State transition in *Arabidopsis thaliana* and *Chlamydomonas reinhardtii*

Gemma Roest
S1690655
July 9th, 2010

Electron microscopy
Prof. Dr. R. Croce
I.E. Wientjes, MSc.

Contents

Pictures front page:
Arabidopsis thaliana: http://users.rcn.com/jkimball.ma.ultranet/BiologyPages/A/Arabidopsis.html
Chlamydomonas reinhardtii: http://epmb.berkeley.edu/facPage/dispFP.php?I=25

Abstract

Photosynthesis is the process by which plants and green algae store light energy in the form of ATP and NADPH and use this to fixate CO_2. It demands an efficient cooperation between PSII and PSI, since electrons generated by photosystem II are transported to photosystem I via a plastoquinone pool and cytochrome b_6f. The efficiency of electron transport is at stake when light conditions change, since photosystem I and photosystem II do not absorb light with the same wavelength. State transitions, the migration of a mobile LHCII pool between PSI and PSII, make sure that under conditions where photosystem I and photosystem II are differentially excited the cooperation still is as efficient as possible. In plants state transitions seem to function to balance the energy absorption by PSI and PSII, while in green algae it is a switch between linear and cyclic electron flow around PSI, and thus a way to regulate the ATP production. The redox state of the plastoquinone pool is the determinant for state transition.

Arabidopsis thaliana serves as a model organism for state transition in plants. In green algae *Chlamydomonas reinhardtii* fulfils this role. In *A. thaliana* about 15-20% of the total amount of LHCII serves as a mobile pool, while in *C. reinhardtii* it is as much as 80%. In *A. thaliana* the migrating part is the M-trimer, and especially Lhcb3 is proposed to have an important role. Phosphorylation of LHCII by STN7 induces the transition from PSII to PSI. STN8, which phosphorylates the PSII core, is also important. PPH1 dephosphorylates LHCII, and is thus responsible for the state 2 to state 1 transition.

In *C. reinhardtii* the migrating subunits are CP26, CP29 and LhcbM5. Phosphorylation of the residues Thr17 and Ser103 of CP29 by Stt7 seems to induce the transition. Also CP26 and the core subunits CP43 and D2 are phosphorylated upon state 1 to state 2 transition. Phosphorylation is proposed to lead to the dissociation of PSII megacomplexes into supercomplexes, and additionally to the migration of the LHCII subunits.

Introduction

Photosynthesis

Oxygenic photosynthesis is one of the most fundamental processes on earth. It is carried out by green plants and algae in the chloroplasts. In these organelles a continuous network of thylakoid membranes is enclosing an aqueous space, the stroma. The thylakoid membranes can fold into stacks, called grana, or they can appear as unstacked stroma lamellae.

In the thylakoid membrane several light- and dark reactions and different complexes are cooperating to store light energy as ATP and NADPH. In the light reactions light is absorbed by the light-harvesting complexes (LHC's) of photosystems which contain the pigments chlorophyll A, chlorophyll B and several carotenoids. It is then transferred to the core, which contains chlorophyll A and β-carotene, but no chlorophyll B [1].

Photosystem II (PSII) is the first complex in the cascade. Its outer antenna system absorbs light, and the absorbed excitation energy is transferred via the pigments to the reaction centre (RC) in the core of the complex, where it is used for charge separation. This leads to the oxidation of H_2O to O_2, and to the transport of electrons from PSII via plastoquinol (PQ), cytochrome b_6f (cyt b_6f) and plastocyanin to photosystem I (PSI). PSI also absorbs light through the pigments in its outer antenna system, and also in PSI charge separation takes place in the reaction centre of the core. However, in this case electrons are used to reduce $NADP^+$ to NADPH. As a result from the charge separations and the transport of electrons, there is also a proton gradient formed over the thylakoid membrane. This ATP gradient is

used by ATP synthase to synthesise ATP. In the dark reactions the NADPH and ATP are used to fixate CO_2 in carbohydrates [2].

PSII and PSI

As stated above, both photosystems consist of an outer antenna system and a core. These parts contain several subunits, which differ slightly between plants and green algae.
In *A. thaliana*, the model organism for plants, the outer antenna system of PSII consists of six different gene products, Lhcb1-6. Lhcb1-3 form homo- or heterotrimers of varying composition [3] which are called light-harvesting complex II (LHCII). *Lhcb4*, *lhcb5* and *lhcb6* result in the three minor monomeric antennae CP29, CP26 and CP24 respectively [4]. The minor antennae are located on the periphery between the antenna complex and the core of PSII [5;6]. *C.* reinhardtii, the model organism for the green algae, has four major LHCII proteins, type I-IV, which are encoded by different duplicated genes LhcbM1-9 [7], and only the minor antennae CP29 and CP26 [8].
The most common organisation of PSII in wild-type *A. thaliana* is the $C_2S_2M_2$ supercomplex. "C2" indicates two copies of the core. Each core contains the proteins D1, D2, CP47 and CP43 and various smaller proteins, from which D1 and D2 form the reaction centre. The core dimer binds two copies of CP29 and CP26, and a strongly bound LHCII trimer to form the C_2S_2 complex. Additionally it binds two copies of CP24 and a moderately bound LHCII trimer, resulting in the $C_2S_2M_2$complex [9]. The green alga *C. reinhardtii* lacks CP24 [8]. In this alga the main supercomplex found is the C_2S_2complex [10]. PSII is mainly located in the grana of the thylakoids [11].
The light-harvesting complex of PSI consists of 9 peptides, lhca1-9, in C. reinhardtii [12], while only 4 peptides are part of the LHCI, lhca1-4, in plants [13]. The main proteins in the PSI core of both green algae and higher plants are PSI-A and PSI-B which form a heterodimer. Additionally the core consists of 11 other different proteins, PSI-C to PSI-L, PSI-N and PSI-O [14]. PSI is mainly found in the stroma lamellae of the thylakoids [11].

