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Sharing light between two photosystems: mechanism of state transitions

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Highlights

- A protein kinase and an antagonistic phosphatase regulate state transitions
- Phosphorylated antennae detach from PSII and connect at least in part to PSI
- In Chlamydomonas, LhcbM trimers as well as monomeric Lhcb4 and Lhcb5 are mobile
- In Arabidopsis, only trimers of Lhcb1 and Lhcb2 are directly involved
- Conditions that favor state 2 in Chlamydomonas also favor cyclic electron flow
- State transitions and other mechanisms of light regulation are interconnected

Abstract

In the thylakoid membrane, the two photosystems act in series to promote linear electron flow, with the concomitant production of ATP and reducing equivalents such as NADPH. Photosystem I, which is preferentially activated in far-red light, also energizes cyclic electron flow which generates only ATP. Thus, changes in light quality and cellular metabolic demand require a rapid regulation of the activity of the two photosystems. At low light intensities, this is mediated by state transitions. They allow the dynamic allocation of light harvesting antennae to the two photosystems, regulated through protein phosphorylation by a kinase and phosphatase pair that respond to the redox state of the electron transfer chain. Phosphorylation of the antennae leads to remodeling of the photosynthetic complexes.

Introduction

Photosynthetic organisms constantly face the challenge of acclimating to changes in their light environment. Both the intensity of light that is available and its spectral quality may undergo abrupt variations depending on external factors such as the time of day, weather, and shading from other plants. In low light conditions, light capture and photochemistry need to be optimized. Conversely in excess light, photon capture is minimized, mechanisms of energy dissipation are activated, protection against reactive oxygen species is enhanced and repair of photo-damage is stimulated.

Chlorophyll is an intrinsic constituent in many components of the photosynthetic machinery, and its fluorescent properties make it an excellent biophysical probe of the photosynthetic electron transfer chain. Thus historically several aspects of the regulation of photosynthesis in response to changing light were monitored through changes in chlorophyll fluorescence. The energy captured by an excited chlorophyll molecule can have several fates: it can be transferred to another pigment, used to drive the redox reactions of photosynthetic electron transfer, dissipated thermally as heat, or re-emitted as fluorescence. Because these different fates are in competition, the fluorescence yield will be modified by changes in any of the other pathways. Decreases in the yield of chlorophyll fluorescence that are not due to photosynthetic activity are thus described as non-photochemical quenching (NPQ), which is attributed to several mechanisms. Upon exposure to excess light, the most rapid, operating in the range of seconds to minutes, is ascribed to energy dissipation (qE) and is regulated by the pH gradient across the thylakoid membrane. Slower components reflect the enhancement of energy dissipation due to the accumulation of zeaxanthin (qZ) through the xanthophyll cycle, and under very high light intensities, photoinhibition and photodamage to the electron transfer chain (qI). A slow contribution to NPQ was

recently shown to reflect light-avoidance movements of the chloroplasts within the plant cell [1]. Under low light intensities, the state transition component of NPQ operates in the timeframe of a few minutes and reflects the dynamic allocation of light harvesting antennae to the photosystems (qT) [2,3].

The classical model of state transitions

State transitions are regulated by the redox state of the PQ pool, sensed at the Q_o site of the cytochrome b_6f complex [4,5]. In state 1, when the PQ pool is oxidized, the LHCII antenna is connected to PSII. Upon over-reduction of the PQ pool, a transition towards state 2 is induced: a mobile component of the LHCII detaches from PSII and connects to PSI – at least in part, as will be discussed below (Figure 1). This decrease in the cross-section of PSII and increase in that of PSI leads to a re-equilibration of the redox poise of the PQ pool. Conversely, when the PQ pool is over-oxidized, a transition towards state 1 leads to the dissociation of the mobile antenna from PSI and its attachment to PSII. State transitions can thus be seen as a regulatory mechanism that ensures homeostatic regulation of the redox poise of the photosynthetic electron transfer chain. It should be emphasized that state transitions operate in low light, but are largely superseded by other mechanisms such as qE under high light [6]. Mathematical modelling of state transitions and qE indeed suggests that under high light, qE plays the predominant role in maintaining the redox homeostasis of the electron transfer chain [7].

