



Different roles for ApcD and ApcF in *Synechococcus elongatus* and *Synechocystis* sp. PCC 6803 phycobilisomes

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ABSTRACT

The phycobilisome, the cyanobacterial light harvesting complex, is a huge phycobiliprotein containing extra-membrane complex, formed by a core from which rods radiate. The phycobilisome has evolved to efficiently absorb sun energy and transfer it to the photosystems via the last energy acceptors of the phycobilisome, ApcD and ApcE. ApcF also affects energy transfer by interacting with ApcE. In this work we studied the role of ApcD and ApcF in energy transfer and state transitions in *Synechococcus elongatus* and *Synechocystis* PCC6803. Our results demonstrate that these proteins have different roles in both processes in the two strains. The lack of ApcD and ApcF inhibits state transitions in *Synechocystis* but not in *S. elongatus*. In addition, lack of ApcF decreases energy transfer to both photosystems only in *Synechocystis*, while the lack of ApcD alters energy transfer to photosystem I only in *S. elongatus*. Thus, conclusions based on results obtained in one cyanobacterial strain cannot be systematically transferred to other strains and the putative role(s) of phycobilisomes in state transitions need to be reconsidered.

1. Introduction

Plants, algae and cyanobacteria, by harvesting solar energy and converting it into chemical energy, provide food and oxygen that are essential for life on earth. In eukaryotic (plants and algae) and prokaryotic (cyanobacteria) oxygen-evolving organisms, photosynthesis involve the participation of two photosystems operating in series, formed by the photochemical centers and antennae, the light harvesting complexes. While the membrane-bound photochemical centers (including the water-oxidizing complex) are similar in cyanobacteria, algae and plants, a large diversity of antennae appeared along evolution. Two major groups can be distinguished: the membrane-bound light-harvesting complexes (LHCII and LHCI), which binds chlorophylls and carotenoids and are present in green algae and in plants, and the huge extramembrane phycobilisomes (PBSs) which bind bilins and are present in red algae, glaucophytes and cyanobacteria (with the exception of prochlorophytes).

Antennae have evolved to efficiently harvest energy in a large variety of ecological niches and environmental conditions. However, when energy absorption exceeds the rate of carbon fixation, light can become lethal since dangerous reactive oxygen species are generated. Thus, oxygenic photosynthetic organisms have developed photoprotective mechanisms in which the efficient light-harvesting antennae enters into quenching states, in which the excess excitation energy is

converted into heat. Due to the diversity of antennae, these mechanisms also differ in LHC-containing and PBS-containing organisms (reviews [1–3]). In plants and green algae, this photoprotective state is induced by acidification of the thylakoid lumen (under high light conditions), protonation of PbsS, a Photosystem II (PSII) subunit, and LHCII conformational changes (reviews [4–6]). The acidic lumen also induces the synthesis of zeaxanthin from violaxanthin, via the xanthophyll cycle [7,8]. By contrast, in cyanobacteria, strong blue-green light photo-activates a soluble carotenoid protein: the Orange Carotenoid Protein (OCP). The photoactivated protein thermally dissipates the excess of excitation light absorbed by PBSs, through its interaction with them [9,10]. In red algae, the PBS seems not to be involved in thermal dissipation of excess energy [11–13].

The LHCII is also involved in “State transitions”, a mechanism that in plants and green algae controls the relative extent of energy arriving at one or the other reaction center, avoiding an over-reduction or over-oxidation of the intersystem electron transport chain [14]. In this mechanism, two states are defined: State I, which presents a high PSII to Photosystem I (PSI) fluorescence ratio (and is induced by light preferentially absorbed by PSI), and State II, characterized by a low PSII to PSI fluorescence ratio (and induced by light preferentially absorbed by PSII). Reduction of the PQ pool and binding of PQH₂ at the Q_o site of the cyt *b₆f* activate a specific kinase which phosphorylates some LHCII trimers [15]. Phosphorylation induces LHCII detachment from PSII and

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partial (or total) attachment to PSI, triggering the transition to State II [16,17]. Meanwhile, dephosphorylation of LHCII triggers the opposite phenomenon and transition to State I (see review [18]).

The role of PBS in cyanobacteria state transitions is less clear. Several models were proposed for the molecular mechanism of cyanobacterial state transitions. One model considers that PBS movement induces changes in direct energy transfer to PSII and PSI [19–21], such as it was demonstrated for LCHII in plant and alga state transitions [22,23]. In another model called the “spillover model”, the energy transfer from PBS to PSII remains equal in both states, but the excess of energy absorbed by PSII is transferred to PSI in State II (spillover). Spillover was proposed to involve movement of photosystems [24–31]. Lastly, in a recent study, it was proposed that the fluorescence changes in cyanobacterial state transitions are due to a PSII-related quenching in State II, without involvement of spillover and with a little participation of PBS detachment [32]. Nevertheless, the prevalence of one mechanism over the other, or the co-existence of some/all of them, is still a matter of discussion.

The PBSs, attached to the stromal side of the thylakoids, are formed by phycobiliproteins (PBP) (covalently binding linear tetrapyrrole chromophores, the bilins) and linker proteins (mostly non-chromophorylated). The PBPs absorb solar energy and the linker proteins have principally a structural role. The highly ordered structure of the PBS, formed by a core (close to the membranes) from which rods radiate, ensures directional energy transfer with high quantum yield, from the rods to the core and finally to the reaction centers (Fig. 1) (for reviews, see [33–35]).

The rods, which are formed by two to four stacked PBPs hexamers, can contain only phycocyanin (PC, blue) hexamers in most of fresh water strains (like those used in this study: *Synechococcus elongatus* PCC 7942 (hereafter *S. elongatus*) and *Synechocystis* sp. PCC 6803 (hereafter *Synechocystis*)) or PC plus phycoerythrin (PE, red to violet) in marine strains. Meanwhile, the core is formed by two (*S. elongatus*), three (*Synechocystis*) or five (*Nostoc* PCC 7120) cylinders, each composed of 4 trimers of allophycocyanin (APC, blue). Furthermore, each APC trimer is formed by α APC- β APC heterodimers. In the basal cylinders, which are in contact with the membranes, each trimer has a different

composition (Fig. 1C). Trimer 1 is formed by 3 α/β APC dimers containing the linker-core (Lc) protein in the central hole. Trimer 2 is a pure α/β APC trimer. Trimer 3 contains one α/β APC dimer, one dimer formed by an α APC subunit and ApcF (a β APC-like subunit) and finally a dimer formed by a β APC subunit and the bilin-linker domain of ApcE. The bilins attached to ApcF and ApcE are close (20 Å) and have big influences one on the other. In trimer 4, which also contains the Lc linker protein, one α APC subunit is replaced by ApcD. ApcE and ApcD bilins are also relatively close although they are in different APC trimers.

While the APC trimers containing only α APC- β APC heterodimers have a fluorescence maximum at 660 nm, the trimers containing ApcD and ApcE/ApcF emit at 680 nm. ApcE and ApcD transfer the energy absorbed by the PBS to the photosystems. ApcE is also essential for the integrity of the PBS core and the interaction of the PBS with the membrane [36]. The PBS can transfer the absorbed energy to both photosystems. Several models exist showing the interaction of one hemi-discoidal PBS with a PSII dimer [37–39], whereas its interaction with PSI is less clear due to the protuberance of stromal extra-membrane PSI subunits. The energy arriving at each reaction center depends on the cyanobacterial strain and also on environmental conditions: while in *Arthrospira platensis* 80% of absorbed energy arrives to PSI [40], this drops to 50% in *Synechocystis* [41].

It was shown that in *Synechococcus* PCC 7002, lack of ApcD decreases energy transfer from PBS to PSI and lack of ApcF decreases energy transfer to PSII. Thus, it was concluded that ApcD is essential for energy transfer to PSI while the lack of ApcF largely impairs energy transfer to PSII [42,43]. However, analysis of 77 K and room temperature fluorescence in *Synechocystis* suggested that energy transfer from PBSs to both photosystems, is largely affected in the absence of ApcF but only a little in the absence of ApcD [44].