Photo protection mechanisms and state transition

Although both PSI and PSII use energy from light to drive photosynthesis, their spectroscopic properties are not the same. Where free PSI fluoresces around 680 nm at 77 K [15], free PSII fluoresces around 730 nm at 77 K [16]. In situations where the light is not totally "white" this difference in spectroscopic properties can lead to a differential excitation of PSI and PSII. Since PSII donates its electrons to PSI a differential excitation can lead to a "traffic jam" of electrons between PSII and PSI. The electrons can then for instance react with oxygen to form radicals. Radicals are very reactive and can cause damage to the organism, generally known as photodamage.
Plants have different mechanisms to protect themselves from photodamage acting on both short and long term. One of the longer term processes is the adjustment of the number of antennae of the photosystems. Since components of the photo systems are encoded in the nucleus and in the chloroplast genome [17] this requires communication between the nucleus and the chloroplasts in both directions [18], and a regulated synthesis and degradation of proteins [19;20]. However, the synthesis and degradation of proteins are time-consuming processes. To be able to respond to fast-changing conditions, plants make use of other mechanisms.
One of them is state transition, which acts within minutes after the change in light-conditions [21]. As stated before, PSI and PSII do not absorb light of the same wavelength [15;16], so the rate of electron transport between the two complexes can vary under different light conditions. To prevent an imbalance in the excitation rates, the relative light-

harvesting properties of PSI and PSII can be modulated by a mobile pool of LHCII. When the light is rich in wavelengths >700 nm, and PSII is mainly excited, the mobile pool functions as antenna for PSI. When the light is enriched in wavelengths <650 nm and PSI is mainly excited, the mobile LHCII pool functions as antenna complex for PSII [21].

State transition

Arabidopsis thaliana *and* Chlamydomonas reinhardtii

When plants and algae are grown in the dark, or illuminated with light with a wavelength >700 nm, the excitation of PSI is favoured over the excitation of PSII [21]. To ensure a proper cooperation between PSII and PSI and to maintain the electron transport between the two complexes, the absorption capacity of PSII is enhanced by the binding of the mobile LHCII pool. Under these conditions the plants or algae are in the so-called "state 1".
When the organisms are illuminated with light with a wavelength <650 nm the excitation of PSII is favoured over the excitation of PSI [21]. As explained before, this situation might result in an accumulation of electrons between PSII and PSI and to the formation of radicals. To prevent this, the mobile LHCII pool is attached to PSII to enhance its absorption and prevent any imbalance in the excitation of PSI and PSII. Under these conditions the plants or algae are in the so-called "state 2".
State transitions have been studied in both plants and green algae. In plants the model organism is *Arabidopsis thaliana*, while for the algae *Chlamydomonas reinhardtii* is being used. Although the physical result in *A. thaliana* and *C. reinhardtii* is the same (part of the LHCII pool moves between PSI and PSII), the exact mechanism and purpose seem to be different between algae and land plants. Also the exact purpose is proposed to differ. In land plants state transition serves as a mechanism to regulate electron transfer between PSI and PSII [22], while in *C. reinhardtii* the main purpose seems to be the regulation of the cyclic and linear electron flow around PSI [23].
The main method to follow the state transitions is to look at the fluorescence of PSI and PSII. Under state 1 conditions the mobile LCHII pool is bound to PSII, and during the transition from state 1 to state 2 the mobile LHCII moves from PSII to PSI. Since the absorption capacity of PSII decreases and that of PSI increases, this can be seen as a decrease in fluorescence of PSII and an increase in fluorescence of PSI.

Models

Different models have been developed to explain the migration of the mobile LHCII pool between PSII and PSI. However, they all propose the involvement of phosphorylation of LHCII.
One model is the surface charge model. In this model the grana stacks are closely packed due to a relatively low surface charge density. The phosphorylation of LHCII leads to an increase of the negative charge on exposed outer membranes of the grana stacks. This induces the partial unstacking of the grana due to coulombic repulsion of the phosphorylated and thus negatively charged LHCII [24]. These changes in the structure of the thylakoid membrane facilitate the movement of phosphorylated LHCII in the grana to PSI in the unstacked lamellae [24;25]. The migration of the phosphorylated LHCII complexes also reduces the repulsive forces between the LHCII complexes. When these forces are minimised the migration of LHCII and the structural changes of the thylakoid membrane stop [26].
A second model is the molecular recognition model. According to this model the introduction of negative charge by phosphorylation induces structural changes in LHCII [26]. These structural changes then decrease the affinity of the phosphorylated LHCII for PSII relative to non-phosphorylated LHCII. The decreased affinity for PSII causes LHCII to diffuse away from PSII, and the change in its structure increased its affinity for PSI. In this way PSI

interacts with phosphorylated LHCII, and when it gets dephosphorylated it interacts with PSII [26].

Regulation of state transition

The molecules involved in the electron transport chain are the key in inducing the transition of the mobile LHCII pool from PSI to PSII or vice versa. The electrons from PSII are transferred to the plastoquinones QA and QB. After double reduction and protonation of QB this results in PQH2 which leaves PSII and diffuses to cyt b_6f. This complex consist of cytochrome b_6, subunit IV, cytochrome f, Rieske iron-sulphur protein, PetG, PetL, PetM and PetN [27]. PSII supplies electrons and protons to the cyt b_6f complex. The binding of PQH2 to the quinol oxidation (Q0) site of cyt b_6f is involved in measuring the redox state of the PQ pool. The Rieske Fe-S subunit might change its position relative to the Q0 site upon binding of PQH2, and this might cause the release of the LHCII kinase from the cyt b_6f complex [28]. This activates the LHCII kinase [29]. This induces the phosphorylation of various components of LHCII and core subunits of PSII [30], which leads to the state 1 to state 2 transition. The relative increase of the size of the light-harvesting complex of PSI increases the rate of electron transport from PSII to PSI. PQH2 oxidation inactivates the kinase and activates a phosphatase [29]. Dephosphorylation of LHCII turns the system then back to state 1 [29;31].
However, the redox state of the PQ pool seems not to be the only process involved in controlling the level of phosphorylation of LHCII. *In vivo* experiments show phosphorylation only at light intensities even lower than the growth irradiance [32]. At higher intensities the level of phosphorylation of LHCII decreases, even when the plastoquinone pool is reduced [32]. This indicates the involvement of another system in the regulation of the phosphorylation of LHCII and PSII and thus in state transition.
The LHCII kinase has been shown to be inactive when it is bound to cyt b_6f, and to be active when it is not bound to the cyt b_6f complex. But its activity is also regulated by two cystein residues close to the N-terminal which are conserved in both *A. thaliana* and *C. reinhardtii* [29]. The disulfide bridge between these residues appears to be essential for state transitions. When these residues were mutated no phosphorylation of LHCII and no state transition was observed [33]. The disulfide bridge seems to couple the phosphorylation of LHCII with the ferredoxin-thioredoxin system [34]. When the bridge is reduced, the activity of the LHCII kinase is inhibited, even if it is free from the cyt b_6f complex [35]. The interplay between the regulation mechanism involving the redox state of the PQ pool and the mechanism involving the ferredoxin-thioredoxin system make the regulation of the level of phosphorylation of LHCII flexible.
Yet another regulating factor involved in state transition is TSP9. This plant-specific protein [36] interacts with LHCII, the PSII core and the PSI core [37]. This protein seems to destabilise the PSII supercomplexes since in mutants lacking TSP9 an increased number of supercomplexes relative to the monomeric complexes was observed [38]. TSP9 seems also to be involved in the phosphorylation of LHCII peptides since plants lacking TSP9 had a decreased level of phosphorylated LHCII peptides [38]. TSP9 itself is phosphorylated by the LHCII kinase [38].

