Revisiting cyanobacterial state transitions

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Photosynthetic organisms are exposed to a fluctuating environment in which light intensity and quality change continuously. Specific illumination of either photosystem (PSI or PSII) creates an energy imbalance, leading to the reduction or oxidation of the intersystem electron transport chain. This redox imbalance could trigger the formation of dangerous reactive oxygen species. Cyanobacteria, like plants and algae, have developed a mechanism to re-balance this preferential excitation of either reaction center, called state transitions. State transitions are triggered by changes in the redox state of the membrane-soluble plastoquinone (PQ) pool. In plants and green algae, these changes in redox potential are sensed by Cytochrome b₆f, which interacts with a specific kinase that triggers the movement of the main PSII antenna (the light-harvesting complex II). By contrast, although cyanobacterial state transitions have been studied extensively, there is still no agreement about the molecular mechanism, the PQ redox state sensor and the signaling pathways involved. In this review, we aimed to critically evaluate the results published on cyanobacterial state transitions, and discuss the "new" and "old" models in the subject. The phycobilisome and membrane contributions to this physiological process were addressed and the current hypotheses regarding its signaling transduction pathway were discussed.

1. Introduction

Light is essential for photosynthetic organisms that harvest solar energy and convert it into chemical energy (ATP) and reducing power (NADPH). These molecules are used in the assimilation of CO₂ and synthesis of organic carbon molecules, in a process called photosynthesis, in which molecular oxygen is produced as a sub-product. However, light can become dangerous in certain cases. For example, when the light energy reaching the photosystems exceeds the energy consumption by multiple cellular processes, the entire photo-

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Perspective

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cyanobacterial state transitions, based on the results obtained in the last few years.

2. Following photosystem fluorescence changes during state transitions

State transitions can be studied by fluorescence measurements at room temperature or at 77 K. At room temperature, changes in only PSII fluorescence can be measured, while at 77 K, both PSII and PSI fluorescence can be resolved. The fluorescence emission spectra of cyanobacterial cells depend on the wavelength of the excitation light (Fig. 2).

When cells are excited at 430 nm, which is preferentially absorbed by the Chl antennae, fluorescence emitted by Chl is principally observed. Thus, under these conditions, the changes observed are mainly related to processes occurring in the membrane Chl antennae and reaction centers. When cells are excited by light absorbed mainly by the PBS (550 to 620 nm, depending on the presence or absence of PE), fluorescence emitted by phycobiliproteins, phycobilisome final emitters, and Chl is detected. In this case, the fluorescence changes observed are related to processes occurring in the PBS and the reaction center, and to the energy transfer from PBS to the photosystems. Independently of the excitation light, the peak at 695 nm is related to CP47 and Reaction Center II fluorescence, while the peak at 715–740 nm is related to PSI.47,48 Emissions at 575, 650, and 660 nm, related to PE, PC, and APC, respectively, contribute much more to the 77 K fluorescence spectrum when PBS are excited.49,50 In addition, with PBS excitation, the peak at 685 nm is mainly related to the PBS terminal emitter, with a contribution of CP43 emission.48 In contrast, when Chl is excited, the 685 nm peak is mainly related to CP43 emission.

State I is characterized by a high PSII (685 and 695 nm) to PSI (715–720 nm) fluorescence ratio in the 77 K spectra. Conversely, State II is characterized by a low PSII to PSI fluorescence ratio. These differences are visible when the PBS or the Chl are excited (Fig. 2), suggesting that changes in both PBS and Chl-containing complexes are involved in state transitions.51,52 It has been observed that PSII-related fluorescence is lower in State II compared to that in State I (see Tables 1 and 2). However, only one third of the realized studies showed that PSI-related fluorescence is lower in State I com-
pared to that in State II (see Tables 1 and 2, and discussion about spillover below).

State transitions kinetics can be studied using modulated fluorometers, which record the fluorescence resulting from excitation by non-actinic pulses (non-induced charge separation), and measuring changes in fluorescence yield. The most frequently used fluorometers are PAM101–103, dual-PAM-100, and multicolor PAM. The only fluorometer used in state transition studies, i.e., PAM101–103, uses a detecting light of 650 nm, which is absorbed by both PBS and Chl. Thus, changes in fluorescence yield could be a result of a change in the PBS and/or the antenna-Chl emission, or a change in the excitation energy transfer from PBS to PSII. In multicolor PAM, several detecting pulses with different wavelengths (going from blue to red) can be used. Since fluorescence emission spectra of cyanobacterial cells depend markedly on the excitation wavelength, the contribution of PBS and Chl fluorescence will be different depending on the wavelength of the detecting pulses (see ref. 54 for a detailed explanation). Changes at the PBS level will mainly be observed when orange detecting light (590–630 nm) is used, while Chl emission changes will be more prominent when using blue detecting light. In dual-PAM, red, which is the most frequently used, and blue detecting pulses are also available.

Fig. 2 Following state transitions by fluorescence spectra at 77 K. 77 K fluorescence emission spectra of dark (black, State II) and blue-light (red, 85 µmol photons m⁻² s⁻¹, State I) adapted S. elongatus (A and C) and T. elongatus (B and D) cells. The excitation was done at 430 nm (A and B) and 550 nm (C and D). Normalization was done at the peak of Rhodamine B (570 nm; 0.4 µM) in A and B; and at the peak of fluorescein (560 nm; 0.4 µM) in C and D. When Chls are mainly excited (λ_exc = 430 nm), fluorescence changes are related principally to membrane processes. Meanwhile, when PBSs are mainly excited (λ_exc = 550–620 nm), the observed fluorescence changes are related to changes in energy transfer inside the PBS and from the PBS to the photosystems. The peak at 650–660 nm is related to PC and APC; the peak at 685 nm is related to PBS terminal emitters and CP43; the peak at 695 nm is related to CP47 and RCII and PSI related peaks appear at 715–740 nm. While State I is characterized by a high PSII to PSI fluorescence ratio, State II is characterized by a low PSII to PSI fluorescence ratio. The spectra are the average of at least 3 independent biological replicates.
In PAM fluorometers, three levels of PSII fluorescence can be measured (Fig. 3). The minimal fluorescence level, $F_o$, is determined using only the detecting modulated light in dark-adapted cells. No measurable charge separation is induced under these conditions, and all PSII are open. Strong pulses are applied to close all PSII centers and measure the maximal fluorescence level ($F_m$ – in darkness and $F'_m$ – in light) (Fig. 3). Under continuous illumination, the steady-state fluorescence level ($F_m$) is measured, which depends on the relative PSII and PSI activity influencing the PQ pool redox state. The contributions of PBS and Chl fluorescence in $F_o$ and $F_m$ are different.\textsuperscript{54}