Experimental manipulations are typically designed to push the system towards its extremes, state 1 or state 2. However, under more normal conditions, the homeostatic balance will tend to keep the system in an intermediate state – which might be described as an approximate “state 1.5” (although this distortion of the “state” concept may be deemed inappropriate). It can thus be expected that under moderate white light, the LHCII antenna transfers energy not only to PSII but also to PSI. Indeed, energy transfer from LHCII bound to PSI is highly efficient [8,9]. In plants, state transitions are typically induced experimentally by changing the spectral quality of light: far-red light that favors PSI is used to promote state 1, while orange or blue wavelengths that favor PSII can be used to induce state 2. Incubation in the dark also favors state 1 and transfer to moderate white light induces a transition towards state 2. In Chlamydomonas, far-red light is not as effective at promoting state1, but the latter can be induced by treatment with a PSII inhibitor (DCMU) in low light which leads to PQ oxidation. Conversely a shift to anaerobic conditions in the dark strongly induces state 2: reducing equivalents from glycolysis, which cannot be used by respiration for lack of oxygen, are transferred to the PQ pool [10]. Furthermore, the latter can be oxidized neither by PSI for lack of light nor by PTOX (plastid terminal oxidase) for lack of oxygen . These conditions lead to a strong reduction of the PQ pool and a pronounced state 2. In

Chlamydomonas, state 2 promotes cyclic electron flow around PSI (CEF) and the production of ATP (see below). The amplitude of state transitions is much larger in Chlamydomonas, where it involves up to 80 % of LHCII, than in plants where 10-15% is mobilized [11-14].

Reversible phosphorylation of thylakoid proteins

It was observed early on that there is a strong correlation between state 2 and the phosphorylation of LHCII antenna proteins. A genetic screen in Chlamydomonas, based on chlorophyll fluorescence measurements of state transitions, led to the long-sought identification of a protein kinase, named STT7, and of its ortholog in Arabidopsis, STN7 [15-17]. Mutants deficient for this kinase are locked in state 1 and show strongly reduced phosphorylation of LHCII. More recently, the counteracting protein phosphatase, PPH1/TAP38, was identified in Arabidopsis [18,19]. Mutants deficient in the phosphatase tend to be locked in state 2 and are affected in the de-phosphorylation of the LHCII antenna. These genetic investigations clearly established that protein phosphorylation plays an essential, causal role in state transitions. Although it seems plausible that STN7, STT7 and PPH1/TAP38 act directly on antenna proteins, this has to our knowledge not yet been unequivocally demonstrated and a kinase cascade is not entirely excluded.

STN7 and STT7 have paralogs in Arabidopsis and Chlamydomonas, named STN8 and STL1 respectively. While STN7 is required for phosphorylation of the antenna, STN8 is required for the phosphorylation of some of the PSII core subunits [17,20]. However, there is some degree of overlap in the specificity of the two kinases. STN8 is counteracted by a stromal phosphatase, PBCP [21]. The *stn8* and *pbcp* mutants show changes in the organization of thylakoid grana and defects in PSII repair [21-24]. While the effects of phosphorylation and of cations on membrane architecture can be interpreted in terms of electrostatic interactions between PSII complexes (the major targets of STN8 and PBCP), effects through phosphorylation of other protein targets should also be considered, such as the recently discovered CURT proteins which are involved in thylakoid membrane curvature [25,26].

Dynamics of thylakoid membrane complexes.

Strong support for the model of state transitions came from the biochemical isolation of supercomplexes in which components of the LHCII antenna are associated with the PSI-LHCI complex. Methods for purification of the PSI-LHCI-LHCII complex have improved over the years and hence knowledge about its structure [27-31]. The PSI-LHCI-LHCII supercomplex isolated from Chlamydomonas

