Molecular mechanisms behind the adjustment of phototrophic light-harvesting and mixotrophic utilization of cellulosic carbon sources in *Chlamydomonas reinhardtii*

Dissertation zur Erlangung des akademischen Grades
Doktor der Naturwissenschaften (Dr. rer. nat.)

vorgelegt von

Olga Blifernez

Dsheskasgan (Kasachstan)

Angefertigt an der Fakultät für Biologie der Universität Bielefeld
Lehrstuhl für Algenbiotechnologie und Bioenergie
unter der Betreuung von Herrn Prof. Dr. Olaf Kruse

Februar 2012

Erstgutachter: Prof. Dr. Olaf Kruse

Zweitgutachter: Prof. Dr. Volker F. Wendisch

TABLE OF CONTENTS

Summary

Plants, green algae and cyanobacteria perform photosynthetic conversion of sunlight into chemical energy in a permanently changing natural environment, where the efficient utilization of light and inorganic carbon represent the most critical factors. Photosynthetic organisms have developed different acclimation strategies to adapt changing light conditions and insufficient carbon source supply in order to survive and to assure optimal growth and protection. This thesis provides further insights into the molecular mechanisms of the acclimation response of the green algae *Chlamydomonas reinhardtii*.

An important acclimation mechanism to altering light conditions involves the post-transcriptional regulation of the nuclear-encoded photosystem II (PSII)-associated light-harvesting complex (LHCII) genes via cytosolic translational control mediated through the RNA-binding protein NAB1. In the active state, NAB1 represses the cytosolic translation of *LHCII* mRNAs by sequestration into translational silent messenger ribonucleoprotein complexes (mRNPs). The overexpression of NAB1 decreases LHCII protein amount whereas NAB1 knockout leads to an increased level of LHCII proteins. Consequently, NAB1 is part of a control system regulating the size and composition of the LHCII complex at the posttranscriptional level. The repressor activity of this specific factor is controlled by two posttranslational modifications: i) by methylation of arginines in the glycine-arginine rich (GAR) motif of the protein, ii) by the thiol status of two C-terminal cysteines. This work provides evidence that arginine methylation represents a slowly reacting modulator, which is required for the maintenance of the repressor activity of NAB1 and is responsive to the availability of light. At the same time, cysteine modification is regarded as the fine-tuning mechanism that dynamically responds to changes in the cytosolic redox-state. Moreover, the observations suggest that the regulation via arginine methylation operates independently from cysteine-based redox control, with its extent strongly depending on the growth conditions. The high methylation state is found under photoautotrophic, and the low methylation state under heterotrophic growth conditions.

Photosynthetic performance is also dependent on inorganic carbon (C_i) supply, because the light-harvesting capacity and the utilization of captured energy have to be balanced. The insufficient C_i-availability can be compensated by diverse organic carbon sources, since some phototrophs can assimilate acetate, glucose or other sugars for mixothrophic growth. The green alga *C. reinhardtii* was so far reported to grow on acetate, but not on hexoses. Intriguingly, *in silico* analysis of the genome of *C. reinhardtii* revealed that it contains genes encoding glycoside hydrolases of different families, known to be involved in cellulose- and

hemicellulose degradation, even though the cell wall of this alga does not contain cellulose and is solely composed of hydroxyproline-rich glycoproteins. This work characterizes the capability of *C. reinhardtii* for cellulose degradation as seen by the digestion of cellulosic materials such as carboxymethyl cellulose, Avicel and filter paper. Furthermore, the results of the present work indicate an assimilation of the breakdown products, in particular cellobiose. Cellulose degradation into cellodextrins (cellobiose-cellopentaose) was shown to be performed by extracellular cellulases CrCel9B and CrCel9C that belong to glycoside hydrolase family 9 (GHF9/subgroup E2). These enzymes display homology to cellulases from Metazoa and phylogenetic analyses suggested that they originated from an ancient eukaryotic ancestor. Furthermore, a positive effect on specific growth rates was observable under different growth conditions (high, low and very-low CO_2 as well as acetate) after media supplementation with cellulosic material.

In conclusion, this work provides advanced insights into the molecular regulation mechanisms of light-acclimation and utilization of an abundant organic carbon source in *Chlamydomonas reinhardtii*. These new findings could help to achieve higher biomass productivity by improved photosynthetic conversion efficiency and additionally, the use of abundant organic carbon sources as an integral part of new photoheterotrophic cultivation concepts.

Abbreviations

$^1O2$	singlet oxygen
-SNO	S-nitrosothiol
$-SO_3H$	sulfonic acid
µg	microgram
µm	micrometer
•OH	hydroxyl radical
2CP	2-Cysteine peroxiredoxin
2D	two-dimensional
3D	three-dimensional
Å	angstrom
ABC	ATP-binding cassette
ADMA	asymmetric dimethylarginine
AdOx	adenosine-2´-3´-dialdehyde
AMI-1	7,7´-carbonylbis (azanediyl)*bis*(4-hydroxy-naphthalene-2-sulfonic acid) sodium salt hydrate
ARS	arylsulphatase
ATP	adenosine-5´-triphosphate
ATPase	ATP synthase
cAPX	cytosolic ascorbate peroxidase
CB	cellobiose
CBD/M	cellulose-binding domain /motive
CCM	CO_2-concentrating mechanism
CD	cellodextrins
Chl	chlorophyll
C_i	inorganic carbon
CMC	carboxymethyl cellulose
CO_2	carbon dioxide
CP26/29/24	minor LHCII polypeptides
CP43/47	core antenna proteins
CSD	cold shock domain
CSDCS	cold shock domain consensus sequence
Cytb_6f	cytochrome b_6/f complex
D1/D2	core protein of PSII
DNS	3,5-dinitrosalicylic acid assay
DP	degree of polymerization
dsDNA	double-stranded DNA
EJC	exon junction complex
EM	electron microscope
EST	expressed sequence tags
FNR	ferredoxin-$NADP^+$ reductase
FP	filter paper
G	glucose
G2-G5	cellobiose, cellotriose, cellotetraose, cellopentaose
G3P	glyceraldehyde-3-phosphate
GAPDH	glyceraldehyde-3-phosphate dehydrogenase
GAR	glycine-arginine rich
GHF	glycoside hydrolase family
GRX	glutaredoxin
GSH	glutathione
GSSG	disulfide bridge
H_2O	water
H_2O_2	hydrogen peroxide
HCO_3^-	hydrogencarbonate
hnRNP	heterogeneous nuclear ribonucleoproteins
HSM	high salt media
L1/2	lutein's
LHC	light-harvesting complex

LIL	light-harvesting-like
LR3	LHC reduced mutant 3
MFS	major facilitator superfamily
mg	milligram
Mg Proto	magnesium protoporphyrin IX
miRNAs	microRNA
ml	milliliter
MMAω	monomethylarginine
mRNA	messenger RNA
mRNPs	messenger ribonucleoprotein complexes
N	nucleus
NAB1	nucleic acid binding protein
$NADP^+$	nicotinamide adenine dinucleotide phosphate (oxidized)
NADPH	nicotinamide adenine dinucleotide phosphate (reduced)
NDM	NAB1 double mutant
NO	nitric oxide
NPC	nuclear pore complex
NPQ	non-photochemical quenching
NSM	NAB1 single mutant
O_2	oxygen
O_2^-	superoxide
PBR	photo bioreactor
PC	plastocyanin
PET	photosynthetic electron transport
PetF	ferredoxin
PGE	plastid gene expression
PHC-BR	photo-hydrocell-bioreactor
PQ	plastoquinone
PQH_2	plastoquinol
PRMTs	protein arginine methyltransferases
PsbS	LHC-related protein
PSI	photosystem I
PSII	photosystem II
qE	energy depending quenching
qI	photo inhibition
qT	state transitions
RBD	RNA-binding domain
RNA	ribonucleic acid
RNAi	RNA interference
RNP	ribonucleoprotein domain
ROS	reactive oxygen species
RRM	RNA recognition motif
rRNA	ribosomal RNA
Rubisco, Rbc	ribulose-1,5-bisphosphate carboxylase/oxygenase
SDMA	symmetric dimethylarginine
SDS-PAGE	sodium dodecyl sulfate polyacrylamide gel electrophoresis
$-SNO_2$	S-nitrothiol
-S-OH	sulfenic acid
-SOOH	sulfinic acid
ssDNA	single-stranded DNA
-S-SG	mixed disulphide with glutathione
stm3	LHC II state transition mutant 3
TLC	thin layer chromatography
TMH	transmembrane helices
tRNA	transfer RNA
U	unit
V	violaxanthin
WOC	water-oxidizing complex
WT	wild type

I. Introduction

1. Photosynthetic energy conversion

Oxygenic photosynthesis is a process of sunlight capture and conversion into chemical energy by photoautotrophs, which involves the reduction of CO_2 to carbohydrates and removal of electrons from H_2O, resulting in the release of O_2 and protons. In plants and green algae, the photosynthetic reactions occur mainly in the chloroplast and are traditionally divided into two phases - the "light reactions", which consist of electron and proton transfer reactions and the "dark reactions", comprising the biosynthesis of carbohydrates from CO_2 and utilizing reducing equivalents and ATP provided by the light reactions.

The oxygenic light reactions are mediated by four multi-subunit membrane-protein complexes (Figure 1): two photosystems PSI and PSII linked by the cytochrome b_6f complex (Cytb_6f), and the ATP synthase (ATPase). The photosynthetic complexes with exception of Cytb_6f, are not distributed evenly through the thylakoid membrane system. Whereas PSII is mostly confined to the grana regions, PSI and ATP synthase are located in the stroma lamellae (Andersson and Anderson, 1980; Dekker and Boekema, 2005; Hankamer et al., 1997; Kaftan et al., 2002). The primary electron transfer reactions take place in the reaction center of PSII, which absorbs light, oxidizes H_2O to O_2 via the water-oxidizing complex (WOC) (Renger, 2001; Renger and Kuhn, 2007) and extracts electrons from water. During linear electron transport, these electrons are used for the reduction of plastoquinone (PQ) to plastoquinol (PQH_2) and are then transferred to Cytb_6f, which mediates the electron transport to PSI via plastocyanin (PC). PSI transfers electrons across the membrane and reduces $NADP^+$ to form NADPH that is then used as reducing power for biosynthetic reactions. Throughout the whole process, proton translocation across the thylakoid membrane generates a concentration gradient enabling ATP formation via the ATP synthase (Figure 1).

Additionally, for the generation of ATP, photosynthesis provides an alternative route, the cyclic electron transport around PSI (Arnon et al., 1954), through which light energy can be used to generate a proton gradient. Cyclic electron transport leads to a return of the resulting electrons from the acceptor side of PSI to the Cytb_6f complex, thus enabling the maintenance of photophosphorylation without a synthesis of NADPH and without inclusion of PSII (Allen, 2003; Alric, 2010; Joliot et al., 2006). Two major functions for cyclic electron flow are presumed, ATP synthesis and the control and protection of light harvesting (Joliot and Johnson, 2011). It is assumed that linear electron transport alone probably generates insufficient amounts of ATP to balance the ATP/NADPH consumption of the Calvin cycle

and the deficit is probably supplied by cyclic electron flow (Allen, 2003; Alric et al., 2010; Joliot et al., 2006; Seelert et al., 2000). In the green alga *C. reinhardtii*, the migration of the mobile antenna system of PSII (LHCII complex) to PSI (*StateI-StateII-Transition*) and the onset of cyclic electron transport were proposed to be connected (Cardol et al., 2009; Finazzi et al., 2002). In addition, cyclic electron flow is essential for photosynthesis, since its absence will lead to excessive accumulation of NADPH in the stroma and thereby, its over-reduction (Munekage et al., 2004).

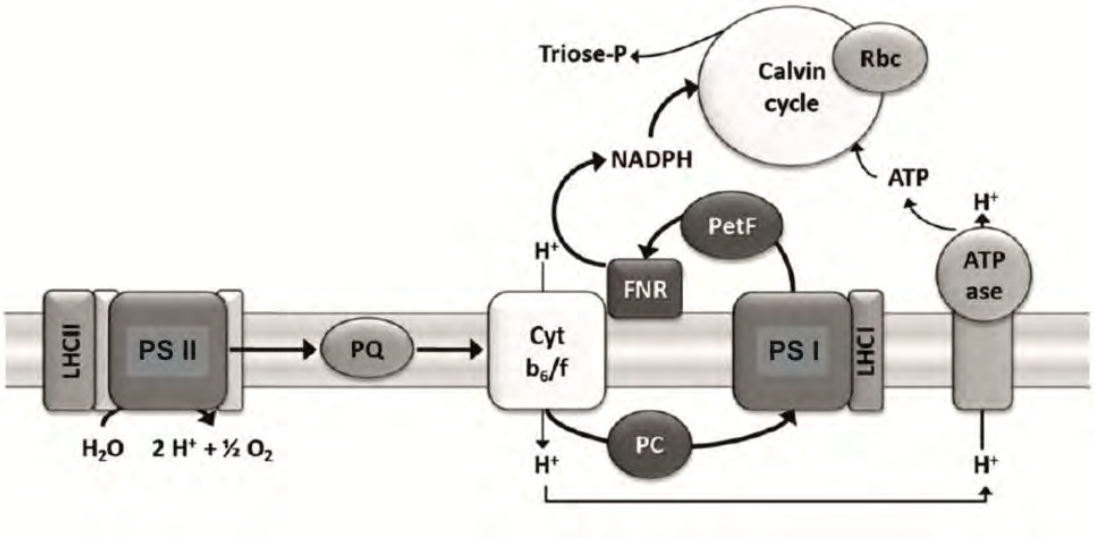

Figure 1: Scheme of linear photosynthetic electron transport pathways in *C. reinhardtii* (Figure was modified after (Hemschemeier and Happe, 2011)). Electrons extracted from water at PSII and transported to PSI via plastoquinone (PQ), Cytb_6f and plastocyanin (PC). PSI donates electrons to $NADP^+$ via ferredoxin (PetF) and ferredoxin-$NADP^+$ reductase (FNR). A proton gradient is built up during the electron transport and used by an ATP-synthase (ATPase) to generate ATP. Both NADPH and ATP are needed for CO_2-fixation and triose-phosphate (Triose-P) generation by the Calvin cycle (Rbc = Rubisco).

The Calvin cycle uses ATP and the reducing equivalent NADPH to convert carbon dioxide into an organic compound, glyceraldehyde-3-phosphate (Triose-P or G3P). The key enzyme of the Calvin cycle, Rubisco (ribulose-1,5-bisphosphate carboxylase/oxygenase) catalyzes the carboxylation of ribulose-1,5-bisphosphate. G3P can then be used for the synthesis of hexose molecules, which subsequently can be converted into starch (Buchanan et al., 2000).

1.1. The light harvesting antennae

The photosystems, where the photochemical conversion of light into chemical energy occurs, consist of two closely associated components, the reaction centers and light harvesting complexes (LHC), a superfamily of chlorophyll (chl *a* and *b*) and carotenoid-binding proteins which absorb sunlight (Minagawa, 2009). Light energy captured by the light harvesting

systems of PSII (LHCII) and PSI (LHCI) is transmitted to the respective reaction centers triggering charge separation.

The *Chlamydomonas* genome sequence predicts 9 *LHCA* and 12 *LHCB* genes encoding LHC polypeptides associated with PSI and PSII (LHCI and LHCII, respectively), and 9 *LHC*-like genes (Elrad and Grossman, 2004; Minagawa and Takahashi, 2004; Nield et al., 2004). Both LHCI and LHCII proteins share significant homology and form an extended light-harvesting complex superfamily together with other LHC-like proteins, suggesting mono-phyletic origin with a common architecture (Neilson and Durnford, 2010; Wolfe et al., 1994).

The light-harvesting antenna for PSI in *C. reinhardtii* is formed by nine polypeptides including LHCA1, LHCA3-LHCA8 in stoichiometric amounts, and LHCA2 and LHCA9 in sub-stoichiometric amounts (Bassi et al., 1992; Hippler et al., 2001; Stauber et al., 2003; Takahashi et al., 2004; Tokutsu et al., 2004). Among them, LHCA1 and LHCA3 are the most abundant LHCI proteins in *Chlamydomonas* (Hippler et al., 2001) and might function as proximal antennae for PSI and remained, in contrast to all the other LHCA proteins conserved in green algae and higher plants (Takahashi et al., 2004; Tokutsu et al., 2004). Up to 20% of the total chlorophyll in the thylakoid membrane is bound to the several LHCI proteins attached as peripheral light-harvesting antennae to the core of the PSI-LHCI supercomplex (Minagawa, 2009), which is formed by the PsaA/PsaB heterodimer and small subunits, essential for plastocyanin-ferredoxin oxidoreductase activity (Melkozernov et al., 2006).

The PSII reaction center, composed of a heterodimer of the D1 and D2 proteins and a few low molecular weight polypeptides, is surrounded by a light-harvesting complex (Figure 2a), basically constituted by a core antenna, minor antenna and the major antenna complex (LHCII) at the periphery of the PSII supercomplex (Minagawa, 2009). The core antenna (CP43 and CP47) plays an essential role in energy transfer from LHCII to the reaction center and binds 13-17 Chl *a*-molecules (Ferreira et al., 2004) and all-*trans* β-carotene, which has a function in the quenching of harmful triplet chlorophyll.

The PS II light harvesting antenna consists of six LHCII protein-types including type I (LHCBM3, LHCBM4, LHCBM6), type II (LHCBM5), type III (LHCBM2, LHCBM7), and type IV (LHCBM1), as well as the two minor LHCII proteins, LHCB5 (CP26) and LHCB4 (CP29) (Stauber et al., 2003; Takahashi et al., 2006). LHCII proteins (LHCBM = Major LHCII), whose nomenclature follows a rough order of their abundance in the *Chlamydomonas* EST database (Asamizu et al., 1999), are the most integral membrane proteins of the plastids (Liu et al., 2004) and bind in addition to Chl *a* also Chl *b*, accounting for up to 50% of the

total chlorophyll in plant and algal thylakoid membranes (Minagawa, 2009; Minagawa and Takahashi, 2004). The three-dimensional structure of the *Chlamydomonas* PS II–LHCII supercomplex revealed that the supercomplex comprises two LHCII trimers and four LHCII monomers per PS II core dimer (Nield et al., 2000) (Figure 2), and corresponds to the C_2S_2-type supercomplex observed in higher plants (Boekema et al., 1995). Each LHCII monomer (Figure 2b) has three transmembrane helices (TMH) and binds 14 chlorophylls (8 Chl *a* and 6 Chl *b*) as well as two luteins and one 9`-*cis*-neoxanthin (Kuhlbrandt et al., 1994; Liu et al., 2004; Yan et al., 2007).

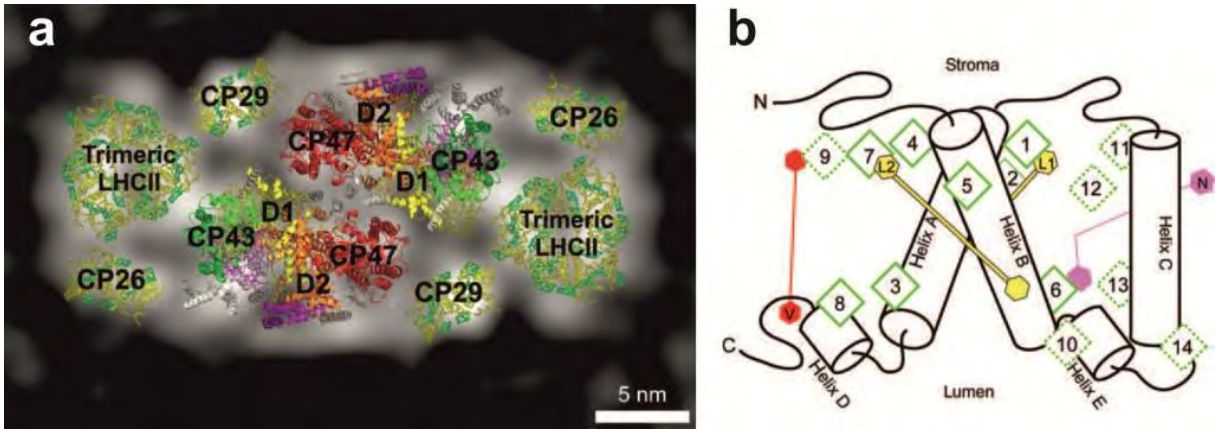

Figure 2: PS II-LHCII supercomplex and the monomeric LHCII protein-model (Harris, 2009; Nield and Barber, 2006). **a.** Top view of the PSII-LHCII supercomplex. Overlaid X-ray structures of the PS II core (Ferreira et al., 2004) without extrinsic OEC proteins, and the spinach LHCII complex (Liu et al., 2004), onto the lumenal top view of the 3D structure of the spinach PS II –LHCII supercomplex derived from cryo-EM and single particle analysis (Nield et al., 2002). The transmembrane helices of the D1 (yellow), D2 (orange), CP43 (green), CP47 (red), and α - and β -subunits of cytochrome b_{559} (purple) subunits are displayed as ribbons **b.** Schematic of the LHCII protein. Transmembrane (A–C) and amphipathic (D–E) helices are shown as cylinders (Liu et al., 2004). Chlorophyll chl *a* (Chl 1–8) and chl *b* (Chl 9–14), represented by diamonds and dotted diamonds, respectively. Two luteins (L1 and L2, yellow), one 9′-cis-neoxanthin (N, pink), and one violaxanthin (V, red) are displayed.

In contrast to the major LHCII, the minor LHCII proteins (polypeptides CP26 and CP29), existing only in monomeric form, bind less chlorophyll and show higher Chl *a*/Chl *b* ratio and a higher amount of violaxanthin (Bassi et al., 1993; Bassi and Wollman, 1991; Green and Durnford, 1996; Minagawa et al., 2001). While these two minor antenna proteins are conserved throughout the Chlorophyta, a homolog for the higher plant minor antenna protein, CP24, has not been found in algae (Minagawa and Takahashi, 2004).

Although the primary role of the LHC proteins is collecting light energy, they are also known to have another crucial role in photosynthesis, i. e. protection of the photosynthetic apparatus (Melkozernov and Blankenship, 2005; Niyogi, 1999). However, the isoform diversity of LHC complexes in higher plants is less pronounced, since they are constituted by only three isoforms Lhcb1-3 (Ganeteg et al., 2004), which can form different trimeric compositions (Jansson, 1994). Though, the amino acid sequences of the LHCBM proteins are

nearly identical and can only be distinguished from each other by the combination of 2D gel analysis and tandem mass spectrometry (Stauber et al., 2003), it is supposed that the isoforms differ in their ability to collect light and their protective properties (NPQ) (Müller et al., 2001). Elrad et al. (2002) showed that the absence of a functional *LHCBM1*-gene in *C. reinhardtii* cannot be compensated by increased expression of other isoforms and results in a reduced capacity for energy-dependent fluorescence quenching (Elrad et al., 2002). Experiments with *Lhcb1* and *Lhcb2* antisense plants showed that no compensation by other isoforms is possible. Even though a normal structure of the PSII-LHCII supercomplex in these plants was retained, their growth was, however, greatly reduced compared to wild type (Ruban et al., 2003). Additionally, there are several distant relatives of the LHC superfamily collectively called LIL (light-harvesting-like) proteins, although their primary roles may not be light-harvesting but rather dissipation of absorbed energy in the form of heat or fluorescence (Adamska et al., 2001; Jansson, 1999). The stress-related LHC proteins – LHCSR (formerly called LI818) are Chl *a*/*b*- and xanthophyll-binding proteins (Bonente et al., 2011a), which are suggested to function in a photo protective manner, for instance LHCSR3 was shown to be essential for energy depending quenching (qE) (Bonente et al., 2011a; Bonente et al., 2011b; Peers et al., 2009).

1.2. Efficient light capture and photoprotection

The photosynthetic light and dark reactions are closely interconnected, the ATP and NADPH provided by the electron-proton transfer system driving the fixation of CO_2 into carbohydrate. However, solar light availability is not constant and changes dramatically during the day, thus light harvesting of photoautotrophs needs to be adjusted to the actual light amounts (Huner et al., 1998) in order to prevent photo-inhibition and even damage in the case of highlight, or to maximize photon capture efficiency in case of low-light levels (Anderson et al., 1995; Durnford and Falkowski, 1997; Falkowski and LaRoche, 1991). Photoautotrophs possess various response mechanisms (photoacclimation) to regulate light capture and conversion, to adapt to changing light qualities and quantities by modulation of their photosynthetic apparatus (Allen, 1992; Lemeille and Rochaix, 2010; Pesaresi et al., 2009; Ruban, 2009; Wollman, 2001). This complex dynamic regulation process of photoacclimation adjusts the cellular activities on short-term (seconds to minutes) and long-term (hours) time scales, thus attempting to maintain a constant photosynthetic efficiency under a variety of light intensities by fine-tuning the capacity of the photosynthetic organism to harvest and utilize light (Chow et al., 1990).

When the absorbed light energy exceeds the requirement for photochemical activity, short-term mechanisms of non-photochemical quenching (NPQ) are triggered in order to prevent ROS production (Demmig-Adams and Adams, 1992; Eberhard et al., 2008; Szabo et al., 2005). NPQ entails three different components, namely energy dependent quenching (qE), state transitions (qT) and photoinhibition (qI). The very slow mechanism of photo-inhibitory quenching qI is more ambiguous than qE or qT. qI is commonly associated with the damage of the D1 protein that leads to photoinhibition and lower photosynthetic capacity (Aro et al., 1993). The impaired PSII reaction centers are capable of quenching fluorescence directly (Horton et al., 1996), but the responsible mechanisms are still unknown. The energy dependent quenching qE is considered to be a very fast process, possibly mediated through the pH-dependent increase in cellular de-epoxidation of violaxanthin to zeaxanthin via the xanthophyll cycle (Demmig-Adams et al., 1996; Niyogi, 1999). Conventionally, three essential factors are supposed for the activation of qE: the xanthophyll cycle, the pH gradient and proteins of LHC superfamily. The LHC-related protein *PsbS*, which is closely associated with the PSII core, was shown to be required for NPQ in *Arabidopsis* (Li et al., 2000). Although a *psbS* gene is present in *C. reinhardtii*, no evidence of the corresponding protein could be found (Bonente et al., 2008), suggesting an alternative form of induction of qE in green algae. The *C. reinhardtii* mutant *npq4*, lacking LHCSRs isoforms resembles the NPQ phenotype of the *Arabidopsis npq4* (Li et al., 2000; Peers et al., 2009). Furthermore recent studies of Bonente et al. revealed the LHCSR3 protein to function as pH sensor and a strong quencher for chlorophyll exited states in *Chlamydomonas* (Bonente et al., 2011a; Bonente et al., 2011b). The NPQ-component, qT, operates in the order of minutes and decreases the PSII absorption cross-section through state transitions (Bonaventura and Myers, 1969; Murata, 1969; Wollman, 2001). It redistributes excitation energy between the photosystems by lateral movement of parts of the light-harvesting complex of PSII (LHCII) (Allen and Forsberg, 2001; Haldrup et al., 2001; Wollman, 2001). The redox-sensitive kinases, STT7 in *C. reinhardtii* (Depege et al., 2003) and STN7 in *A. thaliana* (Bellafiore et al., 2005), that specifically phosphorylate the mobile LHCII are activated upon reduction of the plastoquinone (PQ) pool, which carries the electrons from PSII to the Cytb_6f, resulting in the dissociation of the mobile antenna from PSII and its subsequent attachment to PSI (state II) (Allen et al., 1981; Allen and Horton, 1981; Bennett, 1977; Wollman, 2001). This migration and the resulting increase of the antenna system of PSI results in the balanced excitation of both photosystems and the deactivation of the kinase activity by re-oxidation of the PQ pool and constutively expressed phosphatases (Shapiguzov et al., 2010). The LHCII is or becomes

dephosphorylated, dissociates from PSI and relocates to PSII (stateII-to-stateI) (Allen and Forsberg, 2001). Both the major and minor LHCII polypeptides were proposed to be subjects phosphorylation and dephosphorylation in *C. reinhardtii* (Kargul and Barber, 2008; Lemeille et al., 2010; Takahashi et al., 2006; Turkina et al., 2006; Wollman and Delepelaire, 1984). The dephosphorylation of LHCII was shown to be accomplished by PPH1 phosphatase in *A. thaliana* (Shapiguzov et al., 2010).

Long-term photoacclimation occurs when the short-term changes are insufficient for coping with the changes in light intensity, thus resorting to extensive changes in enzyme activity and gene expression that lead to structural changes in the thylakoid membrane and alterations in the photosynthetic apparatus (Anderson et al., 1995; Falkowski and LaRoche, 1991). The adjustment of the antenna size to changing light and other environmental stress conditions involves the regulation of gene expression of individual components of the PSII associated light-harvesting complex LHCII, which is influenced by many factors (Elrad and Grossman, 2004). At the level of transcriptional regulation it has been shown that circadian rhythm (Jacobshagen et al., 1996; Kindle, 1987), chlorophyll synthesis (Johanningmeier and Howell, 1984) and intensity of incident light (Durnford et al., 2003; Elrad et al., 2002; McKim and Durnford, 2006; Teramoto et al., 2002) influence the LHCII expression in *C. reinhardtii*. However, an exclusive role of transcription regulation for LHC antenna control in plants, as earlier suggested, was ruled out after transformation experiments with antisense tobacco constructs (Flachmann and Kuhlbrandt, 1995) and after studies of light responsiveness on *Lhcb* translation in tobacco seedlings (Petracek et al., 1997). Additionally, further examples demonstrated that the synthesis and the expression of the photosynthetic proteins (*LHCB* genes) underlies the regulation on the translational level, thus illustrating the importance of posttranscriptional regulation processes in *C. reinhardtii* (Durnford et al., 2003; McKim and Durnford, 2006; Mussgnug et al., 2005).

1.3. Plastid-to-nucleus retrograde signalling

Photoacclimation causes a series of reactions that require signal transduction pathways and feedback mechanisms to link the gene expression to the apparent light intensity (Durnford and Falkowski, 1997). To be able to assemble the complete photosynthetic complex during photoacclimation, the expression of the nuclear-encoded *LHC* genes must be coordinated with the expression of chloroplast-encoded components. This suggests a mechanism for the communication between the chloroplast and the nucleus to coordinate photoacclimation. Signals from the nucleus to the organelles are called anterograde, whereas the transmission of

informations from chloroplast or mitochondria (excluded here) to the nucleus is referred to as retrograde signaling (Pfannschmidt, 2010; Woodson and Chory, 2008). The existence of a 'plastid factor' to coordinate gene expression in the two compartments has long been proposed (Oelmuller et al., 1986; Surpin and Chory, 1997), but so far no true signalling molecule leaving the plastid has been identified, nor is the retrograde signalling mechanism fully understood (Pfannschmidt, 2010). However, a variety of potential plastid sensors (Figure 3), including chlorophyll precursors, reactive oxygen species (ROS), and the plastoquinone pool have been suggested to induce or mediate signals from the chloroplast to the nucleus (Dietz, 2003; Gray, 2003; Nott et al., 2006; Pfannschmidt et al., 2003; Rodermel, 2001; Woodson and Chory, 2008).

Among the retrograde signals under debate (Figure 3), is the chlorophyll precursor magnesium protoporphyrin IX (Mg Proto), which was described to accumulate under reduced photosynthesis and represses *LHCB* gene expression (Johanningmeier and Howell, 1984), and represents the best characterized signalling molecule candidate. Though, recent publications have clearly demonstrated that the accumulation of Mg Proto does not correlate with changes in *LHCB* expression and, instead metabolite fluxes were proposed to be responsible for the signal generation (Mochizuki et al., 2008; Moulin et al., 2008).

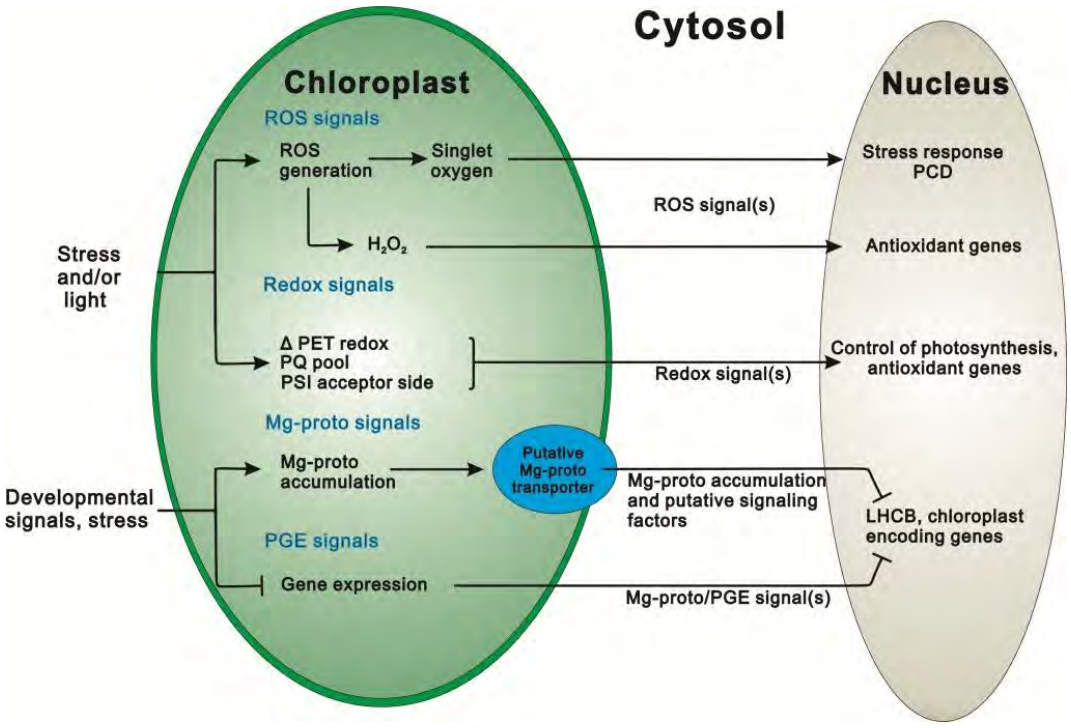

Figure 3: Retrograde signalling pathways from the chloroplast to the nucleus. (Figure modified after (Woodson and Chory, 2008); PGE, plastid gene expression, PET photosynthetic electron transport chain, PQ plastoquinone, PSI photosystem I.

Plastid gene expression (PGE) signals, which were proposed to provide a ‘‘plastid factor’’ that leaves the plastid and represses nuclear photosynthesis genes (Oelmuller et al., 1986), seem to play a role only during the early developmental stages. Though the signal itself is not known yet and no experimental evidence for protein or RNA export from the plastid has been obtained (Nott et al., 2006), an integration of Mg Proto and PGE signals is presumed. Another group of signals, triggered by changes in the chloroplastic redox milieu could be represented by the increased amounts of ROS, because they not only constitute a threat to cellular components, they can also act as retrograde signals (Suzuki et al., 2012). Thereby, the signalling pathways might be induced by ROS independently or together due to diverse reactivities of the species (Møller et al., 2007). For instance, the gene expression of cytosolic ascorbate peroxidase (cAPX) was reported to be initiated by a changed redox state of the PQ pool, and to maintained high due to the following increase in the H_2O_2 concentration (Yabuta et al., 2004). However, most ROS are very short-lived and rather unspecific signalling molecules; therefore they could initiate signalling cascades within the organelle, which then pass the envelope by unknown means (Apel and Hirt, 2004). Additionally, other photosynthetic redox signals, derived most obviously from the redox state of the PQ pool (Pfannschmidt et al., 1999), seem to be converted into a phosphorylation cascade (Pesaresi et al., 2009; Steiner et al., 2009). For instance, it could also be shown that in green algae, the nucleus encoded *LHCB* genes are transcriptionally regulated by the PQ pool redox state (Escoubas et al., 1995; Teramoto et al., 2002). However, the studies of Humby et al., using an LHC:Arylsulphatase (ARS) reporter system in *Chlamydomonas reinhardtii*, revealed that neither the PQ pool nor Q_A redox state were directly involved in short-term retrograde signalling (Humby et al., 2009). Additionally, there were also a few studies, which suggested that the PQ pool in plants is not the primary sensor for LHC regulation (Piippo et al., 2006; Pursiheimo et al., 2001). On the other hand, a detailed analysis of the repression of the *lhcb1* transcript of *Dunaliella tertiolecta* showed that the trans-thylakoid membrane potential appears to be a critical determinant of gene expression on short time scales (0.5-4 h), whereas on time scales of 8 h or longer, the redox state of the plastoquinone pool becomes increasingly more important (Chen et al., 2004). Furthermore, studies on a PSI-deficient barley mutant with a constitutively reduced PQ pool and normal mRNA-amounts for photosynthetic genes, suggested that the PQ pool redox state plays a role in long-term regulation of the expression of antenna proteins mainly at the post-transcriptional level (Frigerio et al., 2007).