When State II adapted cells are illuminated with far-red light (absorbed by PSI) or low intensities of blue light (principally absorbed by Chl), an increase in all levels of fluorescence yield is observed, which is related to State II to State I transition (Fig. 3). The Chl antenna of PSI is almost three times larger than that of PSII, i.e., 100 and 35 Chl molecules, respectively.$^{55,56}$ Thus, illumination with light principally absorbed by Chl results in a higher activity of PSI compared to that of PSII, and oxidation of the PQ pool occurs.

When State I adapted cells are illuminated with orange or green light (principally absorbed by phycobiliproteins), a decrease in all levels of fluorescence related to State I to State II transition is induced (Fig. 3). Since PBS is the principal antenna of PSII,$^{57}$ this illumination increases PSII activity, resulting in reduction of the PQ pool. However, PSI is still active under orange and green light, because PBS can transfer energy directly to both photosystems.$^{58,59}$ Moreover, PSI can also receive energy from PSII (known as spillover), when the excess energy arriving at PSI is transferred to PSI via the Chl antennae.$^{60-66}$ The percentage of energy arriving at one or the other photosystem depends on the cyanobacterial strain and the environmental conditions.$^{59,67}$

It is worth mentioning that upon illumination of dark-adapted cells with any kind of light (including orange light), PSI is activated and the PQ pool becomes more oxidized. Consequently, as mentioned previously, an increase in fluorescence yield related to State I transition is observed. However, blue light is more effective than orange light in inducing this transition.$^{46}$ Thus, both dark to light and orange to blue transitions induce an increase in fluorescence related to State I. Although majority of published results suggest that the mechanisms involved in these transitions are identical, one publication proposed that while changes in light quality induce only PBS movement, dark-light transition involves changes in concentration of PSI trimers and photosystem movements as well.$^{68}$

In the dark, in contrast to plants and green algae, cyanobacterial cells are in State II.$^{59,70}$ In cyanobacteria, both photosynthesis and respiration take place in the thylakoid membranes, and PQ, Cyt $b_{6}f$ and plastocyanin (or cytochrome $c_{6}$) are common to both electron transport chains (see review$^{70}$). Homologs of mitochondrial Complex I (NDH-1) and Complex II (succinate dehydrogenase, SDH) reduce the PQ pool, and terminal oxidases oxidize it via the respiratory chain.$^{71}$ Since
the PQ pool reduction is generally faster than its oxidation via respiration, the PQ pool is reduced in the dark.59,76 However, due to differences in this redox state balance between cyanobacterial strains, the level of PQ reduction state varies, leading to different levels of State II fluorescence in the dark.52,72 Mutants in the respiratory process also affect state transitions. For instance, NDH-1 and SDH mutants are in State I in the dark,73–76 while terminal oxidase mutants have a more pronounced dark State II.75

The different “levels” of State II in the dark lead to different amplitudes of fluorescence changes during dark to light transitions. In Synechocystis,51,52,61,77–80 Synechococcus PCC 700251,51,81 and Spirulina platensis,68,82,83 these changes are smaller than in S. elongatus or T. elongatus (ref. 52 and Fig. 3). Interestingly, both S. elongatus and T. elongatus lack the protective mechanism OCP-related NPQ.84 Thus, studying cyanobacterial state transitions in S. elongatus could lead to a better understanding of the PBS and membrane contributions to the process, which are further discussed in the following sections.

3. PBS contribution to state transitions

PBS contribution in cyanobacterial state transitions has been largely studied due to the role of LHClI in plant and green alga state transitions. First, it was shown that PBS mutations affect this process in different cyanobacterial strains.40,41,51,85 Then, FRAP experiments showing that PBS are capable of moving along the surface of the thylakoid membranes,86–88 and that the same chemicals that inhibit PBS movement inhibit state transitions,68,83,89 seemed to confirm that PBS movement from one photosystem to the other could be involved in state transitions. However, more recent experiments and results showed that the recovery of fluorescence after PBS bleaching is not only due to PBS diffusion but also to internal processes in this macromolecular complex.90 The role of PBS in cyanobacterial state transitions remains a matter of discussion.

3.1 PBS mutants impaired in state transitions

Mutations in ApcD, ApcE,40,41,51,85 and a particular domain of ApcE44 affect state transitions in several cyanobacterial strains (Table 3). Dong et al. (2009)46 suggested that in the ΔApcD Synechococcus PCC 7002 mutant, the inhibition of state transitions was due to a permanent decrease in energy transfer from PBS to PSI. However, it has recently been shown that a lack of ApcD also decreases the energy transfer from PBS to PSI in S. elongatus, without impairing state transitions.43 Furthermore, in Synechocystis, the lack of ApcD does not decrease energy transfer from PBS to any of the photosystems, but it inhibits state transitions.41,43 In the ApcE-C190S and the PB-loop ApcE Synechocystis mutants, the energy transfer from PBS to the photosystems is significantly affected.33,44 However, state transitions in the former are not impaired,91 while they appear to be decreased in the latter.44

