Chinese Science Bulletin

ApcD is required for state transition but not involved in blue-light induced quenching in the cyanobacterium *Anabaena* sp. PCC7120

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Pbycobilisomes (PBS) are able to transfer absorbed energy to photosystem I and II, and the distribution of light energy between two photosystems is regulated by state transitions. In this study we show that energy transfer from PBS to photosystem I (PSI) requires ApcD. Cells were unable to perform state transitions in the absence of ApcD. The *apcD* mutant grows more slowly in light mainly absorbed by PBS, indicating that ApcD-dependent energy transfer to PSI is required for optimal growth under this condition. The *apcD* mutant showed normal blue-light induced quenching, suggesting that ApcD is not required for this process and state transitions are independent of blue-light induced quenching. Under nitrogen fixing condition, the growth rates of the wild type and the mutant were the same, indicating that energy transfer from PBS to PSI in heterocysts was not required for nitrogen fixation.

cyanobacteria, photosystem, phycobilisome, state transition

Cyanobacteria and plants have two photosystems, both performing oxygenic photosynthesis. Most cyanobacteria use phycobilisomes (PBS) as major light-harvesting complexes (1). PBS are water-soluble, supramolecular complexes composed of phycobiliproteins and colorless linker proteins[1−4]. PBS are typically associated with photosystem II (PSII) and deliver most light energy absorbed by phycobiliproteins to PSII reaction centers[5,6]. However, when photosystem I (PSI) receives too little light energy, the light energy absorbed by PBS can also be delivered to PSI[7]. The process of redistributing light energy to the two photosystems to produce an optimal rate of electron transfer is called "state transition"[8,9].

Under excessive light conditions, both cyanobacteria and plants are subject to photoinhibition. One of the responses to a high light condition by both plants and cyanobacteria is non-photochemical quenching (NPQ). In cyanobacteria, NPQ is mainly composed of state transitions[10,11] and high blue-light induced quenching[12]. The relationship between state transition and blue-light induced quenching is not known. Whether state transitions are involved in this process is not clear. We studied the roles of ApcD in state transitions in a filamentous cyanobacterium *Anabaena* sp. PCC 7120 (hereafter *Anabaena* 7120). The cyanobacterium *Anabaena* 7120 is able to fix nitrogen when combined nitrogen is absent in the growth medium and the site for nitrogen fixation is a specialized cell called heterecyst. Although some PBS are present in heterocysts[13], their role in heterocysts are not understood. Little is known of whether light energy absorbed by PBS in heterocysts could be transferred to PSI, which is the only photosystem in heterocysts because PSII is absent in these differentiated cells.

ApcD is one of the two terminal transmitters in the core of PBS and it was suggested to be critical to energy transfer[14]. A mutant lacking *apcD* (PKU211) was con-

Received August 8, 2008; accepted August 22, 2008
doi: 10.1007/s11434-008-0482-3
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Supported by the National Natural Science Foundation of China (Grant No. 30230040) and National High-Tech Research and Development of China (Grant No. 2007AA021405)

structed in *Anabaena* 7120 and it was confirmed by Southern hybridization (data not shown). Figure 1 shows the fluorescence emission spectra of the wild type and the mutant under either state 1 or state 2 conditions. While it is evident that the wild type was able to perform state transitions, PKU211 showed no state transitions as the emission from PSII and PSI remained the same under either condition. These results support the conclusion that the ApcD subunit of PBS is required for state transitions observed in unicellular cyanobacteria[15—17], as has been confirmed by the study of room-temperature fluorescence induction with modulated fluorometer (Figure 2). The wild-type cells of *Anabaena* 7120 had the expected state transitions: dark-adapted cells were in state 1 and a state 2 transition was observed when these cells were illuminated with low intensity blue light, which induced a higher maximum fluorescence yield (F_m'). High intensity blue-light induced non-photochemical quenching (NPQ), as the F_m' value decreased significantly (Figure 2(a)). When strain PKU211 (*apcD*$^-$) was treated in a similar fashion, the fluorescence yield of the dark-adapted cells was unchanged when cells were illuminated with a low intensity blue light (Figure 2(b)). Because the high blue-light induced NPQ in PKU211 was the same as that in the wild type, ApcD is not required for the blue-light dependent NPQ and the two processes are likely independent of each other.

The *apcD* mutant grew normally in the presence of combined nitrogen under white light illumination as compared with the wild type cells (Figure 3(a)). Under a green light which is preferentially absorbed by PBS, the mutant grew more slowly than the wild type (Figure 3(b)), suggesting that the energy transfer from PBS to PSI is required for optimal growth when excitation of the two photosystems is unbalanced. In the absence of combined nitrogen, PKU211 was able to form heterocysts and fix nitrogen (data not shown), and the growth rate of PKU211 was similar to that of the wild type un-

