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The Roles of Cytochrome b_{559} in Assembly and Photoprotection of Photosystem II Revealed by Site-Directed Mutagenesis Studies

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Cytochrome b_{559} (Cyt b_{559}) is one of the essential components of the Photosystem II reaction center (PSII). Despite recent accomplishments in understanding the structure and function of PSII, the exact physiological function of Cyt b_{559} remains unclear. Cyt b_{559} is not involved in the primary electron transfer pathway in PSII but may participate in secondary electron transfer pathways that protect PSII against photoinhibition. Site-directed mutagenesis studies combined with spectroscopic and functional analysis have been used to characterize Cyt b_{559} mutant strains and their mutant PSII complex in higher plants, green algae, and cyanobacteria. These integrated studies have provided important *in vivo* evidence for possible physiological roles of Cyt b_{559} in the assembly and stability of PSII, protecting PSII against photoinhibition, and modulating photosynthetic light harvesting. This mini-review presents an overview of recent important progress in site-directed mutagenesis studies of Cyt b_{559} and implications for revealing the physiological functions of Cyt b_{559} in PSII.

Keywords: photosynthesis, photosystem II, cytochrome b_{559}, site-directed mutagenesis, photoprotection, photoinhibition

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INTRODUCTION

Cytochrome b_{559} is one of the essential components of Photosystem II in all oxygenic photosynthetic organisms (Whitmarsh and Pakrasi, 1996; Stewart and Brudvig, 1998; Guskov et al., 2009; Umena et al., 2011). Cyt b_{559} is a heme-bridged heterodimer protein comprising one α- and one β- subunit (encoded by the *psbE* and *psbF* genes) of 9 and 4 kDa, respectively (see **Figure 1**). Each subunit provides a His ligand (His-22 residue of the α- or β-subunit of Cyt b_{559} in *Synechocystis* sp. PCC 6803, corresponding to His-23 residue of α- or His-24 residue of the β-subunit of Cyt b_{559} in *Thermosynechococcus elongatus*) for the non-covalently bound heme, which is located near the stromal side of PSII. In addition, Cyt b_{559} has different redox potential forms depending on the type of PSII preparations and treatments: a HP form with a midpoint redox potential of about +400 mV, an IP form of about +200 mV, and a LP form with a midpoint redox potential of about 0–80 mV (Stewart and Brudvig, 1998; Roncel et al., 2001 and references therein). In intact PSII preparations, Cyt b_{559} is mostly in the reduced HP form under

Abbreviations: APC, allophycocyanin; Car, β-carotene; Cyt b_{559}, cytochrome b_{559}; HP, high potential; IP, intermediate potential; LP, low potential; NPQ, non-photochemical fluorescences quenching; PQ, plastoquinone; PQH_2, plastoquinol; PSII, Photosystem II; Q_A, primary quinone electron acceptor in PSII; Q_B, the secondary quinone electron acceptor in PSII; Q_C, the third plastoquinone-binding site in PSII.

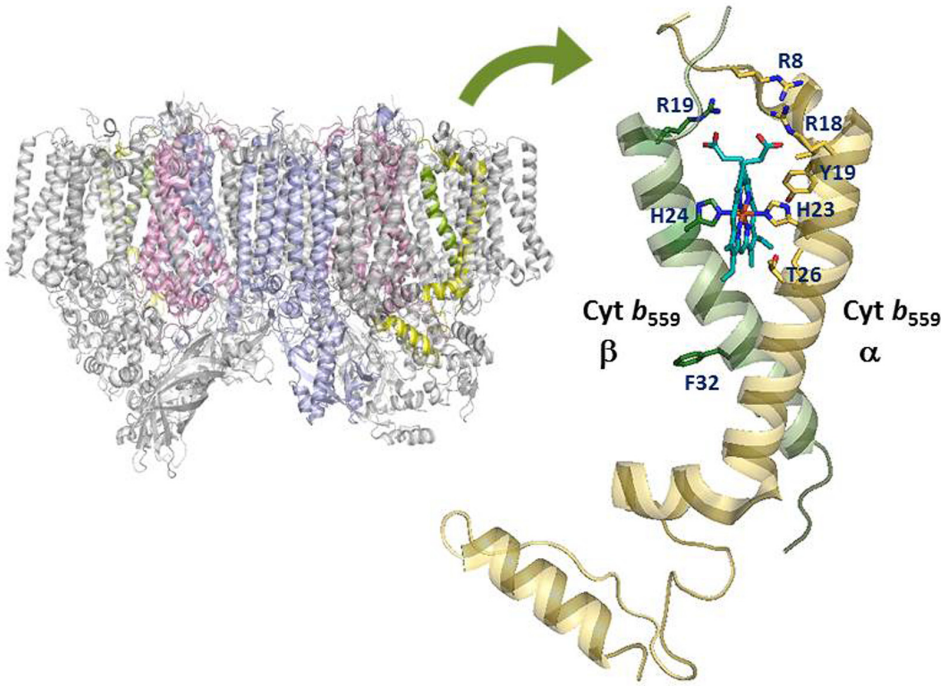

FIGURE 1 | Structural model of the cytochrome b_{559} in Photosystem II. Amino acid residues of cytochrome b_{559} targeted by recent site-directed mutagenesis work are labeled by using amino acid sequences of cytochrome b_{559} from *Thermosynechococcus elongatus*. The figure was created using PyMol and the PDB file 4UB8.

ambient conditions. In inactive or less intact PSII preparations, Cyt b_{559} is typically in the LP or IP form and mostly oxidized (presumably by molecular oxygen) under ambient conditions (Barber and De Las Rivas, 1993; Poulson et al., 1995; Pospíšil et al., 2006).

