton Motive Force (pmf) in Vitro and in Vivo. Control of pmf Parsing into Δψ and ΔpH by Ionic Strength. *Biochemistry,* 40, 1226–1237.

DalCorso, G., Pesaresi, P., Masiero, S., Aseeva, E., Schünemann, D., Finazzi, G., … Leister, D. (2008). A Complex Containing PGRL1 and PGR5 Is Involved in the Switch between Linear and Cyclic Electron Flow in Arabidopsis. *Cell*, 132, 273–285.

Dang, K.-V. (2015). Regulation of photosynthetic cyclic electron transport (Dissertation). Retrieved from: http://www.theses.fr/2015AIXM4004.

Davis, G. A., Kanazawa, A., Schöttler, M. A., Kohzuma, K., Froehlich, J. E., Rutherford, A. W., … Sayre, R. (2016). Limitations to photosynthesis by proton motive force-induced photosystem II photodamage. *eLife,* 5, 27–39.

Day, A., & Goldschmidt-Clermont, M. (2011). The chloroplast transformation toolbox: selectable markers and marker removal. *Plant Biotechnology Journal*, 9, 540–553.

Debuchy, R., Purton, S., & Rochaix, J. D. (1989). The argininosuccinate lyase gene of Chlamydomonas reinhardtii: an important tool for nuclear transformation and for correlating the genetic and molecular maps of the ARG7 locus. *The EMBO Journal*, 8, 2803–9.

Delosme, R., Olive, J., & Wollman, F.-A. (1996). Changes in light energy distribution upon state transitions: an in vivo photoacoustic study of the wild type and photosynthesis mutants from Chlamydomonas reinhardtii. *Biochimica et Biophysica Acta (BBA) - Bioenergetics*, 1273, 150–158.

Dent, R. M., Han, M., & Niyogi, K. K. (2001). Functional genomics of plant photosynthesis in the fast lane using Chlamydomonas reinhardtii. *Trends in Plant Science*, 6, 364–371.

Desplats, C., Mus, F., Cuiné, S., Billon, E., Cournac, L., & Peltier, G. (2009). Characterization of Nda2, a plastoquinone-reducing type II NAD(P)H dehydrogenase in chlamydomonas chloroplasts. *The Journal of Biological Chemistry,* 284, 4148–57.

Doebbe, A., Rupprecht, J., Beckmann, J., Mussgnug, J. H., Hallmann, A., Hankamer, B., & Kruse, O. (2007). Functional integration of the HUP1 hexose symporter gene into the genome of C. reinhardtii: Impacts on biological H2 production. *Journal of Biotechnology*, 131, 27–33.

Drop, B., Yadav K.N., S., Boekema, E. J., & Croce, R. (2014). Consequences of state transitions on the structural and functional organization of Photosystem I in the green alga Chlamydomonas reinhardtii. *The Plant Journal*, 78, 181–191.

Drop, B., Webber-Birungi, M., Fusetti, F., Kouřil, R., Redding, K. E., Boekema, E. J., & Croce, R. (2011). Photosystem I of Chlamydomonas reinhardtii contains nine light-harvesting complexes (Lhca) located on one side of the core. *The Journal of Biological Chemistry*, 286, 44878–87.

Dubini, A., & Ghirardi, M. L. (2015). Engineering photosynthetic organisms for the production of biohydrogen. *Photosynthesis Research*, 123, 241–253.

Eilenberg, H., Weiner, I., Ben-Zvi, O., Pundak, C., Marmari, A., Liran, O., … Yacoby, I. (2016). The dual effect of a ferredoxin-hydrogenase fusion protein in vivo: successful divergence of the photosynthetic electron flux towards hydrogen production and elevated oxygen tolerance. *Biotechnology for Biofuels*, 9, 182.

Endo, T., & Asada, K. (1996). Dark Induction of the Non-Photochemical Quenching of Chlorophyll Fluorescence by Acetate in Chlamydomonas reinhardtii. *Plant and Cell Physiology*, 37, 551–555.

Ermakova, M., Battchikova, N., Richaud, P., Leino, H., Kosourov, S., Isojärvi, J., … Aro, E.-M. (2014). Heterocyst-specific flavodiiron protein Flv3B enables oxic diazotrophic growth of the filamentous cyanobacterium Anabaena sp. PCC 7120. *PNAS*, 111, 11205–10.

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

Eroglu, E., & Melis, A. (2016). Microalgal hydrogen production research. *International Journal of Hydrogen Energy*, 41, 12772–12798.

Fatihi, A., Latimer, S., Schmollinger, S., Block, A., Dussault, P. H., Vermaas, W. F. J., … Basset, G. J. (2015). A Dedicated Type II NADPH Dehydrogenase Performs the Penultimate Step in the Biosynthesis of Vitamin K1 in Synechocystis and Arabidopsis. *The Plant Cell*, 27, 1730–41.

Finazzi, G., Petroutsos, D., Tomizioli, M., Flori, S., Sautron, E., Villanova, V., … Seigneurin-Berny, D. (2015). Ions channels/transporters and chloroplast regulation. *Cell Calcium*, 58, 86–97.

Forestier, M., King, P., Zhang, L., Posewitz, M., Schwarzer, S., Happe, T., … Seibert, M. (2003). Expression of two [Fe]-hydrogenases in Chlamydomonas reinhardtii under anaerobic conditions. *European Journal of Biochemistry*, 270, 2750–2758.

Gaffron, H., & Rubin, J. (1942). Fermentative and photochemical production of hydrogen in algae. *The Journal of General Physiology*, 26(2). 219.

Gerotto, C., Alboresi, A., Meneghesso, A., Jokel, M., Suorsa, M., Aro, E.-M., & Morosinotto, T. (2016). Flavodiiron proteins act as safety valve for electrons in Physcomitrella patens. *PNAS*, 113, 12322–12327.

Ghirardi, M. L. (2015). Implementation of photobiological H2 production: the O2 sensitivity of hydrogenases. *Photosynthesis Research*, 125, 383–393.

Ghirardi, M. L., Dubini, A., Yu, J., & Maness, P.-C. (2009). Photobiological hydrogen-producing systems. *Chem. Soc. Rev.*, 38, 52–61.