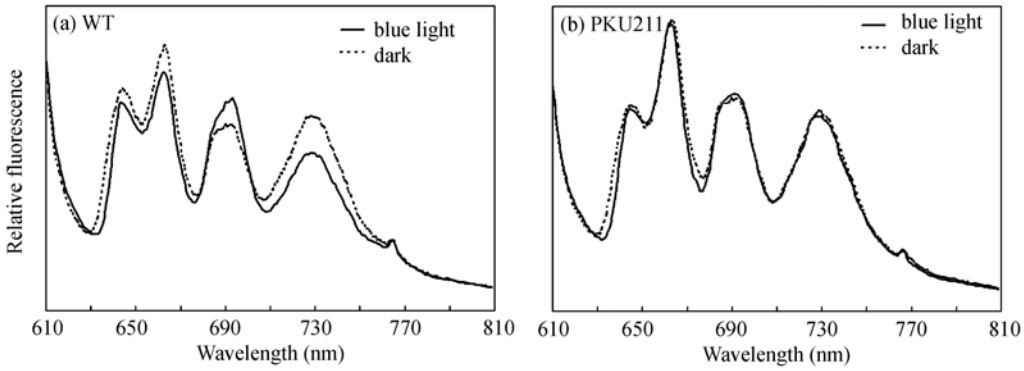

Figure 1 Fluorescence emission spectra of *Anabaena* sp. PCC 7120 cells at 77 K. Cells of the wild type (a) and PKU211 (*apcD*$^-$) (b) at a concentration equivalent to 5 μg Chl/mL were incubated in the dark to establish state 2 (solid line), or illuminated with blue-light in the presence of 5 μmol/L 3-(3,4-dichlorophenyl)-1,1-dimethyl urea (DCMU) to establish state 1 (dashed line) prior to freezing in liquid nitrogen. A glass tube containing the frozen cells was inserted into a dewar filled with liquid nitrogen for measurement of emission spectra at 77 K. The excitation wavelength was 580 nm. Each curve is an average of 4 individual measurements.

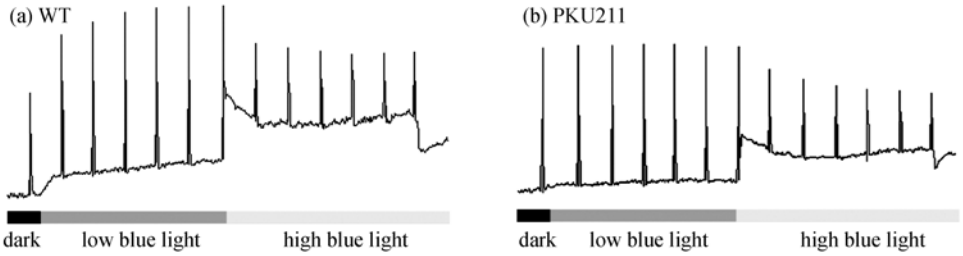

Figure 2 Fluorescence yields and induction curves of for *Anabaena* sp. PCC 7120 wild type and PKU211. Fluorescence yields were measured with a PAM fluorometer in dark-adapted cells of the wild type (a) and PKU211 (*apcD*$^-$) (b). Cells were first incubated in the dark for 5 min, and then a low intensity blue light (50 μmol photons·m^{-2}·s^{-1}) and a high intensity blue light (560 μmol photons·m^{-2}·s^{-1}) were successively switched on as indicated in the figures. The time duration between the saturating white light flashes at 3000 μmol photons·m^{-2}·s^{-1} was 30 s.

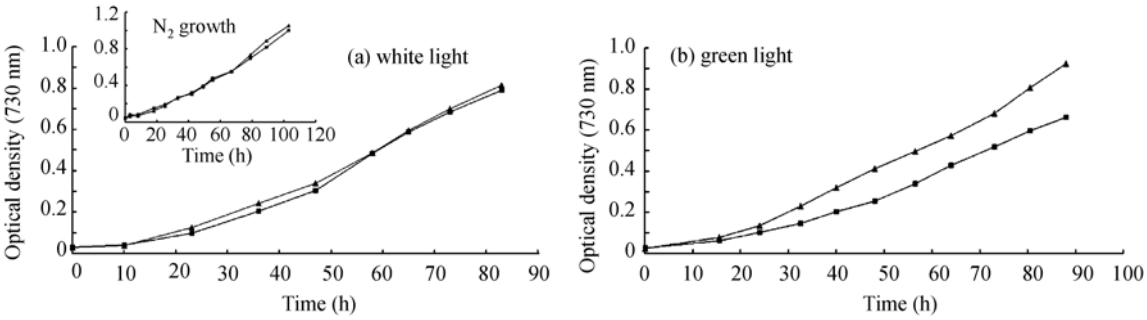

Figure 3 Growth measurement of the wild type (triangles) and PKU211 (squares) under white light (a) or green light (b). Growth was performed at 28℃ and the cultures were bubbled with air supplemented with 1% CO_2. The light intensity was 35 μmol photons·m^{-2}·s^{-1}. The inset of (a) was growth measurement in a medium without combined nitrogen.

der white light (inset of Figure 3(a)). This indicates that energy transfer from the phycobiliproteins to PSI is not important to nitrogen fixation in heterocysts because heterocysts are the only sites for nitrogen fixation and they only have PSI. We speculate that the phycobiliproteins observed in heterocysts are to be degraded in the process of further heterocyst maturation.

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