Additionally, metabolic pool changes could also represent a part of the retrograde signalling, since many metabolites have been suggested to pass the plastidial envelope via several transporters and to be essential and intrinsic part in the metabolism of the cell (Weber and Fischer, 2007). However, further research focusing specifically on links between the chloroplast, mitochondria and cytosol such as the production, export and metabolism of triose phosphates and NADPH via the malate-oxaloacetate shuttle, could help to elucidate the key components in photosynthetic stress mediated gene signalling.

1.4. *C. reinhardtii* as a model organism for the acclimation response studies

Organisms can resist even dramatic changes in their environmental surrounding due to the development of complex sensing and signaling acclimation mechanisms, which involve different cellular compartments, several types of biomolecules and various signaling mechanisms. The green unicellular eukaryotic alga *Chlamydomonas reinhardtii*, also referred to as *´green yeast´* (Rochaix, 1995), emerged in the past years as a model organism for studying of adaptive responses in photosynthetic organisms. Furthermore the research on this organism provides a lot of insights into the molecular biology from the basics of photosynthesis (Eberhard et al., 2008), carbon fixation (Spalding, 2008) and circadian clock (Matsuo and Ishiura, 2011) to the understanding the molecular basis of human diseases (Christensen et al., 2007; Duquesnoy et al., 2009; Redding and Cole, 2008; White and Quarmby, 2008) and production of therapeutic proteins (Rasala et al., 2010), which renders this alga an important model system of both photosynthetic and non-photosynthetic eukaryotes.

The ellipsoid-shaped cell of *Chlamydomonas reinhardtii* has a size of about 10 μm and includes a large cup-shaped chloroplast, which has a single large pyrenoid surrounded by starch bodies (Figure 4) (Harris, 2001). The centrally located nucleus with a prominent nucleolus is partially enclosed by the chloroplast and the nuclear membrane is continuous with the endoplasmic reticulum, and Golgi bodies are situated nearby. Multiple mitochondria with 0.2-0.3 μm in cross-section are dispersed throughout the cytosol. Two anterior flagella (10 to 12 μm in length) arise from a pair of basal bodies located just beneath the apical end of the cell and are used for motility. The red pigment “eyespot” senses light direction and intensity, and triggers phototactic responses (Harris, 2001). The algal cell is enclosed within a wall consisting primarily of hydroxyproline-rich glycoproteins with arabinose, mannose, galactose, and glucose being the predominant sugars (Harris, 2009; Miller et al., 1972).

The ability of *C. reinhardtii* to grow in various environments like freshwater habitats, but also soils (Proschold et al., 2005) and bogs (Kroes, 1972) was so far explained by the capability of this species to grow not only photoautotrophically but also mixotrophically by photosynthetic carbon dioxide assimilation and simultaneously uptake of an external organic carbon source (Heifetz et al., 2000). Additionally, *C. reinhardtii* grows in the dark (heterotrophically) with acetate as the sole carbon source while maintaining a functional photosynthetic apparatus (Dent et al., 2005).

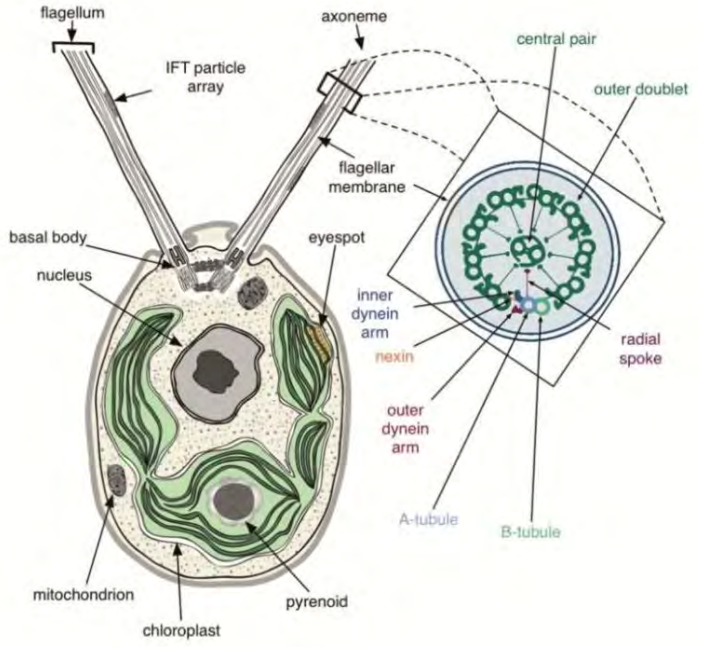

Figure 4: Schematic of the cellular structure of the unicellular green microalga *C. reinhardtii* (Merchant et al., 2007). This photoheterotroph possesses one large chloroplast with a pyrenoid, mitochondria, nucleus and two flagellae for motility as well as an eyespot for light sensing.

The *Chlamydomonas* genome project reported the complete sequences of the chloroplast genome (GenBank accession number BK000554) (Grossman et al., 2003; Maul et al., 2002) and mitochondrial genome (GenBank accession U03843) (Michaelis et al., 1990) as well as the whole nuclear genome (Grossman, 2005; Merchant et al., 2007; Shrager et al., 2003) accomplished by Joint Genome Institute of the US Department of Energy, http://www.jgi.doe.gov/chlamy). The comparative phylogenomic analyses of the nuclear ~120-megabase genome allows the identification of genes encoding uncharacterized proteins (Merchant et al., 2007). Expressed sequence tags (ESTs; (Shrager et al., 2003)), microarray information and other genomic resources can be found at http://www.chlamy.org and http://genome.jgi-psf.org/. Additionally, numerous methods for genetic manipulation of *C. reinhardtii* have been established, including nuclear transformation via the glass beads method (Kindle, 1990; Mayfield and Kindle, 1990), transformation methods for the chloroplast (Bateman and Purton, 2000; Ramesh et al., 2004) and mitochondria (Remacle et al., 2006). Several RNAi approaches have recently been established for the possibility of down-regulation of specific nuclear genes (Cerutti and Casas-Mollano, 2006; Molnar et al.,

2007; Schroda, 2006). So, *Chlamydomonas reinhardtii* is potentially useful for studies of adaptive responses at cellular, physiological, biochemical and molecular levels, since various molecular and genetic tools are available for this organism and several mutant strains can be relatively easily created or are already available.

2. Acclimation to natural varying conditions in light and carbon

The complex process of photosynthesis is affected by a variety of environmental stresses such as temperature, nutrient limiting conditions of carbon, nitrogen, sulfate and phosphate (Grossman et al., 2007) and light stress. The ability to acclimate to fluctuations in light quality and quantity as well as CO_2 availability is crucial for photosynthetic organisms in order to maintain a balance between light-harvesting capacity and the utilization of captured energy, since the light-harvesting by the photosystems occurs on a much faster time scale than the intersystem electron transport or carbon fixation (Huner et al., 1998). At low light intensities, an increase in the photon flux density correlates with increased photosynthetic carbon fixation (Elrad et al., 2002). However, with elevating light intensity, photosynthesis eventually reaches a light-saturated maximum rate that is limited by the rate of carbon fixation in the Calvin cycle (Sukenik et al., 1987). Situations of excessive excitation of the reaction centers of the photosystems can lead to the formation of the reactive oxygen species (ROS) like highly reactive, cytotoxic singlet oxygen ($^1O2$), superoxide (O_2^-), hydroxyl radical ($^{\bullet}OH$) and hydrogen peroxide (H_2O_2) (Asada, 2006; Krieger-Liszkay, 2005; Niyogi, 1999), which spontaneously react with all biomolecules in their surroundings and can cause photo-oxidative damage. For instance, ROS formation is the main cause of damage to the D1 protein under high light conditions (Aro et al., 1993; Keren et al., 1997; Ohnishi et al., 2005; Vass et al., 1992).

2.1. The factor light – translational regulation of *LHC* gene expression via NAB1

The expression regulation of the nuclear-encoded light-harvesting chlorophyll-binding proteins of PSII (LHCII) is an essential long-term acclimation strategy (Walters, 2005), which is known to be controlled at many levels from transcription initiation (Durnford and Falkowski, 1997; Escoubas et al., 1995; Maxwell et al., 1995) to post-transcriptional mechanisms (Durnford et al., 2003; Flachmann and Kuhlbrandt, 1995; McKim and Durnford, 2006). Besides their role in light energy capturing, LHCII proteins facilitate the dissipation of light energy as heat or fluorescence via ‘energy-dependent non-photochemical quenching’ (NPQ) of chlorophyll fluorescence, when solar irradiation exceeds photosynthetic capacity.

This strategy is reported to protect the cell from oxidative damage (Müller et al., 2001), but with a drawback that a large proportion of the absorbed photons (~80–95%) are wasted as fluorescence and heat under high light levels (Polle et al., 2002; Polle et al., 2003), limiting the overall photosynthetic conversion efficiency (Kruse et al., 2005). With the identification of *C. reinhardtii* nuclear light acclimation mutant *stm3* (Kruse et al., 1999), which does not express the RNA-binding protein NAB1 and shows a phenotype that is characterized by an increased light-harvesting PSII antenna size, an important control mechanism of the light harvesting complex (*Lhcb*) genes at the translational level in the cytosol (Mussgnug et al., 2005) was described.

The cytosolic translation repressor protein NAB1was shown to recognize mRNAs of the light-harvesting proteins (LHCII) *in vitro* and *in vivo* (Mussgnug et al., 2005) and binds in particular *LHCBM6* transcripts with a high affinity at a mRNA sequence (CSDCS) that was discovered on mRNA targets of the NAB1 homologous translation repressor FRGY2 from *Xenopus laevis* (Manival et al., 2001). RNA-binding by NAB1 prevents LHCBM translation via sequestration of the message in translationally silent messenger ribonucleoprotein complexes (mRNPs). The *cis*-acting element within the LHCBM mRNA, which is recognized by NAB1 shows different degrees of identity to the CSDCS (cold shock domain consensus sequence) region contained in FRGY2 target mRNAs, if compared between the 9 distinct isoforms encoded in the *C. reinhardtii* genome. Inspection of the *LHCBM6*-CSDCS and comparison to other isoform CSDCS sequences implies that the tetranucleotide GCCA, present in the FRGY2 target sequence as well as *LHCBM6*, represents an important determinant of binding affinity. For the two isoforms *lhcbm6* and *lhcbm4, in vitro* RNA binding studies and *in vivo* RNA co-immunoprecipitation experiments revealed that NAB1 binds the isoform 6 with higher affinity (Mussgnug et al., 2005). Moreover, only the CSDCS motif of *LHCBM6* matches exactly to the FRGY2 target sequence within this 4 base pair stretch. This selective recognition of distinct isoform messages supports the view that the existence of several nearly identical LHCBM isoforms in plants and algae does not represent functional redundancy (Elrad et al., 2002; Ganeteg et al., 2004), but rather points towards isoform specific functions in response to a highly variable natural environment.

This cytosolic factor is composed of two RNA-binding motifs, the N-terminal CSD (cold shock domain) and the C-terminal RRM (RNA recognition motif) domain (Mussgnug et al., 2005). These two domains are connected with each other by a GAR (glycine-arginine rich) motif which contains several RGG boxes known to be also involved in RNA binding (Burd and Dreyfuss, 1994; Kiledjian and Dreyfuss, 1992). The CSD domain is highly

homologous to the CSD region of FRGY2 a well characterized translation repressor found in *Xenopus laevis* (Matsumoto et al., 1996; Tafuri and Wolffe, 1990). Proteins containing CSD domains are commonly referred to as "Y-box" proteins and represent one of the most ancient and well conserved nucleic acid binding domains from bacteria to higher animals and plants, which facilitates binding to RNA, ssDNA and dsDNA and plays an important role in development and stress adaptation mechanisms (Chaikam and Karlson, 2010; Sommerville, 1999; Wolffe, 1994; Wolffe et al., 1992). The CSD motif of eukaryotic Y-box proteins is combined with a second RNA-binding domain (auxiliary domain) which modulates the RNA affinity of the protein and is in case of the Y-box proteins FRGY2 and YB-1 dispensable for selective RNA recognition (Bouvet and Wolffe, 1994). In case of NAB1 the CSD motif is combined with a C-terminal RRM domain that was shown to be not essential for selective RNA recognition, since it was demonstrated by means of *in vitro* studies that the isolated CSD of NAB1 is sufficient for selective and specific mediation of RNA recognition (Mussgnug et al., 2005). However, the RRM may also play an important role in RNA binding. The RNA recognition motif (RRM), also known as RNA-binding domain (RBD) or ribo-nucleoprotein domain (RNP) is the most abundant RNA-binding domain in higher vertebrates (present in about 0.5–1% of human genes) (Venter et al., 2001). RRM-containing proteins are involved in most post-transcriptional gene expression processes like mRNA and rRNA processing, RNA export and stability (Dreyfuss et al., 2002). Extensive biochemical and structural studies of this RNA-binding domain (Maris et al., 2005) have shown that this domain is not only involved in RNA/DNA recognition but also in protein–protein interaction (Cléry et al., 2008). Though the common feature of the RRM and CSD-type domains is a frequent coupling to auxiliary RNA-binding domains and thus enhance their functional activity (Manival et al., 2001). For instance, a Y-box protein from *Xenopus laevis* FRGY2 comprises one CSD and one 'basic/aromatic (B/A)-island' containing C-terminal domain (Philippe et al., 1995; Wolffe, 1994) and an ubiquitous abundant nucleolar protein nucleolin comprises four RRM- and GAR-domain (Ginisty et al., 1999). Remarkably, the combination of RRM-, GAR- and CSD-binding domains seems not to be ubiquitous, because so far only the genomes of *C. reinhardtii*, *C. incerta* and *V. carteri* were shown to contain NAB1 type proteins (Nematollahi et al., 2006; Popescu et al., 2006).

So far, apart from NAB1, only one additional cytosolic factor CHLAMY1 has been identified in *C. reinhardtii*. CHLAMY1 forms a hetero-trimeric complex consisting of the subunits C1 and C3 and controls in a circadian manner the cytosolic translation of the $UG_{(\geq 7)}$ repeat sequence containing mRNAs (Mittag, 1996; Zhao et al., 2004). Some of these mRNAs

encode for enzymes from the carbon and nitrogen assimilation pathways of the cell (Waltenberger et al., 2001). Therefore the regulation via NAB1 is the first case of cytosolic control of photosynthetic gene translation with a specific factor (Mussgnug et al., 2005; Wobbe et al., 2008). Whereas translation control in the cytosol is well characterized in the animal kingdom (Moore, 2005), the knowledge about translation regulation in the cytosol of photosynthetic organisms via specific factors is limited.

2.1.1. Translation control mediated by RNA-binding proteins

The expression of biologically active proteins in eukaryotic cells is controlled at multiple points during the process of gene expression (Moore, 2005). Translation, occurring in the cytosol of eukaryotes, is the mechanism by which ribosomes read the genetic code in mRNA and synthesize a protein product based on a specific genetic code (Day and Tuite, 1998). Translational processes may be influenced by global controls, where some mRNAs may be sequestered in the nucleus or cytoplasm, and translation is delayed until a signal is received to turn on translation (Gebauer and Hentze, 2004). Small non-coding RNAs (miRNAs) and mRNAs (mRNAs) in dynamic association with proteins are referred as to messenger ribonucleoprotein complexes (mRNPs) (Hieronymus and Silver, 2004). In yeast, it is estimated that circa 570 different proteins are able to bind RNA (Costanzo et al., 2001). In addition to the localization of mRNAs in specific subcellular regions for the purpose of translation, the degradation of mRNAs in yeast and mammals appears to take place in specific cytosolic areas, the so-called “p-bodies” (Buchan and Parker, 2009; Teixeira et al., 2005). While p-bodies usually mean the end of an mRNA, sequestration into eukaryotic p-body-like structures, called “stress granules” leads to a preservation or protection of a transcript under stress conditions (Gilks et al., 2004) such as cellular insults, heat shock, energy deprivation, oxidative stress, and glucose starvation (DeLeeuw et al., 2007; Fujimura et al., 2009; Groušl et al., 2009).

Little is known about the mechanisms of translational regulation such as silencing and storage of mRNA, translation initiation and mRNA degradation in algae or plants. In higher eukaryotes, a large proportion of mRNA is localized to ribonucleoprotein (RNP) complexes, where it is translationally inactive (Evdokimova et al., 2001; Sommerville, 1999). These highly dynamic complexes contain RNA-binding proteins, which influence the structure and interactions of the RNA as well as their biogenesis, stability, function, transport and cellular localization, as well as degradation (Figure 5), among them a unique RNP stands for each RNA (Glisovic et al., 2008).

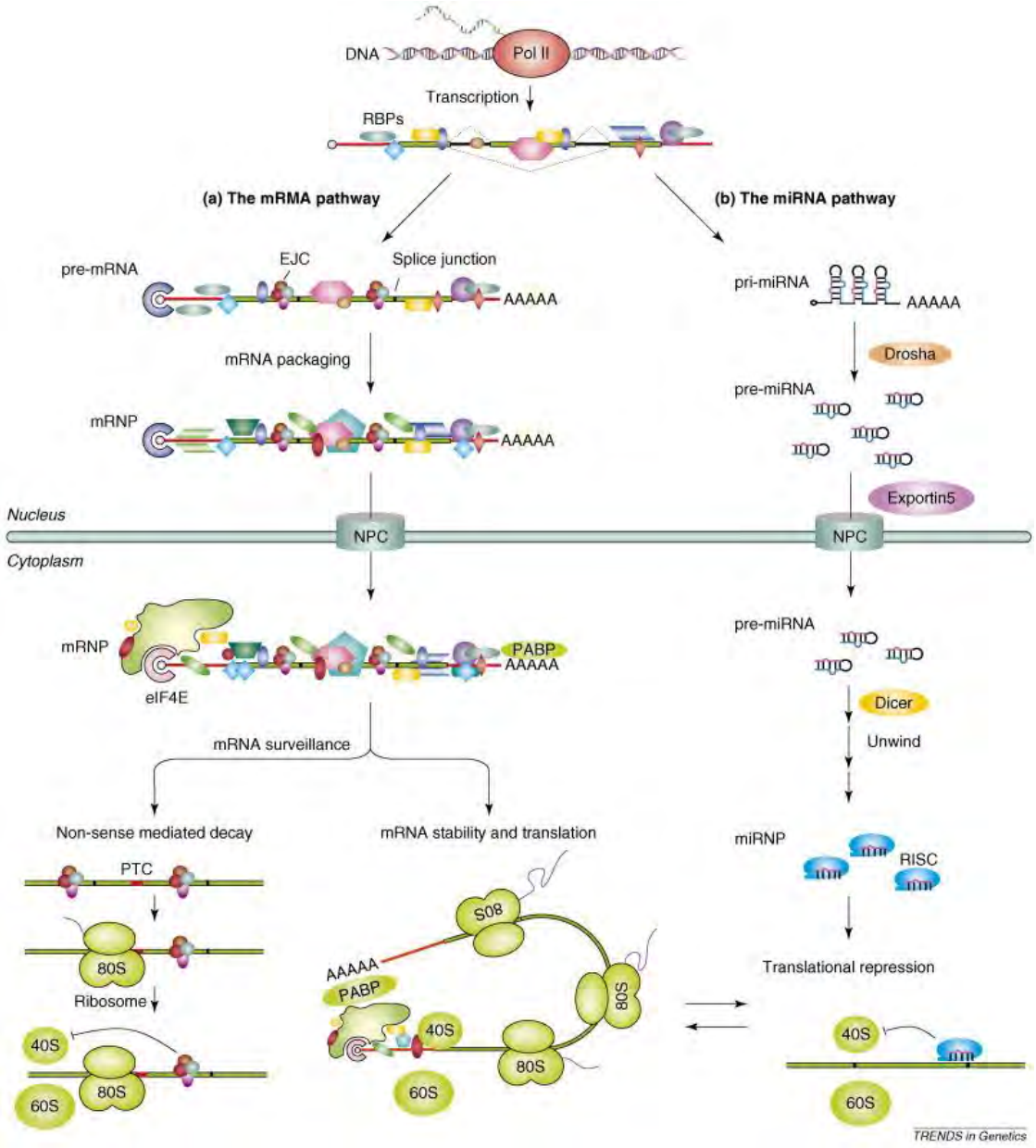

Figure 5: The functions of RNA binding proteins in eukaryotes (Lukong et al., 2008). (**a**) The mRNA pathway. pre-mRNAs transcripts, generated transcriptionally by RNA polymerase II (Pol II), are processed into mature mRNAs by a series of steps including the removal of introns during pre-mRNA splicing and the addition of a 3′ polyadenylation tail. Thereby, RBPs bind in a dynamic manner to pre-mRNAs and mRNAs. The splice junctions are bound by the exon junction complex (EJC), and thus regulating the cytoplasmic fate of the mRNAs (Le Hir and Séraphin, 2008). The mRNAs bound with RBPs and other factors are assembled into mature mRNPs (Glisovic et al., 2008) and exit the nucleus via the nuclear pore complex (NPC). In the cytoplasm, new cytoplasmic components are added to the mRNPs. In nonsense-mediated decay, the first translational round ends with the ribosomes terminating at the premature termination codon (PTC) (Chang et al., 2007), whereby EJC is involved in preventing the protein translation and the generation of deleterious proteins (Le Hir and Séraphin, 2008). A functional mRNA has its 7-methylguanosine cap structure bound by eIF4E and its polyadenylation tail bound by the poly(A) binding protein (PABP), followed by mRNA circularization and protein translation (Wendel et al., 2007). (**b**) The miRNA pathway. The biogenesis of miRNAs starts with nascent primary microRNAs (pri-miRNA) transcripts produced by RNA Pol II and processed by the RNase III enzyme Drosha complex into precursor miRNAs (pre-miRNAs) (Filipowicz et al., 2008). The pre-miRNA is exported by Exportin 5 and in the cytoplasm, pre-miRNAs are further processed by RNase III enzyme/dsRNA binding protein Dicer into mature miRNAs. One strand of the miRNAs is incorporated into the effector complex RNA-induced silencing complex (RISC) forming a miRNP that recognizes specific targets through imperfect base-pairing and induces posttranscriptional gene silencing.

RNA-binding proteins (RBPs) play key roles in post-transcriptional control of RNAs, which can occur at many different steps in RNA metabolism, including splicing, polyadenylation, mRNA stability and localization as well as translation (Curtis et al., 1995; Glisovic et al., 2008; Johnstone and Lasko, 2001).

RBP are capable of regulating translation initiation and translational repression by a variety of mechanisms, as they have one or more known RNA binding domains such as the RNA Recognition Motif (RRM, also known as RBD or RNP domain), K Homology (KH) domain, Zinc finger (mainly C-x8-C-x5-C-x3-H type), RGG box, DEAD/DEAH box, Pumilio/FBF (PUF) domain, double-stranded RNA binding domain (DS-RBD), Piwi/ Argonaute/Zwille (PAZ) domain, Sm domain, etc. (Burd and Dreyfuss, 1994; Lunde et al., 2007; Maris et al., 2005; Moore, 2005). Many RBPs have one or more copies of the same RNA binding domain while others have two or more distinct domains (Burd and Dreyfuss, 1994). Several RNA binding domains are suggestive for the molecular function of the RBP, for instance, PAZ domain for short single-stranded RNA binding in RNAi or microRNAs (miRNA) processes, and Sm domain for snRNA binding in splicing and possibly in tRNA processing (Lunde et al., 2007). However, other domains only predict RNA binding and do not specifically indicate in which aspect of RNA metabolism they may participate.

So far, translational control has been reported in regulating the expression of photosynthetic genes for light regulated responses in plants and green algae (Dickey et al., 1998; Helliwell et al., 1997; Kim et al., 2003; McKim and Durnford, 2006; Mussgnug et al., 2005; Petracek et al., 1997; Piques et al., 2009; Sherameti et al., 2002). Additionally, Liu et al. reported very recently that the photomorphogenesis in *Arabidopsis* was clearly impacted by the translational control of thousands of genes, in addition to transcriptomic changes (Liu et al., 2012). Furthermore, considerable efforts have been also invested in studies of genome-wide translational control in plants, with special focus on plants responding to abiotic stresses, including dehydration, elevated temperature, high salinity, oxygen deprivation, sucrose starvation and heavy metal (Branco-Price et al., 2008; Branco-Price et al., 2005; Kawaguchi et al., 2004; Matsuura et al., 2010; Nicolai et al., 2006; Sormani et al., 2011), revealing that plants show a global repression of translation under abiotic stresses.

2.1.2. Posttranslational modifications of RNA- binding proteins

Generally, RNA-binding proteins are known to play key roles in posttranslational regulation of gene expression and contain several RNA-binding motifs, whose conserved structures and diversity affect their function (Burd and Dreyfuss, 1994). The posttranslational

modifications allow the regulation of the biological activity of a protein and are thus beside the expression regulation of a protein, an additional mechanism for fine regulation of biological processes. They were previously reported to affect protein-protein interactions between mRNPs (Barlat et al., 1997; Ceman et al., 2003), and to play important roles in the assembly and remodeling of stress granules (Anderson and Kedersha, 2002; Guil et al., 2006; Kedersha et al., 2002; Mokas et al., 2009; Tsai et al., 2008; Tsai and Wei, 2010). Posttranslational modifications such as phosphorylation (Mazroui et al., 2007), acetylation (Kwon et al., 2007), arginylation (Carpio et al., 2010) and methylation (Dolzhanskaya et al., 2006a) were shown to be crucial for the function of the involved proteins.

RNA-binding proteins that harbor RGG boxes, or more precisely, RG-rich regions, also referred to as GAR motifs, were shown to play important roles in many aspects of RNA processing, in two interrelated ways: i) glycine-flanked arginine residues within RGG repeats serve as target sites for Type I protein arginine methyltransferases (PRMTs) (Figure 6), and methylation of specific arginine residues can have varying effects on a protein's RNA-binding activity, its ability to interact with other proteins and its intracellular localization (Aletta and Hu, 2008); ii) alternative splicing, in and around RG-rich domains has been shown to modulate both nucleic acid binding (Dempsey et al., 1998) and protein methylation (Dolzhanskaya et al., 2006b).

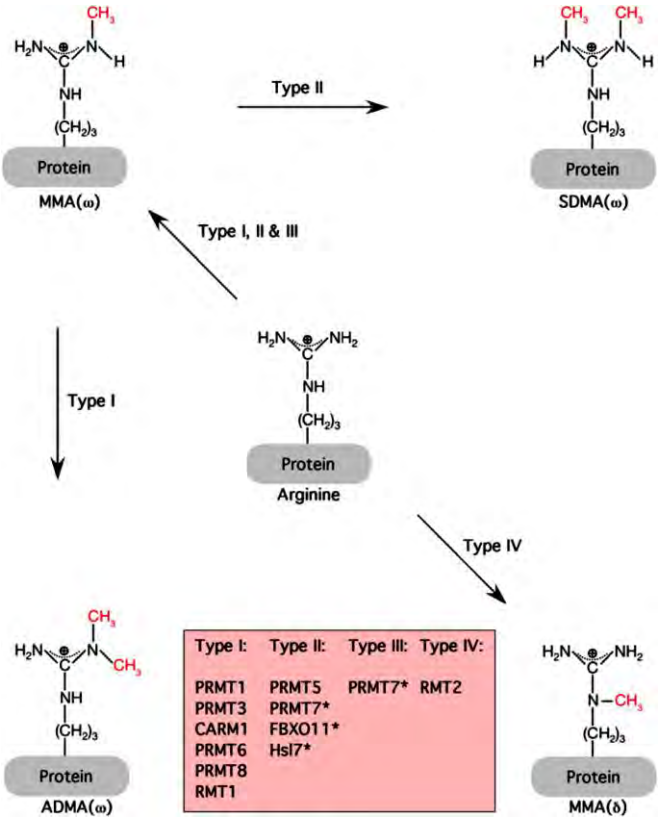

Figure 6: Types of methylation on arginine residues. Figure from (Bedford and Clarke, 2009). Types I, II, and III PRMTs generate monomethylarginine (MMAω) on one of the terminal (ω) guanidino nitrogen atoms. These two nitrogen atoms are equivalent. The generation of asymmetric dimethylarginine (ADMA) is catalyzed by type I enzymes, and the creation of symmetric dimethylarginine (SDMA) is catalyzed by type II enzymes. Type III PRMTs only monomethylate. Type IV enzyme activity that catalyzes the monomethylation of the internal guanidino nitrogen atom has only been described in yeast. The type of methylation reactions catalyzed by PRMT7, FBXO11, and Hsl7 are still being established and are, thus, marked with an asterisk (Bedford, 2007; Cook et al., 2006; Krause et al., 2007).

Protein arginine methylation, one of the most abundant and important post-translational modifications, is involved in a multitude of biological processes in eukaryotes, such as mRNA splicing, transcriptional control, signal transduction, DNA repair, RNA processing and protein translocation (for review see (Bachand, 2007; Bedford and Clarke, 2009; Bedford and Richard, 2005; Nicholson et al., 2009). In mammalian cells, the modification of arginine side chain guanidino groups is quantitatively one of the most extensive protein methylation reactions with a large number of modified proteins (Pahlich et al., 2006; Paik and Kim, 1980), thereby is omega-N^G,N^G-dimethylarginine the most prevalent one, where two methyl groups are placed on one of the terminal nitrogen atoms of the guanidine group; this derivative is commonly referred to as asymmetric dimethylarginine (ADMA) (Figure 6). The other derivatives, the symmetric dimethylated derivative, where one methyl group is placed on each of the terminal guanidino nitrogens (omega-N^G,N^G-dimethylarginine; SDMA) and the monomethylated derivative with a single methyl group on the terminal nitrogen atom (omega-N^G-monomethylarginine; MMA), occur at levels of about 20% to 50% that of ADMA (Paik and Kim, 1980). These derivatives are known to be present on a multitude of distinct protein species in the cytoplasm, nucleus, and organelles of mammalian cells (Bedford and Richard, 2005). Methylated arginine residues in proteins are often flanked by one or more glycine residues (Gary and Clarke, 1998), but there are many exceptions to this general rule.

Another type of posttranslational modification on regulatory proteins represents the chemical modifications of the cysteine thiol group, which was long considered to be only important for protein stabilization, in particular in the extracellular space (Hogg, 2003). The formation of the disulphide bond is a reversible and highly regulated process, where the thiol group undergoes not only the oxidative modification, but also S-glutathionlyation, S-thiolation, and S-nitrosylation under certain conditions (Figure 7), thereby activating or inactivating the protein (Paget and Buttner, 2003). Sulphur- and selenium containing amino acids are cysteine, methionine, selenocysteine and selenomethionine, however cysteines have a crucial role in stability and biochemical function, since they are the most conserved and a highly reactive amino acid in their thiol form even under physiological conditions.

The cysteine residues of cytosolic proteins are maintained in the reduced thiol state by action of thiol-based redox buffer systems (glutathione/glutaredoxins; thioredoxins/ thioredoxin reductase) which create a reducing environment under stress-free conditions (Michelet et al., 2006; Schafer and Buettner, 2001). Under certain conditions, where the photosynthetic machinery is not optimally adjusted to the environment, chloroplasts produce ROS, which oxidize protein thiols and affect protein function (Foyer and Noctor, 2005;

Noctor et al., 1997). Reversible oxidation of protein dithiols to disulfides (intramolecular, intermolecular or mixed with glutathione) can result in structural changes which in turn can modulate the biological activity of certain proteins (Fedoroff, 2006).

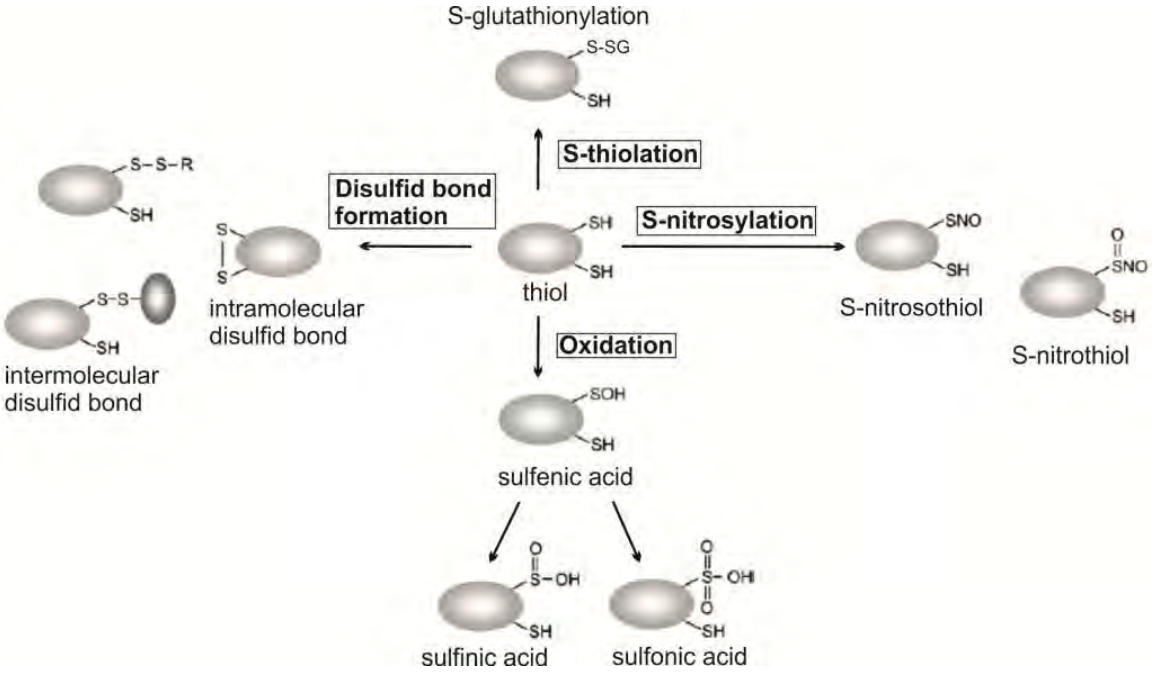

Figure 7: Oxidative modifications of the thiol group (Figure created on the basis of (Paget and Buttner, 2003)). Main mechanism are the primary oxidation to sulfenic acid (-SOH) and the subsequent processes like S-thiolation, S- nitrosylation, further oxidation to sulfinic and sulfonic acid or the formation of intra- or intermolecular disulphide bonds. Abbreviations: -S-OH: sulfenic acid; -SOOH: sulfinic acid; $-SO_3H$: sulfonic acid-S-SG: mixed disulphide with glutathione; -SNO: S-nitrosothiol; $-SNO_2$: S-nitrothiol).

Hyperoxidation of the sulfenic acid to sulfinic acid form was regarded to be a new kind of posttranslational modification (Rhee et al., 2005), resulting in a reversible inactivation like in the case of 2-Cysteine peroxiredoxin (2CP) or in an irreversible inactivation process as for glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (Rhee et al., 2005). The highly specific and reversible process of S-nitrosylation, which is promoted by nitric oxide (NO), was suggested to have a special function in regulating signalling cascades in plants (Romero-Puertas et al., 2007). The process of S-thiolation includes S-glutathionylation of the thiol group (Figure 7) and can exert a protective function for the thiol group under oxidative stress, mostly accompanied by decreased activity (Ghezzi, 2005). At the same time S-glutathionylation is a mechanism to prevent oxidation of the cellular GSH redox buffer itself, to avoid the exclusion of the oxidized form GSSG from the cell. A linkage between photosynthesis and glutathione biosynthesis via photosynthetic carbon skeleton provision is discussed as a possible mechanism for retrograde plastid-to-cytosol signalling in plant systems (Mullineaux and Rausch, 2005).