Arabidopsis thaliana

Function of state transition in A. thaliana

In A. thaliana about 15-20% of LHCII moves between PSII and PSI [39]. This pool determines the relative absorption properties of PSI and PSII under different light conditions. However, state transitions do not seem to be essential for survival in A. thaliana. [40]. Even mutants that are unable to perform state transition do not show a changed phenotype relative to the wild-type plants, and photosynthetic properties are also very similar [41]. Apparently the ability to balance the excitation energy of PSI and PSII is not crucial.

M-trimer migrates between PSI and PSII

Wild-type *A. thaliana* in state 1 has been shown that PSII appears mainly in $C_2S_2M_2$ supercomplexes, while in state 2 the relative amount of $C_2S_2M_2$ complexes increases [42]. This indicates the involvement of the M-trimer in state transitions.

Evidence for this is provided by an *A. thaliana* ΔLhcb6 mutant, which lacks CP24. CP24 is located between the M-trimer and the PSII core in the supercomplex [43]. In the *A. thaliana* mutant lacking CP24 only C_2S_2 supercomplexes were observed [44], and the rate of state 1 to state 2 transition was increased [44]. This indicates a role of CP24 in the binding of the M-trimer to the PSII core in state 1. In the absence of CP24, the M-trimers are not bound to a supercomplex, but free in the thylakoid membrane. This may make it easier for the trimer to shift to PSI, and thus explain the increased rate of state transition. Additionally, the amount of LHCII bound to PSI in state 2 was the same as in wild-type *A. thaliana* [44]. Apparently CP24 is not essential for the binding of the mobile LHCII trimer to PSI.

Also the composition of the M-trimer seems to be important for state transitions. ΔLhcb3 *A. thaliana* mutants showed a faster state transition rate than the wild-type *A. thaliana*, but the extent of transition was the same as in the wild-type *A. thaliana* [43]. Despite the lack of Lhcb3, the ΔLhcb3 mutant still showed $C_2S_2M_2$ supercomplexes with complete M-trimers like the wild-type. Apparently the role of Lhcb3 is taken over by Lhcb1 or Lhcb2, which were up-regulated in the mutant [43]. However, the supercomplexes in the ΔLhcb3 mutant did show a structural rearrangement which was not seen in either a ΔLhcb1 or ΔLhcb2 mutant. The M-trimers in the supercomplexes were shown to be rotated about 21° with respect to the position of the M-trimer in wild-type *A. thaliana* [43], as shown in figure 1.

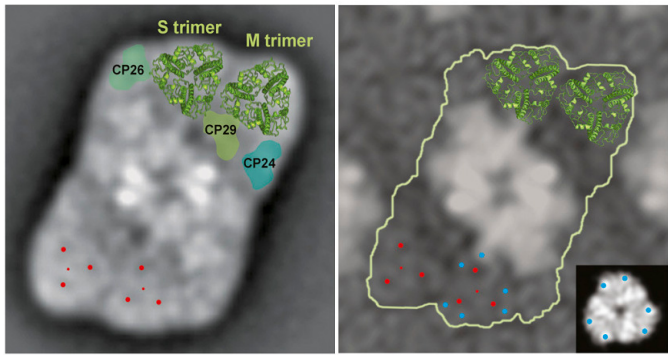


**Figure 1. Electron microscopy image showing the $C_2S_2M_2$ complex of PSII in wild-type (A) and ΔLhcb3 (B)* A. thaliana*. Blue dots show a comparable relative electron density with a LHCII trimer (inset). Red dots show positions with high contrast in the LHCII trimers. The M-trimer of ΔLhcb3 shows a rotation of 21° relative to the wild-type. Figure adapted from [43].*

Apart from a structural change in the supercomplex, the ΔLhcb3 mutant also showed an increased level of phosphorylation of the LHCII trimers [43]. This increase in phosphorylation was proportional to the amount of changed Lhcb3 [43]. Lhcb1 and Lhcb2 contain phosphorylation sites but Lhcb3 does not. When the lack of Lhcb3 is compensated by Lhcb1 or Lhcb2 this may lead to an increase in phosphorylation sites in the LHCII trimers. This may introduce more repulsive forces between the trimers and the PSII core than when Lhcb3 would be part of the trimer. The supercomplex may be destabilised in this way, thus increasing the rate of the transition from state 1 to state 2.
Also the change in conformation of the $C_2S_2M_2$ supercomplex may cause a less stable supercomplex when Lhcb3 is changed into Lhcb1 or Lhcb2 [43]. The rotation of the M-trimer in the absence of Lhcb3 may decrease the interaction between the PSII core and the trimer. This looser binding of the trimer to the PSII core may then facilitate an easier transition from state 1 to state 2 relative to an M-trimer containing Lhcb3.

Binding of LHCII to PSI

Electron microscopy data show that the mobile LHCII binds at the PSI-H, -L, -A, -K site of PSI [42]. To determine which subunits of PSI interact with the mobile LHCII several mutants lacking one of the components were used. Plants lacking the PSI-H subunit of PSI showed the same antenna size under state 1 and state 2 conditions as the wild-type under state 1 light [40]. This indicates the essential role of PSI-H in state transition. The fluorescence properties of PSII in this mutant did not change under state 1 or state 2 conditions, so presumably the mobile LHCII stays attached to PSII even under state 2 conditions. Another feature of the ΔPSI-H plants was the reduction of the level of PSI-L. The mutants lacking PSI-H had only half of the wild-type PSI-L. Plants lacking PSI-L showed only 20% of the wild-type level of PSI-H [40].
Also plants lacking the PSI-O subunit were impaired in state transition. Although state transition was not totally inhibited, it was reduced to half the level of state transition in the wild-type [45]. Additionally, plants lacking PSI-H or PSI-L also showed a 70%-90% reduction of the level of PSI-O relative to the wild-type, but the PSI-O mutant did not show a reduction in the levels of PSI-H or PSI-L [45]. All these observations do not only indicate interaction of the subunits with each other, but also with LHCII.
Cross-linking experiments confirmed that Lhcb1 of LHCII interacts with the PSI-H, PSI-I, PSI-L and PSI-O subunits [46]. All of these subunits are located on the opposite side of the site where LHCI binds to the PSI core [47] as shown in Figure 2.