In addition, the absence of ApcD and ApcF affects state transitions. The Δ ApcD mutants of *Synechocystis*, *Synechococcus* PCC 7002 and *Anabaena* sp. PCC 7120 lack state transitions [42,44–46]. These cells present a high PSII fluorescence in darkness and under blue and orange illumination, which is related to State I [42,44,45]. It was proposed that this phenotype was due to the lack of energy transfer from PBSs to PSI

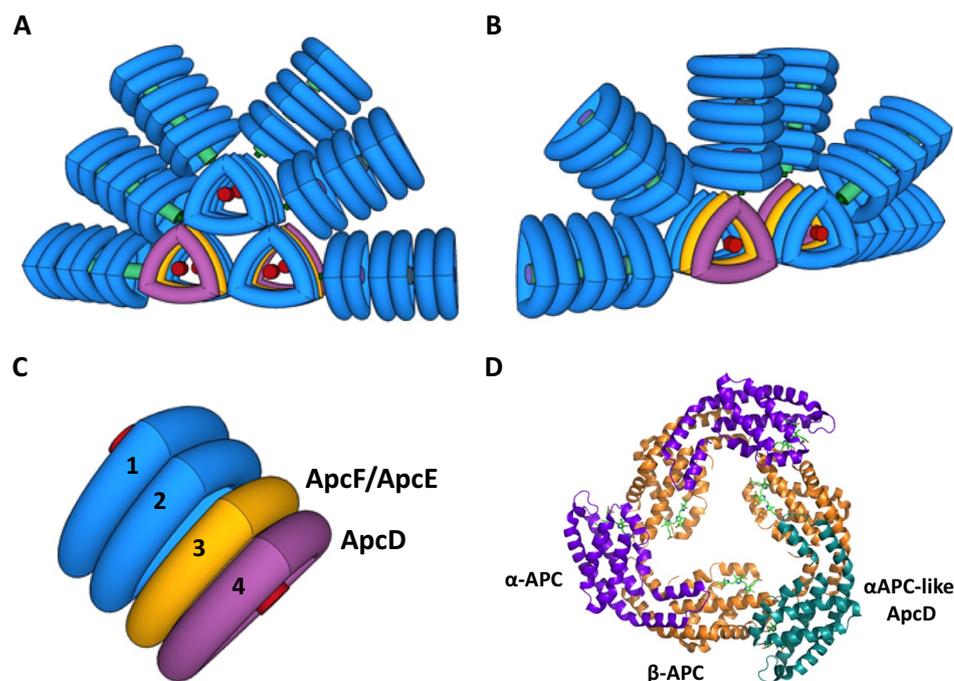


Fig. 1. The model of *Synechocystis* PCC 6803 (A) and *Synechococcus elongatus* PCC 7942 (B) phycobilisomes. In these strains, the PBSs are constituted by a tricylindric (*Synechocystis*) or a bicylindric core (*S. elongatus*) surrounded by six rods. Each rod is formed by three hexamers of blue phycocyanin (PC). The rods are stabilized by non-chromophorylated linker proteins (in green). The rod-core linker (LRC) protein (small cylinder in green) stabilizes the binding of the rods to the core. In *Synechocystis* the upper core cylinder is formed by 4 trimers of allophycocyanin (APC), composed by a α APC- β APC heterodimer which binds two phycocyanobilins (maximal fluorescence 660 nm). In addition, 2 core linker (Lc) proteins (in red) present in the external trimers stabilize each cylinder. In both strains, in both basal cylinders, in one of the external trimers (in violet in C), one α APC subunit is replaced by a special α APC-like subunit called ApcD. In the adjacent trimer (in orange in C), a β APC-like subunit named ApcF replaces one β -subunit, and another dimer is formed by a β -APC subunit and the bilin-linker domain of an α APC-like domain (ApcE). The bilins attached to ApcF and ApcE are close (20 Å) and have big influences one on the other. Both ApcD and ApcF/ApcE proteins were described as terminal

energy acceptors of the PBS, presenting a maximal fluorescence at 680 nm. In (D) the X-ray tridimensional structure of the APC trimer containing ApcD is shown. The pdb file used in (D) was 4PO5. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

[42] and/or a permanent PBS binding to PSII [44] in the Δ ApcD mutant. Meanwhile, the lack of ApcF inhibits state transitions in *Synechocystis* [44] but not in *Synechococcus* PCC 7002 [42,44].

The contradictory results already obtained in the ApcD and ApcF *Synechocystis* and *Synechococcus* 7002 mutants, with regard to energy transfer and state transitions, suggested that the role of these proteins could be different in different strains [42–44]. However, none of these studies directly compared the strain-dependence effect of these mutations. To further understand the role of these proteins, we compared in the present study the phenotype of Δ ApcD and Δ ApcF mutants of *S. elongatus* and *Synechocystis* cells. We show that lack of these proteins alters the energy transfer from PBSs to PSII and PSI in different ways in these strains. Surprisingly, the lack of ApcD or ApcF was found to not impair state transitions in *S. elongatus*.

2. Materials and methods

2.1. Culture conditions

The cyanobacteria *Synechocystis* sp. PCC 6803 and *Synechococcus elongatus* PCC 7942, wild-type (WT), Δ ApcD and Δ ApcF strains were grown photo-autotrophically in BG11 medium [47]. Cells were kept in a rotary shaker (120 rpm) at 31 °C and illuminated by fluorescence white lamps (50 μ mol photons $m^{-2} s^{-1}$) under a CO₂-enriched atmosphere (50 mL/min CO₂ flow into the incubator). Cells were maintained in their logarithmic phase of growth for all the experiments. The *Synechocystis* Δ ApcD, Δ ApcF and ApcE-C190S strains were obtained by [48], and the CK mutant strain from [49].

2.2. Δ ApcD and Δ ApcF *S. elongatus* mutants construction

To construct the Δ ApcD mutant, a 2.2 kb DNA region surrounding the *Synpcc7942_0240* locus, encoding the *apcD* gene, was amplified by PCR using genomic DNA of *S. elongatus*. The two restriction sites-creating oligonucleotides used (*XhoI*_{apcD_fw} and *NotI*_{apcD_rv}) are presented in Supplementary Table S1. The resulting PCR product was digested by *NotI* and *XhoI* restriction enzymes and cloned in the pBluescript SK+ plasmid. Then, the plasmid was digested by *SmaI* and ligated to a 1.3 kb kanamycin resistance cassette, interrupting the *apcD* gene. This plasmid was named “*interrupted apcD*”.

To construct the Δ ApcF mutant, a 1.2 kb DNA region surrounding the *Synpcc7942_2158* locus, encoding the *apcF* gene, was amplified using genomic DNA of *S. elongatus*. The two restriction sites-creating oligonucleotides (*XhoI*_{apcF_fw} and *NotI*_{apcF_rv}) are shown in Supplementary Table S1. The resulting PCR product was digested by *NotI* and *XhoI* restriction enzymes and cloned in the pBluescript SK+ plasmid. A new *SmaI* restriction site was introduced 240 pb downstream of the ApcF start-codon, by site directed mutagenesis, using the Quickchange XL kit of Stratagene and the oligonucleotides ApcF_{smaI_fw} and ApcF_{smaI_rv} (Supplementary Table S1). An enzymatic digestion by *SmaI* and *BstAPI* was used to replace the initial 200 nucleotides of *apcF* gene by a spectomycin/streptomycin (Sm/Sp) resistance cassette, inactivating the *apcF* gene. This plasmid was called Δ apcF since it lacks a large portion of the *apcF* gene.

The “*interrupted apcD*” and Δ apcF plasmids were used to transform *S. elongatus* as described in [50]. Selection was made at 33 °C and under dim light (30 μ mol photons $m^{-2} s^{-1}$), on plates containing BG11 medium supplemented with 1% agarose and different antibiotics: 40 μ g/mL kanamycin or 25 μ g/mL spectomycin and 50 μ g/mL streptomycin (for Δ ApcD and Δ ApcF, respectively). Genomic DNA was isolated from the colonies growing in the presence of antibiotics as described in [50]. PCR analysis and DNA sequencing were performed in order to confirm complete segregation (Supplementary Fig. S1).