Ghirardi, M. L., Posewitz, M. C., Maness, P.-C., Dubini, A., Yu, J., & Seibert, M. (2007). Hydrogenases and Hydrogen Photoproduction in Oxygenic Photosynthetic Organisms. *Annual Review of Plant Biology*, 58, 71–91.

Ghirardi, M. L., Zhang, L., Lee, J. W., Flynn, T., Seibert, M., Greenbaum, E., & Melis, A. (2000). Microalgae: a green source of renewable H2. *Trends in Biotechnology*, 18, 506–511.

Ghirardi, M. L., Togasaki, R. K., & Seibert, M. (1997). Oxygen Sensitivity of Algal H2-Production. In Biotechnology for Fuels and Chemicals (pp. 141–151). Totowa, NJ: Humana Press.

Ghysels, B., Godaux, D., Matagne, R. F., Cardol, P., & Franck, F. (2013). Function of the Chloroplast Hydrogenase in the Microalga Chlamydomonas: The Role of Hydrogenase and State Transitions during Photosynthetic Activation in Anaerobiosis. *PLoS ONE*, 8, e64161.

Gonçalves, V. L., Saraiva, L. M., & Teixeira, M. (2011). Gene expression study of the flavodi-iron proteins from the cyanobacterium Synechocystis sp. PCC6803. *Biochemical Society Transactions*, 39. 216-218.

Gorman, D. S., & Levine, R. P. (1965). Cytochrome f and plastocyanin: their sequence in the photosynthetic electron transport chain of Chlamydomonas reinhardi. *PNAS*, 54, 1665–9.

Graham, L. E. (1996). Green algae to land plants: An evolutionary transition. *Journal of Plant Research,* 109, 241–251.

Grewe, S., Ballottari, M., Alcocer, M., D'Andrea, C., Blifernez-Klassen, O., Hankamer, B., … Kruse, O. (2014). Light-Harvesting Complex Protein LHCBM9 Is Critical for Photosystem II Activity and Hydrogen Production in Chlamydomonas reinhardtii. *The Plant Cell*, 26, 1598–1611.

Grieco, M., Tikkanen, M., Paakkarinen, V., Kangasjärvi, S., & Aro, E.-M. (2012). Steady-state phosphorylation of light-harvesting complex II proteins preserves photosystem I under fluctuating white light. *Plant Physiology*, 160, 1896–910.

Gupta, V. K. (2014). Bioenergy research : advances and applications. Elsevier.

Haehnel, W. (1984). PHOTOSYNTHETIC ELECTRON TRANSPORT IN HIGHER PLANTS. *Ann. Rev. Plant Physiol*, 35, 659–93.

Happe, T., Hemschemeier, A., Winkler, M., & Kaminski, A. (2002). Hydrogenases in green algae: do they save the algae's life and solve our energy problems? *Trends in Plant Science,* 7, 246–250.

Happe, T., & Kaminski, A. (2002). Differential regulation of the Fe-hydrogenase during anaerobic adaptation in the green alga Chlamydomonas reinhardtii. *European Journal of Biochemistry*, 269, 1022–1032.

Harris, E. H. (2001). CHLAMYDOMONAS AS A MODEL ORGANISM. *Annual Review of Plant Physiology and Plant Molecular Biology*, 52, 363–406.

He, M., Li, L., Zhang, L., & Liu, J. (2012). The enhancement of hydrogen photoproduction in Chlorella protothecoides exposed to nitrogen limitation and sulfur deprivation. *International Journal of Hydrogen Energy*, 37, 16903–16915.

Heifetz, P. B., Förster, B., Osmond, C. B., Giles, L. J., & Boynton, J. E. (2000). Effects of acetate on facultative autotrophy in Chlamydomonas reinhardtii assessed by photosynthetic measurements and stable isotope analyses. *Plant Physiology*, 122, 1439–45.

Helman, Y., Tchernov, D., Reinhold, L., Shibata, M., Ogawa, T., Schwarz, R., … Kaplan, A. (2003). Genes Encoding A-Type Flavoproteins Are Essential for Photoreduction of O2 in Cyanobacteria. *Current Biology*, 13, 230–235.

Hemschemeier, A., & Happe, T. (2011). Alternative photosynthetic electron transport pathways during anaerobiosis in the green alga Chlamydomonas reinhardtii. *Biochimica et Biophysica Acta (BBA) - Bioenergetics*, 1807, 919–926.

Hemschemeier, A., Jacobs, J., & Happe, T. (2008). Biochemical and physiological characterization of the pyruvate formate-lyase Pfl1 of Chlamydomonas reinhardtii, a typically bacterial enzyme in a eukaryotic alga. *Eukaryotic Cell*, 7, 518–26.

Herdean, A., Teardo, E., Nilsson, A. K., Pfeil, B. E., Johansson, O. N., Ünnep, R., … Lundin, B. (2016a). A voltage-dependent chloride channel fine-tunes photosynthesis in plants. *Nature Communications*, 7, 11654.

Herdean, A., Nziengui, H., Zsiros, O., Solymosi, K., Garab, G., Lundin, B., & Spetea, C. (2016b). The Arabidopsis Thylakoid Chloride Channel AtCLCe Functions in Chloride Homeostasis and Regulation of Photosynthetic Electron Transport. *Frontiers in Plant Science*, 7, 115.

Hertle, A. P., Blunder, T., Wunder, T., Pesaresi, P., Pribil, M., Armbruster, U., & Leister, D. (2013). PGRL1 Is the Elusive Ferredoxin-Plastoquinone Reductase in Photosynthetic Cyclic Electron Flow. *Molecular Cell*, 49, 511–523.

Heyno, E., Gross, C. M., Laureau, C., Culcasi, M., Pietri, S., & Krieger-Liszkay, A. (2009). Plastid alternative oxidase (PTOX) promotes oxidative stress when overexpressed in tobacco. *The Journal of Biological Chemistry*, 284, 31174–80.

Hohmann-Marriott, M. F., & Blankenship, R. E. (2011). Evolution of Photosynthesis. *Annual Review of Plant Biology*, 62, 515–548.