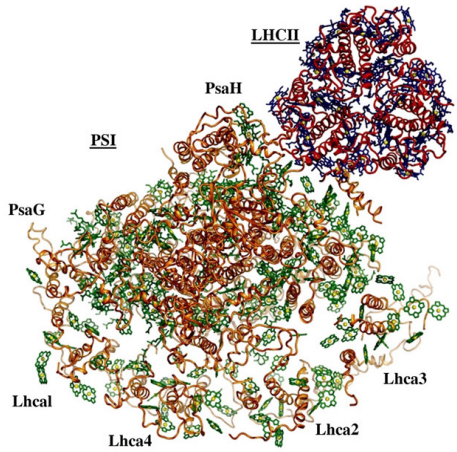


**Figure 2. Model of proposed binding of LHCII to PSI in* A. thaliana*. The mobile LHCII unit binds to the PsaH subunit of PSI, opposite to the binding site of LHCI. Figure adapted from [47].*

Phosphorylation of antenna proteins of PSII

Although the involvement of one or more kinases in state transition has been known for a long time [48], only recently STN7 has been identified to be the LHCII kinase in *A. thaliana* [49]. In mutants lacking this protein LHCII did not show any phosphorylation, and the plants showed only a very small increase in PSI fluorescence in state 2. This indicates that this protein is involved in the phosphorylation of LHCII, and that phosphorylation of LHCII is indeed essential for the state 1 to state 2 transition.

The counteracters of STN7 is the phosphatase PPH1/TAP38 [50;51]. This phosphatase dephosphorylates specifically LHCII, and is essential for the state 2 to state 1 transition [50].

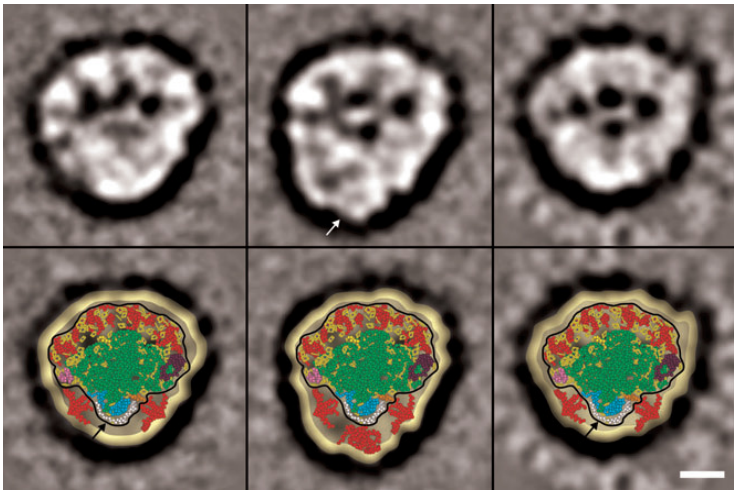
Chlamydomonas reinhardtii

Function of state transition in C. reinhardtii

The function of state transition in the green alga *C. reinhardtii* seems to be different from *A. thaliana*. Where in *A. thaliana* only 15-20% of the LHCII pool migrates between PSI and PSII [39] is it as much as 80% in *C. reinhardtii* [52]. This already indicates that the function of state transitions might be more extensive in *C. reinhardtii* than in *A. thaliana*. It appears that in *C. reinhardtii* it is more a way to regulate the electron flow around PSI and the energy needs than to distribute absorbed energy [53].
Electrons can be transported from H_2O via PSII and PSI to NADPH during the light reactions. This so-called "linear electron transport" yields both NADPH and ATP. On the other hand electrons can cycle around PSI in a "cyclic electron transport". This leads to the formation of ATP, but not to the formation NADPH. Experiments under physiological conditions have shown that normally cyclic and linear electron transport happen at the same time [54].
The involvement of cyt b_6f in the electron transport in both state 1 and state 2 was shown by making use of a cyt b_6f inhibitor [29] in cells in either state 1 or state 2. This inhibitor prevented the reduction of cyt b_6f irrespective of the complex the electrons were originating from. Cells in both state 1 and state 2 showed an increased oxidation of cyt b_6f relative to untreated cells [29], indicating that cyt b_6f is essential in both state 1 and state 2.
To see whether cyt b_6f was involved in cyclic or linear electron transport when cells were in state 1 or state 2, linear electron transport between PSII and PSI was inhibited by adding a PSII inhibitor to the cells [29]. When the inhibitor was added to cells in state 1, the cyt b_6f complex was more oxidised than an untreated cell, indicating that electrons are withdrawn from cyt b_6f but that it is not receiving electrons. When the inhibitor was added to cells in state 2, the oxidation of cyt b_6f showed no difference with an untreated cell [29], indicating that electrons are both withdrawn from and delivered to the cyt b_6f complex. These results show an alternative for linear electron transport in state 2, which is presumably cyclic electron transport. In state 1 on the other hand linear electron transport seems to be the main form of electron transport.

LhcbM5, CP26 and CP29 migrate between PSI and PSII

In contrast to *A. thaliana*, the mobile unit in *C. reinhardtii* is not the major antenna complex of PSII, but the minor antennae LhcbM5, CP26 and CP29 [7]. Isolation of the PSI complex in state 2 and immunoblotting showed that in state 2 most CP29 and a small amount of CP26 was connected to PSI [7]. When the isolated PSI complex in state 2 and the isolated PSII complex in state 2 were digested with Trypsin and characterised by mass spectrometry it was shown that in state 2 LhcbM5 was bound to PSI but not to PSII [7]. CP26 binds very weakly to the PSI-LHI complex, CP29 stronger, and LhcbM5 binds the strongest of the peptides [7].
Electron microscopy data have also shown the involvement of CP29 in state transition [22;55]. Wild-type *C. reinhardtii* showed an additional subunit near the PsaH subunit from the PSI core in state 2. This subunit was absent when the complex was isolated from state 1. Mutants without CP29 in state 2 showed the same phenotype as the wild-type in state 1, as shown in figure 3.



A **B** **C**

**Figure 3. Electron microscopy images and models from LHCI-PSI complex of wild-*type C. reinhardtii *in state 1 (A), in state 2 (B) and of ΔCP29* C. reinhardtii *in state 2 (C). In the wild-type* C. reinhardtii *an additional subunit to the complex is observed in state 2, but not in state 1. In ΔCP29* C. reinhardtii *no additional subunit is observed. Figure adapted from [22].*

CP29 has been shown to be essential for the state 1 tot state 2 transition in *C. reinhardtii*. In mutants lacking this protein a decrease in fluorescence of PSII was shown under state 2 conditions, but the fluorescence of PSI did not increase [56]. Apparently the mobile unit dissociates from PSII, but does not re-associate with PSI. In contrast to this, a mutant lacking CP26 did not show any change in fluorescence, indicating that this peptide is not essential for state transition [56].