| Article | Species | Changes in PSI fluorescence | Changes in PSII fluorescence | Internal standard used | Conclusions about spill-over |
|---------|---------|----------------------------|-----------------------------|------------------------|-----------------------------|
| Calzadilla et al. (2015)92 | S. elongatus and Synechocystis PCC 6803 | Increase in State I | No change | Rhodamine B | No involvement |
| Ranjbar Choubeh et al. (2018)90 | Synechocystis PCC 6803 | Increase in State I | No change | Rhodamine B | No involvement |
| Ogawa & Sekimoto (2013)74 | Synechocystis PCC 6803 | Increase in State I | No change | Rhodamine B | No involvement |
| Aspinwall et al. | Synechocystis PCC 6803 | Increase in State I | No change | Rhodamine B | No normalization at PSI peak |
| Kondo et al. (2009)107 | Synechocystis PCC 6803 | Increase in State I | No change | Rhodamine B | Spillover |
| Li et al. (2004)83 | Synechococcus PCC 7002 | No change | Increase in State I | Rhodamine B | No normalization at 660 nm |
| Mullineaux and Allen, (1990)46 | Synechococcus PCC 603 | This journal is © The Royal Society of Chemistry and Owner Societies 2020 | | | |
| Salehian and Bruce, (1992)158 | Spirulina platensis | Increase in State I | Increase in State I | Rhodamine B | Spillover |
| Li et al. (2006)91 | Synechococcus PCC 6301 | Increase in State I | Decrease in State I | Rhodamine B | Spillover |

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It has been proposed that the impairment of state transitions in the ΔApcD Synechocystis mutant is due to a permanent binding of PBS to PSII, which increases the PSII antenna even in darkness and PSI illumination (when the PQ pool is reduced). This hypothesis can explain the relatively high fluorescence observed in the dark-adapted ΔApcD Synechocystis cells. In addition, a lack of state transitions in the ΔApcD mutants was observed in Synechocystis, Synechococcus PCC 7002 and Anabaena PCC 7120, but not in S. elongatus (Table 3). These results could be due to the different roles of ApcD in the PBS–photosystem interaction in different strains, which could also explain the different roles of ApcD in energy transfer to photosystems.

As mentioned previously, absence of ApcD decreases energy transfer from PBS to PSI in S. elongatus and Synechococcus PCC 7002, but not in Synechocystis, while the ΔApcF mutation affects energy transfer to PSII and PSI only in Synechocystis.

A mutation in the rpaC gene also affects state transitions in Synechocystis, but not in S. elongatus. This gene, which is strongly conserved among different cyanobacteria, codes for a 9 kDa protein, that has been proposed to be localized on the cytoplasmatic side of the thylakoid membrane. The rpaC Synechocystis mutant was suggested to present a stronger PBS–PSII interaction, like the ΔApcD mutant, impairing state transitions. In contrast, a large decrease in the RpaC concentration did not affect this process in S. elongatus, which might again be due to different PBS–photosystem interaction mechanisms in different strains. A complete segregation of this mutation was not viable in S. elongatus, suggesting an essential role of this gene in this strain.

The different effects of the ApcD and RpaC mutants in S. elongatus and Synechocystis could also be explained by a different PBS contribution to state transitions in these strains. If this is the case, membrane processes could have a more important role in S. elongatus than in Synechocystis. It is worth mentioning that in the ΔrpaC Synechocystis, ΔApcD Synechocystis, Synechococcus 7002 and Anabaena PCC 7120 mutants, only the fluorescence changes related to PBS

Fig. 3 Following state transitions by fluorescence measurements at room temperature. The fluorescence changes of S. elongatus (A), T. elongatus (B) and Synechocystis (C) were followed by a PAM101 fluorometer. Dark adapted cells (Chl concentration 2.5 µg mL^{-1}) were successively illuminated with blue light (85 µmol photons per m² per s) and orange light (20 µmol photons per m² per s). Saturating pulses (400 ms × 12 000 µmol photons per m² per s) were applied each 90 s. The PAM used in the measurements has a 650 nm detecting light, which is absorbed by PBS and Chl. Consequently, the changes in fluorescence yield observed under different conditions can be due to changes in the emission of PBS and/or Chl-antenna, or changes in excitation energy transfer between PBS and PSII. Three levels of fluorescence can be observed: F0, the minimal fluorescence level determined by detecting light in dark-adapted cells; Fm, Fm′ or Fm”, the maximal fluorescence observed when saturating pulses are applied to the cells under darkness or light conditions, respectively; Fv/Fm: steady-state fluorescence under continuous illumination. In the dark, cyanobacteria cells are in State II due to reduction of the PQ pool by respiration. Upon illumination of dark adapted cells with blue light (mainly absorbed by Chl), PSI is activated and the PQ pool becomes more oxidized, leading to State I. When State I adapted cells are illuminated with orange light (principally absorbed by phycobiliproteins), PSII activity is increased, resulting in reduction of the PQ pool. Thus, a decrease of Fm related to State I to State II transition is observed.
Table 3
Alterations in state transitions and energy transfer in PBS mutants