Höhner, R., Aboukila, A., Kunz, H.-H., & Venema, K. (2016). Proton Gradients and Proton-Dependent Transport Processes in the Chloroplast. *Frontiers in Plant Science*, 7, 218.

Horton, P., Ruban, A. V., & Walters, R. G. (1994). Regulation of Light Harvesting in Green Plants (Indication by Nonphotochemical Quenching of Chlorophyll Fluorescence). *Plant Physiology*, 106, 415–420.

Houille-Vernes, L., Rappaport, F., Wollman, F.-A., Alric, J., & Johnson, X. (2011). Plastid terminal oxidase 2 (PTOX2) is the major oxidase involved in chlororespiration in Chlamydomonas. *PNAS*, 108, 20820–5.

Hwang, J.-H., Kim, H.-C., Choi, J.-A., Abou-Shanab, R. A. I., Dempsey, B. A., Regan, J. M., … Jeon, B.-H. (2014). Photoautotrophic hydrogen production by eukaryotic microalgae under aerobic conditions. *Nature Communications*, 5, 957–970.

Ilík, P., Pavlovič, A., Kouřil, R., Alboresi, A., Morosinotto, T., Allahverdiyeva, Y., … Shikanai, T. (2017). Alternative electron transport mediated by flavodiiron proteins is operational in organisms from cyanobacteria up to gymnosperms. *New Phytologist*, 214, 967–972.

Iwai, M., Takizawa, K., Tokutsu, R., Okamuro, A., Takahashi, Y., & Minagawa, J. (2010). Isolation of the elusive supercomplex that drives cyclic electron flow in photosynthesis. *Nature*, 464, 1210–1213.

Jans, F., Mignolet, E., Houyoux, P.-A., Cardol, P., Ghysels, B., Cuiné, S., … Franck, F. (2008). A type II NAD(P)H dehydrogenase mediates light-independent plastoquinone reduction in the chloroplast of Chlamydomonas. *PNAS*, 105, 20546–51.

Johnson, G. N. (2011). Physiology of PSI cyclic electron transport in higher plants. *Biochimica et Biophysica Acta (BBA) - Bioenergetics*, 1807, 384–389.

Johnson, X., Steinbeck, J., Dent, R. M., Takahashi, H., Richaud, P., Ozawa, S.-I., … Alric, J. (2014). Proton gradient regulation 5-mediated cyclic electron flow under ATP- or redox-limited conditions: a study of ΔATpase pgr5 and ΔrbcL pgr5 mutants in the green alga Chlamydomonas reinhardtii. *Plant Physiology*, 165, 438–52.

Joliot, P., & Joliot, A. (1989). Characterization of linear and quadratic electrochromic probes in Chlorella sorokiniana and Chlamydomonas reinhardtii. *Biochimica et Biophysica Acta (BBA) - Bioenergetics*, 975, 355–360.

Kawabata, Y., & Takeda, S. (2014). Regulation of xanthophyll cycle pool size in response to high light irradiance in Arabidopsis. *Plant Biotechnology*, 31, 229–240.

Kindle, K. L. (1987). Expression of a gene for a light-harvesting chlorophyll a/b-binding protein in Chlamydomonas reinhardtii: effect of light and acetate. *Plant Molecular Biology*, 9, 547–563.

King, P. W., Posewitz, M. C., Ghirardi, M. L., & Seibert, M. (2006). Functional studies of [FeFe] hydrogenase maturation in an Escherichia coli biosynthetic system. *Journal of Bacteriology*, 188, 2163–72.

Klughammer, C., & Schreiber, U. (2008). Complementary PS II quantum yields calculated from simple fluorescence parameters measured by PAM fluorometry and the Saturation Pulse method. *PAM Application Notes*, 1, 27–35.

Kosourov, S. N., Ghirardi, M. L., & Seibert, M. (2011). A truncated antenna mutant of Chlamydomonas reinhardtii can produce more hydrogen than the parental strain. *International Journal of Hydrogen Energy*, 36, 2044–2048.

Kosourov, S. N., & Seibert, M. (2009). Hydrogen photoproduction by nutrient-deprived Chlamydomonas reinhardtii cells immobilized within thin alginate films under aerobic and anaerobic conditions. *Biotechnology and Bioengineering*, 102, 50–58.

Kosourov, S., Tsygankov, A., Seibert, M., & Ghirardi, M. L. (2002). Sustained hydrogen photoproduction byChlamydomonas reinhardtii: Effects of culture parameters. *Biotechnology and Bioengineering*, 78, 731–740.

Kramer, D. M., Cruz, J. A., & Kanazawa, A. (2003). Balancing the central roles of the thylakoid proton gradient. *Trends in Plant Science*, 8, 27–32.

Kramer, D. M., & Sacksteder, C. A. (1998). A diffused-optics flash kinetic spectrophotometer (DOFS) for measurements of absorbance changes in intact plants in the steady-state. *Photosynthesis Research*, 56, 103–112.

Kruse, O., Rupprecht, J., Bader, K.-P., Thomas-Hall, S., Schenk, P. M., Finazzi, G., & Hankamer, B. (2005). Improved photobiological H2 production in engineered green algal cells. *The Journal of Biological Chemistry*, 280, 34170–7.

Kukuczka, B., Magneschi, L., Petroutsos, D., Steinbeck, J., Bald, T., Powikrowska, M., … Hippler, M. (2014). Proton Gradient Regulation5-Like1-Mediated Cyclic Electron Flow Is Crucial for Acclimation to Anoxia and Complementary to Nonphotochemical Quenching in Stress Adaptation. *Plant Physiology*, 165, 1604–1617.

Kuntz, M. (2004). Plastid terminal oxidase and its biological significance. *Planta*, 218, 896–899.

Lam, M. K., & Lee, K. T. (2012). Microalgae biofuels: A critical review of issues, problems and the way forward. *Biotechnology Advances,* 30, 673–690.

Laurinavichene, T. V., Fedorov, A. S., Ghirardi, M. L., Seibert, M., & Tsygankov, A. A. (2006). Demonstration of sustained hydrogen photoproduction by immobilized, sulfur-deprived Chlamydomonas reinhardtii cells. *International Journal of Hydrogen Energy*, 31, 659–667.