2.2. The factor carbon – adaptation to limiting inorganic carbon availability

Carbon availability is a limiting factor for many organisms. In autotrophs, efficient storage of light energy in carbohydrates is often limited by the availability of inorganic carbon (C_i). This is especially the case for aquatic photosynthetic organisms, since the diffusion of CO_2 in an aqueous solution is 10,000 times slower than the diffusion of CO_2 in air (Badger and Spalding, 2000; Moroney and Ynalvez, 2007). Alga have adapted to these challenges inter alia by inducing CO_2-concentrating mechanism (CCM) that allows them to optimize carbon acquisition (Spalding, 2008). The main role of the CCM is to increase the CO_2-concentration on the site of Rubisco, (Ribulose-1,5-bisphosphate carboxylase/oxygenase**;** EC 4.1.1.39), the enzyme responsible for the initial fixation of CO_2 ((Badger and Price, 2003; Badger and Spalding, 2000; Giordano et al., 2005; Kaplan and Reinhold, 1999). Since this mechanism is driven by active transport of HCO_3^-/CO_2, it is only active under low or insufficient CO_2 conditions and requires considerable changes in the cell (Badger and Price, 2003; Badger and Spalding, 2000; Kaplan and Reinhold, 1999).

However, another possibility to cope with the C_i-limitations might be by mixotrophy. Although most alga and plants are photoautotrophs, some microalgae are facultative phototrophs or photoheterotrophs and can use organic carbon substances as the sources of energy and carbon for mixotrophic cell growth, in which organic carbon is assimilated in the light simultaneously with CO_2 fixation and, hence, both respiratory and photosynthetic metabolism have to operate concurrently (Marquez et al., 1993; Ogawa and Aiba, 1981). Much work has been performed on mixotrophic growth of the green algae *Chlamydomonas reinhardtii* (Chen and Johns, 1996; Heifetz et al., 2000), *Chlorella* (Ip and Chen, 2005; Lalucat et al., 1984), and *Haematococcus pluvialis* (Jeon et al., 2006; Kang et al., 2005). Additionally, certain cyanobacteria such as *Synechococcus* (Kang et al., 2004; Vernotte et al., 1992), *Spirulina platensis* (Marquez et al., 1993), and *Anabaena variabilis* (Mannan and Pakrasi, 1993) were reported to grow mixotrophically when supplied with appropriate carbon sources. Some diatoms such as *Phaeodactylum tricornutum* (Garc a et al., ; Liu et al., 2009), *Navicula saprophila* (Kitano et al., 1997), and *Nitzschia* (Kitano et al., 1997; Wen and Chen, 2000) are able to grow mixotrophically. All these microalgae can use different organic carbon sources, such as glucose, acetate, and glycerol.

The green alga *Chlamydomonas reinhardtii* is a facultative “acetate flagellate”, capable of growing heterotrophically on acetate, but not on glucose or hexoses (Harris, 2009; Sager and Granick, 1953). Nevertheless, the genome of *C. reinhardtii* contains genes encoding

putative glycoside hydrolases (Merchant et al., 2007; Phytozome7.0), known to be involved in the degradation of cellulose (Bayer et al., 2006). Cellulose represents the primary product of photosynthesis, thus plants and other photosynthetic organisms express cellulolytic enzymes in order to maintain the integrity and for remodeling of the cell wall (Gilbert, 2010). However, in contrast to a variety of microalgae species, the green alga *C. reinhardtii* possesses a non-cellulosic wall (Adair and Snell, 1990; Horne et al., 1971) which is solely composed of hydroxyproline-rich glycoproteins (Miller et al., 1972). Many other organisms, which do normally not contain cellulose and prefer a heterotrophic lifestyle, express a variety of different cellulases rendering them capable of growth on cellulosic biomass as the sole carbon source (Bayer et al., 2006; Leschine, 1995; Lynd et al., 2002). Possibly, the existence of cellulolytic enzymes in *C. reinhardtii* enables this green alga to digest cellulose and assimilate the break-down products for mixotrophic growth.

2.3. Cellulose and cellulases

Cellulose is the most abundantly produced biopolymer in terrestrial environments (Holtzapple, 1993; Malhi, 2002) and represents therefore a particularly attractive and sustainable feedstock for biofuel production. While the main part of cellulose production is accomplished by photosynthetic higher plants and algae, non-photosynthetic organisms (certain bacteria, marine invertebrates, fungi, slime molds and amoebae) have also been reported to produce cellulose (Coughlan, 1985; Gilbert, 2010; Jarvis, 2003; Lynd et al., 2002). Cellulose is a linear condensation polymer consisting of glucose units joined by ß-1,4-glycosidic bonds with a degree of polymerization (DP) from 100 to 20,000 depending on substrate origin (Haigler and Weimer, 1991; Ljungdahl and Eriksson, 1985; O'Sullivan, 1997; Zhang and Lynd, 2004). The disaccharide cellobiose (D-anhydroglucopyranose) is regarded as the repeating unit in cellulose inasmuch as each glucose unit is rotated by 180° relative to its neighbor. This rotation causes cellulose to be highly symmetrical, since each side of the chain has an equal number of hydroxyl groups (O'Sullivan, 1997; Zhang et al., 2006; Zhang and Lynd, 2004). Cellulose molecules are strongly associated through inter- and intramolecular hydrogen-bonding and van der Waals forces that result in the formation of microfibrils, which in turn form fibers (Gardner and Blackwell, 1974; Nevell, 1985; Zhbankov, 1992). These molecules form highly ordered crystalline domains interspersed by more disordered, amorphous regions. Generally, the percentage and crystalline form of cellulose within a plant cell wall varies according to cell type and developmental stage (Haigler and Weimer, 1991). The cellulose molecule is very stable, with a half-life of several

million years for spontaneous ß-glucosidic bond cleavage at room temperature, which implicates that cellulose degradation in nature is accomplished by enzymes (Berner, 2003; Wolfenden and Snider, 2001; Zhang et al., 2006).

A widespread group of enzymes, named glycoside hydrolases (GH, EC 3.2.1.-) that hydrolyze the glycosidic bond between two or more carbohydrates or between a carbohydrate and a noncarbohydrate moiety, are classified based on amino acid sequence similarities and hydrophobic cluster analysis (Cantarel et al., 2009; Henrissat, 1991; Henrissat and Davies, 1997), currently separated in 112 GH families. Glycoside hydrolases generally have a modular structure, which consists of a catalytic domain and one or more noncatalytic carbohydrate-binding modules joined by a highly glycosylated peptide linker (Bayer et al., 2006). Deletion of the noncatalytic domains has no effect on the activity against soluble polysaccharides, but reduces activity against complex and insoluble substrates like crystalline cellulose (Raghothama et al., 2000).

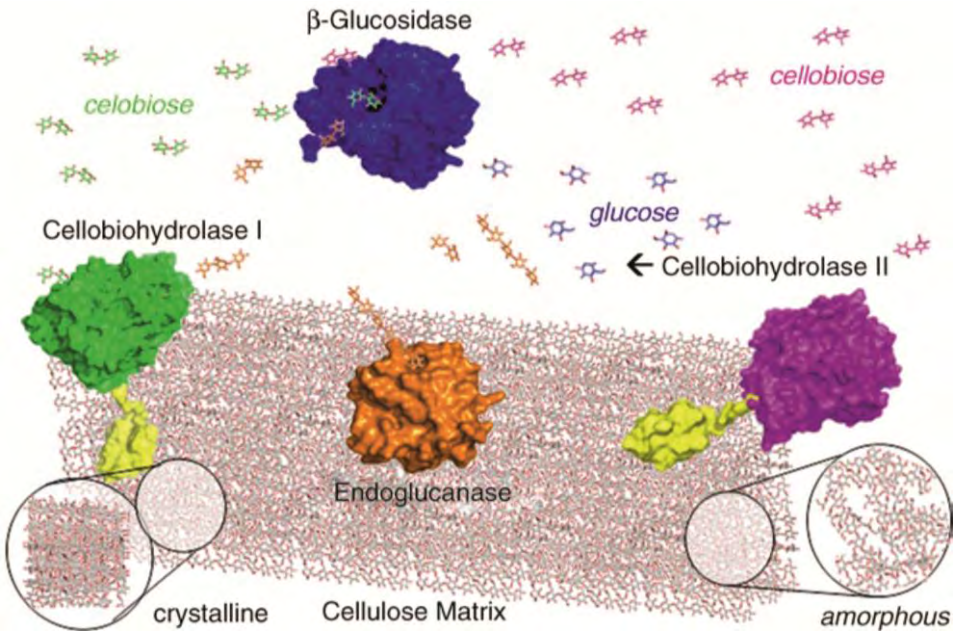

Figure 8: Scheme showing the mechanisms of cellulases enzyme action on the cellulose matrix. Figure from (Serpa and Polikarpov, 2011).

Cellulosic degradation into glucose or low molecular weight soluble saccharides is a complex process and requires the synergistic action of at least three major enzymatic components, also called glycoside hydrolases (GH, EC 3.2.1.-) (Bayer et al., 2006; Serpa and Polikarpov, 2011; Teeri, 1997; Zhang et al., 2006; Zhang and Lynd, 2004) (Figure 8):

(1) Endoglucanase or endo 1,4-ß-glucanase (EG, EC 3.2.1.4) acting randomly on 1,4-ß-linkages. It prefers to degrade amorphous (noncrystalline) rather than crystalline cellulose.

(2) Exoglucanase or exo 1,4-ß-cellobiohydrolase (CBH, EC 3.2.1.91) progressively cleave cellulose chains at the reduced and non-reduced termini to release soluble cellobiose or glucose.

(3) ß-glycosidases or 1,4-ß-glycosidases (EC 3.2.1.21) hydrolyze cellobiose into glucose

The primary hydrolysis, driven by endo- and exoglucanases, also collectively referred to as cellulases, occurs on the surface of solid substrates yielding soluble cellodextrins (DP up to 6), which are then cleaved by ß-glycosidases into terminal, nonreducing ß-D-glucose residues (Bayer et al., 2006; Serpa and Polikarpov, 2011). In particular ß-glycosidases are often associated with the cell surface and hydrolyze particularly cellobiose – the main end product of cellulolytic digestion, but also other cellodextrins to glucose before, during or after the transport process (Bayer et al., 2006). Additionally, ß-glucosidases are assumed to be generally responsible for the regulation of the whole cellulolytic process and its rate-limiting factor during enzymatic hydrolysis of cellulose, as both endoglucanase and exoglucanase activities are often susceptible to cellobiose inhibition, while ß-glucosidases are inhibited by glucose (Harhangi et al., 2002; Kaur et al., 2007).

II. Specific aims

The current demand for renewable energy sources is enormous and in particular the production of CO_2-neutral biofuels represents an important issue. The green algae, among them also *Chlamydomonas reinhardtii* are considered as potential candidates for the generation of renewable energies. One of the major advantages of a photosynthetic microorganism is the capability to convert sunlight and CO_2 into biomass and valuable products. Photosynthetic productivity is directly depending on the solar photon capture and conversion efficiency of the system, and undergoes limitations under fluctuating light conditions and limited carbon availability (Kruse et al., 2005; Prince and Kheshgi, 2005; Zhu et al., 2008). The photosynthetic organisms have developed many acclimation mechanisms in order to prevent damage and to balance the uptake of carbon and the absorption of light. A deeper understanding of the molecular mechanisms behind the regulation of the antenna size and composition as well as the utilization of light and carbon in *C. reinhardtii* is therefore of great importance.

The post-transcriptional regulation of the light harvesting antenna was described to be accomplished via the cytosolic RNA-binding protein NAB1 that represses the translation of the light-harvesting complex proteins of PSII (LHCII) (Mussgnug et al., 2005). The function and the regulation of this protein, strongly depending on posttranslational modifications, and the associated effects on the antenna size and cell growth were within the center of this work.

An additional enhancement of algal growth rates and biomass productivity could be achieved by mixotrophic cultivation. *Chlamydomonas reinhardtii* is considered to be a mixotrophic organism, which grows by photosynthetic carbon dioxide assimilation and simultaneously uptake of an external organic carbon source such as acetate. Additionally, the genome of this phototrophic organism contains genes with putative cellulolytic activities, although its cell wall not composed of cellulose, but hydroxyproline-rich glycoproteins. Cellulose represents the most abundant organic compound in the world and the capability of *C. reinhardtii* to digest and to use the cellulolytic material for growth could be of great biotechnological relevance. An understanding of the basic mechanisms enabling cellulose utilization by this organism represents another aim within this thesis.

III. Discussion and outlook

Phototrophic growth of green microalgae depends mainly on light to energy conversion efficiency and the sufficient availability of inorganic carbon. The present work is dedicated to the further understanding of the molecular mechanisms involved in the acclimation to changing light conditions (manuscript 1, 2) and adaptation to CO_2 deficiency (manuscript 3) in the green algae *C. reinhardtii*.

The assimilation of CO_2 within the Calvin cycle completely relies on the photosynthetic process and requires an efficient capture of sunlight by the light-harvesting systems of both photosystems and also sufficient amounts of inorganic carbon. Modulation of the antenna size and light harvesting is controlled by regulative mechanisms targeting all steps of LHCBM gene expression, including the cytosolic translation control of nuclear-encoded LHC proteins via NAB1. The activity of this factor and hence the extent of translation repression is strongly dependent on its posttranslational modifications, namely arginine methylation (manuscript 2) and cysteine-based redox regulation (manuscript 1). Limitations of inorganic carbon supply, that lead to over-excitations of the photosystems and cause a decrease in photosynthetic efficiency, can be avoided by assimilation of organic carbon for mixotrophic growth. As a microalga inhabiting soil as well as aquatic environments *C. reinhardtii* is surrounded by cellulosic material (e.g. decomposing organic matter). The capability of *C. reinhardtii* to secrete hydrolytic enzymes in order to digest cellulosic material for mixotrophic growth (manuscript 3) provides a new paradigm for the enormous metabolic flexibility of this species and represents a promising mechanism for algal biomass generation.

The three manuscripts presented in this thesis are:

[1] Wobbe, L., **Blifernez, O.**, Schwarz, C., Mussgnug, J. H., Nickelsen, J., Kruse, O. (2009) Cystein modification of a specific repressor protein controls the translational status of nucleus – encoded LHCII mRNAs in *Chlamydomonas*. *PNAS*, **106**(32), 13290-13295.

[2] **Blifernez, O.**, Wobbe, L., Niehaus, K., Kruse, O. (2011) Protein arginine methylation modulates light-harvesting antenna translation in *Chlamydomonas reinhardtii*. *The Plant Journal*, **65**, 119-130

[3] **Blifernez, O.**, Klassen, V., Doebbe, A., Kersting, K., Grimm, P., Wobbe, L., Kruse, O. (2011) Cellulose utilization by the unicellular phototrophic eukaryote *Chlamydomonas reinhardtii*. (Submitted)[1]

[1] The first authors name and the title of the manuscript have changed during the review process.

1. Acclimation to varying light availability – NAB1-mediated antenna size control

For the efficient photoautotrophic conversion of CO_2 into organic material, light is indispensable as an energy source. However, solar light availability is not constant and light harvesting of photoautotrophs needs to be adjusted to the prevailing light condition in order to prevent photodamage under high and to maximize photon capture efficiency low irradiances. Regulated expression of nuclear encoded photosynthetic genes demands retrograde and anterograde signal transduction, but limited knowledge exists about the key factors transducing such signals (Koussevitzky et al., 2007; Pfannschmidt, 2010). It is getting more and more evident that post-transcriptional processes play an essential role in the regulated expression of nuclear encoded photosynthetic genes in lower and higher photosynthetic eukaryotes, with the nuclear encoded *Lhcb* gene family as a prominent paradigm (Durnford et al., 2003; Flachmann and Kuhlbrandt, 1995; Frigerio et al., 2007; McKim and Durnford, 2006; Mussgnug et al., 2005). For instance, the examination of the light-dependence of LHCB translation in tobacco revealed that the light-induced enhancement of LHCB translation was dependent on photosynthetic electron transport (PET) and thus controlled by the redox-state of the electron transport chain (Petracek et al., 1997; Tang et al., 2003). This regulation pattern was also observed for the nuclear encoded PSI-subunits PsaD, PsaL and PsaF in spinach seedlings (Sherameti et al., 2002). Accumulation of LHCSR3, which is a stress-induced light-harvesting protein has also been shown to depend on functional photosynthetic electron transport (Petroutsos et al., 2011).

In *C. reinhardtii*, the cytosolic translational repressor protein NAB1 differentially binds and sequestrates *LHCBM* mRNA in sub-polysomal mRNPs (messenger ribonucleoprotein complexes) separating those transcripts from the factors required for translation initiation (Mussgnug et al., 2005) and is thus a factor controlling the size and composition of the LHCII complexes at the translational level. NAB1 distinguishes specifically between the distinct LHCBM isoforms, binding in particular to *LHCBM6* transcripts with a high affinity. The transcripts encoding LHCBM proteins contain a sequence motif (CSDCS) that was also discovered on mRNA targets of the NAB1-homologous translation repressor FRGY2 from *Xenopus laevis* (Manival et al., 2001; Mussgnug et al., 2005). The CSDCS motif differs between the LHCBM isoform mRNAs, explaining the preference of NAB1 for certain isoforms, which indicates a functional diversity of LHCBM isoforms in *C. reinhardtii*. This view is supported by the phenotype of knock-down or knock-out mutants, which were generated to assign specific functions to individual LHCBMs (Elrad et al., 2002). It is tempting to speculate that selective recognition of *LHCBM6* by NAB1 represents a

mechanism that not only adjusts the overall antenna size of PSII but also its isoform composition, which could be of crucial importance for the proper function of the entire LHCII complex under varying natural and cellular conditions. Since the amount of NAB1 protein was not observed to alter under different light conditions (Claus, 2005), suggesting that the extent of cytosolic LHCII translation repression is not mainly regulated by in- or decreasing levels of NAB1 in the cytosol, it was assumed that this factor is rather controlled by a activity switch based on post-translational modifications. The results of this work demonstrated inter alia that the translational repressor NAB1 is regulated by post-translational methylation of arginine residues within its GAR motifs **[2]** in conjunction with a redox-dependent regulation mechanism **[1]**.

1.1. Cystein modification

The C-terminal RRM motif of NAB1 contains two cysteine residues, Cys181 and Cys226, which are also conserved in the amino acid sequences of NAB1 homologous proteins identified in *Chlamydomonas incerta* (GenBank DQ122905; (Popescu et al., 2006)) and *Volvox carteri* (GenBank EF123075; (Nematollahi et al., 2006)). These cysteines are subject to reversible modification, and the activity state of NAB1 is determined by the prevailing redox-state of the cytosol. Changes in the redox-state of the cysteines represent the fundamental chemical events behind this activity regulation **[1]**. This mechanism includes a shift from oxidized to reduced NAB1 cysteines with the latter thiol state representing the more active form which represses *LHC* translation.

The RNA-binding activity of NAB1 was highly decreased by different kinds of cysteine modification *in vitro,* indicating that the unmodified dithiol state of NAB1 is the high affinity state in the algal cell **[1**, Fig. 1B**]**. Furthermore, this conclusion was supported by the phenotype of the cysteine double mutant of NAB1 (NDM1), which imitated the dithiol state of NAB1 and showed a smaller PS II antenna system, indicated by reduced chlorophyll content, increased *a/b* ratio and lower LHCII accumulation **[1**, Fig. 2A, 3B, C**].** Notably the increase in the Chl *a*/*b* ratio of NDM1 (≈ 21%) was similar to the values reported for the LHCBM1 knock out mutant *npq5* from *C. reinhardtii* (≈ 11%, (Elrad et al., 2002)) or to the ratios determined for *Lhcb2* antisense lines from *A. thaliana* (≈11-30%, (Andersson et al., 2003)). Analysis of both single mutants $NAB1_{Cys181Ser}$ and $NAB1_{Cys226Ser}$ revealed that the effect of the of Cys226 mutation on PSII antenna size was more pronounced than that caused by the Cys181 mutation.

In general, cysteine residues can be engaged in the redox-control of protein activity by different post-translational modifications (Michelet et al., 2006). One common mechanism is the formation of a dithiol-disulfide switch which involves an intramolecular disulfide bridge. However, the data obtained from the *in silico* analysis **[1**, Fig. 1A and S1**]**, peptide mapping **[1**, Fig. S2**]** and in vitro RNA-binding studies **[1**, Fig. 1B NEM, 4-vinylpyridine**]** indicate that intramolecular disulfide formation is not the mechanism underlying NAB1 regulation. The cysteine residues of cytosolic proteins are maintained in the reduced thiol state by action of thiol-based redox buffer systems (glutathione/glutaredoxins; thioredoxins/thioredoxin reductase), whereas the total concentration of glutathione and the ratio of reduced to oxidized glutathione defines the cytosolic redox-state and undergoes considerable changes in response to a variety of environmental stresses (Schafer and Buettner, 2001). Disulfide bridge formation in proteins can be frequently correlated to the oxidation state of the glutathione redox buffer (Schafer and Buettner, 2001). NAB1 cysteines were extensively modified by the cellular thiol compound glutathione disulfide, which had a strong negative impact on RNA affinity *in vitro* **[1**, Fig. 1B, C**]**. However, future experiments are needed to clarify whether glutathionylation of NAB1 occurs *in vivo*.

Polysome analyses and RNA co-immunoprecipitation experiments demonstrated the interconnection of the NAB1 thiol state and its activity as a translation repressor *in vivo*, showing that NAB1 is fully active in its dithiol state and is reversibly deactivated by modification of the cysteines **[1**, Fig. 4 and 5B**]**. This was further supported by the fact that oxidative stress conditions led to a dramatic decline in the RNA-binding activity of Wt-NAB1 *in vivo*, whereas the effect on the $NAB1_{Cys226Ser}$ mutant (NSM2) was significantly attenuated, confirming the critical importance of Cys226 for this control mechanism **[1**, Fig. 5B**]**. These observations led to the conclusion that the exchange of cysteine to serine in the mutants NDM1, NSM1 and NSM2 prevents cysteine modification and therefore the inactivation of NAB1. The reduced antenna size of the cysteine mutants therefore indicates that the modification of Cys181 or Cys226 in the Wt-NAB1 is a switch to turn-off the repressor activity of NAB1. Consequently the inability of the NAB1-mutants to switch-off NAB1 activity was accompanied by a reduced growth rate under phototrophic low light conditions, where algae cells capable of antenna size enlargement are favored **[1**, Fig. 3**]**.

Altogether these results demonstrate that the cysteines Cys181 and Cys226 of the NAB1-RRM domain are essential components of a redox-control mechanism affecting the translation repressor activity of the protein. The exchange of Cys181 caused a milder phenotype compared to the Cys226Ser mutation. This difference could be explained by the

lower accessibility of Cys181, as predicted by an *in silico* structure of the RRM domain **[1**, Fig. 1A and S1**]** and by the fact that the position of Cys181 is not conserved in RRM domains of mammalian proteins, whereas Cys226 is conserved in the RRM domain of hnRNP M from *H. sapiens*, suggesting that this specific cysteine could play an important role for redox-regulation of RRM domains in the animal and plant kingdom. Mutation of Cys226 creates a phenotype that is as pronounced as that caused by the $NAB1_{Cys181/226Ser}$ double mutation, indicating that the modification of Cys226 alone is sufficient to switch-off the repressor function of NAB1.

In *C. reinhardtii* two plastidic RRM proteins RB47 and RBCL were already characterized as factors controlling photosynthetic gene translation and both are regulated via thiol-mediated conformational switches (Alergand et al., 2006; Cohen et al., 2005; Cohen et al., 2006; Fong et al., 2000; Irihimovitch and Shapira, 2000; Yosef et al., 2004). However, these proteins were shown to be located in the plastid, whereas NAB1 represents a eukaryotic example of a cytosolic RRM protein being subject to cysteine-based redox control. At the present time there is no complete scenario of the retrograde signaling pathway of the NAB1 redox-regulation, since the current knowledge of the interplay between the plastidic redox-state and the redox-state of the cytosolic redox-buffer system is very limited in *C. reinhardtii* and other plant organisms (Pfannschmidt, 2010). There are some reports regarding redox-regulated photosynthetic gene translation in the cytosol of plant cells (Petracek et al., 1997; Sherameti et al., 2002; Tang et al., 2003), however the molecular mechanisms and the involvement of transcript-specific RNA-binding proteins remains unknown and needs to be elucidated. Nevertheless, the finding that activation of NAB1 is linked to the cytosolic redox poise provides further insight into the redox-controlled regulation mechanisms in the cytosol of a photosynthetic cell. Future experiments will have to address the questions of how the cytosolic redox state and the activation/deactivation of NAB1 are coupled to the photosynthetic activity and the prevailing redox state within the chloroplast.

1.2. Arginine methylation

As stated above, the translation efficiency of nucleus-encoded *LHCBM* transcripts is responsive to the cytosolic redox state via cysteine modification of the specific repressor NAB1. However, despite of the strongly reduced *in vivo* RNA-binding activity of NAB1 observable after oxidative modification of both cysteines, a complete inactivation of NAB1 could not be achieved by application of diamide-induced stress to *C. reinhardtii* cells **[1]**.

This finding suggested a second type of posttranslational modification with an equally crucial effect for the regulation of NAB1 activity, possibly working in concert with thiol modification. The N-terminal CSD and the C-terminal RRM domain of NAB1 are connected by a GAR (glycine-arginine rich) motif which contains several RGG boxes **[2**, Fig. 1a**]** known to be preferred arginine methylation sites of PRMT (protein arginine methyltransferases) enzymes (for review see (Bedford and Clarke, 2009)). As implied by the presence of a GAR domain containing RGG methylation boxes NAB1 was found to be methylated *in vitro* and *in vivo* **[2**, Fig. 1b,c**]**. Moreover, according to mass spectrometric analysis the NAB1 protein contains at least two asymmetrically dimethylated sites at Arg90 and Arg92 **[2**, Fig. 2**]**. According to the *in silico* analysis of protein methylation sites by the prediction web tool Memo (Chen et al., 2006) or BPB-PPMS (Shao et al., 2009) additional arginines in the NAB1 protein could be dimethylated in an asymmetrical fashion **[2**, Fig. 1a**]**. More detailed mass spectrometric analysis with additional proteinases like chymotrypsin as well as the generation of mutants, where the arginines in the RGG methylation boxes are exchanged with another amino acid, could be used to distinguish between different methylation sites in regard to their function. However, based on the presence of asymmetrically dimethylated arginines within NAB1 *in vivo* and the *in vitro* methylation of recombinant NAB1 by PRMT1 from rat **[2**, Fig. 1b and 2**]**, it can be concluded that NAB1 must be methylated *in vivo* by a type I arginine methyltransferase, most likely by protein arginine methyltransferase 1 (PRMT1). This enzyme is the predominant type I protein arginine methyltransferase in mammalian cells (for review see (Nicholson et al., 2009), which is thought to be responsible for approximately 85% of all cellular PRMT activity (Tang et al., 2000). Moreover, PRMT1 is characterized by the exclusive formation of asymmetric dimethylarginine (Gary and Clarke, 1998) and is present in both (Herrmann et al., 2005). Further experiments are needed to determine whether NAB1 is methylated by PRMT1 in the cytosol or in the nucleus.

In order to characterize the functional importance of arginine methylation for the function of NAB1, inhibitors AdOx (adenosine-2′-3′-dialdehyde) or AMI-1 (7,7′-carbonylbis (azanediyl)*bis*(4-hydroxy-naphthalene-2-sulfonic acid) sodium salt hydrate) were applied, which are known to prevent methylation of protein arginines (Cheng et al., 2004; Dolzhanskaya et al., 2006a; Feng et al., 2009). The phenotype observable after inhibitor treatment of wild type cells was therefore characterized by an enlarged PSII antenna **[2**, Fig. 3 and S5**]** and resembled the phenotype of the NAB1-deficient mutant *Stm3* (Kruse et al., 1999; Mussgnug et al., 2005), indicating a strong impact on the light harvesting antenna. These results allow the conclusion that insufficient methylation leads to repressor activity losses of

NAB1. A reduced *in vivo* methylation of NAB1 leads thus to an impaired binding to its target mRNA *LHCBM6* **[2**, Fig. 4a**]** and hence revealed translation repression, causing an increased accumulation of light-harvesting proteins which is not due to a higher mRNA expression of *LHCBM* mRNAs **[2**, Fig. S3**]**. Under heterotrophic growth conditions in dark-grown cell cultures low methylation levels of NAB1 were detected. On the other hand, an increased photosynthetic activity under mixotrophic and even more under phototrophic growth conditions induced a higher extent of arginine methylation in NAB1 **[2**, Fig. 6**]**, indicating that the environmental factor light triggers arginine methylation of the RNA-binding protein NAB1, thereby activating translational control of light-harvesting complex proteins in order to adjust the antenna size when cell growth completely depends on efficient photosynthesis.

Arginine methylation was already shown to be involved in the regulation of different aspects of RNA-binding protein function (for review (Blackwell and Ceman, 2011; Yu, 2011)). The GAR or RGG motif represents a well-known arginine-rich RNA binding domain, which is normally found in conjunction with other RNA-binding domains (Burd and Dreyfuss, 1994; Dreyfuss et al., 1993; Kajita et al., 1995) and have been shown to increase the affinity of a protein for RNA (Abdul-Manan and Williams, 1996; Bagni and Lapeyre, 1998; Brown et al., 1998; Shamoo et al., 1994). A modulation of target RNA affinity by arginine methylation has been demonstrated for the GAR motif-containing proteins FMRP (Blackwell et al., 2010; Dolzhanskaya et al., 2006a; Stetler et al., 2006) and RBP16 (Goulah and Read, 2007; Hayman and Read, 1999). Both Y-box proteins, the mitochondrial RBP16 and the cytosolic NAB1contain an N-terminal CSD (cold shock domain) connected to a GAR motif and require methylation of arginines within their GAR motifs for a stable association with their target mRNAs (Goulah and Read, 2007) **[2**, Fig. 4**]**. It is also possible that GAR motifs confer specific RNA binding to a protein, since heterogeneous nuclear ribonucleoprotein hnRNP U has no other discernible RNA binding motif and can discriminate between different RNA sequences, indicating that the GAR motif is both necessary and sufficient for RNA binding activity (Kiledjian and Dreyfuss, 1992). However, in other cases such as with hnRNP A1 and G, as well as NAB1, the GAR motif is found in combination with other RNA-binding elements, suggesting other functions of this domain besides contributing to RNA binding. Although, the GAR motif does not always seem to be involved in sequence-specific recognition of RNA, the auxiliary domains were shown to be essential for translation regulation by either facilitating the assembly of sub-polysomal mRNPs (Matsumoto et al., 1996) or by enabling association of the protein with polysomal mRNPs (Blackwell et al., 2010). Furthermore, it has been shown for several modular RNA-binding proteins, that the

unstructured auxiliary domain is vital for the translation regulating function of the protein (Blackwell et al., 2010; Matsumoto et al., 1996; Nekrasov et al., 2003). For instance, hnRNPs represent a very large group of multifunctional proteins participating in a variety of cellular processes, mostly involved in RNA metabolism, either in the nucleus or the cytoplasm (Chaudhury et al., 2010). The interaction and binding of RNA-binding proteins with mRNAs can inhibit the translation or lead to a stimulated translation (Minich and Ovchinnikov, 1992; Sommerville, 1999). For instance, hnRNP E1 has been shown to function as both a translational co-activator and co-repressor. It functions as an IRES *trans*-activating factor (ITAF) and stimulates translation of *poliovirus* RNA (Gamarnik and Andino, 1997). On the other hand, hnRNP E1 causes translational repression of *15-lipoxygenase* (*15-LOX*) during erythrocyte maturation (Ostareck et al., 1997), human papillomavirus type 16 (HPV-16) *L2* mRNA (Collier et al., 1998), *disabled-2* (*Dab2*), and *interleukin-like EMT inducer* (*ILEI*) mRNAs during transforming growth factor-beta (TGFβ)-induced epithelial–mesenchymal transition (EMT) (Chaudhury et al., 2010). For the cytosolic NAB1 protein, which contains besides the GAR motif two additional RNA binding domains, the RRM and the CSD domains, whereas the latter displayed sequence-specific binding to the CSDCS sequence of *LHCBM6* mRNA *in vitro* and was so far identified to function as a translational repressor (Mussgnug et al., 2005). However, the possibility of NAB1 operating as a translational activator towards specific substrates should be considered to be investigated in the future.

In summary, the data obtained from this work leads to the conclusion that arginine methylation of NAB1 in *C. reinhardtii* is used to discriminate between physiological situations which require antenna size control by NAB1-mediated translation repression and situations under which it becomes dispensable. Methylation of the GAR domain thus represents a key mechanism for the regulation of NAB1 activity. Even though, the posttranslational modification of non-histone proteins by arginine methylation is well characterised in mammalian cells with numerous identified methylated proteins *in vivo* (Bedford and Clarke, 2009; Bedford and Richard, 2005; Lee and Stallcup, 2009), arginine methylation of proteins in photosynthetic organisms is poorly understood and the investigations have just begun. For instance, arginine methylated proteins were reported to be present in the flagellum of *C. reinhardtii*, but the identity of these proteins still needs to be revealed (Schneider et al., 2008; Sloboda, 2009; Sloboda and Howard, 2009). The *Arabidopsis thaliana* protein MDB7 is shown to be a substrate for AtPRMT11 *in vitro* (Scebba et al., 2007). However, recent reports demonstrated that AtPRMT5 in *A. thaliana* is part of the circadian clock (Hong et al., 2010; Sanchez et al., 2010) and is essential for proper

pre-mRNA splicing that impacts diverse developmental processes (Deng et al., 2010). AtPRMT5 methylates various non-histone substrates, including RNA processing factors, U snRNP AtSmD1, D3, and AtLSm4 proteins and thus mutations in this arginine methyltransferase lead to splicing defects in hundreds of genes that are involved in multiple cellular and biological processes (Deng et al., 2010). Therefore, protein arginine methylation in photosynthetic cells represents a new important field of research within plant molecular biology and the results obtained for NAB1 during this thesis make an important contribution to a better understanding of this protein modification in photosynthetic eukaryotes.

1.3. Interplay between the post-translational modifications of NAB1

The results obtained during this PhD thesis demonstrate a direct correlation between the extent of NAB1 arginine methylation and the need to adjust the photosynthetic apparatus of *C. reinhardtii* to optimal light harvesting **[2]**. Additionally, NAB1 activity was shown to be redox-controlled by reversible cysteine modification **[1]**. Inhibition of arginine methylation in the $NAB1_{Cys181Ser/Cys226Ser}$ mutant (NDM1), which was shown to be permanently active, causing a pale green phenotype due to a reduced LHCBM translation **[1]**, led to an increase of ~30% in the total chlorophyll amount **[2**, Fig. 5**]**. Similar behavior was observed for chlorophyll content in the wildtype after inhibitor application, but it still was about 25% higher than in the NDM1 mutant. Therefore, these observations indicate that both post-translational modification contribute to the control of the translation repressor activity of NAB1 and have an equivalent impact on the expression of LHCII proteins (Figure 9). Further analyses are required in order to reveal, if the cysteine redox-control and arginine methylation are working in concert or if they represent independent regulation mechanisms, which are active under different physiological conditions.