2.3. LC-MS/MS analysis

To confirm the absence of ApcD in the PBSs of the Δ ApcD mutant, a LC-MS/MS analysis was performed. PBS isolation was performed as described in [51]. The isolated PBSs of WT and Δ ApcD *S. elongatus* strains were diluted in 50 mM NH₄HCO₃ for enzymatic digestion. Then, peptides were analyzed by LTQ-Orbitrap Velos for the identification of the ApcD protein in the samples. Measurements were done by the SI-CaPS service of the Institut de Biologie Intégrative de la Cellule (I2BC), Gif-sur-Yvette, France.

2.4. Fluorescence measurements

2.4.1. PAM fluorometer

State transitions were monitored using a pulse amplitude modulated fluorometer (101/102/103-PAM; Walz, Effeltrich, Germany), in a 1 × 1 cm square stirred cuvette. All experiments were carried at 31 °C on dark-adapted (15 min) whole cells at a chlorophyll concentration of 2.5 μ g/mL. State I was induced by 85 μ mol photons $m^{-2} s^{-1}$ of blue-green light (halogen white light filtered by a Corion cut-off 550-nm filter; 400 to 500 nm). State II was induced by 25 μ mol photons $m^{-2} s^{-1}$ of orange light (halogen white light filtered by a Melles Griot 03 FIV 046 filter; 600 to 640 nm) or by dark incubation. Saturating flashes (400 ms × 1200 μ mol photons $m^{-2} s^{-1}$) were given to probe the maximum fluorescence level. The fluorescence parameters used in the analysis are the following: F₀, basal fluorescence; F_{md}, maximum fluorescence in darkness; F_m′, maximum fluorescence under illumination; F_{mb}, maximum fluorescence under blue light illumination; F_{mo}, maximum fluorescence under orange light illumination; F_v = variable fluorescence = F_m - F₀; F_{vd}, variable fluorescence in darkness; F_vb, variable fluorescence under blue light illumination.

2.4.2. Fluorescence emission spectra

77 K and room temperature fluorescence emission spectra were monitored in a CARY Eclipse spectrophotometer (Varian). For 77 K measurements, whole cells (5.0 μ g Chl/mL) were dark-adapted for 15 min before the measurements. Then, spectra were recorded corresponding to State II. For State I spectra, cells were illuminated for 5 min by 85 μ mol photons $m^{-2} s^{-1}$ of blue-green light (halogen white light filtered by a Corion cut-off 550-nm filter; 400 to 500 nm). Samples were collected in RMN tubes (5 mm diameter) and frozen by immersion in liquid nitrogen. Excitation was made at 430 nm or 590 nm, and emission was scanned from 620 nm to 800 nm. All spectra were normalized by the signal intensity at 800 nm.

Room temperature fluorescence spectra were recorded at a phycocyanin concentration of 1 μ M, estimated using the formulae expressed in [52]. Whole cells were dark-adapted for 15 min before the measurements, which corresponds to State II conditions. Excitation was made at 590 nm and emission was scanned from 620 nm to 800 nm.

2.4.3. Closure of PSII reaction centers

The closure of reaction centers was followed by the PSI fluorometer (PSI Instruments, Brno, Czech Republic), in the 1-ms to 1-s time range, in dark-adapted (15 min) whole cells (2.5 μ g Chl/mL). Before the measurements, DCMU (10 μ M) and DBMIB (20 μ M) were added to the cells. Blue measuring light (λ = 460 nm) and orange actinic light (35 μ mol photons $m^{-2} s^{-1}$, λ = 630 nm) were used in all cases.

2.5. P700⁺ measurements

P700 absorption changes were measured using the P700 module of a standard PAM (101/102/103-PAM; Walz, Effeltrich, Germany). Measurements were made at 31 °C on whole cells (2.5 μ g Chl/mL) in a 1 × 1 cm square cuvette in the presence of DCMU (15 μ M), DBMIB (100 μ M) and methyl viologen (MV, 300 μ M). Orange light (halogen white light filtered by a Melles Griot 03 FIV 046 filter; 600 to 640 nm)

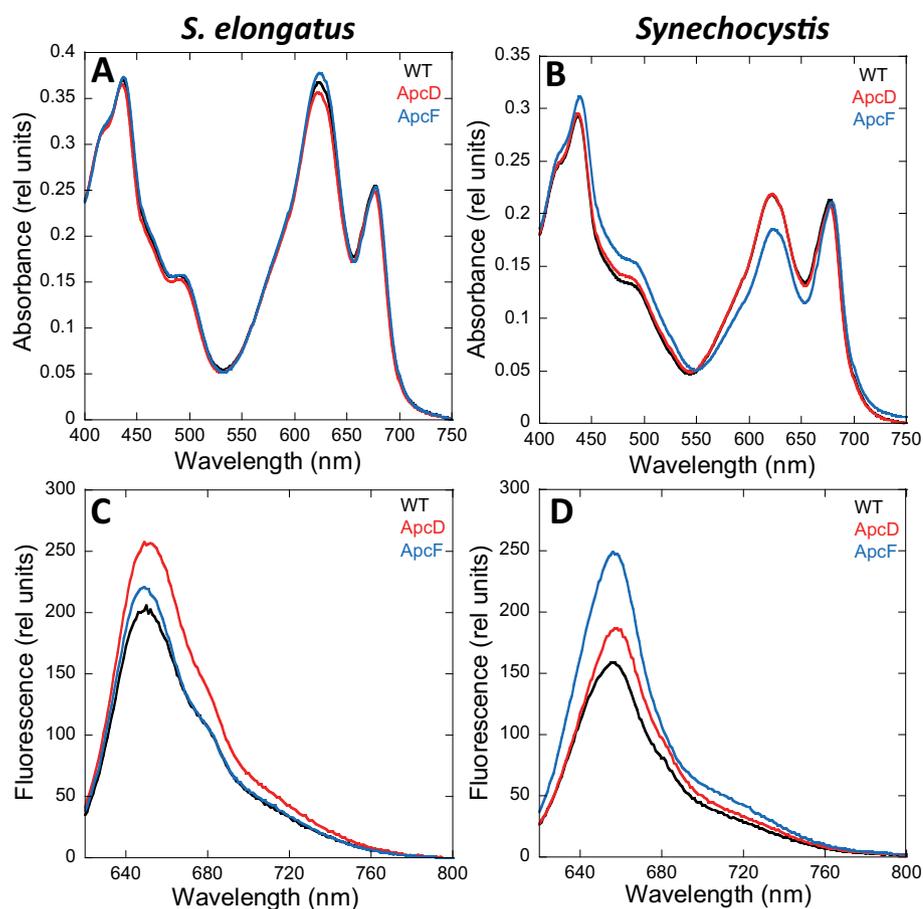


Fig. 2. Absorbance and room temperature fluorescence emission spectra of *S. elongatus* (A and C, respectively) and *Synechocystis* (B and D, respectively) strains. (A and B) Absorbance spectra measurements were recorded at a Chl concentration of 2.5 $\mu\text{g}/\text{mL}$. (C and D) Room temperature fluorescence spectra of dark-adapted (15 min) cells were recorded at a phycocyanin concentration of 1 μM . Black lines, WT strains; red lines, ΔApcD strains; blue lines, ΔApcF strains. The curves shown are the average of three biological replicates. The excitation wavelength was 590 nm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

or blue light (halogen white light filtered by a Corion cut-off 550-nm filter; 400 to 500 nm) were used as actinic lights.

3. Results and discussion

3.1. Energy transfer from PBS to photosystems in the *S. elongatus* and *Synechocystis* ΔApcD and ΔApcF mutants

The construction of the *Synechocystis* ΔApcD and ΔApcF mutants was described in [48]. The construction of the *S. elongatus* mutants is described in Materials and Methods. The phycocyanin (PC, absorbance at 620 nm) to chlorophyll (Chl, absorbance at 680 nm) ratio was higher in *S. elongatus* than in *Synechocystis* cells. *S. elongatus* (Fig. 2). While the PC to Chl ratio was similar between the mutants and WT *S. elongatus* cells, the ΔApcF *Synechocystis* mutant always presented a slightly smaller PC to Chl ratio. The PSI to PSII ratio was also measured in both WT strains by $(F_A, F_B)^-$ and TyrD^+ EPR signals, being 2–3 in *S. elongatus* and 4–5 in *Synechocystis*.