Phosphorylation of antenna proteins and core of PSII

In *C. reinhardtii* the most common supercomplex is the C_2S_2 supercomplex [10]. However, this supercomplex has the tendency to aggregate into megacomplexes [57]. These megacomplexes are proposed to exist in state 1 [58], and to be connected by LHCII trimers [9]. Phosphorylation of LHCII type I, which is the most abundant LHCII peptide, is proposed to result in a change in conformation and to induce the dissociation of the megacomplexes into supercomplexes [59;60]. This leads to the unstacking of the thylakoid membrane and movement of LHCII from PSII to PSI [58]. LHCII type I itself remains attached to the PSII complex in both state 1 and state 2 [58].

The minor antennae CP29 has been shown to be phosphorylated at the Thr7 and Thr33 residues in state 1 [61] when it is bound in the mega- and supercomplexes [58]. However, in the migrating LHCII four residues, Thr7, Thr33, Thr17 and Ser103, are phosphorylated [61]. These residues are all located at the surface between the core of PSII and its LHCII. Thr27 of Lhcbm1 is phosphorylated in state 2 but not in state 1 [61]. Also CP26 is more phosphorylated in state 2 than in state 1 [58], but the exact residues have not been identified. It is proposed that the phosphorylation of CP26 and CP29 induces the migration of LHCII from PSII to PSI during the transition from state 1 to state 2 [58].

The PSII core subunits CP43 and D2 were modified during the state transition too. The N-terminal of the D2 protein was acetylated and phosphorylated in state 2 but not in state 1 [61].

Stl1 has been found to dephosphorylate LHCII, but its exact function and mechanism are not clear yet.

The time-scale of phosphorylation of PSII during the state 1 to state 2 transition is summarised in figure 4. First LHCII Type I is phosphorylated, which leads of the dissociation of the megacomplex in supercomplexes and unstacking of the thylakoid grana. Phosphorylation of the minor antennae CP26 and CP29 and the PSII core occur simultaneously, resulting in the detachment of LHCII from the core of PSII.

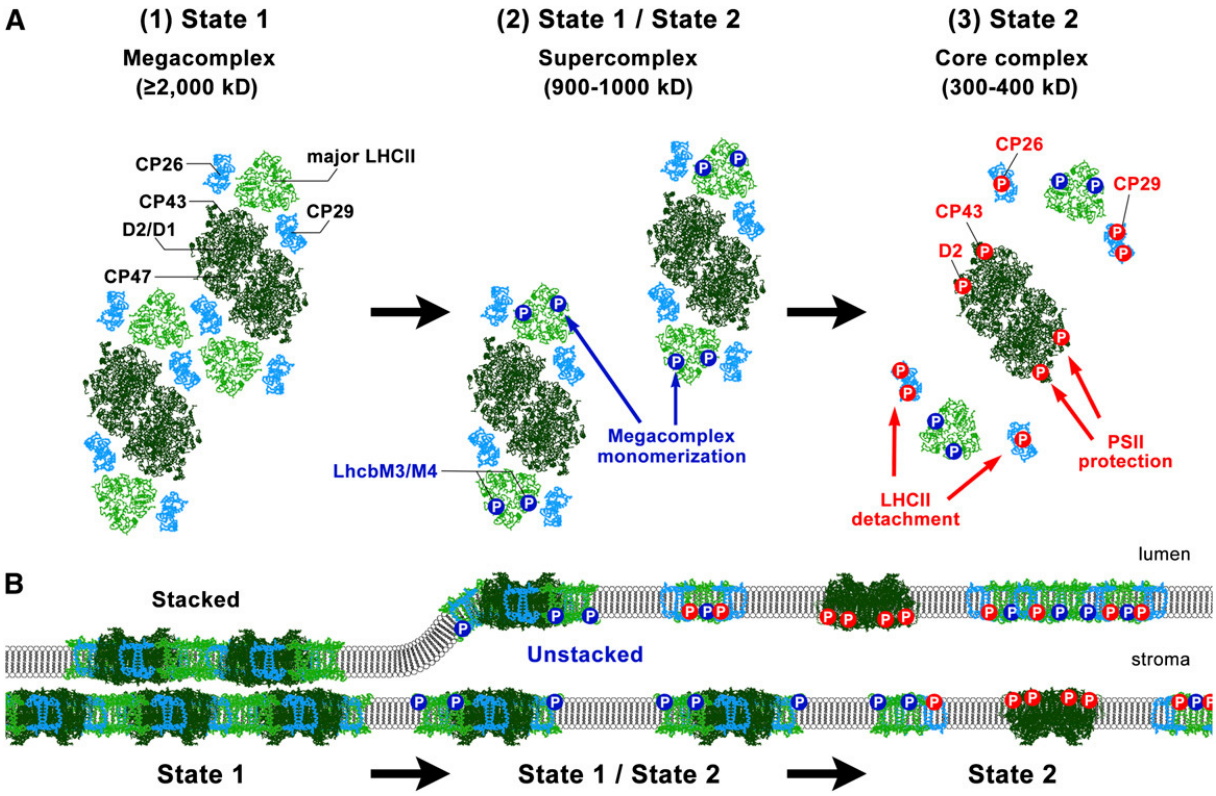


**Figure 4. Model of state transition in* C. reinhardtii*. Phosphorylation of LHCII Type I induces dissociation of the megacomplex into supercomplexes and unstacking of the thylakoid membrane. Phosphorylation of CP26, CP29, CP43 and D2 result in detachment of the mobile LHCII from the supercomplex. Figure adapted from [58].*

Discussion

A. thaliana *versus* C. reinhardtii

The function of state transition in *A. thaliana* seems to differ a lot from its function in *C. reinhardtii* although the underlying mechanism is very similar. Already from the amount of LHCII migrating between PSI and PSII this becomes clear. In *A. thaliana* only 15-20% of the total LHCII content dissociates from PSII and it has been shown not to be essential for the survival of the plant [39;41]. In *C. reinhardtii* up to 80% migrates, but it is not known if state transition is crucial for this organism to survive [52]. Also the subunits that are migrating between PSII and PSI differ between the two organisms. The most important mobile subunit in *A. thaliana* is the M-trimer, and CP24 seems only to be involved in the connection of the M-trimer to the PSII complex [43;44]. However, it is surprising that the extent of state transition in the ΔLhcb6 mutant was the same as in the *A. thaliana* wild-type. When all M-trimers would be free from the PSII supercomplexes, it would have been to be expected that the extent of state 1 to state 2 transition would increase in this mutant. However, still only 15-20% of the LHCII content was migrating [43;44].
In *C. reinhardtii* LhcbM5 and the minor antenna CP29 are the most important moving units. CP26 has a minor role, but its exact role in *C. reinhardtii* is not clear [7;22].
Not only the extent of transition is different, also the final purpose. In *A. thaliana* state transition only seems to function as a means to use the available light energy as efficiently as possible. In *C. reinhardtii* state transition can also be used to adapt its metabolism to the ATP-needs of the organism. When the electron chain switches from mostly linear in state 1 to mostly cyclic in state 2 [29], and the metabolism changes from producing ATP and NADPH to only producing ATP, this might be beneficial during a short ATP depletion.