Generally, RNA-binding proteins, for instance, hnRNP proteins are known to undergo several post-translational modifications, including phosphorylation, sumoylation, methylation and ubiquitination, and such changes regulate their subcellular localization (for review see (Chaudhury et al., 2010)). For example, hnRNP proteins belonging to the A, B, and C groups and hnRNP G, K, and U were all found to be phosphorylated *in vivo* (Dreyfuss et al., 1993) and like in case of hnRNP K, this modification mediates the targeting of this factor to the cytosol, where it subsequently silences translation of target transcripts (Habelhah et al., 2001). Since NAB1 was shown to function in the cytosol (Mussgnug et al., 2005), future experiments should address the possibility of another potential post-translational modification for this factor like phosphorylation.

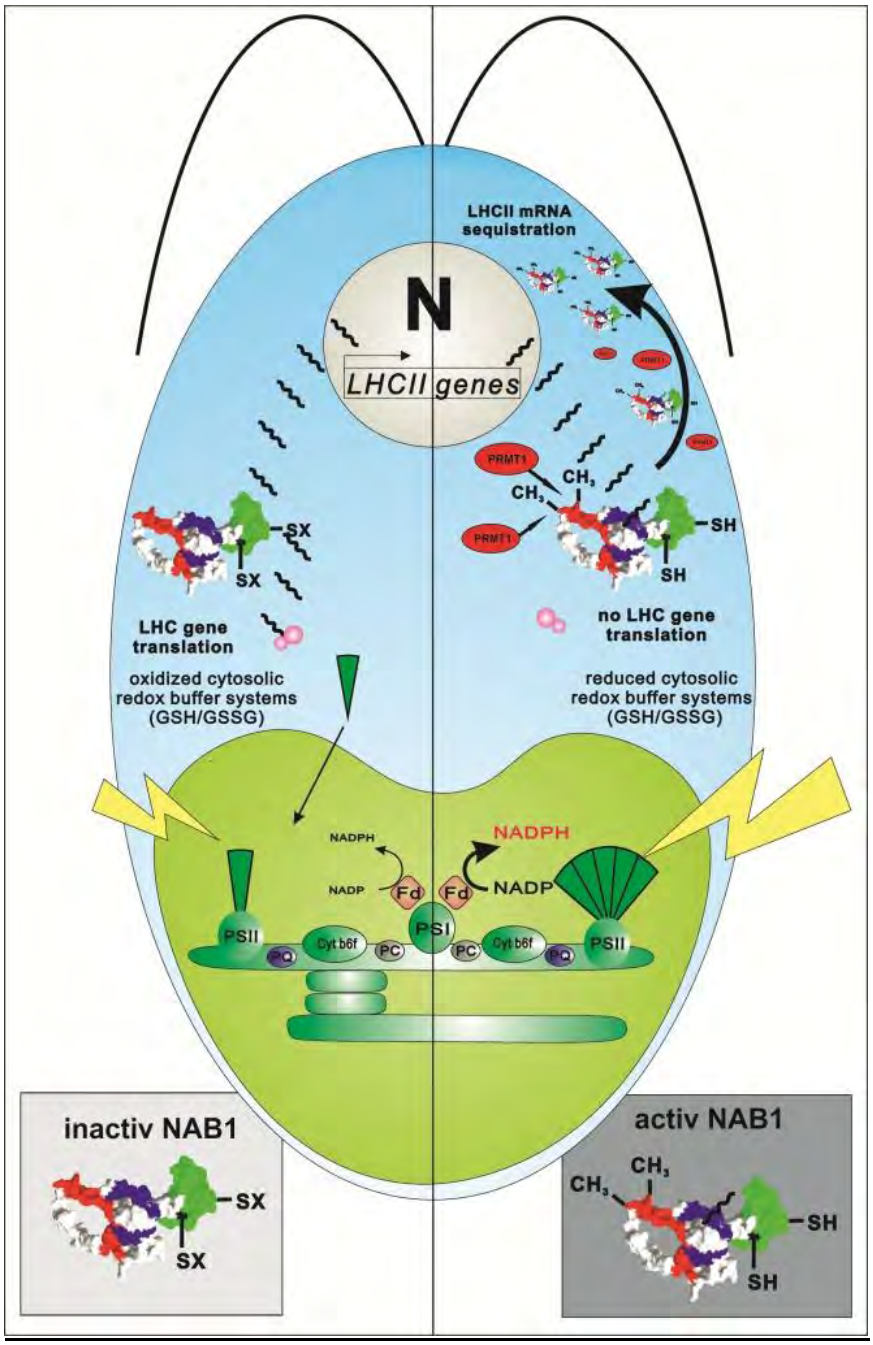

Figure 9: A summarizing model of NAB1activity regulation via post-translational modifications. In a situation of low photosynthetic performance (left side of the figure), characterized by a too small antenna and an increased demand for LHCBM proteins, oxidation of the cytosolic glutathione pool triggers the inactivation of NAB1-mediated translation repression due to cysteine modification. A lack of arginine methylation within the GAR domain causes a further decrease of NAB1 activity. The opposite situation is depicted on the right side. A too large antenna results in a reduced cytosolic redox environment, which triggers arginine methylation as well as cysteine reduction, leading to the activation of NAB1 and hence LHCBM translation repression. *Ab initio* NAB1 protein model generated with the structure prediction server I-TASSER (Zhang, 2008, 2009; Roy et al., 2010). The cold shock domain (CSD) is shown in dark blue and the RNA recognition motif (RRM) domain is shown in dark green. The position of the GAR motifs I and II are indicated in red.

In contrast to other post-translational modifications, arginine methylation seems to be a less dynamic process (Fackelmayer, 2005), with an enormous metabolic cost of 12 ATPs associated with the formation of a methylated product (Atkinson, 1977). The modification of cysteines, on the other hand, was demonstrated to be a highly dynamic process with fast

kinetics, which responds rapidly to changes in the redox-state of the cytosolic glutathione pool (Meyer et al., 2007; Ostergaard et al., 2004). In our current working model arginine methylation represents the slowly responding regulator, which is responsive to the light availability, and cysteine modification is regarded as a dynamic fine-tuning mechanism. This additional redox-based mechanism could respond much faster to transient perturbations of photosynthetic performance and local changes in the plastidic demand for LHCBM synthesis in the cytosol than arginine methylation.

Physiological conditions, characterized by a reduced cytosolic redox state, are connected to an active state of NAB1 and hence efficient translation repression of LHCII transcripts (Figure 9, right panel). Within this model a reduced photosynthetic performance caused by limited light supply in combination with a small antenna system oxidises the cytosolic redox-system (Figure 9, left panel), which in turn deactivates NAB1, thereby stimulating LHCII protein synthesis and facilitating an increase of the photosynthetic performance.

1.4. Candidate signaling pathways and further research on NAB1 regulation

The cytosolic translational repressor NAB1 was shown to regulate the translation of plastid-targeted LHCII proteins and controls therefore light-harvesting capacity in *C. reinhardtii* cells (Mussgnug et al., 2005). Translation control of certain LHCII proteins was determined to be activated through different post-translational mechanisms, the methylation of arginines in the GAR motif of the protein **[2]** and by a cysteine-based redox-control **[1]**. Since the reduced cysteines characterize the active form of NAB1, whereas oxidation of cysteines, especially of Cys226, represents the deactivation of its repressor activity **[1]**, the regulation mechanism NAB1 activation/deactivation could most likely be linked to reducing conditions in the cytosol. Physiological situations, relevant for NAB1 redox-regulation, could be connected with ROS (reactive oxygen species) formation, produced under conditions where excess light is absorbed by a too large antenna system (Dietz, 2003). However, NAB1 activation requires the reduction of protein cysteines **[1]** and ROS production has an opposite effect on cysteines causing their oxidation, indicating the involvement of other signaling molecules or potential signal sources.

The cytosol is in a highly reduced redox state under normal, stress-free conditions (Schafer and Buettner, 2001), with NADPH known to be essential for the maintenance of this reduced environment and supplied by the glucose consuming oxidative pentose phosphate cycle (Foyer and Noctor, 2009) and NAD(P)H exporting shuttle systems in the chloroplast

envelope membrane (Foyer and Noctor, 2009; Kelly and Gibbs, 1973). The redox-state of cytosolic protein cysteines is largely determined by the redox-state of the glutathione pool (Meyer and Hell, 2005). It has already been shown that that glutathione biosynthesis is modulated by light and that certain photosynthates are required for the synthesis of glutathione (Mullineaux and Rausch, 2005; Noctor et al., 1997). A variety of established methods exist for the measurement of the glutathione pool size and its redox-state (Griffith, 1980; Noctor and Foyer, 1998). Studies regarding the glutathione pool size and reduction state under limiting and saturating light conditions should provide further insights as well as SH-labelling experiments applying the SH-specific label mPEG-MAL (Katzen and Beckwith, 2003) **[1]**. Another important question, which has to be addressed, is if thioredoxin and/or glutaredoxin proteins are components of the NAB1 activation pathway. The cysteines in recombinant NAB1 were demonstrated to be glutathionylated by treatment with glutathione disulfide *in vitro* **[1]**, which needs to confirmed *in vivo* using detailed mass spectrometric analysis. The first enzyme identified as a specific glutathionyl-mixed disulfide oxidoreductase was a glutaredoxin (Gravina and Mieyal, 1993) and glutaredoxins (GRXs) were shown to catalyze the re-reduction of glutathionylated protein cysteines much more efficiently than other disulfide oxidoreductases such as thioredoxins or protein disulfide isomerases (Couturier et al., 2009). This fact makes GRXs a likely candidate for NAB1 activation.

Further investigations of NAB1 redox control should comprise systematic analyses of the NAB1 thiol/redox state under different physiological conditions, to gain a deeper understanding of plastid-to-cytosol redox signalling and to analyse the correlation of the NAB1 redox-state and the redox-state of the cell. Furthermore, the questions should be addressed of how the cytosolic redox state and the activation/deactivation of NAB1 are coupled to the photosynthetic activity and the prevailing redox state within the chloroplast. In addition to the analyses regarding the redox-state of NAB1 and its impact on the translation efficiency of LHCII mRNAs, the contribution of LHCII transcription control to antenna size adjustment as well as the role of state-transitions within antenna size regulation should also be studied. Additionally, this thesis provides evidences that NAB1 contains methylated arginines *in vivo* and furthermore that this modification has a significant impact on NAB1 activity **[2]**. Future investigations should therefore include detailed analyses addressing the contribution of arginine methylation to redox signalling and the mutual influence of cysteine and arginine modification as activity switches. Systematical analysis of the methylation status of NAB1 in response to altered external conditions (e.g. light supply, circadian rhythms), in order to discover whether cysteine redox-control and arginine methylation are working in concert or if

they represent independent mechanisms, which are active under different physiological conditions.

Another important aspect of NAB1 research is the determination of a 3D structure of NAB1, which could improve understanding of the regulatory function of NAB1 *in vivo*. With *LHCBM6* one of the prime *in vivo* mRNA binding partners of NAB1 (Mussgnug et al., 2005) **[1, 2]**, crystallization experiments in the presence and absence of the RNA binding partner and the comparison of both structures could reveal, which conformational alterations are required for RNA recognition and provide further information about the structural consequences of cysteine modification and arginine methylation.

Translation repressor proteins from other organisms were shown to bind numerous target RNAs *in vivo* (Chaudhury et al., 2010), thereby representing post-transcriptional operons and regulating certain cellular processes by controlling the translation of respective key factors simultaneously. The identification of additional new mRNA targets of NAB1 is essential in order to get further insights into the function of this factor. The characterisation of NAB1 mRNA-targets can be performed on a whole transcriptome scale, using the mRNAs bound to NAB1-mRNP complexes, which are isolated by co-immunoprecipitation with anti-NAB1 antibodies and subsequently identified via microarray studies or *Illumina Solexa* transcriptome analysis systems. Another equally important investigation represents the identification of other proteins, which are part of NAB1-mRNP complexes in order to understand the signalling network responsible for NAB1 control. This should lead to the identification of essential components, which structure cytosolic mRNP complexes containing photosynthetic gene transcripts. Sub-polysomal mRNP fractions, isolated by sucrose gradient centrifugation and oligo-dT chromatography subsequently applied to enrich intact cytosolic mRNP complexes via the poly-A-tail of bound mRNAs, could be followed proteomic analysis by 2D SDS-PAGE in conjunction with mass spectrometry.

1.5. Optimized antenna for efficient biomass production

The efficiency of light to biomass conversion is a very important factor for various industrial proposals, since the current interest for the potential application of microalgae as a feedstock for synthetic chemistry and biofuels steadily increases (Melis, 2009; Radakovits et al., 2010; Stephens et al., 2010a). The theoretical maxima of solar energy conversion efficiencies and productivity to biomass were calculated to be by 8-10% (Dubinskii and Guida, 1979; Melis, 2009), however the best case solar-to-biomass energy conversion efficiency reported with green microalgae did not exceed the 2-3% value (Ben-Amotz and

Avron, 1990; Borowitzka, 2005; Dismukes et al., 2008; Dubinskii and Guida, 1979). This discrepancy is mainly due to the fact that photosynthesis in wild-type cells operates efficiently with light intensities of up to about 400 µmol photons m^{-2} s^{-1} (Melis, 2009). At higher sunlight intensities, which can reach up to 2500 µmol photons m^{-2} s^{-1} during the course of a sunny day, it may be concluded that more than 80% of absorbed solar irradiance would be wasted in the photosynthetic apparatus (Melis, 2009), since saturating light causes photoinhibition and photodamage (Melis, 1999) triggering photoprotective mechanisms. The assembly of large antenna molecule arrays in the photosystems of photoautotrophs represents a survival strategy and competitive advantage in the wild, where light is often limiting (Kirk, 1994). Capturing more light for self, even if wasted, means maximum competition in the wild and prevents light capture by potential competitor (Melis, 2009). However, this characteristic obviously seems to be detrimental to the yield and productivity in dense culture, where competition for resources including light is not a benefit (Anten, 2005; Ort and Melis, 2011). The minimization of light absorption of the individual cell chloroplast could help to achieve higher performance characteristics, since it would permit greater transmittance of irradiance through a high-density cultivation (Figure 10) and could improve photosynthetic productivity and solar-to-product conversion efficiency (Melis, 2009; Mitra and Melis, 2008).

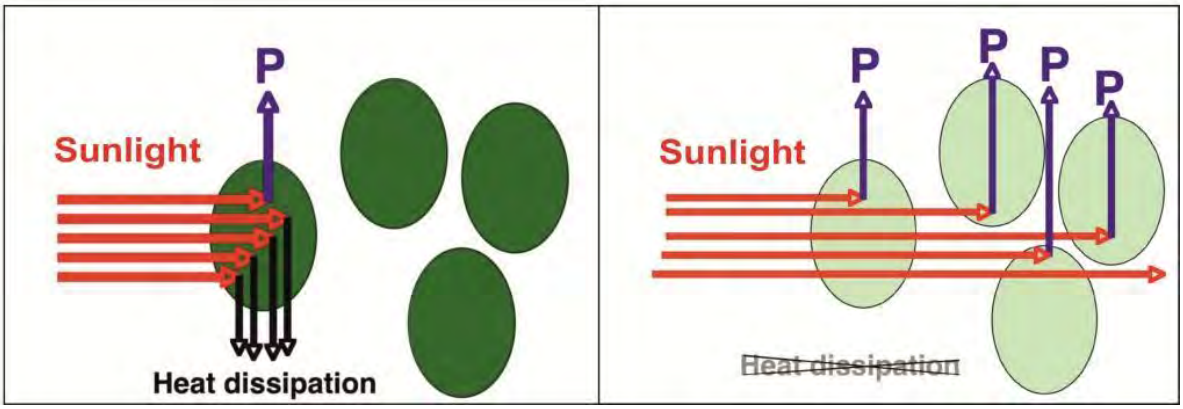

Figure 10: Schematic of microalga cells in a high-density culture. Figure from (Mitra and Melis, 2008). Left panel: The fate of absorbed sunlight in fully pigmented (dark green) microalgae culture. Individual cells at the surface of the culture over-absorb incoming sunlight, and wastefully 'heat dissipate' most of it, limiting culture productivity (P). The high absorption probability by the first layer of cells would cause shading and prevent cells deeper in the culture from being exposed to sunlight. Right panel: The sunlight penetration through cells with a reduced chlorophyll antenna size. Individual cells have a diminished absorption of sunlight, thereby permitting greater penetration of irradiance and enhanced photosynthetic productivity (P) by cells deeper in the culture.

It has been already demonstrated, that a smaller light harvesting antenna of microalgae would result in a relatively higher light intensity for the saturation of photosynthesis in individual cells (Melis, 1999; Neidhardt et al., 1998), while alleviating photoinhibition of photosynthesis (Nakajima et al., 1998) and permitting an overall greater productivity by the

mass culture (Nakajima and Ueda, 1997; Nakajima and Ueda, 1999; Polle et al., 2003). Additionally, Mussgnug et al. reported that the cultures of the *Stm3LR3* RNAi mutants, in which the complete LHC gene family was down-regulated, showed an improved light penetration and photosynthetic efficiency as well as a reduced photo-oxidative damage (Mussgnug et al., 2005). Furthermore, the relevance of the cytosolic translational repressor protein NAB1 was recently demonstrated to have an impact on the efficiency of sunlight to biomass conversion in the high H_2 producing strain *Stm6Glc4* (Doebbe et al., 2007) and was found to have a 10-20% smaller LHC, an increase in photosynthetic efficiency and faster biomass production compared to the parental strain (Beckmann et al., 2009).

The obtained knowledge about the NAB1 repressor activity and its regulation **[1, 2]** (Mussgnug et al., 2005), which was achieved during this PhD thesis, should be used to provide even greater improvement for a higher sunlight to biomass conversion efficiencies and photosynthetic productivity of the algae under high cell density and bright sunlight conditions. Since a reduced light harvesting antenna, mediated and controlled via NAB1, could help to reduce the shading that occurs with normally pigmented wild-type cells, permitting thus a more uniform illumination of the cells in a mass culture (Figure 10) and minimizing efficiency losses, thereby increasing the overall process efficiency (Hankamer et al., 2007; Kruse et al., 2005; Prince and Kheshgi, 2005). Optimized sunlight to biomass conversion in the biotechnologically relevant organism *C. reinhardtii* will be reliant on an advanced understanding of antenna size regulation in this alga, since optimized algal strains with improved antenna systems, which can be obtained by genetic engineering, should have increased growth rates in bioreactor systems paving the way towards an economic feasibility of algal biomass and CO_2 neutral generation of portable energy sources.

2. Acclimation to carbon limitation – the use of alternative carbon sources

An efficient photosynthetic carbon fixation is first of all dependent on the availability of light and inorganic carbon (C_i). Since both factors undergo fluctuations in quantity, photosynthetic organisms need to acclimate to these changes, in order to maintain the balance between the light-harvesting and the carbon fixation capacities. Especially, the photosynthesis in aquatic environments may be limited due to the low solubility and slow diffusion rate of CO_2 in water (Badger and Spalding, 2000). Adaptation mechanisms were evolved to cope with these limitations, by inducing the CO_2 concentrating mechanism (CCM) that allows an optimized carbon acquisition. Another possible adaptation strategy to limiting availability of CO_2, and inorganic carbon in general, represents the assimilation of organic carbon for

heterotrophic or mixotrophic growth. Many green algae, including *C. reinhardtii* are found in quite different environments like bogs (Kroes, 1972) and soils (Proschold et al., 2005) and are known to grow mixotrophically by photosynthetic CO_2 assimilation and simultaneously uptake of an external organic carbon source (Heifetz et al., 2000) and hence, both respiratory and photosynthetic metabolism have to operate concurrently (Marquez et al., 1993; Ogawa and Aiba, 1981). The addition of organic carbon may enhance the growth of certain microalgae, for instance the biomass of *Chlamydomonas humicola* was reported to increase 20-fold when grown on acetate (Laliberte and Delanoue, 1993), while *Scenedesmus acutus* increases 6-fold on molasses (Shamala et al., 1982). Acetate was also shown to enhance growth and ammonium uptake in mixotrophic cultures of *Scenedesmus obliquus* (Combres et al., 1994). Altogether, growth rates reached under mixotrophic conditions represent approximately the same as the sum of the growth rate in the photoautotrophic and heterotrophic cultures, which were observed for *Chlorella regularis* (Endo et al., 1977), *Chlorella vulgaris* (Ogawa and Aiba, 1981), and *Spirulina platensis* (Marquez et al., 1993). However, in contrast to the many other green microalga like *Chlorella sp.* (Tanner, 1969), *C. reinhardtii* is unable to use hexoses as a direct carbon source, probably owed to the fact that this species lacks a functional hexose transport system in the outer plasma membrane (Harris, 2009; Sager and Granick, 1953). Nevertheless, *in silico* analysis of the *C. reinhardtii* genome revealed, that it contains genes encoding glycoside hydrolases like endo-β-1,4-glucanases and β-glucosidases **[3**, Table S1**]**, known to be involved in cellulolytic degradation system (Bayer et al., 2006). Cellulose represents the primary product of photosynthesis and is the most abundant natural compound in the biosphere (Holtzapple, 1993), being crucial for fiber reinforcement in higher plants. All photosynthetic organisms, which possess a cellulose-containing cell wall, express cellulolytic enzymes, since they are required for the rearrangement of the cell wall structure (Gilbert, 2010). And yet cellulose digestion is not required in *C. reinhardtii* as part of the cell wall remodeling mechanism, because in contrast to many other microalgae like *Chlorella* (Braun and Aach, 1975), its cell wall is solely composed of hydroxyproline-rich glycoproteins (Miller et al., 1972) and no evidence for the presence of cellulosic material has been found (Adair and Snell, 1990; Horne et al., 1971).

2.1. Degradation of cellulose and utilization of cellodextrins for growth

The investigation of the cellulose digestive system of the green alga *C. reinhardtii* revealed that this species secretes hydrolytic enzymes, which perform the degradation of

cellulosic material to cellobiose and cellodextrins, and imports them into the cells, where they are converted to glucose by β-glucosidases (Figure 11).

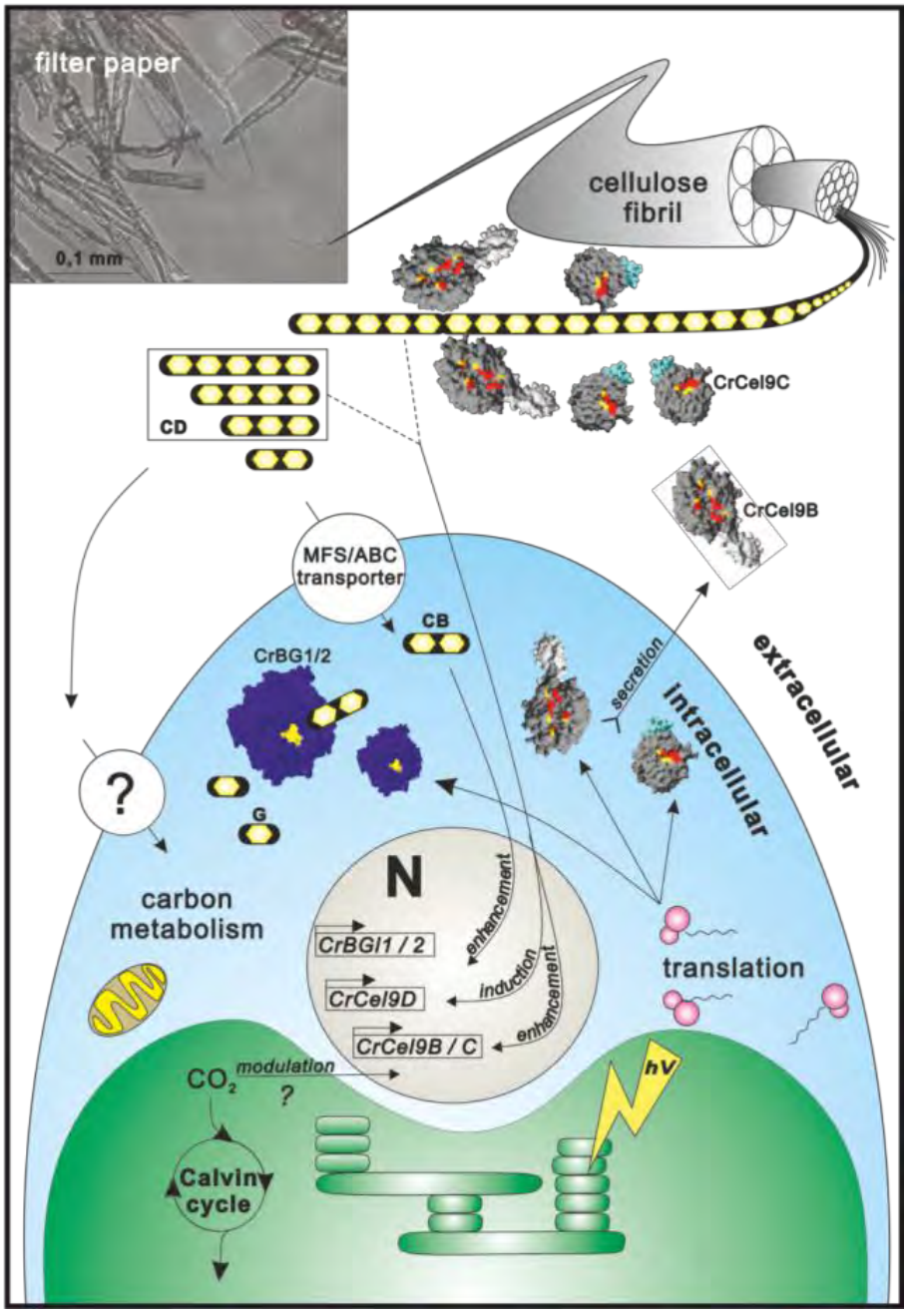

Figure 11: A model illustrating the potential mechanism in *C. reinhardtii* for the use of cellulose as a carbon source for mixotrophic growth (Figure from [3, Fig.4B]). Abbreviations: N: nucleus; CD: cellodextrins; CB: cellobiose; G: glucose

The present work demonstrates that the assimilation of cellulosic material is dependent on photosynthesis within a mixotrophic regime, where cellulose represents an extra carbon source especially under CO_2 limiting conditions. This system is much simpler than those encountered in cellulolytic organisms like fungi or bacteria, which engages a huge number of different glycoside hydrolase families for cellulose and hemicellulose digestion (Bayer et al., 2006; Lynd et al., 2002).

The secretion of active endoglucanases in *C. reinhardtii* into the solid media was firstly observed within Congo red plate assays (Teather and Wood, 1982), where the degradation of the water-soluble cellulose derivative carboxymethyl cellulose (CMC) **[3**, Fig. 1**]** indicated the existence of an enzymatic system for extracellular cellulose digestion in *C. reinhardtii*. Growth experiments under different physiological conditions (high, low and very low CO_2) **[3**, Fig. 2**]**, suggested a special role of cellulosic material as an alternative carbon source. Under very low CO_2 conditions, where *Chlamydomonas* showed the highest specific growth rate increase caused by CMC addition, the growth rate of *Chlorella kessleri* was unaffected, indicating that *Chlorella* does not secrete CMCases **[3**, Fig. 1A, 2B**]**, although this species does contain a cellulosic cell wall and cellulase genes in its genome (Braun and Aach, 1975).

Zymographic and mass spectrometric analysis of the secreted hydrolytic enzymes in the culture supernatant have led to the identification of two highly similar proteins CrCel9B (UniProtKB A8JFG8) and CrCel9C (UniProtKB A8JFH1) **[3**, Fig. 1B, C**]**, which both belong to glycoside hydrolase family 9 (GHF9) and are highly homologous to metazoan cellulases in particular termites. Homology-based modeling (Roy et al., 2010) identified the GHF9 endo/ exocellulase CelE4 from *T. fusca* (Sakon et al., 1997) as a structural homolog of CrCel9B and endoglucanase AaCel9A from *A. acidocaldarius* (Eckert et al., 2009) as a homolog of CrCel9C **[3**, Fig. S1 and Table S1**]**. The enzymes of *C. reinhardtii* harbor an additional unknown type of module at either the C- (CrCel9B) or N-terminus (CrCel9C) in contrast to endogenous termite endoglucanases (Watanabe et al., 1998), which typically have only a catalytic GHF9 domain **[3**, Fig. S1]. Moreover, the enrichment of the algal cellulases via purification on Avicel indicates a cellulose-binding function of the extra modules in CrCel9B/C, since efficient binding to microcrystalline cellulose requires binding modules in addition to the catalytic domain (Irwin et al., 1998). Furthermore the analysis of the hydrolysis products, derived from the *in vitro* hydrolysis assay on different cellulosic substrates (King et al., 2009) using cell-free crude supernatant-enzymes by thin layer chromatography (TLC), displayed the production and an enrichment of cellodextrins (cellotriose, cellotetraose and cellopentaose, G3-G5) as well as cellobiose (G2), but no accumulation of glucose **[3**, Fig. 3A**]**, suggesting an exo/endoglucanase activity of the secreted proteins. Moreover, there was neither hydrolytic activity nor digestion visible for the *C. kessleri* CMC-culture supernatants (Table 1, **[3**, Fig. 3A, *C.k.***]**).

The specific cellulolytic activity of the secreted enzymes from *C. reinhardtii,* induced by the presence of CMC, was determined to be at 0,036 ± 0,001 U/mg crude secreted proteins for CMC, 0,0048 ± 0,0008 U/mg for Avicel, and 0,0056 ± 0,0008 U/mg for filter paper,

respectively. These values are comparably low to the specific activities of other organisms (Table 1), which are considered to be specialists on hydrolytic digestion of cellulosic material (Li and Calza, 1991; Ng and Zeikus, 1981; Robson and Chambliss, 1984; Spiridonov and Wilson, 1998). Then again, other organisms, such as the aerobic bacterium *Pseudomonas fluorescens*, display with 0,029 IU/mg crude protein more comparable activities to *C. reinhardtii* (Bakare et al., 2005).

Table 1. The specific cellulolytic activity of the crude supernatants from *C. reinhardtii* and *C. kessleri* cultures. *In vitro* hydrolysis assay was performed using cell-free supernatant from the cultures grown under CO_2-limiting conditions supplemented with 0.1 % CMC, in addition of an antibiotic (25µg/ml kasugamycin (Day et al., 1990)) together with a fungicide (5µg/ml carbendacime (Mahan et al., 2005)) to exclude any types of contamination. The specific activity of the crude supernatant-enzymes toward the specific substrate was determined via DNS-assay (Ghose, 1987; King et al., 2009; Miller, 1959). The specific activities from other organisms are presented for comparison. (* Na-CMC, **CMC 7HS)

species	organism	specific activity U/mg crude enzyme		
		CMC	Avicel	filter paper
green algae	*Chlamydomonas reinhardtii*	0.036 ± 0.001	0.0048 ± 0.0008	0.0056 ± 0.0008
green algae	*Chlorella kessleri*	ND	ND	ND
anaerobic fungus (Li and Calza 1991)	*Neocallimastix frontalis* EB188	7,76	0,13	0,07
aerobic fungus (Ng and Zeikus 1981)	*Trichoderma reesei* QM9414	7.86* 46.6**	0,37	0,34
aerobic bacteria (Spiridonov and Wilson 1998)	*Thermomonospora fusca*	3,14	-	0,23
aerobic bacteria (Robson and Chambliss 1984)	*Bacillus subtilis* DLG	6,45	ND	0,05
anaerobic bacteria (Ng and Zeikus 1981)	*Clostridium thermocellum* LQRI	4.63* 21.3**	0,09	0,05

An explanation for these findings could be that the main task of the green alga *C. reinhardtii*, which features many bacterial and animal genes (Merchant et al., 2007), lies in the photosynthetic activity, so that the capability of hydrolytic digestion of cellulose and cellulose-like materials may be just of an additive manner. Additionally, *C. reinhardtii* secretes many proteins into the external media under low as well as under high CO_2-conditions **[3**, Fig. 1C, input**]** (Baba et al., 2011), so that it is hard to say, what proportion of the crude supernatant is represented by the cellulases. Further studies with isolated cellulases are required to define the real specific activity and functional capabilities of these enzymes.

The ability of *Chlamydomonas reinhardtii* to degrade soluble (CMC) and crystalline (filter paper, Avicel) cellulosic substrates to small cellodextrins and cellobiose allows the only conclusion, that the secretome of *C. reinhardtii* contains an endo-/exoglucanase system or at least one processive endoglucanase. In addition, the metabolomic data regarding the import of cellobiose **[3**, Fig. 3B**]**, might explain, why in contrast to *Chlorella kessleri*, this green microalga does not possess (or need) a hexose uptake system (Doebbe et al., 2007; Harris, 2009; Sager and Granick, 1953). The uptake of cellobiose and cellodextrins is often accomplished by inducible membrane transporter systems, however, so far only a few cellobiose transporters have been identified belonging to the MFS (major facilitator superfamily) (Galazka et al., 2011) or ABC (ATP-binding cassette) transporter family (Nataf et al., 2009) and members of both are encoded by the *Chlamydomonas* nuclear genome (e.g. UniProtKB A8J114/A8ILE4).

2.2. The origin of cellulolytic enzymes in *Chlamydomonas reinhardtii*

It is well known that plants, some bacteria, fungi, protozoa, and sea squirts, which synthesize cellulose, should also be able to degrade or modify it during growth and development (Gilbert, 2010). However, the existence of cellulase encoding genes in the genome of *Chlamydomonas* is of particular scientific interest, since it cannot be explained by the requirement of cell wall rearrangement, because the cell wall of this organism is solely composed of hydroxyproline-rich glycoproteins (Adair and Snell, 1990; Horne et al., 1971; Miller et al., 1972). *In silico* analysis of amino acid sequences of the identified hydrolytic enzymes (CrCel9B/ CrCel9C) **[3**, Fig. 1B, C**]** revealed that these proteins belong to glycoside hydrolase family 9 (GHF9/subgroup E2) and their catalytic domains show a high homology to metazoan endo-β-1,4-glucanases (Nozaki et al., 2009). There are two distantly related families of the GHF9 gene: subgroup E1 is confined to bacteria (Tomme et al., 1995), whereas subgroup E2 has been found in bacteria, Dictyostelium, termites and other Metazoa (Tokuda et al., 1999; Tomme et al., 1995). Presumable explanations for this high homology between the bacterial, green algal and the metazoan cellulolytic genes could be either a common ancient ancestor or an acquirement by horizontal gene transfer from prokaryotes. Since the degradation of cellulosic material is usually to a large part carried out by bacteria, fungi, and protozoa, most famously as commensals in the guts of herbivorous animals, it is generally assumed (Morris, 2003) that most animals are unable to digest cellulose and that the exceptions in termites and nematodes have acquired their cellulolytic endoglucanases by horizontal gene transfer from prokaryotes (Dehal et al., 2002; Mayer et al., 2011; Smant et al.,

1998). However, the presence of the cellulases in diverse animals, plants and even green algae could alternatively mean that they are derived from genes in an ancient ancestral eukaryote and have persisted only in some metazoan lineages (Davison and Blaxter, 2005; Lo et al., 2003; Matthysse et al., 2004; Yan et al., 1998). .