Room temperature fluorescence spectra of all strains presented a maximum at 660 nm (related to bulk APC) and a shoulder at 680 nm (related to PBS terminal emitters, ApcD and ApcE, and PSII Chl) (Fig. 2C and D). ΔApcD and ΔApcF mutants showed a higher fluorescence at 660 nm (at the same PC concentration) than the WT, in *S. elongatus* as well as in *Synechocystis* cells. This could be related to a defective energy transfer from PBS to one photosystem or the other (or to both photosystems) and/or to a defective energy transfer from bulk APC to terminal emitters and/or a different state in darkness. It is important to remark that while in *Synechocystis*, the highest fluorescence was observed in the ΔApcF mutant, in *S. elongatus*, the ΔApcD mutant showed the highest fluorescence. These results already suggest that the lack of these proteins has different effects on energy transfer in the two

strains.

To investigate whether the PSII effective antenna size varies in the ΔApcD and ΔApcF *S. elongatus* and *Synechocystis* mutants, their fluorescence induction curves in the presence of DCMU and DBMIB were compared to those of their respective WTs. When adding DCMU, the rate of fluorescence increase depends only on the antenna size and it is independent of the dark PQ pool redox state and light PQ reoxidation rates. DBMIB was added to avoid State I transition during the measurements. Fig. 3 shows the kinetics of fluorescence induction induced by non-saturating orange light (preferentially absorbed by phycobilliproteins) in dark-adapted WT and mutants cells of *S. elongatus* and *Synechocystis*. The fluorescence induction kinetics in the ΔApcD *Synechocystis* and *S. elongatus* cells, compared to their respective WTs, were almost identical (Fig. 3), indicating that the absence of ApcD does not affect energy transfer from PBS to PSII in neither of these strains. In contrast, the absence of ApcF slowed down the kinetics of fluorescence induction in *Synechocystis* cells, but not in *S. elongatus* (Fig. 3). Thus, the absence of ApcF seems to affect energy transfer from PBS to PSII only in *Synechocystis* cells, with multiphasic kinetics suggesting some heterogeneity in the coupling of PBS to PSII (see discussion below).

Then, to investigate whether the absence of ApcD or ApcF affects the energy transfer from PBS to PSI, we measured the oxidation kinetics of P700 in the presence of DCMU (15 μM), DBMIB (100 μM) and MV (300 μM) (Fig. 4). The presence of DCMU prevents the reduction of P700⁺ by electrons coming from PSII. DBMIB inhibits reduction of luminal electron donors by cyclic/respiratory electron transport and, in addition, MV largely inhibits charge recombination in PSI. In the presence of these inhibitors, P700 photo-oxidation exhibits no or a negligible lag phase. This indicates that luminal electron donors are pre-oxidized in darkness, due presumably to cytochrome oxidase activity. Under these conditions, the initial rate of P700 photooxidation depends

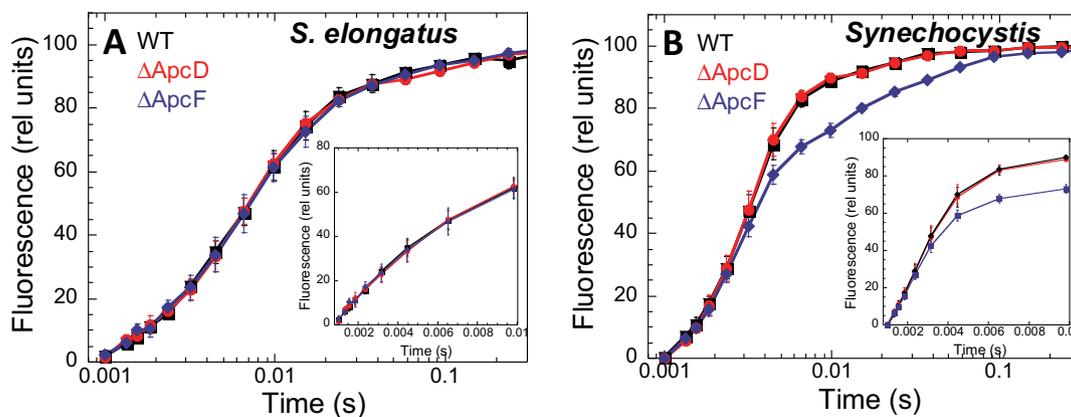


Fig. 3. Chlorophyll *a* fluorescence induction transients. Measurements were done in *S. elongatus* (A) and *Synechocystis* (B) WT, Δ ApcD and Δ ApcF strains (black, red and blue lines, respectively). Cells ($2.5 \mu\text{g Chl/mL}$) were dark-adapted to state II for 15 min before the measurements, and the following inhibitors were added subsequently: $10 \mu\text{M DCMU}$ and $20 \mu\text{M DBMIB}$. Measurements were done with orange actinic light ($35 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) and blue measuring light. The detection wavelength was $\geq 695 \text{ nm}$ (far-red). The curves are the average of at least 3 independent biological experiments. The errors bars correspond to the standard deviation of the data shown. Insets shows the first 0.01 s of each measurement on a linear scale. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

only on the antenna size. The measurements were done using orange or blue actinic light (Fig. 4 and Supplementary Fig. S2, respectively).

Under blue light illumination, the kinetics of P700 oxidation were rather similar in WT and mutants strains. This was expected since the Chl antennae were not modified in these mutants (Supplementary Fig. S2). In contrast, under orange light illumination, the rates of P700 oxidation were slower in the Δ ApcD *S. elongatus* and the Δ ApcF *Synechocystis* mutants, than in their respective WT (Fig. 4 and Table 1).

Therefore, less energy transfer from PBS to PSII occurred in these strains (Fig. 4A and D). By contrast, no significant differences were observed in the rates of P700 oxidation in the Δ ApcD *Synechocystis* and the Δ ApcF *S. elongatus* mutants, when compared to their respective WT (Table 1, Fig. 4B and C).

Altogether, these experiments show that the absence of ApcD does not affect energy transfer to PSII in any of the strains studied (*Synechocystis* and *S. elongatus* (this work) and *Synechococcus* PCC7002