cells in state 2 contains major LHCII subunits (LhcbM) as well as minor subunits CP29 and CP26 (Lhcb4 and Lhcb5) (Figure 1) [27]. Biochemical quantification showed two Lhcbm5, two CP29 and one CP26 subunits per PSI proteins in one preparation [28]. Electron microscopy of a different preparation, purified by PSI affinity chromatography, showed a complex that could contain two LHCII trimers and one or two monomeric LHCII proteins in addition to the PSI core and its nine associated LHCI subunits [29]. The PSI-LHCI-LHCII complex purified from higher plants contains one LHCII trimer in addition to the PSI core and its four associated Lhcba subunits [31] (Figure 2). The mobile LHCII trimer binds on the side opposite to the Lhcba belt, in proximity to the PsaH, PsaL and PsaO subunits [9,32]. This PSI-associated trimer is enriched in specific isoforms of the major LHCII subunits Lhcb1 and Lhcb2 but lacks Lhcb3, indicating that when this trimer is associated with PSII it is the loosely bound, extra trimer [9,11,33]. Consistently, the analysis of Arabidopsis *amiLhcb1* and *amiLhcb2* knock-down lines and of the *lhcb3* mutant showed that Lhcb1 and Lhcb2 are required for state transitions, while Lhcb3 is dispensable but indirectly influences their rate [34,35] (Figure 3).

The reversible allocation of the mobile LHCII antenna to PSI or PSII can be seen as a dynamic equilibrium, where the relative binding affinities of LHCII components for the two photosystems are modified by their phosphorylation status [36]. This raises the question whether the assembly state of PSII and its phosphorylation also influence its binding affinity for the mobile antenna, and consequently state transitions. In Arabidopsis, a role for PSII supercomplex remodeling in state transitions is contradicted by observations that PSII supercomplexes are not disassembled in state 2 [33,34]. Different levels of de-stabilization of PSII supercomplexes were obtained by knocking down individual Lhcb components of the PSII antenna system (Damkjaer et al 2009, de Bianchi et al. 2008, 2011, Andersson et al 2003) showing that (i) depletion of Lhcb components and PSII supercomplex de-stabilization make state transitions faster; (ii) the total amplitude of state-transition-dependent fluorescence changes never exceed those of the WT or are lower (Fig 3). These observations suggest that migration to PSI-LHCI is determined by the capacity of specific LHCII proteins to migrate and connect rather than by the stability of PSII supercomplexes. This conclusion is consistent with the specific inhibition of state transitions in knock-down lines of Lhcb2 (Pietrzykowska et al., 2014). Accelerated rates of state transitions were also observed in Arabidopis mutant lines that show defects in the assembly of PSII supercomplexes, such as in a mutant deficient for PSBW, a 6.1-kDa protein which associates with PS II and is required for its stability in oxygenic eukaryotes , [37]. The analysis of another mutant line of Arabidopsis suggested that remodeling of PSII supercomplexes could facilitate state transitions [38]. Recent work indicates that this line is deficient for both the luminal protein PSB27 and the minor antenna CP26, and that it is the lack of

CP26 that accounts for the decreased formation of PSII supercomplexes while the lack of Psb27 impairs light acclimation [39].

In Chlamydomonas the mechanism may be different, due to the mobility of the inner antenna proteins CP29 and CP26 upon phosphorylation and their association with PSI in state 2 [27-30]. Thus, because of the requirement for the minor antennas for supercomplex assembly, it is not unexpected that in the alga the PSII supercomplexes were observed to dissociate in state 2 [40]. Similar to the role of Lhcb1 and Lhcb2 in Arabidopsis, a specific involvement of LhcbM2 and LhcbM7 in state transitions was observed in Chlamydomonas [41].

How far do the mobile antennae have to move ?

A striking feature of thylakoid membrane organization is the spatial segregation of the photosynthetic complexes in different domains. PSII is confined to grana stacks, PSI and ATPsynthase are found in the domains that are exposed to the stroma, namely grana margins, grana ends and stromal lamellae, while the $cytb_6f$ complex is distributed to both. Thus the dynamic allocation of the mobile antennae between the photosystems may imply their spatial migration, particularly in Chlamydomonas where the amplitude of state transitions is large. Immuno-gold electron microscopy indeed showed that the ratio of LHCII in unstacked versus stacked membrane domains increases two- to three-fold in state 2 compared to state 1 in maize or Chlamydomonas [42,43]. The enrichment of LHCII, but not of PSII, in stroma membrane fractions upon transition to state 2 also implies LHCII may travel to stromal membranes, where its level is consistent with the average change in PSI antenna size [11]. However, it has also been proposed, based on fractionation of membrane domains from spinach thylakoids, that the functional association of mobile LHCII with PSI-LHCI in state2 mainly occurs at grana margins [44]. Indeed, grana margins are a likely site of proximity between PSI and PSII supercomplexes and thus the "mobile LHCII" population might be preferentially exchanged in this domain.