Several studies have proposed that Cyt b_{559} participates in secondary electron transfer pathways that protect PSII against photoinhibition (see **Figure 2**; Heber et al., 1979; Falkowski et al., 1986; Thompson and Brudvig, 1988; Barber and De Las Rivas, 1993; Poulson et al., 1995; Magnuson et al., 1999; Faller et al., 2001; Tracewell and Brudvig, 2008 and references therein). In these models, the HP form of Cyt b_{559} is thought to donate its electron, via a β-carotene molecule (Car_{D2}), to reduce highly oxidized chlorophyll radicals in PSII under donor-side photoinhibitory conditions (e.g., the oxygen-evolving complex is impaired or under assembly). Oxidized Cyt b_{559} may accept an electron from the acceptor side of PSII (Q_B^-, or reduced PQH_2 from the pool), thus forming a cyclic pathway of electron transfer within PSII. On the other hand, when the electron transfer on the acceptor side of PSII is inhibited (e.g., under high-light conditions), the oxidized Cyt b_{559} might accept an electron from the acceptor side of PSII to prevent the formation of damaging singlet oxygen species (Nedbal et al., 1992; Vass et al., 1992; Barber and De Las Rivas, 1993; Bondarava et al., 2010). In addition, several different enzymatic functions of Cyt b_{559} have been proposed, such as superoxide dismutase (Ananyev et al., 1994) and PQH_2 oxidase in intact PSII (Kruk and Strzałka, 1999, Kruk and Strzalka, 2001; Bondarava et al., 2003, 2010) and superoxide oxidase and reductase in tris-washed PSII (Tiwari and Pospísil, 2009; Pospíšil, 2011). Moreover, a novel quinone-binding site (Q_C) was identified close (about 15 Å) to the heme of Cyt b_{559} in the 2.9-Å PSII crystal structure from *T. elongatus* (Guskov et al., 2009). The occupancy of this Q_C site with PQ (or PQH_2) has been proposed to modulate the redox equilibration between Cyt b_{559} and the PQ pool (Kaminskaya et al., 2006, 2007; Kaminskaya and Shuvalov, 2013) or be involved in the exchange of PQ on the Q_B site from the pool (Guskov et al., 2009). However, the Q_C site was not detected in the more recent 1.9-Å PSII crystal structure (Umena et al., 2011). Despite the recent remarkable progress in understanding the structure and function of PSII, the exact function of Cyt b_{559} in PSII remains unclear.

This mini-review gives an overview of important progress in recent site-directed mutagenesis studies performed to reveal the physiological function(s) of Cyt b_{559} in PSII. More comprehensive reviews on the structure and functions of Cyt b_{559} are available (Whitmarsh and Pakrasi, 1996; Stewart and Brudvig, 1998; Faller et al., 2005; Pospíšil, 2011; Shinopoulos and Brudvig, 2012).

FUNCTION IN ASSEMBLY AND STABILITY OF PSII REACTION CENTERS

Prior mutagenesis studies with *Synechocystis* sp. PCC 6803, *Chlamydomonas reinhardtii* or *Nicotiana tabacum* showed no