Endogenous GHF9 genes were reported in the animal phyla Arthropoda (Dismukes et al., 2008; Nozaki et al., 2009; Watanabe et al., 1998), the Mollusca (Suzuki et al., 2003) and Chordata (Dehal et al., 2002), and they also have a wide distribution in angiosperms (flowering plants). In addition they also have been discovered in some fungi (Steenbakkers et al., 2002) and a single amoebozoan (Dictyostelium discoideum; (Libertini et al., 2004)). The phylogenetic analyses of the catalytic domain of the *C. reinhardtii* GHF9-cellulases (Figure 12) has led to the conclusion that the existence of these enzymes in the algal genome might probably be due to a common ancestor, since the cellulase genes of the prokaryotic and eukaryotic species seem to appear in a monophyletic clade (Dupuis, 1984; Envall et al., 2008). However, the results of the similarity searches could be affected by skewed or insufficient sampling because matching homologues from related species (e.g. other green algae) are not yet in the databases or even sequenced. Besides the cellulase from *C. reinhardtii*, only one algal cellulase from *Volvox carteri* (UniProt KB D8UIZ8) appeared in the BLAST-search and initial alignment, probably due to the lack of completely sequenced algal genomes. Although, the phylogenetic tree for GHF9 cellulases, presented here (Figure 12), seems to be poorly resolved, the results are entirely consistent with the model already suggested by Davison and Blaxter, where they proposed that the glycoside hydrolase family 9 genes are present in at least five metazoan phyla, and the monophyly of the metazoan GHF9 genes in the phylogeny suggests a single, ancient origin (Davison and Blaxter, 2005). Lo et al. used in an earlier work the intron positional evidence to argue that GHF9 subgroup E2 genes from termites, abalone, and sea squirt have an ancient, common origin (Lo et al., 2003). Moreover, GHF9 genes from the Viridiplantae and Metazoa are monophyletic, with high support, which is implicating an origin for the gene in an ancient eukaryote (Davison and Blaxter, 2005). Additionally, even when the catalytic domains of the GHF9-members are very similar, there are differences between them in terms of the cellulose-binding domain (CBM). For instance, in contrast to the endogenous glycoside hydrolase of termites (Watanabe et al., 1998), which typically contain only a catalytic GHF9 domain (Fig. S1, NtEG) the algal enzymes possess an additional unknown type of cellulose-like-binding module at either the C- (CrCel9B) or N-terminus (CrCel9C).

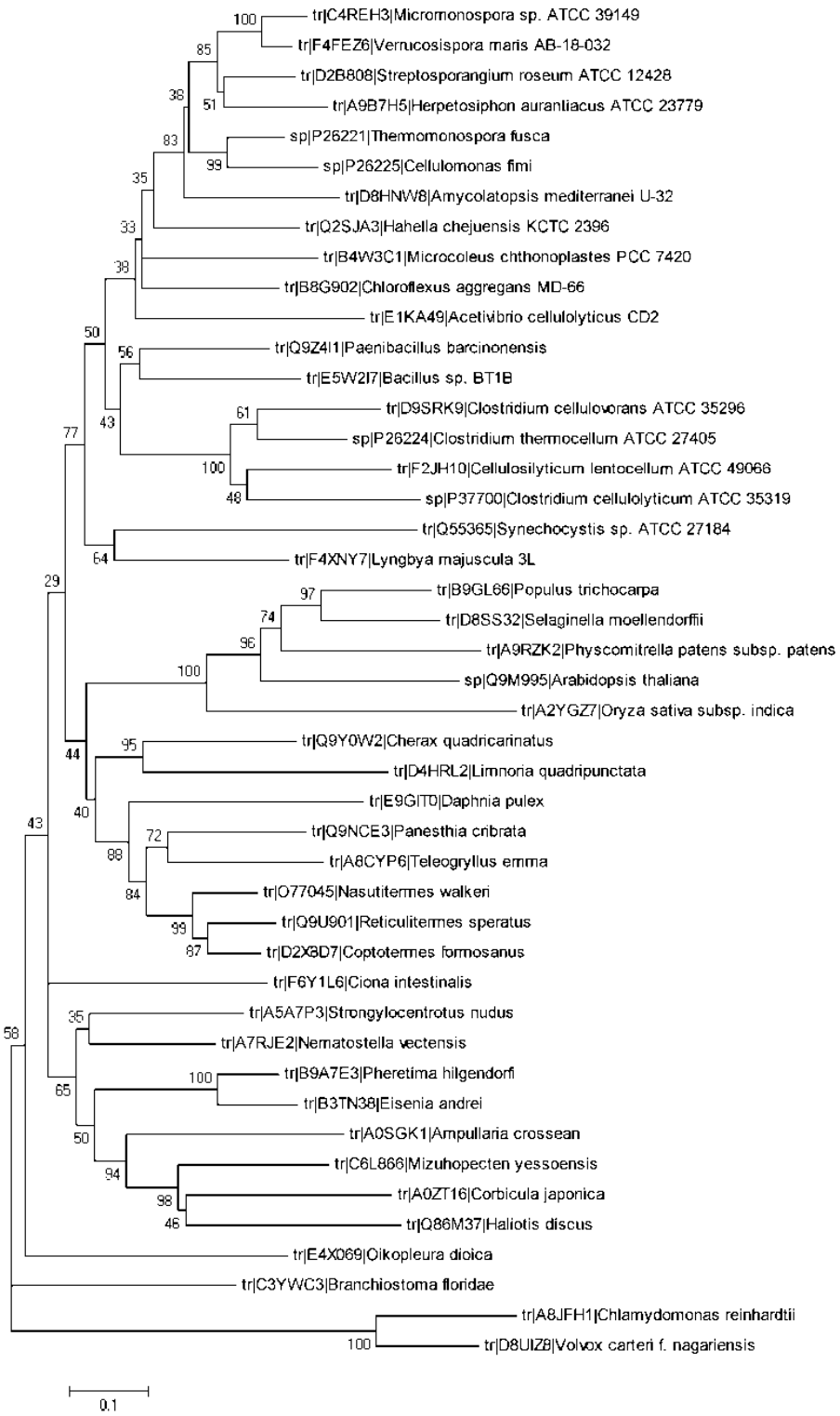

Figure 12: Unrooted phylogenetic tree of 45 GHF9-members based on amino acid sequences of the catalytic domain (293 characters, UniProt KB numbers are shown next to each enzyme). Initial alignment was made in Clustal W (Thompson et al., 1994) and positions containing gaps and missing data were excluded from the analysis. The evolutionary history was inferred using the Neighbor-Joining method (Saitou and Nei, 1987). The bootstrap consensus tree inferred from 1000 replicates is taken to represent the evolutionary history of the taxa analyzed (Felsenstein, 1985), whereas branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the JTT matrix-based method (Jones et al., 1992) and are in the units of the number of amino acid substitutions per site. Evolutionary analyses were conducted in MEGA5 (Tamura et al., 2011).

In summary, the monophyly and diversity of eukaryotic glycoside hydrolases of the family 9, and the corresponding rareness of the GHF9 subgroup E2 in prokaryotes, in conjunction with the conserved intron position between plants and animals (Davison and Blaxter, 2005; Lo et al., 2003), represent implicative evidence for the presence of an ancestral GHF9 cellulase gene in an early eukaryote, predating the divergence between eukaryotic kingdoms.

2.3. Relevance of cellulolytic enzymes of *C. reinhardtii* in its natural habitats

According to the observations gained during this work, the unicellular green microalga *Chlamydomonas reinhardtii* is capable of GHF9 endo-ß-1,4-glucanases secretion, which results in digestion of exogenous cellulose, followed by cellobiose (cellodextrin) uptake and assimilation **[3]**. Cellulosic substrates like CMC, Avicel and filter paper (FP) were shown to be digested to cellodextrins *in vitro* as well as *in vivo* **[3**, Fig.3**]** (Table 1), yielding higher growth rates during cultivation **[3**, Fig.3**]**. However, substrates, like the water-soluble cellulose derivative CMC or insoluble microcrystalline Avicel cellulose do not exist in nature, or are relatively rare like filter paper, which is produced from pure cotton cellulose. Natural cellulosic substrates also referred to as lignocellulose include wood, grass, forestry waste, agricultural residues, and municipal solid waste and are composed of three types of biopolymers – cellulose, hemicellulose and lignin (Martinez et al., 2005; Perez et al., 2002). These macromolecules are strongly intermeshed and chemically bonded by non-covalent forces and covalent cross-linkages in native substrates that hinders their biodegradation and require for a successful fragmentation a battery of hydrolytic or oxidative enzymes (Bayer et al., 2006; Eriksson et al., 1990; Pauly and Keegstra, 2008; Perez et al., 2002; Serpa and Polikarpov, 2011). Whereas cellulose makes up about 45-50 % of the total dry weight of wood, hemicellulose represented by up to 25-30 % (Jeffries and Kurtzman, 1994) and demands for its degradation many different hydrolytic enzymes (hemicellulases) including xylanases, mannanases, and galactosidases (Bayer et al., 2006; Perez et al., 2002). According to a BLAST survey the genome of *C. reinhardtii* contain, not only the already described candidate glycoside hydrolases of the families 1 and 9 (GHF 1: CrBGl1 and CrBGl2; GHF 9: CrCel9B, CrCel9C and CrCel9D [3, Table S1]), but also 15 additional families of glycoside hydrolases (Table 2). The annotated genes belong to different families and open up the possibility of the digestion not only of cellulose but also of other substrates, for instance, hemicellulose via functional enzymes with ß-xylosidase- (GHF 3), α/ß-mannosidase- (GHF 5, 38 and 47) and α/ß-galactosidase (GHF 2 and 31) – activities (Table 2).

Table 2. A BLAST survey of the *C. reinhardtii* nuclear genome revealed the existence of several glycoside hydrolases. UniProtKB data base accession numbers (UniProtKB) as well as the Phytozome 7.0 locus names (Phytozome7.0) are given for available protein/gene annotations. Glycoside hydrolases family (GHF) classification was performed using the Carbohydrate-Active Enzymes (CAZy) server (Cantarel et al., 2009; CAZy; Henrissat, 1991; Henrissat and Davies, 1997).

GHF	possible function	candidates in *C. reinhardtii*	
		Phytozome	UniProtKB
GHF1	ß-glucosidase (EC 3.2.1.21)	Cre07.g320850	A8I718
	Thioglucosidase (EC 3.2.1.147)	Cre03.g171050	A8IEM7
GHF2	ß-galactosidase (EC 3.2.1.23)	Cre08.g379450	A8IZ85
GHF3	ß-xylosidase (EC 3.2.1.37)	Cre19.g752200	-
GHF5	ß-mannosidase (EC 3.2.1.25) mannan endo-β-1,4-mannosidase (EC 3.2.1.78)	Cre01.g048350	A8JCF5
		Cre02.g098000	A8I258
		Cre07.g343950	A8ITT2
		Cre12.g492800	A8JDR6
		Cre02.g141600	A8J0P1
GHF9	endo-1,4-ß-glucanase (EC 3.2.1.4) cellobiohydrolase (EC 3.2.1.91)	Cre17.g730550	A8JFH1
		Cre17.g730600	A8JFG8
		Cre06.g270500	A8HW25
GHF13	α-amylase (EC 3.2.1.1)	Cre08.g362450	A8J4D3
		Cre08.g385500	A8IYY5
GHF14	ß-amylase (EC 3.2.1.2)	Cre06.g307150	A8IMV2
		Cre06.g270350	A8HW34
		Cre01.g044100	A8HMV0
GH16	endo-ß-1,3-glucanase (EC 3.2.1.39) endo-ß-1,3-1,4-glucanase (EC 3.2.1.6)	Cre02.g115950	A8I2Q2
		Cre03.g170700	A8IEP7
		Cre03.g173100	A8IED3
GHF18	chitinase (EC 3.2.1.14) endo-β-N-acetylglucosaminidase (EC 3.2.1.96)	Cre02.g112400	A8I335
		Cre05.g233200	-
		Cre03.g200650	A8IXP6
		Cre13.g570700	-
		Cre07.g317250	A8I6I8
GHF20	ß-N-acetylhexosaminidase (3.2.1.52)	Cre22.g762950	-
GHF31	α-galactosidase (EC 3.2.1.22) α-glucosidase (EC 3.2.1.20) α-1,3-glucosidase (EC 3.2.1.84)	Cre01.g026250	A8HQ67
		Cre03.g194700	A8IWM1
		Cre03.g190500	A8IRW5
GHF32	ß-fructofuranosidase (EC3.2.1.26)	Cre12.g488050	A8ILB4
		Cre12.g488000	A8ILB2
GHF37	α, α-trehalase (EC:3.2.1.28)	Cre03.g195600	A8IWP9
GHF38	α-mannosidase (EC 3.2.1.24)	Cre10.g437950	A8IG58
GHF47	1,2-α-mannosidase (EC:3.2.1.113)	Cre07.g336600	A8JCH8
GHF63	Mannosyl-oligosaccharide glucosidase (EC 3.2.1.106)	Cre13.g579750	A8HTR3
GHF81	endo-1,3(4)-ß-glucanase (EC 3.2.1.39)	Cre12.g513400	A8JHA2
GHF85	endo-ß-N-acetylglucosaminidase (EC 3.2.1.96)	Cre13.g582250	-

Although not surprising, α/ß-amylases genes were also detected, which are associated with the starch-degrading pathways and well known in *C. reinhardtii*, since starch synthesis and degradation are of potential interest in this alga (Ball et al., 1990; Chochois et al., 2009;

Kruse et al., 2005). Furthermore, the existence of several genes, involved in the chitinolytic degradation (chitinases GHF 18 and endo-ß-N-acetylglucosaminidases GHF 18 and 85), suggests an additional possible organic carbon source, since it was already shown by Park et al., that *C. reinhardtii* is capable of chitin digestion, however the responsible enzymes still need to be characterized (Park et al., 2008). Another interesting glycoside hydrolase family contains genes such as ß-fructofuranosidases (GHF 32), which were shown to be involved in the hydrolysis on fructo-oligosaccharides, consisting of β-2,1-linked fructose to sucrose, and naturally contained in artichoke tubers (Bacon and Edelman, 1951) chicory roots (Ende and Laere, 1996) and burdock roots (Abe et al., 2009; Ishiguro et al., 2009). However, the aerobic degradation of lignocellulosic component lignin requires in addition to oxygen, coenzymes, metals and complexing agents an array of enzymes (e.g. lignin peroxidases (EC 1.11.1.14), manganese peroxidase (EC 1.11.1.13), versatile peroxidases (EC 1.11.1.16) as well as laccases (EC 1.10.3.2)) (Hammel and Cullen, 2008; Hatakka, 1994; Martinez et al., 2005; Munk and Dersch, 2008; Perez et al., 2002). However, so far no enzymes were found in *C. reinhardtii*, known to be involved in the degradation of lignin.

In summary, this large portfolio of hydrolytic enzymes present in the green alga, many of them probably targeting the polysaccharides of the plant cell wall (Bayer et al., 2006), indicates that the cellulose source, digested by *C. reinhardtii* in nature, may be of plant origin. The ability of the green alga *Chlamydomonas reinhardtii* to grow on complex substrates was observed by cultivating the cells on poplar wood, grown without any CO_2 supply or other organic carbon sources than wood in a tightly closed petri dish. The resulting algal carpet (Figure 13), seen after 65 days provides further evidence for the capability of *C. reinhardtii* for mixotrophic assimilation of complex cellulosic substrates as an external carbon source.

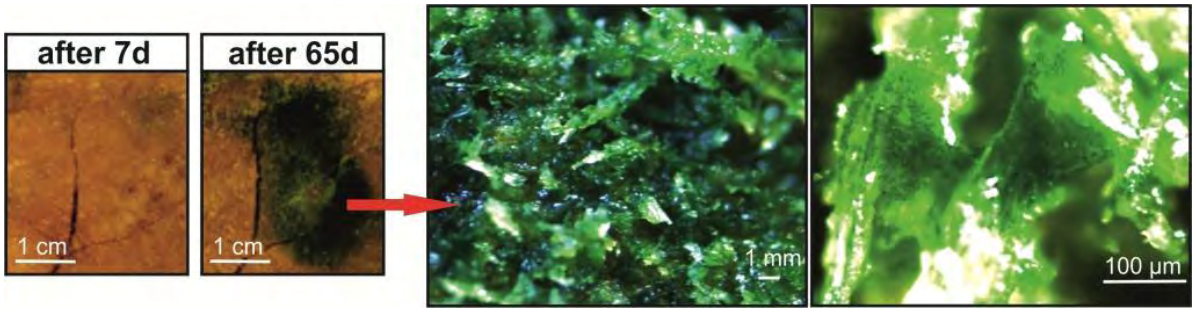

Figure 13: *C. reinhardtii* secretes cellulolytic active enzymes for cellulose degradation and utilizes the hydrolysis products for growth. (A) Growth of *C. reinhardtii cc124* cells on autoclaved poplar wood in a tightly closed petri dish. The wood was inoculated $1x10^5$ cells and the growth under continuous white light (100 µmol m^{-2} s^{-1}) without any CO_2 was monitored for 65 days.

Further research should address the degradation of lignocellulose and the participation of the different hydrolytic enzymes in this process. The fact that enzymes involved in

lignolysis have not been identified in *C. reinhardtii* thus far, does not challenge the observed utilization of wood-derived cellulose, since the sawing of wood increases the accessibility of the lignin-free polysaccharides and resembles a sort of a pretreatment, known to be required in the industrial degradation of lignocellulose (Mosier et al., 2005; Zhu et al., 2010). Additionally, a significant part of wood is derived from non-lignified tissues (e.g. parenchyma and collenchyma) (Berg and McClaugherty, 2008; Wei et al., 2009), which is also accessible without the aid of delignifying enzymes. The identification and characterization of all secreted glycoside hydrolases involved in the degradation of cellulose and hemicellulose needs to be performed. Moreover, the transport and the metabolic assimilation of the hydrolysis products need to be investigated.

2.4. Optimized cellulolytic/lignocellulolytic degradation for biotechnological use

Plant biomass represents a renewable energy source, which is a result of solar energy conversion during the process of photosynthesis. Photosynthetic fixation of CO_2 yields more than 10^{11} tons of dry plant material worldwide per year (Berner, 2003; Malhi, 2002; Schlesinger, 1991; Zhang and Lynd, 2004) consisting to 40-50 % of cellulose, 15-35 % hemicellulose and 10–30 % lignin (Bayer et al., 2006). Together, these substances represent a composite material of rigid cellulose fibers embedded in a cross-linked matrix of lignin and hemicellulose and minor components of the plant cell wall such as proteins, lipids, soluble sugars and minerals, that bind the fibers (Eriksson et al., 1990; Gilbert, 2010; Mohnen, 2008; Pauly and Keegstra, 2008). Despite its recalcitrance, plant biomass is considered a promising feedstock provided that strategies can be devised, which enable the conversion of cell wall polysaccharides into fermentable sugars (Buckeridge and Goldman, 2011; Himmel and Bayer, 2009; Sticklen, 2008). Although a number of microorganisms, including fungi and bacteria, have the capacity to degrade plant biomass, most commercially available enzymes are currently produced by genetically engineered strains of filamentous fungi *Trichoderma reesei* (*Hypocrea jecorina*) and *Aspergillus niger* (Cherry and Fidantsef, 2003; Kirk et al., 2002). However, since a significant fraction of the biofuel production costs is determined by the efficiency of biomass degradation, the hydrolytic activities of individual enzymes should therefore be enhanced. Knowledge of the molecular mechanisms underlying enzymatic hydrolysis of cellulosic feedstocks is one of the important parts of developing cost-effective processes for biomass to biofuel conversion.

The green alga *Chlamydomonas reinhardtii* is an unicellular photosynthetic organism with a fully sequenced genome (Merchant et al., 2007) that is nowadays, like many other

microalga considered to be a potential producer of an alternative energy resource in the form of biomass. It produces bio-hydrogen, lipids, proteins and carbohydrates in large amounts over a short period of time, which can be processed into biofuels and value-added products (Brennan and Owende, 2010; Kruse and Hankamer, 2010; Morweiser et al., 2010; Mussgnug et al., 2010). Additionally, according to the data provided in this work, *C. reinhardtii* is capable of cellulose degradation and assimilation of the breakdown-products like cellobiose **[3]** for mixotrophic growth. There is also the possibility that this microalga is also capable of hemicellulose or maybe even lignocellulose degradation, however, this hypothesis needs to be elucidated. Furthermore, the hydrolytic activities observed *in vitro* when *C. reinhardtii* crude supernatants were used (Table 1), appeared to be low in comparison to the best-performers in cellulose degradation, and therefore need to be enhanced in order to be competitive.

Moreover, it is generally assumed, that the primary challenge in biomass conversion to biofuels is to achieve yields that make it cost-competitive with the current fossil-based fuels (Dashtban et al., 2009; Martinez et al., 2005; Perez et al., 2002). Cellulose in the plant cell wall is not readily available to enzymatic hydrolysis because of the low accessibility of (micro-) crystalline cellulose fibers and the presence of lignin (mainly) and hemicellulose on the surface of cellulose, which prevents cellulases from accessing the substrate efficiently (Zhang et al., 2007; Zhang et al., 2006). Thus, pretreatment of lignocellulosic residues before hydrolysis is required, using high temperatures and acid, which is expensive, slow and inefficient (Rubin, 2008) and decreases the overall fermentation yields because of the released inhibitors such as weak acids, furan and phenolic compounds during this process (Palmqvist and Hahn-Hagerdal, 2000). Some of these problems could be overcome by applying microorganisms such as aerobic fungi or bacteria. An alternative option could be represented by the application of genetically manipulated green algae *C. reinhardtii* (Kindle, 1990; Mayfield and Kindle, 1990), which contain an aerobic lignin degrading system in addition to efficient cellulose and hemicellulose degradation activity.

3. Bioenergy concept – an outlook

The recognition of the increasing energy demand, limited fossil fuel sources and the threat of anthropogenic global warming has created an enormous effort in the development of renewable energy sources (IPCC, 2007; Martinot et al., 2007; REN21, 2011; Verbruggen and Al Marchohi, 2010). Most systems used nowadays, for instance, photovoltaic, solar thermal, nuclear and wind power, mainly produce electricity, but the current energy request accounts two-third as fuel (Rifkin, 2002).

The use of plant biomass, obtained by the photosynthetic process, which harnesses sunlight in order to convert carbon dioxide into energy-rich fermentable compounds, is generally regarded a resource alternative to fossil fuels (Brennan and Owende, 2010; Chisti and Yan, 2011; Costa and de Morais, 2011; Dragone et al., 2010; Singh et al., 2011; Stephens et al., 2010b). Microalgae can grow on non-arable land using saline or waste water and produce lipids, proteins and carbohydrates in large amounts over short periods of time, which can be processed into biofuels (e. g. biodiesel, bio-ethanol, methane, hydrogen) and valuable co-products (Brennan and Owende, 2010; Gouveia, 2011; Gouveia and Oliveira, 2009; Kruse and Hankamer, 2010; Li et al., 2008; Mata et al., 2010; Posten and Schaub, 2009; Rupprecht, 2009; Schenk et al., 2008; Scott et al., 2010). Nevertheless some bottlenecks remain to a marketable application of microalgae, since these organisms are not naturally adapted to be efficient biomass-producers (Douskova et al., 2009; Fon Sing et al., 2011; Kumar et al., 2010).

Even though, the use of microalga as a sustainable feedstock for the production of biodiesel and biogas as well as for nutraceutical and pharmaceutical purposes is of great interest (Perez-Garcia et al., 2011; Vazhappilly and Chen, 1998; Xu et al., 2006), but it requires a highly effective cultivation modes, low production costs and the efficient conversion of light energy into biomass and product (Norsker et al., 2011). At the present time, microalgal biomass has mainly been produced by photoautotrophic cultivation, where the production rate is directly correlated to the photon conversion efficiency of the system. Fluctuating light conditions as well as fluorescence and heat losses (up to 80 %) under highlight radiation (Kruse et al., 2005; Prince and Kheshgi, 2005; Zhu et al., 2008) limit biomass productivity. The productivity is also limited by impaired light diffusion due to strong light absorbance of the outmost layers of the mass culture (Melis, 2009). These limitations are conquerable by the development and design of special photo-bioreactor (PBR) systems (Morweiser et al., 2010) as well as by the generation of engineered algal strains with truncated antenna systems. In order to avoid the waste of light energy and to increase the light penetration in the PBR by photoautotrophic cultivation, algal mutants with small or reduced antenna size can be used, obtained, for instance, by overexpression of permanently active NAB1 (Beckmann et al., 2009; Melis, 1978; Mussgnug et al., 2007; Mussgnug et al., 2005). Therefore, the deeper understanding of the molecular mechanisms behind the regulation of antenna size and composition in *C. reinhardtii* **[1, 2]**, gained during this work is of great biotechnological relevance and interest, and can be used in future approaches for the creation of high biomass producing mutants.

To ensure a sufficient carbon supply for varying light intensities, mixotrophic cultivation conditions might be helpful, using an organic carbon source such as cellulose. Agronomic residues such as corn stover, sugarcane waste, wheat or rice straw, forestry, and paper mill discards, the paper portion of municipal waste and dedicated energy crops, also contain cellulose in large quantities, which can be made available for the production of biomass (or biofuel) (Lin and Tanaka, 2006). The capability of *C. reinhardtii* to hydrolyse and assimilate cellulosic material, which has been described in this work **[3]**, could be employed for mixotrophic cultivation. The production of algal biomass using mixotrophic cultivation, for which growth rates were postulated to reach the sum of the growth rate in the photoautotrophic and heterotrophic cultures (Blackwell and Ceman, 2011; Endo et al., 1977; Marquez et al., 1993; Ogawa and Aiba, 1981), seems to be more profitable than just the heterotrophic (Isleten-Hosoglu et al., 2012; Perez-Garcia et al., 2011; Vazhappilly and Chen, 1998; Xu et al., 2006). Therefore, feeding of cellulose could result in a mixotrophic approach with significant increase of biomass production rates. Additionally, the use of cellulosic material could represent a much cheaper alternative to glucose in bio-hydrogen production (Doebbe et al., 2007).

Altogether, these findings should have implications for the use of *C. reinhardtii* and other microalgae within bio-refinery concepts. An approach that potentially combines the benefits described within this PhD thesis, of reduced algal light-harvesting antenna and mixotrophic cultivation in one PBR (photo-hydrocell-bioreactor [PHC-BR]) is presented in Figure 14.

In this operation unit the conversion of solar energy could be joined by the simultaneous assimilation of cellulosic material, due to the fact that hydrolytic reactions take place in the supernatant, which are independent of light, the input/storage and degradation of cellulosic maternal can be done in the dark phase of the bioreactor (Figure 14). The separator of the dark and light phases has the function to hold back non-hydrolyzed material in the dark, so that the effect of light reflection on these particles does not decrease light penetration in the light phase of the PHC-BR. The additional reactor volume could be positively reflected in PBR costs, because of lower surface to volume ratio and therefore lower material costs per liter reactor volume (Janssen et al., 2003). Because of the higher light penetration and the benefits of the mixotrophic cultivation, an increase in cell density, and hence higher biomass concentration could be achieved, thus reducing the costs for harvesting and concentration of biomass for further processing.

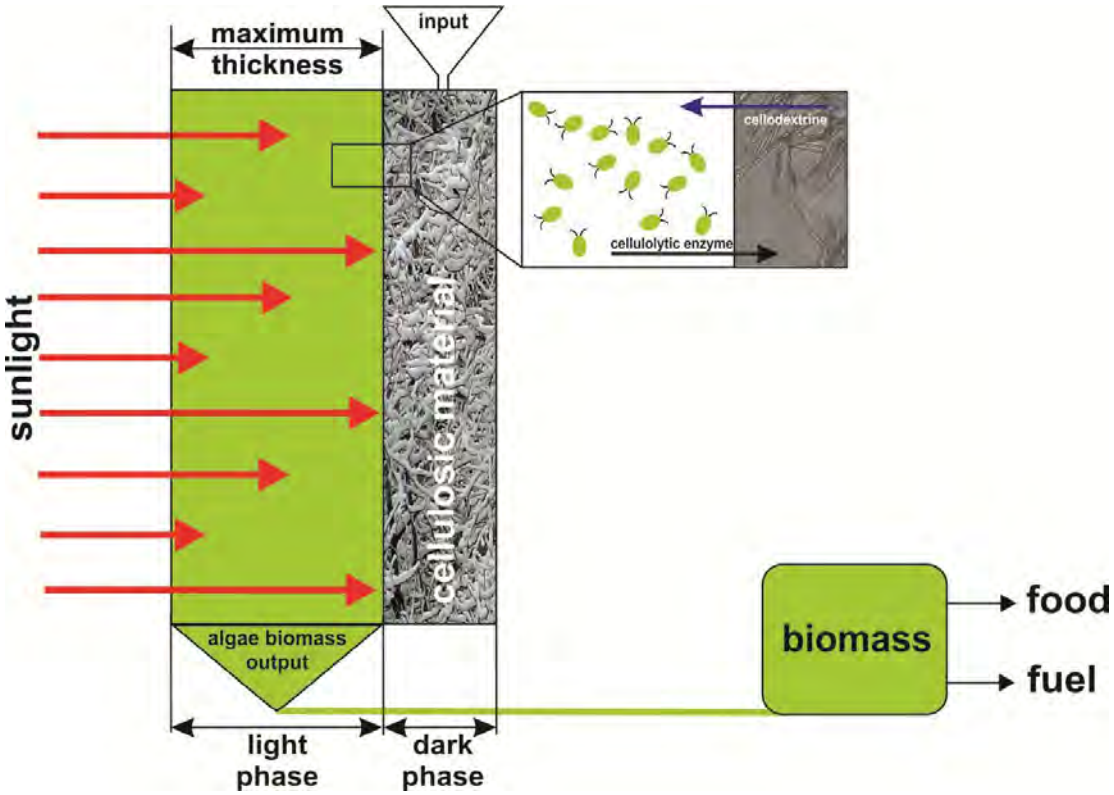

Figure 14: A model of a combined photo-hydrocell-bioreactor (PHC-BR). The bioreactor is fractionated with fine mash separator in two parts the light phase and dark phase. Input of fertilizer and cellulosic material is accomplished on top direct into dark phase. The algae biomass is harvested on the bottom of light phase reactor.

In summary, the combined algal photo- and hydrolysis bioreactor PHC-BR could represent a promising tool for the achievement of high biomass productivities to cope with the future challenges such as lack of food (for animal or human) or fossil fuels. This system provides the possibility of the simultaneously use of sunlight for CO_2 fixation (greenhouse gas) and hydrolysis and assimilation of cellulosic or lignocellulosic material to produce a high value biomass.

IV. References

Abdul-Manan, N. and Williams, K. R. (1996). hnRNP A1 binds promiscuously to oligoribonucleotides: utilization of random and homo-oligonucleotides to discriminate sequence from base-specific binding. *Nucleic Acids Research* **24**, 4063-70.

Abe, M., Ueno, K., Ishiguro, Y., Omori, T., Onodera, S. and Shiomi, N. (2009). Purification, cloning and functional characterization of fructan: Fructan 1-fructosyltransferase from edible burdock (*Arctium lappa* L.). *Journal of Applied Glycoscience* **56**, 239-246.

Adair, W. S. and Snell, W. J. (1990). The *Chlamydomonas reinhardtii* cell wall: structure, biochemistry and molecular biology. In *Organization and Assembly of Plant and Animal Extracellular Matrix*, (ed. R. P. Mecham and W. S. Adair), pp. pp. 15 – 84 Orlando: Academic Press.

Adamska, I., Kruse, E. and Kloppstech, K. (2001). Stable insertion of the early light-induced proteins into etioplast membranes requires chlorophyll a. *J Biol Chem* **276**, 8582-7.

Alergand, T., Peled-Zehavi, H., Katz, Y. and Danon, A. (2006). The chloroplast protein disulfide isomerase RB60 reacts with a regulatory disulfide of the RNA-binding protein RB47. *Plant Cell Physiol* **47**, 540-8.

Aletta, J. M. and Hu, J. C. (2008). Protein arginine methylation in health and disease. *Biotechnol Annu Rev* **14**, 203-24.

Allen, J. F. (1992). Protein phosphorylation in regulation of photosynthesis. *Biochim Biophys Acta* **1098**, 275-335.

Allen, J. F. (2003). Cyclic, pseudocyclic and noncyclic photophosphorylation: new links in the chain. *Trends Plant Sci* **8**, 15-9.

Allen, J. F., Bennett, J., Steinback, K. E. and Arntzen, C. J. (1981). Chloroplast protein phosphorylation couples plastoquinone redox state to distribution of excitation energy between photosystems. *Nature* **291**, 25-29.

Allen, J. F. and Forsberg, J. (2001). Molecular recognition in thylakoid structure and function. *Trends Plant Sci* **6**, 317-26.

Allen, J. F. and Horton, P. (1981). Chloroplast protein phosphorylation and chlorophyll fluorescence quenching. Activation by tetramethyl-p-hydroquinone, an electron donor to plastoquinone. *Biochimica et Biophysica Acta (BBA) - Bioenergetics* **638**, 290-295.

Alric, J. (2010). Cyclic electron flow around photosystem I in unicellular green algae. *Photosynth Res* **106**, 47-56.

Alric, J., Lavergne, J. and Rappaport, F. (2010). Redox and ATP control of photosynthetic cyclic electron flow in *Chlamydomonas reinhardtii* (I) aerobic conditions. *Biochim Biophys Acta* **1797**, 44-51.

Anderson, J. M., Chow, W. S. and Park, Y. I. (1995). The grand design of photosynthesis: Acclimation of the photosynthetic apparatus to environmental cues. *Photosynthesis Research* **46**, 129-139.

Anderson, P. and Kedersha, N. (2002). Stressful initiations. *J Cell Sci* **115**, 3227-34.

Andersson, B. and Anderson, J. M. (1980). Lateral heterogeneity in the distribution of chlorophyll-protein complexes of the thylakoid membranes of spinach chloroplasts. *Biochim Biophys Acta* **593**, 427-40.

Andersson, J., Wentworth, M., Walters, R. G., Howard, C. A., Ruban, A. V., Horton, P. and Jansson, S. (2003). Absence of the Lhcb1 and Lhcb2 proteins of the light-harvesting complex of photosystem II - effects on photosynthesis, grana stacking and fitness. *Plant J* **35**, 350-61.

Anten, N. P. (2005). Optimal photosynthetic characteristics of individual plants in vegetation stands and implications for species coexistence. *Ann Bot* **95**, 495-506.

Apel, K. and Hirt, H. (2004). Reactive oxygen species: metabolism, oxidative stress, and signal transduction. *Annu Rev Plant Biol* **55**, 373-99.

Arnon, D. I., Allen, M. B. and Whatley, F. R. (1954). Photosynthesis by isolated chloroplasts. *Nature* **174**, 394-6.

Aro, E. M., McCaffery, S. and Anderson, J. M. (1993). Photoinhibition and D1 Protein Degradation in Peas Acclimated to Different Growth Irradiances. *Plant Physiol* **103**, 835-843.

Asada, K. (2006). Production and scavenging of reactive oxygen species in chloroplasts and their functions. *Plant Physiol* **141**, 391-6.

Asamizu, E., Nakamura, Y., Sato, S., Fukuzawa, H. and Tabata, S. (1999). A large scale structural analysis of cDNAs in a unicellular green alga, *Chlamydomonas reinhardtii*. I. Generation of 3433 non-redundant expressed sequence tags. *DNA Res* **6**, 369-73.

Atkinson, D. E. (1977). Cellular energy metabolism and its regulation. New York: Academic Press.

Baba, M., Suzuki, I. and Shiraiwa, Y. (2011). Proteomic Analysis of High-CO2-Inducible Extracellular Proteins in the Unicellular Green Alga, *Chlamydomonas reinhardtii*. *Plant and Cell Physiology* **52**, 1302-1314.

Bachand, F. (2007). Protein arginine methyltransferases: From unicellular eukaryotes to humans. *Eukaryotic Cell* **6**, 889-898.

Bacon, J. S. and Edelman, J. (1951). The carbohydrates of the Jerusalem artichoke and other Compositae. *Biochem J* **48**, 114-26.

Badger, M. R. and Price, G. D. (2003). CO2 concentrating mechanisms in cyanobacteria: molecular components, their diversity and evolution. *J Exp Bot* **54**, 609-22.

Badger, M. R. and Spalding, M. H. (2000). CO2 acquisition, concentration and fixation in cyanobacteria and algae. In *Photosynthesis: Physiology and Metabolism.*, (ed. T. S. RC Leegood, S von Caemmerer, eds), pp. pp. 369-397. Dordrecht, The Netherlands: Kluwer Academic Publishers.

Bagni, C. and Lapeyre, B. (1998). Gar1p binds to the small nucleolar RNAs snR10 and snR30 in vitro through a nontypical RNA binding element. *J Biol Chem* **273**, 10868-73.