Does all of the mobile antenna attach to PSI in state 2 ?

At room temperature, most of chlorophyll fluorescence emanates from PSII and its light-harvesting antennae. Thus what is revealed by room-temperature fluorescence measurements of state transitions is the disconnection of a part of the LHCII antenna from PSII. Upon a transition to state 2, when the relative cross-section of PSII decreases, the PSI-LHCI-LHCII supercomplex is formed. However the question arises whether all of the antennae that disconnect from PSII functionally attach to PSI [45]. If state transitions are considered as a dynamic equilibrium, there must necessarily be a pool of mobile

antenna that is not attached to either photosystem, and the question can be rephrased: what is the size of this free pool ? furthermore, what is the fate of light energy that it captures ? In Chlamydomonas, photo-acoustic measurements of the relative cross-sections of the PSI and PSII antennae in state 1 versus state 2 indicated that most of the mobile antenna connects to PSI in state 2 [42]. However, other evidence suggested that a significant part of the mobile antenna may remain disconnected and in a quenched (thermally dissipating) state in this alga. Fluorescence lifetime imaging microscopy (FLIM) showed that upon a transition to state 2, a fraction of the fluorescence showed an increase in lifetime from 170 ps to 250 ps [46]. This longer value approximately corresponds to the fluorescence lifetime in a mutant deficient for both photosystems (PSI and PSII), where the LHCII is necessarily disconnected. A recent analysis of fluorescence lifetimes in Chlamydomonas also indicated that the antenna that detaches from PSII only partly connects with PSI, and that the detached antenna is quenched, possibly in an aggregated state [47]. This view was supported by the observation that low-temperature fluorescence spectra do not show a concomitant increase in PSI emission when PSII emission decreases in state 2 [47]. However low-temperature fluorescence spectra may not provide a quantitative estimate of antenna cross-section because of possible artefacts inherent to the method, and the observation awaits further confirmation. Measurements of electrochromic shift (ECS) absorbance transients indicated an increase of the PSI antenna in state 2 (20%-25%) that is significantly smaller than might be expected from the decrease in PSII antenna (75%) [48,49]. Considering the role of state transitions in acclimation predominantly at low light intensities, it seems somewhat paradoxical that a significant fraction of LHCII would remain disconnected and quenched, rather than productively contributing to photosynthetic electron flow. In contrast to Chlamydomonas, there is to our knowledge no evidence that a pool of disconnected LHCII remains quenched in higher plants in state 2.

Do state transitions contribute to cyclic electron flow ?

Linear electron flow (LEF) involves the two photosystems and generates both NADPH and ATP, while cyclic electron flow (CEF) around PSI only produces ATP. By favoring excitation of PSI, state 2 can be expected to promote the rate of CEF. Beyond this effect on the cross-section of PSI, regulated by STT7, conditions that promote state 2 induce a regulatory switch from LEF to CEF in Chlamydomonas [50]. It is debated whether this switch is triggered by STT7, or by another regulator that also responds to the redox poise of the electron transfer chain [49]. An obvious candidate would be the STL1 kinase, the Chlamydomonas ortholog of STN8. Consistent with this hypothesis, in Arabidopsis STN8 is required for the phosphorylation of PGRL1 (proton gradient regulation like 1) which is a key component of FQR

(Ferredoxin-Plastoquinone Reductase), in the branch of CEF that is sensitive to the inhibitor Antimycin A [51-54]. Whether the role of STN8 in fine-tuning of CEF is through PGRL1 phosphorylation or through other targets is not yet established [54]. In this respect it is of interest that phosphorylation of CaS (calcium sensor protein) is also dependent on STN8 in Arabidopsis [55], while in Chlamydomonas CAS and PGRL1 are part of large complex involved in CEF [53]. This complex also contains PSI, LHCI, mobile LHCII, the cytochrome b_6f complex and FNR (Feredoxin NADPH Reductase) [56].