Role of phosphorylation

Phosphorylation is a widely used mechanism to regulate processes. Also in state transition in *A. thaliana* and *C. reinhardtii* phosphorylation has been given an important role since the discovery of the process [62]. However, the regulation of state transition appears to be more complicated than was thought at first. The basic regulation by a LHCII kinase was assumed for a long time but only recently STN7/Stt7 was identified as the protein involved.
However, this LHCII kinase now seems to be only one part of the regulation of state transition. Also TSP9 is an essential component, and this protein also acts in the phosphorylation of LHCII. It is still unclear which protein is finally responsible for the phosphorylation and which is only part of a phosphorylation cascade.
Also the mechanism by which the state 2 to state 1 transition takes place has not been studied thoroughly. Recently PPH1/TAP38 was discovered to dephosphorylate LHCII in *A. thaliana* [50], but its orthologue in *C. reinhardtii* has not been identified. Also the phosphatase dephosphorylating the core of PSII is not known yet.
The regulation of the level of phosphorylation, and thus the regulation of state transition appears to be interlinked with other processes. In situations where the plastoquinone pool was reduced LHCII was shown to be less phosphorylated than expected [32]. Also *in vitro* experiments showed different results from *in vivo* experiments. It has been suggested that stroma components which are lost when the thylakoid membranes are isolated for *in vitro* experiments might be involved [33]. State transition may also be interlinked with other photo protection mechanisms. When plants were grown under high light the phosphorylation level was shown to decrease even when the plastoquinone pool was reduced [32].

The two existing models which describe the effect of phosphorylation on state transitions are the surface charge model and the molecular recognition model. The surface charge model implies the repulsion of the negative charges of the LHCII complexes introduced by the phosphorylation, which may lead to the migration of LHCII [24]. The molecular recognition model states a conformation change due to the phosphorylation, which may lead to a changed affinity of LHCII for PSII and PSI [26]. However, the only conformational change was seen in the ΔLhcb3 mutant from *A. thaliana* [43], and the importance of the rotation of the M-trimer in this mutant is not very clear. The surface charge model seems therefore more plausible, although this model seems to become a bit too simple, since also the involvement of minor antennae and the PSII core has been shown in *A. thaliana* [44] and *C. reinhardtii* [7;22]. The model as proposed in [58] also includes these factors, and may currently be the best description of the transition from state 1 to state 2.

Role of Lhcb3 in A. thaliana

The absence of Lhcb3 in the ΔLhcb3 mutant of *A. thaliana* showed an increased rate of state transition, and the presence of a rotated M-trimer. It was proposed that Lhcb1 and Lhcb2 took over the role of Lhcb3 [43]. The change in transition rate was attributed to the increase in phosphorylation, since Lhcb1 and Lhcb2 have a phosphorylation site, and Lhcb3 does not have a phosphorylation site. The explanation of the increased rate by an increased level phosphorylation and thus of an increased repulsion between the mobile trimer and the PSII core seems plausible since phosphorylation is known to have a role in state transitions. Lhcb3 may then act to modulate the rate, since a trimer containing Lhcb3 has less phosphorylation sites than a trimer only composed of Lhcb1 and Lhcb2. A trimer containing Lhcb3 has smaller repulsive forces and thus a slower state 1 to state 2 transition rate. The LHCII trimers are known to have different compositions, and to be either homo- or heterotrimers [3]. The modulating function of Lhcb3 in state transitions may be an explanation for this observation and for the relative small mobile LHCII pool [39].
However, the rotation of the M-trimer in the $C_2S_2M_2$ supercomplex in the ΔLhcb3 mutant was not observed in a wild-type *A. thaliana*. If the presence of Lhcb3 in the M-trimer indeed would regulate the coordination of the M-trimer in the supercomplex, this rotation should also have been observed in wild-type *A. thaliana*, since the varying composition of the M-trimers would also in the wild-type result in trimers without Lhcb3. Additionally, no change in the S-trimer of the $C_2S_2M_2$ complex was observed [43]. When the presence of Lhcb3 in the trimers would indeed modulate the binding of the trimer to the PSII core, also differences in orientation or binding affinity of the S-trimer to the PSII core would be expected.

Future research

In short, the phosphorylation of LHCII during the transition from state 1 to state 2 is the most studied topic. However, the regulation of the phosphorylation, its exact role in the state transition, the transition from state 2 to state 1 and individual components still remain to be investigated.
To identify yet unknown proteins, the use bio-informatics could be an approach. By searching for orthologues of proteins in other organisms, like for the orthologue of PPH1 of *A. thaliana* in *C. reinhardtii*, new proteins involved in state transitions may be found. Also the search for proteins that are co-expressed with for instance STN7 and TSP9 in *A. thaliana*, and that are up- or down-regulated under state 1 or state 2 conditions, may result in candidates that may play a role in the regulation of state transition. Alternatively micro-arrays in which the RNA levels under state 1 and state 2 conditions would be compared may also result in the identification of new proteins involved in state transitions.

To see which proteins are interacting with each other and in which order, making use of two-hybrid systems could be an option. In the case of state transition in *A. thaliana* this technique may be used to find the exact interacting partners of STN7 and TSP9. It is not yet clear which of these two proteins is in the end responsible for the phosphorylation of LHCII, or to which part of LHCII is phosphorylated. By using parts of the outer antenna of PSII with a putative phosphorylation site as "bait" and the active sites of STN7 or TSP9 as "prey" it might be possible to demonstrate the interaction between the kinases and the antenna complex. In this way potential gaps in the phosphorylation cascade might also be identified. The same technique may be used to demonstrate interactions between STN7/Stt7 and cyt b_6f, or between STN7/Stt7 and the ferredoxin-thioredoxin system.