Bakare, M. K., Adewale, I. O., Ajayi, A. O., Okoh, A. I. and Shonukan, O. O. (2005). Regulatory mutations affecting the synthesis of cellulase in *Pseudomonas fluorescens*. *African Journal of Biotechnology* **4**, 838-843.

Ball, S. G., Dirick, L., Decq, A., Martiat, J. C. and Matagne, R. F. (1990). Physiology of Starch Storage in the Monocellular Alga *Chlamydomonas-Reinhardtii*. *Plant Science* **66**, 1-9.

Barlat, I., Maurier, F., Duchesne, M., Guitard, E., Tocque, B. and Schweighoffer, F. (1997). A role for Sam68 in cell cycle progression antagonized by a spliced variant within the KH domain. *J Biol Chem* **272**, 3129-32.

Bassi, R., Pineau, B., Dainese, P. and Marquardt, J. (1993). Carotenoid-binding proteins of photosystem II. *Eur J Biochem* **212**, 297-303.

Bassi, R., Soen, S. Y., Frank, G., Zuber, H. and Rochaix, J. D. (1992). Characterization of chlorophyll a/b proteins of photosystem I from *Chlamydomonas reinhardtii*. *J Biol Chem* **267**, 25714-21.

Bassi, R. and Wollman, F.-A. (1991). The chlorophyll-a/b proteins of photosystem II in *Chlamydomonas reinhardtii*. *Planta* **183**, 423-433.

Bateman, J. M. and Purton, S. (2000). Tools for chloroplast transformation in *Chlamydomonas*: expression vectors and a new dominant selectable marker. *Mol Gen Genet* **263**, 404-10.

Bayer, E., Shoham, Y. and Lamed, R. (2006). Cellulose-decomposing Bacteria and Their Enzyme Systems

The Prokaryotes, (ed. M. Dworkin S. Falkow E. Rosenberg K.-H. Schleifer and E. Stackebrandt), pp. 578-617: Springer New York.

Beckmann, J., Lehr, F., Finazzi, G., Hankamer, B., Posten, C., Wobbe, L. and Kruse, O. (2009). Improvement of light to biomass conversion by de-regulation of light-harvesting protein translation in *Chlamydomonas reinhardtii*. *J. Biotech.* **142**, 70-77.

Bedford, M. T. (2007). Arginine methylation at a glance. *J Cell Sci* **120**, 4243-6.

Bedford, M. T. and Clarke, S. G. (2009). Protein Arginine Methylation in Mammals: Who, What, and Why. *Molecular Cell* **33**, 1-13.

Bedford, M. T. and Richard, S. (2005). Arginine methylation an emerging regulator of protein function. *Mol. Cell* **18**, 263–272.

Bellafiore, S., Barneche, F., Peltier, G. and Rochaix, J. D. (2005). State transitions and light adaptation require chloroplast thylakoid protein kinase STN7. *Nature* **433**, 892-5.

Ben-Amotz, A. and Avron, M. (1990). The biotechnology of cultivating the halotolerant alga *Dunaliella*. *Trends in Biotechnology* **8**, 121-126.

Bennett, J. (1977). Phosphorylation of chloroplast membrane polypeptides. *Nature* **269**, 344-346.

Berg, B. and McClaugherty, C. (2008). Plant Litter: Decomposition, Humus Formation, Carbon Sequestration. Berlin New York: Springer.

Berner, R. A. (2003). The long-term carbon cycle, fossil fuels and atmospheric composition. *Nature* **426**, 323-6.

Blackwell, E. and Ceman, S. (2011). A new regulatory function of the region proximal to the RGG box in the fragile X mental retardation protein. *J Cell Sci* **124**, 3060-5.

Blackwell, E., Zhang, X. and Ceman, S. (2010). Arginines of the RGG box regulate FMRP association with polyribosomes and mRNA. *Human Molecular Genetics*, 1-10.

Boekema, E. J., Hankamer, B., Bald, D., Kruip, J., Nield, J., Boonstra, A. F., Barber, J. and Rogner, M. (1995). Supramolecular structure of the photosystem II complex from green plants and cyanobacteria. *Proc Natl Acad Sci U S A* **92**, 175-9.

Bonaventura, C. and Myers, J. (1969). Fluorescence and oxygen evolution from *Chlorella pyrenoidosa*. *Biochim Biophys Acta* **189**, 366-83.

Bonente, G., Ballottari, M., Truong, T. B., Morosinotto, T., Ahn, T. K., Fleming, G. R., Niyogi, K. K. and Bassi, R. (2011a). Analysis of LhcSR3, a protein essential for feedback de-excitation in the green alga *Chlamydomonas reinhardtii*. *PLoS Biol* **9**, e1000577.

Bonente, G., Passarini, F., Cazzaniga, S., Mancone, C., Buia, M. C., Tripodi, M., Bassi, R. and Caffarri, S. (2008). The occurrence of the psbS gene product in *Chlamydomonas reinhardtii* and in other photosynthetic organisms and its correlation with energy quenching. *Photochemistry and Photobiology* **84**, 1359-70.

Bonente, G., Pippa, S., Castellano, S., Bassi, R. and Ballottari, M. (2011b). Acclimation of *Chlamydomonas reinhardtii* to different growth irradiances. *J Biol Chem*.

Borowitzka, M. A. (2005). Cultivation of Microalgae from Village Level to Industrial Scale. *Phycologia* **44**, 11-12.

Bouvet, P. and Wolffe, A. P. (1994). A role for transcription and FRGY2 in masking maternal mRNA within *Xenopus* oocytes. *Cell* **77**, 931-41.

Branco-Price, C., Kaiser, K. A., Jang, C. J., Larive, C. K. and Bailey-Serres, J. (2008). Selective mRNA translation coordinates energetic and metabolic adjustments to cellular oxygen deprivation and reoxygenation in *Arabidopsis thaliana*. *Plant J* **56**, 743-55.

Branco-Price, C., Kawaguchi, R., Ferreira, R. B. and Bailey-Serres, J. (2005). Genome-wide analysis of transcript abundance and translation in *Arabidopsis* seedlings subjected to oxygen deprivation. *Ann Bot* **96**, 647-60.

Braun, E. and Aach, H. G. (1975). Enzymatic Degradation of Cell-Wall of *Chlorella*. *Planta* **126**, 181-185.

Brennan, L. and Owende, P. (2010). Biofuels from microalgae—A review of technologies for production, processing, and extractions of biofuels and co-products. *Renewable and Sustainable Energy Reviews* **14**, 557-577.

Brown, V., Small, K., Lakkis, L., Feng, Y., Gunter, C., Wilkinson, K. D. and Warren, S. T. (1998). Purified recombinant Fmrp exhibits selective RNA binding as an intrinsic property of the fragile X mental retardation protein. *J Biol Chem* **273**, 15521-7.

Buchan, J. R. and Parker, R. (2009). Eukaryotic Stress Granules: The Ins and Outs of Translation. *Molecular Cell* **36**, 932-941.

Buchanan, B. B., Gruissem, W. and Jones, R. L. (2000). Biochemistry and molecular biology of plants.: American society for plant biologists.

Buckeridge, M. S. and Goldman, G. H. (2011). Routes to Cellulosic Ethanol: Springer New York.

Burd, C. G. and Dreyfuss, G. (1994). Conserved structures and diversity of functions of RNA-binding proteins. *Science* **265**, 615-621.

Cantarel, B. L., Coutinho, P. M., Rancurel, C., Bernard, T., Lombard, V. and Henrissat, B. (2009). The Carbohydrate-Active EnZymes database (CAZy): an expert resource for Glycogenomics. *Nucleic Acids Res* **37**, D233-8.

Cardol, P., Alric, J., Girard-Bascou, J., Franck, F., Wollman, F. A. and Finazzi, G. (2009). Impaired respiration discloses the physiological significance of state transitions in *Chlamydomonas*. *Proc Natl Acad Sci U S A* **106**, 15979-84.

Carpio, M. A., Lopez Sambrooks, C., Durand, E. S. and Hallak, M. E. (2010). The arginylation-dependent association of calreticulin with stress granules is regulated by calcium. *Biochem J* **429**, 63-72.

CAZy. Carbohydrate-Active EnZymes (CAZy) database http://www.cazy.org/

Ceman, S., O'Donnell, W. T., Reed, M., Patton, S., Pohl, J. and Warren, S. T. (2003). Phosphorylation influences the translation state of FMRP-associated polyribosomes. *Hum Mol Genet* **12**, 3295-305.

Cerutti, H. and Casas-Mollano, J. (2006). On the origin and functions of RNA-mediated silencing: from protists to man. *Current Genetics* **50**, 81-99.

Chaikam, V. and Karlson, D. T. (2010). Comparison of structure, function and regulation of plant cold shock domain proteins to bacterial and animal cold shock domain proteins. *BMB Rep* **43**, 1-8.

Chang, Y. F., Imam, J. S. and Wilkinson, M. F. (2007). The nonsense-mediated decay RNA surveillance pathway. *Annu Rev Biochem* **76**, 51-74.

Chaudhury, A., Hussey, G. S., Ray, P. S., Jin, G., Fox, P. L. and Howe, P. H. (2010). TGF-beta-mediated phosphorylation of hnRNP E1 induces EMT via transcript-selective translational induction of Dab2 and ILEI. *Nat Cell Biol* **12**, 286-93.

Chen, F. and Johns, M. R. (1996). Heterotrophic growth of *Chlamydomonas reinhardtii* on acetate in chemostat culture. *Process Biochemistry* **31**, 601-604.

Chen, H., Xue, Y., Huang, N., Yao, X. B. and Sun, Z. R. (2006). MeMo: a web tool for prediction of protein methylation modifications. *Nucleic Acids Research* **34**, W249-W253.

Chen, Y. B., Durnford, D. G., Koblizek, M. and Falkowski, P. G. (2004). Plastid regulation of Lhcb1 transcription in the chlorophyte alga *Dunaliella tertiolecta*. *Plant Physiology* **136**, 3737-3750.

Cheng, D. H., Yadav, N., King, R. W., Swanson, M. S., Weinstein, E. J. and Bedford, M. T. (2004). Small molecule regulators of protein arginine methyltransferases. *Journal of Biological Chemistry* **279**, 23892-23899.

Cherry, J. R. and Fidantsef, A. L. (2003). Directed evolution of industrial enzymes: an update. *Current Opinion in Biotechnology* **14**, 438-443.

Chisti, Y. and Yan, J. Y. (2011). Energy from algae: Current status and future trends Algal biofuels - A status report. *Applied Energy* **88**, 3277-3279.

Chochois, V., Dauvillee, D., Beyly, A., Tolleter, D., Cuine, S., Timpano, H., Ball, S., Cournac, L. and 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.

Chow, W. S., Melis, A. and Anderson, J. M. (1990). Adjustments of photosystem stoichiometry in chloroplasts improve the quantum efficiency of photosynthesis. *Proc Natl Acad Sci U S A* **87**, 7502-6.

Christensen, S. T., Pedersen, L. B., Schneider, L. and Satir, P. (2007). Sensory Cilia and Integration of Signal Transduction in Human Health and Disease. *Traffic* **8**, 97-109.

Claus, C. (2005). Molekulare Charakterisierung der Funktion des LHCII-Translationsfaktors NAB1 in *Chlamydomonas reinhardtii*, (ed. Bielefeld: Universität BIelefeld.

Cléry, A., Blatter, M. and Allain, F. H. T. (2008). RNA recognition motifs: boring? Not quite. *Current Opinion in Structural Biology* **18**, 290-298.

Cohen, I., Knopf, J. A., Irihimovitch, V. and Shapira, M. (2005). A proposed mechanism for the inhibitory effects of oxidative stress on Rubisco assembly and its subunit expression. *Plant Physiol* **137**, 738-46.

Cohen, I., Sapir, Y. and Shapira, M. (2006). A conserved mechanism controls translation of Rubisco large subunit in different photosynthetic organisms. *Plant Physiol* **141**, 1089-97.

Collier, B., Goobar-Larsson, L., Sokolowski, M. and Schwartz, S. (1998). Translational inhibition in vitro of human papillomavirus type 16 L2 mRNA mediated through interaction with heterogenous ribonucleoprotein K and poly(rC)-binding proteins 1 and 2. *J Biol Chem* **273**, 22648-56.

Combres, C., Laliberte, G., Reyssac, J. S. and Delanoue, J. (1994). Effect of Acetate on Growth and Ammonium Uptake in the Microalga *Scenedesmus-Obliquus*. *Physiologia Plantarum* **91**, 729-734.

Cook, J. R., Lee, J. H., Yang, Z. H., Krause, C. D., Herth, N., Hoffmann, R. and Pestka, S. (2006). FBXO11/PRMT9, a new protein arginine methyltransferase, symmetrically dimethylates arginine residues. *Biochem Biophys Res Commun* **342**, 472-81.

Costa, J. A. V. and de Morais, M. G. (2011). The role of biochemical engineering in the production of biofuels from microalgae. *Bioresource Technology* **102**, 2-9.

Costanzo, G., Camier, S., Carlucci, P., Burderi, L. and Negri, R. (2001). RNA polymerase III transcription complexes on chromosomal 5S rRNA genes in vivo: TFIIIB occupancy and promoter opening. *Mol Cell Biol* **21**, 3166-78.

Coughlan, M. P. (1985). Cellulose hydrolysis: the potential, the problems and relevant research at Galway. *Biochem Soc Trans* **13**, 405-6.

Couturier, J., Jacquot, J. P. and Rouhier, N. (2009). Evolution and diversity of glutaredoxins in photosynthetic organisms. *Cellular and Molecular Life Sciences* **66**, 2539-2557.

Curtis, D., Lehmann, R. and Zamore, P. D. (1995). Translational regulation in development. *Cell* **81**, 171-8.

Dashtban, M., Schraft, H. and Qin, W. (2009). Fungal bioconversion of lignocellulosic residues; opportunities & perspectives. *Int J Biol Sci* **5**, 578-95.

Davison, A. and Blaxter, M. (2005). Ancient origin of glycosyl hydrolase family 9 cellulase genes. *Mol Biol Evol* **22**, 1273-84.

Day, A., Debuchy, R., van Dillewijn, J., Purton, S. and Rochaix, J. D. (1990). Studies on the maintenance and expression of cloned DNA fragments in the nuclear genome of the green alga *Chlamydomonas reinhardtii*. *Physiologia Plantarum* **78**, 254-260.

Day, D. A. and Tuite, M. F. (1998). Post-transcriptional gene regulatory mechanisms in eukaryotes: an overview. *J Endocrinol* **157**, 361-71.

Dehal, P., Satou, Y., Campbell, R. K., Chapman, J., Degnan, B., De Tomaso, A., Davidson, B., Di Gregorio, A., Gelpke, M., Goodstein, D. M. et al. (2002). The draft genome of *Ciona intestinalis*: insights into chordate and vertebrate origins. *Science* **298**, 2157-67.

Dekker, J. P. and Boekema, E. J. (2005). Supramolecular organization of thylakoid membrane proteins in green plants. *Biochim Biophys Acta* **1706**, 12-39.

DeLeeuw, F., Zhang, T., Wauquier, C., Huez, G., Kruys, V. and Gueydan, C. (2007). The cold-inducible RNA-binding protein migrates from the nucleus to cytoplasmic stress granules by a methylation-dependent mechanism and acts as a translational repressor. *Experimental Cell Research* **313**, 4130–4144.

Demmig-Adams, B. and Adams, W. W. (1992). Photoprotection and Other Responses of Plants to High Light Stress. *Annual Review of Plant Physiology and Plant Molecular Biology* **43**, 599-626.

Demmig-Adams, B., Gilmore, A. M. and Adams, W. W., 3rd. (1996). Carotenoids 3: in vivo function of carotenoids in higher plants. *FASEB J* **10**, 403-12.

Dempsey, L. A., Hanakahi, L. A. and Maizels, N. (1998). A specific isoform of hnRNP D interacts with DNA in the LR1 heterodimer: canonical RNA binding motifs in a sequence-specific duplex DNA binding protein. *J Biol Chem* **273**, 29224-9.

Deng, X., Gu, L., Liu, C., Lu, T., Lu, F., Lu, Z., Cui, P., Pei, Y., Wang, B., Hu, S. et al. (2010). Arginine methylation mediated by the *Arabidopsis* homolog of PRMT5 is essential for proper pre-mRNA splicing. *Proc Natl Acad Sci U S A* **107**, 19114-9.

Dent, R. M., Haglund, C. M., Chin, B. L., Kobayashi, M. C. and Niyogi, K. K. (2005). Functional Genomics of Eukaryotic Photosynthesis Using Insertional Mutagenesis of *Chlamydomonas reinhardtii*. *Plant Physiology* **137**, 545-556.

Depege, N., Bellafiore, S. and Rochaix, J. D. (2003). Role of chloroplast protein kinase Stt7 in LHCII phosphorylation and state transition in *Chlamydomonas*. *Science* **299**, 1572-5.

Dickey, L. F., Petracek, M. E., Nguyen, T. T., Hansen, E. R. and Thompson, W. F. (1998). Light regulation of Fed-1 mRNA requires an element in the 5' untranslated region and correlates with differential polyribosome association. *Plant Cell* **10**, 475-84.

Dietz, K. J. (2003). Redox control, redox signaling, and redox homeostasis in plant cells. *Int Rev Cytol* **228**, 141-93.

Dismukes, G. C., Carrieri, D., Bennette, N., Ananyev, G. M. and Posewitz, M. C. (2008). Aquatic phototrophs: efficient alternatives to land-based crops for biofuels. *Curr Opin Biotechnol* **19**, 235-40.

Doebbe, A., Rupprecht, J., Beckmann, J., Mussgnug, J. H., Hallmann, A., Hankamer, B. and Kruse, O. (2007). Functional integration of the HUP1 hexose symporter gene into the genome of *C. reinhardtii*: Impacts on biological H-2 production. *Journal of Biotechnology* **131**, 27-33.

Dolzhanskaya, N., Merz, G., Aletta, J. M. and Denman, R. B. (2006a). Methylation regulates the intracellular protein-protein and protein-RNA interactions of FMRP. *Journal of Cell Science* **119**, 1933-1946.

Dolzhanskaya, N., Merz, G. and Denman, R. B. (2006b). Alternative splicing modulates protein arginine methyltransferase-dependent methylation of fragile X syndrome mental retardation protein. *Biochemistry* **45**, 10385-93.

Douskova, I., Doucha, J., Livansky, K., Machat, J., Novak, P., Umysova, D., Zachleder, V. and Vitova, M. (2009). Simultaneous flue gas bioremediation and reduction of microalgal biomass production costs. *Applied Microbiology and Biotechnology* **82**, 179-185.

Dragone, G., Fernandes, B., Vicente, A. A. and A., T. J. (2010). Third generation biofuels from microalgae. *Applied Microbiology* **2**, 1355-1366.

Dreyfuss, G., Kim, V. N. and Kataoka, N. (2002). Messenger-RNA-binding proteins and the messages they carry. *Nat Rev Mol Cell Biol* **3**, 195-205.

Dreyfuss, G., Matunis, M. J., Pinol-Roma, S. and Burd, C. G. (1993). hnRNP proteins and the biogenesis of mRNA. *Annu Rev Biochem* **62**, 289-321.

Dubinskii, A. A. and Guida, P. P. (1979). Side-effects of the donator of sulfhydryl groups, unithiol. *Vrach Delo*, 68-71.

Dupuis, C. (1984). Hennig,Willi Impact on Taxonomic Thought. *Annual Review of Ecology and Systematics* **15**, 1-24.

Duquesnoy, P., Escudier, E., Vincensini, L., Freshour, J., Bridoux, A. M., Coste, A., Deschildre, A., de Blic, J., Legendre, M., Montantin, G. et al. (2009). Loss-of-Function Mutations in the Human Ortholog of *Chlamydomonas reinhardtii* ODA7 Disrupt Dynein Arm Assembly and Cause Primary Ciliary Dyskinesia. *The American Journal of Human Genetics* **85**, 890-896.

Durnford, D. G. and Falkowski, P. G. (1997). Chloroplast redox regulation of nuclear gene transcription during photoacclimation. *Photosynthesis Research* **53**, 229-241.

Durnford, D. G., Price, J. A., McKim, S. M. and Sarchfield, M. L. (2003). Light-harvesting complex gene expression is controlled by both transcriptional and post-transcriptional mechanisms during photoacclimation in *Chlamydomonas reinhardtii*. *Physiologia Plantarum* **118**, 193-205.

Eberhard, S., Finazzi, G. and Wollman, F. A. (2008). The Dynamics of Photosynthesis. In *Annual Review of Genetics*, vol. 42, pp. 463-515. Palo Alto: Annual Reviews.

Eckert, K., Vigouroux, A., Lo Leggio, L. and Morera, S. (2009). Crystal structures of *A. acidocaldarius* endoglucanase Cel9A in complex with cello-oligosaccharides: strong -1 and -2 subsites mimic cellobiohydrolase activity. *J Mol Biol* **394**, 61-70.

Elrad, D. and Grossman, A. R. (2004). A genome's-eye view of the light-harvesting polypeptides of *Chlamydomonas reinhardtii*. *Curr Genet* **45**, 61-75.

Elrad, D., Niyogi, K. K. and Grossman, A. R. (2002). A major light-harvesting polypeptide of photosystem II functions in thermal dissipation. *Plant Cell* **14**, 1801-1816.

Ende, W. and Laere, A. (1996). De-novo synthesis of fructans from sucrose in vitro by a combination of two purified enzymes (sucrose: sucrose 1-fructosyl transferase and fructan: fructan 1-fructosyl transferase) from chicory roots (*Cichorium intybus* L.). *Planta* **200**, 335-342.

Endo, H., Sansawa, H. and Nakajima, K. (1977). Studies on *Chlorella regularis*, Heterotrophic Fast-Growing Strain.2. Mixotrophic Growth in Relation to Light-Intensity and Acetate Concentration. *Plant and Cell Physiology* **18**, 199-205.

Envall, I., Erseus, C. and Gustavsson, L. M. (2008). Ultrastructural investigation of coelomocytes in representatives of Naidinae and Rhyacodrilinae (Annelida, Clitellata, Tubificidae). *J Morphol* **269**, 1157-67.

Eriksson, K.-E. L., Blanchette, R. A. and Ander, P. (1990). Microbial and enzymatic degradation of wood and wood components. In *Springer series in wood science*, (ed., pp. IX, 407. Berlin ; Heidelberg etc.: Springer.

Escoubas, J. M., Lomas, M., Laroche, J. and Falkowski, P. G. (1995). Light-intensity regulation of cab gene-transcription is signaled by the redox state of the plastoquinone pool. *Proceedings of the National Academy of Sciences of the United States of America* **92**, 10237-10241.

Evdokimova, V., Ruzanov, P., Imataka, H., Raught, B., Svitkin, Y., Ovchinnikov, L. P. and Sonenberg, N. (2001). The major mRNA-associated protein YB-1 is a potent 5' cap-dependent mRNA stabilizer. *EMBO J* **20**, 5491-502.

Fackelmayer, F. O. (2005). Protein arginine methyltransferases: guardians of the Arg? *Trends in Biochemical Sciences* **30**, 666-671.

Falkowski, P. G. and LaRoche, J. (1991). Acclimation to spectral irradiance in algae. *Journal of Phycology* **27**, 8-14.

Fedoroff, N. (2006). Redox regulatory mechanisms in cellular stress responses. *Ann Bot* **98**, 289-300.

Felsenstein, J. (1985). Phylogenies and the Comparative Method. *American Naturalist* **125**, 1-15.

Feng, Y., Xie, N., Wu, J., Yang, C. and Zheng, Y. G. (2009). Inhibitory study of protein arginine methyltransferase 1 using a fluorescent approach. *Biochemical and Biophysical Research Communications* **379**, 567-572.

Ferreira, K. N., Iverson, T. M., Maghlaoui, K., Barber, J. and Iwata, S. (2004). Architecture of the photosynthetic oxygen-evolving center. *Science* **303**, 1831-8.

Filipowicz, W., Bhattacharyya, S. N. and Sonenberg, N. (2008). Mechanisms of post-transcriptional regulation by microRNAs: are the answers in sight? *Nat Rev Genet* **9**, 102-14.

Finazzi, G., Rappaport, F., Furia, A., Fleischmann, M., Rochaix, J. D., Zito, F. and Forti, G. (2002). Involvement of state transitions in the switch between linear and cyclic electron flow in *Chlamydomonas reinhardtii*. *EMBO Rep* **3**, 280-5.

Flachmann, R. and Kuhlbrandt, W. (1995). Accumulation of plant antenna complexes is regulated by posttranscriptional mechanisms in tobacco. *Plant Cell* **7**, 149-160.

Fon Sing, S., Isdepsky, A., Borowitzka, M. and Moheimani, N. (2011). Production of biofuels from microalgae. *Mitigation and Adaptation Strategies for Global Change*, 1-26.

Fong, C. L., Lentz, A. and Mayfield, S. P. (2000). Disulfide bond formation between RNA binding domains is used to regulate mRNA binding activity of the chloroplast poly(A)-binding protein. *J Biol Chem* **275**, 8275-8.

Foyer, C. H. and Noctor, G. (2005). Redox homeostasis and antioxidant signaling: a metabolic interface between stress perception and physiological responses. *Plant Cell* **17**, 1866-75.

Foyer, C. H. and Noctor, G. (2009). Redox regulation in photosynthetic organisms: signaling, acclimation, and practical implications. *Antioxid Redox Signal* **11**, 861-905.

Frigerio, S., Campoli, C., Zorzan, S., Fantoni, L. I., Crosatti, C., Drepper, F., Haehnel, W., Cattivelli, L., Morosinotto, T. and Bassi, R. (2007). Photosynthetic antenna size in higher plants is controlled by the plastoquinone redox state at the post-transcriptional rather than transcriptional level. *Journal of Biological Chemistry* **282**, 29457-29469.

Fujimura, K., Katahira, J., Kano, F., Yoneda, Y. and Murata, M. (2009). Microscopic dissection of the process of stress granule assembly. *Biochim Biophys Acta* **1793**, 1728-37.

Galazka, Z., Grochowiecki, T., Jakimowicz, T., Kowalczewski, M. and Szmidt, J. (2011). Is severe atherosclerosis in the aortoiliac region a contraindication for kidney transplantation? *Transplant Proc* **43**, 2908-10.

Gamarnik, A. V. and Andino, R. (1997). Two functional complexes formed by KH domain containing proteins with the 5' noncoding region of poliovirus RNA. *RNA* **3**, 882-92.

Ganeteg, U., Kulheim, C., Andersson, J. and Jansson, S. (2004). Is each light-harvesting complex protein important for plant fitness? *Plant Physiol* **134**, 502-9.

J. M. F., Grima, E. M. and Camacho, F. G. (2005). Mixotrophic growth of the microalga *Phaeodactylum tricornutum*: Influence of different nitrogen and organic carbon sources on productivity and biomass composition. *Process Biochemistry* **40**, 297-305.

Gardner, K. H. and Blackwell, J. (1974). The hydrogen bonding in native cellulose. *Biochim Biophys Acta* **343**, 232-7.

Gary, J. D. and Clarke, S. (1998). RNA and protein interactions modulated by protein arginine methylation. In *Progress in Nucleic Acid Research and Molecular Biology, Vol 61*, vol. 61, pp. 65-131. San Diego: Academic Press Inc.

Gebauer, F. and Hentze, M. W. (2004). Molecular mechanisms of translational control. *Nat Rev Mol Cell Biol* **5**, 827-35.

Ghezzi, P. (2005). Oxidoreduction of protein thiols in redox regulation. *Biochem Soc Trans* **33**, 1378-81.

Ghose, T. K. (1987). Measurement of Cellulase Activities. *Pure and Applied Chemistry* **59**, 257-268.

Gilbert, H. J. (2010). The biochemistry and structural biology of plant cell wall deconstruction. *Plant Physiol* **153**, 444-55.

Gilks, N., Kedersha, N., Ayodele, M., Shen, L., Stoecklin, G., Dember, L. M. and Anderson, P. (2004). Stress granule assembly is mediated by prion-like aggregation of TIA-1. *Mol Biol Cell* **15**, 5383-98.

Ginisty, H., Sicard, H., Roger, B. and Bouvet, P. (1999). Structure and functions of nucleolin. *Journal of Cell Science* **112**, 761-772.

Giordano, M., Beardall, J. and Raven, J. A. (2005). CO2 concentrating mechanisms in algae: mechanisms, environmental modulation, and evolution. *Annu Rev Plant Biol* **56**, 99-131.

Glisovic, T., Bachorik, J. L., Yong, J. and Dreyfuss, G. (2008). RNA-binding proteins and post-transcriptional gene regulation. *FEBS Lett* **582**, 1977-86.

Goulah, C. C. and Read, L. K. (2007). Differential effects of arginine methylation on RBP16 mRNA binding, guide RNA (gRNA) binding, and gRNA-containing ribonucleoprotein complex (gRNP) formation. *Journal of Biological Chemistry* **282**, 7181-7190.

Gouveia, L. (2011). Microalgae as a Feedstock for Biofuels, pp. 1-69: Springer Berlin Heidelberg.

Gouveia, L. and Oliveira, A. (2009). Microalgae as a raw material for biofuels production. *Journal of Industrial Microbiology & Biotechnology* **36**, 269-274.

Gravina, S. A. and Mieyal, J. J. (1993). Thioltrasferase is a specific glutathionyl mixed disulfide oxidoreductase. *Biochemistry* **32**, 3368-3376.

Gray, J. C. (2003). Chloroplast-to-nucleus signalling: a role for Mg-protoporphyrin. *Trends Genet* **19**, 526-9.

Green, B. R. and Durnford, D. G. (1996). The Chlorophyll-Carotenoid Proteins of Oxygenic Photosynthesis. *Annu Rev Plant Physiol Plant Mol Biol* **47**, 685-714.

Griffith, O. W. (1980). Determination of glutathione and glutathione disulfide using glutathione-reductase and 2-vinylpyridine. *Analytical Biochemistry* **106**, 207-212.

Grossman, A. R. (2005). Paths toward algal genomics. *Plant Physiol* **137**, 410-27.

Grossman, A. R., Croft, M., Gladyshev, V. N., Merchant, S. S., Posewitz, M. C., Prochnik, S. and Spalding, M. H. (2007). Novel metabolism in *Chlamydomonas* through the lens of genomics. *Curr Opin Plant Biol* **10**, 190-8.

Grossman, A. R., Harris, E. E., Hauser, C., Lefebvre, P. A., Martinez, D., Rokhsar, D., Shrager, J., Silflow, C. D., Stern, D., Vallon, O. et al. (2003). *Chlamydomonas reinhardtii* at the Crossroads of Genomics. *Eukaryotic Cell* **2**, 1137-1150.

ouš T I o P Fydoá I Všoá P J d F Vojtoá J íská K oá I Noákoá L J oškoá D t (9). Robust heat shock induces eIF α-phosphorylation-independent assembly of stress granules containing eIF3 and 40S ribosomal subunits in budding yeast, *Saccharomyces cerevisiae*. *Journal of Cell Science* **122**, 2078-2088.

Guil, S., Long, J. C. and Caceres, J. F. (2006). hnRNP A1 relocalization to the stress granules reflects a role in the stress response. *Mol Cell Biol* **26**, 5744-58.

Habelhah, H., Shah, K., Huang, L., Ostareck-Lederer, A., Burlingame, A. L., Shokat, K. M., Hentze, M. W. and Ronai, Z. (2001). ERK phosphorylation drives cytoplasmic accumulation of hnRNP-K and inhibition of mRNA translation. *Nat Cell Biol* **3**, 325-30.

Haigler, C. H. and Weimer, P. J. (1991). Biosynthesis and biodegradation of cellulose. New York, N.Y.: Dekker.

Haldrup, A., Jensen, P. E., Lunde, C. and Scheller, H. V. (2001). Balance of power: a view of the mechanism of photosynthetic state transitions. *Trends Plant Sci* **6**, 301-5.

Hammel, K. E. and Cullen, D. (2008). Role of fungal peroxidases in biological ligninolysis. *Curr Opin Plant Biol* **11**, 349-55.

Hankamer, B., Barber, J. and Boekema, E. J. (1997). Structure and Membrane Organization of Photosystem II in Green Plants. *Annu Rev Plant Physiol Plant Mol Biol* **48**, 641-671.

Hankamer, B., Lehr, F., Rupprecht, J., Mussgnug, J. H., Posten, C. and Kruse, O. (2007). Photosynthetic biomass and H2 production by green algae: from bioengineering to bioreactor scale-up. *Physiologia Plantarum* **131**, 10-21.

Harhangi, H. R., Steenbakkers, P. J., Akhmanova, A., Jetten, M. S., van der Drift, C. and Op den Camp, H. J. (2002). A highly expressed family 1 beta-glucosidase with transglycosylation capacity from the anaerobic fungus *Piromyces* sp. E2. *Biochim Biophys Acta* **1574**, 293-303.

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

Harris, E. H. (2009). The *Chlamydomonas* Sourcebook: Academic Press.

Hatakka, A. (1994). Lignin-modifying enzymes from selected white-rot fungi: production and role from in lignin degradation. *FEMS Microbiology Reviews* **13**, 125-135.

Hayman, M. L. and Read, L. K. (1999). Trypanosoma brucei RBP16 is a mitochondrial Y-box family protein with guide RNA binding activity. *Journal of Biological Chemistry* **274**, 12067-12074.

Heifetz, P. B., Förster, B., Osmond, C. B., Giles, L. J. and Boynton, J. E. (2000). Effects of Acetate on Facultative Autotrophy in *Chlamydomonas reinhardtii* Assessed by Photosynthetic Measurements and Stable Isotope Analyses. *Plant Physiology* **122**, 1439-1446.

Helliwell, C. A., Webster, C. I. and Gray, J. C. (1997). Light-regulated expression of the pea plastocyanin gene is mediated by elements within the transcribed region of the gene. *Plant J* **12**, 499-506.

Hemschemeier, A. and Happe, T. (2011). Alternative photosynthetic electron transport pathways during anaerobiosis in the green alga *Chlamydomonas reinhardtii*. *Biochim Biophys Acta* **1807**, 919-26.

Henrissat, B. (1991). A classification of glycosyl hydrolases based on amino acid sequence similarities. *Biochem J* **280 (Pt 2)**, 309-16.

Henrissat, B. and Davies, G. (1997). Structural and sequence-based classification of glycoside hydrolases. *Curr Opin Struct Biol* **7**, 637-44.

Herrmann, F., Lee, J., Bedford, M. T. and Fackelmayer, F. O. (2005). Dynamics of human protein arginine methyltransferase 1 (PRMT1) in vivo. *Journal of Biological Chemistry* **280**, 38005-38010.

Hieronymus, H. and Silver, P. A. (2004). A systems view of mRNP biology. *Genes Dev* **18**, 2845-60.

Himmel, M. E. and Bayer, E. A. (2009). Lignocellulose conversion to biofuels: current challenges, global perspectives. *Current Opinion in Biotechnology* **20**, 316-317.

Hippler, M., Klein, J., Fink, A., Allinger, T. and Hoerth, P. (2001). Towards functional proteomics of membrane protein complexes: analysis of thylakoid membranes from *Chlamydomonas reinhardtii*. *Plant J* **28**, 595-606.

Hogg, P. J. (2003). Disulfide bonds as switches for protein function. *Trends Biochem Sci* **28**, 210-4.

Holtzapple, M. T. (1993). Cellulose. In *Encyclopedia of food science food technology and nutrition.*, (ed. R. Macrae R. K. Robinson and M. J. Saddler), pp. p. 758–67. London: Academic Press.

Hong, S., Song, H. R., Lutz, K., Kerstetter, R. A., Michael, T. P. and McClung, C. R. (2010). Type II protein arginine methyltransferase 5 (PRMT5) is required for circadian period determination in *Arabidopsis thaliana*. *Proc Natl Acad Sci U S A* **107**, 21211-6.

Horne, R. W., Davies, D. R., Norton, K. and Gurney-Smith, M. (1971). Electron Microscope and Optical Diffraction Studies on Isolated Cell Walls from *Chlamydomonas*. *Nature* **232**, 493-495.