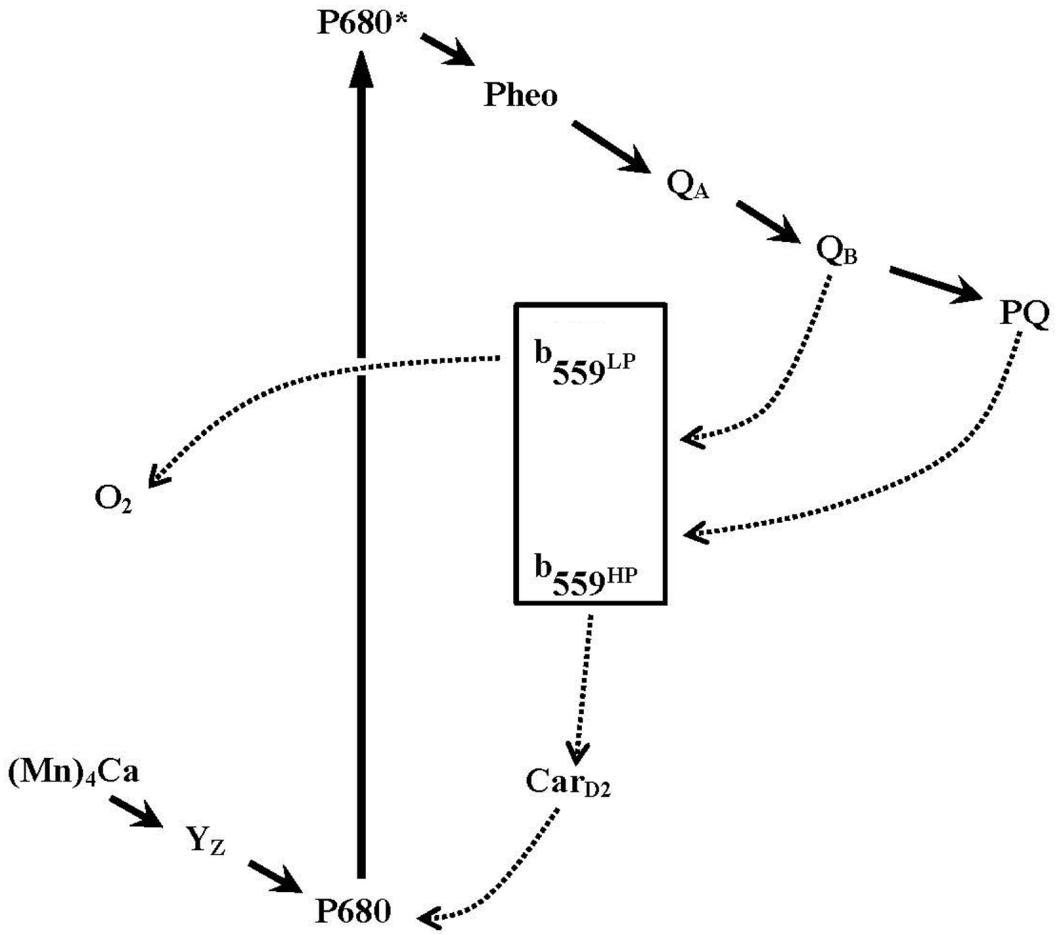

FIGURE 2 | A simplified scheme for electron transfer pathways within Photosystem II reaction centers. Primary electron transfer pathway (bold solid lines with arrows) and possible secondary electron transfer pathways involving cytochrome b_{559} (dotted line with arrows) are shown. The scheme is modified from Whitmarsh and Pakrasi (1996).

stable PSII reaction centers assembled in the absence of either Cyt b_{559} subunit (Pakrasi et al., 1988, 1989, 1990; Morais et al., 1998; Swiatek et al., 2003; Suorsa et al., 2004). Several authors proposed that Cyt b_{559} plays an important structural role, such as being a nucleating factor, during the early stage of PSII assembly (Pakrasi et al., 1988; Morais et al., 1998; Komenda et al., 2004).

In addition, mutagenesis studies with *Synechocystis* sp. PCC 6803 showed that substituting either of the heme axial ligands (His22 of the α-subunit or His22 of the β-subunit of Cyt b_{559} in *Synechocystis* sp. PCC 6803) with Leu, Met, Glu, Gln, Tyr, Lys, Arg, or Cys abolished the photoautotrophic growth and severely diminished the assembly or stability of PSII in the mutant cells, except for H22Kα mutant cells, which were able to grow photoautotrophically and accumulated stable PSII reaction centers (∼81% as compared with wild-type cells; Pakrasi et al., 1991; Hung et al., 2007, 2010). Electron paramagnetic resonance results indicated the displacement of one of the two axial ligands to the heme of Cyt b_{559} in H22Kα mutant reaction centers, at least in isolated reaction centers (Hung et al., 2010). In addition, H22Kα and Y18Sα (corresponding to Y19Sα in *T. elongatus*) in mutant PSII core complexes contained predominately the LP form of Cyt b_{559}. The findings support the concept that the redox properties of Cyt b_{559} are strongly influenced by the hydrophobicity and ligation environment of the heme (Krishtalik et al., 1993; Gadjieva et al., 1999; Roncel et al., 2001; Pospíšil and Tiwari, 2010).

Spectroscopic and functional characterizations of the cyanobacterium *Synechocystis* sp. PCC 6803 with mutation of charged residues on the cytoplasmic side of Cyt b_{559} in PSII have been reported (Chiu et al., 2013). All mutant cells grew photoautotrophically and assembled stable PSII. However, R7Eα, R17Eα, and R17Lβ mutant cells grew significantly slower and were more susceptible to photoinhibition as compared with wild-type cells. In addition, the PSII core complexes from R7Eα and R17Lβ cells contained predominantly the LP form of Cyt b_{559}. Electron paramagnetic resonance results indicated the displacement of one of the two axial ligands to the heme of Cyt b_{559} in the reaction centers of the R7Eα and R17Lβ mutants. In recent PSII crystal structural models (Guskov et al., 2009; Umena et al., 2011), the side chains of

these Arg residues of Cyt b_{559} (corresponding to Arg8 and Arg18 residues of the α-subunit and Arg19 residue of the β-subunit of Cyt b_{559} in *T. elongatus*) are in close contact with the heme propionates of Cyt b_{559} (see **Figure 1**). Thus, the electrostatic interactions between these Arg residues and the heme propionates of Cyt b_{559} may affect the ligation structure and redox properties of the heme in Cyt b_{559} (Chiu et al., 2013).

Furthermore, mutagenesis studies of *C. reinhardtii* showed that the H23Yα, H23Mα, and H23Cα mutant cells were unable to grow photoautotrophically, were sensitive to photoinhibition, accumulated 10–20% of the PSII (compared to wild-type cells), and contained a disrupted heme pocket while still retaining significant O_2 evolution activity (Morais et al., 2001; Hamilton et al., 2014). Thus, the heme of Cyt b_{559} was not required for photosynthetic water oxidation by PSII (Morais et al., 2001). A recent study also presented evidence to ascribe the photoinhibition phenotype of H23Cα mutant cells to a faster rate of photodamage and an impaired PSII repair cycle (Hamilton et al., 2014). Hence, Cyt b_{559} may play important roles in the assembly, repair and maintenance of the PSII complex *in vivo*.