| Species                  | Mutation | State transitions $\lambda_{exc}$ PBS | State transitions $\lambda_{exc}$ Chl | Energy transfer PSII | Energy transfer PSI | Effect on PBS | Effect on PSI |
|--------------------------|----------|--------------------------------------|--------------------------------------|---------------------|-------------------|--------------|--------------|
| *Synechocystis* PCC 6803 | ApcD, ApcF | Inhibited in Synechocystis $\Delta$ApcD, $\Delta$ApcF | Impaired in Synechocystis $\Delta$ApcD, $\Delta$ApcF | Aффected in Synechocystis $\Delta$ApcD, $\Delta$ApcF | Aффected in Synechocystis $\Delta$ApcD, $\Delta$ApcF | Not affected | Not affected |
| *Synechocystis* PCC 6803 | ApcD, ApcF | Inhibited in Synechocystis $\Delta$ApcD, $\Delta$ApcF | Impaired in Synechocystis $\Delta$ApcD, $\Delta$ApcF | Aффected in Synechocystis $\Delta$ApcD, $\Delta$ApcF | Aффected in Synechocystis $\Delta$ApcD, $\Delta$ApcF | Not affected | Not affected |
| *S. elongatus*            |          |                                      |                                      |                     |                   |              |              |
| *Synechocystis* PCC 6803 | ApcD, ApcF | Inhibited in Synechocystis $\Delta$ApcD, $\Delta$ApcF | Impaired in Synechocystis $\Delta$ApcD, $\Delta$ApcF | Aффected in Synechocystis $\Delta$ApcD, $\Delta$ApcF | Aффected in Synechocystis $\Delta$ApcD, $\Delta$ApcF | Not affected | Not affected |
| *S. elongatus*            |          |                                      |                                      |                     |                   |              |              |
| *Synechocystis* PCC 6803 | ApcD, ApcF | Inhibited in Synechocystis $\Delta$ApcD, $\Delta$ApcF | Impaired in Synechocystis $\Delta$ApcD, $\Delta$ApcF | Aффected in Synechocystis $\Delta$ApcD, $\Delta$ApcF | Aффected in Synechocystis $\Delta$ApcD, $\Delta$ApcF | Not affected | Not affected |

3.2 PBS long distance movement versus PBS detachment and small changes in PBS–photosystems interaction

The possible diffusion of PBS along the thylakoid membrane was first showed by fluorescence recovery after photobleaching (FRAP) experiments in *Dactylococcopsis salina* and *S. elongatus*, showing that PBS–photosystem association is a dynamic process which depends on weak interactions with membrane lipids. Based on FRAP experiments and to the possible association of PBSs with PSI and PSII, the hypothesis of a light-harvesting complex moving and modulating the energy reaching each photosystem, analogous to plant and green algae state transitions, has frequently been proposed (Fig. 4). Within this hypothesis, it was suggested that in State II, the low PSII fluorescence is related to a smaller antenna, since most of the PBS are connected to PSI, while in State I, the high PSI fluorescence is related to a bigger PSII antenna, due to the migration of PBS from PSI to PSII. More recently, it was demonstrated by single molecule spectroscopy that PBS can lose (dark state) and recover their fluorescence spontaneously (blinking). Under high light intensities, the “dark state” is formed more often and the PBSs remain longer time in this state. Then, in darkness the PBSs recover their fluorescence. Because this blinking also affects FRAP experiments, the movement of PBS could be largely slower than it was supposed in the first studies.

Another argument for the involvement of PBS movement in state transitions was the fact that PBS mobility is inhibited by high osmotic buffers, which also inhibit state transitions in *S. elongatus* and *Spirulina platensis*. Incubation of cyanobacterial cells with phosphate (>0.5 M), betaine (1 M) or sucrose (1 M) suppresses the fluorescence changes related to state transitions observed at 77 K when PBS are excited ($\lambda_{exc}$ ≈ 590 nm). These results seemed to support the hypothesis that the movement of PBS was the principal mechanism involved in cyanobacterial state transitions. However, it was shown that these hyper-osmotic buffers also impair membrane processes, inhibiting the changes observed in 77 K fluorescence spectra when Chl is excited ($\lambda_{exc}$ ≈ 430). In
addition, these chemical were also shown to inhibit quenching processes in the PBS. Thus, it is not possible to conclude from these experiments that PBS long distance movements are involved in state transitions.

It was also shown that PSI oligomerization affects both state transitions and PBS diffusion. In Δpsal strains of *Synechococcus* 7002 and *S. elongatus*, PSI trimerization is impaired, and State II to State I transition is faster than in the WT. In the *S. elongatus* mutant, it was also shown that the PBS diffusion rate was increased. Although Aspinwall et al. (2004) ascribed the faster kinetics of state transitions to the increase in PBS diffusion rate, the effect of differing distribution of protein complexes in the membrane cannot be discarded.

It has recently been shown that cyanobacterial thylakoid membranes are organized in a mosaic-like structure defined by domains. Three types of domains were described, the first domain was enriched mainly by PSI, the second by PSII and PBS, and in the third domain, the abundance of PSII, PSI and PBS was balanced. These microdomains seem to be very stable and not change with light conditions. The fact that macrocomplexes containing PSII–PSI–PBS were isolated, most probably from the third type of microdomain, suggests that very small position modification of the PBS (and/or photosystems) could induce changes in energy transfer from PBS to one or other photosystem. Thus, small PBS movement could be involved in state transitions. However, although the heterogeneous organization of cyanobacterial thylakoid membranes have been shown in several works, a conclusive model especially concerning the stability of the domains is still not available.

As an alternative to the long distance PBS movement model, recent publications have associated the high PSII fluorescence in State I to PBS detachment from thylakoid membranes (Fig. 4). Kaña et al. (2012) studied chlorophyll fluorescence induction curves and spectrally resolved fluorescence induction (SRFI) in different cyanobacterial strains, and proposed that transition from State II to State I involves PBS decoupling from PSII. Furthermore, Chukhutsina et al. (2015) and Ranjbar Choubeh et al. (2018) demonstrated, by measurements of fast time-resolved fluorescence, that the duration of PBS fluorescence was higher in State I than in State II, correlating with a small population of functionally disconnected PBS. However, in *Synechocystis*, PBS decoupling was estimated to be around 13% of the total PBS pool, once again questioning the relevance of this light-harvesting complex in cyanobacterial state transitions.