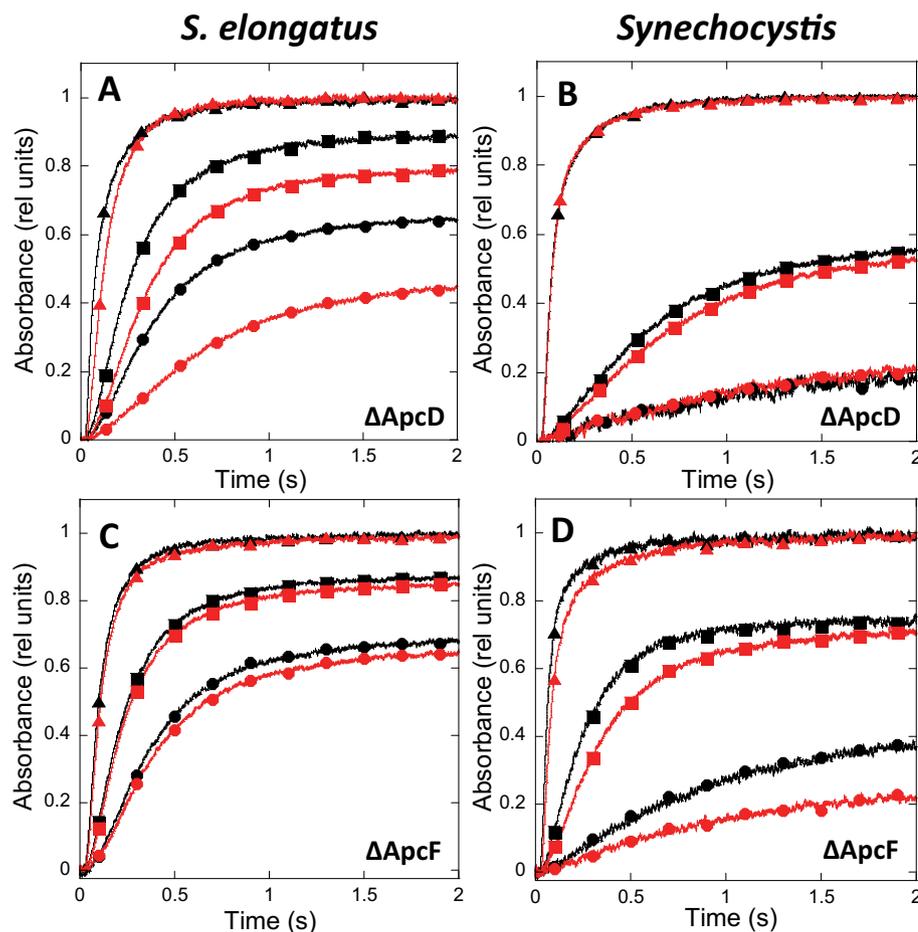


Fig. 4. P700 oxidation absorption kinetics under orange illumination of *S. elongatus* and *Synechocystis* Δ ApcD and Δ ApcF mutants. Whole cells ($2.5 \mu\text{g Chl/mL}$) were dark adapted to state II for 15 min before the measurements. Then the following inhibitors were added subsequently: $15 \mu\text{M DCMU}$, $300 \mu\text{M MV}$ and (after 10 min) $100 \mu\text{M DBMIB}$. Orange actinic light, at an intensity of $50 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ (circles), $100 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ (squares) and $200 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ (triangles) were used for *S. elongatus* (A and C). Regarding *Synechocystis* strains, $5 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ (circles), $7 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ (squares) and $50 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ (triangles) were used when the Δ apcD mutant was tested (B), while $6 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ (circles), $8 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ (squares) and $50 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ (triangles) were used in the case of the Δ apcF mutant (D). WT strains are shown in black and the mutant strains in red. At least three biological independent experiments have been measured and the mean trace for each strain is shown. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1

Initial rates of P700 oxidation kinetics in *S. elongatus* and *Synechocystis* WT, Δ ApcD and Δ ApcF strains under orange light illumination. The rates were determined using a linear fit. The mean and the standard deviation of at least three biological independent experiments are shown. The values are expressed in absorbance (rel units).sec⁻¹. * indicates significant differences (Student's test, $p < 0.05$) between WT and mutant strains, for each strain and in each light condition, respectively. Measurements were performed by pairs (each mutant was measured each time with the WT).

<i>S. elongatus</i>	Light intensity		
	Low	Intermediate	High
WT	1.46 ± 0.05	2.35 ± 0.17	8.99 ± 0.82
Δ apcD	0.94 ± 0.22*	1.87 ± 0.22*	6.30 ± 0.76*
WT	1.70 ± 0.24	2.79 ± 0.13	7.31 ± 0.41
Δ apcF	1.57 ± 0.28	2.77 ± 0.52	6.93 ± 0.93

<i>Synechocystis</i>	Light intensity		
	Low	Intermediate	High
WT	0.75 ± 0.12	1.08 ± 0.08	11.43 ± 3.08
Δ apcD	0.73 ± 0.10	0.92 ± 0.08	10.77 ± 1.19
WT	0.87 ± 0.05	2.62 ± 0.10	13.89 ± 0.03
Δ apcF	0.77 ± 0.04*	1.89 ± 0.01*	9.24 ± 0.37*

[43]), indicating that ApcE is either the major energy donor to PSII or can replace ApcD in this role when ApcD is absent. In agreement with these results, several structural studies showed the interaction of the ApcE-containing trimer with the PSII reaction center and its internal antenna [38,39,53]. In contrast to what happens in the absence of ApcD, the lack of ApcF affects the energy transfer from PBS to PSII in *Synechocystis* (this work) and *Synechococcus* PC7002 [42], but not in *S. elongatus*. Nevertheless, the decrease of energy transfer observed is smaller than that induced by a modification of the bilin-binding Cys in ApcE (Supplementary Fig. S3). In this mutant, the bilin is not attached covalently to the protein and it has an emission at 710 nm instead of at 680 nm [48]. This bilin acts as a competitive trap of the energy absorbed by the PBS. Interestingly, the PBSs having this modified ApcE are still able to transfer energy to PSII with a rather high efficiency, suggesting that in this case energy transfer occurs principally via ApcD. The rate of closure of PSII centers (increase of fluorescence) was largely faster in the ApcE *Synechocystis* mutant than in a mutant in which the PBSs lack the rods (CK strain) (Supplementary Fig. S3).

The lack of ApcF also affects the energy transfer from PBSs to PSI in *Synechocystis* (this work and [44]), but neither in *S. elongatus* (this work), nor in *Synechococcus* PCC 7002 [43]. As already said, in trimer 3 of the basal core cylinders, one β subunit and an α subunit from different dimers are replaced by ApcF and the bilin-binding domain of ApcE, respectively (Fig. 1). The bilins attached to ApcF and ApcE are close (20 Å) and have big influences one on the other. In the Δ ApcF mutant, ApcF is replaced by a β APC subunit. This replacement seems to be very well tolerated in *S. elongatus* PBSs with no changes in energy transfer to any of the photosystems. By contrast, in *Synechocystis* and *Synechococcus* PCC 7002, this change decreases the energy transfer to photosystems, most probably by modifying the interaction between the bilins.

On the contrary, the lack of ApcD affects energy transfer to PSI in *S. elongatus* and *Synechococcus* PCC 7002 but not in *Synechocystis*. This result was unexpected in *Synechocystis* since Liu et al. [39] showed that, in this strain, ApcD can be cross-linked with PSI polypeptides, suggesting its localization on the edge area of PSI. Nevertheless, it has been already suggested that ApcD plays a secondary role (if any) in energy transfer to photosystems in *Synechocystis* [44]. Our results and those of Ashby and Mullineaux do not allow us to discard the possibility that ApcD transfers energy to PSI in WT *Synechocystis*. However, they clearly

demonstrate that in the absence of ApcD, energy transfer to both photosystems is not decreased indicating that ApcE can efficiently transfer the energy absorbed by the PBS to both photosystems. In *S. elongatus* and *Synechococcus* PCC 7002, ApcE can also transfer energy to PSI but less efficiently than ApcD. We can conclude that in different cyanobacteria strains, the role of PBS terminal emitters in energy transfer and/or the structure of the core PBS and/or the interaction PBS/photosystems are different. Thus, the results obtained in one strain cannot be simply extrapolated to all cyanobacteria strains.

3.2. State transitions in WT and ApcD and ApcF mutants of *S. elongatus* and *Synechocystis*

As we already mentioned, it was reported that the lack of ApcD inhibits state transitions in *Synechocystis*, *Synechococcus* PCC 7002 and *Anabaena* PCC 7120 [42,44–46]. In addition, the Δ ApcF *Synechocystis* mutant also seemed to be affected in state transitions [44]. In this work, we studied the effect of the lack of these proteins in *S. elongatus* and *Synechocystis*.

3.2.1. Fluorescence kinetics at room temperature followed by a PAM fluorometer

Fluorescence changes induced by illumination in dark-adapted WT and mutated *S. elongatus* and *Synechocystis* cells were followed at room temperature by a PAM fluorometer. Fig. 5 shows typical traces of fluorescence kinetics in dark-adapted *S. elongatus* (Fig. 5A) and *Synechocystis* (Fig. 5B) cells, successively illuminated by low intensities of blue and orange light. Dark-adapted cells presented a low maximal fluorescence (F_{md}) indicating that cells were in State II. This is typical for cyanobacteria cells in which the PQ pool is reduced in darkness by the respiratory electron transport chain [54]. Upon illumination with blue light, preferentially exciting chlorophyll, a large and rapid increase of F_m' was observed (arriving to a maximal F_{mb}' level), indicating transition to State I. The F_{vb} to F_{vd} ratio (F_v = variable fluorescence = $F_m - F_0$) was around 4.7 in WT *S. elongatus* while being only 1.2 in WT *Synechocystis* cells (Fig. 5, Table 2). Under orange illumination (preferentially exciting phycobiliproteins) of blue-light adapted cells, a decrease of F_m' in both cyanobacterial strains was induced (Fig. 5). In *Synechocystis* cells, the steady state F_{mo} level was similar to the F_{md} level, while in *S. elongatus* the F_{mo} level was higher.