In *A. thaliana* CP24 seems to act as a linker protein between the mobile M-trimer and the PSII core. However, also CP29 interacts with both the M-trimer and the PSII core. It would be interesting to see whether CP24 still bind the M-trimer to the PSII core in the absence of CP29, or that CP29 also has some role in the binding of the M-trimer.

The suggestion of different functions of LHCII trimers with different composition is also interesting. To investigate the role of Lhcb3 in *A. thaliana* more extensively, and to determine whether it acts as a modulator of the rate of state transition, it could be an option to use a double mutant lacking both Lhcb1 and Lhcb2. Provided that this mutant would still form stable LHCII trimers, these trimers would only contain Lhcb3. If the lack of a phosphorylation site in Lhcb3 really is the moderating factor, than in the double mutant no state transition at all should be observed. A comparable experiment could be performed in *C. reinhardtii*. *C. reinhardtii* contains 9 different LhcbM proteins in four classes, while *A. thaliana* has only three major Lhcb proteins [3]. By knocking the LhcbM genes one by one out, or by eliminating classes of LhcbM proteins, a possible regulation system as proposed for Lhcb3 in *A. thaliana* may be found in *C. reinhardtii*.

References

[1] Ben-Shem A. *Crystal structure of plant photosystem I*. Nature 2003; 426: 630-635.

[2] Barber J, Andersson B. *Revealing the blueprint of photosynthesis*. Nature 1994; 370: 31-34.

[3] Jackowski G. *Identification of Lhcb1/Lhcb2/Lhcb3 heterotrimers of the main light-harvesting chlorophyll a/b-protein complex of Photosystem II (LHC II)*. Biochimica et biophysica acta 2001; 1504: 340-345.

[4] Jansson S. *A guide to the Lhc genes and their relatives in Arabidopsis*. Trends Plant Sci 1999; 4: 236-240.

[5] Boekema EJ. *Supramolecular organization of photosystem II and its light-harvesting antenna in partially solubilized photosystem II membranes*. European journal of biochemistry 1999; 266: 444-452.

[6] Harrer R. *Nearest-neighbor analysis of a photosystem II complex from Marchantia polymorpha L. (liverwort), which contains reaction center and antenna proteins*. European journal of biochemistry 1998; 255: 196-205.

[7] Takahashi H. *Identification of the mobile light-harvesting complex II polypeptides for state transitions in Chlamydomonas reinhardtii*. Proceedings of the National Academy of Sciences of the United States of America 2006; 103: 477-482.

[8] Minagawa J. *Structure, function and assembly of Photosystem II and its light-harvesting proteins*. Photosynthesis research 2004; 82: 241-263.

[9] Dekker JP. *Supramolecular organization of thylakoid membrane proteins in green plants*. Biochimica et biophysica acta 2005; 1706: 12-39.

[10] Nield J. *3D map of the plant photosystem II supercomplex obtained by cryoelectron microscopy and single particle analysis*. Nature structural biology 2000; 7: 44-47.

[11] Andersson B. *Lateral heterogeneity in the distribution of chlorophyll-protein complexes of the thylakoid membranes of spinach chloroplasts*. Biochimica et biophysica acta 1980; 593: 427-440.

[12] Bassi R. *Characterization of chlorophyll a/b proteins of photosystem I from Chlamydomonas reinhardtii*. The Journal of biological chemistry 1992; 267: 25714-25721.

[13] Jansson S. *A guide to the Lhc genes and their relatives in Arabidopsis/IT>*. Trends Plant Sci 1999; 4: 236-240.

[14] Scheller HV, Naver H, Møller BL. *Molecular aspects of photosystem I*. Physiologia plantarum 1997; 100: 842-851.

[15] Nussberger S, Dekker JP, Kühlbrandt W, Van Bolhuis BM, Van Gondele R, Van Amerongen H. *Spectroscopic characterization of three different monomeric forms of the main chlorophyll a/b binding protein from chloroplast membranes*. Biochemistry 1994; 33: 14775-14783.

[16] Croce R. *The Lhca antenna complexes of higher plants photosystem I*. Biochimica et biophysica acta 2002; 1556: 29-40.

[17] Sigiura M. *The chloroplast genome*. Essays in biochemistry 1995; 30: 49-57.

[18] Fey V, V. *Photosynthetic redox control of nuclear gene expression*. Journal of Experimental Botany 2005; 56: 1491-1498.

[19] Yang DH. *Induction of acclimative proteolysis of the light-harvesting chlorophyll a/b protein of photosystem II in response to elevated light intensities*. Plant Physiology 1998; 118: 827-834.

[20] Zelisko A. *AtFtsH6 is involved in the degradation of the light-harvesting complex II during high-light acclimation and senescence*. Proceedings of the National Academy of Sciences of the United States of America 2005; 102: 13699-13704.

[21] Haldrup A. *Balance of power: a view of the mechanism of photosynthetic state transitions*. Trends in Plant Science 2001; 6: 301-305.

[22] Kargul J. *Photosynthetic acclimation: structural reorganisation of light harvesting antenna--role of redox-dependent phosphorylation of major and minor chlorophyll a/b binding proteins*. The FEBS journal 2008; 275: 1056-1068.

[23] Finazzi G. *State transitions, cyclic and linear electron transport and photophosphorylation in Chlamydomonas reinhardtii*. Biochimica et biophysica acta 1999; 1413: 117-129.

[24] Barber J. *Influence of Surface Charges on Thylakoid Structure and Function*. Annual Review of Plant Physiology 1982; 33: 261-295.

[25] Barber J. *Membrane conformational changes due to phosphorylation and the control of energy transfer in photosynthesis*. Photosynthesis research 1983; 5: 181-190.

[26] Allen JF. *How does protein phosphorylation regulate photosynthesis?* Trends in Biochemical Sciences 1992; 17: 12-17.

[27] Stroebel D. *An atypical haem in the cytochrome b(6)f complex*. Nature 2003; 426: 413-418.

[28] Aro E, Ohad E-M. *Redox regulation of thylakoid protein phosphorylation*. Antioxidants & redox signaling 2003; 5: 55-67.

[29] Finazzi G. *The central role of the green alga Chlamydomonas reinhardtii in revealing the mechanism of state transitions*. Journal of Experimental Botany 2005; 56: 383-388.

[30] Wollman FA, Lemaire C. *Studies on kinase-controlled state transitions in Photosystem II and b6f mutants from Chlamydomonas reinhardtii which lack quinone-binding proteins*. Biochimica et Biophysica Acta - Bioenergetics 1988; 933: 85-94.