**Fig. 4** Different models of phycobilisome contribution to cyanobacterial state transitions. (A) PBS-mobile model: During state transitions some PBSs move from one photosystem to the other changing the antenna size of both photosystems. In State I are more attached to PSII (high PSII fluorescence) and in State II to PSI (high PSI fluorescence); (B) PBS detachment model: the PBSs detach from both photosystems or from one of them in State I. The high “PSII” fluorescence in State I is related to the longer fluorescence life of detached PBSs.
As mentioned earlier, PBS contribution to state transitions can be observed by 77 K fluorescence emission spectra. All authors have reported an increase in PSII fluorescence (685 and 695 nm) during transition from State II to State I, on preferential excitation of the PBS ($\lambda_{exc} \approx 590$) (Table 1). Conversely, for PSI fluorescence (715–740 nm), some authors have reported no changes between State I and State II, while others have observed a decrease in its fluorescence during State I to State II transition, and still others have reported an increase during this transition (Table 1). The variability in the results can be ascribed at least partially to the different cyanobacterial strains studied. However different results were obtained with the same strains. Thus, different growth conditions and lights used to trigger state transitions could influence the obtained results. In addition, the normalization method employed in each case could significantly affect the interpretation of these results. In most cases, the fluorescence emission spectra were normalized within the spectra themselves (to PC, APC, PSII or even PSI fluorescence peaks) (Table 1), hindering the analysis of the fluorescence changes observed. For instance, some authors did not observe changes in PSI fluorescence between State II and State I in Synechocystis, but the PSI peak was used for normalization.74,105,107 In addition, several studies cited in Table 1 normalized their results to the APC peak, which was also found to change during state transitions.108

In only two of the papers mentioned in Table 1,65,106 the fluorescence changes were normalized to an internal standard. When these chemicals, such as fluorescein or rhodamine B,65,106 are used, the fluorescence emission values are normalized to a wavelength outside the spectrum, allowing a better calibration of the fluorescence changes observed. Similar results can be obtained when normalization is done to wavelengths outside the spectrum (such as 800 nm), even in the absence of internal standards.52 In summary, the normalization of 77 K fluorescence emission spectra needs to be correctly addressed in order to facilitate the understanding of the PBS contribution to cyanobacterial state transitions. This subject is further discussed in section 4, where the contribution of membrane fluorescence to this physiological process is analyzed.

As a conclusion, although the analysis of PBS fluorescence changes during state transitions has been largely addressed in different cyanobacterial strains, it is still unclear how PBS participates in this process. As it is depicted in Table 1, the use of different experimental procedures to analyze the PBS-fluorescence related changes, hinders the possibility of finding general mechanisms for cyanobacterial state transitions. Nevertheless, based on the more recent results and our own interpretation of them, we propose that long distance movement of PBS is not involved in state transitions. Moreover, changes in the relative interaction of PBS and photosystems (involving slight PBS position changes) are most probably not involved neither. We suggest that only partial PBS detachment from one or both photosystems could contribute to the increase of PSII fluorescence in State I.

4. Membrane processes contribution to state transitions: spillover versus PSII quenching

Membrane participation in cyanobacterial state transitions was first proposed by Bruce et al. (1985) in the cyanobacteria Anacystis nidulans.109 The involvement of the membrane was also suggested in Synechocystis cells, due to the changes in the thylakoid membrane topology of PSII distribution between the two states.61,110,111 In this cyanobacterium, a row disposition was observed for PSI in State I, and a random distribution (favoring spillover) was observed in State II. Further evidence supporting the involvement of the movement of membrane complexes, or changes in the oligomerization state of these complexes was presented by El-Bissati et al. (2000),65 who demonstrated that this process is affected by alterations in membrane fluidity. However, more recently, Maksimov et al. (2017)112 have suggested that the rate of oxidation of the PQ pool depends on membrane fluidity, and therefore on temperature. Thus, the changes observed by Bissati et al. could have been related to slower kinetics of reduction and oxidation of the PQ pool in less fluid membranes.65 Finally, state transitions were also observed in mutants of Synechocystis and Synechococcus 7002 lacking PBS.83,113

It was first proposed that the rearrangement of photosystem positions in the membrane induced changes in spillover (energy transfer from PSII to PSI) during state transitions.61,109 However, although changes in spillover have long been studied (Fig. 5),51,61,77 there are no clear results confirming the participation of this phenomenon in cyanobacterial state transitions. A key evidence supporting the spillover model would be a decrease in PSI fluorescence at 77 K (when Chl is preferentially excited) during State II to State I transition (when photosystems move apart), concomitant with an increase in PSI fluorescence under the same conditions. However, most studies on spillover did not show this result (Table 2). Of the articles listed in Table 2, only one work realized by Bruce et al. (1989) in Synechococcus PCC 7002,81 and two studies on Spirulina platensis,58,81 showed an apparent increase in PSI fluorescence in State II, which can be considered as an evidence for spillover. However, none of these works analyzed the 77 K steady-state fluorescence emission spectra using internal standards (such as fluorescein or rhodamine B). The utilization of these standards allows the study of the absolute variation in the different fluorescence contributions.52,79,80,106 Interestingly, when these internal standards were used, authors lead to the conclusion that due to the constant level of PSI emission between State I and II, spillover is an unlikely mechanism of cyanobacterial state transitions.52,80,106 Moreover, a recent study employing a streak camera to measure fast (ps to ms) fluorescence decays, strongly suggested that at least in S. elongatus (and most probably in Synechocystis) changes in spillover are not involved in state transitions.106 These authors measured fast fluorescence decay kinetics (at 77 K) of cells adapted to State I and II, and subsequently obtained the
decay-associated spectra (DAS) after global analysis of the data. When 430 nm excitation wavelengths were used, PSII emission decreased in State II, however, the DAS showed that PSI emission was similar in State I and State II.