Fig. 5C and E show the variation of fluorescence induced by successive blue and orange illuminations in dark-adapted Δ apcD and Δ apcF *S. elongatus* mutants, respectively. Blue illumination of these cells induced a large and rapid increase of F_m' . Then, illumination by orange light reduced the F_m' value, in a manner equivalent to that observed for WT *S. elongatus* cells. The fluorescence parameters of different independent experiments were averaged and are shown in Table 2. Comparison of the F_{vb} to F_{vd} ratio in the three strains indicates that the absence of ApcD or ApcF did not affect state transitions in *S. elongatus* (Table 2). By contrast, the lack of ApcD completely abolished state transitions in *Synechocystis* (Fig. 5D). The absence of ApcF in the same strain, although it did not completely suppressed state transitions, largely decreased the amplitude of fluorescence changes (Fig. 5F).

3.2.2. State transitions studied by 77 K fluorescence spectra

The 77 K fluorescence emission spectra were measured in WT and mutants cells of *S. elongatus* and *Synechocystis* cells after dark incubation (15 min, State II) and blue light illumination (5 min, State I) (Figs. 6 and 7). When the PBSs were preferentially excited in WT cells (excitation at 590 nm), we observed a large peak at 650–660 nm related to PC and APC fluorescence, a peak at 683 nm related to CP43 and the last emitters of PBS, a peak (or shoulder) at 695 nm corresponding to Reaction Center II and CP47, and finally a peak at 718–725 nm related to PSI fluorescence [55,56]. The PSII-related peaks at 683 and 695 nm were higher in blue-light adapted cells (State I) than in dark-adapted cells (State II), showing the typical fluorescence changes related to state

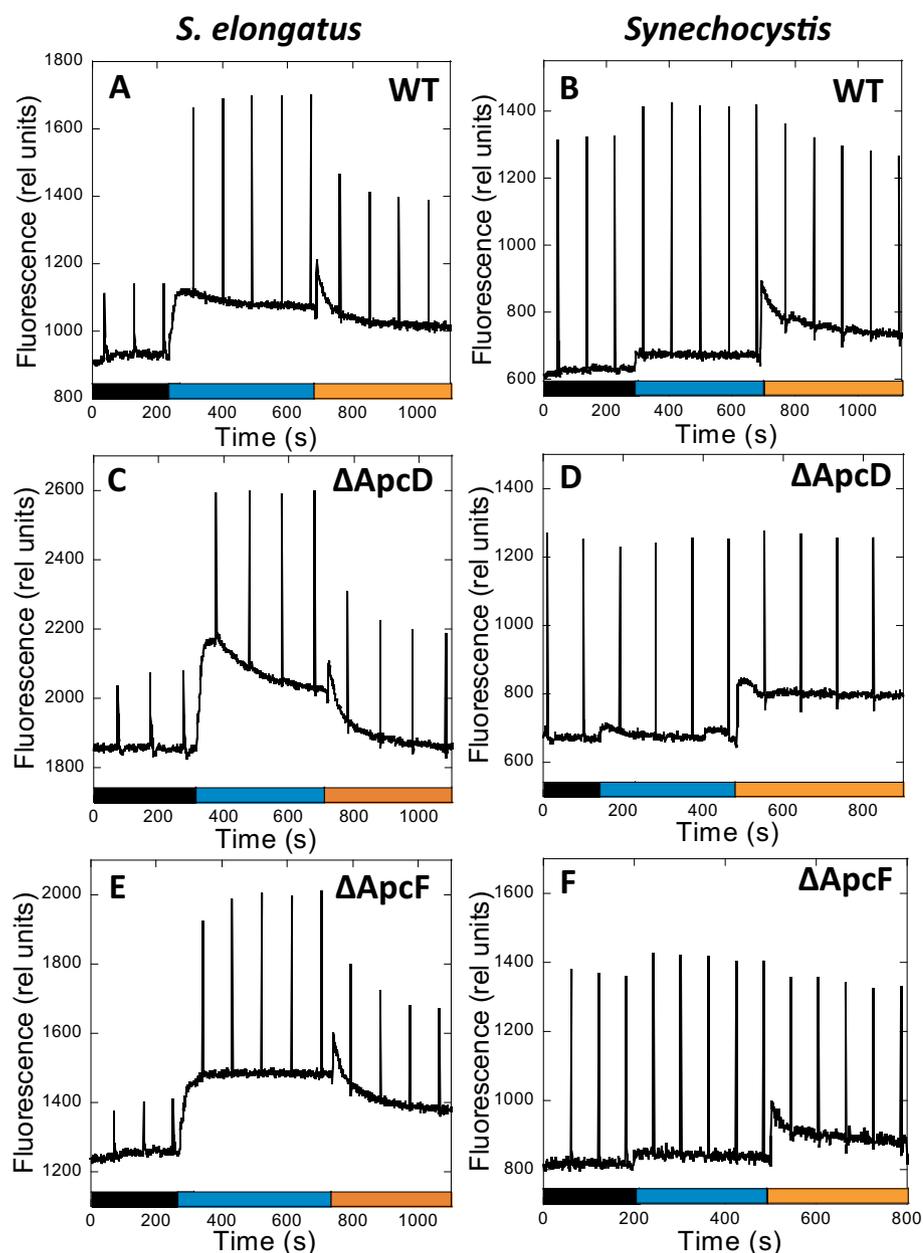


Fig. 5. State transitions in WT and mutants of *S. elongatus* and *Synechocystis*. The fluorescence changes were followed by a PAM fluorometer. Dark adapted cells ($2.5 \mu\text{g Chl/mL}$) were successively illuminated with blue ($85 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) and orange ($20 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) light. Saturating pulses ($400 \text{ ms} \times 1200 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) were applied every 90 s. Typical experiments are shown. (A and B) *S. elongatus* and *Synechocystis* WT, respectively; (C and D) *S. elongatus* and *Synechocystis* ΔapcD , respectively; (E and F) *S. elongatus* and *Synechocystis* ΔapcF , respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 2

Fluorescence parameters of *S. elongatus* and *Synechocystis* obtained from a PAM fluorometer. The meaning of each parameter is defined in Materials and Methods. All the values (\pm SD) are the average of at least 4 biological independent experiments. Values without common letters differ significantly within each strain (Tukey test, $p < 0.05$).

	<i>S. elongatus</i>	<i>Synechocystis</i>
	$F_{\text{vb}}/F_{\text{vd}}$	$F_{\text{vb}}/F_{\text{vd}}$
WT	$4.72 \pm 0.85^{\text{a}}$	$1.24 \pm 0.03^{\text{a}}$
ΔapcD	$4.94 \pm 0.22^{\text{a}}$	$1.01 \pm 0.03^{\text{b}}$
ΔapcF	$5.31 \pm 0.45^{\text{a}}$	$1.12 \pm 0.03^{\text{c}}$

transitions in *S. elongatus* and *Synechocystis*.

When the chlorophyll was preferentially excited (excitation at 430 nm), the PSI emission peak (at 718–725 nm) was the highest one. The PSI fluorescence was similar in dark and blue-light adapted cells. The PSII-related peaks at 683 and 695 nm were higher when cells were incubated under illumination than in darkness. It is worth noting that,

in *S. elongatus*, the peak at 683 nm could be contaminated with some PBS fluorescence, since an emission peak at 660 nm was observed. The relative sizes of the different peaks (related to PBPs, PSII and PSI) were different in *S. elongatus* and *Synechocystis*, which could be due at least partially to differences in PBP to Chl and PSII to PSI ratios. In addition, the differences of 683 and 695 nm emissions between State I and II were much larger in *S. elongatus* than in *Synechocystis*.