Li, X., Zhang, R., Patena, W., Gang, S. S., Blum, S. R., Ivanova, N., … Jonikas, M. C. (2016). An Indexed, Mapped Mutant Library Enables Reverse Genetics Studies of Biological Processes in Chlamydomonas reinhardtii. *The Plant Cell*, 28, 367–87.

Li, X.-P., Muller-Moule, P., Gilmore, A. M., & Niyogi, K. K. (2002). PsbS-dependent enhancement of feedback de-excitation protects photosystem II from photoinhibition. *PNAS*, 99, 15222–7.

Lichtenthaler, H. K. (1987). Chlorophylls and carotenoids: Pigments of photosynthetic biomembranes. *Methods in Enzymology*, 148, 350–382.

Liran, O., Semyatich, R., Milrad, Y., Eilenberg, H., Weiner, I., & Yacoby, I. (2016). Microoxic Niches within the Thylakoid Stroma of Air-Grown Chlamydomonas reinhardtii Protect [FeFe]-Hydrogenase and Support Hydrogen Production under Fully Aerobic Environment. *Plant Physiology*, 172, 264–71.

Maruyama, S., Tokutsu, R., & Minagawa, J. (2014). Transcriptional Regulation of the Stress-Responsive Light Harvesting Complex Genes in Chlamydomonas Reinhardtii. *Plant and Cell Physiology*, 55, 1304–1310.

Mata, T. M., Martins, A. A., & Caetano, N. S. (2010). Microalgae for biodiesel production and other applications: A review. *Renewable and Sustainable Energy Reviews*, 14, 217–232.

Maul, J. E., Lilly, J. W., Cui, L., dePamphilis, C. W., Miller, W., Harris, E. H., & Stern, D. B. (2002). The Chlamydomonas reinhardtii plastid chromosome: islands of genes in a sea of repeats. *The Plant Cell*, 14, 2659–79.

Mehler, A. H. (1951). Studies on reactions of illuminated chloroplasts. *Archives of Biochemistry and Biophysics*, 33, 65–77.

Mekala, N. R., Suorsa, M., Rantala, M., Aro, E.-M., & 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 Physiology*, 168, 721–34.

Melis, A. (2007). Photosynthetic H2 metabolism in Chlamydomonas reinhardtii (unicellular green algae). *Planta*, 226, 1075–1086.

Melis, A., & Happe, T. (2001). Hydrogen production. Green algae as a source of energy. *Plant Physiology*, 127, 740–8.

Melis, A., Zhang, L., Forestier, M., Ghirardi, M. L., & Seibert, M. (2000). Sustained photobiological hydrogen gas production upon reversible inactivation of oxygen evolution in the green alga Chlamydomonas reinhardtii. *Plant Physiology*, 122, 127–36.

Merchant, S. S., Prochnik, S. E., Vallon, O., Harris, E. H., Karpowicz, S. J., Witman, G. B., … Grossman, A. R. (2007). The Chlamydomonas Genome Reveals the Evolution of Key Animal and Plant Functions. *Science*, 318, 245-250.

Meuser, J. E., D'Adamo, S., Jinkerson, R. E., Mus, F., Yang, W., Ghirardi, M. L., … Posewitz, M. C. (2012). Genetic disruption of both Chlamydomonas reinhardtii [FeFe]-hydrogenases: Insight into the role of HYDA2 in H2 production. *Biochemical and Biophysical Research Communications,* 417, 704–709.

Minagawa, J. (2011). State transitions—The molecular remodeling of photosynthetic supercomplexes that controls energy flow in the chloroplast. *Biochimica et Biophysica Acta (BBA) - Bioenergetics*, 1807, 897–905.

Minagawa, J., & Takahashi, Y. (2004). Structure, function and assembly of Photosystem II and its light-harvesting proteins. *Photosynthesis Research*, 82, 241–263.

Mitchell, P. (1975). The protonmotive Q cycle: A general formulation. *FEBS Letters*, 59, 137–139.

Mitchell, P., & Moyle, J. (1967). Respiration-driven proton translocation in rat liver mitochondria. *Biochemical Journal*, 105, 1147-1162.

Miyake, C., & Asada, K. (1992). Thylakoid-Bound Ascorbate Peroxidase in Spinach Chloroplasts and Photoreduction of Its Primary Oxidation Product Monodehydroascorbate Radicals in Thylakoids. *Plant and Cell Physiology*, 33, 541–553.

Moroney, J. V, & Ynalvez, R. A. (2007). Proposed carbon dioxide concentrating mechanism in Chlamydomonas reinhardtii. *Eukaryotic Cell*, 6, 1251–9.

Müller, P., Li, X. P., & Niyogi, K. K. (2001). Non-photochemical quenching. A response to excess light energy. *Plant Physiology*, 125, 1558–66.

Munekage, Y., Hashimoto, M., Miyake, C., Tomizawa, K.-I., 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.

Murata, N. (1969). Control of excitation transfer in photosynthesis I. Light-induced change of chlorophyll a fluoresence in Porphyridium cruentum. *Biochimica et Biophysica Acta (BBA) - Bioenergetics*, 172, 242–251.

Mustila, H., Paananen, P., Battchikova, N., Santana-Sánchez, A., Muth-Pawlak, D., Hagemann, M., … 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 and Cell Physiology,* 24, pcw047.

Nagy, G., Ünnep, R., Zsiros, O., Tokutsu, R., Takizawa, K., Porcar, L., … Minagawa, J. (2014). Chloroplast remodeling during state transitions in Chlamydomonas reinhardtii as revealed by noninvasive techniques in vivo. *PNAS,* 111, 5042–7.

Nawrocki, W. J., Santabarbara, S., Mosebach, L., Wollman, F.-A., & Rappaport, F. (2016). State transitions redistribute rather than dissipate energy between the two photosystems in Chlamydomonas. *Nature Plants*, 2, 16031.

Nicolet, Y., de Lacey, A. L., Vernède, X., Fernandez, V. M., Hatchikian, E. C., &. Fontecilla-Camps, J. C. (2001). Crystallographic and FTIR Spectroscopic Evidence of Changes in Fe Coordination Upon Reduction of the Active Site of the Fe-Only Hydrogenase from Desulfovibrio desulfuricans. *J. Am. Chem. Soc*., 123, 1596–1601.