Ranjbar Choubeh et al. (2018) recently proposed that the decrease in PSII fluorescence is related to a reversible PSII-quenching in State II (Fig. 5), at least in *S. elongatus* and *Synechocystis* cells. This quenching has been suggested to take place at the level of PSII, and is not related to the NPQ mechanism described for cyanobacteria, which occurs in the PBS. It is worth noting that higher PSII quenching should lead to extra heat emission in State II. However, optoacoustic studies did not show differences between the heat production in States I and II. As previously discussed, Van Amerongen et al. showed that in both *S. elongatus* and *Synechocystis*, a partial disconnection of PBS occurs in State I, which should increase the conversion of excitation energy into heat. This increase in heat could be adventitiously similar to the heat increase due to PSII quenching in State II, which might explain the absence of heat emission changes during state transitions. Overall, the results of Ranjbar Choubeh et al. (2018) demonstrated that PSI is not quenched by PSI in State II in *S. elongatus*. The quenching mechanism related to PSII remains to be elucidated.

Stadnichuk et al. (2009) also suggested that state transitions do not involve PSI, and could be completely attributed to PSII. In their study, fluorescence emission changes at 77 K were observed for PSII, but not for PSI, when Chl were excited in *Synechocystis* cells. Based on these results, they suggested that spillover is an unlikely mechanism implicated in cyanobacterial state transitions. These observations were made in WT, CK (mutant without PC) and PAL (mutant depleted in PBS) strains, leading to the conclusion that thylakoid membranes participate in state transitions, and their contribution is not negligible.

5. The signal transduction pathways and the role of Cyt *b*_{6}f

Apart from the mechanism involved in cyanobacterial state transitions, the signal transduction pathways leading to State I/II transition remain to be deciphered. It is well established that changes in the redox state of the PQ pool trigger state transitions in cyanobacteria, like in plants and green algae. While a reduced PQ pool induces State II transition, State I transition is induced by its oxidation. As previously discussed, in the dark, cyanobacterial cells are in State II due to PQ pool reduction via respiration. In addition,
chemochemicals oxidizing or reducing the PQ pool also trigger state transitions. Among these chemicals, some quinones, such as p-benzoquinone (PBQ) or 2,6-dimethoxy-1,4-benzoquinone (DBMIB), induce State I transition through oxidation of the PQ pool in dark-adapted cells. In contrast, addition of 2,5-dibromo-3-methyl-6-isopropyl-p-benzoquinone (DBMIB) induces State I to State II transition even under blue-light illumination. DBMIB binds close to the [2Fe–2S] cluster of the Cyt b6f and blocks photosynthetic and respiratory electron transport, inhibiting the re-oxidation of the PQ pool by the complex. Thus, the PQ pool is reduced in its presence.

There are two DBMIB binding sites in Cyt b6f, one with high affinity and the other with low affinity. The high affinity binding site is located at the periphery of the complex, outside the Qo pocket. At low concentrations (0.5–1 µM), DBMIB binds only to the high affinity site, and in darkness, it does not inhibit electron transport in vivo, as reported in Synechococcus 7002. For inhibition of electron transport, the cells must be illuminated to induce the migration of DBMIB from its peripheral position to the low affinity site (close to the [2Fe–2S] cluster). However, at higher concentrations (>2 µM), DBMIB fully inhibits Cyt b re-reduction also in darkness.

Given the relationship between the redox state of the PQ pool and state transitions in both cyanobacteria and plants, and the involvement of Cyt b6f in the signal transduction pathway of plants and algae, the participation of this complex in cyanobacterial state transitions has also been hypothesized. Results suggesting the participation of phosphorylation reactions in this signal transduction pathway strengthened this idea. Hence, Mao et al. (2002) and Huang et al. (2003) proposed Cyt b6f as the redox sensor of the PQ pool in cyanobacterial state transitions. They based their conclusions on the effect of DBMIB on PBQ or DMBQ poisoned cells, suggesting that DBMIB induces transition to State II by binding to the Cyt b6f complex, even when the PQ pool remains oxidized in the presence of the aforementioned quinones. However, they did not measure the redox state of the pool under these conditions.

It has recently been shown, using chlorophyll fluorescence induction curves, that the PQ pool is rapidly reduced by addition of DBMIB, even in the presence of DMBQ.

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**Fig. 6** Effect of different chemicals on the redox state of the PQ pool in cyanobacteria. Cyanobacterial state transitions is triggered by changes in the redox state of the PQ pool. While accumulation of oxidized PQ induces State I transition, increase in the concentration of its reduced form (PQH2) leads to State II. Different chemicals affect the redox state of PQ pool. The quinones p-benzoquinone (PBQ) and 2,6-dimethoxy-1,4-benzoquinone (DBMIB) induces State II transition. DBMIB inhibits Cyt b6f re-oxidation of the PQ pool, and as a consequence PQH2 is accumulated. N,N,N'-Tetramethyl-p-phenylenediamine (TMPD) is capable of oxidizing the PQ pool under blue light illumination, leading to State I, even in the presence of DBMIB. In the figure, the arrows indicate the direction of electron transport between different protein complexes/chemical compounds, and its size is proportional to the quantity of electrons transfer under different conditions. Orange and blue arrows indicate electron transfer under orange or blue light illumination, respectively. Black arrows indicate electron transfer occurring under dark and light conditions. Dashed arrows indicate electrons taken/given by the added chemicals. Protein complexes: PSI, photosystem II; PSII, photosystem I; PQ, plastoquinone pool; Cyt b6f, cytochrome b6f. Chemical compounds: DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethyleurea; DMBQ, 2,6-dimethoxy-1,4-benzoquinone; DBMIB, 2,5-dibromo-3-methyl-6-isopropyl-p-benzoquinone; TMPD, N,N,N',N'-tetramethyl-p-phenylenediamine; PBQ, p-benzoquinone.
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Moreover, the same concentration of DBMIB has different effects on state transitions under light-induced or DMBQ-induced State I-adapted cells. The concentration of DBMIB used were higher than 2 µM. These experiments strongly suggest that DBMIB induces State II transition due to the reduction of the PQ pool, and not due to its binding to Cyt b6f. In another set of experiments, the same authors showed that it was possible to induce State I transition when illuminating DBMIB-poisoned cells, using N,N,N′-tetramethyl-p-phenylene-diamine (TMPD).