Concluding remarks: towards "Evo-Physio" of state transitions

State transitions were highly conserved during the evolution of plants and algae; however they also show interesting differences that are beginning to offer new perspectives on the evolution and physiological significance of the mechanisms of light acclimation. As was already noted above, the amplitude of state transitions is much larger in the green alga Chlamydomonas than in vascular plants. Furthermore, in plants the mobile antenna is constituted of a trimer of the major antenna polypeptides Lhcb1 and Lhcb2, while in Chlamydomonas it also includes CP29 (Lhcb4) and CP26 (Lhcb5) together with the major antenna LhcbM polypeptides [28,29,48]. Lycophytes such a Selaginella exhibit light-regulated phosphorylation of CP24 (Lhcb6) which may contribute, together with LHCII phosphorylation, to the balance of light harvesting by PSI and PSII [57].

In Chlamydomonas, where competence for thermal energy dissipation (qE) only develops in high light with the expression of the LHCSR3 protein, state transitions appear to contribute to the management of excess light [58]. Interestingly, LHCSR3 reversibly associates with PSI or PSII under state2 or state 1 respectively. In Arabidopsis, where qE depends on PSBS which is constitutively expressed, the STN7 kinase is nevertheless important for acclimation to fluctuating light intensities [16,59]. In rice, phosphorylation of CP29 plays a role in enhancing qE, in contrast with dicots such as Arabidopsis where antenna phosphorylation is primarily involved in state transitions [60]. These observations are examples of the close interplay between state transitions and thermal dissipation (qE) in the response to fluctuating light irradiances [61]. It appears likely that future research will further show that state transitions should be considered in the context of other mechanisms of light acclimation, which we could not do within the narrow focus of this review.

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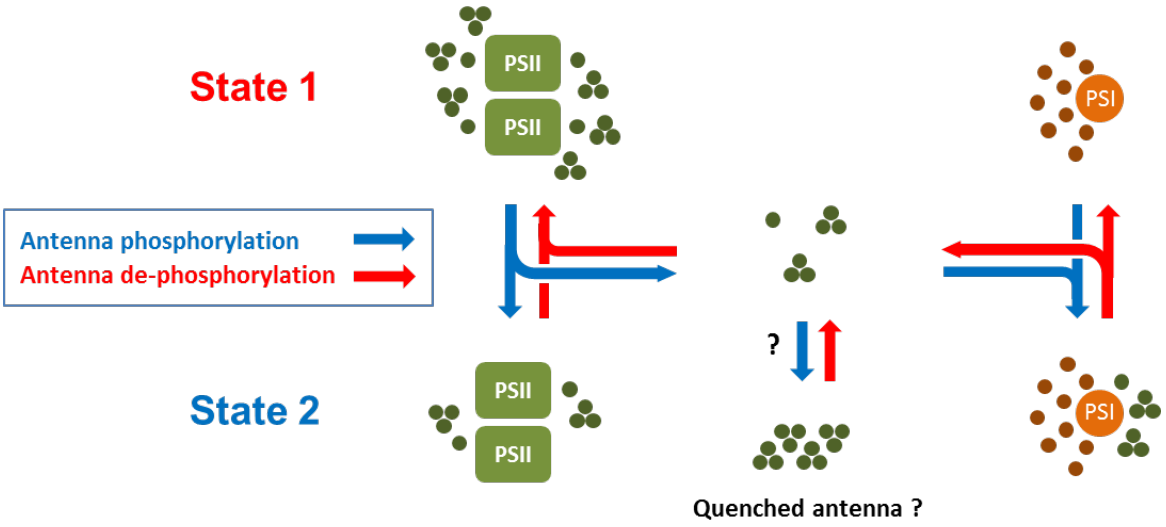

Figure 1. State transitions in Chlamydomonas.