In the other recent mutant study of *T. elongatus* that took advantage of the robustness of the PSII variant with PsbA3 as the D1 subunit, the four constructed Cyt b_{559} mutants (H23Aα, H23Mα, Y19Fα, and T26Pα) grew photoautotrophically (*T. elongatus* is an obligate photoautotroph; Sugiura et al., 2015). Although the H23Aα and H23Mα mutants assembled only an apo-Cyt b_{559}, the steady-state level of active PSII was comparable to that in the wild-type control. The results suggest that the heme has no structural role in the assembly of PSII in the presence of α- and β-subunits of Cyt b_{559}. This finding is in strong contrast to the *Synechocystis* sp. PCC 6803 mutant showing that proper coordination of the heme cofactor in Cyt b_{559} is important to the assembly or stability of PSII (Pakrasi et al., 1991; Hung et al., 2007). In addition, Cyt b_{559} mutant cells of *T. elongatus* showed no correlation between the rate of photoinhibition and the redox potential of the heme. However, the recovery of the oxygen-evolving activity of PSII after photoinhibition was significantly slower in these mutant cells. PsbA3 is the D1 isoform expressed in *T. elongatus* under high-light conditions (Nakamura et al., 2002; Kós et al., 2008). The high-light D1 isoform in cyanobacteria has a Glu instead of a Gln residue (for the low-light D1 isoform) at position 130 in the D1 protein sequence (for a review, see Mulo et al., 2009) and this Glu residue forms hydrogen-bonding interactions with pheophytinD1 (Dorlet et al., 2001; Shibuya et al., 2010). Cyanobacterial PSIIs with the high-light D1 isoform showed increased photo-tolerance and accelerated non-radiative charge recombination (Tichy et al., 2003). This phototolerant property has been attributed to a photoprotection mechanism involving the redox potential of pheophytinD1, which enhances the probability for non-radiative recombination of the singlet radical pair and prevents the formation of potentially damaging ^{3}P680 and singlet oxygen species (Vass and Cser, 2009; Sugiura et al., 2014). Further investigation could determine whether PsbA3 may compensate the photoprotective function of Cyt b_{559} in the assembly and stability of PSII in these Cyt b_{559} mutant cells.

FUNCTION IN PROTECTING PSII AGAINST PHOTOINHIBITION

Numerous site-directed mutagenesis studies have investigated the role of Cyt b_{559} in protecting PSII against photoinhibition under high light. In the photoprotective models, oxidized Cyt b_{559} may accept an electron from the acceptor side of PSII (Q_B^-, Q_C, or reduced PQH_2 from the pool). Previous mutant studies of dark-adapted leaves of the F26Sβ Cyt b_{559} tobacco mutant showed a greatly reduced PQ pool, conversion of the redox-potential form of Cyt b_{559} to the LP form, and photosynthetic activities sensitive to high light (Bondarava et al., 2003, 2010). In addition, R7Eα and R17Lβ Cyt b_{559} mutant cells of *Synechocystis* sp. PCC 6803 and R18Sα Cyt b_{559} mutant cells of *T. elongatus* showed markedly reduced PQ pools, altered redox-potential forms of Cyt b_{559}, and high susceptibility to light stress (Chiu et al., 2013; Guerrero et al., 2014). A defect in PQH_2 oxidase activity of Cyt b_{559} due to altered redox-potential forms of Cyt b_{559} in these mutant strains could explain their high susceptibility to strong light and greatly reduced PQ pools. Therefore, Cyt b_{559} may function as a PQH_2 oxidase to keep the PQ pool and the acceptor side of PSII oxidized in the dark, thereby preventing PSII from acceptor-side photoinhibition (Kruk and Strzałka, 1999, Kruk and Strzalka, 2001; Bondarava et al., 2003, 2010). However, one recent study reported no defect in PQH_2 oxidation in the dark in H23Cα mutant cells of *C. reinhardtii*, even though H23Cα mutant cells contained a disrupted heme-binding pocket of Cyt b_{559} and were sensitive to photoinhibition (Hamilton et al., 2014). Further studies are required to clarify this discrepancy. In addition, study of the 2.9-Å resolution PSII crystal structure reported the binding of Q_C at a hydrophobic cavity near Cyt b_{559} (Guskov et al., 2009). Several spectroscopic studies have provided evidence that the occupancy of the Q_C site by PQ (or PQH_2) may modulate the redox potential of Cyt b_{559} and mediate the redox equilibration between Cyt b_{559} and the PQ pool (Kaminskaya et al., 2006, 2007; Kaminskaya and Shuvalov, 2013). A recent study provided evidence of a possible one-electron oxidation of PQH_2 by Cyt b_{559} at the Q_C site involved in the formation of a superoxide anion radical (Yadav et al., 2014). The above results are consistent with Cyt b_{559} possibly accepting an electron from PQH_2 via the Q_C site in PSII. However, the Q_C site was not present in the more recent 1.9-Å PSII crystal structure (Umena et al., 2011). Further investigations are needed to solve this important issue.

Spectroscopic and functional characterization of the H22Kα and Y18Sα Cyt b_{559} mutant cells of *Synechocystis* sp. PCC 6803 showed that both mutants have functional PSII and exhibited the normal period-four oscillation in oxygen yield (Hung et al., 2010). However, both mutants were more susceptible to photoinhibition than the wild type under high-light conditions. In addition, PSII core complexes from the H22Kα and Y18Sα

mutants predominantly contained the oxidized LP form of Cyt b_{559} (∼79 and 86%, respectively). A defect in the photoprotective function of Cyt b_{559} in H22Kα and Y18Sα mutants could explain their high susceptibility to strong light. Furthermore, H22Kα and Y18Sα Cyt b_{559} mutants in a D1-D170A genetic background that prevented assembly of the Mn cluster showed almost completely abolished accumulation of PSII even under normal-growth-light conditions. The data support an important redox role of Cyt b_{559} in protecting PSII under donor-side photoinhibition conditions (Hung et al., 2010).

Furthermore, under low light, the H23Cα Cyt b_{559} mutant showed more rapid assembly of the Mn_4CaO_5 cluster than the wild-type control in *C. reinhardtii* (Hamilton et al., 2014). However, the photoactivation of oxygen-evolving PSII in the H23Cα mutant was inhibited under high light. The results suggest that reduction of $P680^+$ via cyclic electron flow within PSII (via Cyt b_{559} and Car_{D2}, **Figure 2**) may compete with the photoactivation process and provides important *in vivo* evidence for a photoprotective role of Cyt b_{559} in photo-assembly of the Mn_4CaO_5 cluster in PSII (Hamilton et al., 2014).