This chemical was shown to restore photosynthetic electron transport, even when Cyt b6f was inhibited by DBMIB. Thus, the oxidation of the PQ pool triggers State I transition, through a signal transduction pathway that does not involve Cyt b6f.

It has also been proposed that the Chl a molecule of the Cyt b6f complex is the signal transmitter in cyanobacterial state transitions. In this model, the Chl a senses the changes in the Qo site, and participates in this signal transduction pathway by altering its volume. This hypothesis proposed by Vladkova (2016) was based on the analysis of various Cyt b6f X-ray structures, differing in the volume of Chl a. Changes in the Chl a volume, along with changes in the redox states of the PQ pool, would affect the hydrophobic thickness of Cyt b6f, which would induce a hydrophobic mismatch in the membrane. This hydrophobic mismatch is the difference between the hydrophobic thickness of the protein and the hydrophobic thickness of the lipid bilayer, and would be the driving force behind membrane reorganization during the progression of state transitions.

If the Chl a volume is the signal transmitter in cyanobacterial state transitions, it would be expected that inhibition of Cyt b6f would have an irreversible effect on this process. The volume of Chl a must not change in DBMIB-poisoned cells, however, addition of TMPD was found to induce state transitions. This suggests that the involvement of this Chl a molecule is also unlikely. It is worth mentioning that under the conditions tested by Calzadilla et al. (2019) (DBMIB concentration >2 µM), the two DBMIB binding sites in Cyt b6f were occupied, fully inhibiting the complex and photosynthetic electron transport.

The possible participation of phosphorylation reactions in cyanobacterial state transitions has also been studied. First reports showing a correlation between changes in thylakoid phosphorylation patterns, PQ pool redox changes and state transitions came from studies conducted on Synechococcus 6301. A model was proposed, in which protein phosphorylation and PBS migration were requisites for State I/II transition (ref. 121, see review). However, none of these studies showed a direct connection between the changes in phosphorylation and state transitions. Moreover, the results of Roberts et al. (1984) suggest that state transitions in organisms with PBS do not involve reversible protein phosphorylation. This has been discussed in detail by Allen (1992).

In more recent works, it has been observed that PBS and photosystem proteins can undergo phosphorylation, indicating the participation of these reactions in cyanobacterial state transitions. However, only Chen et al. (2015) directly addressed this possible relationship. These authors showed that four residues of the β subunits of PC (Ser22, Ser49, Thr94 and Ser154) were phosphorylated. Mutation of these amino acids avoiding phosphorylation seemed to affect state transitions. A slower and smaller increase of fluorescence was observed upon illumination of dark-adapted mutant cells. However, these cells were not extensively characterized, for instance, alterations in the energy transfer within the PBS were suggested, which might affect PBS concentration and/or PSI/PSI ratio between strains. Moreover, the dark PQ pool redox state was not measured; in addition, the differences between mutants cannot be ignored. Consequently, the observed impairment in state transitions could not conclusively be linked to the lack of PBS phosphorylation.

Ser/Thr kinases have been widely described as key factors in the signal transduction pathways of cyanobacteria (ref. 131 and 132, see review). Synechocystis presents 12 genes coding for Ser/Thr kinases (Spk), of which seven are classified in the PKN2 subfamily (spkA to spkG), and the rest have been assigned to the ABC1 subfamily (spkH to spkL). These kinases have been shown to participate in different cell functions, such as cell motility, oxidative stress response, salt and low temperature stress, and regulation of carbon and nitrogen metabolism. In addition, the GroES chaperone protein and ferredoxin were also reported to be targets of some Spk.

As previously mentioned, Chen et al. (2015) suggested the possible participation of Ser/Thr kinases in cyanobacterial state transitions, like in plants and green algae. Recently, Calzadilla et al. (2019) addressed this issue by constructing single mutants for all the Ser/Thr kinases and phosphatases identified in Synechocystis. None of them were impaired in state transitions. In addition, experiments with kinase (Staurosorpin and K252a) and phosphatase inhibitors (NaF and Na3VO4) were also performed. Incubation with these chemicals had no effect on state transitions in Synechocystis and S. elongatus, although they affected other processes in the cells. Thus, these results lead to the conclusion that phosphorylation reactions are not essential in cyanobacterial state transitions.

6. Conclusions and perspectives

Despite the large number of studies conducted on cyanobacterial state transitions, the mechanism behind this physiological process has not yet been determined. Four different mechanisms (PBS movement, PBS detachment, spillover and PSI quenching), and combinations of them, were proposed to be related to the changes of fluorescence observed when PSI or PSII is specifically illuminated. Moreover, different models were proposed for the same cyanobacteria strain (Table 4).

Nevertheless, considering the literature included in the present review, it is possible to make some conclusions about the molecular mechanism and the signal transduction path-
ways involved. Our proposed model is described in Fig. 7. First, it is clear that cyanobacterial state transitions cannot be reduced to changes in the energy transfer from PBS to the photosystems, as was proposed several years ago. Although it was demonstrated that PBS could move on the thylakoid surface, there is no direct evidence showing that this movement has a physiological role in state transitions. Furthermore, recent results suggest that only a partial detachment of PBS from one or both photosystems is involved in state transitions. However, we cannot totally discard small changes in their position favoring a better energy transfer to one or other photosystem, at least in some cyanobacteria strains. Finally, PBS contribution seems to depend on the cyanobacterial strain and growth conditions.