Horton, P., Ruban, A. V. and Walters, R. G. (1996). Regulation of Light Harvesting in Green Plants. *Annu Rev Plant Physiol Plant Mol Biol* **47**, 655-684.

Humby, P. L., Cunningham, M. L., Saunders, H. L., Price, J. A. and Durnford, D. G. (2009). Compartmental cross-talk in the regulation of light harvesting complex transcription under short-term light and temperature stress in *Chlamydomonas reinhardtii*. *Botany* **87**, 375-386.

Huner, N. P. A., Öquist, G. and Sarhan, F. (1998). Energy balance and acclimation to light and cold. *Trends in Plant Science* **3**, 224-230.

Ip, P.-F. and Chen, F. (2005). Production of astaxanthin by the green microalga *Chlorella zofingiensis* in the dark. *Process Biochemistry* **40**, 733-738.

IPCC. (2007). Climate change 2007. In *Fourth Assessment Report*, (ed. Intergovernmental Panel on Climate Change: IPCC.

Irihimovitch, V. and Shapira, M. (2000). Glutathione redox potential modulated by reactive oxygen species regulates translation of Rubisco large subunit in the chloroplast. *J Biol Chem* **275**, 16289-95.

Irwin, D., Shin, D.-H., Zhang, S., Barr, B. K., Sakon, J., Karplus, P. A. and Wilson, D. B. (1998). Roles of the Catalytic Domain and Two Cellulose Binding Domains of *Thermomonospora fusca* E4 in Cellulose Hydrolysis. *J. Bacteriol.* **180**, 1709-1714.

Ishiguro, Y., Ueno, K., Abe, M., Onodera, S., Fukushi, E., Benkeblia, N. and Shiomi, N. (2009). Isolation and structural determination of reducing fructooligosaccharides newly produced in stored edible burdock. *Journal of Applied Glycoscience* **56**, 159-164.

Isleten-Hosoglu, M., Gultepe, I. and Elibol, M. (2012). Optimization of carbon and nitrogen sources for biomass and lipid production by *Chlorella saccharophila* under heterotrophic conditions and development of Nile red fluorescence based method for quantification of its neutral lipid content. *Biochemical Engineering Journal* **61**, 11-19.

Jacobshagen, S., Kindle, K. L. and Johnson, C. H. (1996). Transcription of CABII is regulated by the biological clock in *Chlamydomonas reinhardtii*. *Plant Mol Biol* **31**, 1173-84.

Janssen, M., Tramper, J., Mur, L. R. and Wijffels, R. H. (2003). Enclosed outdoor photobioreactors: Light regime, photosynthetic efficiency, scale-up, and future prospects. *Biotechnology and Bioengineering* **81**, 193-210.

Jansson, S. (1994). The light-harvesting chlorophyll a/b-binding proteins. *Biochim Biophys Acta* **1184**, 1-19.

Jansson, S. (1999). A guide to the Lhc genes and their relatives in Arabidopsis. *Trends Plant Sci* **4**, 236-240.

Jarvis, M. (2003). Chemistry: cellulose stacks up. *Nature* **426**, 611-2.

Jeffries, T. W. and Kurtzman, C. P. (1994). Strain Selection, Taxonomy, and Genetics of Close-Fermenting Yeasts. *Enzyme and Microbial Technology* **16**, 922-932.

Jeon, Y.-C., Cho, C.-W. and Yun, Y.-S. (2006). Combined effects of light intensity and acetate concentration on the growth of unicellular microalga *Haematococcus pluvialis*. *Enzyme and Microbial Technology* **39**, 490-495.

Johanningmeier, U. and Howell, S. H. (1984). Regulation of light-harvesting chlorophyll-binding protein mRNA accumulation in *Chlamydomonas reinhardi*. Possible involvement of chlorophyll synthesis precursors. *J Biol Chem* **259**, 13541-9.

Johnstone, O. and Lasko, P. (2001). Translational regulation and RNA localization in *Drosophila* oocytes and embryos. *Annu Rev Genet* **35**, 365-406.

Joliot, P. and Johnson, G. N. (2011). Regulation of cyclic and linear electron flow in higher plants. *Proc Natl Acad Sci U S A* **108**, 13317-22.

Joliot, P., Johnson, G. N. and Joliot, A. (2006). Cyclic electron transfer around Photosystem I. In *Photosystem I: The Light-Driven Plastocyanin:Ferredoxin Oxidoreductase*, (ed. J. H. Golbeck), pp. 639–656. Berlin: Springer.

Jones, D. T., Taylor, W. R. and Thornton, J. M. (1992). The rapid generation of mutation data matrices from protein sequences. *Computer Applications in the Biosciences* **8**, 275-82.

Kaftan, D., Brumfeld, V., Nevo, R., Scherz, A. and Reich, Z. (2002). From chloroplasts to photosystems: in situ scanning force microscopy on intact thylakoid membranes. *EMBO J* **21**, 6146-53.

Kajita, Y., Nakayama, J., Aizawa, M. and Ishikawa, F. (1995). The UUAG-specific RNA binding protein, heterogeneous nuclear ribonucleoprotein D0. Common modular structure and binding properties of the 2xRBD-Gly family. *J Biol Chem* **270**, 22167-75.

Kang, C. D., Lee, J. S., Park, T. H. and Sim, S. J. (2005). Comparison of heterotrophic and photoautotrophic induction on astaxanthin production by *Haematococcus pluvialis*. *Applied Microbiology and Biotechnology* **68**, 237-241.

Kang, R., Wang, J., Shi, D., Cong, W., Cai, Z. and Ouyang, F. (2004). Interactions between organic and inorganic carbon sources during mixotrophic cultivation of *Synechococcus* sp. *Biotechnology Letters* **26**, 1429-1432.

Kaplan, A. and Reinhold, L. (1999). Co2 Concentrating Mechanisms in Photosynthetic Microorganisms. *Annu Rev Plant Physiol Plant Mol Biol* **50**, 539-570.

Kargul, J. and Barber, J. (2008). Photosynthetic acclimation: structural reorganisation of light harvesting antenna--role of redox-dependent phosphorylation of major and minor chlorophyll a/b binding proteins. *FEBS J* **275**, 1056-68.

Katzen, F. and Beckwith, J. (2003). Role and location of the unusual redox-active cysteines in the hydrophobic domain of the transmembrane electron transporter DsbD. *Proceedings of the National Academy of Sciences of the United States of America* **100**, 10471-10476.

Kaur, J., Chadha, B. S., Kumar, B. A., Kaur, G. S. and Saini, H. S. (2007). Purification and characterization of beta-glucosidase from *Melanocarpus* sp MTCC 3922. *Electronic Journal of Biotechnology* **10**, 260-270.

Kawaguchi, R., Girke, T., Bray, E. A. and Bailey-Serres, J. (2004). Differential mRNA translation contributes to gene regulation under non-stress and dehydration stress conditions in *Arabidopsis thaliana*. *Plant J* **38**, 823-39.

Kedersha, N., Chen, S., Gilks, N., Li, W., Miller, I. J., Stahl, J. and Anderson, P. (2002). Evidence that ternary complex (eIF2-GTP-tRNA(i)(Met))-deficient preinitiation complexes are core constituents of mammalian stress granules. *Mol Biol Cell* **13**, 195-210.

Kelly, G. J. and Gibbs, M. (1973). Mechanism for Indirect Transfer of Photosynthetically Reduced Nicotinamide Adenine-Dinucleotide Phosphate from Chloroplasts to Cytoplasm. *Plant Physiology* **52**, 674-676.

Keren, N., Berg, A., van Kan, P. J., Levanon, H. and Ohad, I. (1997). Mechanism of photosystem II photoinactivation and D1 protein degradation at low light: the role of back electron flow. *Proc Natl Acad Sci U S A* **94**, 1579-84.

Kiledjian, M. and Dreyfuss, G. (1992). Primary structure and binding activity of the hnRNP U protein: binding RNA through RGG box. *EMBO J* **11**, 2655-64.

Kim, J. Y., Song, H. R., Taylor, B. L. and Carre, I. A. (2003). Light-regulated translation mediates gated induction of the *Arabidopsis* clock protein LHY. *EMBO J* **22**, 935-44.

Kindle, K. L. (1987). Expression of a Gene for a Light-Harvesting Chlorophyll-a-Binding Chlorophyll-B-Binding Protein in *Chlamydomonas-Reinhardtii* - Effect of Light and Acetate. *Plant Molecular Biology* **9**, 547-563.

Kindle, K. L. (1990). High-frequency nuclear transformation of *Chlamydomonas reinhardtii*. *Proc Natl Acad Sci U S A* **87**, 1228-32.

King, B. C., Donnelly, M. K., Bergstrom, G. C., Walker, L. P. and Gibson, D. M. (2009). An optimized microplate assay system for quantitative evaluation of plant cell wall-degrading enzyme activity of fungal culture extracts. *Biotechnol Bioeng* **102**, 1033-44.

Kirk, J. T. O. (1994). Light and photosynthesis in aquatic ecosystems. Cambridge England ; New York, NY, USA: Cambridge University Press.

Kirk, O., Borchert, T. V. and Fuglsang, C. C. (2002). Industrial enzyme applications. *Current Opinion in Biotechnology* **13**, 345-351.

Kitano, M., Matsukawa, R. and Karube, I. (1997). Changes in eicosapentaenoic acid content of *Navicula saprophila*, *Rhodomonas salina* and *Nitzschia sp*. under mixotrophic conditions. *Journal of Applied Phycology* **9**, 559-563.

Koussevitzky, S., Nott, A., Mockler, T. C., Hong, F., Sachetto-Martins, G., Surpin, M., Lim, I. J., Mittler, R. and Chory, J. (2007). Signals from chloroplasts converge to regulate nuclear gene expression. *Science* **316**, 715-719.

Krause, C. D., Yang, Z. H., Kim, Y. S., Lee, J. H., Cook, J. R. and Pestka, S. (2007). Protein arginine methyltransferases: evolution and assessment of their pharmacological and therapeutic potential. *Pharmacol Ther* **113**, 50-87.

Krieger-Liszkay, A. (2005). Singlet oxygen production in photosynthesis. *J Exp Bot* **56**, 337-46.

Kroes, H. W. (1972). Growth Interactions Between *Chlamydomonas globosa* Snow and *Chlorococcum ellipsoideum* Deason and Bold: The Role of Extracellular Products. *Limnology and Oceanography* **17**, 423-432.

Kruse, O. and Hankamer, B. (2010). Microalgal hydrogen production. *Curr Opin Biotechnol* **21**, 238-43.

Kruse, O., Nixon, P. J., Schmid, G. H. and Mullineaux, C. W. (1999). Isolation of state transition mutants of *Chlamydomonas reinhardtii* by fluorescence video imaging. *Photosynthesis Research* **61**, 43-51.

Kruse, O., Rupprecht, J., Mussgnug, J. H., Dismukes, G. C. and Hankamer, B. (2005). Photosynthesis: a blueprint for solar energy capture and biohydrogen production technologies. *Photochem Photobiol Sci* **4**, 957-70.

Kuhlbrandt, W., Wang, D. N. and Fujiyoshi, Y. (1994). Atomic model of plant light-harvesting complex by electron crystallography. *Nature* **367**, 614-21.

Kumar, A., Ergas, S., Yuan, X., Sahu, A., Zhang, Q., Dewulf, J., Malcata, F. X. and van Langenhove, H. (2010). Enhanced CO(2) fixation and biofuel production via microalgae: recent developments and future directions. *Trends Biotechnol* **28**, 371-80.

Kwon, S., Zhang, Y. and Matthias, P. (2007). The deacetylase HDAC6 is a novel critical component of stress granules involved in the stress response. *Genes Dev* **21**, 3381-94.

Laliberte, G. and Delanoue, J. (1993). Autotrophic, Heterotrophic, and Mixotrophic Growth of *Chlamydomonas humicola* (Chlorophyceae) on Acetate. *Journal of Phycology* **29**, 612-620.

Lalucat, J., Imperial, J. and Pares, R. (1984). Utilization of light for the assimilation of organic matter in *Chlorella* sp. VJ79. *Biotechnol Bioeng* **26**, 677-81.

Le Hir, H. and Séraphin, B. (2008). EJCs at the Heart of Translational Control. *Cell* **133**, 213-216.

Lee, Y. H. and Stallcup, M. R. (2009). Minireview: protein arginine methylation of nonhistone proteins in transcriptional regulation. *Molecular Endocrinology* **23**, 425-33.

Lemeille, S. and Rochaix, J. D. (2010). State transitions at the crossroad of thylakoid signalling pathways. *Photosynth Res* **106**, 33-46.

Lemeille, S., Turkina, M. V., Vener, A. V. and Rochaix, J. D. (2010). Stt7-dependent phosphorylation during state transitions in the green alga *Chlamydomonas reinhardtii*. *Mol Cell Proteomics* **9**, 1281-95.

Leschine, S. B. (1995). Cellulose degradation in anaerobic environments. *Annu Rev Microbiol* **49**, 399-426.

Li, X. L. and Calza, R. E. (1991). Fractionation of cellulases from the ruminal fungus *Neocallimastix frontalis* EB188. *Appl Environ Microbiol* **57**, 3331-6.

Li, X. P., Bjorkman, O., Shih, C., Grossman, A. R., Rosenquist, M., Jansson, S. and Niyogi, K. K. (2000). A pigment-binding protein essential for regulation of photosynthetic light harvesting. *Nature* **403**, 391-5.

Li, Y., Horsman, M., Wu, N., Lan, C. Q. and Dubois-Calero, N. (2008). Biofuels from Microalgae. *Biotechnology Progress* **24**, 815-820.

Libertini, E., Li, Y. and McQueen-Mason, S. J. (2004). Phylogenetic analysis of the plant endo-beta-1,4-glucanase gene family. *Journal of Molecular Evolution* **58**, 506-515.

Lin, Y. and Tanaka, S. (2006). Ethanol fermentation from biomass resources: current state and prospects. *Appl Microbiol Biotechnol* **69**, 627-42.

Liu, M. J., Wu, S. H. and Chen, H. M. (2012). Widespread translational control contributes to the regulation of *Arabidopsis* photomorphogenesis. *Mol Syst Biol* **8**, 566.

Liu, X., Duan, S., Li, A., Xu, N., Cai, Z. and Hu, Z. (2009). Effects of organic carbon sources on growth, photosynthesis, and respiration of *Phaeodactylum tricornutum*. *Journal of Applied Phycology* **21**, 239-246.

Liu, Z., Yan, H., Wang, K., Kuang, T., Zhang, J., Gui, L., An, X. and Chang, W. (2004). Crystal structure of spinach major light-harvesting complex at 2.72 A resolution. *Nature* **428**, 287-92.

Ljungdahl, L. G. and Eriksson, K. E. (1985). Ecology of Microbial Cellulose Degradation. *Advances in Microbial Ecology* **8**, 237-299.

Lo, N., Watanabe, H. and Sugimura, M. (2003). Evidence for the presence of a cellulase gene in the last common ancestor of bilaterian animals. *Proceedings of the Royal Society of London Series B-Biological Sciences* **270**, S69-S72.

Lukong, K. E., Chang, K.-w., Khandjian, E. W. and Richard, S. (2008). RNA-binding proteins in human genetic disease. *Trends in Genetics* **24**, 416-425.

Lunde, B. M., Moore, C. and Varani, G. (2007). RNA-binding proteins: modular design for efficient function. *Nat Rev Mol Cell Biol* **8**, 479-90.

Lynd, L. R., Weimer, P. J., van Zyl, W. H. and Pretorius, I. S. (2002). Microbial cellulose utilization: fundamentals and biotechnology. *Microbiol Mol Biol Rev* **66**, 506-77, table of contents.

Mahan, K. M., Odom, O. W. and Herrin, D. L. (2005). Controlling fungal contamination in *Chlamydomonas reinhardtii* cultures. *Biotechniques* **39**, 457-8.

Malhi, Y. (2002). Carbon in the atmosphere and terrestrial biosphere in the 21st century. *Philos Transact A Math Phys Eng Sci* **360**, 2925-45.

Manival, X., Ghisolfi-Nieto, L., Joseph, G., Bouvet, P. and Erard, M. (2001). RNA-binding strategies common to cold-shock domain- and RNA recognition motif-containing proteins. *Nucleic Acids Research* **29**, 2223-2233.

Mannan, R. M. and Pakrasi, H. B. (1993). Dark heterotrophic growth conditions result in an increase in the content of photosystem II units in the filamentous cyanobacterium *Anabaena variabilis* ATCC 29413. *Plant Physiol* **103**, 971-7.

Maris, C., Dominguez, C. and Allain, F. H. T. (2005). The RNA recognition motif, a plastic RNA-binding platform to regulate post-transcriptional gene expression. *FEBS Journal* **272**, 2118-2131.

Marquez, F. J., Sasaki, K., Kakizono, T., Nishio, N. and Nagai, S. (1993). Growth characteristics of *Spirulina platensis* in mixotrophic and heterotrophic conditions. *Journal of Fermentation and Bioengineering* **76**, 408-410.

Martinez, A. T., Speranza, M., Ruiz-Duenas, F. J., Ferreira, P., Camarero, S., Guillen, F., Martinez, M. J., Gutierrez, A. and del Rio, J. C. (2005). Biodegradation of lignocellulosics: microbial, chemical, and enzymatic aspects of the fungal attack of lignin. *Int Microbiol* **8**, 195-204.

Martinot, E., Dienst, C., Weiliang, L. and Qimin, C. (2007). Renewable Energy Futures: Targets, Scenarios, and Pathways. *Annual Review of Environment and Resources* **32**, 205-239.

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

Matsumoto, K., Meric, F. and Wolffe, A. P. (1996). Translational repression dependent on the interaction of the Xenopus Y-box protein FRGY2 with mRNA - Role of the cold shock domain, tail domain, and selective RNA sequence recognition. *Journal of Biological Chemistry* **271**, 22706-22712.

Matsuo, T. and Ishiura, M. (2011). *Chlamydomonas reinhardtii* as a new model system for studying the molecular basis of the circadian clock. *FEBS Letters* **585**, 1495-1502.

Matsuura, H., Ishibashi, Y., Shinmyo, A., Kanaya, S. and Kato, K. (2010). Genome-wide analyses of early translational responses to elevated temperature and high salinity in *Arabidopsis thaliana*. *Plant Cell Physiol* **51**, 448-62.

Matthysse, A. G., Deschet, K., Williams, M., Marry, M., White, A. R. and Smith, W. C. (2004). A functional cellulose synthase from ascidian epidermis. *Proc Natl Acad Sci U S A* **101**, 986-91.

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

Maxwell, D. P., Laudenbach, D. E. and Huner, N. (1995). Redox Regulation of Light-Harvesting Complex II and cab mRNA Abundance in Dunaliella salina. *Plant Physiol* **109**, 787-795.

Mayer, W., Schuster, L., Bartelmes, G., Dieterich, C. and Sommer, R. (2011). Horizontal gene transfer of microbial cellulases into nematode genomes is associated with functional assimilation and gene turnover. *BMC Evolutionary Biology* **11**, 13.

Mayfield, S. P. and Kindle, K. L. (1990). Stable nuclear transformation of *Chlamydomonas reinhardtii* by using a *C. reinhardtii* gene as the selectable marker. *Proc Natl Acad Sci U S A* **87**, 2087-91.

Mazroui, R., Di Marco, S., Kaufman, R. J. and Gallouzi, I. E. (2007). Inhibition of the ubiquitin-proteasome system induces stress granule formation. *Mol Biol Cell* **18**, 2603-18.

McKim, S. M. and Durnford, D. G. (2006). Translational regulation of light-harvesting complex expression during photo acclimation to high-light in *Chlamydomonas reinhardtii*. *Plant Physiology and Biochemistry* **44**, 857-865.

Melis, A. (1978). Oxidation-reduction potential dependence of the two kinetic components in chloroplast system II primary photochemistry. *FEBS Lett* **95**, 202-6.

Melis, A. (1999). Photosystem-II damage and repair cycle in chloroplasts: what modulates the rate of photodamage ? *Trends Plant Sci* **4**, 130-135.

Melis, A. (2009). Solar energy conversion efficiencies in photosynthesis: Minimizing the chlorophyll antennae to maximize efficiency. *Plant Science* **177**, 272-280.

Melkozernov, A. N., Barber, J. and Blankenship, R. E. (2006). Light harvesting in photosystem I supercomplexes. *Biochemistry* **45**, 331-45.

Melkozernov, A. N. and Blankenship, R. E. (2005). Structural and functional organization of the peripheral light-harvesting system in photosystem I. *Photosynth Res* **85**, 33-50.

Merchant, S. S. Prochnik, S. E. Vallon, O. Harris, E. H. Karpowicz, S. J. Witman, G. B. Terry, A. Salamov, A. Fritz-Laylin, L. K. Marechal-Drouard, L. et al. (2007). The *Chlamydomonas* genome reveals the evolution of key animal and plant functions. *Science* **318**, 245-50.

Meyer, A. J., Brach, T., Marty, L., Kreye, S., Rouhier, N., Jacquot, J. P. and Hell, R. (2007). Redox-sensitive GFP in *Arabidopsis thaliana* is a quantitative biosensor for the redox potential of the cellular glutathione redox buffer. *Plant Journal* **52**, 973-986.

Meyer, A. J. and Hell, R. (2005). Glutathione homeostasis and redox-regulation by sulfhydryl groups. *Photosynthesis Research* **86**, 435-457.

Michaelis, G., Vahrenholz, C. and Pratje, E. (1990). Mitochondrial DNA of *Chlamydomonas reinhardtii*: the gene for apocytochrome b and the complete functional map of the 15.8 kb DNA. *Mol Gen Genet* **223**, 211-6.

Michelet, L., Zaffagnini, M., Massot, V., Keryer, E., Vanacker, H., Miginiac-Maslow, M., Issakidis-Bourguet, E. and Lemaire, S. D. (2006). Thioredoxins, glutaredoxins, and glutathionylation: new crosstalks to explore. *Photosynth Res* **89**, 225-45.

Miller, D. H., Lamport, D. T. and Miller, M. (1972). Hydroxyproline heterooligosaccharides in *Chlamydomonas*. *Science* **176**, 918-20.

Miller, G. L. (1959). Use of Dinitrosalicylic Acid Reagent for Determination of Reducing Sugar. *Analytical Chemistry* **31**, 426-428.

Minagawa, J. (2009). Light harvesting proteins. In *The Chlamydomonas sourcebook. Organellar and Metabolic Processes.*, vol. 2, pp. 503-540. San Diego: Academic Press.

Minagawa, J., Han, K.-C., Dohmae, N., Takio, K. and Inoue, Y. (2001). Molecular characterization and gene expression of lhcb5 gene encoding CP26 in the light-harvesting complex II of *Chlamydomonas reinhardtii*. *Plant Molecular Biology* **46**, 277-287.

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

Minich, W. B. and Ovchinnikov, L. P. (1992). Role of cytoplasmic mRNP proteins in translation. *Biochimie* **74**, 477-83.

Mitra, M. and Melis, A. (2008). Optical properties of microalgae for enhanced biofuels production. *Opt. Express* **16**, 21807-21820.

Mittag, M. (1996). Conserved circadian elements in phylogenetically diverse algae. *Proceedings of the National Academy of Sciences* **93**, 14401-14404.

Mochizuki, N., Tanaka, R., Tanaka, A., Masuda, T. and Nagatani, A. (2008). The steady-state level of Mg-protoporphyrin IX is not a determinant of plastid-to-nucleus signaling in *Arabidopsis*. *Proc Natl Acad Sci U S A* **105**, 15184-9.

Mohnen, D. (2008). Pectin structure and biosynthesis. *Current Opinion in Plant Biology* **11**, 266-277.

Mokas, S., Mills, J. R., Garreau, C., Fournier, M. J., Robert, F., Arya, P., Kaufman, R. J., Pelletier, J. and Mazroui, R. (2009). Uncoupling stress granule assembly and translation initiation inhibition. *Mol Biol Cell* **20**, 2673-83.

Møller, I. M., Jensen, P. E. and Hansson, A. (2007). Oxidative Modifications to Cellular Components in Plants. *Annual Review of Plant Biology* **58**, 459-481.

Molnar, A., Schwach, F., Studholme, D. J., Thuenemann, E. C. and Baulcombe, D. C. (2007). miRNAs control gene expression in the single-cell alga Chlamydomonas reinhardtii. *Nature* **447**, 1126-1129.

Moore, M. J. (2005). From birth to death: The complex lives of eukaryotic mRNAs. *Science* **309**, 1514-1518.

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

Morris, S. C. (3). Life's solution: inevitable humans in a lonely universe. Cambridge, United Kingdom: Cambridge University Press,.

Morweiser, M., Kruse, O., Hankamer, B. and Posten, C. (2010). Developments and perspectives of photobioreactors for biofuel production. *Appl Microbiol Biotechnol* **87**, 1291-301.

Mosier, N., Wyman, C., Dale, B., Elander, R., Lee, Y. Y., Holtzapple, M. and Ladisch, M. (2005). Features of promising technologies for pretreatment of lignocellulosic biomass. *Bioresour Technol* **96**, 673-86.

Moulin, M., McCormac, A. C., Terry, M. J. and Smith, A. G. (2008). Tetrapyrrole profiling in *Arabidopsis* seedlings reveals that retrograde plastid nuclear signaling is not due to Mg-protoporphyrin IX accumulation. *Proc Natl Acad Sci U S A* **105**, 15178-83.

Müller, P., Li, X. P. and Niyogi, K. K. (2001). Non-Photochemical Quenching. A Response to Excess Light Energy. *Plant Physiology* **125**, 1558-1566.

Mullineaux, P. M. and Rausch, T. (2005). Glutathione, photosynthesis and the redox regulation of stress-responsive gene expression. *Photosynthesis Research* **86**, 459-474.

Munekage, Y., Hashimoto, M., Miyake, C., Tomizawa, K., Endo, T., Tasaka, M. and Shikanai, T. (2004). Cyclic electron flow around photosystem I is essential for photosynthesis. *Nature* **429**, 579-82.

Munk, K. and Dersch, P. (2008). Mikrobiologie. In *Taschenlehrbuch Biologie*, (ed., pp. 618 S. Stuttgart: G. Thieme.

Murata, N. (1969). Control of excitation transfer in photosynthesis. I. Light-induced change of chlorophyll a fluorescence in *Porphyridium cruentum*. *Biochim Biophys Acta* **172**, 242-51.

Mussgnug, J. H., Klassen, V., Schluter, A. and Kruse, O. (2010). Microalgae as substrates for fermentative biogas production in a combined biorefinery concept. *J Biotechnol* **150**, 51-6.

Mussgnug, J. H., Thomas-Hall, S., Rupprecht, J., Foo, A., Klassen, V., McDowall, A., Schenk, P. M., Kruse, O. and Hankamer, B. (2007). Engineering photosynthetic light capture: impacts on improved solar energy to biomass conversion. *Plant Biotechnology Journal* **5**, 802-814.

Mussgnug, J. H., Wobbe, L., Elles, I., Claus, C., Hamilton, M., Fink, A., Kahmann, U., Kapazoglou, A., Mullineaux, C. W., Hippler, M. et al. (2005). NAB1 is an RNA binding protein involved in the light-regulated differential expression of the light-harvesting antenna of *Chlamydomonas reinhardtii*. *Plant Cell* **17**, 3409-3421.

Nakajima, Y., Tsuzuki, M. and Ueda, R. (1998). Reduced photoinhibition of a phycocyanin-deficient mutant of *Synechocystis* PCC 6714. *Journal of Applied Phycology* **10**, 447-452.

Nakajima, Y. and Ueda, R. (1997). Improvement of photosynthesis in dense microalgal suspension by reduction of light harvesting pigments. *Journal of Applied Phycology* **9**, 503-510.

Nakajima, Y. and Ueda, R. (1999). Improvement of microalgal photosynthetic productivity by reducing the content of light harvesting pigment. *Journal of Applied Phycology* **11**, 195-201.

Nataf, Y., Yaron, S., Stahl, F., Lamed, R., Bayer, E. A., Scheper, T. H., Sonenshein, A. L. and Shoham, Y. (2009). Cellodextrin and laminaribiose ABC transporters in *Clostridium thermocellum*. *J Bacteriol* **191**, 203-9.

Neidhardt, J., Benemann, J. R., Zhang, L. and Melis, A. (1998). Photosystem-II repair and chloroplast recovery from irradiance stress: relationship between chronic photoinhibition, light-harvesting chlorophyll

antenna size and photosynthetic productivity in *Dunaliella salina* (green algae). *Photosynthesis Research* **56**, 175-184.

Neilson, J. A. D. and Durnford, D. G. (2010). Structural and functional diversification of the light-harvesting complexes in photosynthetic eukaryotes. *Photosynthesis Research* **106**, 57-71.

Nekrasov, V. S., Smith, M. A., Peak-Chew, S. and Kilmartin, J. V. (2003). Interactions between centromere complexes in *Saccharomyces cerevisiae*. *Molecular Biology of the Cell* **14**, 4931-46.

Nematollahi, G., Kianianmomeni, A. and Hallmann, A. (2006). Quantitative analysis of cell-type specific gene expression in the green alga *Volvox carteri*. *Bmc Genomics* **7**, 19.

Nevell, T. P. (1985). Cellulose chemistry and its applications. In *Ellis Horwood series in chemical science*, (ed., pp. 552. Chichester: E. Horwood.

Ng, T. K. and Zeikus, J. G. (1981). Comparison of Extracellular Cellulase Activities of *Clostridium thermocellum* LQRI and *Trichoderma reesei* QM9414. *Appl Environ Microbiol* **42**, 231-40.

Nicholson, T. B., Chen, T. and Richard, S. (2009). The physiological and pathophysiological role of PRMT1-mediated protein arginine methylation. *Pharmacol Res* **60**, 466-74.

Nicolai, M., Roncato, M. A., Canoy, A. S., Rouquie, D., Sarda, X., Freyssinet, G. and Robaglia, C. (2006). Large-scale analysis of mRNA translation states during sucrose starvation in arabidopsis cells identifies cell proliferation and chromatin structure as targets of translational control. *Plant Physiol* **141**, 663-73.

Nield, J., Balsera, M., De Las Rivas, J. and Barber, J. (2002). Three-dimensional electron cryo-microscopy study of the extrinsic domains of the oxygen-evolving complex of spinach: assignment of the PsbO protein. *J Biol Chem* **277**, 15006-12.

Nield, J. and Barber, J. (2006). Refinement of the structural model for the Photosystem II supercomplex of higher plants. *Biochim Biophys Acta* **1757**, 353-61.

Nield, J., Kruse, O., Ruprecht, J., da Fonseca, P., Buchel, C. and Barber, J. (2000). Three-dimensional structure of *Chlamydomonas reinhardtii* and *Synechococcus elongatus* photosystem II complexes allows for comparison of their oxygen-evolving complex organization. *J Biol Chem* **275**, 27940-6.

Nield, J., Redding, K. and Hippler, M. (2004). Remodeling of light-harvesting protein complexes in *Chlamydomonas* in response to environmental changes. *Eukaryot Cell* **3**, 1370-80.

Niyogi, K. K. (1999). PHOTOPROTECTION REVISITED: Genetic and Molecular Approaches. *Annu Rev Plant Physiol Plant Mol Biol* **50**, 333-359.

Noctor, G., Arisi, A. C. M., Jouanin, L., Valadier, M. H., Roux, Y. and Foyer, C. H. (1997). Light-dependent modulation of foliar glutathione synthesis and associated amino acid metabolism in poplar overexpressing gamma-glutamylcysteine synthetase. *Planta* **202**, 357-369.

Noctor, G. and Foyer, C. H. (1998). Simultaneous measurement of foliar glutathione, gamma-glutamylcysteine, and amino acids by high-performance liquid chromatography: Comparison with two other assay methods for glutathione. *Analytical Biochemistry* **264**, 98-110.

Norsker, N. H., Barbosa, M. J., Vermue, M. H. and Wijffels, R. H. (2011). Microalgal production--a close look at the economics. *Biotechnology Advances* **29**, 24-7.

Nott, A., Jung, H. S., Koussevitzky, S. and Chory, J. (2006). Plastid-to-nucleus retrograde signaling. *Annu Rev Plant Biol* **57**, 739-59.

Nozaki, M., Miura, C., Tozawa, Y. and Miura, T. (2009). The contribution of endogenous cellulase to the cellulose digestion in the gut of earthworm (*Pheretima hilgendorfi*: Megascolecidae). *Soil Biology and Biochemistry* **41**, 762-769.

O'Sullivan, A. (1997). Cellulose: the structure slowly unravels. *Cellulose* **4**, 173-207.

Oelmuller, R., Dietrich, G., Link, G. and Mohr, H. (1986). Regulatory Factors Involved in Gene-Expression (Subunits of Ribulose-1,5-Bisphosphate Carboxylase) in Mustard (Sinapis-Alba L) Cotyledons. *Planta* **169**, 260-266.

Ogawa, T. and Aiba, S. (1981). Bioenergetic analysis of mixotrophic growth in *Chlorella vulgaris* and *Scenedesmus acutus*. *Biotechnology and Bioengineering* **23**, 1121-1132.

Ohnishi, N., Allakhverdiev, S. I., Takahashi, S., Higashi, S., Watanabe, M., Nishiyama, Y. and Murata, N. (2005). Two-step mechanism of photodamage to photosystem II: step 1 occurs at the oxygen-evolving complex and step 2 occurs at the photochemical reaction center. *Biochemistry* **44**, 8494-9.

Ort, D. R. and Melis, A. (2011). Optimizing antenna size to maximize photosynthetic efficiency. *Plant Physiology* **155**, 79-85.

Ostareck, D. H., Ostareck-Lederer, A., Wilm, M., Thiele, B. J., Mann, M. and Hentze, M. W. (1997). mRNA silencing in erythroid differentiation: hnRNP K and hnRNP E1 regulate 15-lipoxygenase translation from the 3' end. *Cell* **89**, 597-606.

Ostergaard, H., Tachibana, C. and Winther, J. R. (2004). Monitoring disulfide bond formation in the eukaryotic cytosol. *Journal of Cell Biology* **166**, 337-345.

Paget, M. S. and Buttner, M. J. (2003). Thiol-based regulatory switches. *Annu Rev Genet* **37**, 91-121.

Pahlich, S., Zakaryan, R. P. and Gehring, H. (2006). Protein arginine methylation: Cellular functions and methods of analysis. *Biochim Biophys Acta* **1764**, 1890-903.

Paik, W. K. and Kim, S. (1980). Natural occurrence of various methylated

amino acid derivatives. New York: John Wiley & Sons.

Palmqvist, E. and Hahn-Hagerdal, B. (2000). Fermentation of lignocellulosic hydrolysates. I: inhibition and detoxification. *Bioresource Technology* **74**, 17-24.

Park, J. K., Kim, J. D., Patil, S. S., Kim, S. J. and Lee, C. G. (2008). Expression and Characterization of Chitinolytic Enzymes in *Chlamydomonas reinhardtii*. *Journal of Chitin and Chitosan* **13**, 95-100.

Pauly, M. and Keegstra, K. (2008). Cell-wall carbohydrates and their modification as a resource for biofuels. *Plant J* **54**, 559-68.

Peers, G., Truong, T. B., Ostendorf, E., Busch, A., Elrad, D., Grossman, A. R., Hippler, M. and Niyogi, K. K. (2009). An ancient light-harvesting protein is critical for the regulation of algal photosynthesis. *Nature* **462**, 518-21.

Perez-Garcia, O., Escalante, F. M., de-Bashan, L. E. and Bashan, Y. (2011). Heterotrophic cultures of microalgae: metabolism and potential products. *Water Res* **45**, 11-36.

Perez, J., Munoz-Dorado, J., de la Rubia, T. and Martinez, J. (2002). Biodegradation and biological treatments of cellulose, hemicellulose and lignin: an overview. *Int Microbiol* **5**, 53-63.