A recent mutant study involving *T. elongatus* showed that the midpoint redox potential of the HP form of Cyt b_{559} was significantly destabilized (converted to the IP form) in mutant PSII core complexes of Cyt b_{559} mutant strains (I14Aα, I14Sα, R18Sα, I27Aα, I27Tα, and F32Yβ; Guerrero et al., 2014). When the oxygen-evolving complex was inactive, the yield of dark-reduction of Cyt b_{559} was lower and the kinetics was slower in the R18Sα mutant than in wild-type cells. The results support the concept that the HP form of Cyt b_{559} may function as a PQH_2 oxidase to keep the PQ pool oxidized and also as an electron reservoir for the cyclic electron flow within PSII when the donor-side of PSII is impaired (Guerrero et al., 2014).

Moreover, a previous spectroscopic study showed that different spectral forms of Car were oxidized in PSII samples containing different redox forms of Cyt b_{559} (Tracewell and Brudvig, 2008). The authors proposed that the quenching properties of PSII may be controlled by the redox form of Cyt b_{559} by modulating the different type of oxidized Car species (radical cation or neutral radical) formed in PSII. Future study could investigate the quenching properties of PSII in the wild type versus Cyt b_{559} mutant strains of cyanobacteria with different redox forms of Cyt b_{559} to validate this proposal.

EFFECTS ON PHOTOSYNTHETIC LIGHT HARVESTING

Recent mutant studies revealed a novel role of Cyt b_{559} in modulating photosynthetic light harvesting in PSII reaction centers. A spontaneously generated mutant from *Synechocystis* sp. PCC 6803 wild-type cells grown in BG-11 agar plates containing 5 mM Glu and 10 μM DCMU carried an Arg7 to Leu mutation on the alpha-subunit of Cyt b_{559} in PSII (Chiu et al., 2009). Results of 77-K fluorescence and room-temperature chlorophyll *a* fluorescence spectra indicated that the energy transfer from phycobilisomes to PSII reaction centers was partially inhibited or uncoupled in this mutant. In addition, the cytoplasmic side of Cyt b_{559} is located within the predicted contact sites in PSII for the APC core complex of the phycobilisome (Barber et al., 2003). The Arg7 to Leu mutation of Cyt b_{559} may alter the interaction between the APC core complex and PSII reaction centers, thereby reducing energy delivery from the antenna to the reaction center and protecting mutant cells against DCMU-induced photo-oxidative stress (Rutherford and Krieger-Liszkay, 2001).

Many cyanobacteria including *Synechocystis* sp. PCC 6803 have a novel blue-green light-induced NPQ mechanism to protect PSII reaction centers against photodamage under high-light stress (Kirilovsky and Kerfeld, 2012). Under high-light conditions, a soluble orange carotenoid protein is able to absorb blue–green light and undergoes photo-conversion into the active red form, which interacts with the APC core of the phycobilisome and dissipates excess excitation energy from the phycobilisome as heat. Interestingly, several *Synechocystis* sp. PCC 6803 mutant cells (e.g., R7Lα and R17Lβ) with mutations on the cytoplasmic side of Cyt b_{559} in PSII showed significant inhibition of the effects of blue–green light-induced NPQ and apparent acceleration on its recovery (Chiu et al., 2013). These results are consistent with the proposal that the mutations on Cyt b_{559} may alter the interaction between the phycobilisome and PSII reaction centers, thereby affecting the regulation of photosynthetic light harvesting in *Synechocystis* sp. PCC 6803.

CONCLUSION AND PERSPECTIVES

Site-directed mutagenesis studies combined with spectroscopic and functional characterization have revealed multiple roles of Cyt b_{559} in the assembly and photoprotection of PSII reaction centers. The findings provide convincing evidence for the physiological role(s) of Cyt b_{559} in a photoprotective secondary electron transfer pathway within PSII reaction centers, as was suggested from earlier studies of isolated PSII complexes (reviews in Whitmarsh and Pakrasi, 1996; Stewart and Brudvig, 1998; Shinopoulos and Brudvig, 2012). In the near future, site-directed mutagenesis studies combined with advanced high-resolution protein crystallography and spectroscopic and functional analysis will provide further new insights (e.g., structure and function relationships for different redox forms of Cyt b_{559}) and possibly the final proof of the molecular mechanisms of Cyt b_{559} in PSII.

AUTHOR CONTRIBUTIONS

H-AC wrote the major part of the manuscript. Y-FC wrote the minor part of the manuscript, contributed the **Figure 1** and edited the references.

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REFERENCES

Ananyev, G., Renger, G., Wacker, U., and Klimov, V. (1994). The photoproduction of superoxide radicals and the superoxide dismutase activity of Photosystem II. The possible involvement of cytochrome b559. *Photosynth. Res.* 41, 327–338. doi: 10.1007/BF00019410

Barber, J., and De Las Rivas, J. (1993). A functional model for the role of cytochrome b559 in the protection against donor and acceptor side photoinhibition. *Proc. Natl. Acad. Sci. U.S.A.* 90, 10942–10946. doi: 10.1073/pnas.90.23.10942

Barber, J., Morris, E. P., and da Fonseca, P. C. A. (2003). Interaction of the allophycocyanin core complex with Photosystem II. *Photochem. Photobiol. Sci.* 2, 536–541. doi: 10.1039/b300063j

Bondarava, N., De Pascalis, L., Al-Babili, S., Goussias, C., Golecki, J. R., Beyer, P., et al. (2003). Evidence that cytochrome b559 mediates the oxidation of reduced plastoquinone in the dark. *J. Biol. Chem.* 278, 13554–13560. doi: 10.1074/jbc.M212842200