When comparing the 77 K fluorescence emission spectra of the WT and mutants cells of *S. elongatus*, some differences were observed (Fig. 6 and Supplementary Fig. S4). When the excitation was done at 590 nm, the peak at 681–683 nm was largely higher in the ΔapcD mutant than in the WT suggesting a partial functional disconnection of PBPs in the mutant. This is in agreement with the observation that there is less energy transfer from PBS to PSI in this mutant.

In the ΔapcF strain, the 660 nm to 683/695 nm emission ratio was higher than in the WT (Fig. 6 and Supplementary Fig. S4), although the ratio PC to Chl was similar in both strains (or even slightly lower in the mutant) (Fig. 2). This suggests that energy transfer from the bulk APC (emission 660 nm) to terminal PBS emitters could be affected in this

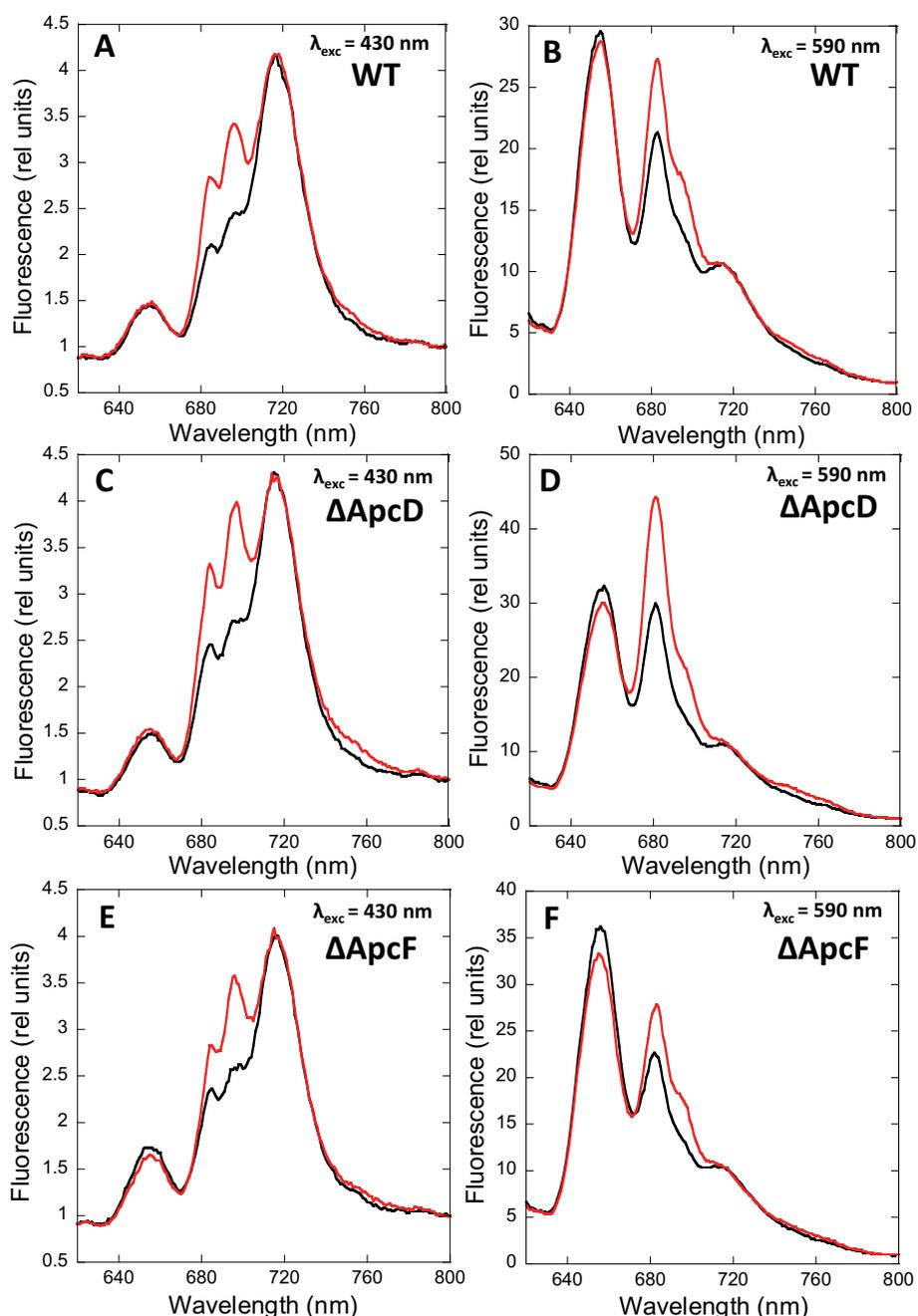


Fig. 6. State transitions in WT and mutants of *S. elongatus*. 77 K fluorescence emission spectra of dark (black, State II) and blue-light (red, 85 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$, State I) adapted WT (A and B), ΔapcD (C and D) and ΔapcF (E and F) cells. The excitation was done at 430 nm (A, C and E) or 590 nm (B, D and F). Normalization was done at 800 nm, and the spectra are the average of at least 3 biological independent experiments. The measurements were done at a chlorophyll concentration of 5 $\mu\text{g/mL}$. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

mutant. Nevertheless, this decrease in energy transfer must be small since alterations of energy transfer from PBS to photosystems were not detected in the ΔApcF strain. The 77 K spectrum of the ΔApcF *Synechocystis* mutant also presented big differences compared to the WT *Synechocystis* spectrum: the 660 nm to 683/695 nm emission ratio was largely larger in the mutant (Fig. 7). This agrees with the affected energy transfer from PBSs to photosystems detected in ΔApcF *Synechocystis* cells.

Both ΔApcD and ΔApcF mutants of *S. elongatus* also presented large differences in 77 K fluorescence emission spectra between dark adapted (State II) and blue-light adapted (State I) conditions (Fig. 6C–F). The emission peaks at 683 and 695 nm (PBS and PSII emission) were largely higher in State I (blue-light illumination) than in State II (darkness), not

only when the cells were excited at 590 nm but also when excited at 430 nm. The emission increase was similar (or even slightly larger in ΔApcD) in the mutants than in the WT, confirming that the absence of ApcD and ApcF does not affect at all state transitions, as shown by the PAM measurements. The PSI emission peaks were similar in both states. It is worth mentioning that PBSs of the WT and ΔapcD *S. elongatus* strains were isolated to confirm the absence of ApcD in the mutant strain. A LC-MS/MS analysis was carried out and no ApcD protein was detected in the ΔApcD *S. elongatus* PBS, while its presence was confirmed in the WT PBS.

By contrast, in ΔApcD and ΔApcF *Synechocystis* mutants, state transitions were largely affected. In ΔApcD *Synechocystis*, the 695 to 720 nm emission ratio was similar in darkness and under blue