In this schematic representation, PSII is shown in light green, and the LHCII proteins are represented by dark green circles: monomeric (Lhcb4/CP29 and Lhcb5/CP26) and trimeric (LhcbM). PSI is shown in orange, with the nine Lhca subunits of its LHCI antenna depicted by dark orange circles. The binding of the mobile LHCII antenna to the two photosystems is in a regulated equilibrium: antenna phosphorylation by the kinase STT7 (blue arrows) promotes its connection to PSI and state 2, while its de-phosphorylation (presumably by the ortholog of PPH1/TAP38 from Arabidopsis) favors its connection to PSII and state 1. In the center of the scheme, the possible existence of a free pool of antenna (presumably quenched) is indicated.

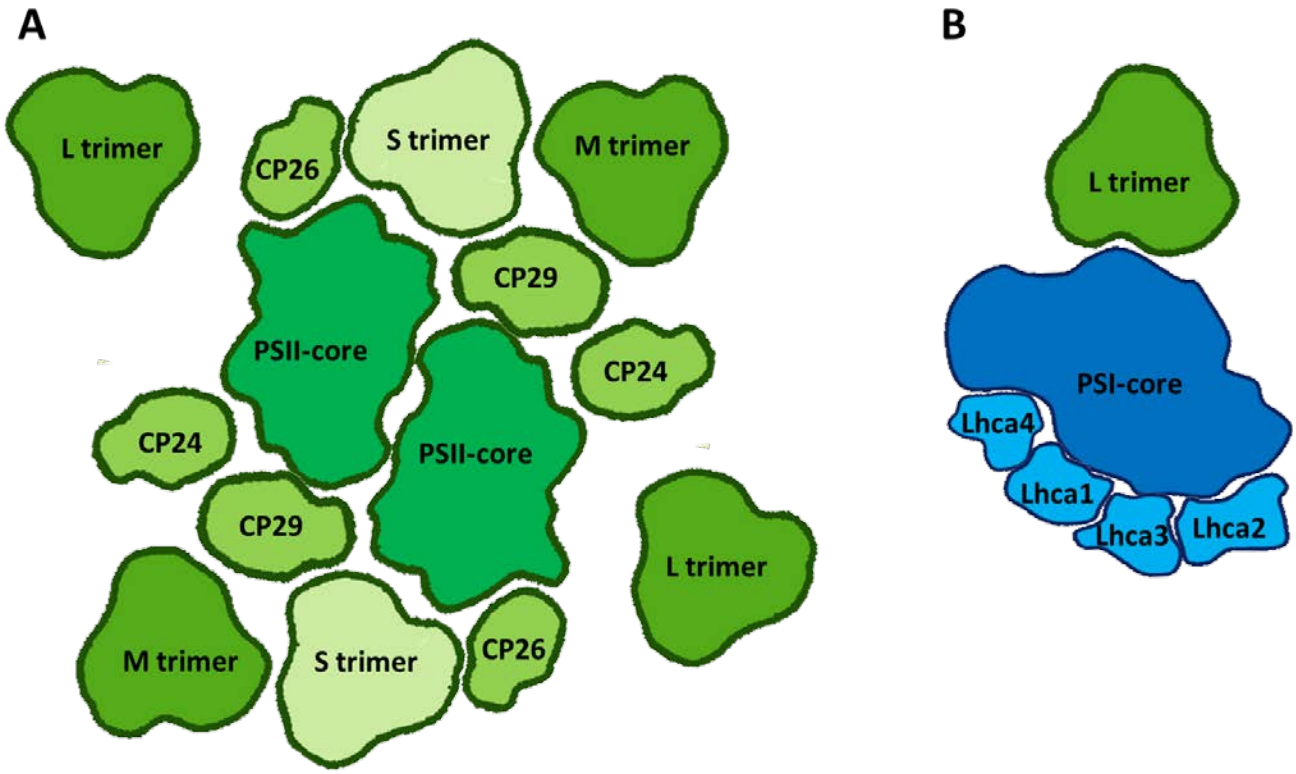

Figure 2. Organization of plant photosynthetic complexes and associated light harvesting antennae.

In these schematic representations, photosynthetic complexes from a higher plant are viewed perpendicular to the thylakoid membrane.

(A). A PSII supercomplex is represented with the dimer of PSII-cores in its center, surrounded by the LHCII antennae: the "minor" monomeric antennae CP29 (Lhcb4), CP26 (Lhcb5) and CP24 (Lhcb6), and trimers of the "major" subunits (Lhcb1, Lhcb2 and Lhcb3). S and M refer to the strong and medium binding of the respective trimers to the PSII supercomplex and L to the loosely bound trimer, part of which can be transferred to PSI in state 2 [62].