Bondarava, N., Gross, C. M., Mubarakshina, M., Golecki, J. R., Johnson, G. N., and Krieger-Liszkay, A. (2010). Putative function of cytochrome b559 as a plastoquinol oxidase. *Physiol. Plant.* 138, 463–473. doi: 10.1111/j.1399-3054.2009.01312.x

Chiu, Y.-F., Chen, Y.-H., Roncel, M., Dilbeck, P. L., Huang, J.-Y., Ke, S.-C., et al. (2013). Spectroscopic and functional characterization of cyanobacterium *Synechocystis* PCC 6803 mutants on the cytoplasmic-side of cytochrome b559 in Photosystem II. *Biochim. Biophys. Acta* 1827, 507–519. doi: 10.1016/j.bbabio.2013.01.016

Chiu, Y.-F., Lin, W.-C., Wu, C.-M., Chen, Y.-H., Hung, C.-H., Ke, S.-C., et al. (2009). Identification and characterization of a cytochrome b559 *Synechocystis* 6803 mutant spontaneously generated from DCMU-inhibited photoheterotrophical growth conditions. *Biochim. Biophys. Acta* 1787, 1179–1188. doi: 10.1016/j.bbabio.2009.05.007

Dorlet, P., Xiong, L., Sayre, R. T., and Un, S. (2001). High field EPR study of the pheophytin anion radical in wild type and D1-E130 mutants of Photosystem II in *Chlamydomonas reinhardtii*. *J. Biol. Chem.* 276, 22313–22316. doi: 10.1074/jbc.M102475200

Falkowski, P. G., Fujita, Y., Ley, A., and Mauzerall, D. (1986). Evidence for cyclic electron flow around Photosystem II in *Chlorella pyrenoidosa*. *Plant Physiol.* 81, 310–312. doi: 10.1104/pp.81.1.310

Faller, P., Fufezan, C., and Rutherford, A. W. (2005). "Side-path electron donors: cytochrome b559, chlorophyll Z and β-Carotene," in *Photosystem II*, eds T. J. Wydrzynski and K. Satoh (Dordrecht: Springer), 347–365.

Faller, P., Pascal, A., and Rutherford, A. W. (2001). Beta-carotene redox reactions in Photosystem II: electron transfer pathway. *Biochemistry* 40, 6431–6440. doi: 10.1021/bi0026021

Gadjieva, R., Mamedov, F., Renger, G., and Styring, S. (1999). Interconversion of low- and high-potential forms of cytochrome b559 in tris-washed Photosystem II membranes under aerobic and anaerobic conditions. *Biochemistry* 38, 10578–10584. doi: 10.1021/bi9904656

Guerrero, F., Zurita, J. L., Roncel, M., Kirilovsky, D., and Ortega, J. M. (2014). The role of the high potential form of the cytochrome b559: study of *Thermosynechococcus elongatus* mutants. *Biochim. Biophys. Acta* 1837, 908–919. doi: 10.1016/j.bbabio.2014.02.024

Guskov, A., Kern, J., Gabdulkhakov, A., Broser, M., Zouni, A., and Saenger, W. (2009). Cyanobacterial Photosystem II at 2.9-Å resolution and the role of quinones, lipids, channels and chloride. *Nat. Struct. Mol. Biol.* 16, 334–342. doi: 10.1038/nsmb.1559

Hamilton, M. L., Franco, E., Deák, Z., Schlodder, E., Vass, I., and Nixon, P. J. (2014). Investigating the photoprotective role of cytochrome b559 in Photosystem II in a mutant with altered ligation of the haem. *Plant Cell Physiol.* 55, 1276–1285. doi: 10.1093/pcp/pcu070

Heber, U., Kirk, M. R., and Boardman, N. K. (1979). M.R. Kirk a, N.K. BoardmanPhotoreactions of cytochrome b559 and cyclic electron flow in Photosystem II of intact chloroplasts. *Biochim. Biophys. Acta* 546, 292–306. doi: 10.1016/0005-2728(79)90047-1

Hung, C.-H., Huang, J.-Y., Chiu, Y.-F., and Chu, H.-A. (2007). Site-directed mutagenesis on the heme axial-ligands of cytochrome b559 in Photosystem II by using cyanobacteria *Synechocystis* PCC 6803. *Biochim. Biophys. Acta* 1767, 686–693. doi: 10.1016/j.bbabio.2007.02.016

Hung, C.-H., Hwang, H.-J., Chen, Y.-H., Chiu, Y.-F., Ke, S.-C., Burnap, R. L., et al. (2010). Spectroscopic and functional characterizations of cyanobacterium *Synechocystis* PCC 6803 mutants on and near the heme axial ligand of cytochrome b559 in Photosystem II. *J. Biol. Chem.* 285, 5653–5663. doi: 10.1074/jbc.M109.044719

Kaminskaya, O., Shuvalov, V. A., and Renger, G. (2006). Evidence for a novel quinone-binding site in the Photosystem II (PS II) complex that regulates the redox potential of cytochrome b559. *Biochemistry* 46, 1091–1105. doi: 10.1021/bi0613022

Kaminskaya, O., Shuvalov, V. A., and Renger, G. (2007). Two reaction pathways for transformation of high potential cytochrome b559 of PS II into the intermediate potential form. *Biochim. Biophys. Acta* 1767, 550–558. doi: 10.1016/j.bbabio.2007.02.005

Kaminskaya, O. P., and Shuvalov, V. A. (2013). Biphasic reduction of cytochrome b559 by plastoquinol in Photosystem II membrane fragments: evidence for two types of cytochrome b559/plastoquinone redox equilibria. *Biochim. Biophys. Acta* 1827, 471–483. doi: 10.1016/j.bbabio.2013.01.007