Nishio, J. N., & Whitmarsh, J. (1993). Dissipation of the Proton Electrochemical Potential in Intact Chloroplasts (II. The pH Gradient Monitored by Cytochrome f Reduction Kinetics). *Plant Physiology*, 101, 89–96.

Niyogi, K. K., & Truong, T. B. (2013). Evolution of flexible non-photochemical quenching mechanisms that regulate light harvesting in oxygenic photosynthesis. *Current Opinion in Plant Biology*, 16, 307–314.

Niyogi, K. K., Li, X.-P., Björkman, O., Shih, C., Grossman, A. R., Rosenquist, M., & Jansson, S. (2000). A pigment-binding protein essential for regulation of photosyntheticlight harvesting. *Nature*, 403, 391–395.

Niyogi, K. K., Bjorkman, O., & Grossman, A. R. (1997). Chlamydomonas Xanthophyll Cycle Mutants Identified by Video Imaging of Chlorophyll Fluorescence Quenching. *The Plant Cell,* 9, 1369–1380.

Noone, S., Ratcliff, K., Davis, R., Subramanian, V., Meuser, J., Posewitz, M. C., … Ghirardi, M. L. (2017). Expression of a clostridial [FeFe]-hydrogenase in Chlamydomonas reinhardtii prolongs photo-production of hydrogen from water splitting. *Algal Research*, 22, 116–121.

Noth, J., Krawietz, D., Hemschemeier, A., & Happe, T. (2013). Pyruvate: ferredoxin oxidoreductase is coupled to light-independent hydrogen production in Chlamydomonas reinhardtii. *The Journal of Biological Chemistry*, 288, 4368–77.

Oey, M., Sawyer, A. L., Ross, I. L., & Hankamer, B. (2016). Challenges and opportunities for hydrogen production from microalgae. *Plant Biotechnology Journal*, 14, 1487–1499.

Oey, M., Ross, I. L., Stephens, E., Steinbeck, J., Wolf, J., Radzun, K. A., … Hankamer, B. (2013). RNAi Knock-Down of LHCBM1, 2 and 3 Increases Photosynthetic H2 Production Efficiency of the Green Alga Chlamydomonas reinhardtii. *PLoS ONE*, 8, e61375.

Ogawa, T. (1991). A gene homologous to the subunit-2 gene of NADH dehydrogenase is essential to inorganic carbon transport of Synechocystis PCC6803. *PNAS*, 88, 4275–9.

Ort, D. R., & Baker, N. R. (2002). A photoprotective role for O2 as an alternative electron sink in photosynthesis? *Current Opinion in Plant Biology*, 5, 193–198.

Papazi, A., Gjindali, A.-I., Kastanaki, E., Assimakopoulos, K., Stamatakis, K., & Kotzabasis, K. (2014). Potassium deficiency, a "smart" cellular switch for sustained high yield hydrogen production by the green alga Scenedesmus obliquus. *International Journal of Hydrogen Energy*, 39, 19452–19464.

Peden, E. A., Boehm, M., Mulder, D. W., Davis, R., Old, W. M., King, P. W., … Dubini, A. (2013). Identification of global ferredoxin interaction networks in Chlamydomonas reinhardtii. *The Journal of Biological Chemistry*, 288, 35192–209.

Peers, G., Truong, T. B., Ostendorf, E., Busch, A., Elrad, D., Grossman, A. R., … Niyogi, K. K. (2009). An ancient light-harvesting protein is critical for the regulation of algal photosynthesis. *Nature*, 462, 518–521.

Peltier, G., Aro, E.-M., & Shikanai, T. (2016). NDH-1 and NDH-2 Plastoquinone Reductases in Oxygenic Photosynthesis. *Annual Review of Plant Biology*, 67, 55–80.

Peltier, G., Tolleter, D., Billon, E., & Cournac, L. (2010). Auxiliary electron transport pathways in chloroplasts of microalgae. *Photosynthesis Research*, 106, 19–31.

Peltier, G., & Cournac, L. (2002). CHLORORESPIRATION. *Annual Review of Plant Biology*, 53, 523–550.

Peng, L., Shimizu, H., & Shikanai, T. (2008). The chloroplast NAD(P)H dehydrogenase complex interacts with photosystem I in Arabidopsis. *The Journal of Biological Chemistry*, 283, 34873–9.

Petroutsos, D., Tokutsu, R., Maruyama, S., Flori, S., Greiner, A., Magneschi, L., … Minagawa, J. (2016). A blue-light photoreceptor mediates the feedback regulation of photosynthesis. *Nature*, 537, 563–566.

Petroutsos, D., Terauchi, A. M., Busch, A., Hirschmann, I., Merchant, S. S., Finazzi, G., & Hippler, M. (2009). PGRL1 participates in iron-induced remodeling of the photosynthetic apparatus and in energy metabolism in Chlamydomonas reinhardtii. *The Journal of Biological Chemistry*, 284, 32770–81.

Philipps, G., Happe, T., & Hemschemeier, A. (2012). Nitrogen deprivation results in photosynthetic hydrogen production in Chlamydomonas reinhardtii. *Planta*, 235, 729–745.

Pinto, T. S., Malcata, F. X., Arrabaça, J. D., Silva, J. M., Spreitzer, R. J., & Esquível, M. G. (2013). Rubisco mutants of Chlamydomonas reinhardtii enhance photosynthetic hydrogen production. *Applied Microbiology and Biotechnology*, 97, 5635–5643.

Pribil, M., Pesaresi, P., Hertle, A., Barbato, R., & Leister, D. (2010). Role of Plastid Protein Phosphatase TAP38 in LHCII Dephosphorylation and Thylakoid Electron Flow. *PLoS Biology*, 8, e1000288.

Quaas, T., Berteotti, S., Ballottari, M., Flieger, K., Bassi, R., & Goss, R. (2015). Non-photochemical quenching and xanthophyll cycle activities in six green algal species suggest mechanistic differences in the process of excess energy dissipation. *Journal of Plant Physiology*, 172, 92–103.