(B). A PSI-LHCI-LHCII supercomplex is depicted, with the belt of LHCI (Lhca1-4) on one side of PSI, and an LHCII trimer (L) docked on the opposing side [9,31].

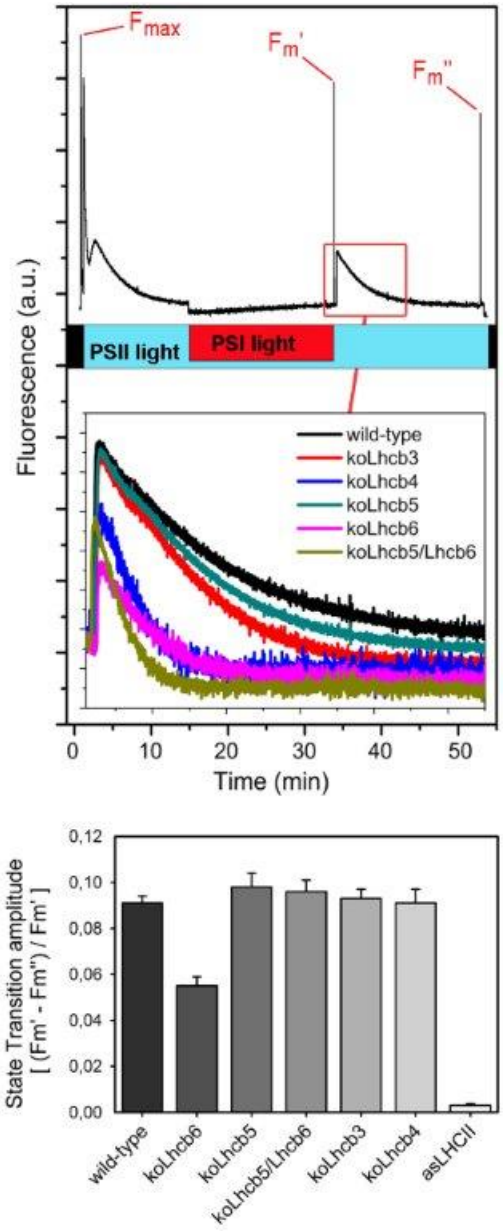

Figure 3. Fluorescence analysis of state transitions in wild-type and mutant genotypes lacking specific components of the PSII antenna system.

Wild-type Arabidopsis is compared to knock-out mutants lacking specific LHCII polypetides (*koLhcb3* [35], *koLhcb4* (CP29)[63], *koLhcb5* (CP26)[64], *koLhcb6* (CP24)[65], *koLhcb5*/*koLhcb6*, or an antisense line where all Lhcb1 and Lhcb2 isoforms are down-regulated (*asLHCII*) [66].

A) Fluorescence kinetics measured upon state transition induction in *lhcb* mutants. Upon illumination of leaves with far-red light (PSI light), chlorophyll fluorescence slowly rises until State I is reached: at this point, the mobile LHCII has re-associated with PSII (maximum fluorescence Fm' is measured with a saturating flash). Turning off the PSI light induces a rapid increase in fluorescence, because background PSII-light now preferentially excites PSII causing the reduction of the PQ pool. The fluorescence rise phase is rapidly followed by a decay phase, as the transition to State II is induced and completed

(Fm”measured with a saturating flash). Thus the decay phase is highly informative, its slope reflects the transition rate from state 1 to state 2.

B) Kinetics of the transition from state 1 to state 2 (enlarged from panel A as indicated with a red box). Several mutants (e.g. *koLhcb4* and *koLhcb5*/*Lhcb6*) show faster fluorescence decay, indicating that their transition from state 1 to state 2 occurs more quickly than the in wild-type.

(C) State transition amplitude measured as the difference between maximum fluorescence emission of PSII in State I and State II (Fm’-Fm’’/Fm’). The overall amplitude of state transitions never exceeds that of the wild type despite the de-stabilization of PSII supercomplexes in these mutants.