Kirilovsky, D., and Kerfeld, C. A. (2012). The orange carotenoid protein in photoprotection of Photosystem II in cyanobacteria. *Biochim. Biophys. Acta* 1817, 158–166. doi: 10.1016/j.bbabio.2011.04.013

Komenda, J., Reisinger, V., Muller, B. C., Dobakova, M., Granvogl, B., and Eichacker, L. A. (2004). Accumulation of the D2 protein is a key regulatory step for assembly of the Photosystem II reaction center complex in *Synechocystis* PCC 6803. *J. Biol. Chem.* 279, 48620–48629. doi: 10.1074/jbc.M405725200

Kós, P. B., Deák, Z., Cheregi, O., and Vass, I. (2008). Differential regulation of psbA and psbD gene expression, and the role of the different D1 protein copies in the cyanobacterium *Thermosynechococcus elongatus* BP-1. *Biochim. Biophys. Acta* 1777, 74–83. doi: 10.1016/j.bbabio.2012.04.010

Krishtalik, L. I., Tae, G.-S., Cherepanov, D. A., and Cramer, W. A. (1993). The redox properties of cytochromes b imposed by the membrane electrostatic environment. *Biophys. J.* 65, 184–195. doi: 10.1016/S0006-3495(93)81050-6

Kruk, J., and Strzałka, K. (1999). Dark reoxidation of the plastoquinone-pool is mediated by the low-potential form of cytochrome b559 in spinach thylakoids. *Photosynth. Res.* 62, 273–279. doi: 10.1023/a:1006374319191

Kruk, J., and Strzalka, K. (2001). Redox changes of cytochrome b559 in the presence of plastoquinones. *J. Biol. Chem.* 276, 86–91. doi: 10.1074/jbc.M003602200

Magnuson, A., Rova, M., Mamedov, F., Fredriksson, P.-O., and Styring, S. (1999). The role of cytochrome b559 and tyrosineD in protection against photoinhibition during in vivo photoactivation of Photosystem II. *Biochim. Biophys. Acta* 1411, 180–191. doi: 10.1016/s0005-2728(99)00044-4

Morais, F., Barber, J., and Nixon, P. J. (1998). The chloroplast-encoded α subunit of cytochrome b559 is required for assembly of the Photosystem Two complex in both the light and the dark in *Chlamydomonas reinhardtii*. *J. Biol. Chem.* 273, 29315–29320. doi: 10.1074/jbc.273.45.29315

Morais, F., Kuhn, K., Stewart, D. H., Barber, J., Brudvig, G. W., and Nixon, P. J. (2001). Photosynthetic water oxidation in cytochrome b559 mutants containing a disrupted heme-binding pocket. *J. Biol. Chem.* 276, 31986–31993. doi: 10.1074/jbc.M103935200

Mulo, P., Sicora, C., and Aro, E.-M. (2009). Cyanobacterial psbA gene family: optimization of oxygenic photosynthesis. *Cell. Mol. Life Sci.* 66, 3697–3710. doi: 10.1007/s00018-009-0103-6

Nakamura, Y., Kaneko, T., Sato, S., Ikeuchi, M., Katoh, H., Sasamoto, S., et al. (2002). Complete genome structure of the thermophilic cyanobacterium *Thermosynechococcus elongatus* BP-1. *DNA Res.* 9, 123–130. doi: 10.1093/dnares/9.4.123

Nedbal, L., Samson, G., and Whitmarsh, J. (1992). Redox state of a one-electron component controls the rate of photoinhibition of Photosystem II. *Proc. Natl. Acad. Sci. U.S.A.* 89, 7929–7933. doi: 10.1073/pnas.89.17.7929

Pakrasi, H. B., Ciechi, P. D., and Whitmarsh, J. (1991). Site directed mutagenesis of the heme axial ligands of cytochrome b559 affects the stability of the Photosystem II complex. *EMBO J* 10, 1619–1627.

Pakrasi, H. B., Diner, B. A., Williams, J. G. K., and Arntzen, C. J. (1989). Deletion mutagenesis of the cytochrome b559 protein inactivates the reaction center of Photosystem II. *Plant Cell* 1, 591–597. doi: 10.2307/3868946

Pakrasi, H. B., Nyhus, K. J., and Granok, H. (1990). Targeted deletion mutagenesis of the beta subunit of cytochrome b559 protein destabilizes the reaction center of Photosystem II. *Z. Naturforsch. C* 45, 423–429.

Pakrasi, H. B., Williams, J. G., and Arntzen, C. J. (1988). Targeted mutagenesis of the psbE and psbF genes blocks photosynthetic electron transport: evidence for a functional role of cytochrome b559 in Photosystem II. *EMBO J.* 7, 325–332.

Pospíšil, P. (2011). Enzymatic function of cytochrome b559 in Photosystem II. *J. Photochem. Photobiol. B* 104, 341–347. doi: 10.1016/j.jphotobiol.2011.02.013

Pospíšil, P., Šnyrychová, I., Kruk, J., Strzałka, K., and Nauš, J. (2006). Evidence that cytochrome b559 is involved in superoxide production in Photosystem II: effect of synthetic short-chain plastoquinones in a cytochrome b559 tobacco mutant. *Biochem. J.* 397, 321–327. doi: 10.1042/BJ20060068

Pospíšil, P., and Tiwari, A. (2010). Differential mechanism of light-induced and oxygen-dependent restoration of the high-potential form of cytochrome b559 in Tris-treated Photosystem II membranes. *Biochim. Biophys. Acta* 1797, 451–456. doi: 10.1016/j.bbabio.2009.12.023