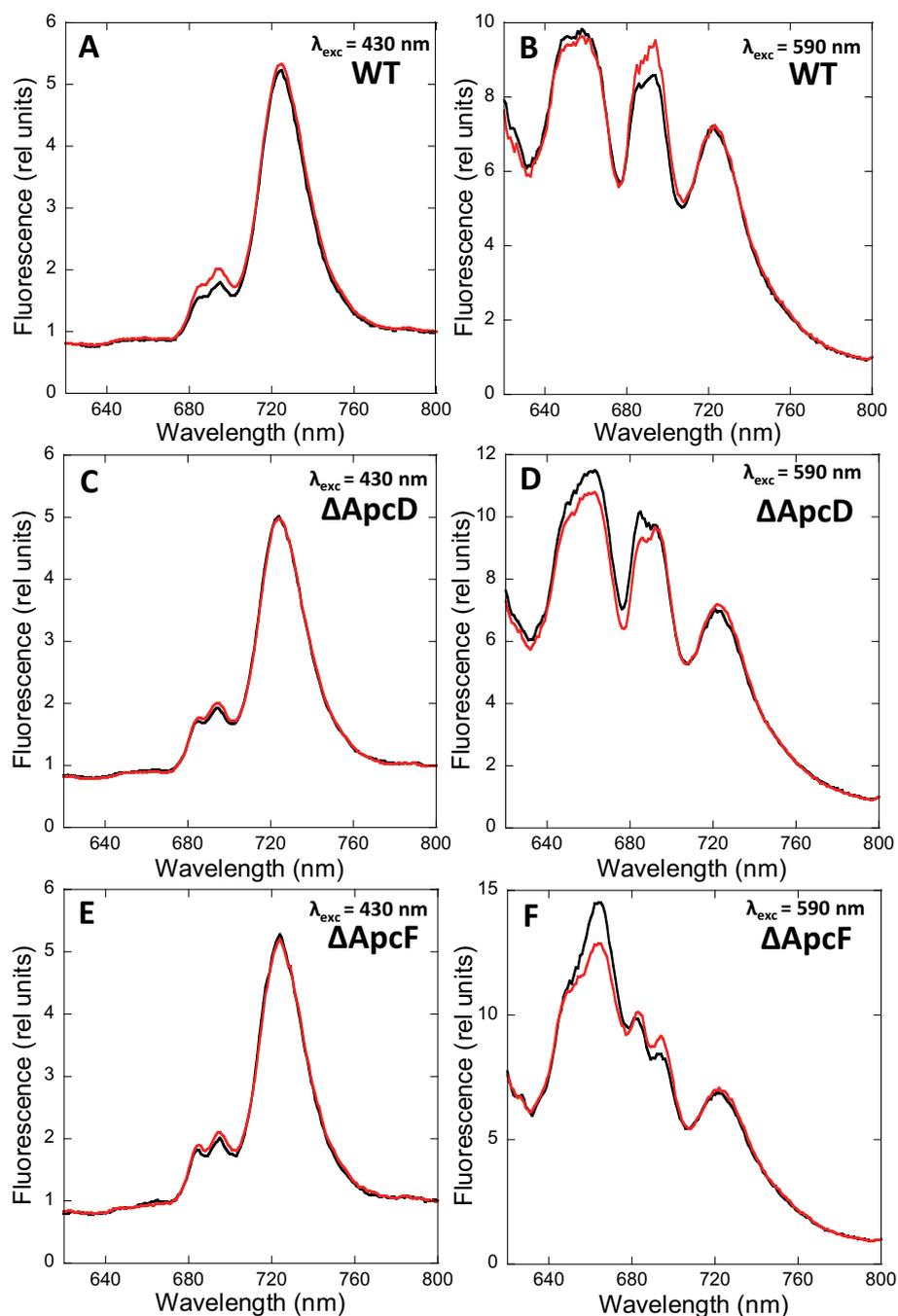


Fig. 7. State transitions in WT and mutants of *Synechocystis*. 77 K fluorescence emission spectra of dark (black, State II) and blue-light (red, $85 \mu\text{mol photons m}^{-2} \text{s}^{-1}$, State I) adapted WT (A and B), ΔapcD (C and D) and ΔapcF (E and F) cells. The excitation was done at 430 nm (A, C and E) or 590 nm (B, D and F). Normalization was done at 800 nm, and the spectra are the average of at least 3 biological independent experiments. The measurements were done at a chlorophyll concentration of $5 \mu\text{g/mL}$. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

illumination indicating complete inhibition of state transitions. In ΔApcF , a slight increase of 695 nm emission was detected but smaller than that observed in the WT indicating partial state transitions inhibition. When ΔApcD and ΔApcF cells were excited at 430 nm, a very small (smaller than in WT) but reproducible fluorescence increase of the 695 nm peak was observed in State I (Fig. 7). This confirms previous observations done by other laboratories [44,46].

From the above results we can hypothesize that PBSs are not or very little involved in *S. elongatus* state transitions, and/or that ApcD has a different role in *S. elongatus*. In the past, it was proposed that the impairment of state transitions in the ΔApcD *Synechocystis* mutant was due to alterations in the relative binding constants between PBSs and

photosystems [44]. In its absence, the PBS would remain attached to PSII and the cells would be permanently in State I as suggested by the higher PSII fluorescence in darkness in the mutant. ApcD is located at the bottom of the PBSs core, together with ApcE in adjacent trimers [57]. The replacement of ApcD with an α -APC subunit, could alter the PBS core structure and/or interaction with the membrane affecting PBS movement and/or detachment/attachment. This could occur in *Synechocystis*, *Synechococcus* PCC 7002 and *Anabaena*, but clearly does not occur in *S. elongatus*.

Other authors proposed that the inhibition of state transitions in the ΔApcD mutant was due to the incapacity of the PBS to transfer energy to PSI [42]. Thus, the PBS could move but the energy transfer to PSI

would not increase. The results obtained in *S. elongatus* do not agree with this proposal. The energy transfer from PBSs to PSI is decreased in *S. elongatus* as it is the case in *Synechococcus* PCC7002 [42]. However, while state transitions are inhibited in *Synechococcus* PCC7002, they are not altered in *S. elongatus*.

The deletion of the *rpaC* gene coding a 9 kDa protein localized in the cytoplasmic side of thylakoids also has different effects in *Synechocystis* and *S. elongatus* cells [58–60]. In *Synechocystis*, the lack of this protein inhibits state transitions [58]. It was suggested that RpaC stabilizes the PBS-PSII interaction [60]. In *S. elongatus*, a large decrease of RpaC did not affect state transitions [61]. In this strain, the *rpaC* gene seems to be essential since complete segregation of the mutant was never obtained [61].

State-transitions related fluorescence changes are also observed when Chl is preferentially excited, showing that state transitions also involve processes that take place in membranes. These processes are not inhibited in any of the strains studied in this work. McConnell et al. [46] also showed that in the Δ ApcD *Synechococcus* sp. PCC 7002 mutant, small fluorescence differences were observed between States I and II when Chl was excited. These Chl changes are larger in *S. elongatus* than in *Synechocystis* and *Synechococcus* PCC 7002, suggesting that the membrane processes involved in state transitions could be more important in *S. elongatus* than in the other strains. Indeed, Ranjbar et al. [32] showed that a large PSII fluorescence quenching (not related to spillover) is involved in *S. elongatus* transition to State II. This quenching was also observed in *Synechocystis* cells, although its amplitude was largely smaller [32].

Using time-resolved fluorescence and streak camera, Chukhutsina et al. [62] showed that in *Synechocystis* WT cells, fluorescence changes are mainly related to PBS uncoupling in State I. A very small PBS uncoupling was also observed in *S. elongatus* [32]. The smaller role of PBSs in *S. elongatus* state transitions could partially explain the fact that this process is not inhibited in the Δ ApcD mutant. Our results strongly suggest that the relative involvement of PBSs and membrane processes in state transitions could vary in different cyanobacterial strains.

4. Conclusions

The lack of ApcD and ApcF affects differently energy transfer and state transitions in different cyanobacteria strains, indicating that conclusions based on results obtained in one cyanobacterial strain cannot be systematically transferred to other strains. ApcD is clearly involved in energy transfer from PBS to PSI in *S. elongatus* and in *Synechococcus* PCC7002. In *Synechocystis*, it is either not involved in energy transfer to any of the photosystems or it can be efficiently replaced by ApcE. By contrast, the lack of ApcF affects energy transfer to both photosystems in *Synechocystis* but not in *S. elongatus*. Surprisingly, the lack of ApcD does not hinder state transitions in *S. elongatus* while it inhibits state transitions in all other studied cyanobacterial strains. This could be explained by a different role of ApcD in the interaction between the PBS and the photosystems and/or a different role of PBSs in *S. elongatus* and *Synechocystis* cells. The contribution of membrane processes could be larger in *S. elongatus* than in *Synechocystis* and other studied strains. In any case, the putative roles of PBSs in state transitions need to be reconsidered.

Author contributions

P.C. performed most of the experiments and analyzed the data; F.M. constructed the cyanobacterial mutants; P.S. designed, performed, analyzed and supervised experiments; D.K. conceived the project, designed and supervised experiments, analyzed data; the article was written by D.K., P.S. and P.C.

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Transparency document

The Transparency document associated this article can be found, in online version.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.bbabi.2019.04.004>.

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