Pesaresi, P., Hertle, A., Pribil, M., Kleine, T., Wagner, R., Strissel, H., Ihnatowicz, A., Bonardi, V., Scharfenberg, M., Schneider, A. et al. (2009). Arabidopsis STN7 Kinase Provides a Link between Short- and Long-Term Photosynthetic Acclimation. *The Plant Cell Online* **21**, 2402-2423.

Petracek, M. E., Dickey, L. F., Huber, S. C. and Thompson, W. F. (1997). Light-regulated changes in abundance and polyribosome association of ferredoxin mRNA are dependent on photosynthesis. *Plant Cell* **9**, 2291-2300.

Petroutsos, D., Busch, A., Janssen, I., Trompelt, K., Bergner, S. V., Weinl, S., Holtkamp, M., Karst, U., Kudla, J. and Hippler, M. (2011). The chloroplast calcium sensor CAS is required for photoacclimation in *Chlamydomonas reinhardtii*. *Plant Cell* **23**, 2950-63.

Pfannschmidt, T. (2010). Plastidial retrograde signalling--a true "plastid factor" or just metabolite signatures? *Trends Plant Sci* **15**, 427-35.

Pfannschmidt, T., Nilsson, A., Tullberg, A., Link, G. and Allen, J. F. (1999). Direct transcriptional control of the chloroplast genes psbA and psaAB adjusts photosynthesis to light energy distribution in plants. *IUBMB Life* **48**, 271-6.

Pfannschmidt, T., Schutze, K., Fey, V., Sherameti, I. and Oelmuller, R. (2003). Chloroplast redox control of nuclear gene expression--a new class of plastid signals in interorganellar communication. *Antioxid Redox Signal* **5**, 95-101.

Philippe, B., Matsumoto, K. and Wolffe, A. P. (1995). Sequence-specific RNA Recognition by the *Xenopus* Y-box Proteins. *Journal of Biological Chemistry* **270**, 28297-28303.

Phytozome7.0. http://www.phytozome.net

Piippo, M., Allahverdiyeva, Y., Paakkarinen, V., Suoranta, U. M., Battchikova, N. and Aro, E. M. (2006). Chloroplast-mediated regulation of nuclear genes in *Arabidopsis thaliana* in the absence of light stress. *Physiol Genomics* **25**, 142-52.

Piques, M., Schulze, W. X., Hohne, M., Usadel, B., Gibon, Y., Rohwer, J. and Stitt, M. (2009). Ribosome and transcript copy numbers, polysome occupancy and enzyme dynamics in *Arabidopsis*. *Mol Syst Biol* **5**, 314.

Polle, J. E., Kanakagiri, S., Jin, E., Masuda, T. and Melis, A. (2002). Truncated chlorophyll antenna size of the photosystems-a practical method to improve microalgal productivity and hydrogen production in mass culture. *International Journal of Hydrogen Energy* **27**, 1257-1264.

Polle, J. E., Kanakagiri, S. D. and Melis, A. (2003). tla1, a DNA insertional transformant of the green alga *Chlamydomonas reinhardtii* with a truncated light-harvesting chlorophyll antenna size. *Planta* **217**, 49-59.

Popescu, C. E., Borza, T., Bielawski, J. P. and Lee, R. W. (2006). Evolutionary rates and expression level in *Chlamydomonas*. *Genetics* **172**, 1567-76.

Posten, C. and Schaub, G. (2009). Microalgae and terrestrial biomass as source for fuels—A process view. *Journal of Biotechnology* **142**, 64-69.

Prince, R. C. and Kheshgi, H. S. (2005). The photobiological production of hydrogen: potential efficiency and effectiveness as a renewable fuel. *Crit Rev Microbiol* **31**, 19-31.

Proschold, T., Harris, E. H. and Coleman, A. W. (2005). Portrait of a species: *Chlamydomonas reinhardtii*. *Genetics* **170**, 1601-10.

Pursiheimo, S., Mulo, P., Rintamaki, E. and Aro, E. M. (2001). Coregulation of light-harvesting complex II phosphorylation and lhcb mRNA accumulation in winter rye. *Plant J* **26**, 317-27.

Radakovits, R., Jinkerson, R. E., Darzins, A. and Posewitz, M. C. (2010). Genetic engineering of algae for enhanced biofuel production. *Eukaryot Cell* **9**, 486-501.

Raghothama, S., Simpson, P. J., Szabo, L., Nagy, T., Gilbert, H. J. and Williamson, M. P. (2000). Solution structure of the CBM10 cellulose binding module from *Pseudomonas* xylanase A. *Biochemistry* **39**, 978-84.

Ramesh, V. M., Bingham, S. E. and Webber, A. N. (2004). A simple method for chloroplast transformation in *Chlamydomonas reinhardtii*. *Methods Mol Biol* **274**, 301-7.

Rasala, B. A., Muto, M., Lee, P. A., Jager, M., Cardoso, R. M. F., Behnke, C. A., Kirk, P., Hokanson, C. A., Crea, R., Mendez, M. et al. (2010). Production of therapeutic proteins in algae, analysis of expression of seven human proteins in the chloroplast of *Chlamydomonas reinhardtii*. *Plant Biotechnology Journal* **8**, 719-733.

Redding, K. E. and Cole, D. G. (2008). *Chlamydomonas*: a sexually active, light-harvesting, carbon-reducing, hydrogen-belching /`planimal/'. *EMBO Rep* **9**, 1182-1187.

Remacle, C., Cardol, P., Coosemans, N., Gaisne, M. and Bonnefoy, N. (2006). High-efficiency biolistic transformation of *Chlamydomonas* mitochondria can be used to insert mutations in complex I genes. *Proc Natl Acad Sci U S A* **103**, 4771-6.

REN21. (2011). Renewables In *Global Status Report*, (ed. Paris.

Renger, G. (2001). Photosynthetic water oxidation to molecular oxygen: apparatus and mechanism. *Biochimica et Biophysica Acta (BBA) - Bioenergetics* **1503**, 210-228.

Renger, G. and Kuhn, P. (2007). Reaction pattern and mechanism of light induced oxidative water splitting in photosynthesis. *Biochim Biophys Acta* **1767**, 458-71.

Rhee, S. G., Kang, S. W., Jeong, W., Chang, T. S., Yang, K. S. and Woo, H. A. (2005). Intracellular messenger function of hydrogen peroxide and its regulation by peroxiredoxins. *Curr Opin Cell Biol* **17**, 183-9.

Rifkin, J. (2002). The hydrogen economy : the creation of the world-wide energy web and the redistribution of power on earth, (ed., pp. 294. New York: J.P. Tarcher/Putnam.

Robson, L. M. and Chambliss, G. H. (1984). Characterization of the cellulolytic activity of a *Bacillus* isolate. *Appl Environ Microbiol* **47**, 1039-46.

Rochaix, J. D. (1995). *Chlamydomonas reinhardtii* as the photosynthetic yeast. *Annu Rev Genet* **29**, 209-30.

Rodermel, S. (2001). Pathways of plastid-to-nucleus signaling. *Trends Plant Sci* **6**, 471-8.

Romero-Puertas, M. C., Laxa, M., Matte, A., Zaninotto, F., Finkemeier, I., Jones, A. M., Perazzolli, M., Vandelle, E., Dietz, K. J. and Delledonne, M. (2007). S-nitrosylation of peroxiredoxin II E promotes peroxynitrite-mediated tyrosine nitration. *Plant Cell* **19**, 4120-30.

Roy, A., Kucukural, A. and Zhang, Y. (2010). I-TASSER: a unified platform for automated protein structure and function prediction. *Nature Protocols* **5**, 725-738.

Ruban, A. V. (2009). Plants in light. *Commun Integr Biol* **2**, 50-5.

Ruban, A. V., Wentworth, M., Yakushevska, A. E., Andersson, J., Lee, P. J., Keegstra, W., Dekker, J. P., Boekema, E. J., Jansson, S. and Horton, P. (2003). Plants lacking the main light-harvesting complex retain photosystem II macro-organization. *Nature* **421**, 648-52.

Rubin, E. M. (2008). Genomics of cellulosic biofuels. *Nature* **454**, 841-845.

Rupprecht, J. (2009). From systems biology to fuel—*Chlamydomonas reinhardtii* as a model for a systems biology approach to improve biohydrogen production. *Journal of Biotechnology* **142**, 10-20.

Sager, R. and Granick, S. (1953). Nutritional studies with *Chlamydomonas reinhardi*. *Ann N Y Acad Sci* **56**, 831-8.

Saitou, N. and Nei, M. (1987). The Neighbor-Joining Method - a New Method for Reconstructing Phylogenetic Trees. *Molecular Biology and Evolution* **4**, 406-425.

Sakon, J., Irwin, D., Wilson, D. B. and Karplus, P. A. (1997). Structure and mechanism of endo/exocellulase E4 from *Thermomonospora fusca*. *Nat Struct Biol* **4**, 810-8.

Sanchez, S. E., Petrillo, E., Beckwith, E. J., Zhang, X., Rugnone, M. L., Hernando, C. E., Cuevas, J. C., Godoy Herz, M. A., Depetris-Chauvin, A., Simpson, C. G. et al. (2010). A methyl transferase links the circadian clock to the regulation of alternative splicing. *Nature* **468**, 112-6.

Scebba, F., De Bastiani, M., Bernacchia, G., Andreucci, A., Galli, A. and Pitto, L. (2007). PRMT11: a new *Arabidopsis* MBD7 protein partner with arginine methyltransferase activity. *Plant J* **52**, 210-22.

Schafer, F. Q. and Buettner, G. R. (2001). Redox environment of the cell as viewed through the redox state of the glutathione disulfide/glutathione couple. *Free Radic Biol Med* **30**, 1191-212.

Schenk, P., Thomas-Hall, S., Stephens, E., Marx, U., Mussgnug, J. H., Posten, C., Kruse, O. and Hankamer, B. (2008). Second Generation Biofuels: High-Efficiency Microalgae for Biodiesel Production. *BioEnergy Research* **1**, 20-43.

Schlesinger, W. H. (1991). Biogeochemistry: an analysis of global change, (ed., pp. XI, 443. San Diego ; New York etc.: Academic Press.

Schneider, M. J., Ulland, M. and Sloboda, R. D. (2008). A Protein Methylation Pathway in *Chlamydomonas* Flagella Is Active during Flagellar Resorption. *Molecular Biology of the Cell* **19**, 4319-4327.

Schroda, M. (2006). RNA silencing in Chlamydomonas: mechanisms and tools. *Curr Genet* **49**, 69-84.

Scott, S. A., Davey, M. P., Dennis, J. S., Horst, I., Howe, C. J., Lea-Smith, D. J. and Smith, A. G. (2010). Biodiesel from algae: challenges and prospects. *Curr Opin Biotechnol* **21**, 277-86.

Seelert, H., Poetsch, A., Dencher, N. A., Engel, A., Stahlberg, H. and Muller, D. J. (2000). Structural biology. Proton-powered turbine of a plant motor. *Nature* **405**, 418-9.

Serpa, V. I. and Polikarpov, I. (2011). Enzymes in Bioenergy. In *Routes to Cellulosic Ethanol*, (ed. M. S. Buckeridge and G. H. Goldman), pp. 97-113. New York: Springer

Shamala, T. R., Drawert, F. and Leupold, G. (1982). Studies on *Scenedesmus acutus* growth. I effect of autotrophic and mixotrophic conditions on the growth of Scenedesmus acutus. *Biotechnology and Bioengineering* **24**, 1287-99.

Shamoo, Y., Abdul-Manan, N., Patten, A. M., Crawford, J. K., Pellegrini, M. C. and Williams, K. R. (1994). Both RNA-binding domains in heterogenous nuclear ribonucleoprotein A1 contribute toward single-stranded-RNA binding. *Biochemistry* **33**, 8272-81.

Shao, J., Xu, D., Tsai, S. N., Wang, Y. and Ngai, S. M. (2009). Computational identification of protein methylation sites through bi-profile Bayes feature extraction. *PLoS One* **4**, e4920.

Shapiguzov, A., Ingelsson, B., Samol, I., Andres, C., Kessler, F., Rochaix, J. D., Vener, A. V. and Goldschmidt-Clermont, M. (2010). The PPH1 phosphatase is specifically involved in LHCII dephosphorylation and state transitions in *Arabidopsis*. *Proc Natl Acad Sci U S A* **107**, 4782-7.

Sherameti, I., Nakamura, M., Yamamoto, Y. Y., Pfannschmidt, T., Obokata, J. and Oelmuller, R. (2002). Polyribosome loading of spinach mRNAs for photosystem I subunits is controlled by photosynthetic electron transport. *Plant J* **32**, 631-9.

Shrager, J., Hauser, C., Chang, C. W., Harris, E. H., Davies, J., McDermott, J., Tamse, R., Zhang, Z. and Grossman, A. R. (2003). Chlamydomonas reinhardtii genome project. A guide to the generation and use of the cDNA information. *Plant Physiol* **131**, 401-8.

Singh, A., Nigam, P. S. and Murphy, J. D. (2011). Renewable fuels from algae: An answer to debatable land based fuels. *Bioresource Technology* **102**, 10-16.

Sloboda, R. D. (2009). Posttranslational protein modifications in cilia and flagella. *Methods Cell Biol* **94**, 347-63.

Sloboda, R. D. and Howard, L. (2009). Protein Methylation in Full Length *Chlamydomonas* Flagella. *Cell Motility and the Cytoskeleton* **66**, 650-660.

Smant, G., Stokkermans, J. P. W. G., Yan, Y. T., de Boer, J. M., Baum, T. J., Wang, X. H., Hussey, R. S., Gommers, F. J., Henrissat, B., Davis, E. L. et al. (1998). Endogenous cellulases in animals: Isolation of beta-1,4-endoglucanase genes from two species of plant-parasitic cyst nematodes. *Proceedings of the National Academy of Sciences of the United States of America* **95**, 4906-4911.

Sommerville, J. (1999). Activities of cold-shock domain proteins in translation control. *Bioessays* **21**, 319-25.

Sormani, R., Delannoy, E., Lageix, S., Bitton, F., Lanet, E., Saez-Vasquez, J., Deragon, J. M., Renou, J. P. and Robaglia, C. (2011). Sublethal cadmium intoxication in *Arabidopsis thaliana* impacts translation at multiple levels. *Plant Cell Physiol* **52**, 436-47.

Spalding, M. H. (2008). Microalgal carbon-dioxide-concentrating mechanisms: *Chlamydomonas* inorganic carbon transporters. *Journal of Experimental Botany* **59**, 1463-1473.

Spiridonov, N. A. and Wilson, D. B. (1998). Regulation of biosynthesis of individual cellulases in *Thermomonospora fusca*. *J Bacteriol* **180**, 3529-32.

Stauber, E. J., Fink, A., Markert, C., Kruse, O., Johanningmeier, U. and Hippler, M. (2003). Proteomics of *Chlamydomonas reinhardtii* light-harvesting proteins *Eukaryotic Cell* **2**, 978-994.

Steenbakkers, P. J., Freelove, A., Van Cranenbroek, B., Sweegers, B. M., Harhangi, H. R., Vogels, G. D., Hazlewood, G. P., Gilbert, H. J. and Op den Camp, H. J. (2002). The major component of the cellulosomes of anaerobic fungi from the genus Piromyces is a family 48 glycoside hydrolase. *DNA Seq* **13**, 313-20.

Steiner, S., Dietzel, L., Schroter, Y., Fey, V., Wagner, R. and Pfannschmidt, T. (2009). The role of phosphorylation in redox regulation of photosynthesis genes psaA and psbA during photosynthetic acclimation of mustard. *Mol Plant* **2**, 416-29.

Stephens, E., Ross, I. L., King, Z., Mussgnug, J. H., Kruse, O., Posten, C., Borowitzka, M. A. and Hankamer, B. (2010a). An economic and technical evaluation of microalgal biofuels. *Nat Biotechnol* **28**, 126-8.

Stephens, E., Ross, I. L., Mussgnug, J. H., Wagner, L. D., Borowitzka, M. A., Posten, C., Kruse, O. and Hankamer, B. (2010b). Future prospects of microalgal biofuel production systems. *Trends Plant Sci* **15**, 554-64.

Stetler, A., Winograd, C., Sayegh, J., Cheever, A., Patton, E., Zhang, X., Clarke, S. and Ceman, S. (2006). Identification and characterization of the methyl arginines in the fragile X mental retardation protein Fmrp. *Human Molecular Genetics* **15**, 87-96.

Sticklen, M. B. (2008). Plant genetic engineering for biofuel production: towards affordable cellulosic ethanol (Retracted article. See vol 11, pg 308, 2010). *Nature Reviews Genetics* **9**, 433-443.

Sukenik, A., Falkowski, P. G. and Bennett, J. (1987). Potential enhancement of photosynthetic energy conversion in algal mass culture. *Biotechnol Bioeng* **30**, 970-7.

Surpin, M. and Chory, J. (1997). The co-ordination of nuclear and organellar genome expression in eukaryotic cells. *Essays Biochem* **32**, 113-25.

Suzuki, K., Ojima, T. and Nishita, K. (2003). Purification and cDNA cloning of a cellulase from abalone *Haliotis discus hannai*. *Eur J Biochem* **270**, 771-8.

Suzuki, N., Koussevitzky, S., Mittler, R. O. N. and Miller, G. A. D. (2012). ROS and redox signalling in the response of plants to abiotic stress. *Plant, Cell & Environment* **35**, 259-270.

Szabo, I., Bergantino, E. and Giacometti, G. M. (2005). Light and oxygenic photosynthesis: energy dissipation as a protection mechanism against photo-oxidation. *EMBO Rep* **6**, 629-34.

Tafuri, S. R. and Wolffe, A. P. (1990). Xenopus Y-box transcription factors: molecular cloning, functional analysis and developmental regulation. *Proc Natl Acad Sci U S A* **87**, 9028-32.

Takahashi, Iwai, M., Takahashi, Y. and Minagawa, J. (2006). Identification of the mobile light-harvesting complex II polypeptides for state transitions in *Chlamydomonas reinhardtii*. *Proc Natl Acad Sci U S A* **103**, 477-82.

Takahashi, Yasui, T. A., Stauber, E. J. and Hippler, M. (2004). Comparison of the subunit compositions of the PSI-LHCI supercomplex and the LHCI in the green alga *Chlamydomonas reinhardtii*. *Biochemistry* **43**, 7816-23.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. and Kumar, S. (2011). MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol Biol Evol* **28**, 2731-9.

Tang, G., Reinhart, B. J., Bartel, D. P. and Zamore, P. D. (2003). A biochemical framework for RNA silencing in plants. *Genes Dev* **17**, 49-63.

Tang, J., Frankel, A., Cook, R. J., Kim, S., Paik, W. K., Williams, K. R., Clarke, S. and Herschman, H. R. (2000). PRMT1 is the predominant type I protein arginine methyltransferase in mammalian cells. *J. Biol. Chem* **275**, 7723–7730.

Tanner, W. (1969). Light-driven active uptake of 3-O-methylglucose via an inducible hexose uptake system of Chlorella. *Biochemical and Biophysical Research Communications* **36**, 278-283.

Teather, R. M. and Wood, P. J. (1982). Use of Congo red-polysaccharide interactions in enumeration and characterization of cellulolytic bacteria from the bovine rumen. *Appl Environ Microbiol* **43**, 777-80.

Teeri, T. T. (1997). Crystalline cellulose degradation: new insight into the function of cellobiohydrolases. *Trends in Biotechnology* **15**, 160-167.

Teixeira, D., Sheth, U., Valencia-Sanchez, M. A., Brengues, M. and Parker, R. (2005). Processing bodies require RNA for assembly and contain nontranslating mRNAs. *RNA* **11**, 371-82.

Teramoto, H., Nakamori, A., Minagawa, J. and Ono, T. (2002). Light-intensity-dependent expression of Lhc gene family encoding light-harvesting chlorophyll-a/b proteins of photosystem II in *Chlamydomonas reinhardtii*. *Plant Physiology* **130**, 325-333.

Thompson, J. D., Higgins, D. G. and Gibson, T. J. (1994). Improved Sensitivity of Profile Searches through the Use of Sequence Weights and Gap Excision. *Computer Applications in the Biosciences* **10**, 19-29.

Tokuda, G., Lo, N., Watanabe, H., Slaytor, M., Matsumoto, T. and Noda, H. (1999). Metazoan cellulase genes from termites: intron/exon structures and sites of expression. *Biochim Biophys Acta* **1447**, 146-59.

Tokutsu, R., Teramoto, H., Takahashi, Y., Ono, T. A. and Minagawa, J. (2004). The light-harvesting complex of photosystem I in *Chlamydomonas reinhardtii*: protein composition, gene structures and phylogenic implications. *Plant Cell Physiol* **45**, 138-45.

Tomme, P., Warren, R. A. and Gilkes, N. R. (1995). Cellulose hydrolysis by bacteria and fungi. *Adv Microb Physiol* **37**, 1-81.

Tsai, N. P., Ho, P. C. and Wei, L. N. (2008). Regulation of stress granule dynamics by Grb7 and FAK signalling pathway. *EMBO J* **27**, 715-26.

Tsai, N. P. and Wei, L. N. (2010). RhoA/ROCK1 signaling regulates stress granule formation and apoptosis. *Cell Signal* **22**, 668-75.

Turkina, M. V., Kargul, J., Blanco-Rivero, A., Villarejo, A., Barber, J. and Vener, A. V. (2006). Environmentally modulated phosphoproteome of photosynthetic membranes in the green alga *Chlamydomonas reinhardtii*. *Mol Cell Proteomics* **5**, 1412-1425

UniProtKB. UniProt Knowledgebase http://www.uniprot.org/

Vass, I., Styring, S., Hundal, T., Koivuniemi, A., Aro, E. and Andersson, B. (1992). Reversible and irreversible intermediates during photoinhibition of photosystem II: stable reduced QA species promote chlorophyll triplet formation. *Proc Natl Acad Sci U S A* **89**, 1408-12.

Vazhappilly, R. and Chen, F. (1998). Eicosapentaenoic acid and docosahexaenoic acid production potential of microalgae and their heterotrophic growth. *Journal of the American Oil Chemists' Society* **75**, 393-397.

Venter, J. C. Adams, M. D. Myers, E. W. Li, P. W. Mural, R. J. Sutton, G. G. Smith, H. O. Yandell, M. Evans, C. A. Holt, R. A. et al. (2001). The Sequence of the Human Genome. *Science* **291**, 1304-1351.

Verbruggen, A. and Al Marchohi, M. (2010). Views on peak oil and its relation to climate change policy. *Energy Policy* **38**, 5572-5581.

Vernotte, C., Picaud, M., Kirilovsky, D., Olive, J., Ajlani, G. and Astier, C. (1992). Changes in the photosynthetic apparatus in the cyanobacterium *Synechocystis sp.* PCC 6714 following light-to-dark and dark-to-light transitions. *Photosynthesis Research* **32**, 45-57.

Waltenberger, H., Schneid, C., Grosch, J. O., Bareiss, A. and Mittag, M. (2001). Identification of target mRNAs for the clock-controlled RNA-binding protein Chlamy 1 from *Chlamydomonas reinhardtii*. *Mol Genet Genomics* **265**, 180-8.

Walters, R. G. (2005). Towards an understanding of photosynthetic acclimation. *J. Exp. Bot.* **56**, 435-447.

Watanabe, H., Noda, H., Tokuda, G. and Lo, N. (1998). A cellulase gene of termite origin. *Nature* **394**, 330-331.

Weber, A. P. and Fischer, K. (2007). Making the connections--the crucial role of metabolite transporters at the interface between chloroplast and cytosol. *FEBS Lett* **581**, 2215-22.

Wei, H., Xu, Q., Taylor Ii, L. E., Baker, J. O., Tucker, M. P. and Ding, S.-Y. (2009). Natural paradigms of plant cell wall degradation. *Current Opinion in Biotechnology* **20**, 330-338.

Wen, Z. Y. and Chen, F. (2000). Heterotrophic production of eicosapentaenoid acid by the diatom *Nitzschia laevis* effects of silicate and glucose. *Journal of Industrial Microbiology & Biotechnology* **25**, 218-224.

Wendel, H. G., Silva, R. L., Malina, A., Mills, J. R., Zhu, H., Ueda, T., Watanabe-Fukunaga, R., Fukunaga, R., Teruya-Feldstein, J., Pelletier, J. et al. (2007). Dissecting eIF4E action in tumorigenesis. *Genes Dev* **21**, 3232-7.

White, M. and Quarmby, L. (2008). The NIMA-family kinase, Nek1 affects the stability of centrosomes and ciliogenesis. *BMC Cell Biology* **9**, 29.

Wobbe, L., Schwarz, C., Nickelsen, J. and Kruse, O. (2008). Translational control of photosynthetic gene expression in phototrophic eukaryotes. *Physiologia Plantarum* **133**, 507-515.

Wolfe, G. R., Cunningham, F. X., Durnfordt, D., Green, B. R. and Gantt, E. (1994). Evidence for a common origin of chloroplasts with light-harvesting complexes of different pigmentation. *Nature* **367**, 566-568.

Wolfenden, R. and Snider, M. J. (2001). The depth of chemical time and the power of enzymes as catalysts. *Acc Chem Res* **34**, 938-45.

Wolffe, A. P. (1994). Structural and functional properties of the evolutionarily ancient Y-box family of nucleic acid binding proteins. *Bioessays* **16**, 245-251.

Wolffe, A. P., Tafuri, S., Ranjan, M. and Familari, M. (1992). The Y-box factors: a family of nucleic acid binding proteins conserved from *Escherichia coli* to man. *New Biol* **4**, 290-8.

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

Wollman, F. A. and Delepelaire, P. (1984). Correlation between changes in light energy distribution and changes in thylakoid membrane polypeptide phosphorylation in *Chlamydomonas reinhardtii*. *J Cell Biol* **98**, 1-7.

Woodson, J. D. and Chory, J. (2008). Coordination of gene expression between organellar and nuclear genomes. *Nat Rev Genet* **9**, 383-95.

Xu, H., Miao, X. and Wu, Q. (2006). High quality biodiesel production from a microalga *Chlorella protothecoides* by heterotrophic growth in fermenters. *J Biotechnol* **126**, 499-507.

Yabuta, Y., Maruta, T., Yoshimura, K., Ishikawa, T. and Shigeoka, S. (2004). Two distinct redox signaling pathways for cytosolic APX induction under photooxidative stress. *Plant Cell Physiol* **45**, 1586-94.

Yan, H., Zhang, P., Wang, C., Liu, Z. and Chang, W. (2007). Two lutein molecules in LHCII have different conformations and functions: Insights into the molecular mechanism of thermal dissipation in plants. *Biochemical and Biophysical Research Communications* **355**, 457-463.

Yan, Y., Smant, G., Stokkermans, J., Qin, L., Helder, J., Baum, T., Schots, A. and Davis, E. (1998). Genomic organization of four beta-1,4-endoglucanase genes in plant-parasitic cyst nematodes and its evolutionary implications. *Gene* **220**, 61-70.

Yosef, I., Irihimovitch, V., Knopf, J. A., Cohen, I., Orr-Dahan, I., Nahum, E., Keasar, C. and Shapira, M. (2004). RNA binding activity of the ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit from *Chlamydomonas reinhardtii*. *J Biol Chem* **279**, 10148-56.

Yu, M. C. (2011). The Role of Protein Arginine Methylation in mRNP Dynamics. *Mol Biol Int* **2011**, 163827.

Zhang, G., Zeng, G., Cai, X., Deng, S., Luo, H. and Sun, G. (2007). *Brachybacterium zhongshanense sp. nov.*, a cellulose-decomposing bacterium from sediment along the Qijiang River, Zhongshan City, China. *Int J Syst Evol Microbiol* **57**, 2519-24.

Zhang, Y. H., Cui, J., Lynd, L. R. and Kuang, L. R. (2006). A Transition from Cellulose Swelling to Cellulose Dissolution by o-Phosphoric Acid: Evidence from Enzymatic Hydrolysis and Supramolecular Structure. *Biomacromolecules* **7**, 644-648.

Zhang, Y. H. and Lynd, L. R. (2004). Toward an aggregated understanding of enzymatic hydrolysis of cellulose: noncomplexed cellulase systems. *Biotechnol Bioeng* **88**, 797-824.

Zhao, B., Schneid, C., Iliev, D., Schmidt, E. M., Wagner, V., Wollnik, F. and Mittag, M. (2004). The Circadian RNA-Binding Protein CHLAMY 1 Represents a Novel Type Heteromer of RNA Recognition Motif and Lysine Homology Domain-Containing Subunits. *Eukaryotic Cell* **3**, 815-825.

Zhbankov, R. G. (1992). Hydrogen-Bonds and Structure of Carbohydrates. *Journal of Molecular Structure* **270**, 523-539.

Zhu, J., Pan, X. and Zalesny, R. (2010). Pretreatment of woody biomass for biofuel production: energy efficiency, technologies, and recalcitrance. *Applied Microbiology and Biotechnology* **87**, 847-857.

Zhu, X. G., Long, S. P. and Ort, D. R. (2008). What is the maximum efficiency with which photosynthesis can convert solar energy into biomass? *Curr Opin Biotechnol* **19**, 153-9.

V. Publications

The three manuscripts presented in this thesis are:

[1] Cystein modification of a specific repressor protein controls the translational status of nucleus – encoded LHCII mRNAs in *Chlamydomonas*

Lutz Wobbe, Olga Blifernez, Christian Schwarz, Jan H. Mussgnug, Jörg Nickelsen and Olaf Kruse

PNAS, 106(32), 2009: 13290-13295, doi: 10.1073/pnas.0900670106

[2] Protein arginine methylation modulates light-harvesting antenna translation in *Chlamydomonas reinhardtii*

Olga Blifernez, Lutz Wobbe, Karsten Niehaus and Olaf Kruse

The Plant Journal, 65, 2011: 119-130, doi: 10.1111/j.1365-313X.2010.04406.x

[3] Cellulose degradation and assimilation by the unicellular phototrophic eukaryote *Chlamydomonas reinhardtii*

Olga Blifernez-Klassen[2], Viktor Klassen, Anja Doebbe, Klaudia Kersting, Philipp Grimm, Lutz Wobbe and Olaf Kruse

Nature Communications 3, 2012:1214, doi: 10.1038/ ncomms2210

[2] The first authors name and the title of the manuscript have changed during the review process.

Curriculum vitae

Persönliche Daten

Vor- und Zuname	Olga Blifernez-Klassen, geb. Blifernez
Geburtsdatum	07. März 1980
Geburtsort	Dsheskasgan (Kasachstan)
Nationalität	deutsch
Familienstand	verheiratet, seit 25.02.2012

Promotion

08/2007 - 04/2012	Promotion am Lehrstuhl Algenbiotechnologie und Bioenergie, Prof. Dr. Olaf Kruse, Fakultät für Biologie, Universität Bielefeld
01/2010 - 06/2011	Promotionsstipendium des Rektorats der Universität Bielefeld

Studium

10/2001 - 03/2007	Studium der Molekularen Biotechnologie an der Universität Bielefeld Abschluss: Diplom (Sehr Gut) Thema der Diplomarbeit: „Regulation der IgE-Synthese durch löslichen IgE Rezeptor (sCD23) an humanen Peripheren Blut Mononukleären Zellen (PBMC)“

Schulbildung

08/1999 - 07/2000	Elisabeth-Knipping-Schule in Kassel Beruf: Staatlich-geprüfte biologisch technische Assistentin Abschluss: Berufsfachschule (Fachrichtung: Biologietechnik)
08/1996 - 07/1999	Berufliches Gymnasium der Elisabeth-Knipping-Schule in Kassel Abschluss: Allgemeine Hochschulreife
08/1993 - 07/1996	Gesamtschule Bad Emstal Abschluss: Mittlere Reife
09/1987 - 05/1992	Grund- und Mittelschule Dsheskasgan Kasachstan

Acknowledgements

Eigene Publikationen

Wobbe, L., **Blifernez, O.**, Schwarz, C., Mussgnug, J. H., Nickelsen, J., Kruse, O. (2009) Cystein modification of a specific repressor protein controls the translational status of nucleus – encoded LHCII mRNAs in *Chlamydomonas*. *PNAS*, **106**(32), 13290-13295.

Blifernez, O., Wobbe, L., Niehaus, K., Kruse, O. (2011) Protein arginine methylation modulates light-harvesting antenna translation in *Chlamydomonas reinhardtii*. *The Plant Journal*, **65**, 119-130.

Nguyen, A.V., Toepel, J., Burgess, S., Uhmeyer, A., **Blifernez, O.**, Doebbe, A., Hankamer, B., Nixon, P., Wobbe, L., Kruse, O. (2011) Time-Course Global Expression Profiles of *Chlamydomonas reinhardtii* during Photo-Biological H_2 Production. *PLoS ONE*, **6**(12): e29364. doi:10.1371/ journal.pone.0029364

Blifernez-Klassen, O., Klassen, V., Doebbe, A., Kersting, K., Grimm, P., Wobbe, L., Kruse, O. (2012) Cellulose degradation and assimilation by the unicellular phototrophic eukaryote *Chlamydomonas reinhardtii*. *Nature Communications* **3**:1214, doi:10.1038/ ncomms2210

Teilnahme an Tagungen, Workshops und Symposien

Symposium der DFG Forschergruppe FOR387 (2007) in Bielefeld

Solar BioFuels Tagung (2008) in Bielefeld

17th Photosynthesis Workshop (2008) in Bochum, Vortrag

Botanikertagung “Plant for the future” (2009) in Leipzig, Posterpräsentation

14th International Conference on the Cell and Molecular Biology of *Chlamydomonas* (2010) in Norton, MA, USA, Posterpräsentation

European Science Foundation Research Conferences (2011) in Bielefeld, Posterpräsentation

Danksagung

An dieser Stelle möchte ich allen, die mich im Laufe meiner Promotion unterstützt haben und zum Gelingen dieser Arbeit beigetragen haben herzlich danken. Mein besonderer Dank gilt dabei:

Prof. Dr. Olaf Kruse für die interessante Aufgabenstellung und die Möglichkeit, am Lehrstuhl für Algenbiotechnologie und Bioenergie der Universität Bielefeld meine Dissertation anfertigen zu können, sowie für die exzellente Betreuung.

Prof. Dr. Volker F. Wendisch für die freundliche Übernahme des Zweitgutachtens.

Dem Rektorat der Universität Bielefeld für die Folgefinanzierung meiner Promotion.

Allen Mitgliedern der „Arbeitsgruppe Kruse“ für eine freundliche Aufnahme in den Arbeitskreis, Kollegialität, außergewöhnlich familiäre und motivierende Arbeits-atmosphäre, in der ich mich stets sehr wohl gefühlt habe.

Lutz, Viktor und Christian für die Korrektur dieser Arbeit und hilfreiche Tipps sowie interessante Analysegespräche.

Prof. Dr. Armin Hallman für die Hilfestellung bei den phylogenetischen Analysen.

Carola Eck und Manuela Meyer für die Hilfe bei den MALDI-Messungen.

Meiner **Familie** für Ihre Hilfe und Beistand sowie liebevolle Fürsorge und Unterstützung.

Zuletzt und doch zuerst meinen Eltern Valentina und Waldemar Blifernez, die mir diesen Weg erst ermöglicht haben und immer für mich da waren. Ohne Euch wäre die Erstellung dieser Arbeit niemals möglich gewesen.

Спасибо Вам огромное за то что вы есть!

Erklärung

Hiermit erkläre ich, dass ich die vorliegende Arbeit selbst angefertigt habe und nur die angegebenen Quellen und Hilfsmittel verwendet habe. Alle aus der Literatur ganz oder annähernd entnommenen Stellen habe ich als solche kenntlich gemacht.

Weiterhin erkläre ich, dass die vorliegende Dissertation weder vollständig noch teilweise einer anderen Fakultät mit dem Ziel vorgelegt worden ist, einen akademischen Titel zu erwerben. Hiermit bewerbe ich mich erstmals um den Doktorgrad der Naturwissenschaften der Universität Bielefeld.

Bielefeld, den 3. Februar 2012