Raines, C. A. (2003). The Calvin cycle revisited. *Photosynthesis Research*, 75, 1–10.

Rochaix, J.-D. (2013). Fine-Tuning Photosynthesis. *Science*, 342(6154).

Rochaix, J.-D. (2011). Assembly of the photosynthetic apparatus. *Plant Physiology*, 155, 1493–500.

Rodrigues, R., Vicente, J. B., Félix, R., Oliveira, S., Teixeira, M., & Rodrigues-Pousada, C. (2006). Desulfovibrio gigas flavodiiron protein affords protection against nitrosative stress in vivo. *Journal of Bacteriology*, 188, 2745–51.

Rumberg, B., & Siggel, U. (1969). pH changes in the inner phase of the thylakoids during photosynthesis. *Die Naturwissenschaften*, 56, 130–132.

Sacksteder, C. A., Kanazawa, A., Jacoby, M. E., & Kramer, D. M. (2000). The proton to electron stoichiometry of steady-state photosynthesis in living plants: A proton-pumping Q cycle is continuously engaged. *PNAS*, 97, 14283–8.

Sambusiti, C., Bellucci, M., Zabaniotou, A., Beneduce, L., & Monlau, F. (2015). Algae as promising feedstocks for fermentative biohydrogen production according to a biorefinery approach: A comprehensive review. *Renewable and Sustainable Energy Reviews*, 44, 20–36.

Samol, I., Shapiguzov, A., Ingelsson, B., Fucile, G., Crèvecoeur, M., Vener, A. V, … Goldschmidt-Clermont, M. (2012). Identification of a photosystem II phosphatase involved in light acclimation in Arabidopsis. *The Plant Cell*, 24, 2596–609.

Scaife, M. A., Nguyen, G. T. D. T., Rico, J., Lambert, D., Helliwell, K. E., & Smith, A. G. (2015). Establishing Chlamydomonas reinhardtii as an industrial biotechnology host. *The Plant Journal*, 82, 532–546.

Schreiber, U., & Klughammer, C. (2008). New accessory for the DUAL-PAM-100: The P515/535 module and examples of its application. *PAM Application Notes*, 1, 1–10.

Scoma, A., Krawietz, D., Faraloni, C., Giannelli, L., Happe, T., & Torzillo, G. (2012). Sustained H2 production in a Chlamydomonas reinhardtii D1 protein mutant. *Journal of Biotechnology*, 157, 613–619.

Seedorf, H., Hagemeier, C. H., Shima, S., Thauer, R. K., Warkentin, E., & Ermler, U. (2007). Structure of coenzyme F420H2 oxidase (FprA), a di-iron flavoprotein from methanogenic Archaea catalyzing the reduction of O2 to H2O. *FEBS Journal*, 274, 1588–1599.

Shapiguzov, A., Ingelsson, B., Samol, I., Andres, C., Kessler, F., Rochaix, J.-D., … Goldschmidt-Clermont, M. (2010). The PPH1 phosphatase is specifically involved in LHCII dephosphorylation and state transitions in Arabidopsis. *PNAS*, 107, 4782–7.

Sheen, J. (1994). Feedback control of gene expression. *Photosynthesis Research*, 39, 427–438.

Shepard, E. M., Mus, F., Betz, J. N., Byer, A. S., Duffus, B. R., Peters, J. W., & Broderick, J. B. (2014). [FeFe]-Hydrogenase Maturation. *Biochemistry*, 53, 4090–4104.

Shikanai, T., & Yamamoto, H. (2017). Contribution of Cyclic and Pseudo-cyclic Electron Transport to the Formation of Proton Motive Force in Chloroplasts. *Molecular Plant*, 10, 20–29.

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 Physiology*, 173, 1636–1647.

Shimakawa, G., Shaku, K., Nishi, A., Hayashi, R., Yamamoto, H., Sakamoto, K., … Miyake, C. (2015). FLAVODIIRON2 and FLAVODIIRON4 proteins mediate an oxygen-dependent alternative electron flow in Synechocystis sp. PCC 6803 under CO2-limited conditions. *Plant Physiology*, 167, 472–80.

Silaghi-Dumitrescu, R., Coulter, E. D., Das, A., Lars G. Ljungdahl, L. G., Jameson, G. N. L., Boi Hanh Huynh, B. H., & Kurtz, D. M. J. (2003). A Flavodiiron Protein and High Molecular Weight Rubredoxin from Moorella thermoacetica with Nitric Oxide Reductase Activity. *Biochemistry*, 42, 2806–2815

Spetea, C., Herdean, A., Allorent, G., Carraretto, L., Finazzi, G., & Szabo, I. (2017). An update on the regulation of photosynthesis by thylakoid ion channels and transporters in Arabidopsis. *Physiologia Plantarum*. https://doi.org/10.1111/ppl.12568

Steigmiller, S., Turina, P., & Gräber, P. (2008). The thermodynamic H+/ATP ratios of the H+-ATPsynthases from chloroplasts and Escherichia coli. *PNAS*, 105, 3745–50.

Steinbeck, J., Nikolova, D., Weingarten, R., Johnson, X., Richaud, P., Peltier, G., … Hippler, M. (2015). Deletion of Proton Gradient Regulation 5 (PGR5) and PGR5-Like 1 (PGRL1) proteins promote sustainable light-driven hydrogen production in Chlamydomonas reinhardtii due to increased PSII activity under sulfur deprivation. *Frontiers in Plant Science*, 6, 892.

Stephens, E., Ross, I. L., & Hankamer, B. (2013). Expanding the microalgal industry – continuing controversy or compelling case? *Current Opinion in Chemical Biology*, 17, 444–452.

Stiebritz, M. T., Reiher, M., Gogonea, V., Armstrong, F. A., Lubitz, W., Happe, T., … Rousset, M. (2012). Hydrogenases and oxygen. *Chemical Science*, 3, 1739.

Stripp, S. T., Goldet, G., Brandmayr, C., Sanganas, O., Vincent, K. A., Haumann, M., … Happe, T. (2009). How oxygen attacks [FeFe] hydrogenases from photosynthetic organisms. *PNAS*, 106, 17331–6.