In contrast, membrane processes seem to be an intrinsic part of cyanobacterial state transitions. Chl fluorescence changes can be ascribed to the proposed PSI-template, and/or changes in spillover involvement. The different membrane topologies observed in State I and State II, favoring or hindering direct energy transfer from PSII to PSI, supported changes in spillover during state transitions. However, recent studies employing a streak camera to measure fast (ps to ms) fluorescence decays, strongly suggested that changes in spillover are not involved in state transitions.106

Thus, many questions remain to be answered: How changes in membrane topology participate in cyanobacterial state transitions? Do these membrane changes also affect PBS energy transfer to photosystems? What is the mechanism involved in PSII-specific quenching? Regarding this last question, it has recently been shown that the thermal phase of the O–J–I–P curve can be ascribed to a light-induced conformational change in PSII, triggered by saturating multiple turnover flashes (ref. 147–149, see review150). This increase in the fluorescence yield of Chl a is temperature dependent,147 and its kinetics depends on the length, rather than the intensity, of the flashes.148 This increase was further found to be related with changes in the interaction between the PSI reaction center and the antenna proteins CP43 and CP47.149,151 Although it has been established that this phenomenon is not related with state transitions, because it takes place at the ms scale and can relax and be regenerated without changes in the redox state of the PQ pool,147 similar PSII conformational changes can be associated with the PSI-specific quenching suggested by Ranjbar Choubeh et al. (2018).106 If the relevance of this quenching is confirmed, the definition of cyanobacterial state transitions as a re-balance of the excitation energy reaching the photosystems needs to be reconsidered.

The different contributions of PBS and membrane processes in cyanobacterial state transitions seem to be dependent on the cyanobacterial strain. Hence, comparing the same processes in different strains would lead to more conclusive results regarding state transitions. Along these lines, the utilization of *S. elongatus* and *T. elongatus* as model organisms can facilitate this work, due to the larger fluorescence differences observed between State I and II, compared to the other organisms used in the past (such as *Synechocystis* or *Synechococcus* 7002). It is also worth mentioning that state transitions could be dependent on growth conditions of the cultured strains. If this is the case, analyzing the fluorescence changes of cells acclimated to different conditions would allow a better understanding of the relative contributions of PBS and membrane processes to state transitions.

In case of the signal transduction pathways, showing that Cyt b$_{56}$ and phosphorylation reactions are not involved in cyanobacterial state transitions was a step forward. However, how changes in the redox state of the PQ pool could be transmitted to the PBS and/or photosystems remains to be discovered. If we consider that one of the main fluorescence changes between State I and II might come from the membrane contribution, and might involve a PSII-specific quenching, one tempting hypothesis would be that PSI itself is able to sense the redox state of the PQ pool. Accumulation of Q$_{a}$ cannot be this redox sensor, because while its accumulation due to 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) addition induces State I, its accumulation due to the reduction of the PQ pool induces State II.

| Organism | Model proposed | References |
|----------|----------------|------------|
| *S. elongatus* | PBS-mobile | Sarcina *et al.* (2001)87; Joshua and Mullineaux (2004)95; Aspinwall *et al.* (2004)99; Joshua and Mullineaux (2005)92; Ranjbar Choubeh et al. (2018)106 |
| | PBS-detachment | Ranjbar Choubeh et al. (2018)106 |
| | PSII-quenching | Ranjbar Choubeh et al. (2018)106 |
| *Synechocystis PCC 6803* | PBS-mobile | Ashby and Mullineaux (1999)14; Joshua and Mullineaux (2005)92 |
| | PBS-mobile/spillover | McConnell *et al.* (2002)51 |
| | Spillover | Chukhutsina *et al.* (2015)79; Kaňa *et al.* (2012)105; Ranjbar Choubeh *et al.* (2018)106 |
| | PSII-quenching | Olive *et al.* (1997)81; Folea *et al.* (2008)111; El-Bissati *et al.* (2000)83; Vernotte *et al.* (1990)77 |
| | | Ranjbar Choubeh *et al.* (2018)106; Stadnichuk *et al.* (2009)80 |
| *Synechococcus PCC 7002* | PBS-mobile | Dong *et al.* (2009)90 |
| | PBS-mobile/spillover | McConnell *et al.* (2002)51 |
| | Spillover | Bruce *et al.* (1989)81 |
| *Spirulina platensis* | PBS-mobile/spillover | Li *et al.* (2006)86; Li *et al.* (2004)83 |
| *Dactylococcopsis salina* | PBS-mobile | Mullineaux *et al.* (1997)80 |
It was suggested that a Qc channel between Cyt b559 and PsbJ could exist.\textsuperscript{152} This channel, which is connected to the Qb site, has been suggested to have a quinone-binding function, and can participate in PSII regulation. Thus, a possible hypothesis would be to consider the Qc hydrophobic tunnel as the redox sensor of the PQ pool. However, the existence of this Qc site is still unclear, since its presence was not confirmed in the most recent high resolution PSII structure model.\textsuperscript{56} Notwithstanding, it was shown that mutations in Cyt b559, PsbJ, and near the putative Qc site, can alter the kinetics and amplitude of state transitions in \textit{Synechocystis} cells.\textsuperscript{153} These results present an interesting starting point for further studies, although the participation of novel factors or known proteins in this signal transduction pathway cannot be discarded. New approaches, such as the utilization of mutant library screenings,\textsuperscript{154} need to be developed to shed light on the mechanism connecting the redox state of the PQ pool and the fluorescence changes observed in cyanobacterial state transitions.

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This work was supported by grants from the Agence Nationale de la Recherche (ANR projects RECYFUEL (ANR-16-CE05-\textsuperscript{Fig. 7 A proposed model for cyanobacterial state transitions. Based on all the literature revised in the present article, we believed that the most probable mechanism involved in cyanobacterial state transitions involves a specific PSII-quenching during State I to State II transition (A), and a partial detachment of PBS during State II to State I transition (B). Nevertheless, small PBS movements that might affect the energy transfer to either of both photosystems cannot be ruled out. In addition, regarding the signal transduction pathways, it was demonstrated that neither Cyt b6f nor phosphorylation reactions are involved in cyanobacterial state transitions. The participation of known or unknown proteins in this signaling pathway needs to be elucidated.
Perspective

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Conflicts of interest

There are no conflicts to declare.

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