Poulson, M., Samson, G., and Whitmarsh, J. (1995). Evidence that cytochrome b559 protects Photosystem II against photoinhibition. *Biochemistry* 34, 10932–10938. doi: 10.1021/bi00034a027

Roncel, M., Ortega, J. M., and Losada, M. (2001). Factors determining the special redox properties of photosynthetic cytochrome b559. *Eur. J. Biochem.* 268, 4961–4968. doi: 10.1046/j.0014-2956.2001.02427.x

Rutherford, A. W., and Krieger-Liszkay, A. (2001). Herbicide-induced oxidative stress in Photosystem II. *Trends Biochem. Sci.* 26, 648–653. doi: 10.1016/S0968-0004(01)01953-3

Shibuya, Y., Takahashi, R., Okubo, T., Suzuki, H., Sugiura, M., and Noguchi, T. (2010). Hydrogen bond interaction of the pheophytin electron acceptor and its radical anion in Photosystem II as revealed by Fourier transform infrared difference spectroscopy. *Biochemistry* 49, 493–501. doi: 10.1021/bi9018829

Shinopoulos, K. E., and Brudvig, G. W. (2012). Cytochrome b559 and cyclic electron transfer within Photosystem II. *Biochim. Biophys. Acta* 1817, 66–75. doi: 10.1016/j.bbabio.2011.08.002

Stewart, D. H., and Brudvig, G. W. (1998). Cytochrome b559 of Photosystem II. *Biochim. Biophys. Acta* 1367, 63–87. doi: 10.1016/s0005-2728(98)00139-x

Sugiura, M., Azami, C., Koyama, K., Rutherford, A. W., Rappaport, F., and Boussac, A. (2014). Modification of the pheophytin redox potential in *Thermosynechococcus elongatus* Photosystem II with PsbA3 as D1. *Biochim. Biophys. Acta* 1837, 139–148. doi: 10.1016/j.bbabio.2013.09.009

Sugiura, M., Nakamura, M., Koyama, K., and Boussac, A. (2015). Assembly of oxygen-evolving Photosystem II efficiently occurs with the apo-Cyt b559 but the holo-Cyt b559 accelerates the recovery of a functional enzyme upon photoinhibition. *Biochim. Biophys. Acta* 1847, 276–285. doi: 10.1016/j.bbabio.2014.11.009

Suorsa, M., Regel, R. E., Paakkarinen, V., Battchikova, N., Herrmann, R. G., and Aro, E.-M. (2004). Protein assembly of Photosystem II and accumulation of subcomplexes in the absence of low molecular mass subunits PsbL and PsbJ. *Eur. J. Biochem.* 271, 96–107. doi: 10.1046/j.1432-1033.2003.03906.x

Swiatek, M., Regel, R. E., Meurer, J., Wanner, G., Pakrasi, H. B., Ohad, I., et al. (2003). Effects of selective inactivation of individual genes for low-molecular-mass subunits on the assembly of Photosystem II, as revealed by chloroplast transformation: the psbEFLJ operon in *Nicotiana tabacum*. *Mol. Genet. Genomics* 268, 699–710. doi: 10.1007/s00438-002-0791-1

Thompson, L. K., and Brudvig, G. W. (1988). Cytochrome b559 may function to protect Photosystem II from photoinhibition. *Biochemistry* 27, 6653–6658. doi: 10.1021/bi00418a002

Tichy, M., Lupinkova, L., Sicora, C., Vass, I., Kuvikova, S., Prasil, O., et al. (2003). *Synechocystis* 6803 mutants expressing distinct forms of the Photosystem II D1 protein from *Synechococcus* 7942: relationship between the psbA coding region and sensitivity to visible and UV-B radiation. *Biochim. Biophys. Acta* 1605, 55–66. doi: 10.1016/S0005-2728(03)00064-1

Tiwari, A., and Pospísil, P. (2009). Superoxide oxidase and reductase activity of cytochrome b559 in Photosystem II. *Biochim. Biophys. Acta* 1787, 985–994. doi: 10.1016/j.bbabio.2009.03.017

Tracewell, C. A., and Brudvig, G. W. (2008). Characterization of the secondary electron-transfer pathway intermediates of Photosystem II containing low-potential cytochrome b559. *Photosynth. Res.* 98, 189–197. doi: 10.1007/s11120-008-9360-8

Umena, Y., Kawakami, K., Shen, J. R., and Kamiya, N. (2011). Crystal structure of oxygen-evolving Photosystem II at a resolution of 1.9 *Å*. *Nature* 473, 55–60. doi: 10.1038/nature09913

Vass, I., and Cser, K. (2009). Janus-faced charge recombinations in Photosystem II photoinhibition. *Trends Plant Sci.* 14, 200–205. doi: 10.1016/j.tplants.2009.01.009

Vass, I., Styring, S., Hundal, T., Koivuniemi, A., Aro, E., and Andersson, B. (1992). Reversible and irreversible intermediates during photoinhibition of Photosystem II: stable reduced QA species promote chlorophyll triplet formation. *Proc. Natl. Acad. Sci. U.S.A.* 89, 1408–1412. doi: 10.1073/pnas.89.4.1408

Whitmarsh, J., and Pakrasi, H. (1996). "Form and function of cytochrome b559," in *Oxygenic Photosynthesis: The Light Reactions*, eds D. Ort and C. Yocum (Dordrecht: Springer), 249–264.

Yadav, D. K., Prasad, A., Kruk, J., and Pospíšil, P. (2014). Evidence for the involvement of loosely bound plastosemiquinones in superoxide anion radical production in Photosystem II. *PLoS ONE* 9:e115466. doi: 10.1371/journal.pone.0115466

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