[31] Bennett J. *Chloroplast phosphoproteins. Evidence for a thylakoid-bound phosphoprotein phosphatase*. European journal of biochemistry 1980; 104: 85-89.

[32] Rintamäki E, Salonen N, Suoranta UM, ndersson B, Aro EM. *Phosphorylation of light-harvesting complex II and photosystem II core proteins shows different irradiance-dependent regulation in vivo. Application of phosphothreonine antibodies to analysis of thylakoid phosphoproteins*. The Journal of biological chemistry 1997; 272: 30476-30482.

[33] Rintamäki EM, Martinsuo P, Pursiheimo S, Aro E-M. *Cooperative regulation of light-harvesting complex II phosphorylation via the plastoquinol and ferredoxin-thioredoxin system in chloroplasts*. Proceedings of the National Academy of Sciences of the United States of America 2000; 97: 11644-11649.

[34] Lemeille S, Willig A, Depège-Fargeix N, Delessert C, Bassi R, Rochaix J-D. *Analysis of the chloroplast protein kinase Stt7 during state transitions*. PLoS biology 2009; 7: e45-e45.

[35] Martinsuo P. *Dithiol oxidant and disulfide reductant dynamically regulate the phosphorylation of light-harvesting complex II proteins in thylakoid membranes*. Plant Physiology 2003; 133: 37-46.

[36] Carlberg I, I. *A novel plant protein undergoing light-induced phosphorylation and release from the photosynthetic thylakoid membranes*. Proceedings of the National Academy of Sciences of the United States of America 2003; 100: 757-762.

[37] Hansson M. *The mobile thylakoid phosphoprotein TSP9 interacts with the light-harvesting complex II and the peripheries of both photosystems*. The Journal of biological chemistry 2007; 282: 16214-16222.

[38] Fristedt R. *Intrinsically unstructured phosphoprotein TSP9 regulates light harvesting in Arabidopsis thaliana*. Biochemistry 2009; 48: 499-509.

[39] Allen JF. *Protein phosphorylation in regulation of photosynthesis*. Biochimica et biophysica acta 1992; 1098: 275-335.

[40] Lunde C. *The PSI-H subunit of photosystem I is essential for state transitions in plant photosynthesis*. Nature 2000; 408: 613-615.

[41] Lunde C. *Plants impaired in state transitions can to a large degree compensate for their defect*. Plant & Cell Physiology 2003; 44: 44-54.

[42] Kouril R. *Structural characterization of a complex of photosystem I and light-harvesting complex II of Arabidopsis thaliana*. Biochemistry 2005; 44: 10935-10940.

[43] Damkjaer JT. *The photosystem II light-harvesting protein Lhcb3 affects the macrostructure of photosystem II and the rate of state transitions in Arabidopsis*. Plant Cell 2009; 21: 3245-3256.

[44] Kovcs L. *Lack of the light-harvesting complex CP24 affects the structure and function of the grana membranes of higher plant chloroplasts*. Plant Cell 2006; 18: 3106-3120.

[45] Jensen PE. *The PSI-O subunit of plant photosystem I is involved in balancing the excitation pressure between the two photosystems*. The Journal of biological chemistry 2004; 279: 24212-24217.

[46] Zhang S, Scheller HV. *Light-harvesting complex II binds to several small subunits of photosystem I*. The Journal of biological chemistry 2004; 279: 3180-3187.

[47] Amunts A. *Functional organization of a plant Photosystem I: evolution of a highly efficient photochemical machine*. Plant physiology and biochemistry 2008; 46: 228-237.

[48] Bennett J. *Chloroplast phosphoproteins. Phosphorylation of polypeptides of the light-harvesting chlorophyll protein complex*. European journal of biochemistry 1979; 99: 133-137.

[49] Bellafiore S, Barneche F, Peltier G, Rochaix J-D. *State transitions and light adaptation require chloroplast thylakoid protein kinase STN7*. Nature 2005; 433: 892-895.

[50] Shapiguzov A. *The PPH1 phosphatase is specifically involved in LHCII dephosphorylation and state transitions in Arabidopsis*. Proceedings of the National Academy of Sciences of the United States of America 2010; 107: 4782-4787.

[51] Pribil M. *Role of plastid protein phosphatase TAP38 in LHCII dephosphorylation and thylakoid electron flow*. PLoS biology 2010; 8: e1000288-e1000288.

[52] Delosme R, Olive J, Wollman FA. *Changes in light energy distribution upon state transitions: an in vivo photoacoustic study of the wild type and photosynthesis mutants from Chlamydomonas reinhardtii*. Biochimica et Biophysica Acta - Bioenergetics 1996; 1273: 150-158.

[53] Vallon O. *Lateral redistribution of cytochrome b6/f complexes along thylakoid membranes upon state transitions*. Proceedings of the National Academy of Sciences of the United States of America 1991; 88: 8262-8266.

[54] Forti G. *In vivo changes of the oxidation-reduction state of NADP and of the ATP/ADP cellular ratio linked to the photosynthetic activity in Chlamydomonas reinhardtii*. Plant Physiology 2003; 132: 1464-1474.

[55] Kargul J, Turkina MV, Nield J, Benson S, Vener AV, Barber J. *Light-harvesting complex II protein CP29 binds to photosystem I of Chlamydomonas reinhardtii under State 2 conditions*. The FEBS journal 2005; 272: 4797-4806.

[56] Tokutsu R. *CP29, a monomeric light-harvesting complex II protein, is essential for state transitions in Chlamydomonas reinhardtii*. The Journal of biological chemistry 2009; 284: 7777-7782.

[57] Dekker JP. *Supramolecular organization of thylakoid membrane proteins in green plants*. Biochimica et biophysica acta 2005; 1706: 12-39.

[58] Iwai M. *Molecular remodeling of photosystem II during state transitions in Chlamydomonas reinhardtii*. Plant Cell 2008; 20: 2177-2189.

[59] Sprang SR. *Structural changes in glycogen phosphorylase induced by phosphorylation*. Nature 1988; 336: 215-221.

[60] Sprang SR. *Phosphorylation controls the three-dimensional structure of plant light harvesting complex II*. The Journal of biological chemistry 1997; 272: 18350-18357.

[61] Turkina MV. *Environmentally modulated phosphoproteome of photosynthetic membranes in the green alga Chlamydomonas reinhardtii*. Molecular & cellular proteomics 2006; 5: 1412-1425.

[62] Steinback KE. *Phosphorylation of the light-harvesting chlorophyll-protein regulates excitation energy distribution between photosystem II and photosystem I*. Archives of biochemistry and biophysics 1982; 216: 356-361.