Sueoka, N. (1960). MITOTIC REPLICATION OF DEOXYRIBONUCLEIC ACID IN CHLAMYDOMONAS REINHARDI. *PNAS*, 46, 83–91.

Sugimoto, K., Okegawa, Y., Tohri, A., Long, T. A., Covert, S. F., Hisabori, T., & Shikanai, T. (2013). A Single Amino Acid Alteration in PGR5 Confers Resistance to Antimycin A in Cyclic Electron Transport around PSI. *Plant and Cell Physiology*, 54, 1525–1534.

Suorsa, M., Järvi, S., Grieco, M., Nurmi, M., Pietrzykowska, M., Rantala, M., … Aro, E.-M. (2012). PROTON GRADIENT REGULATION5 is essential for proper acclimation of Arabidopsis photosystem I to naturally and artificially fluctuating light conditions. *The Plant Cell*, 24, 2934–48.

Surzycki, R., Cournac, L., Peltier, G., & Rochaix, J.-D. (2007). Potential for hydrogen production with inducible chloroplast gene expression in Chlamydomonas. *PNAS*, 104, 17548–53.

Takahashi, H., Clowez, S., Wollman, F.-A., Vallon, O., & Rappaport, F. (2013). Cyclic electron flow is redox-controlled but independent of state transition. *Nature Communications*, 4, 1107–1125.

Takahashi, Y., Yasui, T., Stauber, E. J., & Hippler, M. (2004). Comparison of the Subunit Compositions of the PSI–LHCI Supercomplex and the LHCI in the Green Alga Chlamydomonas reinhardtii. *Biochemistry*, 43, 7816–7823.

Takahashi, Y., Rahire, M., Breyton, C., Popot, J. L., Joliot, P., & Rochaix, J. D. (1996). The chloroplast ycf7 (petL) open reading frame of Chlamydomonas reinhardtii encodes a small functionally important subunit of the cytochrome b6f complex. *The EMBO Journal*, 15, 3498–506.

Takahashi, Y., Hansson, Ö., Mathis, P., & Satoh, K. (1987). Primary radical pair in the Photosystem II reaction centre. *Biochimica et Biophysica Acta (BBA) - Bioenergetics*, 893, 49–59.

Terashima, M., Petroutsos, D., Hüdig, M., Tolstygina, I., Trompelt, K., Gäbelein, P., … Hippler, M. (2012). Calcium-dependent regulation of cyclic photosynthetic electron transfer by a CAS, ANR1, and PGRL1 complex. *PNAS*, 109, 17717–22.

Tibiletti, T., Auroy, P., Peltier, G., & Caffarri, S. (2016). Chlamydomonas reinhardtii PsbS Protein Is Functional and Accumulates Rapidly and Transiently under High Light. *Plant Physiology*, 171, 2717–30.

Tikhonov, A. N. (2014). The cytochrome b6f complex at the crossroad of photosynthetic electron transport pathways. *Plant Physiology and Biochemistry*, 81, 163–183.

Tikkanen, M., Grieco, M., & Aro, E.-M. (2011). Novel insights into plant light-harvesting complex II phosphorylation and "state transitions." *Trends in Plant Science*, 16, 126–131.

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

Tokutsu, R., Teramoto, H., Takahashi, Y., Ono, T., & Minagawa, J. (2004). The Light-Harvesting Complex of Photosystem I in Chlamydomonas reinhardtii: Protein Composition, Gene Structures and Phylogenic Implications. *Plant and Cell Physiology*, 45, 138–145.

Tolleter, D., Ghysels, B., Alric, J., Petroutsos, D., Tolstygina, I., Krawietz, D., … Peltier, G. (2011). Control of hydrogen photoproduction by the proton gradient generated by cyclic electron flow in Chlamydomonas reinhardtii. *The Plant Cell*, 23, 2619–30.

Torzillo, G., Scoma, A., Faraloni, C., Ena, A., & Johanningmeier, U. (2009). Increased hydrogen photoproduction by means of a sulfur-deprived Chlamydomonas reinhardtii D1 protein mutant. *International Journal of Hydrogen Energy*, 34, 4529–4536.

Trivedi, J., Aila, M., Bangwal, D. P., Kaul, S., & Garg, M. O. (2015). Algae based biorefinery—How to make sense? *Renewable and Sustainable Energy Reviews*, 47, 295–307.

Trouillard, M., Shahbazi, M., Moyet, L., Rappaport, F., Joliot, P., Kuntz, M., & Finazzi, G. (2012). Kinetic properties and physiological role of the plastoquinone terminal oxidase (PTOX) in a vascular plant. *Biochimica et Biophysica Acta (BBA) - Bioenergetics*, 1817, 2140–2148.

Tsygankov, A. A., Kosourov, S. N., Tolstygina, I. V., Ghirardi, M. L., & Seibert, M. (2006). Hydrogen production by sulfur-deprived Chlamydomonas reinhardtii under photoautotrophic conditions. *International Journal of Hydrogen Energy*, 31, 1574–1584.

Umena, Y., Kawakami, K., Shen, J.-R., & Kamiya, N. (2011). Crystal structure of oxygen-evolving photosystem II at a resolution of 1.9 Å. *Nature*, 473, 55–60.

Ünlü, C., Drop, B., Croce, R., & van Amerongen, H. (2014). State transitions in Chlamydomonas reinhardtii strongly modulate the functional size of photosystem II but not of photosystem I. *PNAS,* 111, 3460–5.

Vicente, J. B., Carrondo, M. A., Teixeira, M., & Frazão, C. (2008a). Chapter 1 – Structural Studies on Flavodiiron Proteins. *In Methods in Enzymology* 437, 3–19.

Vicente, J. B., Justino, M. C., Gonçalves, V. L., Saraiva, L. M., & Teixeira, M. (2008b). Chapter 2 – Biochemical, Spectroscopic, and Thermodynamic Properties of Flavodiiron Proteins. *In Methods in Enzymology* 437, 21–45.

Vicente, J. B., Scandurra, F. M., Rodrigues, J. V., Brunori, M., Sarti, P., Teixeira, M., & Giuffrè, A. (2007). Kinetics of electron transfer from NADH to the Escherichia coli nitric oxide reductase flavorubredoxin. *FEBS Journal,* 274, 677–686.

Vicente, J. B., Gomes, C. M., 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. *Biochemical and Biophysical Research Communications*, 294, 82–87.

Volgusheva, A., Kukarskikh, G., Krendeleva, T., Rubin, A., Mamedov, F., Radzun, K. A., … Hankamer, B. (2015). Hydrogen photoproduction in green algae Chlamydomonas reinhardtii under magnesium deprivation. *RSC Adv*, 5, 5633–5637.

Volgusheva, A., Styring, S., & Mamedov, F. (2013). Increased photosystem II stability promotes H2 production in sulfur-deprived Chlamydomonas reinhardtii. *PNAS*, 110, 7223–8.

Vollmar, M., Schlieper, D., Winn, M., Büchner, C., & Groth, G. (2009). Structure of the c14 rotor ring of the proton translocating chloroplast ATP synthase. *The Journal of Biological Chemistry*, 284, 18228–35.

Wang, C., Yamamoto, H., & Shikanai, T. (2015). Role of cyclic electron transport around photosystem I in regulating proton motive force. *Biochimica et Biophysica Acta (BBA) - Bioenergetics*, 1847, 931–938.

Wasserfallen, A., Ragettli, S., Jouanneau, Y., & Leisinger, T. (1998). A family of flavoproteins in the domains Archaea and Bacteria. *European Journal of Biochemistry*, 254, 325–332.

Whitney, L. A. S., Loreti, E., Alpi, A., & Perata, P. (2011). Alcohol dehydrogenase and hydrogenase transcript fluctuations during a day-night cycle in Chlamydomonas reinhardtii: the role of anoxia. *New Phytologist,* 190, 488–498.

Winkler, M., Hemschemeier, A., Jacobs, J., Stripp, S., & Happe, T. (2010). Multiple ferredoxin isoforms in Chlamydomonas reinhardtii – Their role under stress conditions and biotechnological implications. *European Journal of Cell Biology*, 89, 998–1004.

Wodniok, S., Brinkmann, H., Glöckner, G., Heidel, A. J., Philippe, H., Melkonian, M., & Becker, B. (2011). Origin of land plants: Do conjugating green algae hold the key? *BMC Evolutionary Biology,* 11, 104.

Wollman, F. A. (2001). State transitions reveal the dynamics and flexibility of the photosynthetic apparatus. *The EMBO Journal,* 20, 3623–30.

Wu, S., Xu, L., Huang, R., & Wang, Q. (2011). Improved biohydrogen production with an expression of codon-optimized hemH and lba genes in the chloroplast of Chlamydomonas reinhardtii. *Bioresource Technology*, 102, 2610–2616.

Wykoff, D. D., Davies, J. P., Melis, A., & Grossman, A. R. (1998). The regulation of photosynthetic electron transport during nutrient deprivation in Chlamydomonas reinhardtii. *Plant Physiology*, 117, 129–39.

Xu, F.-Q., Ma, W.-M., & Zhu, X.-G. (2011). Introducing pyruvate oxidase into the chloroplast of Chlamydomonas reinhardtii increases oxygen consumption and promotes hydrogen production. *International Journal of Hydrogen Energy*, 36, 10648–10654.

Yacoby, I., Pochekailov, S., Toporik, H., Ghirardi, M. L., King, P. W., & Zhang, S. (2011). Photosynthetic electron partitioning between [FeFe]-hydrogenase and ferredoxin: NADP+-oxidoreductase (FNR) enzymes in vitro. *PNAS,* 108, 9396–401.

Yadav, K. N. S., Semchonok, D. A., Nosek, L., Kouřil, R., Fucile, G., Boekema, E. J., & Eichacker, L. A. (2017). Supercomplexes of plant photosystem I with cytochrome b6f, light-harvesting complex II and NDH. *Biochimica et Biophysica Acta (BBA) - Bioenergetics*, 1858, 12–20.

Yamamoto, H., Takahashi, S., Badger, M. R., & Shikanai, T. (2016). Artificial remodelling of alternative electron flow by flavodiiron proteins in Arabidopsis. *Nature Plants*, 2, 16012.

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. *The Plant Cell,* 23, 1480–93.

Yamori, W., Makino, A., & Shikanai, T. (2016). A physiological role of cyclic electron transport around photosystem I in sustaining photosynthesis under fluctuating light in rice. *Scientific Reports*, 6, 20147.

Yen, H.-W., Hu, I.-C., Chen, C.-Y., Ho, S.-H., & Lee, D.-J. (2013). Microalgae-based biorefinery – From biofuels to natural products. *Bioresource Technology*, 135, 166–174.

Yeremenko, N., Jeanjean, R., Prommeenate, P., Krasikov, V., Nixon, P. J., Vermaas, W. F. J., … Matthijs, H. C. P. (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 and Cell Physiology*, 46, 1433–1436.

Yoon, H. S., Hackett, J. D., Ciniglia, C., Pinto, G., & Bhattacharya, D. (2004). A Molecular Timeline for the Origin of Photosynthetic Eukaryotes. *Molecular Biology and Evolution,* 21, 809–818.

Zhang, P., Eisenhut, M., Brandt, A.-M., Carmel, D., Silén, H. M., Vass, I., … Aro, E.-M. (2012). Operon flv4-flv2 provides cyanobacterial photosystem II with flexibility of electron transfer. *The Plant Cell*, 24, 1952–71.

Zhang, P., Allahverdiyeva, Y., Eisenhut, M., Aro, E.-M., & Higgins, D. (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, L., Happe, T., & Melis, A. (2002). Biochemical and morphological characterization of sulfur-deprived and H 2 -producing Chlamydomonas reinhardtii (green alga). *Planta*, 214, 552–561.

Annales Universitatis Turkuensis

Turun yliopisto
University of Turku

Painosalama Oy, Turku, Finland 2017

ISBN 978-951-29-6887-9 (PRINT)
ISBN 978-951-29-6888-6 (PDF)
ISSN 0082-7002 (Print) | ISSN 2343-3175 (Online)